Welfare Indicators for farmed Atlantic salmon:

tools for assessing fish welfare

Even in a school, there are individuals. Photo: Lars H. Stien

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Contents

Guide to the handbook

Do you want…

..more information on welfare?

- Definition of animal welfare — A.1.1
- Welfare standards, risk assessments and assessment protocols — A.1.4-A.1.8
- Welfare needs of Atlantic salmon — A.2
- Direct Animal-based WIs, LABWIs and OWIs — A.3
- Indirect Environment based WIs, LABWIs and OWIs — A.4
- Classifications and schemes to assess WIs, LABWIs and OWIs — A.5

…to evaluate welfare?

- Flow through systems — B.1
- RAS (recirculating aquaculture systems) — B.2
- Sea cages — B.3
- Submersible cages — B.4
- Snorkel cages — B.5
- Semi-closed containment systems in the sea — B.6
- Cages with liceskirts — B.7

…to evaluate welfare during different routines and operations?

- Crowding — C.1.1
- Pumping — C.1.2
- Stunning and killing in connection with slaughter — C.1.3
- Euthanising — C.1.4
- Bathing and medicinal treatments — C.1.5
- Anaesthesia — C.1.6
- Vaccination — C.1.7
- Transport — C.1.8
- Feed management — C.1.9
- System sanitation — C.1.10
- Grading — C.1.11
- Examination of live fish — C.1.12
- Monitoring welfare with regard to new and emerging technologies — C.2

Introduction to the handbook

Fish welfare is a key issue in commercial farming and is central to many decisions that farmers take during their daily husbandry practices and longer term production planning. It is also a prominent topic for NGO's, animal welfare organisations and charities, regulatory bodies, policy makers and consumers. Farmers have long been interested in optimising the welfare of their animals and actively employ strategies that address fish welfare concerns and attempt to minimise threats to fish welfare. Independent third party organisations have even developed fish welfare standards and certification schemes for certain aquaculture species (e.g. RSPCA welfare standards for farmed Atlantic salmon and rainbow trout, RSPCA, 2018a, b).

The topic of fish welfare has also been covered in numerous aquaculture research and review papers over the years, both from a fundamental and also applied perspective. This wealth of information and documentation can be spread over a wide range of sources that may not be easily accessible for the farmer and other end users. In many cases the wealth of information requires interpretation and re-presentation before it is suitable for use out on the farm.

Once the farmer has information on fish welfare, they need to implement it in their production systems and daily husbandry practices. This can be a serious challenge as even measuring fish welfare can be challenging and the tools available for measurement may not be suitable for all species or all life stages. To assess the overall welfare status of the fish we use Welfare Indicators (**WIs**). Welfare indicators can either be direct animal-based (something you get from the fish), or indirect resource-based (e.g. rearing environment, infrastructure etc.). However, some WIs may be too complex or too difficult to apply on a farm. WIs that are appropriate for on-farm use are termed Operational Welfare Indicators (**OWIs**). WIs that can be sampled on the farms, but need to be sent to a laboratory or other remote analytical facility are termed Laboratory-based Welfare Indicators (**LABWIs**). There are other potential WIs that cannot currently be classified as either OWIs or LABWIs, these are mainly used in research but may be useful in the future or under specific circumstances at present.

From the suite of appropriate OWIs or LABWIs available, the end user then needs to apply these to different production systems and husbandry routines. **This is the goal of this handbook – to assemble a farm-friendly toolbox of fit for purpose Operational Welfare Indicators (OWIs) and Laboratory-based Welfare Indicators (LABWIs) for use out on fish farms in different production systems and husbandry routines. It also includes advice on their implementation and interpretation.**

The FISHWELL welfare indicator handbook is the primary output of the Norwegian Seafood Research Fund (Fiskeri - og Havbruksnæringens Forskningsfond, FHF) project «FISHWELL: Kunnskapssammenstilling om fiskevelferd for laks og regnbueørret i oppdrett». The project group included a diverse range of welfare scientists and veterinarians from Nofima, the Institute of Marine Research, Nord University, the Norwegian Veterinary Institute (all Norway) and the University of Stirling (UK). For a list of authors see each specific section of the handbook.

The authors would like to say a huge thank you to the steering group of the FISHWELL project (Olai Einen, Cermaq; Solveig Gaasø, Marine Harvest Norway; Lene Høgset, Fishguard; Bjarne Johansen, Nordlaks; Berit Seljestokken, Grieg Seafood) for their valuable inputs and guidance, especially during the evolution, preparation and drafting of the handbook. We also wish to thank Susanna Lybæk and her colleagues at Dyrevernalliansen for their thorough and valuable comments and feedback on an earlier version of this handbook.

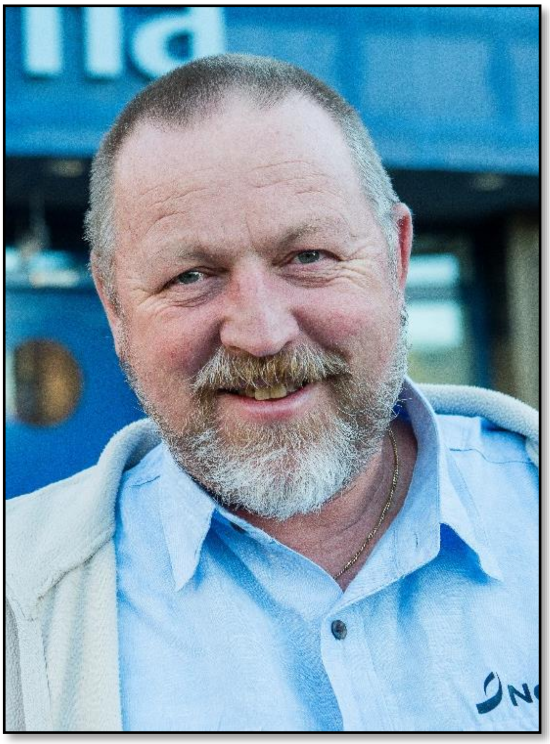
Many thanks also to Lars Speilberg of Scanvacc for kindly providing the pictures for the Speilberg Scale, Alistair Smart of Smart Aqua for providing the pictures and text for the crowding intensity scale and Tim Ellis of CEFAS for permission to reproduce the table summarising the key factors affecting non-invasive methods of cortisol monitoring. Tony Wall of the Fish Vet Group also kindly gave permission to reproduce the morphological scheme for diagnosing and classifying eye cataracts. Thanks also to John Avizienius of the RSPCA for good discussions and for kindly providing permission to reproduce data and text from the RSPCA welfare standards for farmed Atlantic salmon. Many thanks also to Reidar Handegård of ILAB for inputs regarding TGP and nitrogen supersaturation. We would also like to thank Barbo Klakegg and Renate Andersen of Åkerblå, Per Anton Sæther of Marin Helse AS, Ida-Kathrin G. Nerbøvik and Britt Tørud of the Norwegian Veterinary Institute, Ioan Simion of HaVet Fiskehelsetjeneste AS and Christian Karlsen and Kjell J. Merok of Nofima for kindly providing pictures for the FISHWELL morphological scoring system.

The FISHWELL handbook cites scientific literature in two different formats. Part A utilises an in-text citation (author/authors and year), whereas Parts B and C cite references using a numeric style.

This handbook is dedicated to our dear friends and colleagues Kjell Ø. Midling and Thomas Torgersen, who unfortunately passed away before the handbook was completed.

Kjell was a world leader in operational fish welfare, both in aquaculture and fisheries and really helped put applied fish welfare on the map for both the research community and the industry. His incredibly infectious enthusiasm, energy, creativity, humour, laughter and comprehensive knowledge and expertise are deeply missed and never forgotten.

Thomas was an exceptionally intelligent and knowledgeable researcher whose models and experiments showed how farmed fish were influenced by and adapted to varying environments, and where the thresholds lay for their coping abilities and welfare. Thomas had a great appreciation and rich knowledge for life's many qualities. His enthusiastic stories, clever humour and warm laughter made life richer for all who knew him. He left us far too early and will be deeply missed.



Kjell Ø. Midling

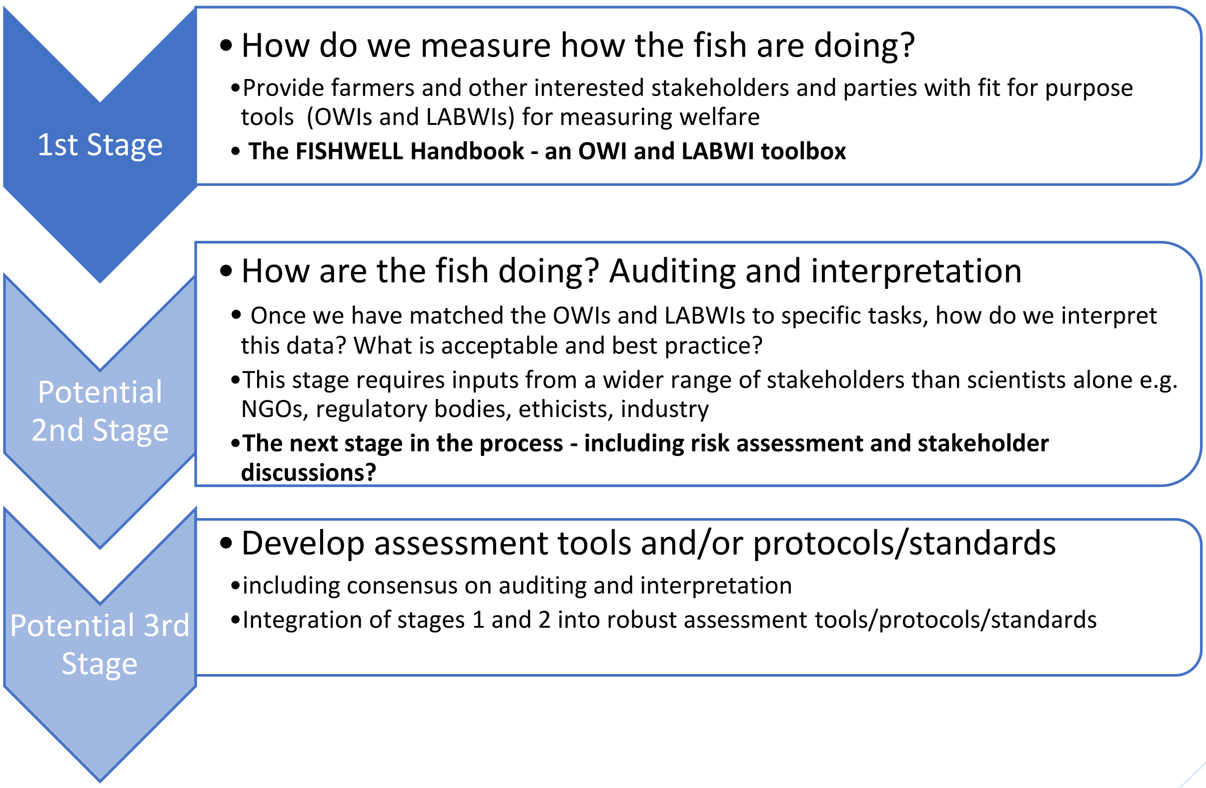
Thomas Torgersen

Objectives of the handbook

Our handbook has three key objectives:

1. Provide the user with an updated scientific summary of the welfare of Atlantic salmon in relation to its welfare needs at different life stages. We also link welfare indicators to specific welfare needs. We describe how each indicator can be used, important parameters or thresholds to look for, the pro's and con's of using it and evaluate whether it's an Operational Welfare Indicator (OWI) or a Laboratory-based Welfare Indicator (LABWI). **See Part A of the handbook.**
2. Provide the user with information on which OWIs and LABWIs are appropriate and fit for purpose in different production systems. **See Part B of the handbook.**
3. Provide the user with information on which OWIs and LABWIs are appropriate and fit for purpose for different husbandry routines and operations. **See Part C of the handbook.**

The goals of putting together the toolbox are to provide the Norwegian Atlantic salmon aquaculture industry and other interested stakeholders with the correct, science based fit-for-purpose tools (OWIs and LABWIs) for measuring and documenting welfare. For Norwegian salmon production we have viewed this as a three stage process (see below). The FISHWELL handbook is the first stage in this process – scientific justification for choosing which OWIs and LABWIs are most appropriate and where (in relation to welfare needs, life stages, rearing systems and routines). We hope that the next phase, **in an open process, involving a much wider stakeholder group (e.g. NGOs, ethicists, biologists, fish vets, regulators and the industry) will include discussion and development of consensus on what is acceptable and unacceptable regarding fish welfare**. The third stage would be developing/refining welfare assessment tools or protocols, based upon stage 1 and 2. These latter two stages are conceptual at this time, but we present this as a road map to where, in our opinion, operational fish welfare in Norway should be. Some certification schemes already adopt similar approaches e.g. the RSPCA in the UK.



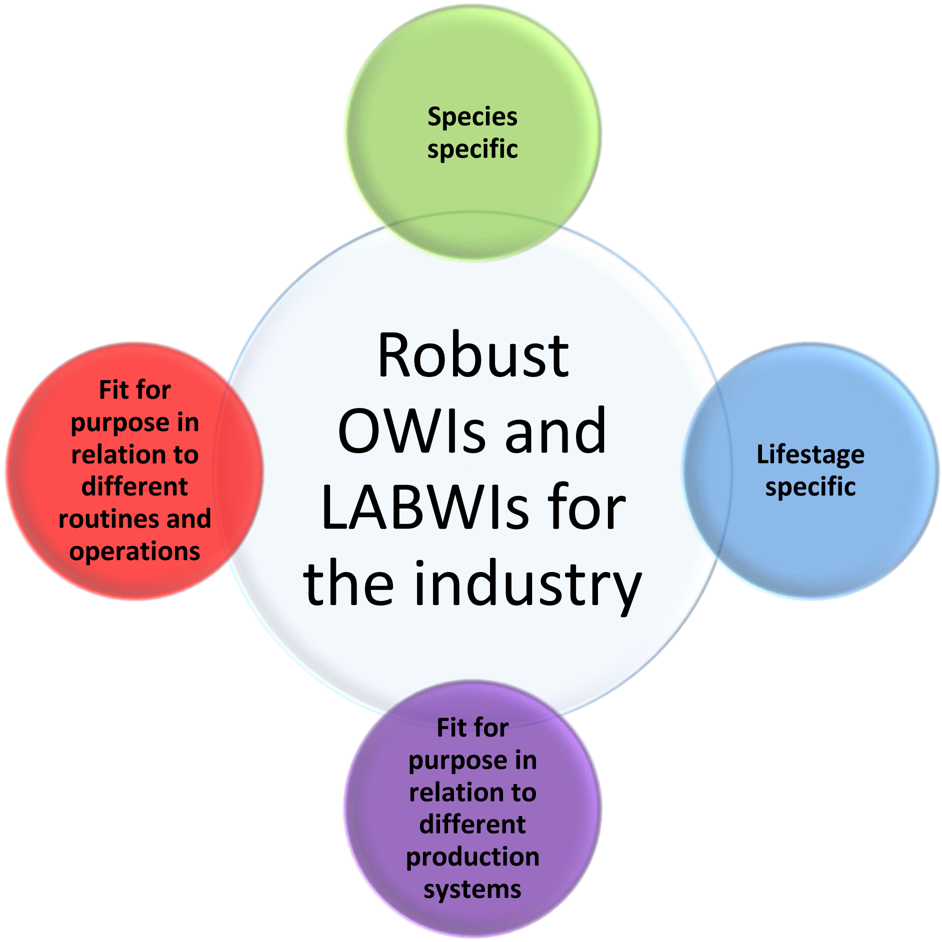
The OWIs and LABWIs have been evaluated in terms of their:

- **Relevance –** their relevance in relation to the fish.
- **Usability –** their ease of use on the farm.
- **Reliability** – is the data they produce repeatable? Is it good enough to make informed decisions on the fish's welfare?
- **Suitability for aquaculture** – are they appropriate and fit for purpose indicators for the fulfilment of the welfare needs of the fish in specific production systems or husbandry routines?

The validation of the OWIs and LABWIs for assessing fish welfare are based upon scientific literature and also existing welfare assessment and assurance schemes and we state the source of this validation. This will allow the reader to identify the sources of the relevant information if they require more detailed information regarding the topic.

Where an OWI and LABWI is potentially suitable for assessing welfare under different farming situations, but where scientific data is lacking and it is not included in existing welfare assessment schemes, we highlight this as a potential tool for assessing welfare. This is especially relevant with new and emerging husbandry routines, technologies and production systems.

It is not within the remit of this handbook for the authors to give an opinion on what is good/acceptable – bad/unacceptable in terms of welfare. Recommendations are only provided where they are supported by science. This is to provide policy makers or regulatory bodies with concrete information upon which to base their decisions.



The goals of the FISHWELL handbook are to provide fit for purpose species and life stage specific OWIs and LABWIs in relation to different production systems and husbandry routines. (Figure: Chris Noble and Jelena Kolarevic)

Welfare Indicators for farmed Atlantic salmon – Part A. Knowledge and theoretical background

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What is fish welfare? Photo: Lars H. Stien

Contents

1. Introduction to fish welfare

1.1. Animal welfare

The term 'welfare' addresses the "*physical and mental health*" and wellbeing of an individual or group (cited from Cambridge Dictionary © Cambridge University Press 2018 https://dictionary.cambridge.org/). We therefore think of good animal welfare as making sure that the animals are treated well, that the animals have a life worth living and that they experience a good quality of life. In particular, we want to avoid animal suffering and cruelty against animals, which most people feel is unethical and wrong.

There are many benefits to improving animal welfare in food production systems and fish farming is no different. Fish farmers know this and have directly or indirectly tried to optimise fish welfare over the years; they want their animals to thrive, grow and stay healthy, all of which are usually correlated with good welfare. In addition to good farm husbandry and stock person ethics, animals in Norway and most European countries are protected by laws and regulations, e.g. the Norwegian Animal Welfare Act (2009) that protects all vertebrates.

To protect and assure welfare, we need to define it in current terms. There is no consensus or universal definition of animal welfare, and the control of fulfilment of laws and regulations are hampered by this lack of conceptual clarity. You can adopt a functions-based approach to defining welfare that equates welfare with biological functioning; a healthy animal with good growth and performance is said to have good welfare. Nature-based definitions state that an animal has a high level of welfare if it is given a natural environment and allowed to perform innate species-specific behaviours. A third feelings-based approach emphases affective states (emotions) and suggests an animal has a high level of welfare if it is free from long lasting negative emotions (such as pain, fear and distress) and can also experience pleasure (Duncan 1993, 1996, 2005; Torgersen et al., 2011). In practice, there is great deal of overlap among the three approaches, but when including physiological function, feelings and living conditions into the same concept it becomes very complex and difficult to know how to best measure and assess animal welfare.

Most animal welfare scientists and laypeople agree that animal welfare relates to what the individual animals experience and perceives, and in the following handbook we will use the following definition:

Animal welfare = the quality of life as perceived by the animal itself (after Stien et al., 2013)

1.2. Fish Cognition

To fulfil their needs, survive and reproduce, fish must interact with their environment and sense the properties of their surroundings. Fish have a rich toolbox of sensory organs adapted to their specific habitats. Naturally, there are big differences in sensory abilities between species. The most common senses are smell, taste, vision, hearing, sense of vibration, touch, temperature, water movement, body position and movement and various types of nociceptors (touch, heat, acid, etc.). Every second millions of signals from the sensory systems arrive at the brain. There is no benefit in collecting all this information if the fish cannot make any sense of it. From the myriads of signals collected, they must make an inner representation of their outer world and what is going on there. Their experienced "Umwelt" (von Uexküll, 1921) or world view from their own perspective is most probably very different from ours, and also the different species must have a different "world view" depending on their sensory systems and brains. Without the ability of some kind of perception, learning, memory, cognition fish could not behave and live as they clearly do from our observations.

We know animals can perform complex behaviours by instinct or innate abilities. The presence of awareness or learning is based on evidence of behaviours or responses which change or adapt to situations and are persistent. In fish there is clear evidence of learned and adaptive behaviours across a wide range of species. In order to learn and adapt it is necessary to integrate neural process into an experienced whole and the ability to know what is potentially beneficial and potentially harmful is dependent upon learning and memory. What is sensed and observed in the present must be put into context with past experiences to interpret and be potentially acted upon. Millions of photons reaching the retina result in signals to the brain which are modelled into entities and movement. These models of objects and movements made by the visual system in the brain must build on past experiences of similar objects and movements. Objects must also be put into categories of concepts, to be the same or similar or different from previous observed objects, otherwise all new objects will be different and unknown.

Many studies have shown that fish have a qualitative experience of the world, have a good ability to learn and remember, have anticipations of the future, have a sense of time, can associate time and place, can make mental maps of their surroundings, can know their group members and can cooperate with them (Brown et al., 2011; Brown, 2015; Nilsson et al., 2010). Fish can also learn by observing others, and some fish can even make innovations and use tools (Bratland et al., 2010; Nilsson et al., 2010; Millot et al., 2014).

The question of whether fish are conscious is still subject to debate, which is not surprising since science has no clear consensus on how consciousness emerges in the brain-body, even in humans. The main opponents against the existence of consciousness in fish claim that since the fish's brain lacks the neocortex they cannot be conscious or feel pain since the neocortex is essential for consciousness in humans and higher primates (Rose, 2002; Key, 2016). However, other scientists claim that this argument is flawed as other parts of the brain can have analogue functions and that the neocortex is not essential for consciousness even in humans, but rather defines the quality of the consciousness (Balcombe, 2016; Braithwaite and Huntingford, 2004; Merker, 2016). It is also very difficult to explain the advanced behaviour and abilities of fish which are apparently dependent on consciousness (Braithwaite and Huntingford, 2004; Broom, 2016).

1.3. Welfare Needs

All animals need access to resources to gain enough energy to survive, grow and reproduce. They also need to protect themselves from dangers such as predators or harmful environments. **An animal's needs can be divided into ultimate or proximate needs. Ultimate needs are necessary for its immediate survival, whilst proximate needs improve its ability to succeed in the long term (Dawkins, 1983).** Ultimate needs include respiration, nutrition, thermoregulation, maintenance of osmotic balance and body integrity. Examples of proximate or behavioural needs are i) behaviours that improve body control and strength (like jumping in salmon or play in juvenile mammals), ii) exploratory behaviours that improves the chances of finding food, or iii) social behaviours that increase connections between individuals and increase e.g. the probability of detecting predators.

The emotional reward systems in the brain generate feelings (e.g. pain, hunger, fear, aggression, anticipation, satisfaction) to guide an animal's behaviour towards fulfilling its needs (Panksepp 2005; Spruijt et al., 2001). When a need is not satisfied, it can cause frustration and suffering and reducing welfare irrespective of whether it is ultimate or proximate (Dawkins, 1990). Some needs are not monitored and acted upon by the emotional system. These can be related to the animal's resources, such as vitamins or minerals they are unlikely to lack in their diet, or to the sensing of potentially harmful chemicals they are unlikely to encounter or cannot do anything to avoid.

If welfare needs are compromised, or conditions become worse, it is detrimental to welfare and the animal can experience negative feelings. If welfare needs are fulfilled, or conditions improve, the animal can experience rewarding or pleasurable feelings.

1.4. Different types of Welfare Indicators

We cannot simply ask a fish how it is feeling. We must therefore use welfare indicators (WIs) to get information about the state of its welfare. Welfare indicators can either be direct, **animal based indicators**, centred on observations of attributes with the animal itself or indirect **environment based indicators**, centred on the resources and environment the animals are subjected to (Duncan, 2005; Stien et al., 2013), see text box below.

Animal based WIs are attributes from the animal itself that indicate that one or more welfare needs have not been fulfilled. They can be indicators of prior welfare problems e.g. results of previously poor nutrition or feeding response which can be identified by the condition factor of the fish or the degree of emaciation. They can also indicate that the fish will not be able to fulfil its welfare needs, e.g. damaged gill tissue. This is not only evidence of a direct injury to living tissue, but may also limit the respiratory capacity of the fish. This in turn will be related to other factors and damage to gills may not result in respiratory distress unless oxygen levels are low or the fish's oxygen demand is increased through stress or exercise. Behavioural indicators may tell an observer about the welfare of the fish at the point of observation. For example, high ventilation rates and gasping at the surface may indicate inadequate oxygen levels or damage to the respiratory system. Animal based WIs are also sometimes called outcome based WIs emphasising that these WIs measure the result of the treatment on the animals themselves.

Animal based indicators are more directly linked to the state of the fish than environmental indicators. However, environmental indicators may predict a problem whilst animal based indicators may only become apparent once the animal is already experiencing poor welfare. An exception is where the observation of reduced welfare in a proportion of the individuals within a group may predict a problem in individuals that are currently unaffected.

Environment based WIs include many aspects of the farming system from water quality to management processes. In terms of water quality, we can assess environmental factors to determine when they are outside a known tolerance or preference range, with the risk of poorer welfare. Examples of these include water temperature and oxygen levels that have to be within a certain range for the fish to fulfil their metabolic requirements for thermoregulation and respiration. As environment based indicators describe the environment rather than the animals themselves, they are classified as indirect welfare indicators. However, as they describe factors that are known to indirectly influence welfare, they are still an important set of indicators in the welfare toolbox. They are also often easy and quick to measure. In addition, environmental indicators may also give indications of future welfare problems caused by long-term exposure to suboptimal conditions before they are visible on the animal.

Whilst many animal and environment based WIs are good for quantifying fish welfare in research or in controlled studies, they are not all are straightforward and easy to use on a fish farm. **WIs that can be used in an on-farm welfare assessment are termed Operational Welfare Indicators, OWIs** (see Noble et al., 2012a) and must:

i) provide a valid reflection of fish welfare,

ii) be easy to use on the farm,

iii) be reliable,

iv) be repeatable,

v) be comparable,

vi) be appropriate and fit for purpose indicators for specific rearing systems or husbandry routines.

Further, to compare between cages or farms or between time points it is important that the indicators are measured in a standardised manner.

Some WIs, already in use and still being developed, satisfy the majority of OWI requirements, but have to be sent to a laboratory or other remote analytical facility. Provided these WIs give the farmer a robust indication of the welfare state of the fish in an acceptable timeframe they are termed **Laboratory-based Welfare Indicators (LABWIs)**.

While environment based WIs are useful for assessing the potential risk to welfare rather than the actual welfare of the animal, we need to have animal based indicators wherever possible.

Definitions of welfare indicators used in this handbook

Animals are assumed to have good welfare when they have their welfare needs fulfilled.

- Welfare needs include: **ultimate needs** (or basic needs) which are necessary for immediate survival and good health (including respiration and nutrition) and **proximate needs** (or behavioural needs) which are necessary for long terms success (including social contact).
- **Welfare indicators** (**WIs**) are observations or measurements that provide information about the extent to which the animal's welfare needs are met.
- **Operational Welfare Indicators** (**OWIs**) are WIs that can realistically be used on the farm.
- **Laboratory Based Welfare Indicators** (**LABWIs**) are WIs that require access to a laboratory or other analytical facilities to provide useful information.
- Welfare Indicators can be:
    - **Animal based** – observations made on or from the animal (also known as Direct WIs or Outcome WIs),
    - **Environment based** – Observation made on the environment, infrastructure and processes (also known as Indirect WIs or Resource-based WIs).

1.5. Welfare standards

There are several standards promoting more welfare friendly aquaculture. One of the most prominent that is specifically and solely aimed at welfare assurance is the RSPCA welfare standard for farmed Atlantic salmon (RSPCA, 2018a) that was originally developed for Atlantic salmon in 2002. A corresponding welfare standard for farmed rainbow trout (RSPCA, 2018b) was also developed in 2014 (Anon, 2014). They give detailed and comprehensive species-specific welfare requirements for husbandry practices, environmental quality, feeding, health management, grading, vaccination, transport, slaughter/killing and crowding. Information of life-stage specific welfare requirements is also given. The standards are based on scientific, veterinary and practical industry expertise and utilise numerous animal based WIs (outcome WIs) and also indirect, environment WIs. More than 70 % of UK salmon production is certified, and the RSPCA report that the standards have contributed to an improvement in fish welfare in UK fish farms (Anon, 2014). Numerous excerpts from the RSPCA welfare standards are presented in this handbook (with kind permission from the RSPCA) especially with regard to some environment based OWIs e.g. oxygen and routines such as feed withdrawal, crowding, grading and transport, amongst others. For further details on the RSPCA welfare standards we recommend the reader refer directly to the original documents, which are regularly updated in consultation with scientists, veterinarians and the industry using the latest scientific findings and also key practical experience (https://science.rspca.org.uk/sciencegroup/farmanimals/standards/salmon).

Another prominent standard that addresses fish welfare is the Aquatic Animal Health Code developed by the World Organization for Animal health (OIE) to ensure safety from infectious agents in international trade in aquatic animals (OIE, 2015a). This code includes some general guiding principles on fish welfare and lists of requirements for minimizing any possible negative welfare effects of transport, stunning and killing. Similarly, the GLOBALG.A.P. aquaculture standard provides extensive checklists for ensuring that measures for maintaining fish welfare are in place (GLOBALG.A.P., 2016). Many of the criteria in the checklist refer back to the Aquatic Animal Health Code. GLOBALG.A.P. offers training courses on understanding and complying with the standard. Fish farming companies must also be inspected annually and approved by an accredited body in order to become GLOBALG.A.P. certified. Most major salmon farming companies have GLOBALG.A.P. certification. However, the focus of the standard is mainly on whether the staff are trained, if records are kept and if the equipment and farming routines are judged appropriate for the situation. The GLOBALG.A.P. standard is therefore primarily a list of environment or resource based indicators, and has very limited details on how to assure animal welfare. This is partly remedied in the Code of Good Practice for Scottish Finfish Aquaculture (Scottish Salmon Producers Organisation, 2016), which is similar to the GLOBALG.A.P. standard, but with many of the checkpoints including more specific requirements for fish welfare. Typical checkpoints, such as those that cover the rearing environment include, water quality, monitoring recommendations and water flow. Compliance with the code is audited by independent certification bodies and about 90 % of Scottish salmon production is covered by the code.

Another standard that addresses fish welfare comes from the Aquaculture Stewardship Council (ASC), which was established by the WWF and IDH (Dutch Sustainable Trade Initiative) in 2010. After a number of roundtable discussions involving a wide range of stakeholders including aquaculturists, scientists, NGOs, retailers, and governmental bodies, the ASC published a standard for salmon aquaculture in 2012 (ASC, 2012). The standard is primarily aimed at limiting environmental impacts from aquaculture, but also has some criteria related to fish welfare demanding regular visits from a designated veterinarian, health management plans, disease monitoring and limits for mortality. This standard is gaining popularity and more and more fish farms are becoming ASC certified; in 2015 there were 84 ASC certified fish farms in Norway. The Best Aquaculture Practices (BAP) Standards and

Guidelines for Salmon Farms is an international certification programme developed by the Global Aquaculture Alliance (BAP, 2016). Although the standard predominantly focuses on environmental responsibility, the standard also covers fish welfare. Its requirements for fish welfare are relatively brief, but are accompanied by an introductory text defining fish welfare and providing a list of behavioural indicators, colour changes and morphological abnormalities that can be used to identify and mitigate against potential welfare problems.

1.6. EFSA - Risk Assessments

The Scientific Panel for Animal Health and Welfare (AHAW) of the European Food Safety Authority (EFSA) has issued expert opinions on the welfare of farmed Atlantic salmon and rainbow trout in relation to different life stages and under different rearing systems (EFSA, 2008a, b). For each life stage and husbandry system they identified potential fish health and welfare hazards, ranking them according to severity, the proportion of the population affected, the probability of their occurrence and also their duration. Farmers or producers can use these lists to get an overview of where to focus their efforts to protect or improve welfare. AHAW grouped the hazards into environment, animal, husbandry, feeding and disease hazards. Environment hazards included: i) rapid changes in water temperature, ii) excessive water temperature, iii) excessive water flow, iv) low water oxygen content, v) excessive carbon dioxide content (recirculating systems), vi) excessive ammonium (recirculating systems), vii) inappropriate light regimes, viii) inappropriate salinity and ix) lack of vertical support (alevins). Animal hazards included: i) aggression and ii) low/high stocking intensity. Husbandry hazards included: i) lack of biosecurity, ii) lack of staff training, iii) lack of grading, and iv) handling. Feeding hazards included: i) unbalanced diet, ii) feed deprivation (long term), iii) deficiency of nutrients, and iv) vegetable proteins. Disease hazards included: i) saprolegnia, ii) eye Lesions, iii) IPN, iv) furunculosis, and v) sea lice. Welfare indicators related to crowding included: i) dorsal skin colour changed from grey-black to blue-green, ii) burst swimming close to the surface, iii) fish swimming on their side, iv) fish gulping at surface, v) fish exposed to air and vi) presence of exhausted fish (see EFSA, 2008a,b for full details). AHAW also published an expert opinion on the welfare aspects of the main systems for stunning and killing of farmed Atlantic salmon and rainbow trout (EFSA 2009a, b). Welfare indicators related to stunning included: i) excessive tail flapping and ii) signs of consciousness as evidence of inappropriate stunning.

1.7. Welfare assessment protocols

In order to encapsulate the different aspects of animal welfare, most animal welfare assessment protocols and researchers use a combination of environment and animal WIs. They typically define a set of WIs that they believe are appropriate for detecting potential effects and which are practical and affordable to use. This can include indicators describing the rearing environment, the physical state of the fish, its behaviour and its appearance. Mortality may be also used as an indicator in such contexts. After the treatment, the measurements are then discussed individually or analysed together using statistical techniques. Examples include, the monitoring program for physical damage or deformity suggested in the RSPCA welfare standards for farmed Atlantic salmon (RSPCA, 2018a), the welfare assessment protocol developed by the Norwegian Veterinary Institute (NVI) (Grøntvedt et al., 2015; Gismervik et al., 2016, 2017) and the Salmon Welfare Index (SWIM) (Stien et al., 2013; Pettersen et al., 2014). These protocols score the welfare of individual fish based on a set of welfare indicators describing their appearance (Table 1.7-1). Each welfare indicator is divided into levels from good to bad welfare and the results are typically represented as the distribution of sampled fish before and after treatment. In the SWIM-protocol the levels are not only ranked from good to bad, but also weighted according to their suggested welfare impact on the fish. The welfare of the fish is calculated as an aggregated score from 0 (worst) to 1 (best). The advantage of using animal WI measurements, such as in these protocols, is that they are largely system and treatment independent and can be used in most situations. The protocols can be used as an early warning system, alerting the farmer that something is potentially wrong and warrants further investigation, preferably before mortality starts to increase.

Table 1.7-1. *Welfare indicators describing the appearance of individual fish in the RSPCA welfare standards for farmed Atlantic salmon (RSPCA, 2018a), the welfare assessment protocol by The Norwegian Veterinary Institute, NVI (Grøntvedt et al., 2015; Gismervik et al., 2016, 2017) and in SWIM 1.1 (Stien et al., 2013; Pettersen et al., 2014)*

RSPCA protocol	NVI Protocol	SWIM 1.1
Eye loss/damage	Eye damage	Eye status
Snout injury	Snout injury	Snout jaw wound
Jaw deformity	Cataract	Upper jaw deformity
Operculum deformity	Fin damage	Lower jaw deformity
Dorsal fin damage	Scale loss	Opercula status
Pectoral fin damage	Skin haemorrhage	Fin condition
Tail fin damage	Wounds	Skin condition
Scale loss/skin damage	AGD gill score	Spine deformity
Spine deformity	Gill score (pale spots)	Sea lice per cm^2
Sea lice damage	Gill paleness	Gill status
		Condition factor
		Emaciation status
		Sexual maturity
		Smoltification state

2. Welfare Needs of salmon

Broadly speaking the welfare needs of salmon can be categorised into needs directly linked to its available resources, water environment, health and behavioural freedom (Fig. 2-1). The list of welfare needs utilised in this handbook are adapted from Mellor et al., (2009) and Stien et al., (2013). Fulfilling or increased fulfilment of the needs are rewarded by the systems in the brain releasing opioids that give pleasurable emotions and feelings, telling the animal that their actions were appropriate or good (Dawkins, 1990; Spruijt et al., 2001; Panksepp and Biven, 2012). When their state of needs gets worse their "punishment circuits" release neurotransmitters that give unpleasant emotions and feelings of e.g. frustration, fear, aggression, depression or pain (Dawkins, 1990; Spruijt et al., 2001; Panksepp and Biven, 2012).

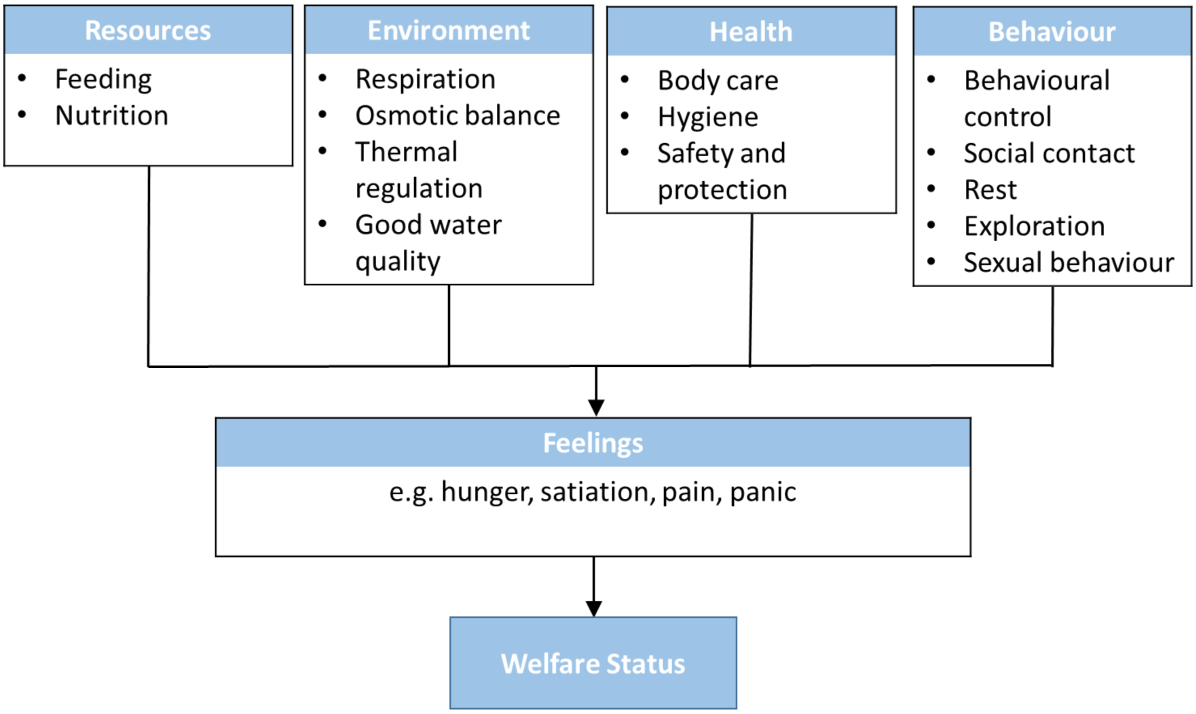


Fig. 2-1. *The welfare needs of salmon can broadly be categorised into available resources, a suitable water environment, good health and freedom to express behaviours. The degree of fulfilment of these needs affects their mental state and thereby the welfare status of the animals. Adapted from "Mellor, D. J., Patterson-Kane, E. & Stafford, K. J. (2009) The Sciences of Animal Welfare. John Wiley & Sons Ltd, Oxford, UK, 212 pp. Copyright 2009" with permission from Wiley-Blackwell.*

Suggested welfare needs for salmon (based upon Stien et al., 2013)

Feeding and nutrition
Regular access to nutritious and healthy food

Respiration
Pumping water over the gills to allow for the uptake of oxygen and the release of carbon dioxide

Osmotic balance
Access to water with salinities and pH to which they can adapt.

Thermal regulation
Access to temperatures to which they can adapt. Allowing the fish to optimise their metabolism and temperature, including thermal comfort

Good water quality
Absence of deleterious concentrations of gasses and ions, metabolites, toxins, and particles

Body care
Ability to clean and maintain their body, scratch or remove parasites

Hygiene
Exposed to environments with low concentrations of harmful organisms (e.g. parasites, bacteria and virus)

Safety and protection
Possibility to avoid perceived danger and potential injuries

Behaviour control
Possibility to stay balanced and move as they wish

Social contact
Access to companions and partners

Rest
Chance to recover from high levels of activity and rest/sleep

Exploration
Fish are given the opportunity to search for resources and information if required

Sexual behaviour
Ability to perform sexually behaviour

While some needs are essential for welfare and survival for all fish species at all life stages, some of the behavioural needs may be more important during, or restricted to, one or more life stages (e.g. sexual behaviour), or as a form of training for a later life stage (e.g. salmon jumping behaviour which may be training for later successful passing of waterfalls on their way to the spawning grounds). Some needs are always relevant (e.g. respiration) while other needs may be irrelevant during shorter acute events such as handling (e.g. feeding and exploration). In the case of respiration, the need must be continuously fulfilled or the fish can die. Other welfare needs, such as exploration, are not crucial for survival but the fish's welfare may still be reduced if they are not fulfilled.

2.1. Feeding and Nutrition

Hunger can be defined as "*the feeling you have when you need to eat*" (Cambridge Dictionary © Cambridge University Press 2018 https://dictionary.cambridge.org/). It motivates animals to search for food and eat, and successful feeding is rewarded both by i) the feeling of satiation and the end of hunger, and ii) the taste and smell of the preferred food. Salmon are adapted to variable and seasonal food availability. The intake of food with the right content is a fundamental need and essential for growth, physiological functioning and health. Feeding motivation, food preferences and aversion are therefore strong motivational factors. Various conditioning experiments have shown that fish show strong anticipatory behaviour for their preferred food sources, indicating an emotional qualitative component of wanting and liking, and an internal 'image' of what they anticipate (Warburton, 2003). Feeding motivation, anticipatory behaviour and feed intake can also increase when fish are deprived of food, indicating emotional states of hunger and an urge to eat, and that access to food is emotionally rewarding. For all animals, it is important to avoid food with a low nutritional value or that can be potentially harmful. This can already be observed at the larval stage where the fish show strong food preference. Fish also show food aversion towards food associated with sickness (Manteifel and Karelina, 1996).

Feeding, when defined in terms of satisfying a need, can be interchangeable with the term appetite "*a natural desire to satisfy a bodily need, especially for food*" (OxfordDictionaries.com © Oxford University Press, 2018). A key goal in relation to satisfying welfare needs would therefore be to feed the fish a species and life stage specific ration that satisfies its appetite requirements. In practice, this goal can be difficult to achieve as the appetite of both individual and group held fish can fluctuate both hourly and daily (Juell et al., 1993; Noble et al., 2008) and variability in appetite for a given life stage of Atlantic salmon may not always be an indicator of poor welfare. For example, juvenile Atlantic salmon may exhibit natural adaptive anorexia in winter and choose not to feed and low appetite may therefore not be an indicator of poor welfare (Huntingford et al., 2006). Appetite and the motivation to feed may therefore be dependent upon life stage or an individual's energy reserves (Huntingford et al., 2006).

The obvious welfare impacts of not fulfilling the need to feed arise when fish are not fed to satiation. However, the exact effects upon the fish are unclear, and are affected by prior history, the individual's energy reserves, the species and the life stage. It can also be affected by the degree of underfeeding, also termed feed restriction (fish are fed, but at reduced amounts) or whether the fish are fasted and food is withdrawn (fish are deprived of feed). Salmon can be more competitive when hungry (Jobling et al., 2012; Damsgård and Huntingford, 2012) and underfeeding has been shown to cause more fighting and injuries than no feeding at all (Ellis et al., 2008). Therefore, for salmon, in the short term, a complete withdrawal of food may result in better welfare than underfeeding.

Fasting, where feed is withheld from fish for a number of days does occur in aquaculture prior to husbandry practices such as slaughter, transport, grading and during the transfer from freshwater to seawater or during a fish health routine or operation (Branson, 2008). Challenging environmental conditions, such as high temperatures or low oxygen levels can also lead to the withdrawal of feed to limit welfare and mortality risks. Furthermore, the outbreak of an infectious disease or agent can also be alleviated by a temporary period of feed withdrawal (Branson, 2008). Underfeeding, where fish are fed at a level that is below satiation, can also occur in a commercial farming situation if the farmers i) have problems assessing satiation levels in large groups, or ii) feed the fish to feed tables, which do not consider both short- and long-term variability in group appetite satiation levels (Noble et al., 2008), or iii) when technical or environmental conditions prevent the farmer feeding the fish to satiation within any given day. In juvenile Atlantic salmon, both short (ca. 10 days) and long (ca. 30 days) periods of feed restriction can be detrimental for fish welfare by increasing aggression and fin damage (Cañon Jones et al., 2010; 2017).

2.2. Respiration

The uptake of oxygen and the release of carbon dioxide is essential for aerobic metabolism and to maintain pH in the body. A salmon will die within minutes without it (see Stien et al., 2013). The *standard metabolic rate*, i.e. the metabolism of fasted and resting fish, cannot be maintained below a certain dissolved oxygen saturation level (S_{crit}, which is dependent on temperature). Metabolism is higher for satiated and/or active fish and the lowest oxygen saturation allowing aerobic metabolism in fed and active fish is called the *limiting oxygen saturation* (LOS). In practical terms, farmed fish are only rarely or never fully fasted and resting, and activity levels are usually high. LOS is therefore the most relevant lower limit for oxygen saturation in fish farms. When oxygen saturation is below the level required for aerobic metabolism (hypoxia) the fish switch to anaerobic glycolysis (Neill and Bryan, 1991; Remen et al., 2012). Anaerobic metabolism will eventually deplete the substrates available for glycolysis and can also lead to a build-up of anaerobic by products, which can lead to death (van den Thillart and van Waarde, 1985; van Raaij et al., 1996; Remen et al., 2012). Hypoxia can also cause a stress response in salmonids (McNeill and Perry, 2006; Remen, 2012). Efficient respiration and sufficient diluted oxygen in the water is therefore a crucial welfare need for salmon. In addition to hypoxia in the holding water, respiration may be limited by air exposure during handling and slaughter, and by non-functional gills which may be the result of injuries, diseases or parasites.

2.3. Osmotic balance

Salmonids are anadromous, meaning they live parts of their life in both freshwater and seawater. In freshwater, salmonids are hyperosmotic, meaning their bodily fluids have higher salinity than the surrounding water and that water diffuses in and salt ions out. This loss of ions is counteracted by the active uptake of ions (Na^+ and Cl^-) through the gills. In freshwater the gills' filtration rate and reabsorption of salt is high, and the fish excrete excess water through diluted urine. In seawater, salmonids are hypoosmotic, meaning that their bodily fluids have lower salinity than the surrounding water. This constitutes a constant threat of dehydration through the loss of bodily fluids and increased ion inflow. The water loss to the surroundings is countered by drinking seawater and low blood filtration rates by the kidneys. The surplus of ions (Na^+, Cl^-, Mg^{2+} and Ca^{2+}) is excreted through the gills and kidneys. During the smoltification process, the activity of the gill enzyme Na^+, K^+-ATPase (NKA) is increased. This enzyme is important for salmonids to maintain their osmotic balance (Jonsson and Finstad, 1995) and to be able to survive in salt water the salmon parr must be able to tolerate the hyper osmotic seawater. There is also a danger that the smolt revert back to their freshwater physiology if they are kept in freshwater too long. Small fish are more sensitive to inappropriate salinities and small salmon that are not smoltified will suffer from dehydration and die within days if released too early into the sea.

2.4. Thermal regulation

Temperature is one of the most important environmental factors influencing salmon biology. Salmon are poikilotherms, meaning their body temperature is regulated by the ambient water temperature. Temperature consequently influences factors like growth rate, the timing of migration, smoltification, immunity and metabolism. The thermal preference of a species often coincides with the species' thermal optimum for physiological functioning and this may shift with age and among different life stages (Sauter et al., 2001).

Poikilothermic animals can only regulate their body temperature through their behaviour. In other words, salmon can only react to inappropriate water temperatures by swimming to another area (Sauter et al., 2001). This behavioural thermoregulation helps salmonids adapt through increased fitness and survival. Water temperature can serve as a cue in a behavioural response (Sauter et al., 2001). The effect of thermal stress upon the fish depends upon the severity and duration of its exposure, which can in turn affect long-term survival (Ligon et al., 1999). Salmonids commonly respond to acute temperature fluctuations via short-term physiological responses including elevated oxygen consumption, and also behaviourally by increasing activity levels (Peterson and Anderson, 1969; Beitinger et al., 2000; Jason et al., 2006; Bellgraph et al., 2010; Folkedal et al., 2012a, b). Temperature fluctuations also induce physiological and behavioural acclimation, with these processes taking days to weeks (Brett and Groves, 1979; Jobling, 1994).

In sea cages it has been shown that Atlantic salmon are attracted by temperatures up to around 17 °C and try to avoid higher temperatures above 18 °C (Oppedal et al., 2011a, b). Reduced feed intake and growth of Atlantic salmon post-smolt reared in tanks are reported at 18 °C (Handeland et al., 2008) and 19 °C (Hevrøy et al., 2012). This corresponds well with the behavioural avoidance of temperatures >18 °C of farmed salmon reared in temperature stratified sea cages (Oppedal et al., 2011a, b), and suggests an upper limit for sustainable thermal conditions in Atlantic salmon post-smolts. Both wild and farmed post-smolts are exposed to temperatures that fluctuate in both the long (e.g. seasonally) and short-term (either via abrupt fluctuations, or by voluntarily changes in swimming depth) (Oppedal et al., 2011a, b). Diurnal migration patterns expose salmon held in sea cages to temperatures spanning 6 – 18 °C (Johansson et al., 2006). Deep dives (100 to 1000 m) undertaken by homing kelts (Lacroix, 2013) imply that salmon are highly flexible within their zone of thermal comfort.

2.5. Good water quality

All fish need to live in water that contains appropriate concentrations of gases and ions, metabolites, toxins and particles. Depending on the substance, concentrations that are too high or too low can be harmful. In aquaculture conditions, salmon are confined to rearing units and optimal water quality conditions must be provided to avoid any potentially negative effects on their performance and welfare. The minimum and maximum recommended concentrations of the most important water quality parameters are provided by the Norwegian Food Safety Authority. Water quality and its variation over time is a major factor that determines the production potential and welfare of fish in different rearing systems and practices (Kristensen et al., 2009).

2.6. Hygiene

Harmful pathogens (parasites, bacteria, fungi, virus and others) can cause a variety of disease conditions. Open fish cages are especially vulnerable to organisms spread by currents and the high density of fish provides the organisms with a good opportunity to find new hosts and spread. Closed or semi-closed systems are also vulnerable to pathogenic outbreaks if there is poor biosecurity or water screening or disinfection procedures. Handling and treatment of the fish may also cause wounds that reduce the fish's external barriers and immune defences, leaving it open for potential infections. Diseases is a clear sign of poor welfare and potentially suffering. However, the harmful effect of diseases will vary in their impact on the welfare of fish, and the intensity, duration and the proportion of fish affected must be considered.

2.7. Safety and Protection

For fish and other animals, the safety from danger and protection of their body against injuries is of utmost importance for survival. The fish skin is the main barrier against infections, but is usually soft and vulnerable for mechanical damage, even if salmon and many other fish are protected by fish scales. A bite from another competing fish or predator may therefore be fatal and fish may be fearful of attack.

2.8. Behaviour control

Fish must have the freedom to control their bodily movements, the ability to move away from danger and also have buoyancy control (Stien et al., 2013). The ability to move away from danger is a fundamental need for all animals, and also to learn to predict danger and learn from aversive incidents. This can be seen in wild fish that panic when they get entangled in fish nets or that can struggle and fight to get loose from a fishing hook. In fish farming, this is also seen when fish are crowded and handled; we can see avoidance behaviour, increased oxygen consumption, catecholamine, cortisol and serotonin levels, all indicating stress and potential fear.

2.9. Social contact

The majority of farmed fish species live in groups, at least for certain parts of their life cycle, and in the wild groups size can vary from pairs, e.g. the European seabass (*Dicentrarchus labrax*), to schools of billions of fish like Atlantic herring (*Clupea harengus*). The need for social contact is related to the need for safety, where the fish can seek safety among equals, the need for information sharing about food and dangers, and to find spawning mates. The social need can also vary through different life stages, and this is the case for salmon that can be territorial and aggressive during the freshwater period, but change to a schooling fish at smoltification. Juvenile salmon can also be aggressive in small groups, but can become less aggressive when held in greater numbers/higher densities (Fernö and Holm, 1986) and exhibit schooling like behaviours even during the fresh water stage. When held in both tanks and sea cages, farmed salmonids normally aggregate in relation to environmental conditions and swim in structured groups. In sea cages, the fish normally develop a circular school structure a few weeks after sea transfer, and maintain this group structure throughout the remaining production period, although it is dependent on the prevailing hydrology. The school can become more "loose" or disperse at dusk, which may prevent physical contact with other fish during darkness (Juell, 1995). Individual salmon may also be very reluctant to break out of the school, where sub-groups and bi-modal vertical distributions of fish may be established by several hundred individuals, suggesting that social contact is important (O. Folkedal, unpublished data).

2.10. Rest

Numerous factors can affect a fish's metabolic scope and its need for rest/physiological restitution. These include water velocity, body size, water temperature, the temperature acclimation state of the fish, as well as feed satiation level. Although salmonids can sustain swimming for long periods at relatively high current velocities that are within their scope for aerobic activity, having the opportunity to reduce activity levels can be important for maintaining normal body functionality (Farrell et al., 1991; Thorarensen et al., 1993). For example, in salmon post-smolts (~100 g) that were reared over 6 weeks in constant velocities of 0.2, 0.8 or 1.5 body lengths per second, fish under the highest water velocities exhibited signs of poor welfare including reduced growth, high ventricular mass, skin and fin damage, as well as a lower expression of the behavioural repertoire observed in fish at lower velocities (Solstorm et al., 2016a, b). Caged salmon can cope with tidal-driven high current periods by switching their behaviours from circular schooling to maintain position against the current, resembling a peloton of cyclists, and thus possibly offering 'shelter' within the fish group (Johansson et al., 2014). Fish in circular tank systems can normally select their preferred velocity in a horizontal current gradient and schooling fish in sea cages may have a similar opportunity from reduced velocities in the inner part of the circular school (Gansel et al., 2014). Sea farming sites are, however, very diverse in both the strength and pattern of water currents they are exposed to (Holmer, 2010).

As fish lack eyelids, fish do not conform to the common definition of sleeping as resting with shut eyes. However, many fish species can qualify as 'sleepers' in terms of fulfilling behavioural and physiological criteria with regard to inactivity, resting postures, circadian activity rhythms and arousal thresholds. These criteria may differ between life stages and be absent during periods like migration and spawning (Reebs, 2008-2014). Farmed salmon schools 'loosen' or disperse at dusk and reduce swimming speeds (Juell, 1995), with night time swimming speed reported to be about 30% slower than during daytime schooling (Korsøen et al., 2009). The reduction of speed is more likely to be a behavioural adjustment for a reduction of visual input, rather than expression of rest, and salmon choose to maintain daytime swimming speeds when artificial lightning is used (Oppedal et al., 2011a). Little information exists on the basal resting mechanisms or 'sleep' in salmonids. However, anecdotal evidence indicates states of resting. During night time with artificial light in sea cages, subgroups of salmon have been observed aggregating away from sub surface lamps and 'hanging' almost motionless with low responsiveness to sudden stimuli (O. Folkedal, pers. obs.). Moreover, wild individual salmon may position themselves in the littoral zone at night, where they are seemingly in a state of sleep (J. Nilsson, pers. obs.).

2.11. Exploration

The fish's natural environment, as in aquaculture rearing units (especially sea cages), shows both spatial and temporal variation in some environmental variables such as current speed, temperature and light level (Oppedal et al., 2011a), but the aquaculture environment shows less variation in e.g. physical constructions. Roaming the environment to explore environmental gradients is important for optimizing factors such as temperature and current velocity, and acquiring information regarding hazards, feed acquisition, etc.

2.12. Body care

Refers to the need an animal has to clean its body, scratch and remove parasites. For fish this need is demonstrated in that they have evolved several symbiotic relationships between cleaner fish or cleaner shrimp that remove ectoparasites, diseased or necrotic tissue from the host fish (which in many cases are large predatory species). Salmonids may also visit fresh water rivers in order to remove lice (Birkeland and Jakobsen, 1997), and jumping has also been suggested as a mechanism for removing lice (Samsing et al., 2015).

2.13. Sexual behaviour

Maturing Atlantic salmon have an inherent need to migrate back to their rivers, swim upstream and perform courtship, choose mates and finally spawn (Thorstad et al., 2011). This behaviour involves a considerable risk of injury and less than 10 % of fish typically survive (Fleming and Reynolds, 2004). Upon entering the river, the salmon typically swim steadily up stream with alternating resting periods, before they start to search for a place to nest. The *"tail - beating motion"* (Fleming and Einum, 2011) that the females use to dig nests can potentially lead to epidermal damage and scale loss. The males will often be aggressive and dominance hierarchies cannot often form around the females when they are nesting. The most dominant male salmon will perform the majority of the courting and mating behaviours with the female (Fleming and Einum, 2011). However, other males, including mature male parr, may also sneak in to get access to spawning females. After the eggs have been fertilised, the female immediately starts to cover them, while at the same time creating a pit for a new nest. Following spawning, the surviving spent salmon either leave immediately or wait for the spring when circumstances are more suitable for migration back to the sea (Fleming and Einum, 2011).

3. Animal based welfare indicators

This chapter describes animal based welfare indicators. Some of these are at the group level and do not involve handling or other disturbances of the fish. Other indicators are at the individual level, which in most cases involves handling and the examination or sampling of individual fish.

Table 3-1. *List of animal based welfare indicators and their relationship to different welfare needs.*

	Needs	Environment				Health			Behaviour					Reso-urces	
	Welfare indicators	Respiration	Osmotic balance	Thermal reg.	Good water q.	Body care	Hygiene	Safety and prot.	Beh. control	Social contact	Rest	Exploration	Sexual beh.	Feeding	Nutrition
Group	Mortality rate	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Group	Behaviour	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Group	Surface activity					×	×		×			×			
Group	Appetite	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Group	Growth	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Group	Scales or blood in the water	×	×					×	×						
Group	Disease	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Individual	Gill beat rate	×			×			×	×						
Individual	Sea lice	×	×			×	×	×							
Individual	Gill bleaching and gill status	×	×				×				×				
Individual	Condition factor													×	×
Individual	Emaciation state		×				×							×	×
Individual	Sexual maturity stage		×										×		
Individual	Smoltification stage		×												
Individual	Vertebral deformation								×		×				
Individual	Fin damage (non-active)								×		×				
Individual	Fin status		×				×	×							
Individual	Scale loss and skin condition		×				×	×							
Individual	Eye damage and status						×	×	×					×	×
Individual	Deformed opercula	×													
Individual	Abdominal organs						×	×							×
Individual	Vaccine-related pathology													×	×
Individual – Blood	Cortisol		×					×	×	×		×		×	
Individual – Blood	Osmolality		×												
Individual – Blood	Ionic composition		×												
Individual – Blood	Glucose							×						×	×
Individual – Blood	Lactate							×	×		×				

3.1. Group based welfare indicators

3.1.1. Mortality rate

Mortality rate is perhaps the most commonly used health related WI. High or increased mortality rates certainly indicate that there is a welfare problem on a farm or in a rearing unit. However, it is necessary to first confirm what is normal then identify the causes of the observed mortality in order to take preventive actions. A low mortality rate does not necessarily mean that there is no welfare problem on a farm. Diseases and other issues may reduce welfare without causing death.

Mortality as a welfare indicator can either be based on long-term mortality or short-term mortality. Short-term mortality is a snapshot of current mortality compared with previous data, some standard or a control. Several standard mortality curves for salmon have been developed (Soares et al., 2011, 2013; Stien et al., 2016). In an analysis of post smolt salmon mortalities form all Norwegian salmon farmers, Stien et al., (2016) reported that the standard (median) mortality curve is highest during the weeks following sea cage transfer, and then gradually declines and stabilises at around 0.2% per month (Stien et al., 2016). Benchmarking of mortality is used in other industries to identify unusual patterns of mortality before any serious loss has occurred and for tracing and tracking diseases (Soares et al., 2011). An obvious weakness with this approach is that many problems only result in mortality after a variable period, making it difficult to identify the true cause of the increased mortality (Soares et al., 2013). However, several authors (Soares et al., 2011; Salama et al., 2016) have been able to link abnormalities in short-term mortality to the development of disease in salmon populations on farms.

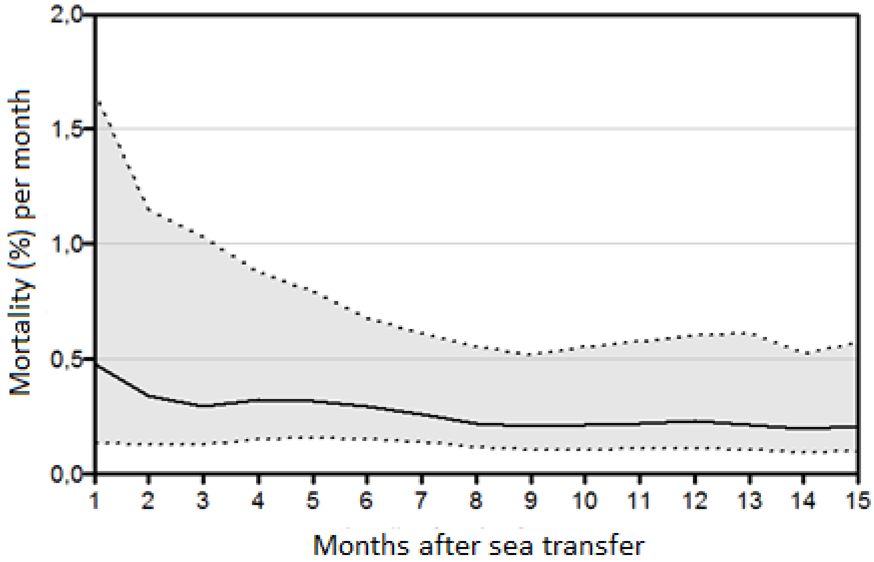


Fig. 3.1.1-1. *Standard mortality curve for the 15 first months of on-growing in cages based on reported data from all Norwegian salmon farmers from 2009-2015. The curve gives the median monthly mortality rate, in addition to the 25- and 75-percentiles.*

Long-term mortality, or accumulated mortality, is a retrospective welfare indicator typically used to assess the welfare of the entire or long parts of animal production cycles. An assessment of the whole production cycle is necessary if the goal is to assess a production method, a production system or a production site. Stien et al., (2016) used the distribution of total mortality after 15 months, based on reported monthly mortality data from all Norwegian salmon farmers from 2009-2015, to classify production cycles into five welfare classes: (1) dark green (better than normal), (2) green, (3) yellow,

(4) orange and (5) red (worse than normal). The reasoning behind classifying the 20 % of production cycles with highest long-term mortality as worse than normal is because the mortality curve is far from normally distributed (Figure 3.1.1-2); it has a long tail to the right indicating that these high mortality production cycles represent abnormalities. These abnormalities can be due to intrinsic properties of the sites, but may also be due to episodic events such as disease outbreaks and fatal accidents during handling (e.g. lice treatments). Kristiansen et al., (2014) showed that fish farms with high average mortality rates generally also had high variation in mortality between production cycles.

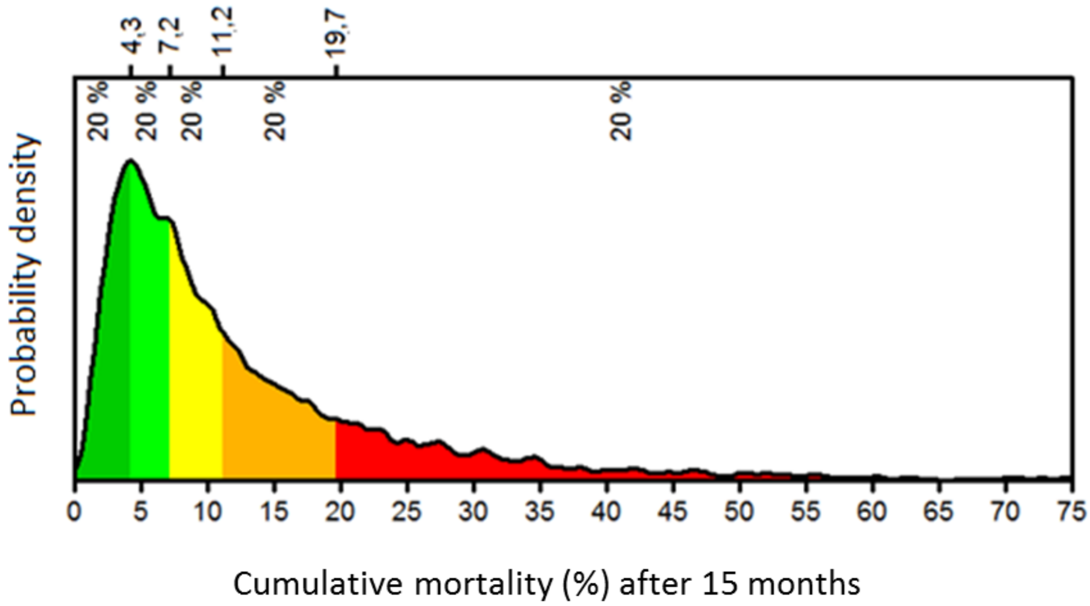


Fig. 3.1.1-2. *Mortality distribution after 15 months of on-growing in sea cages. 0-4.3 % (dark green, better than normal welfare), 4.3-7.2 % (green), 7.2-11.2 % (yellow), 11.2-19.7 % (orange), >19.7 % (red, worse than normal welfare).*

Sampling and analytical considerations

Long-term mortality (e.g. cumulative mortality or survival) rates may be utilised as a retrospective welfare indicator and short-term mortality (daily mortality) rates can be used as an OWI (e.g. Ellis et al., 2012a). It is important to determine cause of death to enable action to be taken to avoid and prevent further mortality. It is also important to consider not only rates but trends of mortality since an increasing trend may indicate a problem before normal thresholds are reached.

Strength of indicator

Simple and already part of daily routines on commercial salmon production facilities. If combined with causes of death (pathology) it can be a valid tool to identify problems and prevent or at least identify further problems.

Weakness of indicator

Ellis et al., (2012a) state "*Mortality is admittedly a crude welfare indicator for farmed fish: it is only measurable at the level of the population, rather than individual*" and by the time a fish has died and contributed to the statistic is it too late to respond. One cannot assume that zero or low mortality is an indicator of good welfare, as welfare may be affected with leading to mortality (Ellis et al., 2012a).

3.1.2. Behaviour

The behaviour of the fish is probably one the best welfare indicators available to the farmer or observer and the only one where we have some degree of access to the subjective experience of the fish. Fish farmers use behaviour as a key tool for monitoring fish welfare, and a large number of salmon cages are equipped with underwater cameras. Behaviour can give an immediate indication of the state of the fish and is in most situations a non-invasive measure. Even if it is claimed that because the fish lack facial expressions it can make it difficult to interpret a fish's experiences, fish do have a rich "body language" that is expressed by differing swimming modes, fin displays, gill ventilation frequencies, skin pigment patterns and colouration, their response to food and also where they position themselves in the water (e.g. Martins et al., 2012). For example, exploratory behaviour or feed anticipatory activity can all be indicators of good welfare in addition to their normal schooling behaviour and daily activity (Martins et al., 2012). A poor or absent response to feed or novel objects and stereotypic or slow swimming can be indicators of disease, stress and poor welfare. Another indicator of poor welfare may be "freezing behaviour" where an individual does not move (Vilhunen and Hirvonen, 2003). This behaviour may be a strategy for avoiding predation (Vilhunen and Hirvonen, 2003) or it could also be a fear response (Yue et al., 2004, see Sneddon et al., 2016 for more information). Other behaviours such as escape type behaviours, hiding, burrowing, seeking shelter or increased group "clumping" may also be related to potential fight-or-flight strategies (Sneddon et al., 2016). In the aquaculture environment, this fleeing behaviour can manifest itself as burrowing behaviour when the fish burrow into the bottom of the holding net or tank. Aggressive behaviour such as chases, nips and attacks can also manifest itself during certain routines or life stages (Cañon Jones et al., 2010). Body rocking behaviours and also the fish rubbing against surfaces has also been observed during nociception (Sneddon, 2006; Sneddon et al., 2016).

Observations of fish behaviour at the group level can also be a good welfare indicator. Different behaviours can include the structure of the shoal, its polarisation, the fish's swimming speed and direction, and the horizontal and vertical distribution of the group as a whole (e.g. Martins et al., 2012). Unstructured swimming at the bottom of the cage or tank can also be an indicator of acute stress. In an operational setting, these indicators require careful interpretation. For example, if fish increase swimming speed and approach the feed delivery area prior to, or at the start of the meal, it can be an indicator of good welfare (Martins et al., 2012). However, if the behaviour persists during a meal or over a number of days, it can also indicate competition for a potentially limited resource (e.g. Noble et al., 2007b) e.g. the fish may be underfed.

Sampling and analytical considerations

Qualitative changes in fish behaviour can easily be assessed by manual observation on the farm or during a routine or husbandry practice, making behaviour a key OWI for detecting welfare threats. Qualitative assessments can be done simply by standing next to a rearing system and looking at the fish (although this may offer a limited field of view in wide, deep or turbid production systems). Widely used underwater cameras (such as those used for feeding in sea cages) offer a better perspective of fish behaviour and can be winch mounted and mobile, covering a wider range of depths within the rearing system in real time. However, they do require active monitoring by the observer. Echo sounders provide a more objective measurement of fish behaviour in sea cages, providing data on the position and the vertical distribution of the fish in the cage. The signal from the echo sounder transducer spreads out in a cone shape, meaning that the echo sounder monitors a very small area in the first few meters from its location and this field on view then increases with distance from the transducer. The transducer is therefore often positioned below, or deep in the sea cage, pointing upwards to be able to get a good record of the fish near the surface. The echo signal from the salmon is mostly from their swim bladders, although this is dependent on the type of sonar used. A weak signal may therefore be that the salmon have deflated swim bladders (Korsøen et al., 2009). Another source of potential error, is the "near field error" where objects near the transducer shade objects further away.

Strength of indicator

Martins et al., (2012) stated "*changes in foraging behaviour, ventilatory activity, aggression, individual and group swimming behaviour, stereotypic and abnormal behaviour have been linked with acute and chronic stressors in aquaculture*" and deviations from normal behaviour are established signs of disease and poor welfare. Both underwater cameras and echo sounder technology are relatively inexpensive and provide the opportunity for real time observation of the fish.

Weakness of indicator

Many of the behavioural indicators are difficult to quantify and are very dependent on the motivation and skills of the observer. Quantitative changes in fish behaviour (absolute changes in swimming speed, aggression levels, and gill beat frequency) are mostly only achievable by later analysis of e.g. collected video data, thus making quantitative analysis of this kind of fish behaviour laborious. Relying on a manual subjective detection of abnormal behaviour requires that the observer must know what is normal given the specific life stage, production system and water environment. The observer may also have difficulty explaining and quantifying what the abnormal behaviour consists off, making it difficult to train new staff. As mentioned above, some behaviours such as an enthusiastic feeding response may be indicators of both positive and negative welfare.

To turn quantitative behavioural analysis into an OWI, technological advances are required. New and emerging technological solutions that offer real-time, objective automated and continuous monitoring of fish behaviour need to be developed and adapted to the farm environment and the demands of welfare monitoring. These might include machine vision solutions or biotelemetry and bio loggers. For sea cages echo-sounder technology recording vertical position and distribution of the fish is already available and in frequent use in scientific small scale experiments. It is, however, challenging to get accurate representations of fish distribution in commercial cages with a large biomass of fish.

3.1.3. Appetite

The need to feed and have access to food is a well-established welfare requirement for farmed fish. However, whether a fish chooses to consume food when it is given access to it, or how much food is consumed can be dependent upon a number of inter-related behavioural and physiological factors, a key one being appetite (e.g. Jobling et al., 2012). Appetite in itself is the result of an array of factors, with three prominent drivers being i) the nutritional status of the fish including its energy reserves and ii) the fullness of the stomach at the time of potential feeding, and iii) seasonal adaptations and the fish's motivation to feed (see Jobling et al., 2012 and references therein). Once a fish makes the decision to feed, appetite can also be regulated by behavioural factors such as competition (Reebs et al., 2002) and also by the nutritional composition of the food. Environmental factors can also dictate and influence appetite, with a key factor being water temperature (Austreng et al. 1987), both in terms of its absolute values and rate of change in the variable. Other factors include daylength, both natural (Noble et al., 2007a) and artificial (Oppedal et al. 2003), oxygen saturation (Remen et al. 2016a) and the health status of the fish (Damsgård et al., 1998; 2004), including its ectoparastic level (Costello, 2006).

Life stage is also a well-established factor influencing appetite in salmonids. For example, juvenile salmon in the wild that choose to delay migration from river to sea have low appetite levels during winter and may enter a state of anorexia (Metcalfe and Thorpe, 1992). Larger salmon also undergo a period of anorexia prior to spawning, often preceded by an appetite surge before the fish enter their anorexic state (Kadri et al., 1996).

Management practices and the exposure to stressors such as repeated disturbance can have a profound impact upon appetite. As a result, the time it takes for appetite to return after e.g. handling, can also be used as an OWI in aquaculture. The effects of this complex inter-relationship of biotic and abiotic factors upon appetite both within and between species and life stages, and within and between individuals and groups of differing sizes mean it is difficult to give absolute operational recommendations on the appetite of fish. Indeed, due to the inherent variability in appetite, giving absolute values may be potentially detrimental to the welfare of the fish and also the performance of the farm. For example, it is very well established that individual and group appetite levels of salmon vary within and between days (Noble et al., 2007a; Jobling et al., 2012) even under stable environmental conditions, with minimal disturbance. If salmon farmers were to feed a fixed ration level according to a theoretical appetite threshold, they would run the risk of either underfeeding the fish (delivering too little food), or overfeeding fish (delivering too much).

Fish have evolved in a highly variable environment where feed availability can be unpredictable. Fish are therefore able to tolerate long-term periods of feed withdrawal and feed restriction (e.g. Huntingford et al., 2006) although this tolerance is dependent upon their nutritional status and energy reserves. The welfare consequences of feed withdrawal and restriction are also dependent upon life stage and species, but their general impacts can be described. The potential welfare consequences of not giving fish sufficient food to satisfy their appetite in the short-term are increased competition for a limited feed resource, which can increase aggression (Cañon Jones et al., 2010), injury (Cañon Jones et al., 2010) and increase stress levels in juvenile salmon. Prolonged feeding of maintenance rations to maintain fish size or limit growth rate can lead to a marked deterioration of welfare, including increased competition and injury (Cañon Jones et al., 2017). The prolonged consequences of not feeding to appetite can be depletion of energy reserves and nutritional status leading to reduced condition factor and even emaciated fish (Jobling et al., 2012). Overfeeding, where fish are fed more than their appetite requirements can lead to reduced water quality due to excess uneaten food pellets or the excretion of nutrient rich faeces by the fish (e.g. EFSA, 2008a, b). This can be especially important in closed- or semi-closed containment rearing systems.

A key recommendation is therefore to feed fish a diet that has an appropriate composition and in amounts that are sufficient to meet their appetite. This can be achieved by feeding the fish a regime that responds to changes in appetite (as many salmon farmers already do). For this approach to be successful, the farmers need robust indicators of hunger and satiation for the size and type of fish within their rearing system, and this is a challenge in both salmon and trout farming.

Sampling and analytical considerations

The farmer usually has daily records of how much feed has been delivered to a tank or cage. If the farmer is confident that this ration size represents the short- and long-term appetite of the fish, or employs e.g. underwater cameras to monitor changes in appetite, then appetite can be used as a welfare indicator. For example, although groups of salmon can show marked differences in appetite within and between days, visual observations of abrupt drops in appetite and a lack of feeding motivation (both short- and longer-term) on farms can be used as a qualitative OWI (Huntingford et al., 2006) and are an indication of poor performance (Stien et al., 2013). However, changes in appetite are also context specific (Huntingford and Kadri, 2014); long-term changes in appetite may be related to water temperature, daylength and season (Kadri et al., 1991; Noble et al., 2007a) and not poor welfare.

Strength of indicator

A reduction or loss of appetite can be caused by the initiation of a stress response (Huntingford and Kadri, 2014). The time it takes for appetite to return after e.g. handling, can therefore also be used as an OWI as it can reflect how well the fish have coped with the stressor. Appetite is easy to measure qualitatively by observing the fish when feed is offered. It is also used as a key early warning system for the farmer; it is quick and does not require further analysis.

Weakness of indicator

Quantitative data on changes in appetite (e.g. abrupt or prolonged drops in group feed intake from expected appetite levels) are difficult to evaluate, primarily due to the inherent variations in daily feed intake and appetite of fish, even when the fish are in good health and exhibit good welfare. This means it is difficult to look for quantifiable deviations from 'expected' or 'normal' appetite levels. A drop in appetite can also be indicative of several threats, requiring further investigation to identify the origin and intensity of the problem.

3.1.4. Growth

Growth and growth rate have long been used as a welfare indicators in animal production (Broom, 1986) including fish (Huntingford and Kadri, 2009). Growth is intrinsically linked to the feeding and nutritional welfare needs of the fish; when these needs are not met, the fish can exhibit poor growth performance.

Growth rates, like appetite, are variable in relation to e.g. life stage and fish size (Jobling, 1983) and may be affected by several factors, such as appetite, the nutritional content of the feed (Jobling, 1983), diseases, social interactions (Adams et al., 2000) and water quality. Low growth can also be symptomatic of a tertiary stress response (e.g. Ellis et al, 2002; Huntingford et al., 2006). However, growth can be affected by factors that are not related to welfare, such as when fish enter a state of anorexia as juveniles (Metcalfe and Thorpe, 1992) or as adults (Kadri et al., 1996), leading Turnbull et al., (2005) to term it an "imprecise" welfare indicator. To clarify if a poor or reduced growth rate is linked to a welfare problem rather than other factors, it has to be coupled with other WIs such as indicators of physiological stress or others indicative of hunger (Ellis et al, 2002). Inter-individual variation in growth rate may also be a useful indicator of welfare as increase size variation within the rearing group can result from underfeeding and increased competition (Johansen and Jobling, 1998; Noble et al., 2008)

Irrespective of this, reduced growth rate (both short- and long-term) may indicate fish are facing a welfare problem (Huntingford et al., 2006) and farmers use it to identify the need for further investigations into the cause.

Sampling and analytical considerations

For growth rate to be a suitable OWI, the farmer requires accurate data and information on fish weight and changes in fish weight over time. Regular weighing gives the farmer a better overall picture of growth performance and means any sudden deviations from expected growth rate can be acted upon if required. Long term deviations from expected growth rate may also be used as an indicator of a chronic problem. Further, both short- and long-term monitoring of growth can be used in retrospective analysis of welfare problems. For size variation within the rearing group to be an OWI, robust data on the weight of individual fish is needed (i.e. this cannot be assessed by bulk weighing).

Growth auditing, in its simplest form, usually requires the farmer to capture a group of fish from each production unit (sample size is usually dictated by experience, labour/time/equipment) and the farmer can then take a batch weight which provides average weight only or individual weights providing mean

± SD. Weighing individuals is time consuming, labour intensive and can disturb both the fish and existing husbandry tasks such as feeding.

Numerous existing and emerging technologies are being developed to help farmers robustly monitor biomass without handling. Existing technologies currently in use primarily fall into two categories: i) rectangular biomass frames, that calculate fish size and condition factor by optically scanning the fish as they swim through the frame, or ii) stereo camera based systems, where fish size is estimated from images captured of the fish as they swim past the cameras. Other biomass auditing approaches are being developed or are available that use acoustic or imaging sonar or laser systems such as Lidar based biomass estimation systems, but these are either still in development or not widely used.

Using growth rate as an OWI depends upon obtaining a good, representative sample of the fish and growth rate may be quantified as i) absolute weight gain, ii) relative or percentage weight increase, iii) specific growth rate (SGR) and/or iv) thermal growth coefficient (TGC).

As stated above, long-term growth rates vary according to fish strain, season, life stage, rearing system, diet etc., so it may be better to use acute changes in growth rate as an OWI within a specific rearing unit or system.

Strength of indicator

It is an OWI that is already regularly monitored on the farms. Changes in growth rate can be used as an early warning system for potential problems, particularly when the farmer has robust growth monitoring practices. It is a quick indicator, and if passive biomass monitoring systems are used, it requires no handling of the fish. It also requires little further analysis for the farmer to get an answer they can act upon. Passive monitoring technologies can give the farmer daily updates on weight gain and growth within their rearing systems.

Weakness of indicator

To use reduced growth rate or deviations from expected growth rate as an OWI the farmer must be confident that the sample weight data they are using is accurate and representative of the group. This can be difficult when using manual sampling (due to small sample size which may not be representative) and also when using passive technologies if the farmer does not trust the data. Manual sampling requires handling the fish and can interfere with daily husbandry routines. A reduction in growth rate may not always be indicative of a welfare threat, meaning the origin and intensity of the potential problem must be investigated further. It is also difficult to audit the performance of individual fish without tagging.

3.1.5. Disease and disease control

Health indicators may be monitored on individual fish or at the group/farm/industry level. Some diseases or conditions may be diagnosed by simply observing the fish (e.g. cataracts) whereas others need an autopsy (e.g. peritonitis after vaccination) or even laboratory tests (e.g. histopathology, bacteriology, etc.). Although health may be one of the most commonly used welfare measures, health indicators can be challenging to interpret when identifying potential causal relationships (Segner et al., 2012). For example, stressful husbandry conditions or poor water quality may lead to secondary infectious disease by impairing the immune system or primary barriers to infection (Huntingford and Kadri, 2014; Segner et al., 2012).

A disease is an abnormal condition, a disorder of a structure or function, which can affect part of or an entire organism. Infectious diseases are caused by various infectious agents including viruses, bacteria, fungi, parasites or others. Diseases may also be caused by internal dysfunctions (e.g. genetic or autoimmunity). As with any animal, disease can have a marked effect on fish welfare, because they frequently result in negative experiences such as pain or discomfort.

Important diseases in Norway affecting fish welfare are summarized in Tables 3.1.5-1, 2 and 3. At the time of preparation some major bacterial diseases (furunculosis, vibriosis) have been effectively controlled by vaccination and the need for medical treatment with antibiotics is generally very low. Viral diseases are a larger challenge, among other things due to the lack of effective vaccines against important disease such as Pancreas Disease (PD). PD is a major viral disease in the seawater stage, causing lasting circulatory problems and reduced growth due to pancreas degeneration for those individuals which survive initial infection. In 2016, 138 outbreaks of PD were reported in Norway (Hjeltnes et al., 2017). For other viral diseases such as Cardiomyopathy Syndrome (CMS) and Heart and Skeletal Muscle Inflammation (HSMI), there are no vaccines available. CMS often has an acute onset of clinical signs and can also cause heart ruptures, mostly in large fish close to harvest. HSMI usually affects salmon in their first year in the sea, causing circulatory failure and muscle degeneration with mortality in some cases. Both diseases often appear in connection with potentially stressful events, like grading, transport or delousing. Even if there is an effective vaccine, vaccination may cause side effects such as abdominal adhesions, due to the adjuvant, which can be a significant welfare problem. Gill disorders can be widespread in aquaculture and are considered a serious welfare problem as respiration, osmoregulation, nitrogenous waste excretion and electrolyte balance can be impaired. Gill disorders can be caused by inorganic particles, plankton, bacteria, parasites (e.g. *Neoparamoeba* sp., microsporidia) and viruses such as Salmon gill poxvirus disease – SGPVD. Further details are given by the Norwegian Veterinary Institute which publishes a yearly report "The Health Situation in Norwegian Aquaculture" covering the key existing, new and emerging diseases, see www.vetinst.no.

Sampling and analytical considerations

Checking for some infectious diseases already forms part of the required inspections routinely performed by fish health service personnel. This routine disease monitoring is risk based and may range from simple visual inspection of the fish to full post-mortem and laboratory examinations.

Strength of indicator

Health constitutes a significant part of animal welfare and disease is therefore a highly relevant OWI (e.g. scoring of cataracts and AGD) or LABWI. Reduced fish welfare should be considered when assessing the impact of any disease (Murray and Peeler, 2005). Early diagnosis could stop an outbreak and potentially prevent reduced welfare.

Weakness of indicator

The absence of disease does not imply good welfare *per se*. However, detecting a disease is a good indication of compromised welfare. As with mortality, detection of diseases can only be used retrospectively. However, eDNA methods (environmental DNA) are being developed that may be able to quantify the presence of microorganisms in water, predicting outbreaks of infectious disease.

Evidence of comprehensive health or disease prevention plans is a useful resource based WI. While frequent treatments may indicate poor disease control and a welfare problem they can also indicate an effective monitoring and response to disease problems they therefore have to be considered in context.

Table 3.1.5-1a. *Important infectious virus diseases in farmed salmon in Norway and their welfare impact. FW = freshwater, SW = seawater.*

	Virus	FW	SW	Welfare impact
Pancreas disease (PD)	Salmonid alphavirus (SAV) / Salmon Pancreas Disease Virus (SPDV)	(×)	×	• First signs of disease are often an abrupt drop in appetite and sick fish cluster at the water surface towards the current direction (NVI, 2017) • Often severe muscle damage, oesophagus- and heart muscle damage, causes circulatory problems (NVI, 2017). • Severe loss of exocrine pancreatic tissue, reduces enzyme production, causes reduced appetite and growth • Outbreaks can cause high mortality and be long lasting (1-32 weeks) (OIE, 2015b) • Subclinical infections are also reported, and can be activated during stress (NVI, 2017) • Welfare impact can be reduced by minimizing stress, euthanizing sick individuals (and those chronically affected), early slaughter • PD is considered to be one of the most important viral diseases in Norway, with 138 registered outbreaks in 2016 (Hjeltnes et al., 2017). SAV2 is most common in central Norway, SAV3 most common in southern parts. In Norway, the disease is notifiable (Hjeltnes et. al., 2018).
Infectious salmon anaemia (ISA)	Infectious salmon anaemia virus (ISAV)	(×)	×	• The virus attacks the surface within all blood vessels and the heart, producing a severe anaemia and circulatory disturbances that can be seen in gills, heart, liver, kidney, spleen etc. (Aamelfot et al., 2014) • Only a few cases have been reported in the freshwater phase, including a case in yolk-sac larvae (Rimstad et al., 2011). • Often low mortality and a chronic progression, daily mortality typically 0.05-0.1% in affected cages, however high mortality is also reported (OIE, 2015b) • Early detection and rapid slaughtering of net pens of clinical ISA may prevent spread at the site. ISA is notifiable and the slaughter of the farm population is the Norwegian strategy for dealing with an outbreak. Much focus is put into hygiene and movement restrictions to prevent its spread (Rimstad et al., 2011; NVI, 2017)
Infectious pancreatic necrosis (IPN)	Infectious pancreatic necrosis virus (IPNV)	×	×	• The virus attacks the pancreas, which is essential for digestion of food, and can also give necrotic enteritis, and fish that survives the acute phase may starve to death (EFSA, 2008a) • Mortality outbreaks are often higher in FW than SW, it can vary from insignificant to 90%, and fry and post-smolt are most susceptible (NVI, 2017). An increase in the number of emaciated fish following an IPN outbreak is commonly described as the most significant after-effect of an outbreak (Bornø & Linaker, 2015) • A large proportion of fish develop a lifelong persistent infection, which can be activated during stress (like transfer to sea) (EFSA, 2008a; NVI, 2017) • Stress can also increase mortality during outbreaks. Hence, with very small fish euthanizing the whole population may be the most welfare friendly strategy (EFSA, 2008a). Fish surviving IPN have higher susceptibility to other diseases like HSMB and PD (NVI, 2017). • The use of QTL eggs that are more resistant to IPN, as well as combating "house strains" of the virus in the infestation phase has probably helped reduce the number of IPN outbreaks registered in the last couple of years (Hjeltnes et al., 2017). Vaccines are reported to have limited effect, and the disease is non-notifiable.

Table 3.1.5-1b. *Important infectious virus diseases in farmed salmon in Norway and their welfare impact. FW = freshwater, SW = seawater.*

	Virus	FW	SW	Welfare impact
Heart and skeletal muscle inflam. (HSMI)	Piscine orthoreo virus (PRV)	×	×	• PRV was first identified from HSMB diseased fish in 2010. The risk factors of developing disease are not fully understood as the virus is widely distributed, also in fish without disease. Different genetic susceptibilities and different genetic variants of the actual PRV virus are likely to be relevant (Hjeltnes et al., 2017). • Inflammation of the heart lining, heart muscle and also red skeletal muscle in the acute phase, causing circulatory problems, but the disease is often overlooked because of slow progression and heart injuries can persist for many months (NVI, 2017). • HSMB is considered to be quite common, primarily occurring during the first year the fish are at sea, but can also occur later. The risk for HSMB in O+ salmon transferred to sea in autumn is considered to be twice as high as 1+ salmon transferred to sea in spring. HSMB is also seen in hatcheries, but saltwater is probably the main reservoir (Hjeltnes et al., 2017; NVI, 2017). • Mortality varies (typically from insignificant to 20% in sea cages), stressors such as grading, transport or other management procedures are often reported to increase mortality (NVI, 2017). • No treatment or vaccine is available, and it`s a real challenge to avoid stress related mortality in diseased fish, for instance during delousing which may cause severe welfare impacts to diseased fish. • Non-notifiable
Cardiomyopathy syndrome (CMS)	Piscine myocarditis virus (PMCV)	×		• Piscine myocarditis virus (PMCV), discovered in 2010, appears to be closely associated with CMS that causes heart rupture and circulatory disturbances in large salmon. • Develops slowly and mortality is often low, but can be long lasting. • Sudden death without prior clinical signs is common, and is often seen in connection with stressors such as grading, transport and delousing. • After PMCV is diagnosed, handling should be reduced to a minimum until slaughter (NVI, 2017) • No vaccine is available, but CMS-QTL- smolts are on the market. • Non-notifiable.
Salmon gill poxvirus (SGPV)	Salmon gill poxvirus (SGPV)	×	×	• Can have an acute development with high mortalities in the hatchery phase. Respiratory problems are a typical symptom as the virus attacks the gills. The disease affects most of the fish in a tank, and often spreads from tank to tank on the farm, and you see a drop in appetite (NVI, 2017). • The disease may also occur during the sea phase. • To reduce the risk of mass mortalities if a SGPV outbreak is suspected, stop feeding, increase oxygen levels, and avoid operations that may be stressful for the fish (NVI, 2017). • Non-notifiable.

Table 3.1.5-2. *Important infectious bacterial diseases in farmed salmon in Norway and their welfare impact. FW = freshwater, SW = seawater.*

	Bacteria	FW	SW	Welfare impact
Yersinosis	*Yersinia ruckeri*	×	×	• Most common in the fresh water stage, where acute septicaemia with high mortality can be seen in salmon fry (Poppe et al., 1999) • Some facilities have reported up to 10% mortalities, during 1–3 months following sea transfer (Bornø & Linaker, 2015). Outbreaks in the sea phase in bigger salmon are becoming a problem and have been seen in connection with de-lousing or handling (Hjeltnes et al., 2018). • Yersinosis has been seen in recirculating aquaculture systems, and "house strains" in biofilm are seen as a problem that have caused recurring episodes of acute cases, some with high mortality (Bornø & Linaker, 2015; Hjeltnes et al., 2017) • Outbreaks of yersinosis are often stress related (handling, transport, sudden osmotic changes, bad water quality etc.), and are often seen together with other infections like saprolegnia or gill infections (Poppe et al., 1999) • Several hatcheries vaccinate against yersinosis (Bornø & Linaker, 2015) • Yersinosis is not a notifiable disease
Flavobacteriosis	*Flavo-bacterium psychrophilum*	×	(×)	• *F. psychrophilum* is mainly associated with fresh water production, and in salmon, systemic infection has been detected at some hatcheries in winter (systemic infection is a bigger problem in rainbow trout and they are more susceptible to the disease, Hjeltnes et al., 2018). • In addition, *F. psychrophilum* is associated with ulcers and fin erosion, which can have severe welfare impacts on salmon (NVI, 2017) • In Norway there have been different strains affecting rainbow trout and salmon (NVI, 2017) • Outbreaks can be associated with a suboptimal environment and stress (NVI, 2017). Not notifiable for salmon.
Winter ulcer	*Moritella viscosa, Tenaci-baculum* spp., *Aliivibrio (Vibrio) wodanis*		×	• Ulcers on the head, flanks and fins is a typical welfare problem in autumn and winter, and can lead to increased mortality and also a reduction in harvest quality (Bornø & Linaker, 2015) • The main welfare aspects of winter ulcers is related to osmo-regulatory problems in connections with the ulcers (Tørud & Håstein, 2008), and the chronic and often long lasting period of probably painful disease where ulcers sometimes penetrate the abdominal cavity or cause sepsis • *Moritella viscosa* is a major contributor, and although nearly all farmed salmon are vaccinated against it, there may be variable effects. Other bacteria that are frequently identified in fish with winter ulcer are *Tenacibaculum* spp. and *Aliivibrio (Vibrio) wodanis*, and the dynamics, if any, are unclear (Bornø & Linaker, 2015) • Low water temperatures at sea water transfer are a potential risk factor, where ulcers develop and mortality occurs after a few weeks (Bornø & Linaker, 2015) • Outbreaks of winter ulcer have also been noted in land based smolt production units that utilise seawater (Hjeltnes et al., 2017) • So-called «non-classical» winter ulcers are less common and are characterized by high mortalities and deep wounds around the mouth (mouth rot) / head, tail and fins. Different *Tenacibaculum* spp. can occur in virtually clean bacteria cultures (Hjeltnes et al., 2017) • Mechanical injuries during lice treatment or other handling are known risks for developing winter ulcers, and ulcers are sometimes treated with antibiotics with varying success (Bornø & Linaker, 2015)

Table 3.1.5-3. *Important parasites and fungal diseases in farmed salmon in Norway and their welfare impact. FW = freshwater, SW = seawater.*

	Parasite/ Fungi	FW	SW	Welfare impact
Sea lice infectio	Salmon louse *Lepeophtheirus salmonis* and *Caligus elongatus*		×	• Lice may damage the fish skin when feeding on the surface, and cause ulcers when numerous. There are welfare challenges associated with delicing (Hjeltnes et al., 2017). For a more detailed description see the sea lice section 3.2.3.
Parvicapsulosis	*Parvicapsula pseudo-branchicola*		×	• Mainly a problem in the most northerly counties in Norway, both concerning morbidity and mortality (Bornø & Linaker, 2015) • High parasite densities and significant pathological changes are observed in the pseudobranch (under the gill cover). The psuedobranchs, whose tasks involve delivering oxygen to the eye and also the control of ion balance, can completely degraded or be severely damaged (NVI, 2017). • Fish with advanced parvicapsulosis are commonly thin, anaemic and have eye haemorrhages (Bornø & Linaker, 2015; NVI, 2017) • The parasite was identified in 39 farms in 2016 (Hjeltnes et al., 2017). *P. psuedobranchicola* have a complex life cycle where polychaetes are the main host and fish are intermediate hosts. Parvicapsulosis is not notifiable.
Amoebic Gill Disease (AGD)	*Paramoeba perurans*		×	• AGD is an emerging serious disease affecting farmed salmon in Norway (Bornø & Linaker, 2015). • The amoebic parasites affects the gills, causing respiratory problems and macroscopically visible gill changes including increased mucus production, which can be used for classification of the disease in a gill scoring system (Taylor et al., 2009) • In addition to respiratory problems, fish can exhibit poor appetite, reduced swimming activity & slow reactions (NVI, 2017). • Early detection is considered important for the treatment efficacy, and it is treated using freshwater or H_20_2. Freshwater is considered less damaging and more effective than H_20_2, but the potential limited availability of well-boats and the freshwater itself have been factors limiting its use (Bornø & Linaker, 2015). • In 2014, minimum 63 salmon farms experienced AGD, but since AGD is not a notifiable disease there were probably a much higher number (Bornø & Linaker, 2015). • AGD fish often have low stress tolerance due to respiratory problems and the treatment itself can be a welfare problem as the disease progresses.
FUNGI, Saprolegniosis	*Saprolegnia parasitica* *Saprolegnia diclina* + others	×		• Mainly a problem in fish eggs, but also seen in fry and parr as complications to gill infections, fin erosion or mechanical injuries and stress. Sexually mature fish in breeding facilities in fresh water can also get infected • Saprolegnia can damage the epidermis, leading to osmotic imbalance and also death • In order for an infection to develop, the fish usually have reduced immune functions, for example due to stress, or have injuries to the mucus or skin layer (NVI, 2017). The infection often starts in areas that are not covered by scales; around the base of the fins, or the head/operculum. If the gills are affected it affects respiration, which can lead to "suffocation" and death (NVI, 2017). • In the case of roe, the presence of dead eggs is essential for saprolegniosis to be established, and the fungus can then spread to living eggs (NVI, 2017). • Saprolegniosis is not notifiable. Preventative measures include avoiding stressing the fish, treating it as gently as possible during handling such as grading and vaccination. It is important to have good hygiene and water quality, so that the formation of spores in the farms water system is avoided. For eggs, it is important to remove dead eggs to prevent its establishment.

3.1.6. Scales or blood in the water

Scale loss and damage to the skin or gills may sometimes be seen as scales floating in the surface of the water and as blood in the water, so called “red water”. Although “red water” does not necessarily mean that the fish will die from the treatment (J. Nilsson, pers. obs.), it should be avoided as it represents damage to the fish. Gill bleeding can be caused by sudden physical or chemical damage (Poppe et al., 1999) and has been observed in connection with the use of mechanical delicing (Gismervik, 2017). Without going into the causes, observations from histopathology samples in 2016 suggest that gill bleeding has become more common (Hanne Skjelstad, Norwegian Veterinary Institute, pers. comm.). Histopathological evidence of gill bleeding can also be seen as artefacts associated with catching/ euthanizing fish (Poppe et al., 1999).

Sampling and analytical considerations

Observed manually but easier to see if the fish are in closed, small containers that have a light colour. Investigation is important to try and find its source.

Strength of indicator

This is an immediate indication that there is a problem such as damage.

Weakness of indicator

Can be difficult to assess how severe the bleeding and the damage to the fish is. It may take some time to process samples and determine the cause of the bleeding.

3.1.7. Bulk oxygen uptake ($\dot{M}O_2$)

Flow-through respirometry can be used as a WI during transport by wellboats to measure bulk oxygen uptake rate ($\dot{M}O_2$) e.g. Tang et al., (2009). We have classified bulk oxygen uptake as a group based WI as it is respiration-based and calculated from changes in oxygen concentration, which itself varies in relation to reduced or increased respiration of the fish. However, the method itself is an indirect assessment of metabolism, and the calculation utilises the OWIs oxygen, water velocity and density, which are all environmental WIs. Table 3.1.7-1 shows how $\dot{M}O_2$ various from species of salmon under various conditions.

Sampling and analytical considerations

Bulk $\dot{M}O_2$ can be calculated from following equation (Farrell, 2006; Tang et al., 2009):

$$\dot{M}O_2 = \frac{V_w \times (C_w O_{2in} - C_w O_{2out})}{BM}$$

Where V_W = the water velocity through the respirometer (well boat) (m^3 min^{-1}); C_WO_{2in} = inflow water O_2 content into the forward compartment (well boat) (mg O_2 min^{-1} L^{-1}); C_WO_{2out} = outflow water O_2 content out of the compartment (well boat) (mg O_2 min^{-1} L^{-1}) and BM = fish mass in respirometer (well boat) (kg) (after Tang et al., 2009).

Strength of indicator

All commercial well boats have equipment to measure oxygen in the different compartments, and the method does not involve any handling or interaction with the fish. With accurate measurements of biomass, oxygen and water velocity this can give a good indication of acute stress. $\dot{M}O_2$ will increase during strenuous and stressful events during transport (Farrell, 2006; Tang et al., 2009).

Weakness of indicator

Can be affected by various factors including handling, water temperature and the nutritional and social status of the fish (Sloman et al., 2000).

Table 3.1.7-1. *Showing a comparison of reported $\dot{M}O_2$ values from differing salmonid species under various conditions. Reproduced from "Tang, S., Brauner, C. J. & Farrell, A. P. (2009) Using bulk oxygen uptake to assess the welfare of adult Atlantic salmon, Salmo salar, during commercial live-haul transport. Aquaculture 286, 318-323. Copyright 2009" with permission from Elsevier.*

Salmonid species	Activity level	$\dot{M}O_2$ (Mg O_2 min^{-1} kg^{-1})	Temp. (°C)	Reference
Atlantic salmon (*S. salar*) (1.5-5.5 kg)	Transport start	2.98±0.13	7.8–15.0	Tang et al., 2009
	Transport end	2.00±0.06		
	Routine[a]	1.32±0.13	12.1±0.2	
	Routine[a]	1.5–4.5	5.5–10.3	Bergheim et al., 1991
	Routine[a]	1.7–3.5	7.2–9.1	Bergheim et al., 1993
	Routine[a]	0.89–2.15	8.5	Forsberg, 1997
Sockeye salmon (*O. nerka*) (1.9–3.3 kg)	Routine[b]	2.99±0.23	16.3±0.3	Farrell et al., 2003
	Maximum[b]	12.28±0.75		
Pink salmon (*O. gorbuscha*) (1.3–1.9 kg)	Routine[b]	4.25±0.69	11.8±0.2	Farrell et al., 2003
	Maximum[b]	12.63±0.44		
Coho salmon (O. kisutch) (2.1–2.5 kg)	Routine[b]	2.23±0.09	5–12	Lee et al., 2003
	Maximum[b]	8.77±0.0		
Chinook salmon (*O. tshawytscha*) (3.7 - 6.4 kg)	Routine[b]	1.99±0.15	8–17	Geist et al., 2003
	Maximum[b]	10.94±0.52		

a=bulk measure; b=individual measure

3.1.8. Surface activity

Surface activity, in terms of the number of rolls and jumps the fish make, is often used after lifting submerged sea cages to the surface in order to determine if salmon have been able to maintain buoyancy in when the cage is submerged. Salmonids have physostomous (open) swim bladders that they fill by swimming to the water surface and gulping air. As air is lost from the bladder they must also refill the bladder regularly to maintain buoyancy (Dempster et al., 2009; Korsøen et al., 2009). Salmon appear to adjust buoyance by changing the bladder content and volume. Smolts and post-smolts swim higher in the water than fry and parr, as the latter are more semi-demersal, and the relative air volume in the swim bladder is larger in smolts than in parr (Saunders, 1965; Wedemeyer, 1996). Salmon in fast running water have less gas than those in still water, as negative buoyancy gives better control in the current (Saunders, 1965). Without surface access, salmonids swim in an upward tilted posture with rapid thrusts of their tails and at a higher speed to compensate for reduced buoyancy, or if possible they may rest on the tank bottom (Tait, 1960; Korsøen et al., 2009). Reduced buoyancy resulting from lack of surface access therefore restricts behavioural control and rest. Salmon reared without surface access can also have a lower condition factor (Tait, 1960; Korsøen et al., 2009) suggesting that they are expending more energy or not feeding as well. Increased surface activity when given access to the surface after submergence in both fry (Tait, 1960) and post-smolts (Dempster et al., 2011) indicates a motivation for the fish to refill the bladder. The swim bladder starts to lose gas from the first day of submergence (Dempster et al., 2009), and after 22 days salmon can lose 95% of the gas content of the bladder (Korsøen et al., 2009). The first signs of reduced welfare are visible after around 3 weeks without surface access (Korsøen et al., 2012), and after 6 weeks more severe signs such as compressed vertebrae may become evident (Korsøen et al., 2009).

Sampling and analytical considerations

When surface activity is related to refilling the swim bladder, the number of jumps and rolls after the cage has been lifted decreases with time as more and more of the fish have been able refill their bladder. It is therefore important to measure surface activity at a standardised time after resurfacing. Surface activity may also vary due to the behaviour of the school or stressors frightening the fish towards the surface (Bui et al., 2013). It is therefore important to measure surface activity over a sufficient time period for the sample to be representative. The number of jumps and rolls are typically converted to jumps $fish^{-1}$. The simplest way to measure surface activity is by counting the number of jumps and rolls using handheld tally counters, but observation by camera and automatic image analysis have also been developed (Jovanović et al., 2016).

Strength of indicator

Easy and straightforward indicator that measures the state of the entire group.

Weakness of indicator

Surface activity can be driven by other reasons than a need to fill the swim bladder, e.g. lice levels (Furevik et al., 1993) or feeding motivation, and often occurs in bursts and pauses that may result in counts that are too high or too low, especially if the counting period is short.

3.2. Individual based welfare indicators

Some individual based WIs, OWIs and LABWIs may also be applicable at the group level, depending upon how they are used. For example, it is preferable to use certain individual OWIs to give the observer a better picture of how severe and widespread a welfare problem is throughout the population; however, abrupt changes in their presence/absence from a simple observation of the group of fish may be useful as an early warning without quantifiable data. An example of this scenario is emaciation. Passive observations of emaciated fish swimming at the surface can be used as an early warning of potential welfare problems. However, to get an overview of severity of the emaciation and prevalence of affected individuals a systematic sample of fish is required (using it as an individual OWI). The same scenario is applicable to dorsal fin damage in Atlantic salmon parr. Dorsal fin damage can be diagnosed by simple surface observations (noticeable grey fins on fish) as a qualitative group OWI. The damage is then quantifiable from a sample of fish within the rearing unit, to estimate its severity and prevalence in the population, i.e. an individual OWI.

3.2.1. Gill beat rate

The gill beat (breathing) rate of fish increases when the need for oxygen supply increases. This can be due to reduced oxygen levels in the water (Vigen, 2008) or a higher metabolic rate arising from higher activity levels or stress (Knoph, 1996; Vigen, 2008; Erikson et al., 2016, Table 3.2-1). In addition to the frequency of the gill beats, the beat amplitude or power of beat can also increase to improve the water flow over the gills. The latter may, however, be more difficult to observe and quantify. Increased beat rate at higher activity is normal (like when humans breath faster and deeper when running compared with resting) and thus is not necessarily an indicator of stress or reduce welfare, but rates higher than expected may indicate that something is wrong, for instance low oxygen saturation, bad water quality or problems with the gills.

Table 3.2.1-1. *Gill beat rate before and during stress in various procedures.*

Life stage	Threshold level (if any) and reference	% change (calm to stress)	Stressor	Reference
Post-smolt	56 beats min^{-1} (quiet) and 61 beats min^{-1} (stress 30.0-56.2 mg/l ammonia). Higher ammonia levels resulted in death	8.2	High ammonia levels	Knoph, 1996
Post-smolt	108 beats min^{-1} (normoxia), 120 breaths min^{-1} (hypoxia), 162 beats min^{-1} (delousing treatment)	11 (hypoxia) 50 (delousing treatment)	Hypoxia after a skirt was put around the cage and subsequent delousing within the skirt	Vigen, 2008
Slaughter	64±2 - 56±1 beats min^{-1} (bulk); 80±2 and 81±1 beats min^{-1} (crowded)	ca. 25-50	Crowding	Erikson et al., 2016

Sampling and analytical considerations

A qualitative assessment of gill beat rate during routine observation of the fish in both daily farming situations and various husbandry practices can be used as an OWI. Abrupt changes in frequency can be an indicator that welfare is compromised. Such changes can be observed from above the water, if visibility is good, or using underwater cameras (e.g. Erikson et al., 2016). It is best carried out if the fish are swimming slowly or static.

Changes in gill beat rate are difficult to quantify on the farm and usually must be assessed retrospectively from e.g. video footage. If the fish are relatively static, this can also be carried out manually by eye (e.g. with a stopwatch), but the repeatability and robustness of the results may not be good. Quantitative analysis of gill beat rate is therefore a LABWI.

Changes in absolute gill beat rates (see Table 3.2.1-1) can be a problematic LABWI as different water states, velocities, etc., can affect absolute values. We suggest the percentage change in gill beat rate measured before, during and after a routine as a better LABWI as this is less affected by the water status.

Strength of indicator

Gill beat rate is a good indicator of fish welfare (Martins et al., 2012). Abrupt increases in gill beat rate can be a quick, robust OWI of a potential welfare threat. Easy to observe in different procedures, from both above and below the water, so long as the fish is swimming slowly or relatively static.

Weakness of indicator

An increase in gill beat rate may be associated with positive experiences as well as welfare threats (Martins et al., 2012). An increase can also be indicative of several different welfare challenges, as a result the problem must be investigated further to identify its source(s). Quantitative assessment of gill beat rate is time consuming and is therefore classified as a LABWI. Technological advances that passively monitor gill beat frequency, via automated vision-based technology or tag systems may turn this indicator into a quantitative OWI in the future.

3.2.2. Reflex behaviour

Simple reflex indicators such as eye roll and the ability to flip upright can easily be used as direct indicators of stress (Davis, 2010). It has been widely acknowledged that certain reflexes, such as the corneal response, are clearly correlated with brain function and their return is one of the first clear signs of recovery after stunning (Anil, 1991). The animal is classified as insensible if responses to these indicators are lacking (Anil, 1991). The vestibulo-ocular reflex (VER; the "eye roll") appears to be a similar indicator. It is the last reflex the fish loses during anaesthesia and is the first reflex that returns after recovery (Kestin et al., 2002). However, there is a need to develop and validate an array of reflex responses suited to salmonids (rainbow trout and Atlantic salmon). Current reflex responses include: i) the eye roll (VER, the tendency for conscious fish to try and move their eyes into the horizontal plane), ii) the "righting-reflex" (rolling the fish on its back and seeing if it rolls back to the upright position in 3 seconds), and iii) the "tail-grab reflex" grabbing/pinching the fish's tail and seeing if it attempts to escape) (e.g. Davis, 2010).

Sampling and analytical considerations

Reflexes can be evaluated individually or as an index (Davis, 2010). An assessor does not need any custom or specialised equipment for their quantification. More advanced equipment e.g. electroencephalography (EEG) or electrocardiography (ECG) can also be used to monitor electrical activity in the heart or brain. However, this equipment requires expert knowledge, both in its use and interpretation.

Strength of indicator

Prolonged reflex impairment has been used as a mortality predictor for numerous fish species under both controlled laboratory conditions (Davis, 2010) and also under farming conditions (Raby et al., 2015). Reflex indices are simple, rapid and inexpensive and it is relatively easy to train people how to use them (e.g. at the commercial production site). They are not affected by fish size or acclimation (Davis, 2010).

Weakness of indicator

Involve exposing the fish to air without anaesthesia. The mechanisms that link reflexes to mortality prediction have not been identified.

3.2.3. Sea lice

Atlantic salmon in Norway are affected by two species of sea lice; salmon lice (*Lepeophtheirus salmonis*) and *Caligus elongatus*. *L. salmonis* is generally a greater health and welfare problem for salmon than *C. elongatus.* A sea lice infestation involving pre-adult and adult lice can lead to tissue damage, an inflammatory response, reduced immunity, primary stress responses, reduced appetite, changes to the gills and skin, a delayed healing response and osmotic problems (Bowers et al., 2000; Finstad et al., 2000; Ross et al., 2000; Boxaspen, 2006; Skugor et al., 2008). The primary stress response can even occur at the infective copepod stage (when the lice attach to the salmon but have not yet begun to feed, e.g. Finstad et al., 2011). Infections with larger numbers of salmon lice negatively affects swimming performance at high current velocities (Bui et al., 2016). Salmon can exhibit a behavioural response to an infestation of salmon lice by leaping from the water (Furevik et al., 1993).

Previous studies by Grimnes and Jakobsen (1996) and Finstad et al., (2000) reported that extreme infections with lice at the copepod and chalimus stages (>1 lice cm^{-2} fish or >100 lice fish $^{-1}$) did not have a critical impact upon the fish, but host mortality swiftly increased when lice reached the pre-adult stages. For data on infestation rates and their effects upon salmon welfare and mortality, see Table 3.2.3-1. Stien et al., (2013) suggested 0.12 lice cm^{-2} fish as the limit for salmon survival, with higher infestations being lethal, whereas Grimnes and Jakobsen (1996) reported a lethal infestation rate of > 0.15 lice cm^{-2} fish (but suggested that the actual threshold is most likely lower). Long-term infestation rates of 0.05 lice cm^{-2} equate to around 7 lice per fish for a 100g salmon and 35 lice for a 1000g fish. While wild salmonids often have lice levels that can lead to welfare problems and mortality (Holst et al., 2003; Torrisen et al., 2013), lice levels are strictly controlled and regulated in commercial aquaculture and such levels are rarely if ever seen on farmed fish (Folkedal et al., 2016). However, these levels may occur on some individuals, especially emaciated fish. Thus, for farmed salmon, where lice levels are low, frequent handling and treatment associated with delousing may be a more serious welfare issue than the lice themselves (see below).

Table 3.2.3-1. *Infestation rates and their effects on salmon welfare and mortality*

Effect	Infestation rate (cm^{-2})	Reference
Short-term	0.01	Nolan et al., 1999
Long-term	0.05	Nolan et al., 1999
Lethal	0.12	Stien et al., 2013

The other sea lice species affecting Norwegian salmon, *C. elongatus* is, in contrast to *L. salmonis*, not host specific and are found on a large number of different species (Revie et al., 2002 and references therein). They are generally less abundant in Norwegian salmon farms than *L. salmonis* and are smaller and less determined feeders. McKinnon (1993) found little response by the immune system on salmon infested with *C. elongatus.* All stages feed on mucus and epithelial cells but rarely penetrate the dermis, and do not usually cause open wounds on their hosts. However, high numbers of *C. elongatus* have been observed to be associated with wounds on salmon, but as far as the authors are aware, there are no data on the limits at which infestation rates start to cause welfare problems.

Sampling and analytical considerations

A detailed manual on how to count lice is available on http://lusedata.no. We will briefly summarise its key findings here. It is important to make sure that lice counting personnel have undergone adequate training and can correctly identify all of the different life stages of the lice. It is also important to ensure that you have all the necessary equipment for the procedure: a form recording lice counts, a suitable net for catching the salmon, the correct anaesthetic, white tanks for holding the sampled fish, a strainer for filtering the water in the tanks for lice, gloves that do not harm the fish, adequate lighting (a headlight in dark periods of the year) and a dip net for collecting the individual fish. The sampling must be carried out carefully to avoid harming the fish and in such a way that the sampled fish are representative of the group.

A maximum of 5 fish should be sedated at a time. A fish is usually sedated after approximately 1 min and is ready for the lice count when its tail no longer beats when it is lifted from the water. In the case of low air temperatures, the fish should be euthanised instead of sedated or the count can also be carried out with the fish submerged in water. During counting the fish should be held carefully using gloves that do not harm the fish. Each count must be carried out diligently, making sure that the fish are well-lit and against a bright background to ensure accurate counting. The number of lice should be classified into life stages, or at least into sessile, mobile and adult females. The water must be filtered to detect any lice that may have fallen off in the tanks and these lice must be included when calculating the average number of lice on the fish.

Strength of indicator

Given some simple training it is relatively easy to count the lice and classify them into stages. Lice clearly influence fish welfare, as even a few lice can be an irritant to the fish, and many lice can lead to wounds and in the long run, even mortality.

Weakness of indicator

As for all the welfare indicators that rely on sampling individual fish from sea cages, getting a representative sample of fish is often difficult. The sampled fish may therefore not represent the "true" situation in the cage.

3.2.4. Gill bleaching and gill status

The gills may be affected by a wide range of organisms and environmental conditions. Since the gills are not only responsible for gas exchange but also osmoregulation and the excretion of nitrogenous waste, damage can have profound effects on fish health and welfare. Bacterial infections, parasites, virus, fungi and poor water quality can all cause gill problems. The gills can respond in a limited number of ways including enlargement and proliferation of superficial cells which interfere with gill function. Therefore, gill damage can make fish more susceptible to low oxygen levels, stress or exercise. In freshwater, many parasites including *Ichthyobodo necator* (costia), *Trichodina* spp. and *Chilodonella* spp. may infect the gills. However, in many cases the main reason is poor water quality, making the gills vulnerable to parasites.

In the sea phase, chronic gill disease in the autumn of the first year after seawater transfer is a common and serious welfare problem. It is certainly multifactorial and epitheliocysts (gill cysts) are often observed (NVI, 2017). Ca. *Branchiomonas cysticola* has been isolated as the primary agent involved in the formation of epitheliocysts in Norwegian salmon (Steinum et al., 2015), but other Chlamydia-like organisms (Ca. *Syngnamydia salmonis*; Ca. *Piscichlamydia salmonis*) and microsporidia may play a role. Chronic gill disease is still not fully understood, but can certainly impact welfare resulting in mortalities of 10-20%, poor growth and increased susceptibility to stress during handling. Amoebic gill disease (AGD) is triggered by the marine amoeba *Neoparamoeba perurans*. It has occurred in all salmon aquaculture regions in recent years (Oldham et al., 2016) and has become a major problem in Norwegian aquaculture. High temperatures and salinity increase the risk for AGD outbreaks and therefore it is so far not a problem in northern Norway. It is also less frequent in fjords with a brackish (<25 ppt salinity) surface layer and the amoeba do not survive in freshwater (Karlsbakk, 2015). AGD is a gill infection that causes massive inflammation of the gills affecting respiration. Clinical infections are expressed as reduced appetite, lethargy, fish congregating at the surface and an increased gill beat rate (Kent et al., 1989; Munday et al., 1990). In untreated cases or advanced cases that are treated, mortalities may reach extreme levels, such as the Norwegian outbreak in 2006 with >80% mortality (Steinum et al., 2008). AGD infections are initially diagnosed by the scoring of pale mucoid areas on the gills, where 0 indicates no infection and 5 indicates a severe infection (Taylor et al., 2009)

Sampling and analytical considerations

Macroscopic evaluation of the gills can provide some limited information about gill condition and the severity of any damage. This can be supplemented by microscopic examination of fresh smears, but histological confirmation is usually required. The AGD scoring system is usually used to monitor both the severity of the infection and also the efficiency of treatment. This requires training in the handling of the fish and also assessing the score. Any suspected gill disease problem should be investigated by a trained fish health professional at the earliest opportunity.

Strength of indicator

Macroscopic examination is cheap, relatively easy to perform when given appropriate training and can provide an indication of the severity of the gill disease. AGD scoring can be used to guide treatment decisions and evaluations. Histopathological samples provides a definitive diagnosis, and some diagnostic services can provide a report in less than two days.

Weakness of indicator

While macroscopic examination and fresh smears can give some indication of gill damage, definitive evaluation requires histological examination. Delays in treatment especially for AGD can result in very serious mortalities.

3.2.5. Condition factor and other condition indices

Condition factor (*K*) is a well-accepted tool for assessing the nutritional status of fish (Bolger & Conolly, 1989; Nash et al., 2006). It is calculated using the formula $K = 100 \times \text{Weight (g)} \times \text{Length (cm)}^{-3}$ and the higher the *K* value, the rounder the fish. There is a clear positive correlation in Atlantic salmon between condition factor and their total lipid content (Einen et al., 1998, 1999; Hamre et al., 2004). Condition factor may vary throughout the year and tends to be higher during summer and autumn when water is warmer and growth rate is higher than during the colder winter and spring (Juell et al., 1994; Endal et al., 2000; Sutton et al., 2000). Moreover, condition factor decreases during smoltification (Farmer et al., 1978), then increases with fish size from around 1 in smolts to 1.6 nearer slaughter (see Stien et al., 2013 and references therein). Very low condition factor may be an indication of emaciation, but Folkedal et al., (2016) found physical appearance to be a better indicator of emaciation than condition factor, as there was an overlap in condition factor between individuals scored as emaciated and individuals scored as healthy. Extremely high condition factor may be indicative of vertebral deformation (Fjelldal et al., 2009a; Hansen et al., 2010).

As condition factor is variable and changes with both life stage and season it is difficult to define exact values that are indicative of reduced welfare, but < 0.9 is usually indicative of emaciation (Stien et al., 2013). Other related measurements include organosomatic indices, which are the relationship between the size of the fish and specified internal organs e.g. the hepatosomatic index (the relationship between the liver and body weight, HSI), the gonadosomatic index (the relationship between the gonads and body weight, GSI), the viscerosomatic index (the relationship between the entire viscera and body weight, VSI) and the splenosomatic index (the relationship between the spleen and body weight, SSI), see Barton (2002).

Sampling and analytical considerations

Indices range from being relatively non-invasive (e.g. straightforward measurements on anaesthetised fish) to lethal, e.g. for organosomatic indices (Sopinka et al., 2016).

Strength of indicators

They are rapid, simple and inexpensive and provide the user with good indications of the collective condition of the fish (Sopinka et al., 2016). There are some non-lethal options available (e.g. length–weight analysis, condition factor, relative weight) and these are already widely assessed on the farms.

Weakness of indicators

Condition indices can be affected by numerous factors including season, life stage, maturation status and the disease status of the fish (Sopinka et al., 2016). The effect often has to be considerable before abnormalities can be detected. The user can also draw inappropriate conclusions due to the limitations of the various methods (Sopinka et al., 2016). They cannot detect chronic stress but can detect a lack of somatic resources which may be related to stress. Organosomatic indices are lethal.

3.2.6. Emaciation state

In all production systems some individuals may become thin or emaciated. For example, in sea cages, some salmon can have stunted growth, very low condition factor (thin or emaciated) and generally poor appearance (Folkedal et al., 2016, Fig. 3.2.6-1), and are referred to as “losers”. Characteristics for emaciated fish are, in addition to their external appearance, a lack of (or little) perivisceral fat, melanisation in the kidney, and behavioural abnormalities such as slow swimming near the net at the surface, and swimming alone and at distance from the main group. They are often heavily infected with internal parasites, e.g. tapeworm, but it is not clear if this is a cause or consequence of the state of the fish. Salmon may become emaciated for various reasons, including disease (Hjeltnes et al., 2016), failed smoltification (Duston, 1994; Hjeltnes et al., 2016), stress (Huntingford et al., 2006), sea lice (Finstad et al., 2011) and the behavioural environment the fish are exposed to (Adams et al., 2000). Transfer to the sea involves a completely new and fluctuating environment, which is stressful and may make individuals stop feeding or switch to a zooplankton diet. A zooplankton diet results in lower growth than a pellet diet, and plankton diet may also lead to parasitic infections (e.g. Anisakis), which have been found in emaciated, but not healthy, individuals (Levsen and Magge, 2015).

Whatever the reason for stunted growth, fish that eventually become much smaller than the majority of the individuals in the group will potentially be outcompeted for food, or may not be able to feed on the larger pellets provided for the average fish size. Emaciated individuals therefore have poor survival and their prevalence often decreases over time (Folkedal et al., 2016). Vindas et al., (2016) recently found that while the brain serotonin activation is elevated in emaciated fish, the serotonergic system is unresponsive to additional stress, indicating that these fish are in a depression-like state. Emaciated fish are more susceptible to disease and their tendency to stay in the surface water, which contains more pathogens and sea lice larvae (Hevrøy et al., 2003), not only increases their levels of infection but they may also act as a source of infection for the rest of the population. As they are poor feeders, it is also difficult to give them in-feed treatments (Coyne et al., 2006).

Sampling and analytical considerations

It may be difficult to judge whether an individual is only lean but with potential to perform well, or in fact in terminal decline. Emaciated fish are usually small in terms of both length, weight and condition factor as their problems arise shortly after sea transfer. However, fish may start to become emaciated at a later stage, for instance as a result of disease and be similar to the average fish in length. Emaciated fish tend to swim slowly near the surface and are therefore more likely to be caught during sampling, resulting in overestimation of their abundance (Folkedal et al., 2016). As this bias is well-known among farmers emaciated fish are often excluded from samples, for instance during lice counts, as they are not representative for the cage. Such practices bias the sample in the opposite direction and fish with obvious welfare problems must be included in any welfare assessment.

Strength of indicator

Emaciated fish can usually be recognized by their abnormal behaviour and easily be spotted as they isolate themselves from the main school near the surface. The presence of emaciated fish may also function as an indicator that there are other problems in the cage, e.g. a disease outbreak or poor smolt quality (Folkedal et al., 2016).

Weakness of indicator

Estimating the proportion of fish in the cage that is emaciated is virtually impossible as there is no way to take representative samples.

***Fig. 3.2.6-1**. An emaciated salmon swimming slowly outside the school near the surface. Photo: Ole Folkedal*

3.2.7. Sexual maturity state

Salmon may mature both in the freshwater stage before smoltification (precocious maturation) or after sea transfer, sometimes as jacks a few months after sea transfer but predominantly as grilse after around 1.5 years at sea. Precocious maturation only occurs in males, but early sea maturation predominantly occurs in males which mature earlier than females. Precocious sexual maturation of parr inhibits smoltification and thus seawater tolerance (McCormick et al., 1998). Maturation is also associated with increased aggression (Taranger et al., 2010). In the wild, maturing salmon in the sea migrate towards the river for spawning, but it is difficult to answer whether mature or maturing farmed Atlantic salmon also exhibit a behavioural need to undertake a spawning migration (cf. Huntingford et al., 2006). Salmon start to physiologically adapt to a hypo osmotic environment during the maturation process (Persson et al., 1998) and it is possible that maturing salmon can experience some osmoregulatory challenges if they begin to mature in the sea cages (Stien et al., 2013). Changes in the activity of hormones associated with reproduction, e.g. sex steroids, cortisol and growth hormone, can affect the immune system of sexually maturing fish, resulting in increased disease susceptibility and a decrease in their overall health status (Taranger et al., 2010 and references therein). Maturing individuals also show abnormal behaviour in the cages, such as standing high in the water against the current direction (Fig. 3.2.7-1). The reduced immune capacity and ability for osmoregulation, together with behavioural changes may lead to reduced welfare and increased mortality in sexually mature salmon.

Sampling and analytical considerations

As with sampling for fish with other individual based OWIs, it is very difficult to estimate the proportion of fish that are sexually mature as their behaviour may bias samples. Initiation of sexual maturation can be detected as increased levels of hormones during early spring (Pall et al., 2006), while the first visible signs of maturation in cages are usually observed in June as increased GSI in maturing individuals (F. Oppedal, pers. comm.). Maturation at the end of the summer can often be detrimental to both body weight and flesh quality (Aksnes et al., 1986).

Strength of indicator

Sexual maturation may have major effects on fish welfare and a large proportion of the fish may mature if precautions are not taken, i.e. control by additional lights or the slaughter of fish before they are fully mature. If early signs of grilse maturation are detected in the spring, it may be possible to delay the development by use of additional light during the summer.

Weakness of indicator

Early detection of the onset of maturation by hormone analysis requires that blood samples are taken from a sufficient and representative number of individuals and sent to a laboratory for analysis; it is therefore a LABWI. Using GSI to detect the development of gonads requires that the fish are killed (see section 3.2.5).

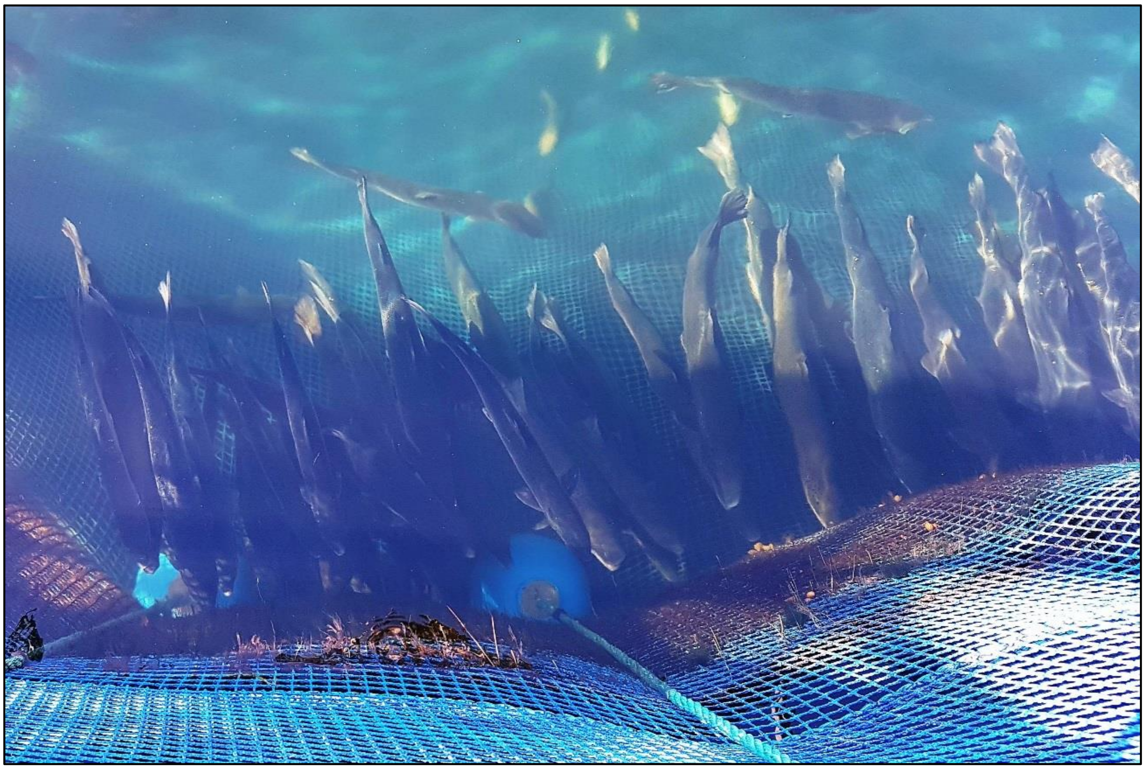


Fig. 3.2.7-1. *Maturing caged salmon that have left the school and stand against the current direction. Photo: Jan Erik Fosseidengen*

3.2.8. Smoltification state

The potentially detrimental physiological effects of transferring poorly smolted fish to seawater are temperature dependent and are greater at high (>14°C) rather than intermediate (10°C) and low (<7°C) temperatures; these challenges may lead to persistent osmotic duress and increased mortalities (Sigholt and Finstad, 1990; Handeland et al., 2000, 2003). In addition, the transfer of poorly smoltified salmon to full strength seawater at intermediate temperatures can also result in increased mortalities and poor growth performance for 1-2 months after transfer (Duston, 1994). These problems are less severe but may still occur if the fish are transferred to brackish water of 20 ppt (Bjerknes et al., 1992; Duston, 1994). Fish that are fully smoltified have few osmoregulatory problems when transferred to full strength sea water (Duston, 1994). It is therefore important to ensure that all fish have smoltified completely and not reverted before they are transferred to sea (Fig. 3.2.8-1).

Sampling and analytical considerations

Sea water adaptation is assessed prior to sea transfer by measuring plasma Cl^- concentrations (111-135 mmol L^{-1} in fresh-water, and 130-160 mmol L^{-1} in seawater), condition factor (decreases during smoltification), morphological indicators (silver colour, parr marks and dark fin edges) and sodium potassium ATPase (NKA) activity/gene expression (increases in smolts, and at approximately 10 µmol ADP mg^{-1} protein * hour, the fish is smoltified). Morphological changes related to smoltification can be scored according existing operational scoring schemes e.g. https://www.pharmaq-analytiq.com/sfiles/75/1/file/v6_prosedyre_010601_vurdering_av_smoltindeks.pdf

Strength of indicator

It is important, easy and inexpensive to collect fish from the tank before sea transfer and at least visually observe that they have the physical smolt characteristics such as silvery colour and darker fins. It is also relatively easy and inexpensive to use physiological or molecular tests for smoltification.

Weakness of indicator

Representative samples may be difficult to take as smoltified and unsmoltified fish have different behaviours such as vertical positioning in the tank. Determination of smoltification state on the basis of sodium potassium ATPase requires that the fish are killed and unless the farm has its own tools for analysis the samples must be sent to a laboratory. A low proportion of poorly smoltified fish may be difficult to detect unless a large number of individuals are examined.

Fig. 3.2.8-1*. Upper picture: A salmon that is not completely smoltified, which can be seen from the yellowish colour on the operculum and the area around the pectoral fin. Lower picture: A completely smoltified salmon. Photos: Jonatan Nilsson*

3.2.9. Vertebral deformities

Vertebral deformities are commonly associated with farmed salmonids. However, they have also been recorded in wild salmonids and non-salmonid populations for many years (Howes, 1894; Sambraus et al., 2014; Boglione et al., 2001; Fjelldal et al., 2009b). Given that wild salmonid populations exhibit vertebral abnormalities, it is reasonable to assume that there will be a background level in farmed fish (Branson and Turnbull, 2008). However, occasionally farmed fish have been severely affected, and despite progress in controlling vertebral deformities they continue to be a problem for the salmonid farming industry (Poppe, 2000; Witten et al., 2005, 2009).

As well as having a potentially significant economic impact, vertebral deformities have welfare implications. Hansen et al., (2010) reported that reduced growth is significantly correlated with an increase in the number of deformed vertebrae in Atlantic salmon. This finding is also supported by previous studies, which have suggested that vertebral malformations in Atlantic salmon are associated with reduced performance, raising concerns regarding the welfare of affected fish (Huntingford et al., 2006; Fjelldal et al., 2009a). It is currently not clear if fish with vertebral deformities experience pain (Branson and Turnbull, 2008). However, those severely affected are undoubtedly inferior swimmers (Powell et al., 2009) and less able to compete for food (Hansen et al., 2010). The vertebrae have a role in calcium and phosphorous homeostasis (Carragher and Sumpter, 1991; Persson et al., 1994), as well as a crucial biomechanical function, by enabling muscle anchoring, propulsion and flexibility during locomotion (Webb, 1975). Deformed fish also appear to have a reduced tolerance to handling and stress (Branson and Turnbull, 2008). There is little published evidence linking vertebral deformities to infectious diseases but it is a reasonable assumption that poor swimming ability could result in greater infection with parasites such as sea lice and displacement to sub-optimal parts of the cage, which could lead to physical damage and associated secondary infections (Samsing et al., 2015).

Although a comprehensive system for the classification of spinal deformities, similar to that in human medicine has not yet been developed for Atlantic salmon, Witten et al., (2009) have developed a 20-type classification system based on x-ray images of the spine which in the future might help establish links between different deformities and specific aetiologies (see Witten et al., 2009 for more information). Previous studies have also suggested methods for the classification of skeletal deformities in other teleost species (e.g. Boglione et al., 2001).

There are an array of potential risk factors for vertebral deformities in fish. These include various nutritional factors (Dabrowski et al., 1990; Cahu et al., 2003; Gorman and Breden, 2007), infectious disease (Kent et al., 1989), the temperatures the eggs are incubated at (Ørnsrud et al., 2004a; Fitzsimmons and Perutz, 2006), rapid growth in underyearling smolts (Fjelldal et al., 2006), water current and quality (Divanach et al., 1997), vaccination (Berg et al., 2006), environmental pollution (Sfakianakis et al., 2006) and triploidy (Fjelldal and Hansen, 2010; Leclercq et al., 2011; Fraser et al., 2012, 2015). It is likely that skeletal malformations, including vertebral deformities, are the result of several contributing factors (Vågsholm and Djupvik, 1998). This makes it difficult to link specific risk factors with specific deformities (Aunsmo et al., 2008b).

Research has shown that vertebral column compression often occurs late in ontogeny (Berg et al., 2006), making it difficult to identify the aetiology and little is known about the underlying biophysiological processes involved. A study by Witten et al., (2005) demonstrated that affected vertebrae in "short tail" Atlantic salmon exhibited altered vertebral end plates, inward bending vertebral edges and structural alterations in vertebral tissues. They also went on to hypothesise that an altered mechanical load could have resulted in the transformation of the bone growth zones and associated replacement of the intervertebral notochord by cartilaginous tissues (Witten et al., 2005).

In another study, Wargelius et al., (2010) showed that Matrix Metallo-Proteinase 13 (MMP-13) was significantly up-regulated in compressed vertebrae, suggesting "*there is a relationship between the development of vertebral compression and increased remodeling activities in farmed Atlantic salmon*".

Sampling and analytical considerations

Vertebral deformation can be graded from minor to severe. X-ray is used in order to detect minor deformations and when more accurate descriptions of the deformation is wanted. The fish are then typically radiographed with a portable X-ray apparatus, and from the digital images one identify the number and type of deformed vertebra.

Strength of indicator

With the exception of minor deformations it is easy to observe and it has a direct impact on the current and future welfare of the fish.

Weakness of indicator

As discussed above, vertebral deformation can be caused by a range of different factors or a combination of factors. It may therefore be difficult for the farmer to find the reason behind the development.

3.2.10. Fin damage and fin status

The fins of Atlantic salmon (as with other teleosts) consist of a fold or layer of epithelium that utilises a number of fin rays for support (see Videler 1993; Noble et al., 2012b).

Fin damage has been classified in many different ways according to the authors' preferences or background (see Noble et al., 2012b). Turnbull et al., (1996) classified fin damage as a) erosion, b) splitting and c) thickening (and also included malformed fins). All types of fin damage can lead to haemorrhaging within or from the tissue of the fin (e.g. Noble et al., 2012b) and this can be classified as an additional type d) haemorrhaging. Turnbull et al., (in prep.) have recently begun classifying fin damage as active or healed. Regardless of the degree of tissue loss, active lesions indicate an ongoing problem that should be addressed, whereas healed fins are evidence of historical damage, see Fig 3.2.13-2-3.

Fin damage is an acknowledged welfare threat as it damages living tissue (Ellis et al., 2008). The fins also possess nociceptors (Becerra et al., 1983) and active fin damage (see Fig. 3.2.13-2-3) can be a route for pathogenic infection (Turnbull et al., 1996; Andrews et al., 2015; Noble et al., 2012b and references therein) as it disrupts the epidermal barrier (Andrews et al., 2015). However, the relationship between the i) severity, ii) frequency and iii) type of fin damage and welfare has not been clearly elucidated in aquaculture environments, especially with regard to different species and life stages (see for example, Ellis et al., 2008; Noble et al., 2012b). The effects of fin damage can also differ according to the life stage of the salmon. For example, in parr, the loss of pectoral fins can reduce their station-holding capacity (Arnold et al., 1991). In smolts and post smolts, active fin damage can subject the fish to osmotic duress (Andrews et al., 2015).

The sampling and analytical considerations and the strengths and weaknesses of using fin damage as a welfare indicator will be summarised at the end of the external morphological WIs section, below.

3.2.11. Scale loss and skin condition

In this handbook we will define epidermal damage as the loss of epidermal tissue to the dermal/subdermal/muscle tissue at any location on the fish's body, which may also be accompanied by haemorrhaging, ulceration or changes in skin colour (Vågsholm and Djupvik, 1998).

The skin with its scales and mucus layer represents a first barrier to infections. Even a small injury can function as a gateway for infection. Further, the presence of nociceptors in the skin suggest skin damage could potentially cause pain and larger wounds/ulcers may compromise osmoregulation. Thus, the condition of the epidermis can have a marked effect upon fish welfare and the relationship between epidermal damage and welfare is outlined in a previous review (Noble et al., 2012b). Epidermal damage can be a key OWI for the farmer, since it is easy to detect and indicates a serious welfare concern. However, the impacts of epidermal injury upon welfare depend not only upon the type, severity and frequency of the injury, but also the potential pathogens that are present in the rearing environment. Noble et al., (2012b) outlined three key bacterial infections i) infectious salmon anaemia (Totland et al., 1996), ii) winter ulcers (Løvoll et al., 2009), and iii) piscirickettsiosis (Smith et al., 1999) that can colonise epidermal lesions and utilize epidermal injuries as a route for infection (Løvoll et al., 2009; Nylund et al., 1994). In fresh water the major risk is from fungal like organisms, e.g. *Saprolegnia* spp. Further, numerous fish health conditions actively impact upon skin condition, as outlined in Tables 3.1.5-1, 2 and 3.

In additional to skin damage, skin colour can change in relation stressful and long lasting crowding events (Mejdell et al., 2007) and is therefore also a suitable qualitative OWI for certain routines. Kittilsen et al., (2009) have shown that the external appearance of Atlantic salmon and rainbow trout reflects their stress responsiveness and their skin patterns can be densely spotted or nearly pattern free. In both species, fish that were densely spotted were also low cortisol-responsive fish. However, more studies need to be done on different domesticated salmon strains to verify whether the trait is stable and persistent and could be used as an OWI.

In terms of effects upon fish welfare, epidermal injuries are damage to live tissue and skin has nociceptors, as the network of free nerve cells in fish run through and in the proximity of the epidermis (Kotrschal et al., 1993). Epidermal injuries affect the physical welfare needs of Atlantic salmon relating to i) osmotic balance, ii) health and the behavioural need of iii) protection. However, their relative importance varies with life stage. Epidermal damage is accounted for in welfare assurance schemes; the RSPCA welfare standards for farmed Atlantic salmon (2018a) state a sample of 150 fish should be taken during slaughter and any epidermal damage and lesions should be noted and acted upon if required. Handling trauma can also impact upon external (and internal) morphological indicators. For example, crush injuries from netting or fish being accidentally trapped in a closing pump valve can be diagnosed via clear damage on the epidermis and also potential subcutaneous damage.

3.2.12. Eye damage and eye status

Eyes can be damaged in numerous ways, with various aetiologies (Table 3.2.12-1) with mechanical injuries being the most frequent (Pettersen et al., 2014). The eyes are especially vulnerable to mechanical trauma, or desiccation during handling, due to their position where they protrude slightly from the head and with no eyelids or self-lubrication for protection. Exophthalmia, also known as "pop eye" is recognized as a non-specific sign of disease that should be investigated further. Behind the eyes, there are numerous of blood vessels (choroid plexus) and also connective tissue and muscle providing mobility for the eyes. Hence, when microorganisms colonize and grow there, the eyes may be pressed out by inflammatory tissue or accumulation of fluid (Poppe, 1999). Eyes can also protrude due to osmoregulatory oedemas and gas bubble disease where gas accumulates in the tissues (Poppe, 1999). Handling fish with exophthalmia can increase the risk of causing even further injuries. It may be a challenge to distinguish between damage that occurs due to the fact that the eyes are protruding and damage resulting in protrusion. In all eye damage it can progress to rupturing of the eye resulting in a shrunken structure (a phthisic eye) and at this stage it is very difficult to determine aetiology. Observation of single fish with darker skin colour can also be a sign of blindness.

A cataract is opacity of the lens (Tröße et al., 2009; Neves and Brown, 2015). Severe cataracts are considered to be irreversible damage of the lens fibres (Waagbø et al., 2003) but opacity of the lens due to osmotic changes can also be reversible (Iwata et al., 1987 in salmonids). Exposure to repetitive stress can increase lens susceptibility to later cataract development (Bjerkås and Sveier, 2004). Cataracts can lead to impaired vision or blindness (Neves and Brown, 2015) which can impact upon avoidance behaviour and also feeding ability, as fish can have problems locating pellets or avoiding potential danger (Noble et al., 2012b). There is also an association with increased susceptibility to secondary diseases and increased mortalities compared with healthy fish (see Pettersen et al., 2014 and references therein e.g. Breck and Sveier, 2001; Ersdal et al., 2001; Waagbø et al., 2010; Remø et al., 2011). Eye condition is also used as a quality indicator and fish that have cataracts often display dark discolouration, and can be downgraded as a result (Neves and Brown, 2015).

A number of factors have been connected to the development of cataracts, such as nutritional deficiencies, osmotic imbalances, water temperature fluctuations (Bjerkås et al., 2001), parasitic infections in the eye, toxic factors, ultraviolet radiation, oxidative stress to the lens fibre, genetic predisposition with rapid growth and rapid change in water salinity (reviewed in Bjerkås and Sveier, 2004). Cataract prevalence in farmed Atlantic salmon has been related to histidine deficiency in salmon feed (Breck et al., 2003, 2005; Waagbø et al., 2010) associated with the removal of blood and bone meal from the feed and also using more vegetable oil in salmon feed (Waagbø et al., 2003; Bjerkås and Sveier, 2004). It has also been shown that cataract development initiated in the freshwater production phase continues after transfer to the seawater (Bjerkås et al., 2001). In a study by Ytrestøyl et al., (2013) the incidence of cataracts in Atlantic salmon post-smolt raised in RAS at different salinities increased over time and was significantly higher for 450g post-smolts raised at 32ppt, compared with the groups raised at 12 and 22 ppt. The molecular basis of cataractogenesis in the salmon lens is still unclear (Tröße et al., 2009).

Table 3.2.12-1. Eye damage, aetiology and risk factors

Eye damage	Risk factors	Effect on welfare	Minimize risk by	References
Injuries-mechanical	Handling Netting Pumping Grading	Potentially painful, skin around eyes and cornea sensitive to nociceptive stimuli. Secondary infections. Can lose vision.	Vacuum pump instead of manually netting/lift nets. Individually netting. Optimize design of handling equipment.	Noble et al., 2012b Pettersen et al., 2014 Gismervik et al., 2016 Chervova, 1997 Sneddon, 2009
Exoph-thalmia	Microorganisms Cardiovascular disorders Oedema Trauma Gas bubble disease (>110% saturation) Generalized illness	Depending on aetiology, but always a sign of welfare at risk. Risk of blindness, due to stretching/ compression optic nerve.	Depending on aetiology.	Poppe, 1999 Noble et al., 2012b Pettersen et al., 2014
Ruptured eyes	Numerous factors e.g. feeding routines	Presumable painful Secondary infections.	Risk factor dependent. If related to feeding then feeding must be optimised (multiple feedings, dispersed areas)	Noble et al., 2012b Sneddon, 2003
Eye flukes	*Diplostomum* spp. Fresh water with piscivorous birds and snails in life cycle	Loss of vision		Poppe, 1999
Haemorr-hages indirect	*Trauma, infections, Parvicapsula pseudobranchicola*.	Affecting Depends on severity and extent.	Avoid trauma, control parasites or infections.	Pettersen et al., 2014 Hjeltnes et al., 2016
Injuries-irritants	Chemical Thermal Toxic UV-light	Pain and reduced sight	Depends on the cause, amongst others, overdosing of medicines	Pettersen et al., 2014

3.2.13. Deformed Opercula

The opercula have an important role in the respiratory mechanisms of fish as they are part of the buccal pump mechanism which increases the respiratory efficiency of teleosts. Deformities such as shortened, missing and warped gill operculum have been associated with the intensive aquaculture production conditions (Koumoundouros et al., 1997).

The aetiology of opercular deformities is largely unknown, but it is primarily attributed to suboptimal rearing conditions, dietary deficiencies and pollution (Eriksen et al., 2007) in particular in earlier life stages. The literature is unclear on aetiology since no studies have examined the pathogenesis of the condition. It has been stated that the deformities occurring after first feeding are more affected by culture conditions than genetic factors (Sadler et al., 2001). A diet that is deficient in phosphorus can lead to abnormally soft opercula in both Atlantic salmon parr and post-smolts (Bæverfjord et al., 1998). In addition, Eriksen et al., (2007) showed that abnormal opercula could be caused by prenatal conditions experienced by the parental generation. Another hypothesis is that the opercula suffer from traumatic injuries during highly competitive feeding. In scramble competition for food a fish that gets a pellet forces out excess water through the open opercula before swallowing the pellet. This leave the opercula susceptible to other fish swimming rapidly towards other pellets with open mouths. Diagnostic case material has demonstrated traumatic damage to the edge of the opercula but there is no empirical evidence to support this hypothesis.

Opercular deformities can lead to a reduced capacity for pumping water over the gills and increases the susceptibility of fish to welfare problems when exposed to inadequate water quality, hypoxic conditions and increased oxygen demand (Ferguson and Speare, 2006). In order to maintain sufficient perfusion of the gills, affected fish have to increase and maintain elevated swimming speeds (Branson, 2008), further increasing the energy cost of respiration. The resulting energy deficit can influence growth performance of the affected fish (Standal and Gjerde, 1987; Burnley et al., 2010). In addition to this, opercular deformities can disturb normal ion uptake balance in freshwater fish (McCormick, 1994).

Missing or shorten opercula (Fig. 3.2.13-1) exposes gill filaments to external trauma, which may be the cause of observed abnormalities in exposed gill tissue (Pettersen et al., 2014). It is not clear if the damage to the gills is the result of contact with external structures or abnormal flow patterns over the gills. Damage to the opercula is associated with increased mortality rates, susceptibility to diseases and therefore reduced animal welfare (Eriksen et al., 2007). However, it has also been shown that Atlantic salmon with shortened opercula can have a significantly lower risk of mortality during an outbreak of bacterial kidney disease compared to fish with a normal opercula (Burnley et al., 2010), although the reason for this association is still not clear.

A higher prevalence of shortened opercula has also been reported for Atlantic salmon smolts produced in flow through systems compared to smolts produced in recirculating aquaculture systems prior to sea water transfer (Kolarevic et al., 2014) and the condition has also been associated with triploidy in juvenile Atlantic salmon parr, smolts and post-smolts (Sadler et al., 2001). Opercular erosion has also been previously used as an OWI in a study on rainbow trout and white-spotted charr in duoculture (Noble et al., 2012c).

Fig. 3.2.13-1. *A salmon parr with shortened operculum. Photo: Jonatan Nilsson*

Sampling and analytical considerations for the morphological WIs fin damage, skin damage, eye damage and opercular injuries

Morphological OWIs can be qualitatively assessed as group OWIs using observations from above the water if visibility is good or the fish are swimming close to the surface. It can also be assessed using cameras in real time. Abrupt changes in prevalence can be an indicator that welfare is compromised. Although the simple presence/absence of these OWIs can be used as an early warning system for welfare threats, this does not allow the severity or frequency of the problem within the population to be accurately estimated.

Quantitative assessments of external OWIs can be carried out relatively rapidly on the farm, but currently depend upon sampling and manually handling the fish. The sampling regime must avoid harming the fish and the operator must make sure that the sampled fish are representative of the population. This is time consuming, labour intensive and can disturb both the fish and existing husbandry tasks such as feeding. Many scoring systems for quantifying morphological OWIs are currently being used by both the industry and researchers, meaning benchmarking, auditing and comparisons between farms and studies can be problematic.

The FISHWELL handbook suggests a unified scoring system (Tables 3.2.13-2-1, 3.2.13-2-2 and 3.2.13-2-3) that is primarily aimed at farmers to help them assess welfare and rapidly detect potential welfare problems out on the farm. It is an amalgamation of the injury scoring schemes used in the Salmon Welfare Index Model (SWIM) (Stien et al., 2013), the injury scoring scheme developed by the Norwegian Veterinary Institute (NVI) (Grøntvedt et al., 2015; Gismervik et al., 2016) and also from other schemes developed by J. F. Turnbull (University of Stirling) and J. Kolarevic and C. Noble (Nofima).

Our suggested scheme standardises scoring for 14 different indicators to a 0-3 scoring system:

i) emaciation, ii) skin haemorrhages, iii) lesions/wounds, iv) scale loss, v) eye haemorrhages, vi) exophthalmia, vii) opercular damage, viii) snout damage, ix) vertebral deformities, x) upper jaw deformity, xi) lower jaw deformity, xii) sea lice infection, xiii) active fin damage, xiv) healed fin damage.

Pictures used in the system represent examples of each scoring category. We suggest dorsal, caudal and pectoral fins as the primary fins to monitor for fin damage. As a comprehensive system for the classification of vertebral deformities, similar to that in human medicine has not yet been developed for Atlantic salmon, we suggest a simplified scoring system similar to that used in the RSPCA welfare standards for farmed Atlantic salmon (RSPCA, 2018a).

Cataract damage is classified using an existing and widely used 0-4 scoring scheme (Wall and Bjerkås, 1999), see Fig. 3.2.13-3. The scoring method records the cataract area in relation to the entire lens surface (looking through the pupil along the pupillary/optic disc axis). You can quickly assess large numbers of fish with minimal equipment to get an impression of the severity of the problem. If possible, a selected number of fish should be inspected under darkened conditions (also with better equipment) to give some indication of position, type, development and aetiology. However, it does not record the density of the cataract which can be important and should be annotated separately (T. Wall pers. comm.)

Strength of external morphological WIs (fin damage, skin damage, eye damage, opercular injuries etc.)

External injuries are an immediate indication or poor fish welfare (Noble et al., 2012b). Abrupt increases in injury frequency and severity can be a quick, robust OWI of poor welfare and an underlying cause that requires urgent investigation. They are easy to observe during a variety of procedures, from both above and below the water, so long as the fish are swimming slowly or relatively static (as group OWIs) and also during routine sampling e.g. sample weighing or lice counting procedures (individual OWIs). Assessment can be carried out relatively rapidly on live fish.

Weakness of external morphological WIs (fin damage, skin damage, eye damage, opercular injuries etc.)

Injuries may have a variety of potential causes and the problem must therefore be investigated further to identify their source(s). Quantitative assessment of external injuries requires handling and sampling of the fish and this can be time consuming, especially in large deep rearing systems where it can take some time to catch the fish. It can also be time consuming to process the individual OWI data and get data the farmers can act upon. Technological advances that passively monitor injuries, via e.g. automated vision-based technology may improve the operational feasibility of morphological OWIs.

Table 3.2.13-2-1. *Morphological scheme for classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. (Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. Nilsson. Photos: K. Gismervik, L. H. Stien, J. Nilsson, C. Noble, J. F. Turnbull, P. A. Sæther, I. K. Nerbøvik, I. Simion, B. Tørud, B. Klakegg, R. Andersen, C. Karlsen, K. J. Merok, F. Gregersen)*

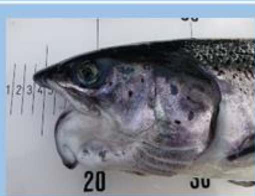
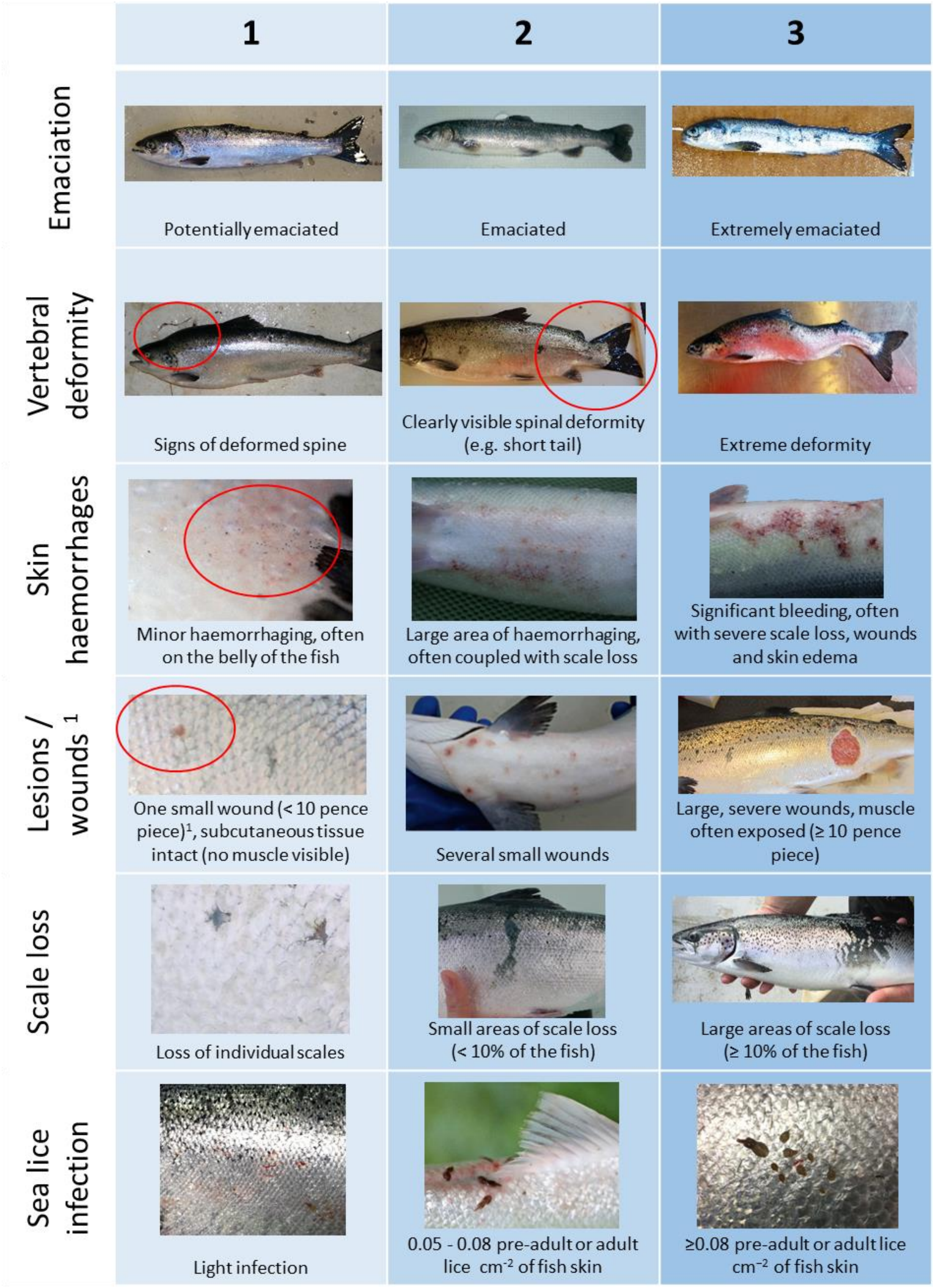
	1	2	3
Eye haemorrhage	 Minor haemorrhages	 Larger haemorrhages, or traumatic injury	 Large haemorrhages / traumatic injury. Eye may be ruptured
Exophthalmia	 Eye protruding a little	 Moderate eye protrusion	 Major eye protrusion
Opercular damage	 Operculum only partly covering gills	 Operculum absent on one of the gills (gill exposed)	 Both opercula absent (both gills exposed)
Snout damage	 Minor wound on snout (either jaw)	 Moderate wound and broken skin on snout	 Large deep and extensive wound. Can cover the whole head
Upper jaw deformity	 Suspected malformation	 Distinct malformation	 Major malformation, jaw pointing backwards
Lower jaw deformity	 Suspected malformation	 Distinct malformation	 Major malformation, jaw pointing backwards

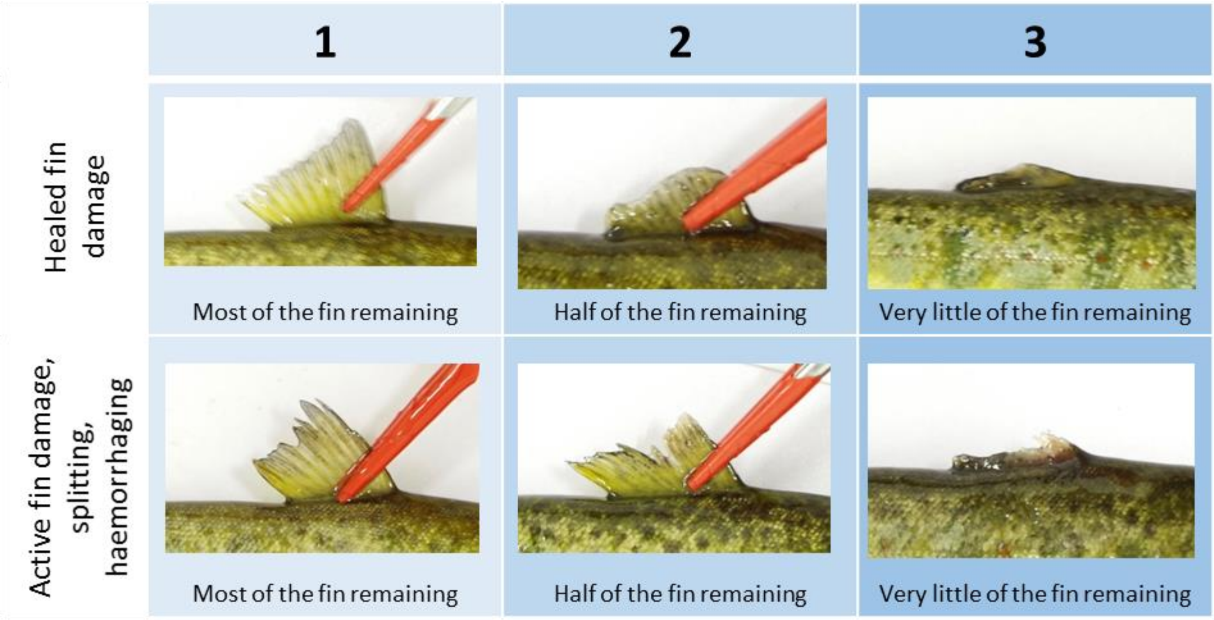
***Table 3.2.13-2-2**. Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. (Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. Nilsson. Photos: K. Gismervik, L. H. Stien, J. Nilsson, C. Noble, J. F. Turnbull, P. A. Sæther, I. K. Nerbøvik, I. Simion, B. Tørud, B. Klakegg, R. Andersen, C. Karlsen, K. J. Merok, F. Gregersen)*



	1	2	3
Emaciation	Potentially emaciated	Emaciated	Extremely emaciated
Vertebral deformity	Signs of deformed spine	Clearly visible spinal deformity (e.g. short tail)	Extreme deformity
Skin haemorrhages	Minor haemorrhaging, often on the belly of the fish	Large area of haemorrhaging, often coupled with scale loss	Significant bleeding, often with severe scale loss, wounds and skin edema
Lesions / wounds [1]	One small wound (< 10 pence piece)[1], subcutaneous tissue intact (no muscle visible)	Several small wounds	Large, severe wounds, muscle often exposed (≥ 10 pence piece)
Scale loss	Loss of individual scales	Small areas of scale loss (< 10% of the fish)	Large areas of scale loss (≥ 10% of the fish)
Sea lice infection	Light infection	0.05 - 0.08 pre-adult or adult lice cm^{-2} of fish skin	≥0.08 pre-adult or adult lice cm^{-2} of fish skin

[1] For pre-smolts "one small wound" should be < 1 cm. NB! Wounds that penetrate the abdominal cavity should be scored as a 3) irrespective of size

Table 3.2.13-2-3. *Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. It is important to differentiate between healed lesions and active lesions. Active lesions indicate an ongoing problem that needs to be addressed (Figure: J. F. Turnbull, C. Noble, D. Izquierdo-Gomez, L. H. Stien, K. Gismervik, J. Nilsson. Photos: J. F. Turnbull)*



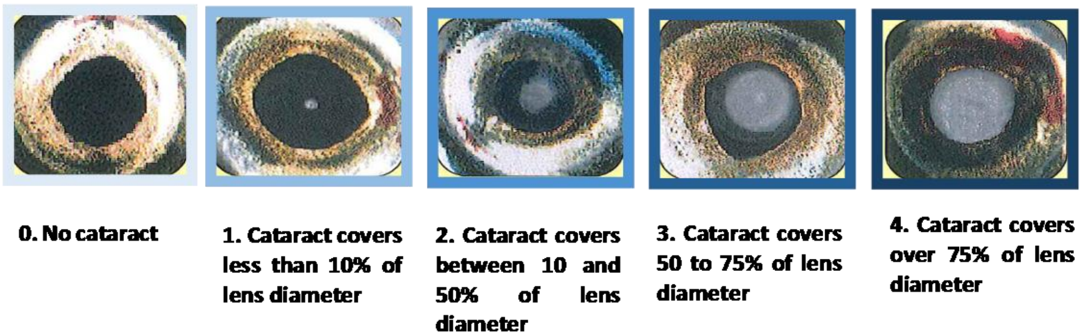


Fig. 3.2.13-3. *Morphological scheme for diagnosing and classifying eye cataracts in Atlantic salmon Text reproduced from "Wall, T. & Bjerkås, E. 1999. A simplified method of scoring cataracts in fish. Bulletin of the European Association of Fish Pathologists 19(4), 162-165. Copyright, 1999" with permission from the European Association of Fish Pathologists. Figure: David Izquierdo-Gomez. Photos reproduced from "Bass, N. and T. Wall (Undated) A standard procedure for the field monitoring of cataracts in farmed Atlantic salmon and other species. BIM, Irish Sea Fisheries Board, Dun Laoghaire, Co. Dublin, Ireland, 2p." with permission from T. Wall.*

3.2.14. Abdominal organs

Inflammation is a non-specific reaction to tissue damage and can be a response to a wide range of factors including, but not limited to, infectious microbes, parasites, mechanical disturbances, exposure to temperature extremes or harmful chemicals (e.g. Roberts and Rodger, 2012; Pettersen et al., 2014). The intestine is a key entry site for infectious agents and these may lead to inflammation and haemorrhaging in the intestine (Poppe, 1999; Lumsden, 2006). Subjecting salmon to different types of, or levels of, nutritional ingredients that they are not adapted to may also lead to inflammation of the intestine (e.g. Bæverfjord and Krogdahl, 1996). Typical indicators for acute inflammation are discoloured and swollen organs, haemorrhages and necrosis (e.g. Pettersen et al., 2014). Melanin deposition is also a sign of a chronic inflammatory response (Agius and Roberts, 2003). Inflammation and reduced organ function can also be linked to illness and negative performance (Pettersen et al., 2014).

Sampling and analytical considerations

The macroscopic evaluation of abdominal organs can give the observer an indicator of specific diseases or parasites, or more generally give some indications of e.g. circulatory failures or peritonitis. Histopathological examination of abdominal organs can be important for aiding diagnosis. Other tests for the presence of pathogens may also be required. While the diagnosis of many diseases requires a diagnostic investigation, trained personnel can often determine the most probable cause of death by carrying out external and internal macroscopic observations during an outbreak of disease or for some endemic diseases (Aunsmo, 2008a).

Strength of indicator

Observation of gross internal abnormalities is a quick and decisive demonstration of a disease condition which will usually have a negative effect on welfare. Histopathology with other sources of information is often required to reach a definitive diagnosis.

Weakness of indicator

Abdominal organs are most easily and usefully inspected and diagnosed on freshly killed fish, meaning the fish have to be killed prior to examination.

3.2.15. Vaccine-related pathology

The vaccination of salmonids in the Norwegian aquaculture industry has dramatically decreased the number of outbreaks of historically important bacterial diseases. As a result, mortalities have decreased considerably, there has been a marked reduction in antibiotic use and animal welfare has improved (e.g. Hjeltnes et al., 2017). However, the vaccine and the vaccination process can have negative impacts on welfare. The general consensus is that the vaccination of fish with current vaccines results in a net benefit for both fish health and welfare (Midtlyng, 1997; Berg et al., 2006; Evensen, 2009). The Norwegian legislation (FOR-2008-06-17-822, Akvakulturdriftsforskriften, §63) also states: "*All juveniles of the species Salmo salar shall at least be vaccinated against furunculosis, vibriosis and cold water vibriosis*".

In Norway, the majority of the current vaccination procedures for Atlantic salmon involve injecting oil-based multivalent vaccines intraperitoneally. The first oil-based vaccines came on the market in the early nineties and each dose had a volume of 0.2 ml, but in recent years new vaccines with lower dosages are becoming more widely used. The oil-based adjuvant operates as a depot of the antigens and an irritant to stimulate the fish's response and thus delivers a long-term effect. However, it can also contribute to potential negative side effects in the fish by its irritant and anti-inflammatory action. The changes in the vaccine formulations over the years are the result of a desire to balance efficacy against the potential side effects.

There is variation in the severity of side effects both between vaccines and with the same vaccine on different occasions (Poppe and Breck, 1997). Factors that can influence the result of a vaccination include: the vaccination technique, water temperature during vaccination (Sommerset et al., 2005; Berg et al., 2006), fish size when subject to vaccination (Berg et al., 2006), hygiene (Olsen et al., 2006), the health status of the fish and individual differences in how fish respond to the vaccine (Midtlyng and Lillehaug, 1998).

The widespread use of vaccines, in addition to their positive and also potentially negative side effects makes vaccination a factor that has a great impact upon the welfare of salmon in Norwegian aquaculture. According to a survey conducted by the Norwegian Veterinary Institute (Hjeltnes et al., 2016), 60.9 % of the respondents reported that vaccine side effects are a minor health problem for fish, and 58.7 % answered that only a few such injuries are ranked above grade 3 on the Speilberg Scale (see Table 3.2.18-1 and Fig. 3.2.18-2). The side effects of vaccination have become milder since the first oil-based vaccines came on the market, but it can still be stressful for the fish to be vaccinated. An example of the potentially severe side effects of vaccination and their implications is presented and discussed in Poppe and Breck (1997). In addition to the visible changes in the fish's abdominal cavity the side effects of vaccination can include: reduced appetite (Sørum and Damsgård, 2004; Bjørge et al., 2011), reduced growth (Midtlyng and Lillehaug, 1998; Sørum and Damsgård, 2004; Aunsmo et al., 2008b), Uveitis (Koppang et al., 2004), vertebral deformities (Aunsmo et al., 2008a), systemic autoimmune symptoms (Koppang et al., 2008; Haugarvoll et al., 2010) and behavioural changes (Bjørge et al., 2011). To minimise the potential side effects of vaccination it is important to monitor the side effects, work on the continuous improvement of vaccine formulation and the optimisation of vaccination routines.

Sampling and analytical considerations

The degree of vaccine side effects in individual fish is often evaluated according to the "Speilberg scale" (Midtlyng et al., 1996), see Table 3.2.15-1 and Fig. 3.2.15-2. The Speilberg Scale is widely used as a welfare indicator in the Norwegian aquaculture industry and is reproduced in Fig. 3.2.15-2 with kind permission from Lars Speilberg. The scale is based on a visual assessment of the extent and location of clinical changes within the abdominal cavity of the fish and it describes changes related to peritonitis; adhesions between organs, between organs and the abdominal wall and melanin deposits (see also Pettersen et al., 2014 and references therein). A Speilberg score of 3 and above is generally regarded as undesirable.

Strength of indicator

Simple, rapid and inexpensive to use.

Weakness of indicator

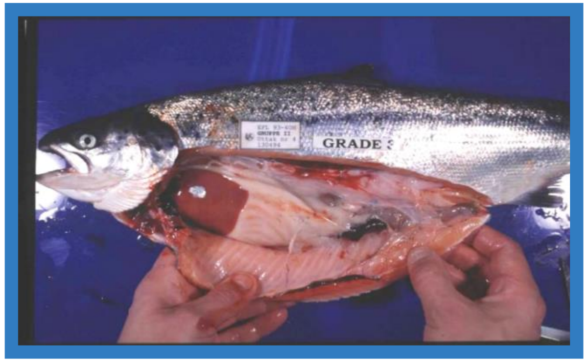
Fish needs to be sacrificed. It can be subjective (rather than objective) and requires adequate training to be reliable or comparable between sites. Different vaccine types may vary in efficacy and side effects, but the same vaccine may also vary in effects and side-effects (Poppe and Breck, 1997).

Table 3.2.15-1*. The Speilberg Scale, reproduced from "Midtlyng et al., 1996, Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (Salmo salar L.) against furunculosis. Fish & Shellfish Immunology 6, 335–350. Copyright 1996" with permission from Elsevier.*

Score	Visual appearance of abdominal cavity	Severity of lesions
0	No visible lesions	None
1	Very slight adhesions, most frequently localized close to the injection site. Unlikely to be noticed by laymen during evisceration	No or minor opaquity of peritoneum after evisceration
2	Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall. May be noticed by laymen during evisceration	Only opasicity of peritoneum remaining after manually disconnecting the adhesions
3	Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall. May be noticed by laymen during evisceration	Minor visible lesions after evisceration, which may be removed manually
4	Major adhesions with granuloma, extensively interconnecting internal organs, which thereby appear as one unit. Likely to be noticed by laymen during evisceration	Moderate lesions which may be hard to remove manually
5	Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may have focal, prominent and/or heavily pigmented lesions or granulomas	Leaving visible damage to the carcass after evisceration and removal of lesions
6	Even more pronounced than 5, often with considerable amounts of melanin. Viscera cannot be removed without damage to fillet integrity	Leaving major damage to the carcass

1. Very slight adhesions, most frequently localized close to the injection site. Unlikely to be noticed by laymen during evisceration.

2. Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall. May be noticed by laymen during evisceration.



3. Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall. May be noticed by laymen during evisceration.

4. Major adhesions with granuloma, extensively interconnecting internal organs, which thereby appear as one unit. Likely to be noticed by laymen during evisceration

5. Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may carry focal, prominent and/or heavily pigmented lesions or granulomas

6. Even more pronounced than 5, often with considerable amounts of melanin. Viscera irremovable without damage to fillet integrity.

Fig. 3.2.15-2*. The Speilberg Scale for intra-abdominal lesions after intraperitoneal vaccination of Atlantic salmon. Photos provided and reproduced with kind permission from Lars Speilberg. Text reproduced from "Midtlyng et al., 1996, Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (Salmo salar L.) against furunculosis. Fish & Shellfish Immunology 6, 335–350. Copyright 1996" with permission from Elsevier.*

3.2.16. Cortisol

Stress is widely defined as "*as a condition in which the dynamic equilibrium of an organism, called homeostasis, is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli, commonly defined as stressors*" e.g. Iversen and Eliassen (2009) and references therein (see also Varsamos et al., 2006; Wendelaar Bonga, 1997, 2011). However, Schreck (2010) preferred a broader interpretation of stress, "*as stress being the physiological cascade of events that occurs when the organism is attempting to resist death or re-establish homeostasis in the face of a threat*". The stress response is categorised into three phases.

- The primary stress response involves the activation of the HPI axis and the secretion of catecholamines (CA) and cortisol into the circulatory system.
- The secondary stress response is the release of glucose into the circulatory system, with increased heart and respiration rate and other physiological changes as a result of the hormones released via the primary response.
- The tertiary stress response is the eventual result (in the whole animal) of excessive, mismanaged or persistent stress and includes adverse effects on growth, immunity and changes in behaviour which can result in lower survival.

It is not always clear what people mean by a stressed animal, since this can be a normal response or a maladaptive tertiary response.

As CA release is rapid and short lived, one cannot use the secretion of CA's as a primary stress response indicator. However, cortisol release in teleosts is relatively slow and the level of circulating plasma cortisol in the fish is therefore used as a measure of the primary stress response. Until recent years neurophysiology and behaviour have been the major tools for investigating the feelings based approach to fish welfare (Chandroo et al., 2004a, 2004b; Rose, 2002; Sneddon, 2006) and cortisol may also be used to evaluate this approach. Early studies by Kestin (1994) linked endocrine stress responses to the neurophysiological aspects of fish welfare. As for humans, cortisol activity in fish is instigated by activity in the brain and changes in plasma cortisol can be linked to negative experiences or the fear response (Schreck, 1981; Ellis et al., 2012b), although its links to positive states cannot be discounted (Ellis et al., 2012b). However, the majority opinion of the authors on the Ellis et al., (2012b) paper was that cortisol elevation is linked to negative feelings in fish.

Despite its use as an indicator for the primary stress response (Barton and Iwama, 1991; Wendelaar Bonga, 1997, 2011) and animal welfare, cortisol levels must be interpreted with caution. A stress response occurs both with positive and negative experiences and only becomes harmful in the tertiary phase if the stress response is excessive, protracted or mismanaged by the animal's physiological processes (Maule et al., 1989; Davis, 2006; Iversen and Eliassen, 2014). It is important to realise that all animals experience various forms of stressors as part of life and there is no such thing as a normal (unstressed) animal just higher, lower and various forms of stress response. Furthermore, cortisol naturally varies throughout the day, at different life stages, individuals and populations. Salmon regulate plasma cortisol within narrow limits, but it has a daily rhythm even in the absence of stressful events (Ebbesson et al., 2008). Therefore, a single cortisol measurement provides little if any information about fish welfare unless linked to other information.

At a group level and with repeated samples, cortisol measurements can provide useful information. Studies carried out by Iversen and Eliassen (2012) reported a link (significant correlation) between high resting levels of plasma cortisol during commercial smolt production and mortality after seawater transfer. They showed that if baseline levels of the hormone were > 50 nM (18.1 ng mL^{-1}) during the smolt production phase, the relative risk for a mortality rate above 5 % after 90 days in the sea significantly increased 3.1 times and the relative risk for disease outbreaks significantly increased 4.9 times. Other studies have reported similar findings and have stated that the normal resting levels of plasma cortisol in fish can be as low as 13.8 nM, while fish with a chronically activated stress response can have a resting level > 27.5 nM (Maule et al., 1987; Pickering and Pottinger, 1989; Van Zwol et al., 2012).

Sampling and analytical considerations

Steroid hormones including cortisol are often measured using either radioimmunoassay (RIA) or enzyme-linked immunoassay (ELISA) in plasma or tissue homogenates (Sopinka et al., 2016). Non-evasive methods can also be used by measuring cortisol in e.g. urine, faeces, scales and water samples (Ellis et al., 2013). However, non-invasive methods are not practical under most circumstances. Further, as plasma cortisol levels can change rapidly in response to challenges, it should be measured pre- and post- stressor to get information on the relative changes in cortisol and information about the individual's state (Ellis et al., 2013; Iversen and Eliassen, 2012; Iversen and Eliassen, 2014; Sopinka et al., 2016).

Strength of indicator

With pre- and post- samples or group averages, cortisol levels can give information on how fish are affected by particular challenges such as handling or differing rearing situations (Barton, 2002; Sapolsky, 2000). Resting cortisol levels can also provide the assessor with information about whether the animal is experiencing chronic stress and can also be predictive of future performance and survival in some cases (Ellis et al., 2012b; Iversen and Eliassen, 2014).

Weakness of indicator

Single cortisol samples are difficult to interpret and it is incorrect to equate high cortisol levels with poor welfare, without additional information. Plasma cortisol analysis can take 1-2 days to complete, even under the best circumstances, making it a LABWI.

Table 3.2.16-1. *Summary of key factors affecting different non-invasive methods of cortisol (steroid) monitoring in fish. Reproduced and modified from "Ellis T., Sanders, M. B. & Scott, A. P. 2013. Non-invasive monitoring of steroids in fishes. Wiener Tierarztliche Monatsschrift 100, 255-269. Crown Copyright & Austrian Society of Veterinarians (ÖGT), 2013" with permission from the authors, Austrian Society of Veterinarians (ÖGT) and Crown Copyright.*

	Mucus and scale	Water sampling Dynamic (Flow-through)	Faeces sampling	Urine sampling
Intrusiveness	Requires capture and handling; potential damage to immune barrier	Non-intrusive	Non-intrusive, but may require capture and handling; pressure to the flanks – method dependent	Requires capture and handling; pressure to the flanks; potential damage to immune barrier
Sample collection	Simple, but standard protocols yet to be developed	Simple, published methods available	Delayed sample collection may allow leaching	Simple, but standard protocols yet to be developed
Expected concentration of target steroid relative to blood	Lower	Much lower	Lower	Similar
Suitability for	Individuals	Population	Individuals	Individuals
Metabolite of target steroid	Free (unconjugated steroid)	Free (unconjugated steroid)	Yet to be determined. Assays have targeted Free (unconjugated) steroid	Yet to be determined. Assays have targeted free and conjugated steroid
Interpretation of Concentration in matrix	Not suitable for commercial systems	Not suitable for commercial systems	Not suitable for commercial systems	Not suitable for commercial systems

3.2.17. Osmolality

Osmolality measures the number of dissolved particles in liquid, and salinity represents the amount of dissolved salt in water. Freshwater has a salinity of 0 ‰ and an osmolality of 0-10 mOsm kg^{-1}, whilst seawater has a salinity of 33-35 ‰ and an osmolality of 1000 mOsm kg^{-1}. Salinity and osmolality are important aspects of the environment for teleosts, and the fish keep their internal blood osmolality within narrow limits irrespective of salinity. To achieve this, water and ions are controlled and regulated via a number of organs in the fish, skin, gills, intestine and kidneys (Marshall et al., 1998; Evans et al., 2005, 2006; Varsamos et al., 2005; Evans and Hyndman, 2006; Evans, 2008). Fish have developed three main strategies for regulating water and salt balance in extracellular fluids such as blood plasma and their intestinal fluid. These three strategies are osmoconform, hyper-osmotic and hypo-osmotic regulation. Osmoconform fish (hagfish) keep the osmolality of their body fluids equal to that of the surrounding environment. Hyper-osmotic (freshwater fish) keep the osmolality of their blood higher than the surrounding environment, whilst hypo-osmotic fish (seawater fish) maintain the osmolality of their internals fluid lower than the surrounding environment. Atlantic salmon are an anadromous species that switch between hypo- and hyper-osmotic environments during migration from fresh to seawater and back (McCormick, 2013). Table 3.2.18-1 shows the ionic composition and osmolality in fish. In general, teleosts attempt to keep an osmolality of between 290-340 mOsm kg^{-1} regardless of the surrounding salinity. Deviations from these levels for prolonged periods will result in mortality (McCormick, 2013). Arnesen et al. (1998) reported that typically osmolality in freshwater was approximately 320 mOsm kg^{-1}, while osmolality ranged from 325 to 345 mOsm kg^{-1} in seawater adapted Atlantic salmon.

Plasma cortisol appears to have an important role directing the hydromineral balance and energy metabolism of fish and any variations in plasma osmolality, magnesium and chloride can be considered part of the secondary stress response (Veiseth et al., 2006). Plasma osmolality and ionic composition can be valuable for examining the osmoregulatory capacity of the fish (Wendelaar Bonga, 1997; Mommsen et al., 1999). Some studies have reported that plasma osmolality and ionic concentrations decrease in fish adapted to freshwater and increase in fish adapted to seawater in response to stressful situations such as handling or confinement (Barton, 2002; Barton and Iwama, 1991; Iversen et al., 1998; Liebert and Schreck, 2006). However, other studies cannot document changes in fish plasma osmolality (Barton and Zitzow, 1995) or chloride levels (Barton et al., 2005) in relation to exposure to stressors. This inconsistency with regard to the effects of stress on osmoregulation is most likely due to the strong compensatory and highly variable mechanism employed by fish in some circumstances (Fiess et al., 2007).

Sampling and analytical considerations

Osmolality is analysed using an osmometer that will measure osmolality to the closest mOsm kg^{-1}. It is available at scientific and commercial laboratories and is therefore a LABWI.

Strength of indicator

Changes in osmolality are a useful indicator of acute stress (Sopinka et al., 2016) and osmolality can be easily and cheaply measured in plasma in commercial laboratories.

Weakness of indicator

Interpreting osmolality in relation to long-term stress exposure can be problematic as it can be affected by a multitude of factors (McDonald and Milligan, 1997; Sopinka et al., 2016). In addition, it requires both capture, anaesthesia and blood sampling to obtain plasma for analyses.

3.2.18. Ionic composition

The transformation of many salmonids, such as Atlantic salmon, from a parr living in freshwater to a smolt adapted to living in seawater includes various morphological, physiological, biochemical and behavioural changes (Björnsson et al., 2011; Folmar and Dickhoff, 1980; McCormick, 2013).

In freshwater, the gill is the site of ion uptake, whilst in seawater it is the site of salt secretion and this allows euryhaline teleosts to maintain control of their internal salt and water balance (Arnesen et al., 1998; Handeland et al., 1998, 2000; Iversen et al., 2009). Specialized cells in the gill, termed ionocytes, chloride cells, or mitochondrion-rich cells (MRC) primarily carry out ion transport.

In the freshwater phase, sodium levels in the fish vary between 130-150 mmol L^{-1} and chloride levels vary between 111-135 mmol L^{-1}. During the period before smoltification, there can be a slight drop below the normal range in freshwater (Folmar and Dickhoff, 1980). In seawater, ion levels increases slightly in post smolts and vary from 140-175 mmol L^{-1} (Na^+) to 130-160 mmol L^{-1} (Cl^-) (Arnesen et al., 1998; Handeland et al., 1998, 2000; Iversen et al., 2009; Sigholt and Finstad, 1990; Staurnes et al., 2001). Seawater challenge tests of 24 to 72 hours (Blackburn and Clarke, 1989) are often used to verify if the salmon are ready for transfer to sea. Sodium and chloride levels below 160 and 150 mmol L^{-1}, respectively are deemed sufficient for salmon to survive and grow in seawater (Blackburn and Clarke, 1989; Finstad et al., 1988). Care should be taken during a seawater challenge test as osmoregulation is affected by changes in sea water temperature (Finstad et al., 1988; Handeland et al., 2000, 2004). Handeland et al., (2000) also showed that high sea water temperatures increased chloride levels and accelerated tissue dehydration, while low temperatures led to a delay in osmotic disturbance but exposed the fish to an extended episode of osmotic stress.

Marine teleosts drink seawater to make up for water lost due to osmotic imbalance and to reduce the risk of dehydration. During this process they actively eliminate divalent ions (e.g. Mg^{2+} and Ca^{2-}) from their body fluids (Redding and Schreck, 1983). The uptake of plasma magnesium (Mg^{2+}) is a function of the gut and its excretion is a function of the kidney (Redding and Schreck, 1983). It appears that the blood plasma magnesium concentrations do not exceed 2 mM in in most cases, and are normally less than 1 mM, regardless of the salinity (Iversen et al., 2009; Iversen and Eliassen, 2014; Liebert and Schreck, 2006). Changes in magnesium balance are a good indicator of acute stress (Iversen et al., 2009; Iversen and Eliassen, 2014; Liebert and Schreck, 2006) and experiments have shown there is a high correlation between increased plasma magnesium and mortality after fish are subjected to stressors (Iversen and Eliassen, 2009; Iversen et al., 2009; Iversen and Eliassen, 2014; Liebert and Schreck, 2006).

Table 3.2.18-1. Reported normal ionic composition ranges of blood plasma in fish (Arnesen et al., 1998; Handeland et al., 1998, 2000; Iversen et al., 2009; Edwards and Marshall 2013).

	Concentration (mM kg water^{-1})						
	Cl^-	Na^+	K^+	Mg^{2+}	Ca^{2+}	SO_4	Osmolality
Seawater	439	513	9.3	50	9.6	29	1050
Seawater fish	180	196	5.1	2.5	2.8	2.7	452
Freshwater fish	130	125	2.9	1.2	2.7	-	262
Salmonids (FW)	111-135	130-150	2.9	0.9-1.5	2.7	-	290 – 320
Salmonids (SW)	135-160	140-175	3.4	1.6-2.0	3.3	-	325 – 345

Sampling and analytical considerations Chloride (Cl-), Sodium (Na^+) and Magnesium (Mg^{2+})

Plasma chloride and sodium analysis is carried out by commercially available titrators or meters that will measure values to the closest mmol L^{-1} (mM). Many smolt plants that conduct 24 to 72 hours seawater challenge tests (Blackburn and Clarke, 1989) have these instruments available and commercial laboratories can also carry out these measurements (Sopinka et al., 2016). Plasma magnesium analysis is carried out by commercially colorimetric assays in plasma or by atom absorption instruments that will measure magnesium to the closest mmol L^{-1} (mM).

Plasma chloride, sodium and magnesium are therefore LABWIs.

Strength of indicators

Changes in ion balance are a useful indicator of acute stress (Sopinka et al., 2016) and can be easily and cheaply measured in plasma in commercial laboratories.

Weakness of indicators

Interpreting changes in ion balance in relation to long-term stress exposure can be problematic as it can be affected by a multitude of factors (McDonald and Milligan, 1997; Sopinka et al., 2016). In addition, it requires both capture, anaesthesia and blood sampling to obtain plasma for analyses.

3.2.19.Glucose

Elevations in plasma cortisol stimulate glycogenolysis, i.e. the conversion of glycogen stored in the tissue to glucose released into the blood (Barton and Iwama, 1991). An increase in plasma glucose is therefore a relatively slow response to a stressor and peaks after around 3-6 hours in salmon (Olsen et al., 2003) although the response is also dependent on the feeding status of the fish. In salmon, plasma glucose levels can increase to twice that of baseline levels 4 h after acute stress (crowding and chasing for 15 min), but can return to baseline levels much faster (2 h) in fasted fish than in fed fish. Fed fish had elevated levels of plasma glucose for more than 12 h due to their higher storage of liver glycogen (Olsen et al., 2003). However, diet composition may not have a marked effect upon glucose levels in salmon (Krogdahl et al., 2004). In addition, plasma glucose levels in the fish blood can exhibit a great deal of variability (especially with regard to carnivorous fish) and may therefore be a poor indicator of secondary stress and of metabolic status (Mommsen et al., 1999).

Increased levels of plasma glucose can be used as a measure of acute stress, but levels should be compared with pre-stress levels rather than any “standard levels”, as plasma glucose is also dependent on feeding status, diet type and other factors (Table 3.2.19-1).

Table 3.2.19-1. *Plasma glucose levels in Atlantic salmon after various feeding regimes and before and after various stress treatments. Most glucose values are read from graphs and are estimated and some values are converted from other units.*

Stage	Feeding status	Treatment	Glucose (mmol L^{-1})	Reference
Freshwater, (parr)	Fed	Pre-stress	3.7	Carey and McCormick, 1998
Freshwater, (parr)	Fed	30 s in air followed by 3 h crowding	5.9	Carey and McCormick, 1998
Freshwater, 200 g	Fed low carb	Pre-stress	4	Krogdahl et al., 2004
Freshwater, 200 g	Fed high carb	Pre-stress	6	Krogdahl et al., 2004
Freshwater, (pre-smolts)	Fed	Pre-stress	4.2	Carey and McCormick, 1998
Freshwater, (pre-smolts)	Fed	30 s in air followed by 3 h crowding	8.1	Carey and McCormick, 1998
Freshwater, (smolts)		Pre-stress	4	Iversen et al., 1998
Freshwater (smolts)		Transport in 1200 L tanks	8.8	Iversen et al., 1998
Freshwater, (smolts)	Fed	Pre-stress	4.6	Carey and McCormick, 1998
Freshwater, (smolts)	Fed	30 s in air followed by 3 h crowding	7.7	Carey and McCormick, 1998
Freshwater (smolts)		Crowding for 3 h followed by pumping	5.7	Espmark et al., 2015
Post-smolts, 150 g	Fed	Pre-stress	3.33	Fast et al., 2008
Sea water, slaughter	Fasted	Pre-stress	4.2	Skjervold et al., 2001
Sea water, slaughter	Fasted	Crowding	7.2	Skjervold et al., 2001

3.2.20. Lactate

Lactate is the product of anaerobic ATP production (glycolysis) in the cells, which occurs when oxygen is not available in sufficient amounts for the cells to utilise aerobic metabolism. The drivers for this could be decreased oxygen levels in the water (Remen et al., 2012) or heavy physical exercise (Milligan and Girard, 1993). As lactate is primarily produced in muscle cells, it takes some time before it appears in the blood and the response is delayed by a few hours. A typical increase in lactate after a stressful event occurs 1-2 hours after the event and in most cases the animal will recover after 6-12 hours (Hatløy, 2015). The peak of plasma lactate during stressors such as transport and handling ranges from 6.4 to 13.3 mmol L^{-1} (Hatløy, 2015; Iversen et al., 2003; Table 3.2.20-1), and this is relatively low compared to levels that have been recorded after intense exercise and air exposure (>20 mmol L^{-1}) in numerous salmonid species (Liebert and Schreck, 2006; Olsen et al., 1995; Pagnotta and Milligan, 1991; Schreck et al., 1976; Wood et al., 1990). Lactate is mainly an indicator of a high level of muscle activity, which is often related to stress.

Table 3.2.20-1. *Plasma lactate levels in Atlantic salmon after various feeding regimes and before and after various stress treatments. Most lactate values are read from graphs and are estimated, and some values are converted from other units.*

Stage	Feeding status	Treatment	Plasma lactate (mmol L^{-1})	Reference
Freshwater, (smolts)	Fasted	Pre-stress	5	Iversen et al., 2005
Freshwater (smolts)		Pre-stress	1.2	Iversen et al., 1998
Freshwater, (pre-smolts)	Fed	Pre-stress	3.4	Carey and McCormick, 1998
Freshwater, (smolts)	Fed	Pre-stress	3	Carey and McCormick, 1998
Seawater, (smolts)	Fasted	Crowding and loading on well boat	9	Iversen et al., 2005
Freshwater, (pre-smolts)	Fed	30 s in air followed by 3 h crowding	3	Carey and McCormick, 1998
Freshwater, (smolts)	Fed	30 s in air followed by 3 h crowding	3.5	Carey and McCormick, 1998
Freshwater (smolts)		Brief air exposure in net followed by 30 min mild crowding	5.3	Espmark et al., 2015
Freshwater (smolts)		Transport in 1200 L tanks	3.6	Iversen et al., 1998

Sampling and analytical considerations regarding glucose and lactate

Glucose and lactate levels may be determined using colorimetric assays on e.g. plasma (Sopinka et al., 2016). They may also be measured from whole blood with hand-held instruments (Sopinka et al., 2016) which have been long validated as a suitable portable tool for measuring these indicators (Wells and Pankhurst, 1999). This means glucose and lactate are classified as OWIs rather than LABWIs

Strength of indicators

Metabolites are good for evaluating the response of fish to numerous routines and stressors (Barton, 2002; Sopinka et al., 2016), such as handling (e.g. by using lactate, Wood et al., 1990). Easy to use out on the farm and cheap to measure using hand-held instruments.

Weakness of indicators

Glucose and lactate levels are also influenced by other factors (not just the stress response). This means the interpretation of results can be challenging and these indicators are best used to evaluate short-term reactions to specific stressors rather than long-term responses.

3.2.21. Haematocrit

Erythrocytes (red blood cells, RBCs) are the cells that are responsible for the transport of oxygen within the blood. Haematocrit refers to the volume of these cells in the blood (expressed as a percentage) e.g. Sopinka et al., (2016). Normal baseline ranges of haematocrit in salmon are 44-49% (Sandnes et al., 1988), but levels may vary with temperature (Sambraus et al., 2017) and between strains (Iversen et al., 1998). Also, most anesthetics will cause an increase in haematocrit (Phuong et al., 2017).

Sampling and analytical considerations

Haematocrit is typically calculated by centrifuging a sample of whole blood to separate the sample into discrete layers. When the resulting volume of red blood cells (RBCs) is divided by the total sample volume you get the packed cell volume (PCV) which is expressed as a percentage (Sopinka et al., 2016). Haematocrit analysis is therefore a LABWI.

Strength of indicator

Haematocrit is relatively low-cost and easy to measure (Sopinka et al., 2016).

Weakness of indicator

Haematocrit values can both increase or decrease when fish are exposed to a stressor (dependent upon the type of stressor) and this means interpreting the results can be challenging (Iversen et al., 1998; Sopinka et al., 2016).

3.2.22. Rigor mortis time and muscle pH

Rigor mortis refers to the stiffness that occurs in any dead animal after death. Rigor lasts until enzymes loosen the tight binding between actin and myosin proteins in the muscle cells. The time until rigor mortis occurs (pre-rigor time) is dependent upon several factors including the stress response. In general, a high stress response as a result of e.g. handling, results in a shorter pre-rigor time. When blood circulation stops after death it results in a complex series of processes in the fish muscle. Immediately after death the muscle is soft and elastic, and the metabolic processes are still active. The catabolic processes of the muscle cells are active as long as energy is available. When the remaining oxygen is used up ATP-dependent anaerobic metabolism takes over. This then leads to the accumulation of lactic acid and a lowering of pH. When the pH-level reaches a certain level, it interferes with the conversation of glycogen to lactic acid which provides energy for new ATP, eventually stopping the production completely (Robb, 2001). The rigor process therefore starts when ATP levels reach a minimum (Robb, 2001). The muscles fibres contract during a primary contractile phase, and this is followed by a secondary stiffening phase where the contractile proteins myosin and actin permanently bind together (Tornberg et al., 2000; Kiessling et al., 2006). In full rigor mortis almost all of the myosin heads form cross-bridges to actin (Schmidt-Nielsen, 1997; Murray, 1999).

The three main factors affecting the timing and intensity of the rigor process are the glycogen reserves in the muscle, the pH-level and the temperature of the muscle (Hulland, 1992). These three factors are dependent on a wide range of pre- and post- slaughter conditions. Both long-term starvation and stress during crowding and pumping can lead to reduced muscle glycogen levels in salmon (Mørkøre et al., 2008; Merkin et al., 2010). Fish can respond to stressor exposure with a classic fight or flight response. This typically involves a rapid contraction of the muscle and can lead to anaerobic metabolism. If the fish is given the opportunity to recover under normal conditions, aerobic metabolism and normal pH will be restored. However, if the fish are subjected to a stressor immediately prior to slaughter, anaerobic circumstances will prevail as the fish will not be given a chance to recover before their circulation fails (Stien et al., 2005). The rigor process in stressed salmon will therefore be initiated from an already acidic muscle state and will progress faster in stressed rather than in unstressed salmon (Stien et al., 2005; Mørkøre et al., 2008; Merkin et al., 2010).

Sampling and analytical considerations

The Rigor Index (Bito et al., 1983) is a simple way to monitor rigor development in whole fish. The fish is placed on a table with the tail half of the fish hanging over the edge. The index is then calculated as the Rigor Index (%) = 100 × (L_0-L_t)/L_0), where L_0 is the distance from the base of caudal fin to the height of the table and L_t is this distance at time t. For completely stiff fish this distance will approach 0. Another method for measuring rigor on whole fish is by probing the hardness of the muscle from the outside. This can be done manually but there are handheld instruments for more objective measurements. In scientific studies, rigor is often measured by tracking the isometric and/or isotonic tension of isolated muscle pieces (Stien et al., 2006). Fillet rigor is often monitored by following how fast and how much it contracts during rigor or by measuring muscle pH by inserting an electrode into the muscle. At the end of rigor, the muscle becomes less hard, the fillet stops contracting and muscle pH stabilises.

Strength of indicator

Acute stress response leads to fast and strong rigor development making exposure to severe stressors before slaughter easy to detect. It can be monitored by cost effective methods such as the Rigor Index, muscle hardness, fillet shrinkage or by simply manually assessing the stiffness of the fish.

Weakness of indicator

The onset and duration of rigor mortis is strongly dependent upon storage temperature. In order to get accurate data the fish has to be tested multiple times to produce a curve of rigor development. Measuring muscle hardness by probing the fish influences muscle texture and frequent probing on the same place may therefore give inaccurate results. The transformation processes starts immediately after slaughter and it is therefore important to begin monitoring immediately to get a correct null point, especially for muscle pH (Kristoffersen et al., 2006). This is a major weakness with using muscle pH after slaughter as a WI on its own.

3.2.23. Mucus

Mucus is a barrier that acts as a "*biochemical interface*" between the fish and its surroundings (Castro and Tafalla, 2015). It covers every body surface that is either i) in contact with the surrounding environment or ii) in contact with items from the external environment, e.g. the gut, gills and skin (Castro and Tafalla, 2015). Mucus has been associated with a variety of functions in fish including respiratory gas exchange, disease resistance, reproduction, ion and water regulation, chemical and physical protection, chemical communication and swimming performance, amongst others (Shephard, 1994). Mucosal tissues share structural similarities, even though its thickness and composition may differ according to its location and also e.g. immunological, physiological and environmental circumstances (Castro and Tafalla, 2015). Although mucosal tissues have varying functions, they all have a similar microanatomical structure (Peterson, 2015).

Mucus is mainly produced by mucous or goblet cells, although other secretory and non-secretory cells can also contribute to its production. Goblet cells produce large internal mucous vacuoles that release their content at the cell surface in the epithelium (Elliott, 2011). The mucus production rate is reliant on the quantity and composition of epidermal mucous cells and also their renewal/turnover rate (Landeira-Dabarca et al., 2014). Mucus is a complex matrix consisting of many components, primarily water (around 95%) and mucins (Salinas and Parra, 2015; Van der Marel et al., 2010). Sanahuja and Ibarz (2015) state mucins are "*glycoproteins densely coated with O-linked oligosaccharides*". In addition, mucus contains other substances in smaller quantities, such as a number of immune factors (Castro and Tafalla, 2015; Easy and Ross, 2009). The composition of mucus varies and can be affected by numerous factors including life stage, stress, acidity, salinity and also infections (Sanahuja and Ibarz, 2015). However, with its high content of cellular and humoral components mucus has a key role in the fish's immune system (Sveen et al., 2016).

The quantity of mucous cells in Atlantic salmon skin also differs with life stage. For example, they decrease by 50% at the beginning of smoltification (O'Byrne-Ring et al., 2003) and variability in certain skin mucus proteins and skin lysozyme activity shows the impact of life stage on mucus production dynamics (Fagan et al., 2003). The size and density and of mucous cells can also be influenced by environmental factors, e.g. increased salinity (Shephard, 1994), high nitrate levels, low oxygen (Vatsos et al., 2010), low pH or acid exposure (Berntssen et al., 1997; Ledy et al., 2003) as well as the presence of pathogens (Nolan et al., 1999) even at low pathogen pressure (Van Der Marel et al., 2010). In response to irritation the number of mucous cells initially increases but eventually there is a decrease or depletion (Roberts, 2012).

With regard to parasites, an analysis of the composition of epidermal mucus proteins of Atlantic salmon infected with sea lice showed increased proteolytic activity (Easy and Ross, 2009) and changes in proteins related to metabolism, translation and number of immune-related proteins (Provan et al., 2013). In addition, an infestation with lice disturbs the mucosal microbiome of the skin of Atlantic salmon, causing a reduction in microbial richness, reduced diversity and a destabilization of the microbial community composition (Llewellyn et al., 2017). These changes in mucus composition could be due to a reduction in the quantity of mucus producing cells in salmon skin following sea louse infestation (Nolan et al., 1999). Another ectoparasite, *Neoparamoeba perurans*, that causes amoebic gill disease (AGD) has been shown to initiate excessive mucous production in gills (Koppang et al., 2015) and affects the protein composition of both skin and gill mucus in salmon (Valdenegro-Vega et al., 2014). With regard to pathogens, Svendsen and Bøgwald (1997) recorded significantly elevated mortalities for fish with skin wounds and in fish without a mucous epidermal layer (in comparison to a control group with an intact mucus layer) when Atlantic salmon were challenged with *Vibrio* and *Aeromonas*.

With regard to husbandry practices, routines such as feed withdrawal can affect the mucus layer. For example, starvation has been shown to negatively affect the abundance of epidermal mucous cells, the quality and quantity of mucins (Landeira-Dabarca et al., 2014) as well as the microbial density and the composition of the microbial community in Atlantic salmon (Landeira-Dabarca et al., 2014). As a consequence, the disease resistance of Atlantic salmon may be affected. In addition, nutritional components have been shown to alter the proteome of the skin mucous barrier (Micallef et al., 2017) and both gut and mucus secretion (Sweetman et al., 2010). Nutrition can also have an effect; Atlantic salmon fed diets containing additional zinc had higher mucous cell coverage of the epidermis and more advanced wound healing progression (Jensen et al., 2015a).

A previous attempt (Easy and Ross, 2010) to correlate handling stress or cortisol to some mucus enzyme/protein profiles showed a weak positive association. However, it also underlined high individual variability in enzyme levels suggesting other possible causes for changes in the composition of mucus protein profiles. A study by Sveen et al., (2016) on Atlantic salmon post-smolt raised at densities of ≥ 100 kg m^{-3} showed structural changes in the skin epithelium as well as changes in the messenger RNA of number of mucus related proteins, while a low specific water flow (≤ 0.3 L kg^{-1} min^{-1}) increased transcriptome of proteins related to the immune and stress responses in skin. Rearing temperature can also affect skin and mucus transcriptomes and composition. In a study by Jensen et al., (2015b) Atlantic salmon reared at 4 ^{o}C had a significantly thicker epidermis, both in dorsal and cranial region and smaller mucous cells coverage compared to individuals raised at 16 ^{o}C. Further, an analysis of the skin transcriptome showed an upregulation of a number of heat-shock proteins at 16 ^{o}C that together with the decrease in epidermal thickness suggest there is a stress response in the skin at this temperature (Jensen et al., 2015b).

Recently, O-Glycan structures (109) of freshwater Atlantic salmon mucins were characterized and showed structural differences between the skin and different parts of the intestine (Jin et al., 2015). As glycan structures provide nutrients for commensal bacterial strains and binding decoys for pathogens, different mucosal environments select for specialized microflora and can play an important role in pathogen adhesion at certain locations (Jin et al., 2015). In Atlantic salmon, both the mucosal density and the mean mucosal cell size in the skin are dependent upon their location, with larger and denser cells located on the dorsolateral side and the smallest, lowest density mucosal cells located on the head region (Pittman et al., 2013). In addition, the general thickness and coverage of the mucous cell layer is greater in the dorsal area compared to the cranial area at three different temperatures: 4, 10 and 16 ^{o}C (Jensen et al., 2015b). Pittman et al., (2013) concluded that "*salmon exhibit a dynamic repeatable pattern of mucous cell development influenced by sex, diet and possibly strain and season*".

Sampling and analytical considerations

In recent years, numerous studies have tried to identify possible mucus biomarkers and techniques that could be used to monitor fish physiology, genetics, health and welfare (Easy and Ross, 2009, 2010; O'Byrne-Ring et al., 2003; Pittman et al., 2013; Provan et al., 2013; Sanahuja and Ibarz, 2015; Valdenegro-Vega et al., 2014; Vatsos et al., 2010). Some of the methods are non-invasive and concentrate mainly on the composition of skin mucus (Easy and Ross, 2009, 2010; Sanahuja and Ibarz, 2015; Valdenegro-Vega et al., 2014) while others require fish euthanasia and preparation of histological skin samples for further quantification of mucous cells and their size (Pittman et al., 2013; Vatsos et al., 2010).

A method for mucosal analysis of different tissues using histological samples is currently available for fish health services and fish farmers that should allow for establishment of cause and effect related to fish mucus and its implications for fish health (Quantidoc, 2017). This method is robust and comparable with regard to time/location, sex etc. (Quantidoc, 2017). In addition, an ELISA kit for the measurements of cortisol in human saliva has been adapted for the determination of cortisol in epidermal mucus in fish and this is available for research purposes (TECOmedical AG, 2016).

As mucous content and the number of mucosal cells are dependent on physiological status, environmental conditions, nutritional status, sex and body location (see above) it is very important that all of these factors are taken into consideration when using mucus as welfare indicator. As an increase in mucous secretion has been correlated with certain stressful situations, e.g. where fish were handled and stunned prior to sampling, the effect of the sampling procedure on mucous secretion has been questioned (Koppang et al., 2015). The same authors therefore conclude that it might be very challenging to examine a mucous layer without disturbing the fish or exposing them to stress. It would be beneficial to further investigate the effect of different sampling methods on mucus composition and the status of mucosal cells. The sampling location of the mucosal tissue also has to be standardized when comparing different treatments or individuals (Pittman et al., 2013). In addition, it has been shown that when quantifying skin mucous cells using histological methodology, mucous cell size can be affected by the section site, decalcification of the sample, the embedding medium and the sectioning plane, whilst mucous cell density was more resilient to the method (Pittman et al., 2011, 2013). As mucosal analysis is dependent on external laboratory analysis and a high level of expertise, we have classified it as a LABWI.

Strength of indicator

Mucus is a physical, biochemical and biological barrier that protects fish from pathogens and is responsive to both endogenous and exogenous factors. The status of mucous layers can provide valuable information about the status of the fish and as such is an important health and welfare indicator. In addition, a recent study indicates that the increased abundance of markers of skin epithelial turnover is a promising indicator of chronic stress in fish (Perez-Sanchez et al., 2017).

Weakness of indicator

The analysis of the mucous barrier layer is currently ongoing in laboratories; it is time consuming and as such has to be classified as a LABWI. In addition to this, detailed knowledge on fish physiological, nutritional, health status, environmental conditions, sex, and size must be documented in order to interpret the data. The sampling procedure also has to be considered as it might affect the results. The only commercially available method for mucous barrier layer characterization requires fish euthanasia and the preparation of histological samples, while more passive methods might be more preferred in the future.

4. Environment based welfare indicators

Fish welfare is closely related to its environment, which in its broadest sense is not just water quality but also infrastructure and handling. Based on scientific knowledge about the animals' preferences and tolerance limits for the various environmental factors, e.g. temperature and oxygen, we can use measurements of environmental factors as indirect welfare indicators. However, much of the literature relates to the effect of environmental parameters on productivity or survival rather than welfare. In addition, many environmental parameters interact with each other and their effects are dependent upon the state of the fish. Therefore, it is often difficult to define limits which either protect welfare or put it at risk. In this handbook, we focus on environment based WIs that are operational, well proven and general, i.e. useful in most farming situations. This includes factors describing water quality and factors also describing the rearing system or rearing practices (Table 4-1).

Table 4-1. *List of environment based welfare indicators and which welfare needs of Atlantic salmon they affect directly. RS & RP = Rearing systems and rearing practices.*

	Needs	Environment				Health			Behaviour					Resour.	
	Welfare indicators	Respiration	Osmotic bal.	Thermal reg.	Good water q.	Body care	Hygiene	Safety and pr.	Beh. control	Social contact	Rest	Exploration	Sexual beh.	Feeding	Nutrition
Water quality	Temperature	×	×	×			×	×							
	Salinity	×	×												
	Oxygen	×	×												
	CO_2	×			×										
	pH	×	×		×										
	Total ammonia nitrogen	×			×									×	
	Nitrite and Nitrate	×	×		×										
	Turbidity and total suspended solids	×			×		×								
RS & RP	Water current speed								×		×				
	Lighting								×	×	×	×		×	
	Stocking density				×				×	×	×				

4.1. Water quality based welfare indicators

4.1.1. Temperature

Fish are poikilothermic and their physiological and metabolic systems therefore need to be adapted to the temperature range they are offered. Temperature affects numerous factors and EFSA (2008a) states "*The major effects of extreme temperatures are changes in metabolic rate, a disturbance in respiration, blood pH imbalance, and a breakdown in osmoregulation and intolerance of handling. Standard behavioural criteria for stress at critical temperatures are associated with equilibrium loss, sudden bursts of activity with frequent collisions with the tank sides, followed by rolling with rapid ventilatory movements (Elliott and Elliott, 1995).*" Further, as the dissolved oxygen content of the water decreases as water temperature increases, some of these physiological responses can be exacerbated.

The preferred temperature for salmon varies with different life stages.

Eggs: A. salmon is naturally an autumn spawner and eggs are normally produced at 4-8°C (Weber, 1997). Higher temperatures increase the risk of gill opercula, fin and jaw deformities (Ørnsrud et al., 2004b)

Fry and parr: have a preferred optimal temperature range of 12-14 °C. Parr naturally live in rivers which may have a wide annual range of temperatures and little possibility to regulate temperature by migration. They can therefore tolerate a wide temperature range from 0 to well above 20°C before feeding ceases (Elliott and Elliott, 2010) but temperatures below 6 °C and above 22 °C can have a negative impact upon growth (Elliott et al., 1997).

Post-smolts: previous studies have stated that caged pre-smolts prefer temperatures around 17 °C and avoid temperatures higher than 18 °C (Johansson et al., 2006; Oppedal et al., 2011a). Temperatures above 17 °C can also have a negative effect upon appetite, performance and mortality. Low temperatures (below 6-7 °C) are avoided by post-smolts, can have a negative impact upon growth, performance and increase the risk for winter ulcers. Post-smolts should therefore have access to temperatures above 6 °C and below 18 °C. Salmon do respond to changes in temperature and especially to increases in temperature. Folkedal et al., (2012) showed that post-smolt salmon displayed less feeding motivation after having experienced a brief period of elevated temperature. Analyses of the oxygen consumption data from this experiment (O. Folkedal, unpublished) showed that the increase in oxygen consumption during the period of elevated temperature was much larger than suggested by the typical relationships between temperature and metabolic rates, e.g. a doubling in rate from a 10 °C increase. A later experiment (O. Folkedal, unpublished) demonstrated that the effects on behaviour (feed anticipation) and metabolic rate of this temperature increase were gradually attenuated after repeated temperature fluctuations, but that this adaptation was slow, requiring weeks.

Warm water treatment: Bathing treatments that utilise warm water (29-34 °C) can be used for delicing salmon. Research indicates that exposing fish to such temperatures can cause pain in salmonids. Ashley et al., (2007) examined the effects of cold and heat upon different types of nociceptors (pain receptors) on the head to the young rainbow trout. The nociceptors did not respond to cold, but did respond to heat. One type of receptor (polymodal) showed an average heat threshold temperature of 29 °C (range 20-37 °C) and another type (mechanothermal) showed an average heat threshold temperature of 33 °C (range 22-40 °C) for transmitting impulses to the brain. Threshold values have also been reported for heat aversion in the goldfish *Carassius auratus* (Nordgreen et al., 2009).

Table 4.1.1-1. *The preferred thermal range for salmon at different life stages.*

	Range (°C)			References
Eggs	4	-	8	Weber, 1997
	4	-	8	Peterson et al., 1977
		<	12	Elliott et al., 1998
	4	-	11	Lightfoot, 2008
				Poxton, 1991
Fry	10	-	14	Poxton, 1991
Parr	12	-	14	Elliott, 1991
Smolts	7	-	14.3	Jonsson and Rudd-Hansen, 1985,
	10	-	13	Duston et al., 1991
				Handeland et al., 2003
Post-	8	-	14	(Marine Harvest, 2016)
smolts	5	-	17	Jones, 2004
	6	-	16	Handeland et al., 2008
	10	-	15	Stien et al., 2013
	10	-	15	Handeland et al., 2003
	16	-	18	Johansson et al., 2009

Sampling and analytical considerations

In tanks the water is generally well mixed and temperature can be measured anywhere in the water. In cages where temperature varies with depth and time (Oppedal et al., 2011a) temperature should be measured throughout the cage depth. Measuring temperature at depths within the cage where no fish are present may give information about the cause for the depth distribution of the fish, as they tend to stay at the most preferred temperatures (Oppedal et al., 2011a). In cages, vertical temperature profiles can be taken with a Conductivity Temperature Depth probe (CTD) together with added sensors for other environment based indicators such as salinity and oxygen.

Strength of indicator

Temperature is cheap and easy to measure and it affects and explains many aspects of behaviour, welfare and the performance of salmon. It also affects other WIs like oxygen, diseases and parasites such as sea lice.

Weakness of indicator

In many production systems it is difficult or even impossible to change the temperature if is too low or too high, although at high temperatures it is possible to use supplemental oxygen.

4.1.2. Salinity

Salmon are osmoregulators and maintain relatively constant blood ion levels at around 250-300 mOsm, or ~10 ppt (McCormick et al., 1989). Salmon fry and parr are raised in freshwater, are hyperosmotic and have an active uptake of ions and excretion of water, while post-smolts in seawater are hypo-osmotic and have to drink water and excrete ions. The growth rate of post-smolts is largely independent of salinity, but newly sea-transferred fish may prefer brackish water and aggregate in or above the halocline in sea cages. Larger post-smolts are relatively unaffected by salinity (with the exception of sexually maturing fish which may seek out fresh water) and it appears that salinity has no significant effect upon their welfare (Stien et al., 2013). When Atlantic salmon begin to spawn they have difficulties osmoregulating in full strength seawater (33-34‰), which can lead to high mortalities if they are held in seawater right through this period (Taranger and Hansen, 1993).

Table 6.2.1. *The optimal salinity levels for salmon at different life stages.*

	Range	References
Fry	Fresh water 0-10 ppm	EFSA, 2008a; Craik and Harvey, 1988
Parr	Fresh water 0-10 ppm	EFSA, 2008a
Smolts	Halocline and brackish water	Duston, 1994; Stien et al., 2013; Handeland et al., 1998; Johansson et al., 2006, 2009
Post-smolts	Close to oceanic levels (33-34‰). 22-28 ppt <20→30	Stien et al., 2013 Bœuf and Payan, 2001 Oppedal et al., 2011a
Brood stock	<10 ppm	Taranger and Hansen, 1993

Sampling and analytical considerations

Although it appears that salinity has no significant effect upon the welfare of adult Atlantic salmon (Stien et al., 2013), access to brackish water may be of benefit. In a sea cage containing 200,000 or more individuals it is possible that some fish may have osmoregulatory challenges, may not be fully smoltified or are maturing (Stien et al., 2013). Fish infected with AGD and sea lice will also benefit from access to a layer of brackish water (Oldham et al., 2016). The best way to measure if there is a layer of brackish water (and also its depth), is by using a CTD. This can normally be done from the barge, as the salinity profile is relatively stable within the area of a fish farm, and will not vary from cage to cage. A CTD deployment provides high resolution data of temperature and salinity calculated from the conductivity measurements, giving the precise positions of any transitions in salinity.

Strength of indicator

Easy to measure and the presence of a layer of brackish water is known to often benefit fish welfare.

Weakness of indicator

Absence of a layer of brackish water does not necessarily mean decreased welfare. Even if there is a layer of brackish water, this layer can often be very cold, which can stop the fish from using it.

4.1.3. Oxygen

As fish are poikilothermic their metabolic rates and oxygen requirements increase at higher temperatures (Brett, 1979; Fry, 1971; Pörtner, 2010; Pörtner and Farrell, 2008; Remen et al., 2013; Barnes et al., 2011). As oxygen saturation declines the metabolic scope is reduced, and when oxygen saturation decreases below a certain level (DO_{maxFI}), appetite is reduced and feed intake declines (Remen et al., 2016a). At oxygen saturations above DO_{maxFI} behaviour and appetite is unaffected, and one can assume that the need for respiration is fully fulfilled. Below the limiting oxygen saturation (LOS) aerobic metabolism can no longer be maintained and saturations below LOS should always be avoided. At oxygen saturations between DO_{maxFI} and LOS, respiration is limited and although the fish will survive, welfare is negatively affected. A shorter period (hours, e.g. during operations) with such levels will not have severe or long lasting effects on welfare, but should be avoided as far as possible. LOS rises at higher activity levels, such as when in panic or during crowding, which may occur during farming operations, and oxygen saturations down to the LOS of moderately active fish should therefore be avoided.

Eggs: Their oxygen requirements of depends upon various aspects including egg size, the developmental stage of the egg and also water temperature and it is therefore difficult to give general statements on the requirements for oxygen supply for eggs (Crisp, 1996). Crisp (1996) suggested that one can expect high egg survival at temperatures below 12.5°C if oxygen saturation is 66% or higher and water velocity past the eggs is at or above 100 cm h^{-1}, while reduced survival rates are possible at lower levels (Crisp, 1996 and references therein). Oxygen levels that are too low during incubation can lead to poor growth, a poor yolk conversion efficiency, premature hatching, a smaller size at hatching and can also have morphological impacts (Crisp et al., 1996 and references therein), which may in turn have a negative effect on the welfare of fish later in life.

Parr and smolts: Detailed data of the oxygen concentrations that appetite and aerobic metabolism are maintained in parr and smolts at different temperatures are not available, but experience does not suggest dramatically different oxygen requirements compared with that of post smolts (see below). For instance, a limiting oxygen saturation (LOS) of 39% O_2 at 12.5°C has been found for parr (Stevens et al., 1998).

Post smolts: The lowest oxygen saturation that does not negatively impact upon appetite (DO_{maxFI}) and the lowest oxygen saturation at which aerobic metabolism can be maintained (LOS) for post-smolt at different temperatures are given in Table 4.1.3-1.

Oxygen levels vary in space and time, especially in sea cages where water exchange is driven by the tide, fish movements and shifting weather conditions and local fish densities (Oppedal et al., 2011b). Post-smolts exposed to cyclic hypoxic conditions with 2 hours hypoxia (60% at 16 °C) every 6th hour respond with a physiological stress response, and appetite is reduced even if they are fed during normoxic periods (Remen et al., 2012, 2014), suggesting that hypoxia may be a longer lasting stressor that has influences beyond the hypoxic period.

Table 4.1.3-1. *Lower limit for oxygen saturation with maximal feed intake (DO_{maxFI}) and limiting oxygen saturation (LOS) for Atlantic salmon post-smolts of 300-500 g. Data from Remen et al., 2016a.*

Temperature	DO_{maxFI}	LOS
7	42%	24%
11	53%	33%
15	66%	34%
19	76%	40%

Sampling and analytical considerations

Oxygen saturation may vary within the body of water in both space and time and measures of oxygen saturation should be done when and where it is expected to be lowest. In tanks, the water at the drain has passed the fish and will normally have the lowest oxygen saturation. In cages, the lowest oxygen saturation is normally found at the depth with highest fish density in the leeward side from the water current, and especially when the current speed is lowest at slack water (e.g. Oppedal et al., 2011a). As both the solubility of oxygen in water and the fish oxygen requirements are dependent upon temperature, temperature should be measured together with oxygen. Ideally, oxygen is measured as a vertical profile by the use of a CTD together with measures of other environment based indicators such as temperature and salinity. Oxygen meters are also integrated in some camera systems used in sea cages. Oxygen probes should be controlled and calibrated regularly, and show 100% saturation when held in air.

Strength of indicator

Easy and rapid to measure and interpret.

Weakness of indicator

Oxygen level may vary greatly in space and time and if measured at the wrong place or at the wrong time, low levels may be missed.

4.1.4. CO_2

High carbon dioxide content is a key concern during the freshwater production phase, where toxic effects of high CO_2 have been observed in the range 20-100 mg L^{-1}, depending of other water parameters and fish metabolism/size (Rosten et al., 2004). When CO_2 dissolves in water it forms carbonic acid, and high levels of CO_2 will reduce the pH of the water, especially if it has low alkalinity (Fivelstad, 2013). Blood concentrations of CO_2 are strongly correlated with water CO_2 (Fivelstad, 2013) and elevated blood concentrations of CO_2 decrease oxygen carrying capability (Wood and Jackson, 1980). Salmon acclimate to elevated plasma CO_2 levels by increasing their plasma bicarbonate concentration, which leads to a reduced concentration of plasma chloride (Fivelstad, 2013).

Parr and smolts: Long-term exposure (weeks and months) to elevated CO_2 levels can have a negative effect on growth in parr (Fivelstad et al., 2007; Hosfeld et al., 2008). Salmon smolts in freshwater respond to elevated CO_2 (~20 mg L^{-1}) by increasing their ventilation frequency (Fivelstad et al., 1999) but this response is transient during chronic exposure, suggesting acclimation to elevated CO_2 (Fivelstad et al., 2003; Hosfeld et al., 2008). This implies physiological adaptation, but swelling of the erythrocytes can be a long term (months) consequence of elevated CO_2 (Fivelstad et al., 2003). The magnitude of the CO_2 effect is dependent on temperature. Fivelstad et al., (2007) found the weight reduction caused by high CO_2 concentrations to be much less at 15 °C (approx. 30% growth reduction) than at 5 °C, where there was almost no growth during 47 days of exposure to 43 mg CO_2 L^{-1}.

Post-smolts: Long-term exposure (weeks and months) to elevated CO_2 levels can also have a negative effect on growth in post-smolts (Fivelstad et al., 1998). For salmon post-smolts held in sea water at 15-16 °C, a CO_2 concentration of 10.6 mg L^{-1} did not affect blood parameters (plasma chloride, plasma sodium and haematocrit) or growth, whereas 26 mg L^{-1} reduced plasma chloride, and 44 mg L^{-1} increased plasma sodium and pH and reduced plasma chloride, oxygen consumption and growth (Fivelstad et al., 1998).

The adverse effects of carbon dioxide are dependent on other factors, especially water alkalinity (Summerfelt et al., 2000) and general safe levels are therefore difficult to state. For Atlantic salmon smolts produced in Norway, the safe criterion is 15 mg L^{-1} (Fivelstad, 2013). Negative effects on growth and/or condition factor have, however, been found at concentrations below 10 mg L^{-1} in salmon parr and smolts (studies summarized in Fivelstad, 2013).

- **Safe maximum levels of CO_2 in the freshwater phase: 15 mg L^{-1} (FOR, 2004)**

Sampling and analytical considerations

CO_2 should be measured on a regular basis during the freshwater phase or during land based production of post-smolts particularly when the biomass is high and water flow in the systems is limited or when the water exchange rate is low. Measurements of CO_2 should preferably be done at the tank outlet. CO_2 can be measured using hand-held instruments or in-line self-standing instruments or probes connected to larger monitoring systems. There are two main ways to measure CO_2: 1) directly, using CO_2 meters, or 2) indirectly, such as calculating it from pH and alkalinity (e.g. Moran et al., 2010, see also references therein). Alternatively, accredited laboratories can provide a "snap-shot" image of the production conditions as a service with a certain time delay to receiving the results. Instruments for the direct measurement of CO_2 are more expensive, have longer response time, are dependent on higher water velocity during measurements but should provide direct and more precise measurements. The indirect method is cheaper but it is dependent on an accurate measurement of pH. In addition, the interference from a number of substances in water that affect alkalinity can reduce the precision of this method, making it unreliable in very soft acidic water.

Strength of indicator

Blood concentrations of CO_2 are strongly correlated with water CO_2 and can provide information on physiological status of the fish.

Weakness of indicator

Irregular or single measurements of CO_2 can only provide a snap-shot of the production conditions without allowing determination of chronic exposure and any long term consequences for the fish.

4.1.5. pH

The pH (hydrogen ions: H^+) of freshwater is in most cases positively correlated with water hardness (dissolved calcium concentration). Fish are vulnerable to acidic water as: 1) high H^+ levels results in loss of calcium in the gill epithelium, causing osmoregulatory problems (loss of Na^+ and Cl^-) and blood acidosis, and 2) Aluminium concentration is inversely correlated with pH, where toxic effects include respiratory problems with Al binding to interlamellar mucous and reduced membrane fluidity, and osmoregulatory problems by loss of ions (Havas and Rosseland, 1995). The toxicity of aluminium is very dependent upon its various forms in which it may be present, its interactions with H^+ and organic acids, and will therefore vary with different water qualities (Havas and Rosseland, 1995).

In Norway, the buffering capacity of freshwater is generally low and 20% of a representative sample of smolt production facilities assessed in 1999-2001 had pH lower than 6 for their inflowing water (Rosten et al., 2004). Measures to increase pH in fish production include adding seawater, lime or silicates (Rosten et al., 2004).

Eggs and larvae: Significant reductions in egg hatching and larval growth have been observed at pH 4.5 and 5 in Atlantic salmon. A pH of 4.5 can increase mortalities in larvae, while a pH of < 6.5 can impair swimming and feeding (Buckler et al., 1995). Mortalities (induced by reduced feeding) were found as high as 70% at pH 5 and 4% at pH 6.1 over 30 days in the early feeding phase (Lacroix et al., 1985). During the earliest life stages, incubation at pH 5 and lower was found to induce sub-lethal effects in Atlantic salmon alevins e.g. on gills and blood vascular structures, while a pH of 4.5 and lower caused injuries to the brain, optic retina and spleen (Daye and Garside, 1980).

Parr and smolts: Smolts are especially vulnerable to low pH, due to their osmoregulatory preparation for seawater. Natural fluctuations in pH caused by rain and snow-melting releasing acid and diluting calcium concentration in the water can boost inorganic monomeric aluminium, and may lead to increased mortalities in pre-smolts (Henriksen et al., 1984). When Atlantic salmon parr were subjected to long-term exposure (3 months) to low pH (pH 4.2-4.7) it impaired both growth performance and smoltification (Saunders et al., 1983). In production units, a build-up of CO_2 will decrease the pH and alter aluminium chemistry, which is a concern. Fivelstad et al. (2003), tested industry relevant CO_2 driven pH levels in Atlantic salmon smolts. Despite low levels of labile aluminium concentrations, fish reared at a low pH of 6 and 5.7 showed 5 and 7 times more gill aluminium deposition compared with that of the control reared at pH 6.6. The low pH reduced plasma chloride and gill Na^+, K^+ -ATPase activity, arrested growth and significantly affected mortality (Fivelstad et al., 2003). Osmoregulatory failure also includes the skin mucous, and smolts exposed to pH 5.6-6.2 for > 80 hours showed a linear relationship between reduced plasma chloride and skin mucous cells as well as high mortalities (Berntssen et al., 1997). For wild salmon smolts in rivers, McCormick et al., (2009) found the smallest physiological response to pH at levels between 6.5 and 7, with the response increasing rapidly with decreasing pH.

Sampling and analytical considerations

Measuring pH in water is an easy process, and can be done using various types of pH-meters. However, it is important that the probe is calibrated in accordance with specifications from the manufacturer. The pH scale is logarithmic.

Strength of indicator

Easy and rapid to measure.

Weakness of indicator

Irregular or single measurements of pH can provide us only with the snap-shot of the production, and the level may vary in space and time. If pH measured at the wrong place or at the wrong time low levels may be missed. A change in pH is often not enough to identify a specific production problem. Additional sampling of other OWIs and LABWIs such as oxygen, heavy metals, CO_2 and total ammonia nitrogen needs to be carried out to ensure some understanding of the potential impact of pH changes.

4.1.6. Total ammonia nitrogen

Ammonia (NH_3) is a consequence of protein catabolism and is often referred to as Unionised Ammonia (UIA) (Thorarensen and Farrell, 2011). Ammonia reacts with water and forms the ion ammonium (NH_4^+). Total ammonia nitrogen (TAN) refers to the sum total of NH_3 and NH_4^+. The reaction between ammonia and ammonium goes both ways and how much of the ammonia that ends up as ammonium depends primarily on pH and to a lesser extent on temperature and salinity, and the NH_3/NH_4^+ ratio decreases with decreasing temperature and pH and increasing salinity (Boyd, 2000). In rearing water and the salmon body fluids, most of the TAN is in the form of ammonium (Thorarensen and Farrell, 2011). In freshwater, most of the ammonia produced by the fish is excreted by diffusion across the gills. However, an accumulation of TAN in the water will reduce the efflux of ammonia across the gills, resulting in elevated levels of TAN in the plasma of the fish (Thorarensen and Farrell, 2011).

Ammonia has a toxic effect upon the central nervous system (CNS), and can be detrimental to the stability of enzymes and membranes, gill health and osmoregulatory performance. An increase in ammonia levels can have a short-term detrimental effect upon feeding and swimming activity, can increase ventilation rate, and lead to a loss of equilibrium and also lead to death (Thorarensen and Farrell, 2011 and references therein). Long term effects are reflected in poor growth performance decreased robustness and fecundity (Thorarensen and Farrell, 2011 and references therein).

For Atlantic salmon, the maximum safe concentration for chronic exposure to UIA is around 0.012 mg L^{-1} (Fivelstad et al., 1995), while the maximum concentration for acute exposure (4 hours) is 0.1 mg L^{-1} (Wedemeyer, 1996).

Safe maximum levels of UIA:

- **Short-term: 0.1 mg L^{-1} (Wedemeyer, 1996)**
- **Long-term: 0.012 mg L^{-1} (Fivelstad et al., 1995)**

Sampling and analytical considerations

While ammonia is more toxic in salt water (mostly due to higher pH) the concentration can be higher in systems with low water turnover, more commonly seem in fresh water. Problems with ammonia can also occur if RAS filtration systems are not working effectively. Ammonia samples should be analysed immediately after sampling or can be fresh frozen at -20°C after filtration for subsequent analysis. Ammonia is commonly measured using "bench top" photometric methods. Alternatively, in-line instruments for measurements of ammonia are available, such as ion-selective electrodes, gas detection or amperometric detection. In-line solutions are mainly based on their application for other industries (drinking water, waste water or sewage) and their accuracy and range of measured values are not always suitable for aquaculture. Photometric methods use substances which react with ammonia and the resulting colour changes are measured. When using photometric methods one should: a) know which form of ammonia is measured, b) make a standard curve using standards of known concentrations, c) account for potential interfering substances (for example filter the sample if Total Suspended Solids (TSS) are > 5 mg L^{-1}) and d) always account for effect of temperature and salinity. Ammonia should be monitored continuously in systems with low water exchange, during transport and in cases when water flow is limited and biomass in the rearing units are high.

Strength of indicator

Ammonia is toxic to Atlantic salmon, can accumulate in blood and tissues and can eventually cause mortalities. Therefore, if levels exceed recommended limits, fish welfare is at risk.

Weakness of indicator

The ammonia balance between the more toxic UIA and ionized ammonia nitrogen (NH_4^+-N) is dependent on pH, temperature and salinity. Measurements of total ammonia nitrogen (TAN) without the other water quality parameters will not provide adequate information on ammonia toxicity.

4.1.7. Nitrite and Nitrate

For freshwater production systems, EFSA (2008a) states "*nitrites are not usually a problem in aquaculture with flow-through (where nitrogenous wastes are adequately flushed away) or in adequately oxygenated water so that oxidation rate of nitrite exceeds the oxidation rate of ammonia*". In RAS systems, the nitrobacter bacteria in the biofilters rapidly convert nitrite to nitrate (which is markedly less toxic) by nitrification (Lewis Jr. and Morris, 1986). Nitrite in blood reacts with iron from haemoglobin and reduces its oxygen carrying capacity (EFSA, 2008a; Thorarensen and Farrell, 2011). Nitrite at high enough concentrations can be toxic for Atlantic salmon in freshwater through its affinity for and interference with gill chloride uptake mechanism (Jensen, 2003). This can lead to chloride depletion and affect gas transport, ion regulation, and cardiovascular, endocrine and excretory processes, the formation of methaemoglobin, and reduced blood oxygen transport (Jensen, 2003; Svobodová et al., 2005). The addition of chloride to freshwater can protect from adverse effects of nitrite toxicity. Nitrite is therefore less harmful and problematic in seawater where chloride levels are naturally high (Thorarensen and Farrell, 2011). It is suggested that a 108:1 Cl:NO_2-N ratio should be used to protect Atlantic salmon parr (Gutierrez et al., 2011). Currently the guidelines for Cl^- requirements in relation to NO^-_2 concentrations are not specified by the Norwegian Food Safety Authority.

Nitrate (NO_3^-) is the end product of nitrification and can also have adverse effects on salmon, but it is considered to be less harmful and it is recommended to keep its concentration in the system to < 100 mg L^{-1} (Bregnballe, 2010). Nitrate can, however, reach higher levels in recirculating systems and must be monitored.

Recommended upper concentrations

- **Nitrite: 0.1 mg L^{-1} (Wedemeyer, 1996; Thorarensen and Farrell, 2011).**
- **Nitrate: 100 mg L^{-1} (Bregnballe, 2010).**

Sampling and analytical considerations

Nitrite nitrogen (NO_2-N) can accumulate in systems with low water exchange (e.g. RAS) and can be toxic to Atlantic salmon. Therefore, NO_2-N should be monitored regularly. Nitrate nitrogen (NO_3-N) is not toxic in current commercial conditions (when up to 25% of the total system volume is exchanged daily) and NO_3-N is diluted.

Both nitrogenous compounds are measured using photometric methods and kits similar to ammonia. Kits use nitrite's reaction with sulphanilamide that produces coloured diazonium v/500-550 nm. For nitrate analysis, it is reduced to nitrite with Cd (i.e. a high background of nitrite can lead to errors). You can improve the precision of nitrite measurements with the use of automated colorimetry methods (0.005-10 mg L^{-1}).

The following recommendations should be followed when measuring nitrite: 1) know which nitrite compound is measured (nitrite or nitrite nitrogen); 2) a standard curve should be made using known concentrations; 3) samples should be filtered if TSS is high; 4) sulphide and metals can interfere with measurements. For nitrate measurements: 1) a standard curve should be used; 2) samples should be filtered if TSS is high; 3) nitrite and Cl^- can interfere which is important when analysing seawater samples.

Strength of indicator

Nitrite is toxic for Atlantic salmon and can cause mortalities. Nitrate indicates the status of the nitrification process in bioreactors in RAS.

Weakness of indicator

Higher concentrations than recommended can be tolerated by salmon when adequate levels of chloride are available. Therefore, chloride should be measured together with nitrite to provide an indication of the threat to fish welfare.

Knowledge gap

Total gas pressure (TGP) and nitrogen supersaturation have been implicated in gas bubble disease (Weitkamp and Katz, 1980), with the same authors also highlighting the "*importance of total gas pressure as opposed to nitrogen partial pressure in causing gas bubble disease*" (Weitkamp and Katz, 1980) and this importance is reiterated by Hosfeld et al., (2010).

- Hjeltnes et al., (2012) state "*supersaturation occurs when the partial pressure of one or more of the gases dissolved in the water becomes greater than the atmospheric pressure. Sudden increases in temperature, decreases in pressure, or excessive oxygenation, are all typical causes of gas supersaturation in aquaculture systems.*"
- Lekang, (2007) also states "*total gas pressure in the water is measured mainly to find not only the total pressure, but also the amount and saturation of dissolved nitrogen gas*" in the water.
- Earlier work states that if nitrogen saturation exceeds 100%, fish can develop gas bubble problems and it appears that this a more of a problem in juveniles compared to adult fish (Weitkamp and Katz, 1980). In salmonids such as Atlantic salmon and rainbow trout, negative effects have been observed when nitrogen levels are over 102% (Lekang, 2007) and Lekang (2007) recommends a limit < 100.5% N_2. Wedemeyer, (1997) also states that N_2 saturation in intensive production systems should be below 110%.
- However, as stated above, nitrogen may just be one of a multitude of factors that can impact upon the welfare of fish subjected to gas supersaturation and that more focus should be paid to TGP than nitrogen saturation (Weitkamp and Katz, 1980).
- According to the Norwegian Food Safety Authority, TGP should not be higher than 100%.
- Since there is little data and a lot of uncertainty and confusion about the tolerance of Atlantic salmon to total gas pressure (TGP) and nitrogen supersaturation in relation to gas bubble disease, we recommend using the above limit values as guidelines and not as absolute limits.

4.1.8.Turbidity and total suspended solids (TSS)

Turbidity refers to the clarity of the water and TSS refers to the suspended material in the water and while these two parameters are related they are not always highly correlated. For example, water clarity may be affected by dissolved as well as suspended substances. Increased turbidity can hinder the observation of fish in tanks and cages. This makes observation of the fish for assessing health difficult and can reduce the farmer's capacity to monitor the feeding response. The effects of turbidity are related to the nature of the substances reducing visibility. The optimal levels of turbidity for salmon are not specified, since acceptable levels would be dependent on the nature of the suspended materials. The concentration of TSS can be described as the mass of particles (both organic and inorganic) above 1 µm in diameter that are found in a known volume of water (e.g. Timmons and Ebeling, 2007). Suspended solids may also contribute to a high chemical or biological oxygen demand and to both biofouling and the formation of sludge deposits in tanks. Fine suspended solids or solids with abrasive particles can have negative effect on gill health and function, compromising oxygen transfer and providing a habitat for the growth of pathogens (Timmons and Ebeling, 2007). A definitive threshold value for an acceptable TSS concentration has not been agreed upon (Timmons and Ebeling, 2007), but an upper limit of 15 mg L^{-1} has been suggested (Thorarensen and Farrell, 2011).

Recommended upper concentration of Total suspended solids:

- **15 mg L^{-1} (Thorarensen and Farrell, 2011).**

Sampling and analytical considerations

Turbidity measures the amount of particles (size range between 0.004 nm and 1.0 mm) that reduce light penetration through the water column. Turbidity can be quantified via, 1) a secchi disk or transparency tubes in e.g. sea cages or, 2) turbidity meters (optoelectronic meters) that measure the intensity of the scattered light at an angle of 90^{o} and provides measures in nephelometric turbidity units (NTU). Samples should be kept in a dark place prior to analysis and a turbidity meter should be calibrated prior to the sample analysis. Turbidity can be measured according to the US EPA method 180.1 "Determination of turbidity by nephelometry": https://www.epa.gov/sites/production/files/2015-08/documents/method_180-1_1993.pdf

TSS is measured using the ESS Method 340.2: Total Suspended Solids, (Dried at 103-105 ^{o}C): http://www.cyanopros.com/refs/epa_tss.pdf. Large submerged or floating particles and seawater can interfere with accurate measurements of TSS. Analytical parallels are recommended.

Strength of indicator

Water turbidity can be correlated with other water quality parameters, e.g. increased turbidity due to organic material can increase water temperatures and decrease DO saturations. The effects of TSS can degrade water quality, clogs equipment and can be damaging to fish gills and harbour pathogens. They should therefore be measured and correlated with other OWIS.

Weakness of indicator

The impact of water turbidity and TSS on fish welfare is dependent on the nature of the suspended particles and this can make it difficult to generalise regarding safe levels.

4.2. Welfare indicators describing rearing systems or rearing practices

4.2.1. Water current speed

In tanks, low water current speed can limit the self-cleaning abilities of the rearing units and the flushing of waste feed and faeces, and with it the water quality fish are exposed to. In sea cages, water current speed influences the rate of water exchange and the effect of current speed upon water quality depends on several factors such as the size of the cage, biomass and biofouling. Hypoxia is correlated with low current speed when there is reduced water exchange in cages (Vigen, 2008). Current speed may also affect the volume of the cage by deformation, although this is related to the nature of the net and cage structure and also the degree of biofouling.

Water current speed influences the swimming performance of fish. Fish maintain their position to a greater or lesser extent relative to the sides or bottom in tanks or swim against the water current velocity. Fish in sea cages swim relative to both the changing water current speed and the net. Water current speeds that are beyond the fishes maximum sustainable swimming speed result in the fish becoming exhausted, failing to hold their position or being displaced into parts of the tank or cage that may be suboptimal. As a given current speed is relative to body size it is often expressed as body length s^{-1} rather than absolute values (cm s^{-1}). While the absolute swimming speed (cm s^{-1}) increases with fish size the relative swimming capacity (body length s^{-1}) generally decreases with fish length. Swimming speed increases with temperature up to a certain thermal optimum; at very high temperatures swimming capacity decreases (Brett, 1964, 1965; Peake, 2008).

Critical swimming speed (U_{crit}) is a measure of maximum aerobic performance, and is measured using incremental velocity protocols in swim tunnel respirometers until the fish fatigues (Brett, 1964; Beamish, 1978; Hammer, 1995; Farrell, 2007). The fish is only able to maintain U_{crit} for short durations (minutes), meaning prolonged swimming only is possible at significant lower speeds (<70% U_{crit}) where the anaerobic component of locomotion does not become too high (Burgetz et al., 1998). U_{crit} is a standardized measure of swimming performance estimated in an extremely artificial environment. It is therefore not directly relevant for farm conditions and should be interpreted with caution. For short periods of time (seconds) fish can burst swim considerably faster than U_{crit}. In practice fish often swim in a burst and glide pattern when current speeds increase, further emphasising the limitations of U_{crit}. However, U_{crit} is frequently discussed in the literature and is therefore included here.

For salmonids, exercise often has positive effects upon the fish and can lead to increased growth and protein deposition, a stronger heart and higher blood flow, and various physiological improvements. However, current velocities that are too high (even if they are well below U_{crit}), may have negative effects on performance, as may current velocities that are too low (Solstorm et al., 2015, 2016a).

Parr: Salmon parr may lie on the bottom and use the water current over the pectoral fins to hold the fish on the substrate (Arnold et al., 1991). They can, therefore, tolerate relatively higher current velocities than free swimming fish. Parr may hold their position against the water current indefinitely up to a threshold where the current is too strong and they must swim actively. Fatigue then occurs within few minutes, resulting in fish drifting backwards in the current (Arnold et al., 1991; Peake et al., 1997). Critical swimming speeds for salmon parr in the temperature range 12.5-19 °C are presented in Fig. 4.2.1-1A, and maximum sustained swimming speeds in Fig. 4.2.1-1B. Parr without pectoral fins have much reduced swimming abilities (Arnold et al., 1991), and it may be assumed that the ability of parr to maintain their position with damaged pectoral fins are lower than what is reported here. This may also apply to parr that do not have access to the substrate.

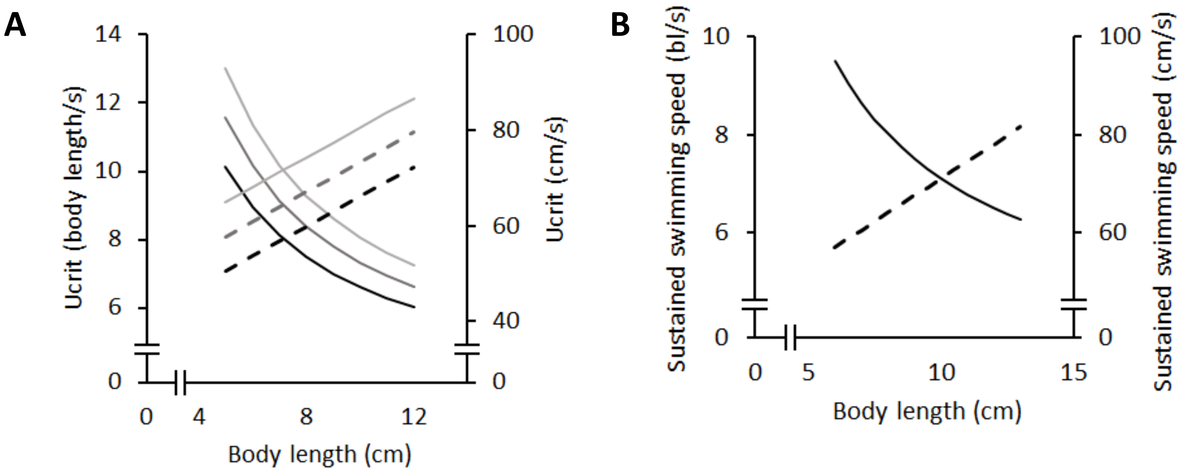


Fig. 4.2.1-1. *A) Critical relative (body lengths s^{-1}, solid lines) and absolute (cm s^{-1}, dashes lines) swimming speed of Atlantic salmon parr as a function of fish length at 12°C (light grey), 16°C (dark grey) and 20°C (black). Data from Peake et al., (1997). B) Maximum sustained swimming speed of Atlantic salmon parr as a function of body length in the temperature range 12.5-19°C. Solid line indicates relative speed (body lengths s^{-1}) and dashed line absolute speed (cm s^{-1}). Data from Peake et al., (1997).*

Smolts: Salmon smolts swim more freely in the water than parr and to a lesser extent maintain a position relative to the bottom (Peake et al., 1997). The effect of body length and temperature on absolute critical swimming speed varies between studies (Peake et al., 1997; Booth et al., 1997). As farmed smolts are usually within a relatively limited size range, the effect of body length is less critical. A linear increase of the absolute critical swimming speed increased from 64 to 109 cm s^{-1} has been found for smolts of 16.5 cm in the temperature range 5-19 °C (Booth et al., 1997).

Absolute sustained swimming speeds of smolts were found to be ca. 50 cm s^{-1} and were not clearly affected by temperature (Tang and Wardle 1992; Booth et al., 1997).

- **Absolute critical swimming speed for salmon smolt: 64-109 cm s^{-1}, increases with body length and temperature.**
- **Absolute sustained swimming speed for salmon smolt: 50 cm s^{-1}.**

Post-smolts: Absolute critical swimming speed for post-smolts is 81 cm s^{-1} (4.1 body lengths s^{-1}) for fish of 20 cm, 91 cm/s (3.2 body lengths s^{-1}) for fish of 29 cm and around 100 cm s^{-1} both for fish of 38 cm (2.6 body lengths s^{-1}, Wagner et al. 2003) and for fish of 51 cm (1.9 body lengths s^{-1}, Remen et al., 2016b).

The maximum sustained swimming speed for Atlantic salmon post-smolt of 30-50 cm body length has been found to be 90 cm s^{-1} (2 body lengths s^{-1}) at 11 °C. However, small (22 cm) post-smolts forced to swim against a current a velocity below this level (1.5 body lengths s^{-1}) for 6 weeks have been found to have restricted growth compared to fish held at 0.8 or 0.2 body lengths s^{-1} (Solstorm et al. 2015; 2016a).

Current velocities that are too low may also have negative effects on the fish. Post-smolts forced to swim against a low current velocity of 0.2 body lengths s^{-1} for 6 weeks gained more fat and less protein (Solstorm et al., 2015), and showed a higher frequency of structural movements, between-individual interactions and aggression which may be stressful for the fish (Solstorm et al., 2016a).

- **Relative critical swimming speed of salmon post-smolts: 2-4 body lengths s^{-1}**
- **Relative sustained swimming speed of salmon post-smolts: 2 body lengths s^{-1}**
- **Welfare may be negatively affected at long-lasting velocities of 1.5 body lengths s^{-1}**
- **Low current speeds increase negative interactions and may impair welfare (Solstorm et al., 2016a)**

Sampling and analytical considerations

In tanks the water current speed varies with the distance to the wall and is at its highest near the wall and is lower towards the centre of the tank. The water is often turbulent and can be difficult to measure with flow meters. An alternative way to measure current speed is to use a floating object and measure the lap time to calculate the speed. During the measurement, one must ensure that the object holds a fairly constant distance from the tank wall during the lap of the tank. A rule of thumb for setting water flow in tanks is that the fish should hold their position relative to the tank wall and if they drift forward, the current is too low whilst if they are driven backwards, the current is too strong.

In sea cages the current speed will vary with the tide, amongst other things and it is not possible to adjust. The flow inside the cage is usually lower than the outside (Johansson et al., 2014) and the degree of damping can be affected by e.g. biofouling. Therefore, current flow and direction should not only be measured outside the cage but also in the cages.

Strength of indicator

Water current speed can be of great importance to the salmon's welfare, especially in cages where the water flow is important for water exchange and where it can vary a lot over time. At low water velocities it can lead to hypoxia, especially at high density and high temperatures. At excessive water velocities it may cause cage deformation, reduce cage volume and also lead to fatigue in the fish, especially in smaller fish that have lower absolute swimming capacities.

Weakness of indicator

Water flow should be measured in the right place at the right time. It varies through the day with the tide cycle and tidal strength also varies with the phase of the moon and is strongest at spring tides. Water flow can also be affected by wind. Obtaining accurate measurements for critical water velocity on the farm can therefore be demanding.

4.2.2. Lighting

Freshwater: Light has an effect upon several endocrine processes in salmonids, including smoltification (Berge et al., 1995) and sexual maturation (Hansen et al., 1992). To control and synchronize the timing of smoltification and to produce out-of-season (0+) smolts, salmon are usually held at simulated long days (constant light) from first feeding. To initialize the smoltification process, day length can then be reduced (12:12; Light:Dark), simulating a winter (“winter signal”, minimum of 4 weeks), followed by another period of long days to simulate spring. This will induce the parr-smolt transformation (Berge et al., 1995; Stefansson et al., 1991). For a successful parr-smolt transformation the parr must reach a certain size before the winter signal is initiated (Skilbrei, 1991; Handeland et al., 2013). This threshold size may vary between populations but Skilbrei (1991) reported it was around 75 mm. The duration of the different light regimes depends on water temperature (Handeland et al., 2004, 2013) but the winter signal is usually 4-6 weeks followed by a similar 4-6 weeks of constant light for the spring signal. The photoperiod regimes for controlling smoltification must be robust as a fish that is not completely smoltified or has begun to reverse the smoltification process is not adapted to seawater and may suffer from osmotic imbalance (see section 3.2.8 “Smoltification state”). Constant light has been found to have negative effects on the neurological development of parr (Ebbesson et al., 2007). When light is suddenly switched on the sudden change in light intensity induces an acute stress response involving panic behaviour (Mork and Gulbrandsen, 1994) and increased oxygen consumption (Folkedal et al., 2010) but salmon can habituate to this response within a week (Folkedal et al., 2010).

Seawater: Ambient light is one of most important parameters driving the vertical positioning of cage-held Atlantic salmon, where vertical gradients of light intensity and temperature are key factors that determine their swimming depth (see Oppedal et al., 2011a for review). When reared under natural light regimes, salmon typically swim closer to the water surface at night and descend at dawn, swimming deeper in the cage during daylight hours (Oppedal et al., 2011a). The use of artificial underwater light can be used to direct swimming behaviour and activity (Juell et al., 2003; Juell and Fosseidengen, 2004) and can also be used to reduce lice load by directing fish to swim deeper in the cage (Frenzl et al., 2014). Underwater light is also used to control sexual maturation in cages. High intensity lights are used from midwinter and during the next 4-6 months to decrease the occurrence of sexual maturation (Hansen et al., 1992; Porter et al., 1999; Oppedal et al., 1997, 2006). However, the artificial extension of the summer by using high intensity light during the autumn can have the opposite effect and induce sexual maturation (Duncan et al., 1999; Oppedal et al., 2006). The use of low intensity lights is sufficient for controlling the swimming depths of salmon during the night (Stien et al., 2014) and weak violet light did not induce sexual maturation.

Salmon can go into an appetite depression lasting for 6-8 weeks after the onset of a continuous light regime, resulting in reduced growth (Oppedal et al., 2003; Hansen et al., 1992; Oppedal et al., 2006). This may be due to “*a stress response of the fish to the changing environment, and a postulated phase advancement of the fish to a circannual growth rhythm that involves reduced winter growth adjusted by photoperiod*” (Oppedal et al., 2006 and references therein).

Table 4.2.2-1. *Light regimes to control development of smoltification and sexual maturation in farmed salmonids*

Stage	Light regime	Time of year	Effect	Reference
Parr	Extended day length	Variable	Avoid smoltification	Berge et al., 1995
Pre-smolt	Reduced day length	Variable	Initiate smoltification	Berge et al., 1995
Pre-smolt-smolt	Extended day length	Variable	Complete smoltification	Berge et al., 1995
Post smolt	24:0	Midwinter and spring	Avoid maturation	Oppedal et al., 2006
Post smolt	Natural	Summer-midwinter	Avoid maturation	Oppedal et al., 2006

Sampling and analytical considerations

The fish's perception of daylength has a major influence on hormonal development, such as smoltification and gender maturation and it is therefore important to use light regimes that do not negatively affect the desired outcomes of these processes. If the purpose of artificial lighting is to influence behaviour, e.g. swimming, an appropriate intensity and spectrum must be used to avoid sexual maturation (Stien et al., 2014). In order for artificial light to have a positive effect on growth, light intensity must be high enough to ensure that the natural photoperiod does not override its effect (Hansen et al., 2017).

Strength of indicator

Light intensity can be manipulated by increasing or decreasing the number of lights on the farm, or changing the strength and / or colour of the lights.

Weakness of indicator

The light intensity the fish experiences can also be affected by the distance from fish to the light source, the clarity of the water and the fish density within the rearing system (how much shading the fish can experience from conspecifics). The fish's interpretation of daylength under artificially extended natural photoperiods is affected by the irradiance of both natural and artificial light (Hansen et al., 2017).

4.2.3. Stocking density

Stocking density (which can also be termed density or rearing density) is typically stated as being the "density of fish at any point in time" within the rearing system (Ellis et al., 2002) and is expressed as kg m^{-3}. The ideal stocking density for Atlantic salmon depends on several variables, such as life stage, water quality, water velocity, social interactions, feed management, management practices and the choice of rearing system (e.g. Turnbull et al., 2008). Interactions between these factors make it challenging to define a specific optimal stocking density but there is little doubt that stocking densities that are too low or high can have negative impacts upon welfare (Adams et al., 2007). However, the potential negative impacts of stocking density upon fish welfare may not always be caused by the density of fish *per se*, but rather from reduced water quality (Hosfeld et al., 2009; Thorarensen and Farrell, 2011) and reduced feed availability (Boujard et al., 2002) caused by higher densities. The welfare needs that are directly or indirectly affected by stocking density include i) hygiene, ii) good water quality, iii) behavioural control, iv) social contact and v) rest. Examples of the potential impacts of stocking density on Atlantic salmon welfare from numerous studies are outlined in Table 4.2.3-1.

Fry and parr: In a laboratory study using 10 fish per group, low stocking density (8 kg m^{-3}) reduced overt aggression and fin damage compared with parr held at 30 kg m^{-3} (Cañon Jones et al., 2011). However, growth and condition were lower at 8 kg m^{-3} compared with 30 kg m^{-3} and the total number of aggressive interactions was higher at the lower density. This suggests the observed effects of density upon welfare are also dependent upon the welfare indicators that are chosen to assess welfare. At the other end of the scale, decreased performance has been found at densities of 146 kg m^{-3} and higher (Soderberg et al., 1993). At densities between 21-86 kg m^{-3} no negative effects of density were found as long as water quality and food rations were kept as recommended (Hosfeld et al., 2009).

Smolt and post-smolt in tanks: As with parr, low stocking densities may increase welfare problems in post-smolts as both skin and fin damage were highest at 15 kg m^{-3} and more severe at 35 kg m^{-3} than at 25 kg m^{-3} (Adams et al., 2007). The growth and condition factor of large post-smolts held at densities of 35-125 kg m^{-3} was not significantly affected by density (Kjartansson et al., 1988). However, another study by Liu et al., (2015) reported lower growth rate and higher cortisol in fish raised at 30-61 kg m^{-3} compared to lower densities.

Post-smolts and adults in sea cages: In sea cages, suboptimal environmental factors such as temperature may make salmon school at much higher local densities (up to 190 kg m^{-3}) even if the mean stocking density (biomass/cage volume) is moderate (32 kg m^{-3}) resulting in local hypoxia (Oppedal et al., 2011b). In a commercial farm study (Turnbull et al., 2005) no clear relationship was found between density and welfare up to ca. 22 kg m^{-3}, but an increase over 22 kg m^{-3} was correlated with poorer welfare.

Table 4.2.3-1. *Effects of stocking density on welfare of Atlantic salmon of different life stages and in different production systems.*

	Density	Comments	Reference
Parr	146 kg m^{-3}	Up to 146 kg m^{-3}: Weight gain lover, no effects on mortality or weight. Over 146 kg m^{-3}: fish growth slower and food conversion was higher	Soderberg et al., 1993
Parr	21, 43, 65, 86 kg m^{-3}	No negative effects as long as water quality and food rations were kept as recommended	Hosfeld et al., 2009
Parr	250 ind/m^2	No effect on swimming, max O_2 consumption, relative ventricle mass. Increased fin damage	Hammenstig et al., 2014
Parr	83 ind/m^2	Lower Hb and Hct	Hammenstig et al., 2014
Parr	30 kg m^{-3}	Better growth and condition. Lower total aggressive interactions (compared to 8 kg m^{-3})	Cañon Jones et al., 2011
Parr	8 kg m^{-3}	Less fin damage and less overt aggression (compared to 30 kg m^{-3})	Cañon Jones et al., 2011
Smolt	100-125 kg m^{-3}	Elevated Hct and lactate. No effect on cortisol, growth or CF (compared to 35-45 and 65-85 kg m^{-3})	Kjartansson et al., 1988
Post-smolt, tank	30.18–61.34 kg m^{-3}	Increased cortisol, ALP activity and maleic dialdehyde content. Lower Immunoglobulin M level and SOD activity (compared to lower densities). No effect on condition factor or mortality	Liu et al., 2015
Post-smolt	25 kg m^{-3}	Better overall welfare than 15 or 35 kg m^{-3}.	Adams et al., 2007
Post-smolt	15 kg m^{-3}	Higher post-feeding aggression (compared to 25 and 35 kg m^{-3})	Adams et al., 2007
Post-smolt, tank	5 kg m^{-3}	Higher mortality after AGD outbreak (compared to 1.7 kg m^{-3})	Crosbie et al., 2010
Adult (13 kg), cage	27 kg m^{-3}	Decreased feed intake, growth rate and feed utilization. Fish suffered from cataract and skin/fin erosions	Oppedal et al., 2011b
Adult, cage	22 kg m^{-3}	Limit density for welfare	Turnbull et al., 2005

Sampling and analytical considerations

Mean density in the aquaculture unit can be easily calculated as biomass (kg) / volume (m^3). However, the fish are often unevenly distributed in the rearing system and local densities can be significantly higher than the average (Oppedal et al., 2011b). In cages, the density in a given depth range can be estimated by hydroacoustics (Oppedal et al., 2011b).

Strength of indicator

Production density can be estimated quite accurately if the farmer has good biomass control and a known water volume.

Weakness of indicator

There is a complex relationship between fish welfare and stocking density and this relationship is influenced by many factors, including water quality, behavioural interactions between the fish and also the availability of feed, amongst others (see Turnbull et al., 2008). Therefore, stocking density must be used in tandem with other indicators when considering fish welfare (Turnbull et al., 2005). Stocking density can also vary widely within a rearing unit and even when fish have a moderate average density, if high local densities were to occur they can increase the risk of local hypoxia (Vigen 2008).

5. OWIs and LABWIs

5.1. How to use OWIs and LABWIs on the farm

The purpose of OWIs are to give the farmer a hands-on tool to use at the production facility, LABWIs are off-site indicators that give the farmer a robust indicator of welfare status in a reasonable amount of time. Since fish welfare is a function of a combination of parameters or dimensions, there are no single OWIs or LABWIs that gives a clear indication of compromised fish welfare. In most cases the sum of several OWIs (also WIs and LABWIs) outside normal ranges will indicate that the fish welfare is in jeopardy in the production facility and that it is time to respond. Figure 5.1-1 shows how OWIs and LABWIs may be used on the farm. The purpose is to be able to recognize negative changes in OWIs and LABWIs as early as possible and make the necessary changes before it becomes a fish welfare issue.

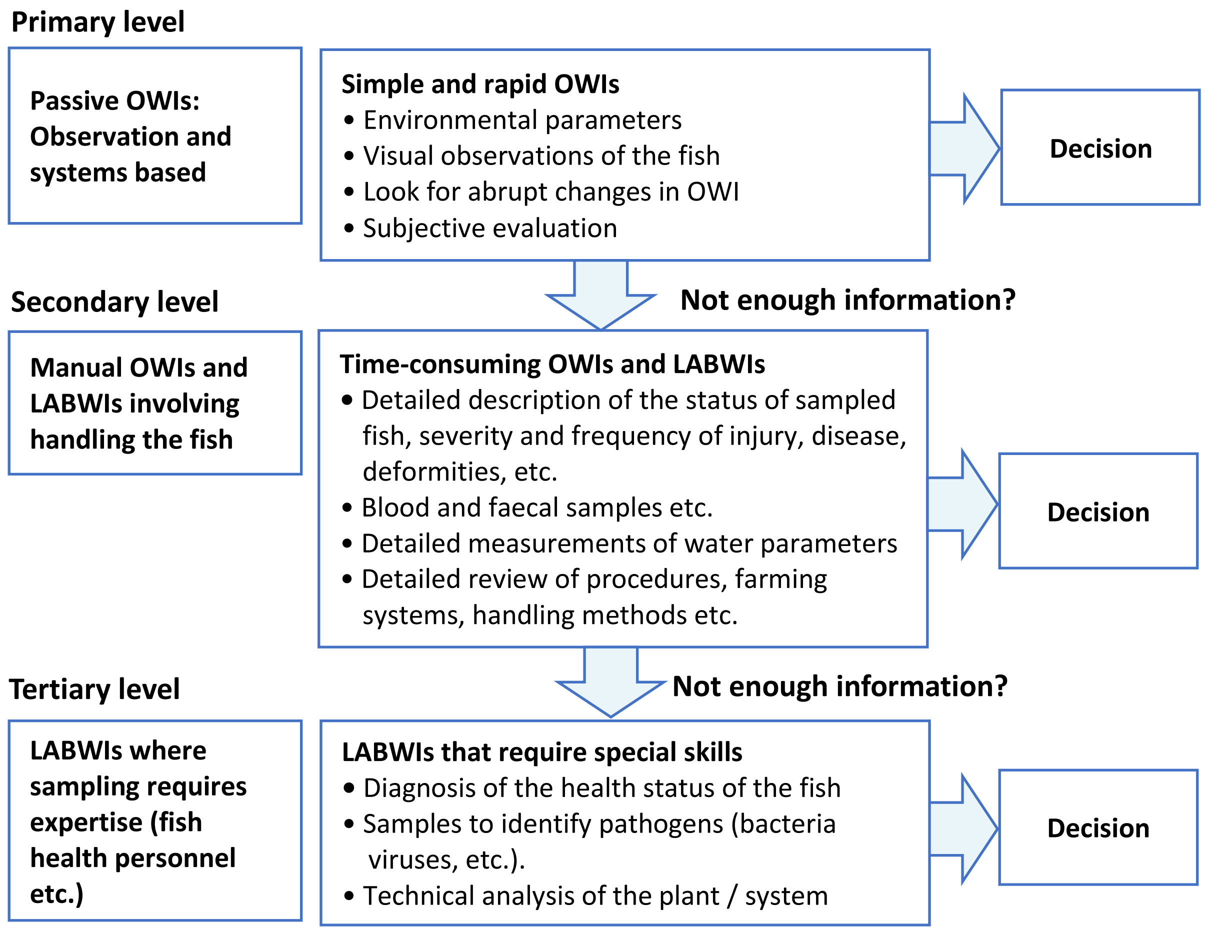


Fig. 5.1-1. *How to use OWIs and LABWIs at the farm as Early Warning Signals for compromised welfare (Figure: C. Noble and M. Iversen).*

5.2. Operational feasibility of WIs

To classify WIs as OWIs or LABWIs, we have made a simplified scoring system based on the sampling and analytical considerations of each WI (reviewed earlier in Part A, sections 3 and 4). 1 = can be used on the farm, 2 = can be used on the farm but needs expertise, requires further data analysis and/or special equipment, 3 = can be sampled on farm but must be analysed in laboratory in a timeframe acceptable to the farmers, 4 = neither on farm or currently requires an extended period of analysis in the laboratory. WIs with score of 2 or less are OWIs, WIs with score of 3 are LABWIs and WIs with score of 4 are neither but may be useful in a research context.

Table 5.2-1 shows the scoring of the environmental WIs, Table 5.2-2 the scoring of the group based WIs and table 5.3-3 the scoring of the individual based WIs. Each table also contains WIs that were put forwards as possible WIs, but that were not included in any of the productions systems or handling practices discussed in Part B and Part C of the handbook, and therefore also not reviewed in Part A (see final column).

Temperature, salinity, oxygen, CO_2, pH, turbidity, lighting and stocking density were all considered to be relative easy to measure (Table 5.2-1). In the case of turbidity, it is often measured using special probes that require considerable maintenance but it can also be measured by lowering a standardised white disk (Secchi disk) into the water and noting how deep the disk can still be seen from the surface.

Table 5.2-1. *Overview of all environmental welfare indicators and whether they are OWIs or LABWIs. See Figure 5.1-1 for further explanation for the simplified scoring system. Used = OWI/LABWI suitable for either Part B or Part C of the handbook. Scoring: 1 = can be used on the farm, 2 = can be used on the farm but needs expertise, requires further data analysis and/or special equipment, 3 = can be sampled on farm but must be analysed in laboratory in a timeframe acceptable to the farmers, 4 = neither on farm or currently requires an extended period of analysis in the laboratory.*

WI	Score (0.0 – 4.0)	OWI	LABWI	Used
Temperature	1.0	×		×
Salinity	1.0	×		×
Oxygen	1.0	×		×
CO_2	1.0	×		×
pH and alkalinity	1.0	×		×
Total ammonia nitrogen	2.0	×		×
Nitrite and Nitrate	2.0	×		×
Turbidity	1.0	×		×
Water current speed	2.0	×		×
Lighting	1.0	×		×
Stocking density	1.0	×		×
Ammonia	2.0	×		×
Total suspended solids	3.0		×	×
Heavy metals	3.0		×	

Mortality rate, surface activity, appetite, growth and observing scales/blood in the water were all rated as being relative straight forward to use (Table 5.2-2), even though e.g. the degree of scales in the water can be difficult to quantify. Observing behaviour can be done via camera and to a degree also from the surface. However, accurately categorising and quantifying the behaviour requires experience.

Table 5.2-2. *Overview of all animal group based welfare indicators and whether they are OWIs or LABWIs. See Figure 5.1-1 for further explanation for the simplified scoring system. Used = OWI/LABWI suitable for either Part B or Part C of the handbook. Scoring: 1 = can be used on the farm, 2 = can be used on the farm but needs expertise, requires further data analysis and/or special equipment, 3 = can be sampled on farm but must be analysed in laboratory in a timeframe acceptable to the farmers, 4 = neither on farm or currently requires an extended period of analysis in the laboratory.*

WI	Score (0.0 – 1.0 – 2.0 – 3.0 – 4.0)	OWI	LABWI	Used
Mortality rate		×		×
Behaviour		×		×
• Abnormal behaviour		×		×
• Aggression		×		×
• Decreasing echo		×		×
Surface activity		×		×
Appetite		×		×
Growth		×		×
Disease / health			×	×
Emaciated fish		×		×
Scales and blood in water		×		×
Bulk oxygen uptake		×		×

Most of the individual WIs are relative easy to assess on the fish (Table 5.2-3). However, cardiovascular responses, NKA1a and NK1b, magnesium and sodium, chloride and osmolality are all considered LABWIs and are also not used in the later sections (Table 5.2-3). Determining killing success by electroencephalography (EEG) or electrocardiography (ECG) require advanced scientific equipment and/or expert knowledge, these indicators are therefore not operational in the daily running of a slaughter house.

Table 5.2-3. *Overview of all individual animal based welfare indicators and whether they are OWIs or LABWIs. See Figure 5.1-1 for further explanation for the simplified scoring system. Used = OWI/LABWI suitable for either part 2 or part 3 of the handbook. Scoring: 1 = can be done on farm, 2 = can be done on farm but needs expertise, requires further data analysis and/or special equipment, 3 = can be sampled on farm but must be analysed in lab in a timeframe acceptable to the farmers, 4 = neither on farm or currently requires an extended period of analysis in the lab.*

WI	Score (0.0–4.0)	OWI	LABWI	Used
Gill beat rate	2.0	×		×
Eye roll (VER)	1.0	×		×
EEG and ECG	4.0			
Sea lice	1.0	×		×
Gill bleaching and status	2.0	×		×
Condition indices	1.0	×		×
• Condition factor	1.0	×		×
• Hepato-somatic index	1.0	×		×
• Cardio-somatic index	1.0	×		×
External morph. WIs	1.0	×		×
• Emaciation state	1.0	×		×
• Sexual maturity state	1.0	×		×
• Smoltification state	2.0	×		×
• Vertebral deformation	1.0	×		×
• Fin damage and fin status	1.0	×		×
• Scale loss and skin cond.	1.0	×		×
• Snout jaw wound	1.0	×		×
• Eye haemor. and status	1.0	×		×
• Opercula deformation	1.0	×		×
• Handling trauma	1.0	×		×
Feed in intestine	1.0	×		×
Skin colour change	1.0	×		×
Abdominal organs	2.0	×		×
Vaccine rel. pathology	2.0	×		×
Blood cortisol	3.0		×	×
Blood ionic composition	3.0		×	×
Blood glucose	2.0	×		×
Blood lactate	2.0	×		×
Blood pH	2.0	×		×
Muscle pH	2.0	×		×
Muscle lactate	2.0	×		
Muscle glucose	2.0			
Rigor mortis time	1.0	×		×
Micro morphology	3.0		×	
Cardiovascular responses	3.0		×	
NKA1a and NK1b	3.0		×	
Magnesium and sodium	3.0		×	
Chloride	3.0		×	
Osmolality	3.0		×	
Haematocrit	3.0		×	

5.3. Welfare assessment example scenario – a guide how to interpret the OWIs and LABWIs

When the farmer starts to observe emaciated fish with a) stunted growth, b) very low condition factor (thin), c) generally poor appearance, and d) behavioural abnormalities such as slow swimming near the net at the surface, swimming alone and at distance from the main group, it is time for the farmer to react. As mentioned in chapter 3.2.6 there are many plausible reasons for this to occur in a rearing facility. The first thing the farmer needs to do is to try to identify the source of this welfare issue. Questions that needs to be asked could be a) was the fish fully smoltified (smolt quality)? b) did this occur after transport to the sea-site (stress related)? If the farmer is able to find the likely source for this welfare issue, a correction of this will improve fish welfare in the cage by reducing numbers of emaciated fish. However, if the problem persists or even escalates, the farmer needs to undertake a secondary level of evaluation, which involves an active investigation of the fish. This stage involves handling a number of emaciated fish to assess its severity, which will give the farmer better quantitative data to make a better-educated decision regarding the welfare issue. If this is not enough and the measures taken by the farmer at the secondary level did not improve the welfare, expertise outside the farm may be required. This could involve autopsy and the sending of various samples to different laboratories and health personnel. It may also involve advanced remediation and treatment to correct the problem (see Figure 5.3-1) or in extreme cases the slaughter of the fish.

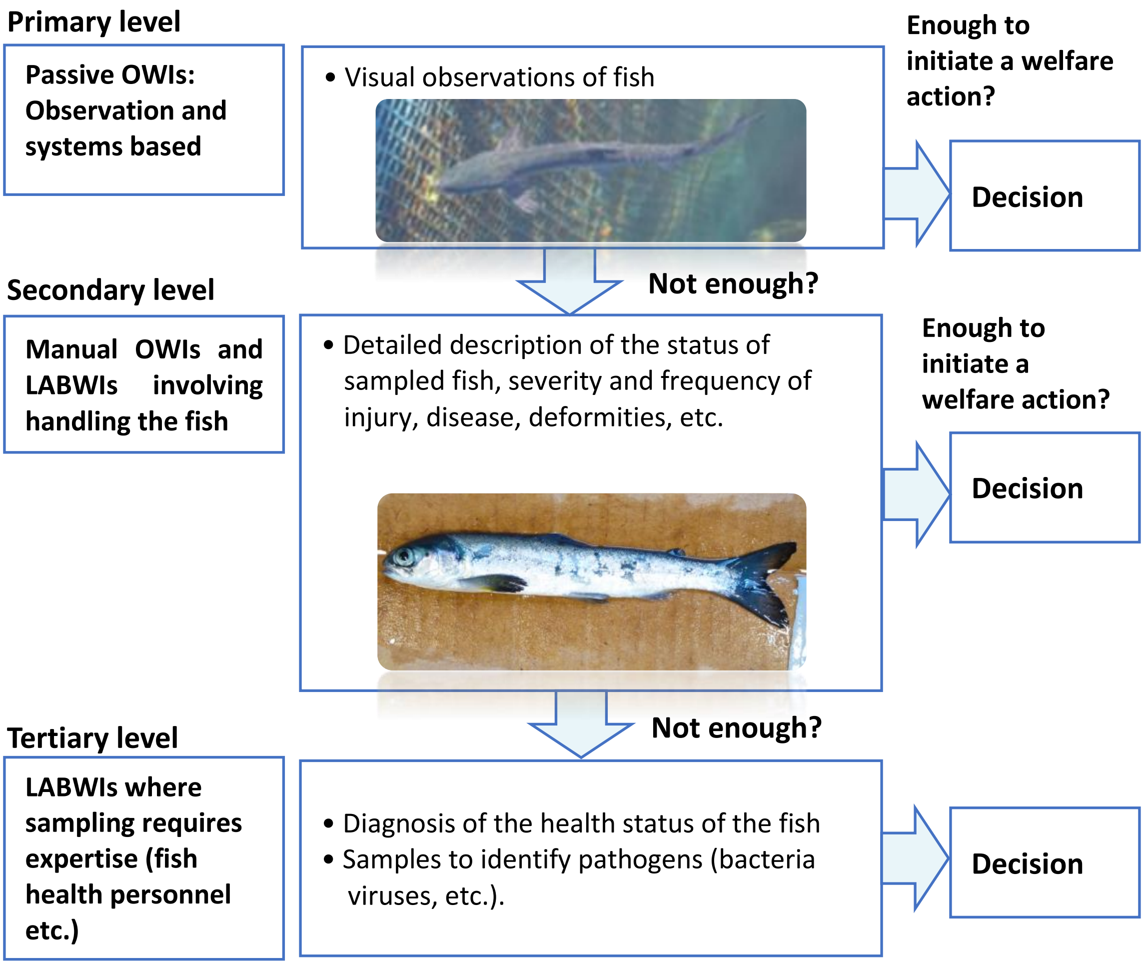


Fig. 5.3.1. *Application of OWIs and LABWIs at the farm as Early Warning Signals: example emaciation state (figure: C. Noble, photos: O. Folkedal)*

5.4. Future OWIs and LABWIs

In this handbook, we have tried to provide an overview of the welfare indicators that can be used for assessing the welfare of farmed Atlantic salmon. Despite the range of OWIs and LABWIs that are currently available to measure and evaluate fish welfare, others are under development or may be developed in the future.

There are a number of steps between the identification of a potential welfare indicator and its application on a farm. What steps do we need to take to turn an existing time consuming or specialist welfare indicator into a LABWI or an OWI? How do we turn some LABWIs into OWIs? How do we make some OWIs more fish- and user-friendly? What new welfare indicators are on the horizon e.g. the use of high throughput -omics techniques (e.g. genomics, proteomics or metabolomics)? Or the operational assessment of metabolic status or remote cardiac activity? Some very valuable individual based OWIs such as those involved in scoring external injuries or fish health still usually require the assessor to catch and handle the fish (and also potentially disturb other individuals during the capture process). This can impact upon the welfare of the fish being assessed and others in the rearing system. The fish may also have to be euthanized to collect samples or complete the analysis. Is there a way to make these processes passive and handling free? Technological advances in machine-based vision systems may mean fish welfare can be assessed and documented in real-time without the need for handling the fish.

Quantitative analysis of behavioural welfare indicators can also be complex and very time consuming. Non-invasive, passive vision- or acoustic-based monitoring systems could potentially monitor changes in fish behavior in real time, however, to the authors knowledge, they have not yet been developed to this level for fish. Telemetry based systems can also provide information on fish behaviour (e.g. evaluate the swimming activity of individual fish with biologgers) although they do involve tagging of the fish and can only monitor a small proportion of the population at present. It may be possible to further develop these technologies through multi-disciplinary researchers working with farmers. The algorithms developed by technologists may also identify factors that are indicative of welfare state that may not be immediately apparent to an observer. Existing, but infrequently used behavioural WIs such as the evaluation of the reflex status of the fish may also be further developed and made more farm friendly.

Physiological welfare indicators, such as glucose and lactate can be measured on the farm using hand-held instruments, although the physiological response to the stressor is not immediate. The further development of handheld meters for measuring other blood parameters could increase the number of physiological indicators that are suitable as OWIs, by making existing LABWIs suitable for use on farms. Other physiological WIs such as cortisol may become more robust for field assessment by assessing cortisol in e.g. the scales (see Part A, Section 3.2.16).

Any of these potential welfare indicators may be included in further editions of this handbook.

5.5. Overview of OWIs and LABWIs covered in Part A & used in Part B and C

What follows is a summary figure outlining all the WIs, OWIs and LABWIs that we have covered in Part A. This figure will be refined into tables in Part B: rearing systems and Part C: routines and operations to provide the farmer with fit for purpose OWIs and LABWIs for different farming situations.

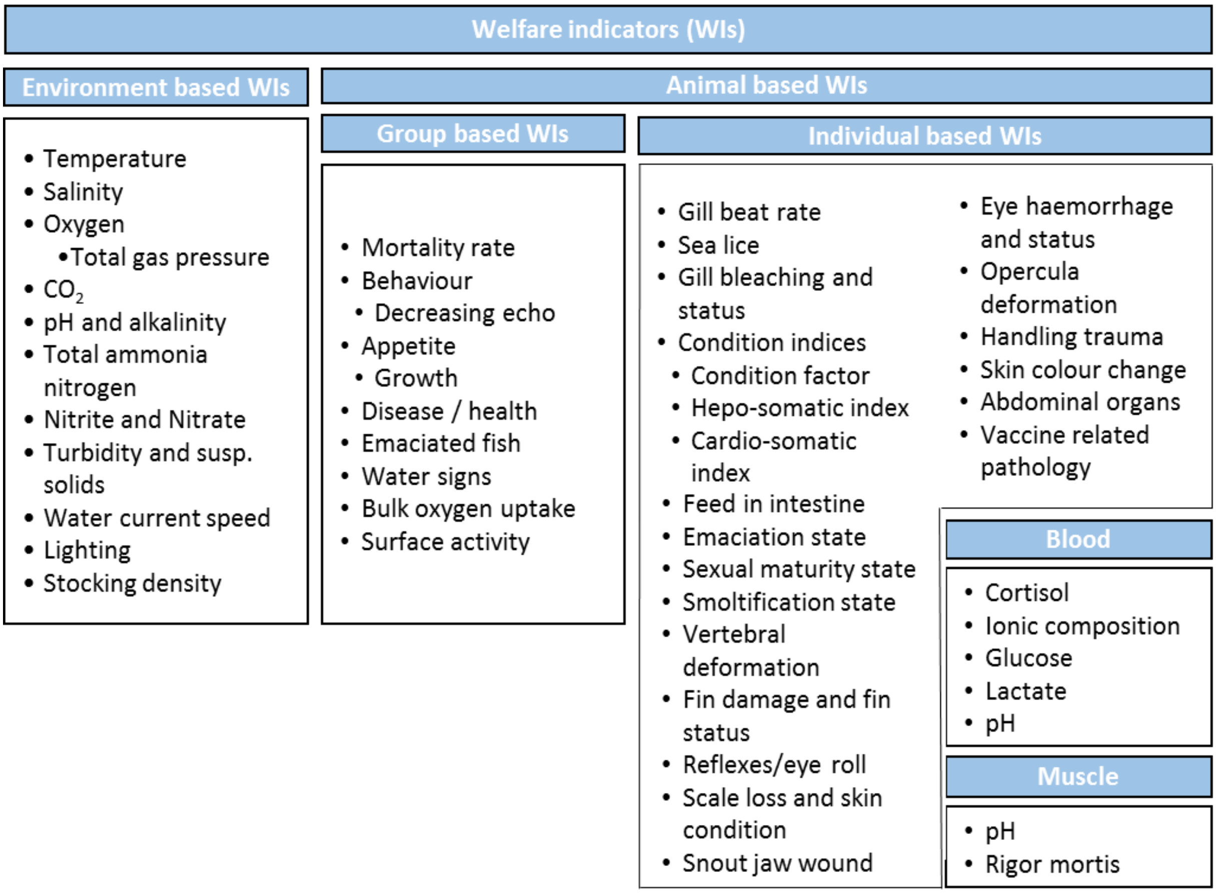


Fig. 5.5-1. *Summary of the WIs, OWIs and LABWIs covered in Part A of the handbook. Indicators are broken down into environment based and animal based WIs. Animal based WIs are further divided into group based and individual based WIs.*

6. Summary of scoring schemes

The following section is a summary of the scoring schemes used in this handbook.

This handbook suggests a unified scoring system (Tables 6.1-1, 6.1-2, 6.1-3) that is primarily aimed at farmers to help them assess welfare and rapidly detect potential welfare problems out on the farm. It is an amalgamation of the injury scoring schemes used in the Salmon Welfare Index Model (SWIM) (Stien et al., 2013), the injury scoring scheme developed by the Norwegian Veterinary Institute (NVI) (Grøntvedt et al., 2015; Gismervik et al., 2016) and also from other schemes developed by J. F. Turnbull (University of Stirling) and J. Kolarevic and C. Noble (Nofima).

Our suggested scheme standardises scoring for 14 different indicators to a 0-3 scoring system:

i) emaciation, ii) skin haemorrhages, iii) lesions/wounds, iv) scale loss, v) eye haemorrhages, vi) exophthalmia, vii) opercular damage, viii) snout damage, ix) vertebral deformities, x) upper jaw deformity, xi) lower jaw deformity, xii) sea lice infection, xiii) active fin damage, xiv) healed fin damage.

Pictures used in the system represent examples of each scoring category. We suggest dorsal, caudal and pectoral fins as the primary fins to monitor for fin damage. As a comprehensive system for the classification of vertebral deformities, similar to that in human medicine has not yet been developed for Atlantic salmon, we suggest a simplified scoring system similar to that used in the RSPCA welfare standards for farmed Atlantic salmon (RSPCA, 2018a).

Cataract damage is classified using an existing and widely used 0-4 scoring scheme (reproduced from Wall and Bjerkås, 1999), see Fig 6.2. The scoring method records the cataract area in relation to the entire lens surface (looking through the pupil along the pupillary/optic disc axis). You can quickly assess large numbers of fish with minimal equipment to get an impression of the severity of the problem. If possible, a selected number of fish should be inspected under darkened conditions (also with better equipment) to give some indication of position, type, development and aetiology. However, it does not record the density of the cataract which can be important and should be annotated separately (T. Wall pers. comm.).

The degree of vaccine side effects in individual fish is often evaluated according to the "Speilberg scale" (Midtlyng et al., 1996), see Table 6.3 and Fig. 6.4. The Speilberg Scale is widely used as a welfare indicator in the Norwegian aquaculture industry. The scale is based on a visual assessment of the extent and location of clinical changes within the abdominal cavity of the fish and it describes changes related to peritonitis; adhesions between organs, between organs and the abdominal wall and melanin deposits (see also Pettersen et al., 2014 and references therein). A Speilberg score of 3 and above is generally regarded as undesirable.

Table 6.1-1. *Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. (Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. Nilsson. Photos: K. Gismervik, L. H. Stien, J. Nilsson, C. Noble, J. F. Turnbull, P. A. Sæther, I. K. Nerbøvik, I. Simion, B. Tørud, B. Klakegg, R. Andersen, C. Karlsen, K. J. Merok, F. Gregersen)*

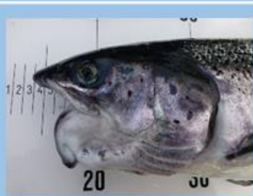
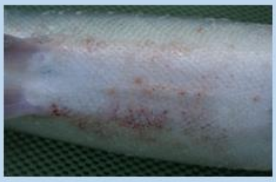
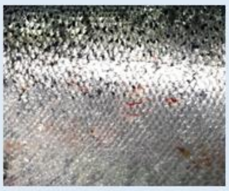
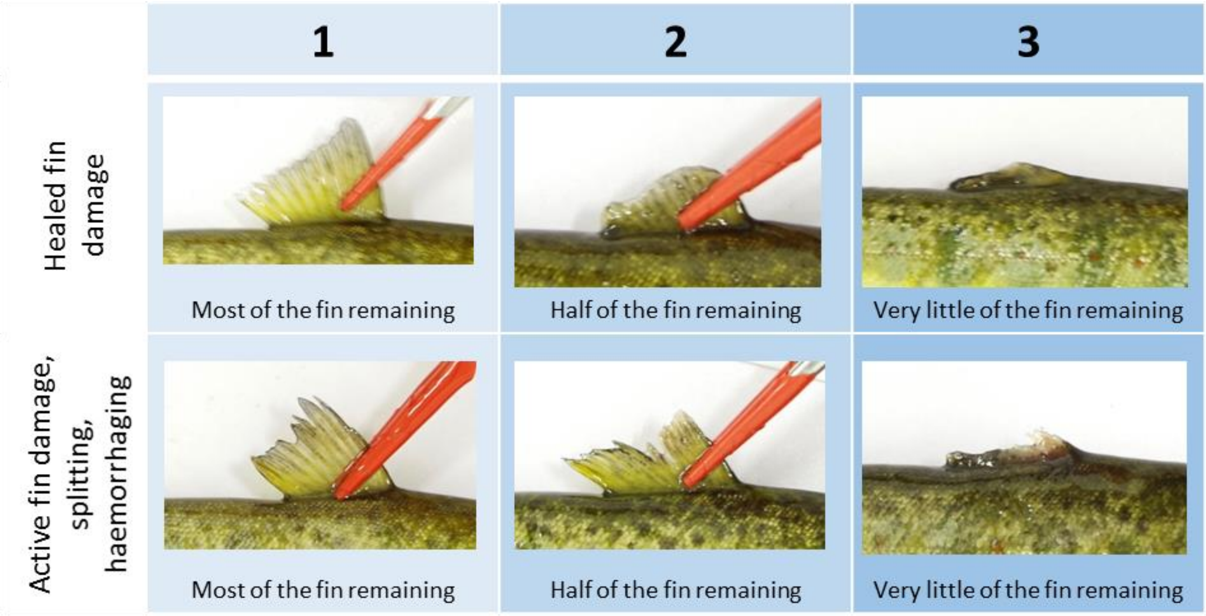
	1	2	3
Eye haemorrhage	 Minor haemorrhages	 Larger haemorrhages, or traumatic injury	 Large haemorrhages / traumatic injury. Eye may be ruptured
Exophthalmia	 Eye protruding a little	 Moderate eye protrusion	 Major eye protrusion
Opercular damage	 Operculum only partly covering gills	 Operculum absent on one of the gills (gill exposed)	 Both opercula absent (both gills exposed)
Snout damage	 Minor wound on snout (either jaw)	 Moderate wound and broken skin on snout	 Large deep and extensive wound. Can cover the whole head
Upper jaw deformity	 Suspected malformation	 Distinct malformation	 Major malformation, jaw pointing backwards
Lower jaw deformity	 Suspected malformation	 Distinct malformation	 Major malformation, jaw pointing backwards

***Table 6.1-2**. Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. (Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. Nilsson. Photos: K. Gismervik, L. H. Stien, J. Nilsson, C. Noble, J. F. Turnbull, P. A. Sæther, I. K. Nerbøvik, I. Simion, B. Tørud, B. Klakegg, R. Andersen, C. Karlsen, K. J. Merok, F. Gregersen)*

	1	2	3
Emaciation	 Potentially emaciated	 Emaciated	 Extremely emaciated
Vertebral deformity	 Signs of deformed spine	 Clearly visible spinal deformity (e.g. short tail)	 Extreme deformity
Skin haemorrhages	 Minor haemorrhaging, often on the belly of the fish	 Large area of haemorrhaging, often coupled with scale loss	 Significant bleeding, often with severe scale loss, wounds and skin edema
Lesions / wounds [1]	 One small wound (< 10 pence piece)[1], subcutaneous tissue intact (no muscle visible)	 Several small wounds	 Large, severe wounds, muscle often exposed (≥ 10 pence piece)
Scale loss	 Loss of individual scales	 Small areas of scale loss (< 10% of the fish)	 Large areas of scale loss (≥ 10% of the fish)
Sea lice infection	 Light infection	 0.05 - 0.08 pre-adult or adult lice cm^{-2} of fish skin	 ≥0.08 pre-adult or adult lice cm^{-2} of fish skin

[1] For pre-smolts "one small wound" should be < 1 cm. NB! Wounds that penetrate the abdominal cavity should be scored as a 3) irrespective of size

Table 6.1-3. *Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. It is important to differentiate between healed lesions and active lesions. Active lesions indicate an ongoing problem that needs to be addressed (Figure: J. F. Turnbull, C. Noble, D. Izquierdo-Gomez, L. H. Stien, K. Gismervik, J. Nilsson. Photos: J. F. Turnbull)*



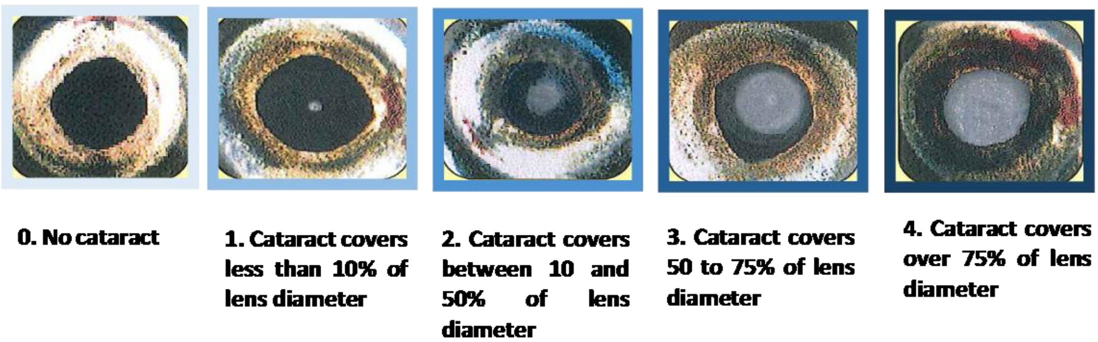


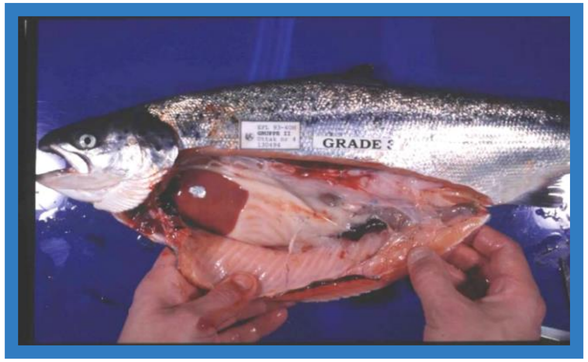
Fig. 6.2. *Morphological scheme for diagnosing and classifying eye cataracts in Atlantic salmon. Text reproduced from "Wall, T. & Bjerkås, E. 1999. A simplified method of scoring cataracts in fish. Bulletin of the European Association of Fish Pathologists 19(4), 162-165. Copyright, 1999" with permission from the European Association of Fish Pathologists. Figure: David Izquierdo-Gomez. Photos reproduced from "Bass, N. and T. Wall (Undated) A standard procedure for the field monitoring of cataracts in farmed Atlantic salmon and other species. BIM, Irish Sea Fisheries Board, Dun Laoghaire, Co. Dublin, Ireland, 2p." with permission from T. Wall.*

***Table 6.3**. The Speilberg Scale, reproduced from "Midtlyng et al., 1996, Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (Salmo salar L.) against furunculosis. Fish & Shellfish Immunology 6, 335–350. Copyright 1996" with permission from Elsevier.*

Score	Visual appearance of abdominal cavity	Severity of lesions
0	No visible lesions	None
1	Very slight adhesions, most frequently localized close to the injection site. Unlikely to be noticed by laymen during evisceration	No or minor opaquity of peritoneum after evisceration
2	Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall. May be noticed by laymen during evisceration	Only opasicity of peritoneum remaining after manually disconnecting the adhesions
3	Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall. May be noticed by laymen during evisceration	Minor visible lesions after evisceration, which may be removed manually
4	Major adhesions with granuloma, extensively interconnecting internal organs, which thereby appear as one unit. Likely to be noticed by laymen during evisceration	Moderate lesions which may be hard to remove manually
5	Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may carry focal, prominent and/or heavily pigmented lesions or granulomas	Leaving visible damage to the carcass after evisceration and removal of lesions
6	Even more pronounced than 5, often with considerable amounts of melanin. Viscera unremovable without damage to fillet integrity	Leaving major damage to the carcass

1. Very slight adhesions, most frequently localized close to the injection site. Unlikely to be noticed by laymen during evisceration.

2. Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall. May be noticed by laymen during evisceration.



3. Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall. May be noticed by laymen during evisceration.

4. Major adhesions with granuloma, extensively interconnecting internal organs, which thereby appear as one unit. Likely to be noticed by laymen during evisceration

5. Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may carry focal, prominent and/or heavily pigmented lesions or granulomas

6. Even more pronounced than 5, often with considerable amounts of melanin. Viscera irremovable without damage to fillet integrity.

***Fig. 6.4**. The Speilberg Scale for intra-abdominal lesions after intraperitoneal vaccination of Atlantic salmon. Photos provided and reproduced with kind permission from Lars Speilberg. Text reproduced from "Midtlyng et al., 1996, Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (Salmo salar L.) against furunculosis. Fish & Shellfish Immunology 6, 335–350. Copyright 1996" with permission from Elsevier.*

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Welfare Indicators for farmed Atlantic salmon: Part B – Fit for Purpose OWIs for different production systems

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Contents

1 Flow-through aquaculture systems

1.1 Rearing Atlantic salmon in flow-through systems on land

This section will outline which OWIs and LABWIs are fit for purpose for land-based intensive flow-through (FT) aquaculture systems. Traditional FT systems are single-pass, meaning the water only passes through the culture system once and is then discharged. The flow of water through the rearing system supplies fish with oxygen and carries dissolved and suspended wastes out of the system. Source water is taken from a river, lake or groundwater wells, circulated through the farm and usually treated before being released back to the aquatic environment. Additional oxygenation of the water is also used. All water in the farm is renewed at least once per day [1]. The majority of Atlantic salmon life stages are produced in FT systems on land (from eggs to post-smolts).

1.2 Challenges to fish welfare

Some of the potential challenges for fish welfare in FT systems are related to biosecurity, water availability, fluctuations in environmental variables and husbandry operations.

Environment:

- **Water supply** in FT systems determines the biomass that can be produced while maintaining all critical water quality parameters. A minimum water exchange rate of 10L m^{-3} tank volume/minute has been suggested [2]. The quality of the intake water (temperature, pH, metal content, particulate content etc.) may change with season and this can affect fish welfare. It is therefore crucial to document and follow changes in the quality of intake water over time to prevent any potential adverse effects on fish health and welfare.
- **Inadequate oxygenation.** Oxygen is the primary water quality indicator that can limit the production of Atlantic salmon in FT systems. This is mainly due to the high oxygen demand and oxygen consumption of salmon in the system, relatively low oxygen solubility in water and a limited supply of dissolved oxygen in the water [2]. In all modern aquaculture facilities, oxygen is added to support the intensity of biomass production. The addition of oxygen should be adapted to any biomass or increase in biomass in the system. Failure to do so might create hypoxic conditions that in time can affect the salmon's growth and welfare. However, the addition of oxygen can create oxygen supersaturated water (> 100% O_2 saturation). In FT systems where specific water flow can be low and where metabolites can accumulate (for example CO_2 and TAN), oxygen supersaturation can lead to decreased ventilation rate and respiratory acidosis. A rapid reduction in the available dissolved oxygen (DO) can lead to metabolic alkalosis and can rapidly impact upon blood pH [3]. Mortality can occur after e.g. the failure of an oxygen supplementation system, or following a transfer of fish from a farm that utilises pure oxygen supplementation, or after 12-24h transport under high levels of DO [3] due to a rapid reduction in available oxygen.
- **CO_2 concentrations.** Ambient dissolved CO_2 concentrations are primarily a consequence of fish metabolism within the FT systems [4] although background CO_2 levels in intake water can also play a role [5]. High concentrations of CO_2 can have a negative effect upon fish production, health and welfare when held in FT systems, but the exact effects depend upon the specific conditions of the system (see [5] and references therein). For Atlantic salmon smolt production in Norway, the legislative limit is 15 mg L^{-1} and maintaining CO_2 concentrations within this limit can be a challenge for many land based FT systems. For example, a water quality survey of 96 water sources of Norwegian smolt production systems showed that 30% of the facilities had average CO_2 concentrations above recommended values [6]. The issue is particularly related to the systems where water aeration has been replaced by the injection of pure oxygen into the intake water, which is a much more effective way of maintaining optimal O_2 levels and

enabling intensive production and associated increases in CO_2. The lack of water degassing, low water exchange rates or background CO_2 concentrations in the intake water (1-2.5 mg L^{-1}; [7]) will lead to the accumulation of CO_2 in the rearing water. In soft Norwegian waters with low alkalinity, the accumulation of CO_2 can lead to a quick reduction of water pH which increases the risk of metal toxicity (for example aluminium toxicity), which in turn can lead to a decrease in blood oxygen carrying capacity and reduced growth [2]. The installation of different CO_2 stripping units within traditional FT systems is an effective welfare action to militate against the risk of high CO_2 upon fish welfare. Whilst initial outlay for the stripping systems may be costly, this investment may pay off in the longer term due to gains in fish performance and production efficiency [5].

- **Water current speed** in tanks used for rearing juvenile Atlantic salmon is usually determined by the amount of water available for exchange [6], self-cleaning requirements and tank oxygenation [8]. Limited access to water can therefore make it difficult to meet the fish's biological requirements for water velocity. The adjustment of water velocity to provide fish with the benefits of e.g. optimal swimming conditions and training is therefore not one of the main requirements during production in FT land based systems. However, velocity can be increased by concentrating and directing the inflow water.
- **Metals,** particularly aluminium and iron, have been known to cause chronic or episodic toxicity problems. A combination of low pH and aluminium is particularly harmful to smoltifying salmon and sub lethal exposure to this metal can increase the susceptibility of salmon to lice infestation [6]. The toxicity of iron is dependent on oxidation of Fe (II) to Fe (III), which is affected by temperature, pH and ionic strength [9]. Both metals can be toxic when fresh water with dissolved metals is mixed with seawater [2]. There are three methods used to treat potential aluminium toxicity: i) the limited addition of seawater, ii) the addition of silica or iii) a combination of both. Iron can be oxidised with oxygen or ozone during an extended retention period [6].

Biosecurity:

- Biosecurity is the exclusion of potential infectious agents and is essential for good health and welfare. Biosecurity risks are common to most production systems with risks being posed by the fish, intermediate hosts and equipment. However, in FT systems there is also the risk of water bringing infectious agents for farmed or wild populations of fish. Each site should have a detailed biosecurity plan coordinated with other users of the water source.

Rearing operations:

- **Monitoring of the environment** on a daily basis can provide necessary insights and knowledge into the environmental parameters that can affect the welfare of Atlantic salmon. The most important water quality parameters that are monitored are oxygen and temperature, while periodical measurements of salinity (where fresh water is supplemented with sea water) and pH are also recommended.
- **Handling** in FT systems includes crowding, pumping, sorting, vaccination and handling in relation to transport. Handling procedures can cause stress and can lead to mechanical injuries and a greater susceptibility to infection. For more information about effect of these procedures on welfare, see Part C of this handbook.

1.3 Operational Welfare Indicators

There are three main groups of OWIs for FT systems: Environment based OWIs, animal group based OWIs and individual based OWIs. The OWIs discussed below refer to Atlantic salmon parr/smolt and post-smolts (Figure 1.3-1).

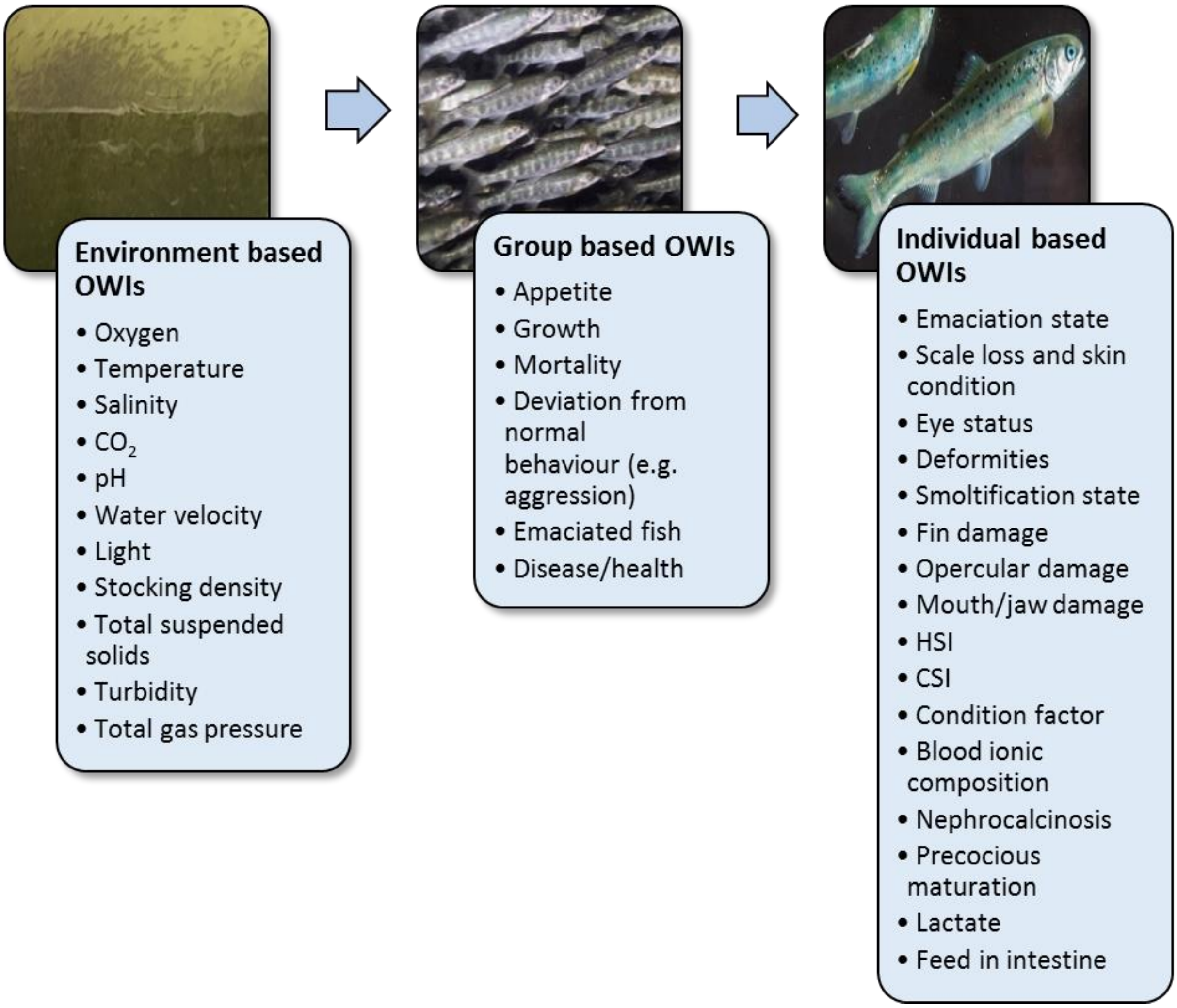


Figure 1.3-1. *Overview of OWIs suitable for flow-through land-based systems. Environment based OWIs address the rearing environment, group based OWIs address the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration Jelena Kolarevic, Frank Gregersen and Terje Aamodt*

1.4 Environment based OWIs

Recommended safe levels of key water quality parameters from the Norwegian Food Safety Authority are given in Table 1.4-1, while relevant life stages for the main environmental OWIs are given in Table 1.4-2.

Table 1.4-1 *Recommended safe levels of key water quality parameters issued by the Norwegian Food Safety Authority* *https://www.mattilsynet.no/fisk_og_akvakultur/akvakultur/drift_av_akvakulturanlegg/vedlegg_til_horingsbrev__vurdering_av_vannkvalitet_etter__22.22205/binary/Vedlegg%20til%20h%C3%B8ringsbrev%20-%20vurdering%20av%20vannkvalitet%20etter%20%C2%A7%2022*

Water quality parameter	Limits
pH (inlet)	6.2 – 7.8
Dissolved oxygen (O_2)	Max. 100 % saturation in tank and 80% in outlet
Carbon dioxide (CO_2)	< 15 mg L^{-1}
Total ammonia nitrogen (TAN = NH_4^+ + NH_3)	< 2 mg L^{-1} (at pH = 6.8, temperature 12^{O}C)
Nitrite	< 0.1 mg L^{-1} (freshwater)
Total organic carbon (TOC)	< 10 mg L^{-1}
Aluminium	< 5 µg L^{-1} (labile) & < 20 µg g^{-1} (gills)

Oxygen is the most important water quality parameter that can limit production in FT systems. Both hypoxia [10] and hyperoxia [11, 12] may cause severe welfare problems in salmon. Oxygen requirements can differ between life stages and are also affected by other factors such as temperature and salinity. The most important factors that will determine oxygen use are body size, temperature, stress, activity (swimming, feeding) and life stage. The recommended optimal oxygen saturation in Atlantic salmon is >80% [13] and feed utilisation and growth can be compromised at lower saturations. Data also suggest that a minimum of 85% air saturation and possibly up to 120% is needed to achieve maximal growth performance in Atlantic salmon [14].

- For eggs, an oxygen saturation above 66% at a temperature >12.5^{o}C and velocity of 100cm h^{-1} gives good survival [15].
- For parr, a minimum O_2 saturation to maintain aerobic metabolism is 39% at 12.5^{o}C [16].
- For post-smolts at 7 and 19^{o}C the minimum O_2 saturation is 24% and 40% respectively [17].

In the majority of cases oxygen is added automatically prior to entering the fish tanks to maintain oxygen saturation >80% (optimal welfare conditions [13]), however saturation at the point of entry into the tank can often be well above 100%. In places where DO-controllers with online probes are used to maintain oxygen at the desired saturation, it is very important that probes are regularly cleaned and calibrated.

Temperature. The optimal temperature for rearing Atlantic salmon can be wide and differs between different life stages. For example, the upper critical range can be between 20-34^{o}C [18] and the lower lethal temperature is around -0.7^{o}C [19]. The optimal temperature for eggs is 4-8°C [20], for growth in parr is 12-14°C, whilst post-smolt fish prefer temperatures around 17^{o}C [21]. Temperatures between 12-13°C help preserve good welfare and health and possibly reduce incidence of sexual maturation in Atlantic salmon parr and post-smolts [22, 23]. High temperatures, especially in the early stages of the salmon's life, can lead to vertebral deformities. Ytteborg et al., [24] documented a high degree of vertebral deformities in fish exposed to 10°C during egg rearing and 16°C during first feeding, compared with fish exposed to 6°C during egg rearing and 10°C during first feeding. Similarly, a study by Bæverfjord [25] also showed that juveniles raised at 18°C did not manage to utilize this elevated rearing temperature for growth and experienced a depression in growth rate.

Salinity is specific for life stages, with fry and parr being grown in fresh water and smolts and post-smolts in seawater or brackish water. For fry and parr, a salinity between 0-10 ‰ is recommended [26]. Smolts show preference for the halocline (<20 ‰ in top layer of water column and >30‰ below; [27]) and can benefit from access to brackish water (28‰ [28]) in particular when water temperature is low (4°C). Post-smolts prefer 33-34 ‰ [13] while brood stock should be subject to a salinity <10 ‰ during the final phases of maturation.

Carbon dioxide is a concern particularly for fresh-water life stages in FT systems. Its solubility decreases with increasing temperature and salinity and salmon's sensitivity to CO_2 may be different for different life stages [29]. There is evidence that the toxicity of CO_2 increases when O_2 saturation is low and also at lower temperatures and low pH (reviewed by Thorarensen and Farrell [14]). The negative effects of CO_2 on salmon are summarized in Part A, section 4.1.4. There are some indications that parr are more tolerant to CO_2 than smolts [29], but in general, concentrations of CO_2 below 15 mg L^{-1} are recommended for use in aquaculture production systems for Atlantic salmon.

pH is problematic for land based FT facilities in Norway where the pH of intake water can be below 6. Such conditions can be very harmful for Atlantic salmon due to the increased toxicity of metals, in particular aluminium in an acidic environment. An increase in pH is achieved by the addition of either seawater, lime or silicate [2]. However, the addition of seawater can compromise biosecurity within the system and the treatment of seawater with filters and UV are important. In addition, seasonal oscillations in pH and metal concentrations in the intake water can occur and the dosing of the chemicals should be adjusted accordingly. Regular pH measurements and historical data would allow for better management of the dosing system. In addition, in Norwegian soft waters with low alkalinity, changes in pH can happen very fast and can have negative effect on the welfare of salmon. pH also decreases as a result of increased CO_2 accumulation in the rearing water, so an appropriate water exchange level is needed to ensure the water has low levels of CO_2. For Atlantic salmon the optimal pH is between 6.5-6.7, while a pH of 5 is limiting and below 5 is not acceptable [2].

Water velocity in tanks is affected by water flow (hydraulic retention time, HRT), by the construction of the inlet and outlet and the presence of fish in the tanks. It can have positive effect on welfare as it exercises the fish, but too high or too low velocities can also have negative effects on health, welfare and performance.

- A velocity of 100 cm h^{-1} has been suggested to ensure good survival of eggs in hatching batches [15].
- The maximum sustained speed for parr with body lengths between 7-13cm at temperatures between 12.5 and 19°C is between 6-10 BL s^{-1} [30].
- The absolute critical swimming speed for salmon smolts is 64-109 cm s^{-1} and this increases with body length and temperature [31].
- The absolute persistent swimming speed for salmon smolt is 50 cm s^{-1} [31, 32].
- It has been reported that velocities of 1.2-1.5 BL sec^{-1} are favourable [33].
- For post-smolts welfare might be negatively affected at long-lasting velocities of 1.5 BL sec^{-1} [34, 35].
- Large changes in water velocity can have a negative effect on fish and a velocity that is too high can result in reduced survival after a pathogen challenge [36].

Light The optimal light quality (intensity and wavelength) for the optimal performance and welfare of Atlantic salmon parr and smolts reared in FT systems is still unclear. However, a study by Handeland et al., [37] reported that an intensity of greater than 43 lux is required to optimise welfare and smolt quality. Inadequate lighting conditions can lead to non-synchronous smoltification and poor smolt quality. Smolt production can involve the use of photo-manipulation to induce the smoltification process. In large deep tanks on land, light placement should be considered in order to ensure optimal light quality.

KNOWLEDGE GAP: The optimal light conditions (both light intensity and light quality) in land-based FT systems is unknown. In addition, the light conditions during smoltification in large commercial rearing tanks is not well documented.

Stocking density is not specified for land-based production and in flow-through systems, it will be limited by type of oxygenation, CO_2 removal, water flow and the size of the fish. The effect of different stocking densities is summarized in part A, chapter 4.2.3.

Table 1.4.1-2 *Environment based operative welfare indicators appropriate for use in FT aquaculture systems.*

OWI	Relevant life stage
Temperature	Egg, fry, parr and smolt. Especially critical during first feeding
Oxygen	Egg, fry, parr and smolt
Velocity	Egg, fry, parr and smolt
pH	Fry, parr and smolt
CO_2	Fry, parr and smolt
Stocking density	Fry, parr and smolt

Turbidity is a measure of water clarity. Increased turbidity prevents observation of fish in the tanks and can potentially have an effect on feeding. Turbidity can also effect water quality as water with high turbidity has less dissolved oxygen.

KNOWLEDGE GAP: The optimal turbidity levels for salmon parr, smolts and post-smolts are not specified.

Total suspended solids (TSS) can be described as the mass of suspended material (both organic and inorganic) above 1 µm in diameter that are found in a known volume of water [38]. Suspended solids contribute to oxygen consumption, biofouling and the formation of sludge deposits and fine suspended solids can have negative effect on gill health and function compromising oxygen transfer and providing habitat for growth of pathogens [38].

KNOWLEDGE GAP: optimal TSS levels for salmon parr, smolts and post-smolts are not specified (also dependent on the type of solids).

Total gas pressure (TGP) and nitrogen supersaturation. According to Hjeltnes et al., [33] "*supersaturation occurs when the partial pressure of one or more of the gases dissolved in the water becomes greater than the atmospheric pressure. Sudden increases in temperature, decreases in pressure, or excessive oxygenation, are all typical causes of gas supersaturation in aquaculture systems*." The temperature increases can be e.g. due to the mixing of water with different temperatures in the tank, and sudden changes in pressure can be e.g. due to weather changes and ice in the source water. Total gas pressure in water is used not only to determine the total pressure in water but also to determine the amount and saturation rate (%) of the dissolved nitrogen in the water. If nitrogen saturation exceeds 100%, earlier work has stated fish can develop gas bubble disease [39]; however, the same authors also state TGP is more important than nitrogen saturation alone [39].

The first external symptoms of exposure to gas supersaturation begin to be visible several hours after exposure and are typically "*bubbles on the fins, tail, opercula and head*" [33]. Their severity is closely linked to percentage supersaturation, the O_2: N_2 ratio and exposure time e.g. [33]. It seems that fry are more vulnerable than adult fish.

With regard to nitrogen supersaturation, negative effects have been observed on the fish at nitrogen saturations above 102% in Atlantic salmon and rainbow trout, [40], and Lekang [40] recommended that N_2 is kept below 100.5%. Wedemeyer [41] also states that N_2 saturation in intensive production systems should be below 110%. Since there is little data and a lot of uncertainty about salmon's tolerance to nitrogen supersaturation, we recommend using the above values as guidelines and not as absolute limits. As the risk of nitrogen supersaturation increases by adding seawater to freshwater, or in spring floods and under severe weather conditions, total gas pressure should be monitored regularly.

However, as stated above, nitrogen may just be one of a multitude of factors that can impact upon the welfare of fish subjected to gas supersaturation and that more focus should be paid to TGP than nitrogen saturation [39]. According to the Norwegian Food Safety Authority, TGP should not be higher than 100%. As there is still a lot of confusion regarding this, it is important to look at TGP and nitrogen supersaturation with regard to gas bubble disease.

KNOWLEDGE GAP: There is a lot of uncertainty about the upper tolerance limits of total gas pressure (TGP) and nitrogen supersaturation in salmon and more knowledge is needed (also see Knowledge gap box at the end of Part A section 4.1.7, Nitrite and nitrate of this handbook)

How to measure water quality (WQ) in FT:

- Monitor continuously by using in-line probes or by point measurements using hand-held instruments, lab equipment and kits and accredited labs
- Monitor at the same time point in relation to the light and feeding conditions in the FT system
- Measure at the same place in the FT system every time
- The correct sampling method is essential
- Follow procedures from the accredited labs
- Plot trends and use active interpretation of the situation
- The proper maintenance of equipment, especially of in-line probes that are exposed to biofouling is essential
- Make sure you know which nitrogen compound is measured by each method (TAN, NO_2-N or NO_2, NH_4^+-N or NH_4^+, NH_3-N or NH_3)

1.5 Group based OWIs

Appetite is a robust, passive OWI for tank rearing and can be an early warning signal for potential welfare problems [42]. Loss in appetite in FT systems can be qualitatively assessed by visually monitoring the feeding behaviour of the fish (poor feed reaction, or even rejection of feed pellets when offered) and can also can be measured by monitoring feed waste [43] and should be monitored continuously. Appetite can be suppressed by i) poor water quality and environmental conditions [44], ii) husbandry routines and choices [45] and iii) outbreaks of disease [46] amongst a multitude of other factors. It can also vary widely within and between days. This variability, in addition to the high number of factors that can impact upon appetite and feeding can make it difficult (and undesirable) to recommend specific daily feed amounts. However, rejection of pellets and low appetite may also mean that fish are satiated (or overfed) or being fed at a time when they do not want to eat, so this must also be considered when using appetite as an OWI. Life stage is also an appetite influencing factor for salmon.

Mortality has to be recorded on a daily basis. Efficient systems for the collection of dead fish from each tank are a prerequisite for the monitoring of fish performance in aquaculture systems. The increase in the size of tanks and a potential inability to visually observe the bottom of the tanks can prove challenging for the accurate daily registration of dead fish. If possible, the cause of mortality should be noted and dead fish are often preserved for further analysis and inspected by fish health personnel.

Growth may be affected by several factors, such as nutrition and diseases, social interactions [47], water quality and chronic stress [48], and may be quantified as e.g. specific growth rate (SGR) and/or thermal growth coefficient (TGC). Using growth rate as an OWI depends upon a good, representative sample of the fish. As stated above, long-term growth rates vary based on the season, life stage, production system and diet. Therefore, it may be better to use acute changes in growth rate as an OWI within a specific rearing unit or system. Acute changes in growth can be used as an early warning system for potential problems, particularly when the farmer has robust growth monitoring practices.

Behaviour. Deviations in behaviour may be an early warning of suboptimal conditions [49]. Behaviour is a general indicator and deviations may be caused by many different factors. Altered swimming behaviour may be because of suboptimal velocity [50], water quality [11] or other stress factors. Aggression can be a problem in salmon parr [51] and can be qualitatively or quantitatively monitored by visual observation of the fish. A better, less labour intensive proxy for monitoring aggression is to note the number of fish with dorsal fin damage, as there is a clear correlation between biting and dorsal fin damage in salmon parr [51]. Abrupt changes in the number of fish with grey thickened dorsal fins can easily be detected by eye when observing fish in FT systems and can be used as an early warning for welfare problems. Changes in the levels of dorsal fin damage are most likely related to inappropriate feeding regimes or underfeeding [51, 52, 53], although other factors may play a role (e.g. handling, water velocity).

Prevalence of emaciated fish. Emaciated fish are often found near the surface, isolated and often around the periphery of the group. They are most notable during the later freshwater stages and early seawater stages. Emaciated fish or “Losers” are fish with stunted growth that are most likely moribund and should be removed during the grading process or any other handling procedure if possible. These fish can experience low welfare for a long time before they die and they can also be a vector for transmitting diseases to other healthier fish [13]. The occurrence of these moribund or emaciated fish should be monitored [13] and any changes in the frequency of their occurrence should be acted upon as a very early warning OWI.

Disease/health status (OWI and LABWI) is followed on a regular basis by fish health personnel to determine the prevalence of certain conditions within the population and the potential causes of mortality or morbidity. Final diagnostics often entail tissue sampling and off site analyses (therefore classified as a LABWI) but some of the external signs of disease or conditions that pose a welfare risk can also be diagnosed on farm by experienced personnel and can lead to a quicker response to potential disease outbreaks. The overview of diseases characteristics for both fresh water and seawater stages of Atlantic salmon are given in Part A, section 3.1.5 of this handbook.

Table 1.5-1 *Group based OWIs appropriate for use in flow-through aquaculture systems*

OWI	Relevant life stage
Appetite and feeding behaviour	Fry, parr and smolt
Growth	Fry, parr and smolt
Mortality	Fry, parr and smolt
Behaviour (swimming, aggression)	Fry, parr and smolt
Emaciated fish	Parr and smolt
Disease / health status	Fry, parr and smolt

1.6 Individual based OWIs

Individual based OWIs and their relevance for different life stages are stated in Table 1.6-1.

Morphological welfare indicators of Atlantic salmon smolts and post-smolts can be examined in FT systems without killing the fish. It is recommended that a number of welfare indicators are followed throughout the production cycle in FT systems, such as fin damage, skin status, eye damage, opercula status, condition factor, vertebral deformities and mouth/jaw wounds.

Emaciation state. Relevant for later freshwater and seawater stages. “Losers” are fish with stunted growth that are most likely moribund and should be removed during the grading process or any other handling procedure if possible during freshwater phase. “Loser” fish are easily recognizable based on their external appearance (thin with low condition factor) and specific behaviour (swimming at the surface).

Scale loss and skin condition. The presence, severity and frequency of scale loss and epidermal damage and wounds should be regularly monitored, especially as the fish approach smolt transfer.

Eye status. Eyes are very vulnerable to mechanical trauma, leading to haemorrhages or desiccation during handling. Exophthalmus (“pop eye”) is often a non-specific sign of disease while cataract or loss of transparency of the eye lens can be caused by number of factors and is more frequent in later life stages, such as smolts and post-smolts. An overview of the different types of eye damage and their effects on fish welfare is included in Part A, section 3.2.12 of this handbook.

Mouth/jaw wounds can occur in relation to handling procedures (crowding, pumping, netting; see Part C of this handbook for more information) or because of contact between the fish and the walls of the tank.

Vertebral deformities. Vertebral deformities may be due to malnutrition [54] or temperature [24] amongst other factors. See Fjelldal et al., [55] for more detailed information and Part A, section 3.2.9 of this handbook.

Opercular damage includes shortening, lack of opercula, warped opercula and “soft” opercula. It is particularly applicable to early life stages in the fresh water phase and can be caused by suboptimal rearing conditions and dietary deficiency.

Fin damage. The effects of fin damage upon welfare are both fin- and life stage specific and the risks can differ according to the life stage of the fish. For example, in parr, the loss of pectoral fins can reduce their station-holding capacity [56]. There is a clear relationship between biting and dorsal fin damage in parr [51]. In smolts and post smolts, active fin damage can subject the fish to osmotic stress [57].

Scoring schemes for i) emaciation, ii) skin haemorrhages, iii) lesions/wounds, iv) scale loss, v) eye haemorrhages, vi) opercular damage, vii) snout damage, viii) vertebral deformities, ix) upper jaw deformity, x) lower jaw deformity, xi) sea lice infection, xii) active and healed fin damage, xiii) cataracts and xiv) the Speilberg scoring scheme for intra-abdominal lesions after the intraperitoneal vaccination of Atlantic salmon are provided at the end of this document.

Smoltification state or adaptation to sea water is evaluated prior to seawater transfer by measuring plasma Cl^- concentrations (111-135 mmol L^{-1} in fresh-water, increasing to 130-160 mmol L^{-1} in post-smolts), condition factor (decreases during smoltification), morphological indicators (silver colour, parr marks and dark fin edges), sodium potassium ATPase (NKA; Na^+ K^+ ATPase) activity/gene expression (increases in the fish, and at approximately 10 µmol ADP/mg prot*t, the fish is smoltified). Smolts tend to swim higher in the water column than parr and a small subsample of individuals taken from the upper part of water column to test smoltification status could underestimate the presence of individuals that are not completely smoltified. Morphological changes related to smoltification can be scored according existing operational scoring schemes e.g. https://www.pharmaq-analytiq.com/sfiles/75/1/file/v6_prosedyre_010601_vurdering_av_smoltindeks.pdf

Blood ionic composition. The composition of ions in blood plasma changes between parr in freshwater and seawater adapted smolts. Normal values in freshwater parr are reported to be between 130-150 mmol L^{-1} Na^+, 111-135 mmol L^{-1} Cl^-, 2.9 mmol L^{-1} K^+, 0.9-1.5 mmol L^{-1} Mg^{2+} and 2.7 mmol L^{-1} Ca^{2+}. Corresponding values in seawater adapted salmon are 140-175 mmol L^{-1} Na^+, 135-160 mmol L^{-1} Cl^-, 3.4 mmol L^{-1} K^+, 1.6-2.0 mmol L^{-1} Mg^{2+} and 3.3 mmol L^{-1} Ca^{2+}.

Feed in the intestine. Feed in the intestine is often an indicator that the fish have eaten in the last 1-2 days [58, 59] but this depends on fish size and temperature. It is easy to check euthanised fish for the presence of feed in the stomach and intestine.

Organ indexes address the relationship between an organ size compared to body size, and may be correlated with welfare (see Part A, section 3.2.5 for more information). Most commonly measured indexes are hepatosomatic index (HSI) - the relationship between liver and body size and cardio somatic index (CSI) – the relationship between heart and body size.

Condition factor (K) is a well-established indicator of the nutritional status of fish and is calculated as 100 x body weight (g) x body length $(cm)^{-3}$ (See Part A, 3.2.5 and references therein). The condition factor for parr should be between 1.0 – 1.3. A condition factor below 0.9 is indicative of emaciation. Condition factor decreases during smoltification, is around 1 for smolts and it increases with fish size in sea.

Precocious maturation. Salmon can mature in freshwater before smoltification (early maturation) or after seawater transfer. Early sexual maturation at the parr stage occurs only in males and inhibits smoltification and thus seawater tolerance. It is also associated with increased aggression. Changes in the activity of various hormones associated with reproduction, such as sex hormones, cortisol and growth hormone, may affect the immune system of sexually mature fish. This can result in increased disease susceptibility and a reduced health status (See Part A, section 3.2.7 of this handbook for more information).

Nephrocalcinosis is a pathology that has so far been related to high concentrations of dissolved CO_2 [60], which involves the formation of mineralized calcium deposits within kidney tissue that are visible to the eye or can be felt when cutting the kidney. This condition can be life-stage dependent as parr exposed to CO_2 levels > 30 mg L^{-1} for 47 days did not show any signs of nephrocalcinosis [29]. A scoring scheme for nephrocalcinosis is currently being validated and kidney morphology should be examined in cases when salmon has been chronically exposed to higher concentrations (>15mg L^{-1}) of dissolved CO_2 during commercial production.

Lactate increases with anaerobic muscle activity and should stay below 6 mmol L^{-1} [61]. It is easily measured with handheld apparatus, but samples should be taken approximately one hour after muscle activity (e.g. handling).

Table 1.6-1 *Individual Operative welfare indicators appropriate for use in flow-through aquaculture systems*

OWI	Relevant life stage
Fin, skin, eye, mouth, opercular damage	Parr and smolt
Smolt index	Smolt
Vertebral deformities	Fry, parr and smolt
Lactate	Parr and smolt
Emaciation state	Parr and smolt
Blood ionic compounds	Parr and smolt
Feed in intestine	Parr and smolt
Organ indexes	Parr and smolt
Condition factor	Parr and smolt
Maturation	Parr and smolt
Nephrocalcinosis	Parr and smolt

1.7 Welfare management scenario: Exposure of Atlantic salmon parr to oxygen supersaturation

Oxygen supplementation is the norm in intensive fresh water aquaculture but there are risks associated with too much oxygen. This scenario summarises an experiment examining the effects of oxygen supersaturation.

In this case study, previously published by Espmark and Bæverfjord [11] Atlantic salmon parr were exposed to normoxic (100% O_2 saturation) and supersaturated (150% and 175% O_2 saturation) water conditions for 25 days. There were other side effects to water quality related to supersaturation and the authors stated that "*fish exposed to 150 and 175% super oxygenated water produced higher levels of carbon dioxide with the subsequent decrease in water pH compared to control fish exposed to 100% O_2*" [11]. By day 7 the fish that were fish exposed to O_2 supersaturated water exhibited greater individual variability in swimming behaviour compared with fish from the control groups (Figure 1.7-1). The individual variation in behaviour decreased from day 7 to day 21. Fish exposed to O_2 supersaturated water also altered their feed consumption half way through the 25 days and exhibited poorer growth and different haematological parameters at day 21 of exposure. Fish subjected to supersaturated water exhibited reduced plasma chloride and decreased haemoglobin levels in relation to increased DO saturations. Plasma cortisol was only higher in the 150% oxygen supersaturation groups at sampling day 21 [11].

This scenario therefore shows that while it is essential that oxygen levels are maintained, oxygen supersaturation can have detrimental effects on welfare and performance.

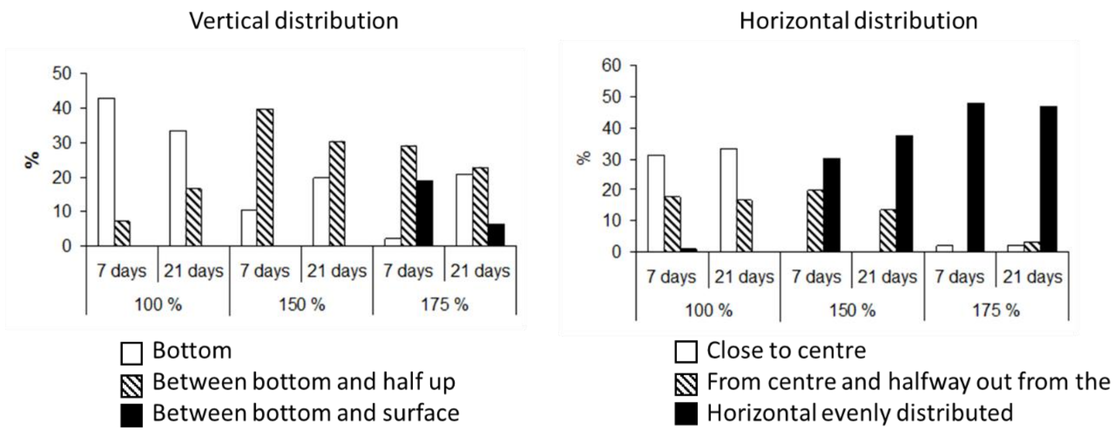


Figure 1.7-1. *Behaviour of salmon parr during exposure to oxygen supersaturation, measured on day 7 and day 21. Reproduced from "Espmark Å. & Bæverfjord G. (2009). Effects of hyperoxia on behavioural and physiological variables in Atlantic salmon (Salmo salar) parr. Aquaculture International 17, 341-353. Copyright 2009" with permission from Springer Nature.*

2 Recirculating aquaculture systems

2.1 Rearing Atlantic salmon in land-based Recirculating Aquaculture Systems

In recirculating aquaculture systems (RAS) water is (at least partially) reused after treatment and waste compounds are removed from the re-used water [62]. Water treatment in RAS typically consists of mechanical filters for particle removal, a biological filter for the nitrification of potentially toxic ammonia and nitrite-nitrogen into nitrate-nitrogen, carbon dioxide stripping and oxygenation of the water prior to return to the fish tanks (Figure 2.1-1). There will also be some form of water disinfection e.g. UV or ozone. In addition, automatic pH regulation, heat recovery and denitrification systems are components that can be added to increase efficiency of the system. RAS offers several advantages that appeal both to the aquaculture industry and society in general [63]. When compared with traditional flow-through systems, the water treatment process of RAS allows for a dramatic reduction in the requirement for new water intake [64]. In addition, environmental conditions may be more stable and controlled in RAS, disease management may be better [65] with enhanced biosecurity [66]. RAS systems may also reduce effluent loads allowing the farmer to meet stringent environmental requirements [62].

However, RAS technology may be vulnerable to a number of challenges, for example it may also be susceptible to failures due to the complexity of the system, it requires more complex management, increased investment and running costs and needs more skilled personnel [67]. In addition, as investment and running costs are higher than the more traditional production systems such as sea cages, production must be intensified to make the system economically viable [68]. This intensification may require farming the fish at higher densities, utilising higher water temperatures and supplemental oxygenation, which can lead to poorer water quality in some instances [14] and can have negative effect on fish welfare. In Norway, RAS were primarily used for smolt production until 2011, when post-smolts were allowed to be produced up to 1 kg in land-based facilities without restrictions on production volume. In 2016, the production of fish to slaughter size was also permitted.

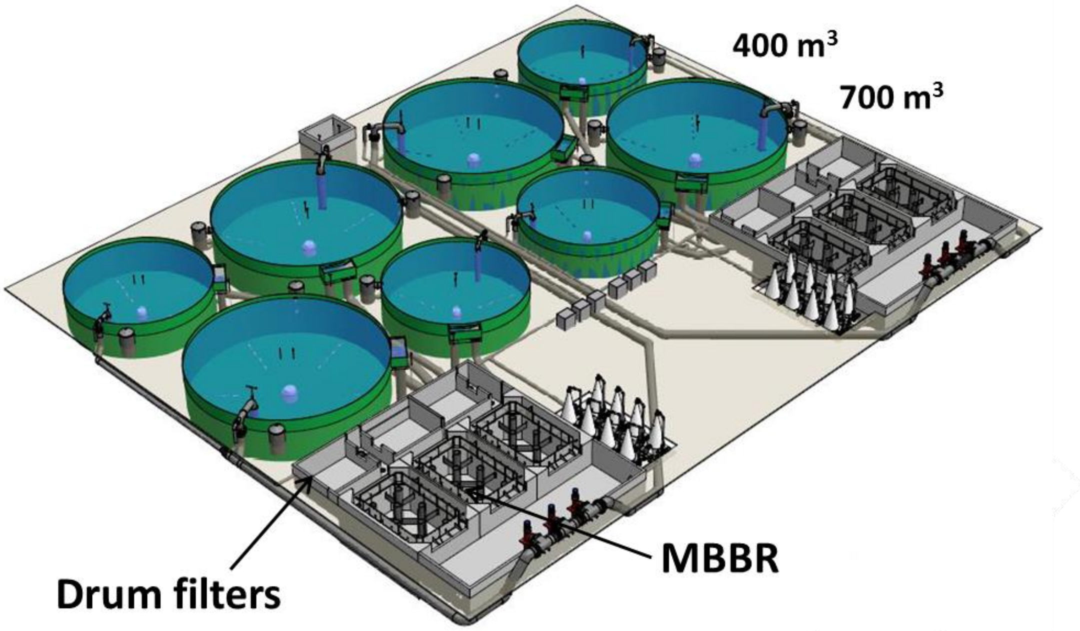


Figure 2.1-1. *Schematic overview of Grieg Seafood's commercial RAS facility at Adamselv with moving bed biofilters (MBBs), drum filters, oxygenation and two tank sizes, 400 and 700 m^3 (Figure: Frode Mathisen, reproduced with permission).*

2.2 Challenges to fish welfare

Environment:

- Flaws in the design and the construction of RAS as well as faulty operations present a high risk for fish welfare as water quality can be adversely affected [33].
- Realistic production plans must be used, taking into account the maximum feeding loads the system was designed for. This is to make sure the operator secures optimal conditions for both the fish and the bacterial community in the biofilters.
- The monitoring of biofilter activity is essential as any disturbance in the nitrification process can lead to an increased concentration of potentially toxic nitrogen compounds (ammonia and nitrite nitrogen) which can affect fish welfare.
- Although the use of RAS systems provides an opportunity for controlled production, long-term chronic exposure to suboptimal water quality could have subclinical and clinical effects on the fish making them more susceptible to diseases [33].
- The accumulation of heavy metals can also occur in RAS with low water exchange rates. Subclinical concentrations of copper (0.056 mg L^{-1}) have been associated with mortalities [69].
- Adequate monitoring of the RAS environment, well established operational routines, alarm systems and back-up systems in cases of emergency and well-trained personal can prevent or at least reduce potentially negative effects on fish welfare.

Biosecurity:

- Good biosecurity is prerequisite for the successful operation of RAS [33].
- The source of potential diseases are biological material (eggs and fish) and make-up water.
- It is difficult to eradicate diseases in RAS due to the effects any remedial treatments may have on the biofilter and its function [33].
- According to Hjeltnes et al., [33] the "*Segregation of different life-stages, an all-in and all-out procedure and disinfection of the production system are considered to be essential in fish health management*".
- Some of the pathogens found in RAS include the parasite *Ichthyobodo* sp. (*Costia*), fungus, *Yersinia* and Infectious Pancreatic Necrosis (IPN) [33].

Rearing Operations:

- Handling in RAS incudes crowding, pumping, sorting, vaccination and handling in relation to transport.
- Response to handling can be size dependent. Ytrestøyl et al., [70] reported that 450 g post-smolts were more sensitive to handling and seawater transfer compared with 250 g and 800 g post-smolts.
- The handling of large fish has to be carefully planned and the use of sedation and products containing PVP and EDTA should be used if netting is a part of fish transfer [23].
- For more information about effect of handling on welfare see Part C of this handbook.

2.3 Operational Welfare Indicators

There are three main groups of OWIs for RAS: environment based OWIs, animal group based OWIs and individual animal based OWIs. The OWIs discussed below refer to Atlantic salmon parr/smolt and post-smolts (Figure 2.3-1).

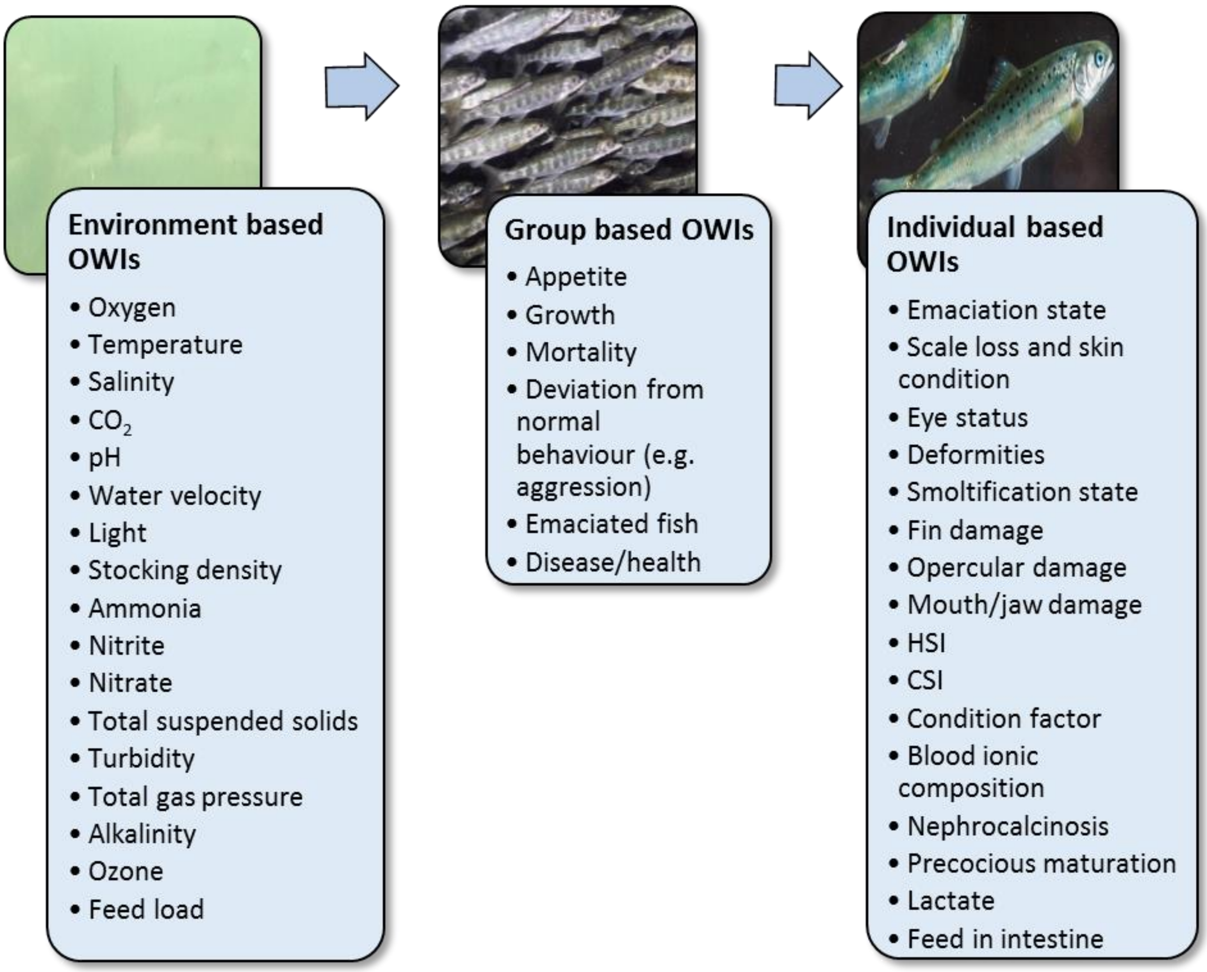


Figure 2.3-1. *Overview of OWIs suitable for recirculating aquaculture systems. Environment based OWIs address the rearing environment, group based OWIs describe the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration Jelena Kolarevic, Frank Gregersen and Terje Aamodt.*

2.4 Environment based OWIs

Water quality in RAS can differ from water quality in traditional flow-through systems (Table 2.4-1). In this chapter, the most important water quality parameters for monitoring of RAS will be mentioned, together with other environmental based indicators.

Table 2.4-1. *Illustration of difference in water quality between freshwater recirculating aquaculture system (RAS) and flow-through system (FT) during production of Atlantic salmon smolt (Kolarevic et al., 2014, reproduced with permission from Jelena Kolarevic). Water quality parameters are presented as tank means ± SD (n= 4 tank for each production system).*

Water quality parameters	**Production system**					
	FT			**RAS**		
pH	6.68	±	1.16	7.28	±	0.12
ΔH^+ (μmoL L^{-1})	0.15	±	0.09	0.04	±	0.02
Alkalinity (mg L^{-1})	17.0	±	1.7	48.0	±	6.6
CO_2 (mg L^{-1})	4.8	±	1.3	4.6	±	1.2
TSS (mg L^{-1})	0.7	±	0.3	3.4	±	1.2
Turbidity (NTU)	0.42	±	0.18	1.38	±	0.43
TAN (mg L^{-1})	0.2	±	0.0	0.3	±	0.1
NO_2-N (mg L^{-1})	0.01	±	0.00	0.06	±	0.04
NO_3-N (mg L^{-1})	0.46	±	0.04	22.73	±	3.43

Oxygen is the most important water quality parameter that requires continuous monitoring in intensive production systems. Both hypoxia [10] and supersaturation [11, 12] may cause severe welfare problems in salmon. However, the effects of oxygen supersaturation conditions in RAS are not well documented. It is the first limiting factor for increased carrying capacity and production in intensive RAS [38]. Oxygen requirements can differ between life stages and are influenced by other factors e.g. temperature and salinity. The most important factors that will determine oxygen use are body size, temperature, stress, activity levels and life stage. The recommended optimal oxygen saturation in Atlantic salmon is 80% [13] and feed utilisation and growth can be compromised at lower saturations. Data also suggest that a minimum of 85% air saturation and possibly up to 120% is needed to achieve maximal growth performance in Atlantic salmon [14].

- For eggs, an oxygen saturation above 66% at a temperature >12.5°C and velocity of 100 cm h^{-1} gives good survival [15].
- For parr, a minimum O_2 saturation to maintain aerobic metabolism is 39% at 12.5°C [16].
- For post-smolts at 7 and 19°C the minimum O_2 saturation is 24% and 40% respectively [17].

Using water aeration as a single source of dissolved oxygen is not enough to maintain densities used in RAS and therefore oxygenation systems that use pure oxygen are used. In the majority of cases oxygen is added automatically prior to entering the fish tanks to maintain oxygen saturation >80% (optimal welfare conditions [13]), however saturation at the point of entry into the tank can often be well above 100%. In places where DO-controllers with online probes are used to maintain oxygen at the desired saturation, it is very important that probes are regularly cleaned and calibrated. Emergency oxygenation systems in tanks are a necessity and should be checked weekly or monthly.

Water temperature. For detailed information about the general requirements and preferences of salmon in relation to temperature, see Part B section 1.4. With regard to RAS, if the system is located inside insulated buildings, water temperature can be > 5°C higher compared with the ambient water temperature (Figure 2.4-1; [71]) due to the heat released from e.g. friction in the pumps and pipes and bacterial activity in the biofilter [72].

- The use of the available temperature gain in RAS can be tempting as it could promote faster growth and a faster return on investment. However, higher temperatures in intensive production can have negative effect on fish welfare (see section 2.7) and can induce early sexual maturation.
- There is an increased risk of vertebral deformities when subjecting eggs and early life stages to freshwater rearing temperatures above 12°C [73].
- High temperatures in intensive RAS can often be a problem, especially in the summer months and adjusting the amount of cool intake water into the system can help regulate temperature in the system [72].
- Research shows that temperatures between 12-13°C in RAS help preserve good welfare and health and possibly reduce incidence of sexual maturation in Atlantic salmon [36, 70].

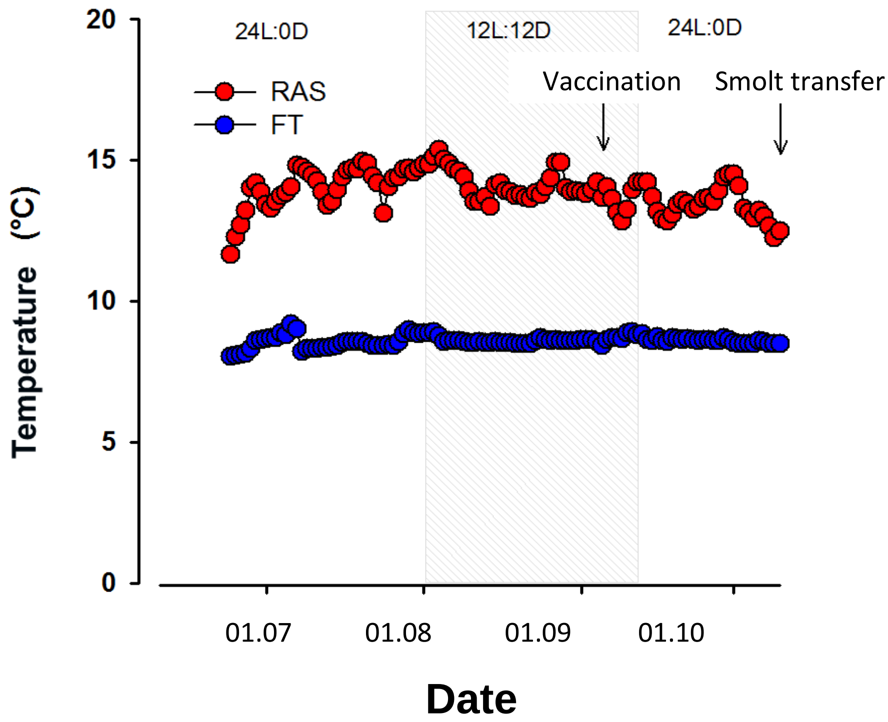


Figure 2.4-1 *Example of a temperature profile of RAS compared to a FT-system, where RAS make-up water comes from the same source as water used in the FT-system. No additional warming of RAS water was carried out (Kolarevic et al., [71], reproduced with permission from Jelena Kolarevic).*

Total ammonia nitrogen (TAN) /unionized ammonia (NH_3). Unionized ammonia is the primary nitrogenous waste product in salmonids (and the majority of teleost species) and it is toxic. It is excreted across the gills where it reacts with water and forms the ammonium ion (NH_4^+), or its ionized form which is less toxic. The proportion of toxic unionized ammonia to the far less toxic ammonium ion is dependent on pH, temperature and to a lesser extent salinity. The accumulation of TAN in the water will reduce the efflux of ammonia across the gills, resulting in elevated levels of TAN in the plasma of the fish [14].

- In properly designed RAS where the feed load accurately matches the carrying capacity of the system and where systems are operated at a pH, temperature and salinity that promotes optimal nitrification and formation of NH_4^+ and water retention times in the tanks allows removal of metabolites, ammonia should be kept at the desired low concentrations.
- If these conditions are not met, salmon in RAS can be exposed to acute ammonia toxicity which can have a short-term detrimental effect upon feeding and swimming activity, can increase ventilation rate, can lead to a loss of equilibrium and can also lead to death [14 and references therein]. Long term effects are reflected in poor growth performance, decreased robustness and fecundity [14 and references therein].
- Atlantic salmon have the capacity to adapt to stable chronic levels of ammonia without long-term effect on performance and welfare [74]. However, any sudden and frequent changes in ammonia concentration could be more demanding.
- RAS for salmon smolt production are built to maintain TAN concentrations < 2mg L^{-1} (at 12°C and pH 6.8-7.2) which is between 3-7µg L^{-1} of NH_3-N. These limits are recommended by Norwegian Food Safety Authority for the commercial production of salmonids and are based upon the results of experiments that were conducted under different circumstances that didn't necessarily focus upon aquaculture production [33]. The maximum safe levels of NH_3-N for salmonid aquaculture in the literature range from 0.012 - 0.030 mg L^{-1} NH_3-N [14].

KNOWLEDGE GAP: all studies have so far been done in flow-through systems and there is a need to verify the safe levels of NH_3-N in a RAS environment.

Carbon dioxide is the second most important and limiting water quality parameter (after oxygen) for intensive production in RAS as it can accumulate in water and be detrimental to fish welfare and performance. In RAS the CO_2 concentration of the water is controlled by the addition of strong bases not containing carbon (e.g. NaOH) or bases containing carbon (e.g. $NaHCO_3$) [38]. The addition of bases does not result in the removal of dissolved inorganic carbon from water but it keeps pH in a range where the majority of CO_2 in water is in the form of HCO_3^-. The active removal of CO_2 via gas exchange must be a part of the water treatment process in RAS. RAS-specific optimal CO_2 concentrations for smolts and post-smolts are still unknown.

KNOWLEDGE GAP: all studies have so far been done in flow-through systems and there is a need to verify the safe levels of CO_2 in the RAS environment.

Nitrite can be potentially toxic for Atlantic salmon in freshwater as it has a high affinity for the gill chloride uptake mechanism. If present in ambient water it can bind to chloride/bicarbonate (Cl^-/HCO_3^-) gill transporters instead of chloride ions [75]. This can lead to chloride depletion, can affect gas transport, ion regulation, and cardiovascular, endocrine and excretory processes, the formation of methaemoglobin and can reduce blood oxygen transport [75, 76]. The addition of chloride to freshwater can protect from adverse effects of nitrite toxicity and it is suggested that a 108:1 $Cl:NO_2$-N ratio should be used to protect Atlantic salmon parr [77].

KNOWLEDGE GAP: Currently the guidelines for Cl- requirements in relation to NO_2 concentrations are not specified by Norwegian Food safety Authority.

Nitrate is the end product of the nitrification process in biofilters. It is considered to be relatively harmless, but it is recommended that its concentration in the system should be kept < 100 mg L^{-1} [72]. In flow through systems nitrite is controlled by daily water exchanges, while in RAS systems, denitrification is necessary to prevent accumulation of nitrate in the system. In Norway, the majority of commercial RAS do not run with zero water exchange and nitrate is eliminated from the system by taking new water into the system.

pH in RAS has to be regulated to account for the nitrification process in the biofilter that causes the acidification of RAS water. In addition, pH controls solubility, reaction equilibria and the toxicity of metals and has to be kept at an optimal level. A common way to control pH is by dosing lime or sodium hydroxide to the system using an automatic dosage system regulated by a pH-meter that has a feed-back to a dosage pump [72]. The recommended inlet pH according to the Norwegian Food Safety authority is between 6.2 and 7.8. However, this pH range is still partly below the optimal pH that is necessary for nitrification, which can range from 7.0 to 9.0 [38]. It is advisable to manage pH in relation to the optimal pH of the nitrifying bacteria and keep the range near the lower threshold in order to limit any potential ammonia stress to the fish. Moreover, rapid fluctuations in pH of > 0.5 to 1.0 units will stress the biofilter, which will need time to adapt to new conditions, affecting the water quality in the system [38].

Turbidity is a measure of water clarity. Increased turbidity in RAS compared with flow-through systems is common, particularly if ozonation is not used. Increased turbidity hinders the observation of fish and feeding in the tanks. Turbidity can also effect water quality. Water with high turbidity levels has less dissolved oxygen.

KNOWLEDGE GAP: The optimal turbidity levels for salmon parr, smolts and post-smolts are not specified and are highly dependent on the type of substances that cause the turbidity.

Total suspended solids (TSS) can be described as the mass of suspended material (both organic and inorganic) above 1 µm in diameter that are found in a known volume of water [38]. Suspended solids in RAS water contribute to oxygen consumption, biofouling and the formation of sludge deposits and fine suspended solids can have negative effect on gill health and function, compromising oxygen transfer and providing a habitat for the growth of pathogens [38]. A definitive value for acceptable TSS concentrations in RAS has not been agreed upon [38]. However, it has been stated that TSS in RAS should be maintained at < 15 mg L^{-1} [14], microfines and colloids can also be negative for post-smolt welfare, health and performance in RAS [78].

KNOWLEDGE GAP: optimal TSS levels for salmon parr, smolts and post-smolts are not specified and are highly dependent on the type of substances resulting in TSS.

Alkalinity in raw water in Norway is low and the use of larger amounts of intake water washes out bases added to RAS. This increases the costs of maintaining the necessary alkalinity for proper nitrification and keeping pH stable within system [79]. The recommended alkalinity for producing Atlantic salmon smolts in RAS is around 70 mg L^{-1} [79].

Salinity directly affects a number of other water quality parameters (ammonia toxicity, dissolved oxygen etc.) as well as water treatment efficiency in the RAS, such as nitrification [80, 81] and CO_2 removal that decreases with increased salinity [82]. Salinity is particularly important for the production of post-smolts to prevent desmoltification and to insure that Atlantic salmon are ready for transfer to seawater. Ytrestøyl et al., [83] showed that using 12 ppt salinity for the production of post-smolts in RAS compared to 22 and 32 ppt leads to better water quality, better growth, FCR, survival, skin health and better filet pigmentation.

Water velocity in tanks is affected by water flow (hydraulic retention time, HRT), by the construction of the inlet and outlet and the presence of fish in the tanks. It can have positive effect on welfare as it exercises the fish, but too high or too low velocities can also have negative effects on health, welfare and performance.

- A velocity of 100 cm h^{-1} has been suggested to ensure good survival of eggs in hatching batches [15].
- The maximum sustained speed for parr with body lengths between 7-13cm at temperatures between 12.5 and 19^{o}C is between 6-10 BL s^{-1} [30].
- The absolute critical swimming speed for salmon smolts is 64-109 cm s^{-1} and this increases with body length and temperature [31].
- The absolute persistent swimming speed for salmon smolt is 50 cm s^{-1} [31, 32].
- It has been shown that swimming speeds of 1-1.5 BL sec^{-1} in RAS can provide good training and can have a good effect on growth for both smolts and post-smolts [36, 83]. However, post-smolt welfare might also be negatively affected at long-lasting velocities of 1.5 BL sec^{-1} [34, 35].
- Sudden changes in water velocity can have a negative effect upon the fish and a velocity that is too high can result in reduced survival after a pathogen challenge [36].

Stocking density in RAS must be supported by the system design, maximum daily feeding loads and efficient water treatment. Stocking density is not specified for land-based production and will be affected by oxygenation, CO_2 removal, water flow and the size of the fish. The effect of different stocking densities is summarized in part A, chapter 4.2.3. Recent results for parr in RAS under optimal water quality conditions suggest densities should be kept below 100 kg m^{-3}, particularly if higher temperatures (> 13°C) are used [36]. For Atlantic salmon post-smolts in RAS, densities over 80 kgm^{-3} in combination with higher CO_2 concentrations (between 20 and 30 mg L^{-1}) caused negative effects on growth, physiological and morphological welfare indicators [84].

Light conditions in RAS can vary between systems where biomass, feed loads, particle removal efficiency, tank size, shape, and the use of ozone can differ. Optimal light conditions (intensity and wavelength) for the optimal performance and welfare of Atlantic salmon smolts and post-smolts in RAS is unknown. Inadequate light conditions and the use of large rearing tanks with high fish densities during smoltification (closing in on 1000 m^3 in newly built RAS) can lead to non-synchronous smoltification and poor smolt quality. Continuous light in combination with higher water temperatures can also cause early maturation in post-smolts.

KNOWLEDGE GAP: The optimal light conditions (both light intensity and light quality) for pre- and post-smolts in RAS is unknown. In addition, the light conditions during smoltification in large commercial rearing tanks is not well documented.

Feed load is closely related to the production capacity of any RAS as it effects water quality in the system [33, 38]. It is recommended that the feed load should be kept relatively steady between days (inter-day variability should be below 10-15%) as acute variations can affect the nitrification efficiency of the biofilters [33, 85], leading to increased nitrite concentrations that can affect fish welfare. This can be a challenge for appetite based feeding as it is known that the day to day variation in appetite may vary by more than 15% [86].

Total gas pressure (TGP) and nitrogen supersaturation. For more information on TGP and nitrogen supersaturation see Part B, section 1.4. In brief, TGP should not be higher than 100% according to the Norwegian Food Safety Authority. However, in RAS with moving bed biofilters TGP can reach 105% due to the depth of the air injection.

KNOWLEDGE GAP: The effect of 100% vs 104-106% TGP on performance and welfare of salmon parr/smolt and post-smolt in RAS is unknown.

Ozone is used in RAS as a disinfectant or as a water treatment agent that improves the coagulation of fine particles. It also reduces levels of TSS, dissolved organic carbon, nitrite and heavy metals and improves the turbidity of the RAS water [87]. It allows the system to operate at lower exchange rates. However, as a strong oxidizing agent "*ozone may cause oxidative stress since the possible formation of reactive oxygen species may cause damage to certain biological molecules*" [33] and is therefore is a health risk for both humans and fish. Indicators that the fish have been exposed to toxic levels of ozone include changes in fish behaviour (fish "gasp" for air and congregate close to the surface, exhibit erratic swimming, darting behaviour) and reduced appetite (fish stop feeding). In the end, fish lose equilibrium and become pale [33]. The risks associated with long-term use of ozone could be one reason why it is not widely used by the industry.

How to measure water quality (WQ) in RAS:

- Measure continuously using in-line probes or by point measurements with hand-held instruments, lab equipment, kits and accredited labs
- Measure at the same time point in relation to the light and feeding conditions and at the same place in the tank
- The correct sampling method is essential
- Follow procedures from accredited labs
- Plot trends and actively interpret the situation
- The proper maintenance of equipment, especially of in-line probes that are exposed to biofouling is essential
- Make sure you know which nitrogen compound is measured by each method (TAN, NO_2-N or NO_2, NH_4^+-N or NH_4^+, NH_3-N or NH_3)
- Monitor biofilter nitrification efficiency
- Make sure you know what water quality the fish is experiencing
- In large tanks check that WQ in the tank is uniform by measuring WQ at different locations within the tank

2.5 Group based OWIs

Appetite is a robust, passive OWI for tank rearing, can be an early warning signal for potential welfare problems [42] and should be closely monitored in RAS. A reduced appetite with a potentially consequential increase in feed wastage can negatively affect the nitrification process in biofilters. The increase in organic matter in the system will promote the growth of heterotrophic bacteria and increase sludge formation. The existence of separators for solids on the side of each tank can help monitor appetite and can help adjustment of feeding load in the system. A loss of appetite in tank rearing systems can be qualitatively assessed by visually monitoring the feeding behaviour of the fish (poor feed reaction, or even rejection of feed pellets when offered, wasted feed on bottom of tanks) but this can sometimes be difficult if the water is turbid. Appetite can be suppressed by i) poor water quality and environmental conditions [44], ii) husbandry routines and choices [45] and iii) after an outbreak of disease [46] amongst a multitude of other factors. It can also vary widely within and between days. This variability, in addition to the high number of factors that can impact upon appetite and feeding can make it difficult (and undesirable) to recommend specific daily feed amounts. However, the rejection of pellets and low appetite may also mean that fish are satiated (or overfed) or being fed at a time when they do not want to eat, so this must also be considered when using appetite as an OWI. Ultimately, the ability to measure daily feed intake in RAS would allow for the optimization of feed management in the system.

Mortality has to be recorded on a daily basis. Efficient systems for the collection of dead fish from each tank are a prerequisite for the monitoring of fish performance in aquaculture systems. The increase in the size of tanks and a potential inability to visually observe the bottom of the tanks can prove challenging for the accurate daily monitoring of dead fish. If possible, the cause of mortality should be determined and recorded. Dead fish are often preserved for further analysis and inspected by fish health personnel.

Growth may be affected by several factors, such as nutrition and diseases, social interactions [47], water quality and chronic stress [48], and may be quantified as specific growth rate (SGR) and/or thermal growth coefficient (TGC). Using growth rate as an OWI depends upon a good, representative sample of the fish. As stated above, long-term growth rates vary according to fish strain, season, life stage, rearing system and diet etc., so it may be better to use acute changes in growth rate as an OWI within a specific rearing unit or system. Acute changes in growth can be used as an early warning system for potential problems, particularly when the farmer has robust growth monitoring practices. Growth of Atlantic salmon parr/smolt in RAS can match growth in flow-through systems [88].

Behaviour can be challenging to monitor in RAS, particularly when the fish are produced at high densities in large rearing tanks and where turbidity can be an issue. Turbidity is a challenge for camera based monitoring technologies and the use of new technologies, such as acoustic telemetry is showing potential for real-time monitoring of salmon swimming activity in RAS [63]. Passive observations of fish behaviour may only be applicable at the water surface and it may be difficult to monitor fish nearer the bottom of the large (up to 1000 m^3) commercial scale tanks. The use of ozone should improve visibility in the system, however in intensive culture systems it is still challenging to monitor the whole group in the tanks. Deviations in behaviour may be an early warning of suboptimal conditions [49], therefore it is important to know what is expected/normal behaviour in order to identify any deviations in behaviour. Behaviour is a general indicator and deviations may be caused by many different factors. Altered swimming behaviour may be because of suboptimal water velocity [50], or quality [11] and a wide range of other stressors. Aggression and biting can be a problem in salmon parr [51] and can be qualitatively or quantitatively monitored by visual observation of the fish. A better, less labour intensive proxy for monitoring aggression is to note the number of fish with dorsal fin damage, as there is a clear correlation between biting and dorsal fin damage in salmon parr [51]. Abrupt changes in the number of fish with grey thickened dorsal fins can easily be diagnosed by eye when observing fish in RAS systems and can be used as an early warning for welfare problems. Changes in the levels of dorsal fin damage are most likely related to inappropriate feeding regimes or underfeeding [51, 52, 53], although other factors may play a role (e.g. handling, water flow).

Prevalence of emaciated fish. Emaciated fish are often found near the surface, isolated and often around the periphery of the group and are most notable during the later freshwater stages and early seawater stages. Emaciated fish or "Losers" are fish with stunted growth that are most likely moribund and should be removed during the grading process or any other handling procedure if possible during freshwater phase. These fish can experience low welfare for a long time before they die and they can also be a vector for transmitting diseases to other healthier fish [13]. The occurrence of these moribund or emaciated fish should be monitored [13] and any changes in the frequency of their occurrence should be acted upon as a very early warning OWI.

Disease/ health status (OWI and LABWI) is followed on a regular basis by fish health personnel to determine the prevalence of certain conditions within the population and the potential causes of mortality or morbidity. Definitive diagnosis often entails tissue sampling and off site analyses (therefore classified as a LABWI) but some of the external signs of disease or conditions that pose a welfare risk can also be diagnosed on farm by experienced personnel and can lead to a quicker response to disease outbreaks. The overview of diseases characteristics for both fresh water and seawater stages of Atlantic salmon are given in Part A, section 3.1.5 of this handbook.

2.6 Individual based OWIs

Morphological welfare indicators of Atlantic salmon smolts and post-smolts can also be examined in RAS without killing the fish. It is recommended that a number of welfare indicators are followed throughout the production cycle in RAS, such as fin damage, skin status, eye damage, opercula status, condition factor, vertebral deformities and mouth/jaw wounds.

Emaciation state. Relevant for later fresh-water and seawater stages. "Losers" are fish with stunted growth that are most likely moribund and should be removed during the grading process or any other handling procedure if possible during fresh-water phase. "Loser" fish are easily recognizable based on their external appearance (thin with low condition factor) and specific behaviour (swimming at the surface) and should be removed from production when possible.

Scale loss and skin condition. The presence, severity and frequency of scale loss and epidermal damage and wounds should be regularly monitored, especially as the fish approach smolt transfer.

Eye status. Eyes are very vulnerable to mechanical trauma, leading to haemorrhages or desiccation during handling. Exophthalmus ("pop eye") is often a non-specific sign of disease while cataract or loss of transparency of the eye lens can be caused by number of factors and is more frequent in later life stages, such as smolts and post-smolts. An overview of types of eye damage and their effects on fish welfare is included in Part A, section 3.2.12 of this handbook.

Mouth/jaw wounds can occur in relation to handling procedures (crowding, pumping, netting; see Part C of this handbook for more information) or because of contact between the fish and the walls of the tank.

Vertebral deformities. Vertebral deformities may be due to malnutrition [54] or temperature [24] amongst other factors. See Fjelldal et al., [55] for more detailed information and Part A, section 3.2.9 of this handbook.

Opercular damage and gill status includes shortening, lack of opercula, warped opercula and "soft" opercula. It is particularly applicable to early life stages in fresh water phase and can be caused by suboptimal rearing conditions and dietary deficiency. Gill bleaching and gill status should also be monitored in relation to turbidity and TSS.

Fin damage. The effects of fin damage upon welfare are both fin- and life stage specific and the risks can differ according to the life stage of the fish. For example in parr, the loss of pectoral fins can reduce their station-holding ability [56]. There is a distinct relationship between biting and dorsal fin damage in parr [51]. In smolts and post smolts, active fin damage can subject the fish to osmotic stress [57].

Scoring schemes for i) emaciation, ii) skin haemorrhages, iii) lesions/wounds, iv) scale loss, v) eye haemorrhages, vi) opercular damage, vii) snout damage, viii) vertebral deformities, ix) upper jaw deformity, x) lower jaw deformity, xi) sea lice infection, xii) active and healed fin damage, xiii) cataracts and xiv) the Speilberg scoring scheme for intra-abdominal lesions after the intraperitoneal vaccination of Atlantic salmon are provided at the end of this document.

Smoltification state or adaptation to sea water is evaluated prior to seawater transfer by measuring plasma Cl^- concentrations (111-135 mmol L^{-1} in freshwater, increasing to 130-160 mmol L^{-1} in post-smolts), condition factor (decreases during smoltification), morphological indicators (silver colour, parr marks and dark fin edges), sodium potassium ATPase (NKA; Na^+ K^+ ATPase) activity/gene expression (increases in the fish, and at approximately 10 µmol ADP/mg prot*t, the fish is smoltified). Smolts tend to swim higher in the water column than parr and a small subsample of individuals taken from the upper part of water column to test smoltification status could underestimate the presence of individuals that are not completely smoltified. Morphological changes related to smoltification can be scored according existing operational scoring schemes e.g. https://www.pharmaq-analytiq.com/sfiles/75/1/file/v6_prosedyre_010601_vurdering_av_smoltindeks.pdf

Blood ionic composition. The composition of ions in blood plasma changes between parr in freshwater and seawater adapted smolts. Normal values in freshwater parr are reported to be between 130-150 mmol L^{-1} Na^+, 111-135 mmol L^{-1} Cl^-, 2.9 mmol L^{-1} K^+, 0.9-1.5 mmol L^{-1} Mg^{2+} and 2.7 mmol L^{-1} Ca^{2+}. Corresponding values in seawater adapted salmon are 140-175 mmol L^{-1} Na^+, 135-160 mmol L^{-1} Cl^-, 3.4 mmol L^{-1} K^+, 1.6-2.0 mmol L^{-1} Mg^{2+} and 3.3 mmol L^{-1} Ca^{2+}.

Organ indexes address the relationship between an organ size compared to body size, and may be correlated with welfare (see Part A, section 3.2.5 for more information). Most commonly measured indexes are hepatosomatic index (HSI) - the relationship between liver and body size and cardio somatic index (CSI) – the relationship between heart and body size. The cardio somatic index and hepatosomatic index are valuable indicators for both smolt and post-smolt, while the gonado-somatic index is in particular important for post-smolts due to potential early maturation issues.

Condition factor (K) is a well-established indicator of the nutritional status of the fish and is calculated as 100 x body weight (g) x body length $(cm)^{-3}$. The condition factor for parr should be between 1.0 – 1.3. A condition factor below 0.9 is indicative of emaciation. Condition factor decreases during smoltification, is around 1 for smolts and it increases with fish size in the sea.

Precocious maturation. Salmon can mature in freshwater before smoltification (early maturation) or after seawater transfer. Early sexual maturation at the parr stage occurs only in males and inhibits smoltification and thus seawater tolerance. It is also associated with increased aggression. Changes in the activity of various hormones associated with reproduction, such as sex hormones, cortisol and growth hormone, may affect the immune system of sexually mature fish. This is something that can result in increased disease susceptibility and a reduced health status (See Part A, section 3.2.7 of this handbook for more information).

Nephrocalcinosis is a pathology that has so far been related to high concentrations of dissolved CO_2 [60] that involves formation of mineralized calcium deposits within kidney tissue that are visible to the eye or can be felt when cutting the kidney. This condition can be life-stage dependent as parr exposed to CO_2 levels > 30 mg L^{-1} for 47 days did not show any signs of nephrocalcinosis [29]. A scoring scheme for nephrocalcinosis is currently being validated and kidney morphology should be examined in cases when salmon has been chronically exposed to higher concentrations (>15mg L^{-1}) of dissolved CO_2 during commercial production.

Lactate increases with anaerobic muscle activity and should stay below 6 mmol L^{-1} [61]. It is easily measured with handheld apparatus, but samples should be taken approximately one hour after muscle activity (e.g. handling).

Feed in the intestine. Feed in the intestine is often an indicator that the fish have eaten in the last 1-2 days [58, 59] but this depends on fish size and temperature. It is easy to check euthanised fish for the presence of feed in the stomach and intestine.

2.7 Welfare management scenario: Exposure of Atlantic salmon parr to high temperatures at high density

There are risks associated with farming at high stocking densities at high temperatures in RAS. This scenario summarises an experiment examining the effects of these two factors.

Atlantic salmon parr (~80 g) were stocked in tanks with RAS water at 30 and 60 kg m^{-3} and at either 13 or 15°C. The intention was to increase stocking density via fish growth at two different temperatures, to monitor and stabilize water quality and to terminate the experiment at any sign of adverse effects. All groups showed similar individual weight development until the high density groups reached 100 kg m^{-3}, when the first signs of depressed growth were observed for the two groups (Figure 2.7-1). Once the density reached 120 kg m^{-3} in the group that was kept at 15°C, feed intake stopped completely overnight and an acute change in behaviour was observed. Fish were vigorously swimming close to the water surface with dorsal fins protruding out of the water. This swimming behaviour was at first periodical, then more or less continuous. The change in behaviour was sequentially followed by mass mortalities (increase in cumulative mortality from 1 to 8%) that occurred 4-5 days after the first signs of the behavioural change [22]. Apart from slightly higher CO_2 concentrations (~16 mg L^{-1}) in the affected tanks, water quality was kept within optimal range of values and was not limiting factor. Fin damage and eye damage were significantly more prevalent in the affected group compared to other treatments, molecular stress markers were indicative of a stress response and branchial homeostasis was compromised. In addition, Atlantic salmon reared in the high density, higher temperature treatment had significantly larger livers and hearts, and the probable cause of mortality was acute stress leading to circulatory failure [36].

In order to avoid potential adverse effects of rearing a high densities at high temperatures a farmer should regularly monitor the suite of environmental and animal based OWIs recommended for RAS and act upon any indicators that suggest a potential problem.

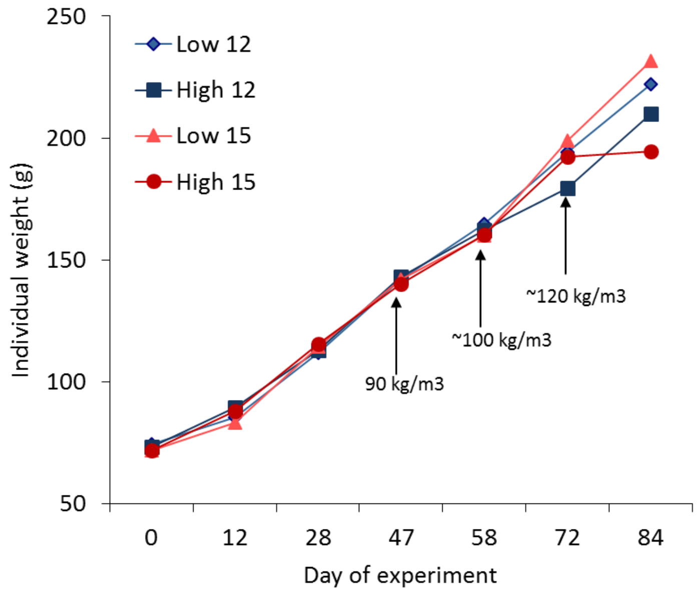


Figure 2.7-1 *.Individual weight of Atlantic salmon parr reared at two temperatures (13 and 15°C) and starting densities of 30 and 60 kg m^{-3} over time. The density of high density groups are indicated in the graph from 90 to 120 kg m^{-3} (Bæverfjord et al., [36]). Reproduced with permission from J. Kolarevic.*

3 Sea cages

3.1 Rearing fish in sea cages

The on-growing of salmon and trout in sea cages has been a huge success, both in terms of production and profitability. Each year more than 1.2 million tonnes, or 250 million farmed salmon and trout are harvested from Norwegian sea farms with a value of more than 40 billion NOK [Norwegian Directorate of Fisheries, http://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur]. Similar production techniques are also used in Australia, Canada, Chile, the Faroe Islands, Iceland, Ireland, Scotland and the U.S. An obvious advantage with rearing fish in sea cages is that natural water currents transport new water into the cages, replenishing oxygen, providing the fish with a natural flowing medium and removing feed particles and faeces. A typical Norwegian sea cage is 40 - 50 m in diameter and has a net that is 10 – 50 m deep (volume 16,000-130,000 m^3). In comparison with fish farmed in land-based tanks, with high fish densities and a relatively uniform water environment, salmon and trout in sea cages have a relatively high degree of freedom of movement and can move up and down within the cage to find their preferred water environment [27]. One of the main difficulties with farming in sea cages is that the farmers have little opportunity to improve the conditions when water quality is sub-optimal and it can also be difficult to treat the fish when they show signs of disease and reduced welfare. However, having a clear understanding of the current welfare state of the fish can guide the farmer when making decisions involving use of lice barrier skirt technology, handling the fish (e.g. de-licing), or postponing or hastening the slaughter of the fish. It can also help shape decisions on whether it is safe to bring in more fish to the site; if the existing fish show signs of reduced welfare or there is a risk of disease, these risks may also endanger the new fish.

3.2 Challenges to fish welfare

Challenging water environment: Smolts are typically transported to sea cages in well-boats and released via pipes into the cages. Here they must cope with a completely new environment and challenges and the first weeks after transfer are often associated with increased mortality [89]. Large losses can be experienced if the fish are sick, have been exposed to challenging transport conditions [90] or if some are not completely smolted. In Norway, salmon transferred to farms in the north of the country can be subject to long periods of very cold water, whilst those transferred to farms further south can be exposed to periods where water is too warm (> 18 °C, Johansson et al., 2006, 2009). The location of the farm, in a fjord on the coast or offshore, also affects the challenges the smolts face after transfer to the sea. The continuous flow of water through the cage means that the salmon have to cope with seasonal changes, due to tidal currents, freshwater runoffs, storms, upwelling and blooms of phytoplankton or zooplankton (see Fig. 3.2-1). Sea cages located in fjords can have strong vertical stratification of water quality and significant daily changes due to tidal currents. Severely hypoxic conditions (down to 30 % saturation) can occur for up to 1 h around slack water periods (Fig. 3.2-2). Coastal farms are usually subjected to water qualities that are relatively consistent but can also be subject to strong and variable water current speeds and upwelling of colder waters that have lower DO levels [27]. In deep fjords with a shallow threshold and poor water exchange, the deep water can even contain toxic hydrogen sulphide. Upwelling can occur in fjords during the winter when an influx of cold water causes the deep water to rise up, or during storms when strong winds push the surface water towards the shore, causing the deep water to rise from beneath.

Harmful organisms: Phytoplankton and zooplankton may cause periods of fluctuating turbidity and oxygen concentrations. For example, although phytoplankton produce oxygen during the day, both phytoplankton and zooplankton can be major consumers of oxygen during the night and can cause substantial depletion of oxygen within the cages (Fig. 3.2-1). Some phytoplankton or zooplankton can also damage the gills of the fish [91] and the influx of new water into the cage can expose the fish to other pathogens or harmful organisms such as viruses, bacteria, parasites or stinging organisms such as jelly fish. Although salmon are normally able to evade jellyfish, swarms of jellyfish can overwhelm the farm and result in high mortality rates [92]. In addition to bacteria and viruses, infectious stages of salmon lice are also a component of the zooplankton and a considerable welfare challenge to farmed salmon. Not only in that lice in large quantities can directly harm the fish, but also in that frequent delicing operations can be highly stressful and can lead to large proportions of the fish being injured or killed [89]. Another parasite that has become a major problem in Norway in recent years is the protozoan *Neoparamoeba* ***perurans*** that causes amoebic gill disease (AGD).

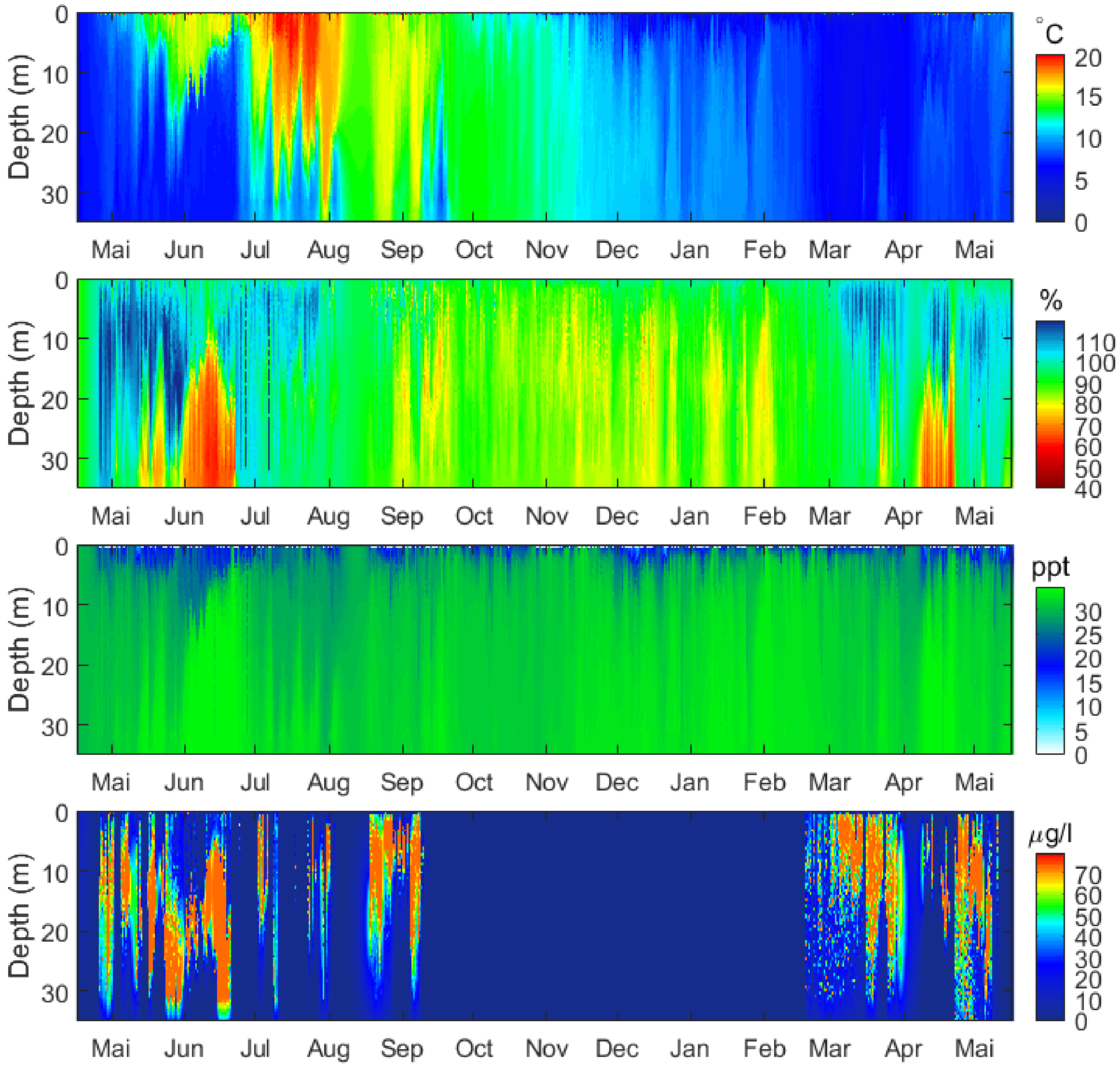


Figure 3.2-1*. Temperature (°C), oxygen saturation (%), salinity (ppt) and fluorescence (µg L^{-1})) measured in a fjord in Western Norway. Upwelling occurred in June and also in April-May, creating sudden and long lasting poor oxygen conditions below 10 m. High concentrations of phytoplankton (measured as fluorescence) in certain parts of the year with long days and high light levels are net producers of oxygen and may lead to oxygen supersaturation, whilst phytoplankton in September are net consumers of oxygen leading to decreased oxygen saturations (data: Kjetil Frafjord- Cargill Innovation). Figure Lars H. Stien, unpublished, reproduced with permission.*

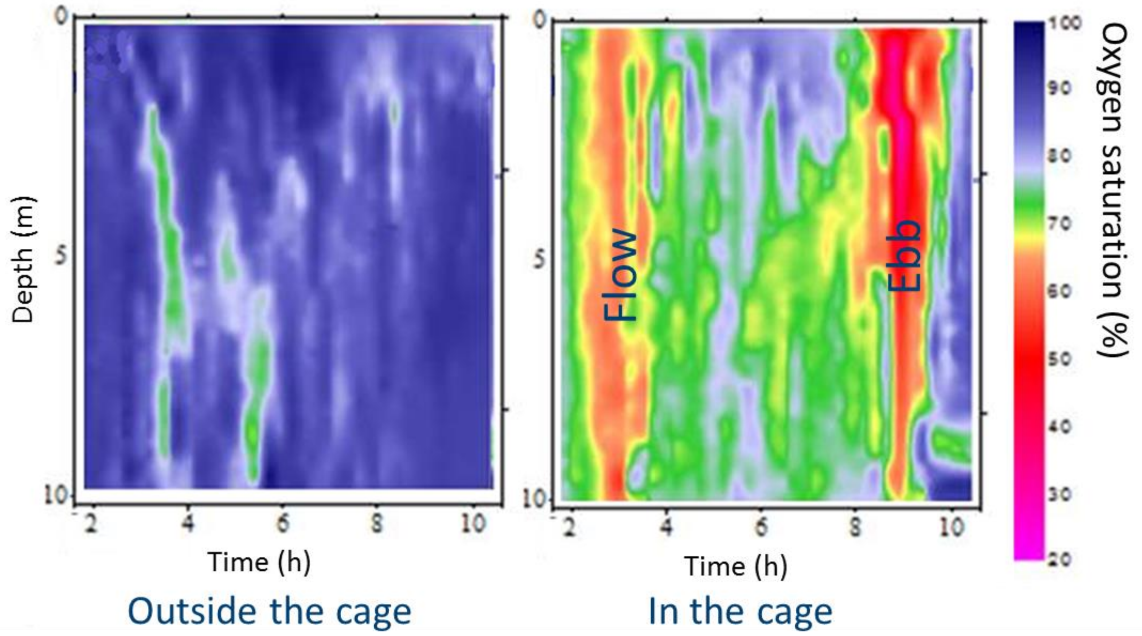


***Figure 3.2-2**. Example of hypoxic conditions inside a sea cage at slack water. Illustration adapted from [93].*

Potentially dangerous environment: Farming out in a natural environment can mean the salmon are vulnerable to predators such as seals and birds. In case of strong currents and insufficient weighting of the net, the net can become deformed, leading to a decreased net volume and potential pockets where the fish can become trapped.

Stressful handling operations: The fish can also be damaged and stressed during rearing operations such as cleaning or changing of nets, crowding, sorting, counting of lice and delicing operations. Wounds from handling can also be a route for infections to enter and their healing can be hindered by lice or cold water conditions, creating a long term welfare issue [94]. See Part C of this handbook for more information on fish welfare in relation to handling and other common husbandry operations.

3.3 Operational Welfare Indicators

There are three main groups of OWIs for sea cages: environment based indirect OWIs, animal group based OWIs and individual animal based OWIs (Figure 3.3-1).

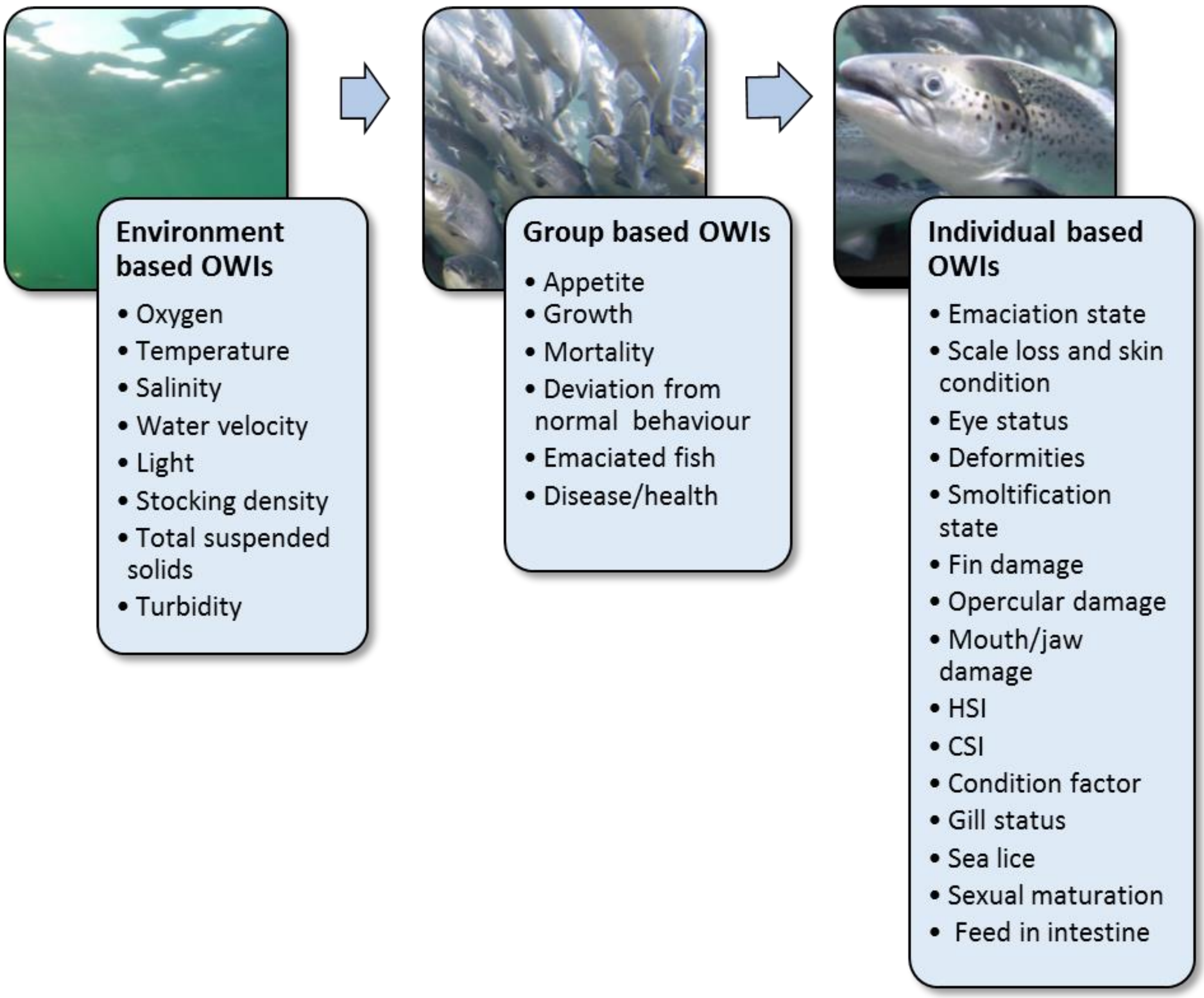


***Figure 3.3-1**. Overview of fit for purpose OWIs for sea cages. Environment based OWIs address the rearing environment, group based OWIs refer to the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration, Lars H. Stien.*

3.4 Environment based OWIs

Temperature is a major factor that influences the vertical distribution of Atlantic salmon held in sea cages [27]. Salmon held in sea cages can prefer temperatures around 17 °C and may actively avoid temperatures above 18 °C [21, 95]. Other studies have found the preferred temperatures to be about 12-14 °C (See Part A section 4.1.1 of this handbook for more information). Low temperatures (below 6-7 °C) are avoided by post-smolts, can have a negative impact upon growth and performance and increase the risk for winter ulcers. Post-smolts should therefore have access to temperatures above 6 °C and below 18 °C. Temperatures outside the preferred range will lead to a significant reduction in growth [96], but salmon can adapt to temperatures from 0 to 23 °C, provided that the oxygen levels are sufficiently high and that there is a gradual transition in temperature [97]. Both high and low temperatures are associated with higher risks for disease outbreaks and increased wound healing time [94, 98].

Oxygen levels within a sea cage depend on the saturation level of the surrounding sea water, how fast the current and fish activity replenishes the cage with new seawater and how much oxygen the fish or plankton inside the cage consume. Salmon increase their metabolic activity with temperature and therefore need more oxygen at higher temperatures. The lowest oxygen saturations that do not affect appetite at various temperatures are presented in Table 3.4-1 [17]. Even short periods of hypoxic conditions, e.g. due to the current standing still at slack water, can lead to physiological stress, reduced appetite and mortalities [13]. When adopting a cautionary approach, Stien et al., [13] stated that oxygen levels should be above 80% in sea cages for optimal rearing.

Temperature	DO_{maxFI}	LOS
7	42%	24%
11	53%	33%
15	66%	34%
19	76%	40%

Table 3.4-1. *Showing lower limits for oxygen saturation with maximal feed intake (DO_{maxFI}) and limiting oxygen saturation (LOS) for Atlantic post-smolts of 300-500 g [17].*

Salinity levels in Norwegian coastal waters are normally around 33 ‰, but sea cages located in fjords can be affected by freshwater runoff causing a halocline consisting of a brackish layer of varying thickness and salinity over water that has a normal salinity below (see Fig 3.2-1, [27 and references therein]). Salinity appears to have little effect on post smolt salmon [21, 95, 99] unless e.g. skin damage or disease impair their osmoregulatory ability [100, 101] and newly transferred fish may prefer brackish water. In addition, a layer of brackish water may give the fish an opportunity to treat themselves against AGD [102] and also to avoid salmon lice [103].

Turbidity and fluorescence are rarely used as welfare indicators in sea cages, but they can give an indication of the presence of plankton and the risk of sudden changes in oxygen saturation (Figure 3.2-1). Some types of particles in the water can also damage the gills of the fish making them vulnerable to infection and some algae and zooplankton are directly harmful to the fish [91]. High turbidity may also impede the farmer's ability to observe the fish and assess how the fish feed.

Water velocity is primarily an indirect WI. As water passes through the cage it replenishes oxygen and can flush out and dilute metabolites and particulate materials such as faecal matter and uneaten feed [13, 104]. Currents that are too high may hinder the fish's ability to maintain their position in the school and in extreme cases can lead to exhausted fish. The length of time that salmon are able to maintain fast swimming primarily depends on their general fitness, water temperature and size. The sustained swimming speed of salmon post-smolts are about 2 body lengths s^{-1} [32], but performance can also be negatively affected at 1.5 body lengths s^{-1} [34, 35].

Stocking density is more of a management practice (a farmer would use WIs and OWIs as assessment tools for deciding whether stocking density is appropriate for their fish) than a welfare indicator. It can be classified as an indirect WI, but this is under discussion. Further, it is dependent upon several variables including life stage, water quality, current speed, feed availability and feeding regime, rearing system and various other husbandry routines and practices [105]. However, there is little doubt that stocking densities that are either too low or too high can impair welfare [106]. Densities below the Norwegian limit of 25 kg m^{-3} are not believed to markedly affect fish welfare [13]. Stocking density in sea cages is therefore primarily an indirect welfare indicator in terms of that increased biomass inside a sea cage increases the risk of hypoxia in periods of high temperature and low water exchange and may make certain operations such as delicing more stressful and last longer. As the water flow will travel a longer distance and thus pass a higher biomass of fish when running through a large cage than a smaller cage, one should pay attention to oxygen saturations to the side of the cage that is leeward of the water current.

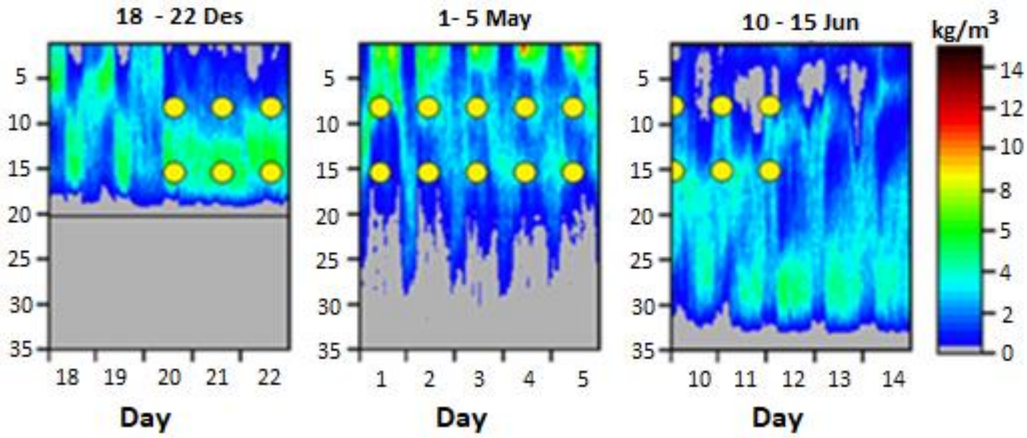


Figure 3.4-1. *Echo sounder data showing the vertical position of salmon in a sea cage at three different time periods [27]. The yellow circles indicate position of the lights. In the first period fish swim deeper during the day and swim closer to the surface at night until the lights are turned on and the fish swim deeper irrespective of time of day. In the second period fish swim near the surface during the day, but swim in deeper colder water at night, near where the lights are positioned. In the third period fish swim deeper, but close to the lights; when the lights are removed, water temperature is the main environmental driver and the salmon swim deeper. Adapted from "Oppedal, F., Dempster, T. & Stien, L. H. (2011) Environmental drivers of Atlantic salmon behaviour in sea-cages: A review. Aquaculture 311, 1–18. Copyright 2011." with permission from Elsevier.*

Light conditions in a sea cage vary with depth, time of day, weather and season. Under natural lighting regimes, salmon can school deeper during the day and ascend to the surface during dusk whilst also reducing swimming speed in relation to the declining light [27]. School structure can also breakdown as light levels fall [27]. Previous work has shown that cage-held salmon avoid the surface during bright daylight periods but can be attracted to underwater and surface lighting during nocturnal periods (Fig. 3.4-1, [27]). Salmon will also maintain diurnal swimming activity and behaviours under when subjected to nocturnal lighting conditions, although this can lead to high densities near the surface in some cases [107]. This can be circumvented by using underwater lights deeper in the cage (e.g. 15 m) so the salmon can distribute themselves both above and below the light [13, 27]. The use of high intensity lights for 4-6 months from midwinter can decrease the frequency of sexual maturation, while their use during the autumn has an opposite effect and induces sexual maturation [108].

How to measure water quality in sea cages

- The goals when measuring water quality in sea cages is to:
    - i) know the water quality that the fish actually experience
    - ii) get an overview of the water quality within the cage as a whole

  It is therefore important to carry out the measurements at the depths where you find the majority of the fish and to get measurements from the surface to the bottom of the cage. The latter goal is crucial for correctly interpreting fish behaviour and e.g. the vertical distribution of fish in the cage.

- Temperature and salinity are not affected by the fish inside the cage and can therefore be measured outside the cage. This can be done either by using a CTD that profiles the entire depth-range of the cage, or by multiple sensors at different depths.
- Oxygen and turbidity can markedly differ inside and outside a sea cage. These parameters should therefore be measured inside the cage. If this is not feasible oxygen should be measured immediately downstream from the cage. As the direction of the current often fluctuates, this demands either moving the sensors around or having sensors at several horizontal positions. A sensible, “good enough” solution may be to always measure in the centre of the cage, and again for the relevant depth range of the sea cage. **As far as the authors are aware, there are no best practice recommendations on how to best measure water quality in existing and emerging large-scale production systems.**
- Turbidity can be easily measured using a Secchi disc. A plain white, circular disc 30 cm (12 in) in diameter is mounted on a pole or line and lowered slowly down in the water. The Secchi depth is the depth at which the disk is no longer visible, and is used as a measure of the transparency of the water.

3.5 Group based OWIs

Appetite or a fish's propensity or willingness to feed [109, 13] is a robust, passive OWI for sea cages and can be an early warning signal for potential welfare problems [42]. However, rejection of pellets and low appetite may also mean that fish are satiated (or overfed) or being fed at a time when they do not want to eat, so this must also be considered when using appetite as an OWI. Amongst a multitude of factors, appetite can be suppressed by i) seawater transfer [110, 111], ii) poor water quality and environmental conditions [44], iii) husbandry routines and choices [45] and iv) after an outbreak of a disease [46]. It is well known that the appetite of salmon in sea cages can vary widely within and between days. This variability, in addition to the high number of factors that can impact upon appetite and feeding can make it difficult (and undesirable) to recommend specific daily feed amounts. Many farmers currently monitor appetite and feeding behaviour using mobile underwater camera's (using combined indicators of fish behaviour and the presence of uneaten pellets) as indicators of appetite and satiation. This is also supplemented with knowledge of feeding based upon previous day(s) and also based upon data on water quality parameters (oxygen, temperature etc.) and water state (current speed, if available).

Poor growth. Although growth rates in fish are flexible, acute periods of poor growth below what is expected/normal (although this is very site specific) can be used as an OWI [42]. The quality of its utility as an OWI is, however, dependent upon robust and regular weighing or biomass estimates. As stated above, long-term growth rates vary, so it may be better to use acute changes in growth rate as an OWI within a specific rearing unit or system. Acute changes in growth can be used as an early warning system for potential problems, particularly when the farmer has robust growth monitoring practices.

Mortality is the most widely used group based welfare indicator for on-growing in sea cages and all Norwegian farmers are required to collect dead fish from the sea cages daily if possible and report the number of dead fish to a database governed by the Norwegian Directorate of Fisheries once a month. These data can be used to benchmark production mortalities and retrospectively assess problems or welfare threats [89, 112, 113]. Based on these data the Institute of Marine Research, Norway has developed mortality curves for Norwegian salmon that the mortality figures in any given production month can be compared against (Figure 3.5-1). The median mortality after 15 months for the industry is 9 % showing that most production predominantly stays in the green area. When mortality is higher than expected (yellow or red zones) especially for prolonged periods, this indicates that something is wrong and the farmer should investigate possible causes to take action.

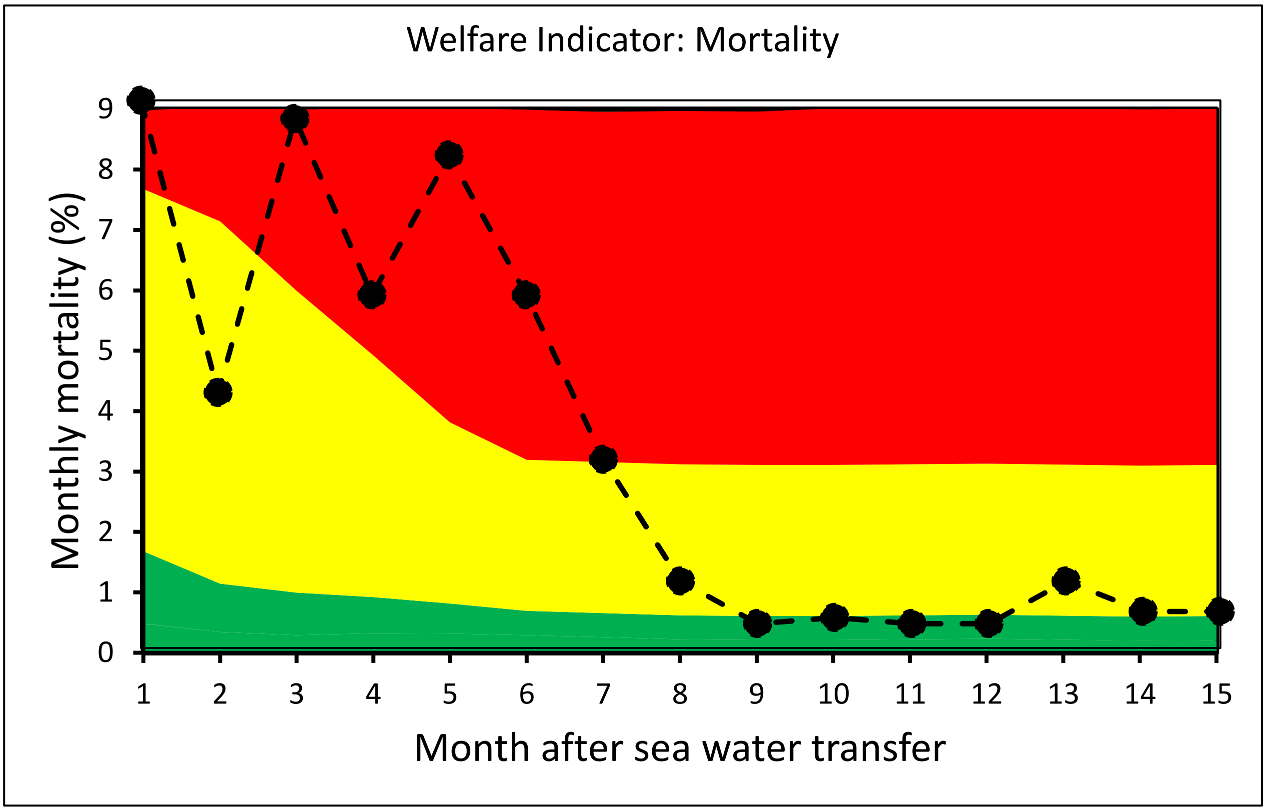


Figure 3.5-1. *Standard mortality curve for the first 15 months after sea transfer [89], based on data reported by the Norwegian salmon industry from 2009-2015. 75 % of all observations are in the green area and can be categorized as "normal", while 5 % of the observations are in the red area and categorized as abnormal.*

Prevalence of emaciated fish. Emaciated fish are often found near the surface, isolated and often around the periphery of the group. They are most notable during the early stages after seawater transfer. These fish can experience low welfare for a long time before they die and they can also be a vector for transmitting diseases to other healthier fish [13]. The occurrence of these moribund or emaciated fish should be monitored [13] and any changes in the frequency of their occurrence should be acted upon as a very early warning OWI.

Deviation and abnormalities from normal expected behaviour are established signs of disease and poor welfare in animals. Emaciated fish at the surface is an example of this, but the changes in behaviour can also be more subtle, and involve the entire population. It is therefore important for fish farmers to monitor behaviour and become familiar with what is normal behaviour for their stock at varying sizes, environmental conditions and seasons.

Salmon behaviour in sea cages:

- Oppedal et al., [27] state "*newly transferred Atlantic salmon smolts show a distinct preference to distribute at the depth of the halocline, independent of the temperature, for the first 2 months in the sea*".
- After the first 2-3 months, salmon typically swim closer to the water surface at night and descend at dawn, swimming deeper in the cage during the day when held under natural lighting conditions [27].

- Submerged lights in the sea cages may disrupt this distribution, allowing the salmon to school in deeper water during the night [13, 27].
- The importance and the effect of suboptimal oxygen saturation on behaviour of cage-held Atlantic salmon is largely unknown.
- Oppedal et al., [27 and references therein] also state that "*at stratified sites where temperature and other environmental variables have been measured in high spatial and temporal resolution, salmon clearly positioned themselves vertically in relation to temperature*".
- In commercial settings, the collective interactions between all individuals cause characteristic circular schooling behaviour [27] that breaks down during feeding (when individual fish swim towards the feed pellets which are usually delivered at the water surface in the centre of the cage). Fish can then leave the feeding arena after they become satiated [114]. However, this behaviour is highly dependent upon feed management, as other studies [115, 116] have shown that feed response, swimming speed and turning angle to capture a pellet were affected when post-smolts were a fed to appetite or fed a fixed ration (and underfed as a consequence of fixed ration feeding). Swimming speeds and turning angles were higher during fixed ration feeding; no differences in swimming speed or angle were observed before, during or after feeding when fed to appetite, meaning swimming speed is a possible OWI of increased competition for a feed resource.
- If fish rise to the surface before feeding begins it may be an indicator of food anticipatory activity (FAA) [117] and these behaviours may develop and be initiated by inadvertent signals such as noise or farm activity around the cages [27].

Disease/health status (OWI and LABWI) is followed on a regular basis by fish health personnel to determine the prevalence of certain conditions within the population and the potential causes of mortality or morbidity. Definitive diagnosis often entails tissue sampling and off site analyses (therefore classified as a LABWI) but some of the external signs of disease or conditions that pose a welfare risk can also be diagnosed on farm by experienced personnel and can lead to a quicker response to disease outbreaks. The overview of diseases characteristics for the seawater stages of Atlantic salmon are given in Part A, section 3.1.5 of this handbook.

Measuring Atlantic salmon behaviour in sea-cages:

- It is possible to get a good overview of fish behaviour using mobile feed cameras. There are numerous works linking e.g. swimming speed and changes in swimming speed to temperature gradients or differences in feeding regimes in cage held post smolts [27, 115, 116]. Swimming speed can also change within a meal in relation to appetite and hunger status. Further, abrupt changes in swimming speed can be in response to predators around the rearing system or adverse water conditions (see Martins et al., [118] and references therein). Therefore, although qualitative changes in fish behaviour can be a good OWI, further detective work needs to be carried out by the farmer to link this change to a specific welfare risk.
- Manually quantifying changes in fish behaviour in cages is labour intensive and would benefit from technological developments to speed this process up and make the data more readily and rapidly available to the farmer for them to act upon. Pinkiwiecz et al., [119] have developed a system for quantifying the swimming speeds of cage-held post-smolts, but this system is not readily available. Other technological developments down the line may make quantified behavioural analysis a robust OWI for the farmer.
- Echo sounder systems, which give the farmer an overview of the vertical distribution of fish within a cage, may offer some benefits to the farmer to generate long term data on fish distributions and deviance from expected behaviour as an OWI. However, generating quantitative data from these systems in a user-friendly manner is labour intensive and they only give a relatively narrow horizontal sample window of behaviour, which may be of limited value in large diameter production systems.

3.6 Individual based OWIs

Individual OWIs describe the welfare of individual fish. In Norway, fish farmers must count and monitor sea lice in their sea cages at least every 7 days when the temperature is equal to or greater than 4 °C, or at least every 14 days at temperatures below 4 °C ([120] Forskrift om bekjempelse av lakselus i akvakulturanlegg, FOR-2012-12-05-1140, NB: Updated version of the regulation is expected in 2018). The lice count involves sampling 10 fish from the cage from 1st June – 31st January or 20 fish from 1st February – 31st May, sedating each fish and carefully counting the lice on the fish and classifying them into different life stages. If the farm has 3 or less cages, then all cages must be sampled each time, otherwise half of the cages must be sampled each time, and in such a way that all cages are sampled after two sampling periods. The regulations also demand that the fish must be caught by a sweep net or another method that secures representative sampling of the fish. Lice counting thereby opens the possibility for not only counting lice, but monitoring welfare indicators based on the appearance of each sampled fish.

Scoring schemes for i) emaciation, ii) skin haemorrhages, iii) lesions/wounds, iv) scale loss, v) eye haemorrhages, vi) opercular damage, vii) snout damage, viii) vertebral deformities, ix) upper jaw deformity, x) lower jaw deformity, xi) sea lice infection, xii) active and healed fin damage, xiii) cataracts and xiv) the Speilberg scoring scheme for intra-abdominal lesions after the intraperitoneal vaccination of Atlantic salmon are provided at the end of this document.

Emaciation state. "Loser" fish are easily recognizable based on their external appearance (thin with low condition factor) and specific behaviour (swimming at the surface) and should be removed from the cage when possible.

Scale loss and skin condition. The presence, severity and frequency of scale loss and epidermal damage and wounds should be regularly monitored.

Eye status. Eyes are very vulnerable to mechanical trauma, leading to haemorrhages or to desiccation during handling. Exophthalmus ("pop eye") is often a non-specific sign of disease while cataract or loss of transparency of the eye lens can be caused by number of factors and is more frequent in later life stages, such as smolts and post-smolts. An overview of types of eye damage and their effects on fish welfare is included in Part A, section 3.2.12 of this handbook.

Mouth/jaw wounds can occur in relation to handling procedures (crowding, pumping, netting; see Part C of this handbook for more information).

Vertebral deformities. Vertebral deformities may be due to malnutrition [54] or temperature [24] amongst other factors. See Fjelldal et al., [55] for more detailed information and Part A, section 3.2.9 of this handbook.

Opercular damage includes shortening, lack of opercula, warped opercula and "soft" opercula. This mostly originates in the fresh water phase but may have welfare implications during periods of low oxygen since fish with damaged opercula have less efficient respiration.

Fin damage. The effects of fin damage upon welfare are both fin- and life stage specific and the risks can differ according to the life stage of the fish. In smolts and post smolts, active fin damage can subject the fish to osmotic stress [57].

Organ indexes address the relationship between an organ size compared to body size, and may be correlated with welfare (see Part A, section 3.2.5 for more information). Most commonly measured indexes are hepatosomatic index (HSI) - the relationship between liver and body size and cardio somatic index (CSI) – the relationship between heart and body size.

Condition factor (K) is a well-established indicator of the nutritional status of the fish and is calculated as 100 x body weight (g) x body length $(cm)^{-3}$. The condition factor for parr should be between 1.0 – 1.3. A condition factor below 0.9 is indicative of emaciation. Condition factor decreases during smoltification, is around 1 for smolts and it increases with fish size in sea.

Gill status can be impaired due to bacterial infections, parasites, viruses or poor water quality. Reduced gill function reduces the fish's ability to exchange gases and excrete waste products and makes the fish more sensitive to stress and the fish can at worst die due to suffocation. Manual scoring of mucous and white spots on the gills is used to monitor amoebic gill disease (AGD).

Sea lice irritate the fish and large numbers of pre-adult and adolescent lice can lead to sores and severe inflammatory reactions. Less than 0.05 lice per cm^2 of skin is considered relatively harmful for the fish, and at levels above 0.12 lice per cm^2, the density of lice on the fish is considered to be potentially fatal [13].

Smoltification state is very important at seawater transfer. Fish that are not smoltified or only partly smoltified will have problems with osmoregulation, growth and in the worst cases can die. Much of the prolonged increased mortality that can be observed in the first few weeks after seawater transfer is attributed to the fact that parts of the population have not been completely smoltified.

Sexual maturation of salmon can occur right after seawater transfer in males, but mainly occurs after 1.5 years at sea. The females mature later and maturing females are usually not a big problem in salmon farming. During maturation, the salmon uses large portions of its energy reserves to build gonads and prepare for the migration back to the river. This preparation includes increased adaptation to freshwater and changes in osmoregulatory capacity. Changes in the activity of various hormones associated with reproduction, such as sex hormones, cortisol and growth hormone, may affect the immune system of sexually mature fish. This is something that can result in increased disease susceptibility and a reduced health status (See Part A, section 3.2.7 of this handbook for more information). Maturing individuals can be identified by certain morphological characteristics: females turn greyer and males turn a rusty colour and can develop a hook-shaped lower jaw. Other less clear signs are behavioural: maturing fish can become aggressive and often swim higher in the water column against the direction of flow.

Feed in the intestine. Feed in the intestine is often an indicator that the fish have eaten in the last 1-2 days [58, 59] but this depends on fish size and temperature. It is easy to check euthanised fish for the presence of feed in the stomach and intestine.

3.7 Welfare management scenario: Monitoring mortalities and injuries

In this example we look at how OWIs may be used as potential tools for guiding production decisions.

A mortality curve benchmarking tool has been developed by Stien et al., [89] (Fig 3.5.1). It benchmarks mortalities in the Norwegian salmon industry for the first 15 months after sea transfer, based on data reported by the industry from 2009-2015. Stien and co-authors classified the lowest 75 % of all reported monthly mortality values as being in the green area of the curve, categorized as “normal”, while the highest 5 % of the mortality values are in the red area and categorized as “abnormal”.

The current scenario addresses a seawater production cycle that started with a 6-month period of high mortality, followed by a long period of low mortality until slaughter. Mortality data was retrospectively plotted against the mortality curve mentioned above (Figure 3.7-1B). The fish had an average weight of 100 g when they were transferred to the sea in November. The elevated mortality during the first month after seawater transfer was attributed to poor smoltification and mortality decreased significantly in December but was still far into the yellow zone. This showed that there was still a need for extra vigilance. Three months after seawater transfer (in January) fish showed clear signs of developing winter ulcers and tenacibaculosis and mortality increased again into the red zone. This situation lasted for four months until May when mortality was back in the yellow zone and from June mortality was in the green zone and the fish had no longer external signs of decreased welfare except for fin damage. Going into the second winter season, the fish had low mortality and did not develop winter ulcers. In this scenario, accumulated mortality after 15 months was 40 %, far above what is normal in Norwegian aquaculture.

The scenario presents a clear fish welfare dilemma: going into month 3 the farmer would have had clear signs of a rising welfare problem and the upcoming months with low water temperature (see Fig. 3.7.1A) would also potentially hinder wound healing and recovery from the disease outbreak. However, conditions improved with higher water temperatures in the spring and the fish experienced low mortalities for the last 12 months of the production cycle (and were slaughtered after 20 months). At month 7 there were discussions on whether the remaining fish should be euthanised based on high cumulative mortalities. However, this would mean that fish were slaughtered after the danger had passed, when mortalities were low and the situation had drastically improved. Nonetheless, many will argue that in this scenario, a decision on euthanasia should have been taken at month 3 to avoid unnecessary suffering to the fish.

Production decisions can be difficult and there may be no right answer; benchmarking data from OWIs such as mortality may help the user better process and evaluate the data they have at hand and use this when reaching their final decision.

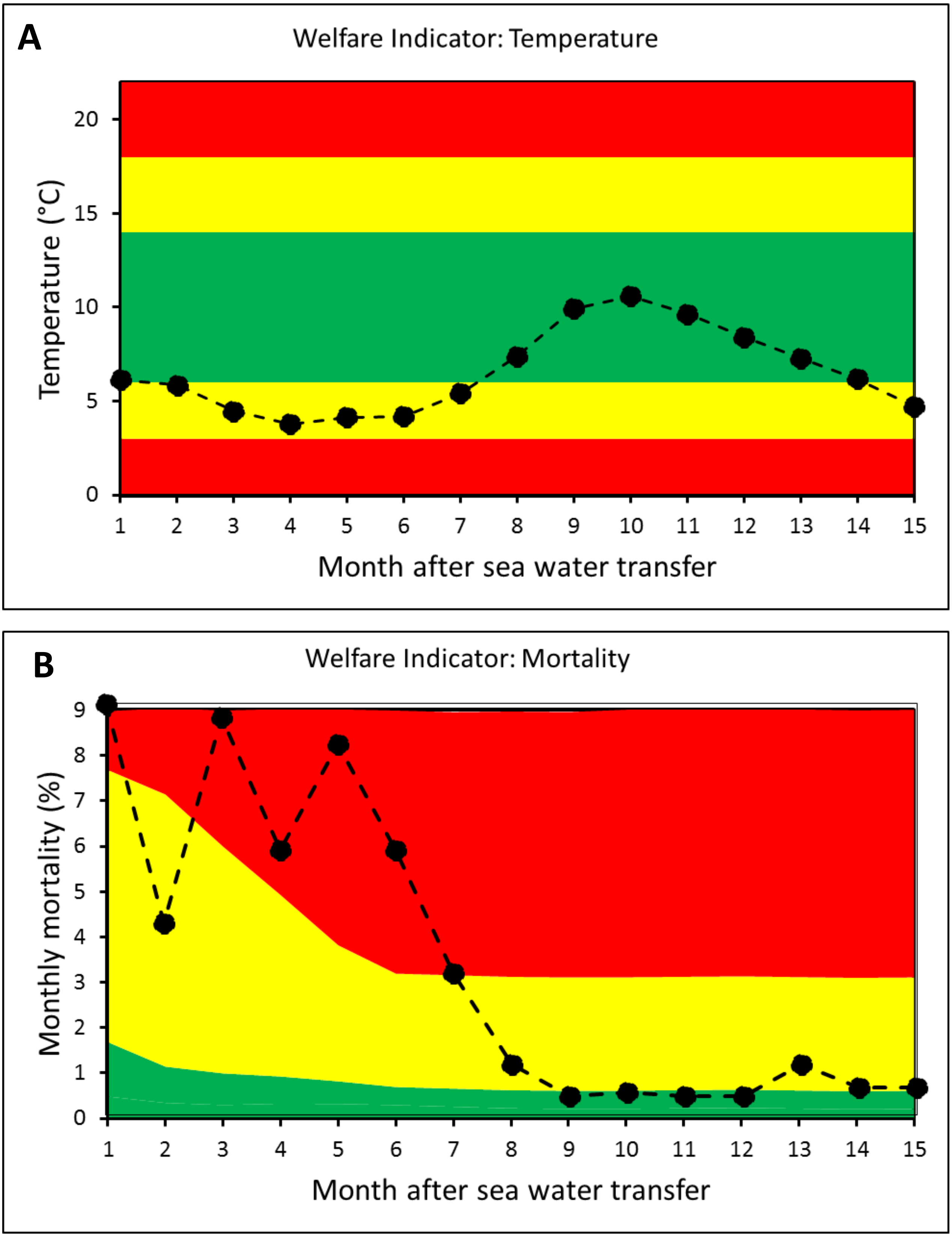


Figure 3.7-1. *Temperature (A) and Mortality curves (B) covering the period from seawater transfer to 15 months at sea. Production month 1 is November, month 6 is May the following year and so on. The colour coding indicates the status of the respective welfare indicators (according to Stien et al., [89]). Lars H. Stien unpublished data.*

4 Submerged sea cages

4.1 Rearing Atlantic salmon in submerged sea cages

Farming salmonids in submerged sea cages may be a way for the industry to avoid surface related problems such as salmon lice and to make new farming sites available where the weather and extreme wave action may have impacts upon both the fish and the farming operation. A submerged sea cage is, in its simplest form, a standard sea cage with a roof sewn into the net keeping the salmon below a given depth. Most of the challenges and WIs for sea cages described in Part B section 3 are therefore also valid for submerged sea cages.

4.2 Challenges to fish welfare

Farming in submerged sea cages adds additional welfare challenges compared with standard sea cages, particularly for salmonids which have a physostomous or open swim bladder and must go to the surface to gulp air to refill the swim bladder and maintain buoyancy [121] (Figure 4.2-1). Typical signs of empty swim-bladders in submerged salmon include tilted 'tail-down, head-up' swimming, exhausted fish and reduced appetite [122, 123, 124]. Producers of submerged sea cages for salmonids try to solve this by either providing some form of underwater air pocket that the salmon can access or by regularly lifting the cages to the surface so the salmon get the opportunity to replenish their swim bladders.

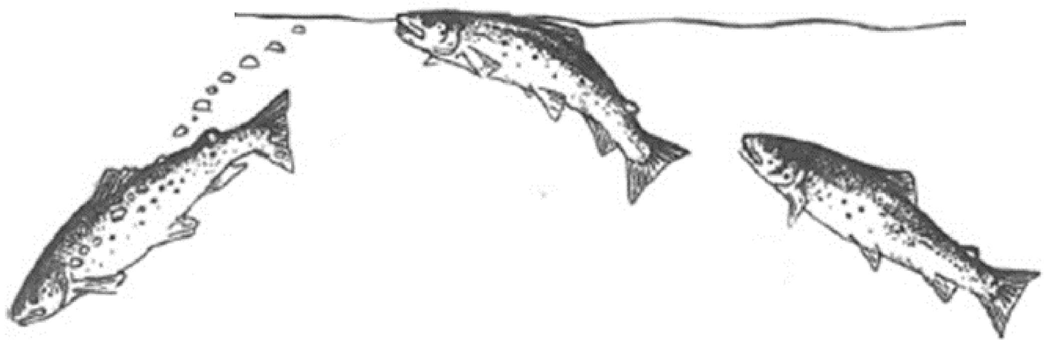


Figure 4.2-1. *Typical swim bladder filling behaviour. The salmon or trout swim to the surface and gulp some air into their swim bladder. Illustration: Stein Mortensen (HI).*

4.3 Operational Welfare Indicators

OWIs for submerged sea cages are similar to those for standard sea cages, but indicators of depleted swim bladders, such as decreased echo strength (when using echo sounder systems), increased swimming speed and swimming at a tilted angle, and surface activity after lifting are added to the grouped based OWIs (Figure 4.3-1). Also, since salmon in submerged sea cages can be restricted from optimising their swimming depth according to water environment and lighting conditions (compared with salmon in standard sea cages), some cues for deviation from normal behaviour are no longer as applicable and the environment based WIs become even more important (as there is limited or no opportunity for the fish to avoid them). Long-term submergence can have detrimental effects on the salmon including a loss of appetite, reduced growth rate and eventually spinal deformities and fin erosion [125].

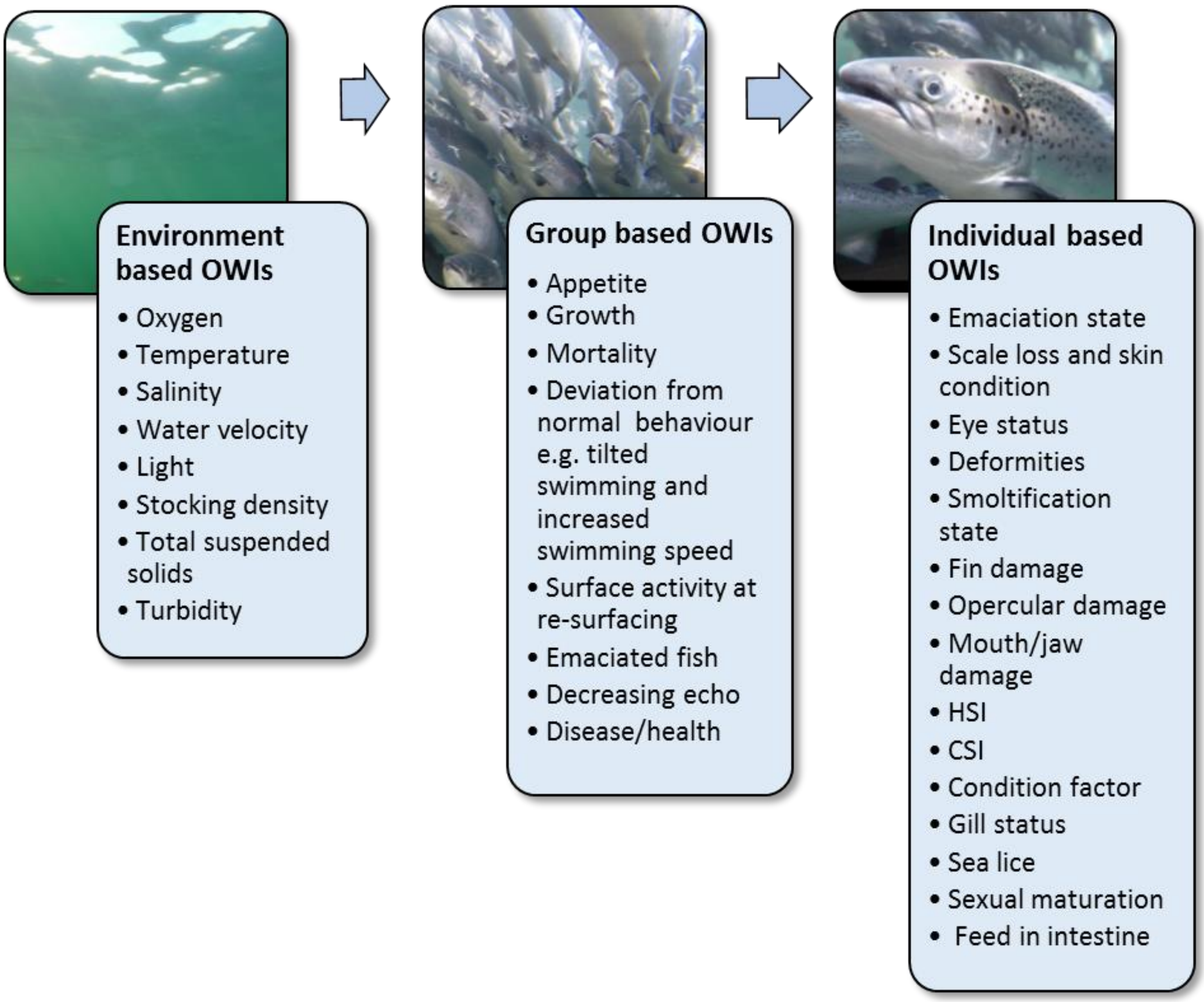


Figure 4.3-1*. Overview of fit for purpose OWIs for submerged sea cages. Environment based OWIs address the rearing environment, group based OWIs attempt to describe the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration, Lars H. Stien.*

4.4 Environment based OWIs

The same environmental OWIs as for sea-cages can be applied to submerged sea cages.

4.5 Group based OWIs

The group based OWIs for sea cages can also be applied for submerged sea cages, but with a little more emphasis on abnormal behaviour and more attention to loss of appetite and reduced growth rate. Special attention should also be paid to the additional submerged sea cage OWIs: abnormal tilt-angle, decreased echo strength, surface activity and increased swimming speed.

- **Abnormal tilt-angles** often occur at night, when the fish reduce their swimming speeds and swimming with an abnormal tilt-angle is a typical sign of depleted swim-bladders. According to Korsøen et al., [125] "*the 'tail-down head-up' swimming position may load the muscles in the tail region to such a degree that some vertebrae become compressed*" and this can also lead to possible deformities in the long-term [125].
- **Decreased echo strength** means that there is less echo reflecting from the salmon. This can either be due to less fish swimming inside the echo beam, fish swimming further from the transducer, fish swimming tilted or that the salmon have less air inside their swim-bladders. Salmon with depleted swim bladders have very little echo signal compared to those with full bladders [125].
- **Surface activity.** Surface activity can be high after bringing the cage to the surface in salmon that have been held in submerged sea cages without the opportunity to fill their swim bladders properly [126].
- **Increased swimming speed** is a response to compensate for loss of buoyancy due to decreased air in their swim bladders [125].

4.6 Individual based OWIs

The individual based OWIs for sea cages can also be applied for submerged sea cages, with special attention to spinal deformities, snout wounds and fin damage.

Scoring schemes for i) emaciation, ii) skin haemorrhages, iii) lesions/wounds, iv) scale loss, v) eye haemorrhages, vi) opercular damage, vii) snout damage, viii) vertebral deformities, ix) upper jaw deformity, x) lower jaw deformity, xi) sea lice infection, xii) active and healed fin damage, xiii) cataracts and xiv) the Speilberg scoring scheme for intra-abdominal lesions after the intraperitoneal vaccination of Atlantic salmon are provided at the end of this document.

4.7 Welfare management scenarios

Submerging salmon for prolonged periods can lead to buoyancy problems (scenario 1). Giving the fish access to air pockets in lieu of surface access may mitigate against this, but it is not without its risks (scenario 2).

Scenario 1: Long term submergence without access to an air pocket

In this scenario, based upon data from [125] fish were submerged for six weeks before the cage was lifted up and the salmon allowed access to the surface again. When submerged the fish started to swim faster (Figure 4.7-1). This response may simply be a consequence of the fish having to maintain buoyancy at a deeper depth. However, after one week the fish also showed clear signs of tilted swimming compared with before submergence (Figure 4.7-1). After three weeks, the tilt became pronounced during the night (with a tile angle of more than 15°) and was easy to spot using IR-cameras. This coincided with a reduction in echo-signal strength by more than 80 % compared with the start of the study (Figure 4.7-1). After re-surfacing the fish were sampled and had reduced growth, poorer fins and more snout injuries compared to controls. In summary, the OWIs used in this scenario showed that the fish had depleted swim bladders from week three, and from then on experienced suboptimal welfare.

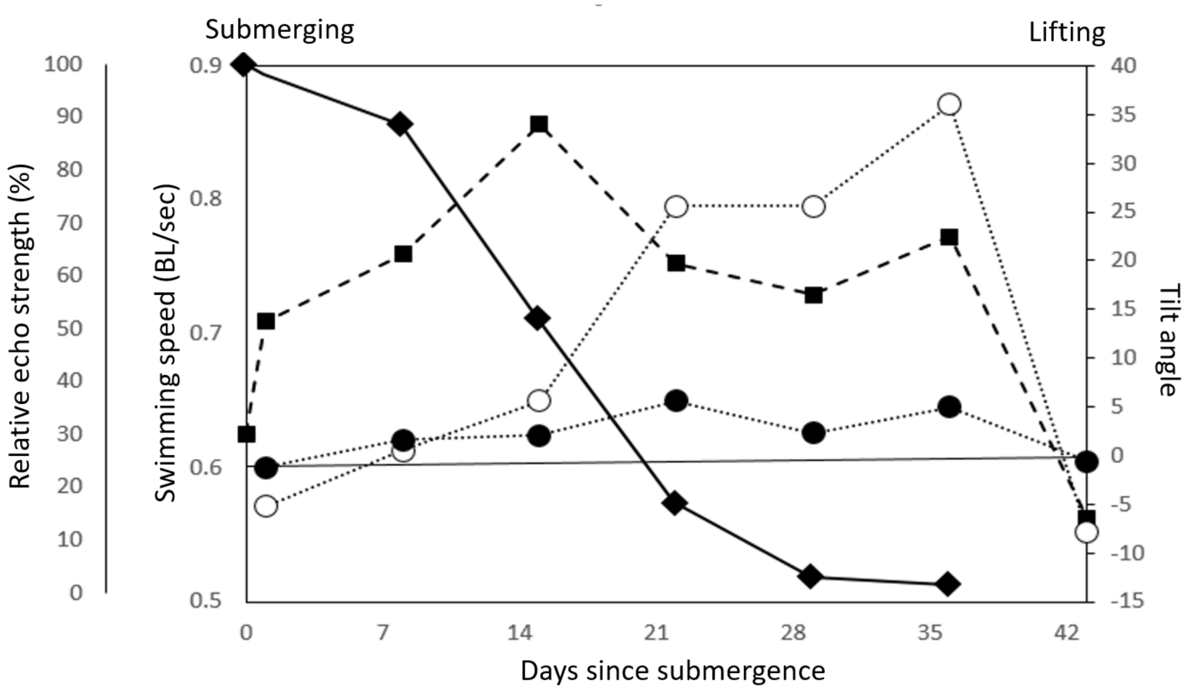


Figure 4.7-1. *Changes in the frequencies of three key OWIs after salmon were submerged in cages without access to an air pocket and after lifting back up to the surface after six weeks (day 42). Figure is based upon data utilised in Korsøen et al., [125]. Relative echo strength (%) compared to before submergence (solid line with filled diamond symbols), swimming speed (BL sec^{-1}) during day time (hashed line with filled square symbols) and swimming tilt angle during the day (dotted line with filled disc symbols) and night (dotted line with open disc symbols).*

Scenario 2: Submergence with access to air pocket

This scenario (unpublished data) evaluated whether fish utilise a submerged air pocket to maintain swim bladder fullness when held in submerged cages. The graph below (Figure 4.7-2) shows the surface activity of fish (number of roll and jumps) during the first two hours after lifting two submerged sea cages with air pockets back to the surface after 3 weeks submergence. Fish in the first cage exhibited low levels of surface activity, indicating the fish had used the air pocket to refill their swim bladders. This data was also supported by echo sounder data; the fish maintained the strength of their echo signal throughout the submergence period. Fish in the second cage exhibited high levels of surface activity and their echo signal was reduced by 40 %, indicating that the fish did not use the air pocket effectively enough to maintain the fullness of their swim bladder.

In summary, not all fish may choose to exploit a submerged air pocket even if they have access to it. Comparing the surface activity of submerged cages after resurfacing can be a good OWI for assessing its utilisation.

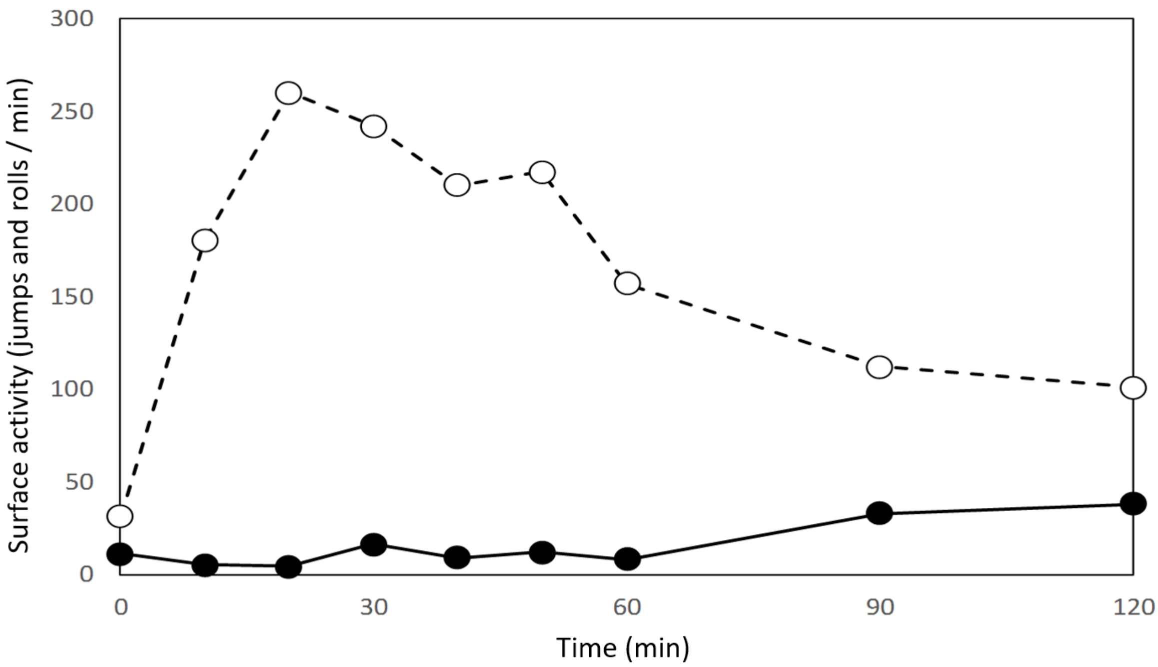


Figure 4.7-2. *The surface activity of salmon upon resurfacing after three weeks of submergence. In the first cage (filled circles with solid line), the salmon had used the air pocket to maintain the fullness of their swim bladder, while in the second cage this has only been partially successful and the salmon had a strong urge to refill the bladder after resurfacing (open circles with dotted lines). Lars H. Stien, unpublished data.*

5 Snorkel sea cages

Photo: Jan Erik Fosseidengen

5.1 Rearing fish in snorkel sea cages

Snorkel sea cages, or Tubenot^{TM}, are defined by Stien et al., [127] as standard "*seacages with a net roof that keep the salmon deep in the water column, while allowing them to access the surface via an enclosed tarpaulin tube (a snorkel)*" (see Figure 5.1-1). They are essentially a special case of a submerged sea cage, where the submerged air pocket is replaced by a tube to the surface that the fish can swim up to gulp air into their swim bladders. All the welfare indicators for submerged sea cages described in section 4 are therefore also valid for snorkel sea cages.

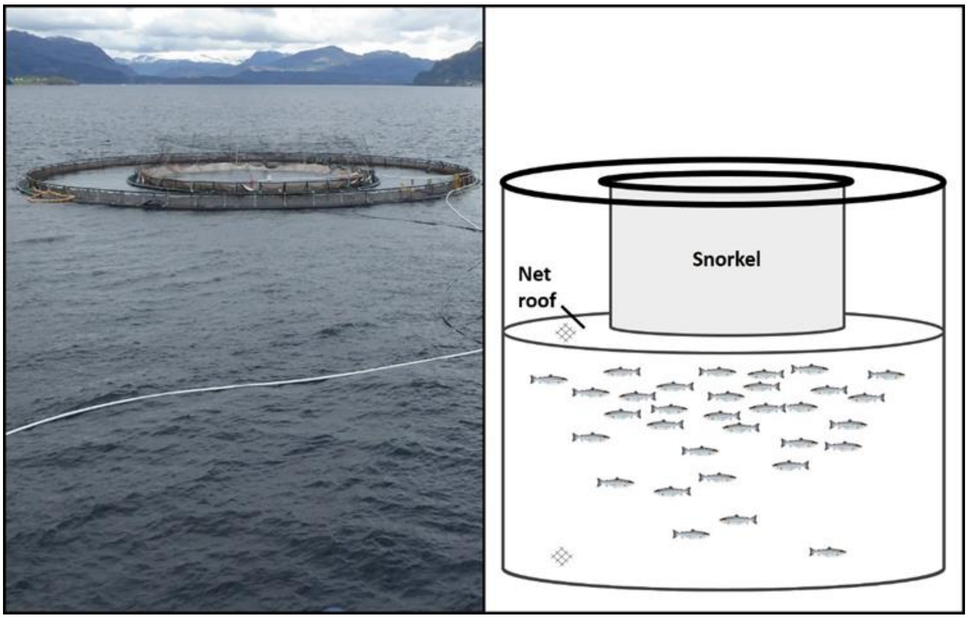


Figure 5.1-1. *Image (left) and schematic (right) of one commercial scale snorkel cage. The purpose of the snorkel is to prevent salmon from coming into contact with surface waters outside of the snorkel whilst giving the salmon access to the surface so they can refill their swim bladder e.g. [102, 128]. The commercial snorkel cages that have been tested so far are 160 m in circumference and had snorkels that were either 60 or 90 m in circumference and 10 m deep. Adapted and reproduced from "Wright, D. W., Stien, L., Dempster, T., Vågseth, T., Nola, V., Fosseidengen, J. -E. & Oppedal, F. [102] 'Snorkel' lice barrier technology reduced two co-occurring parasites, the salmon louse (Lepeophtheirus salmonis) and the amoebic gill disease causing agent (Neoparamoeba perurans), in commercial salmon sea-cages. Preventive Veterinary Medicine 140, 97-105. Copyright 2017" with permission from Elsevier.*

5.2 Challenges to fish welfare

Compared with submerged cages, snorkel sea cages have an advantage in that they can be managed in a similar way as with standard sea cages e.g. the farmers have access to the fish from the surface and can use the same feeding equipment. Currently the industry has opted for snorkels that are 60 m in circumference, 10 m deep and that are mounted inside 160 m circumference sea cages. Although the snorkel technology does not have a negative impact upon fish welfare in small scale studies [89] a range of challenges have been predicted under commercial scale conditions. Water quality inside the confined water volume of the snorkels may be impaired and lead to decreased oxygen saturation and increased turbidity (Figure 5.2.1-1), especially in the more voluminous snorkels where great numbers of fish can aggregate in the snorkel. In situations where lice reach levels that trigger further intervention (> 0.5 mature females fish^{-1} threshold in Norway), treatments that involve removing the snorkel and pulling the net towards the surface become more labour intensive. The relatively slow exchange of water inside the snorkels may lead to an aggregation of particles and pathogens that may harm the fish. To increase water exchange in the snorkel commercial farmers typically pump deep water into the snorkel in order to increase water movement and replenish oxygen conditions [102]. If fish held in snorkel cages were to be infected with AGD (*Neoparamoeba perurans*), the snorkels may have to be removed to allow fish to be bathed with freshwater or hydrogen peroxide (that are currently used to treat AGD outbreaks) [102, 129, 130, 131, 132]. At sites where frequent freshwater bathing is needed, snorkel use may be impractical. A way to circumvent this may be to continuously fill the snorkel with freshwater to exterminate or dislodge *N. perurans* that are attached to the gills as the salmon repeatedly go through the snorkel to access the surface and refill their swim bladder [102].

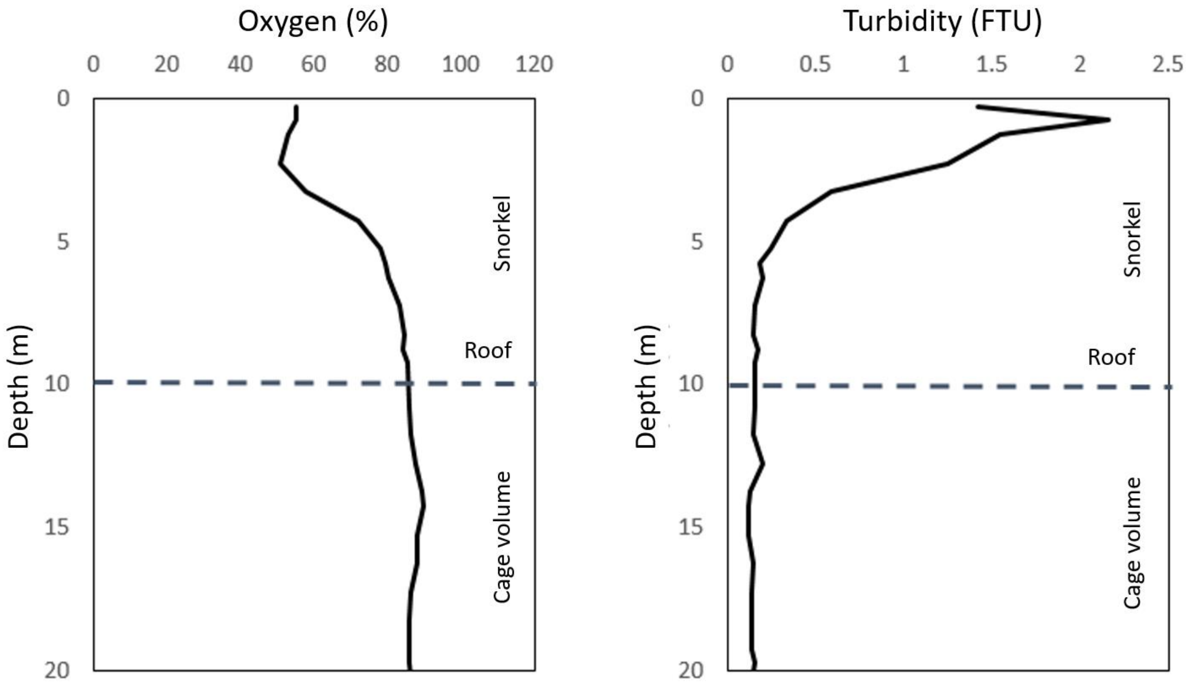


Figure 5.2.1-1. *Oxygen and turbidity measured in a commercial 10 m deep snorkel and into the cage below. Notice the decrease in oxygen in the top of the snorkel and the increase in turbidity.*

In a spring–summer study [102], snorkel cages decreased new sea lice infestations by 84 % without confounding effects on fish welfare. And, in an autumn-winter study at another site, fewer chemical treatments were necessary at a farm with snorkel cages compared to at a neighbouring farm that was used as control. Chemical bathing was, however, labour-intensive as snorkel removal was required [102]. High AGD-related gill scores for fish held at the farm with snorkel cages rapidly declined when the farmer pumped freshwater into the snorkels, which created a low salinity surface layer that the salmon could swim through to refill their swim bladder [102]. Snorkel deployment and periods of intermittent low DO saturations did not have a significant effect upon fish welfare during the study. In summary, the study suggested snorkel cage technology may be a practical tool for helping commercial salmon sea-cage farmers manage co-occurring outbreaks of sea lice and amoebic gill disease [102]. However, the use of snorkel cages does come with operational challenges that need to be addressed in their further development.

5.3 Operational Welfare Indicators

OWIs for snorkel sea cages are generally the same as for submerged sea cages and standard sea cages (Figure 5.3-1). However, commercial trials have suggested that oxygen and turbidity are environment based OWIs that are particularly important to monitor inside the snorkels [102]. One advantage of snorkel compared to submerged sea cages is that fish can be sampled for lice counting and welfare assessment, in a similar way as with standard sea cages.

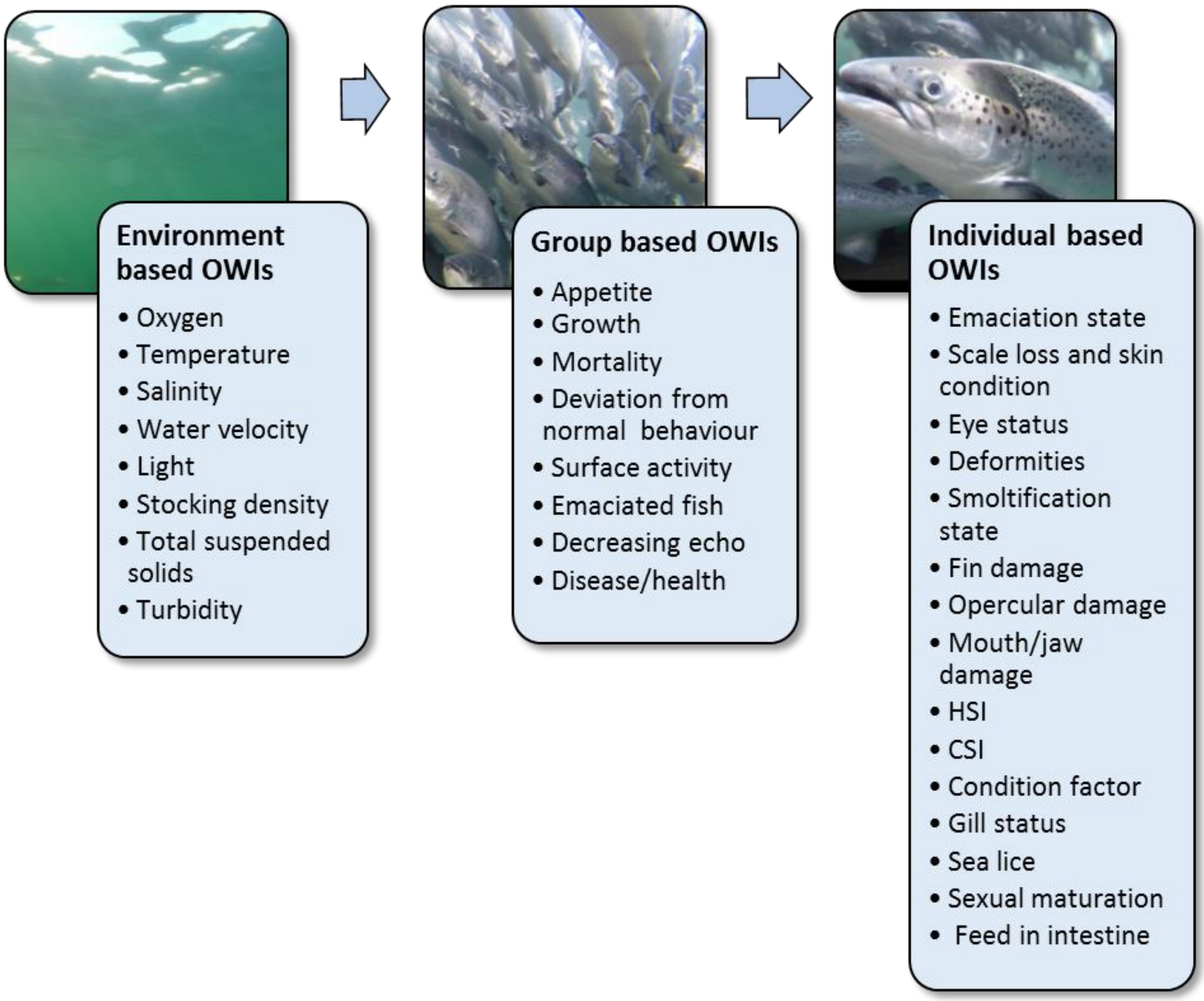


Figure 5.3-1. *Overview of fit for purpose OWIs for snorkel cages. Environment based OWIs address the rearing environment, group based OWIs refer to the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration, Lars H. Stien.*

5.4 Environment based OWIs

Oxygen and turbidity. The same environmental OWIs as for submerged sea cages can also be applied to snorkel sea cages, but with special emphasis on the environment inside the snorkel itself.

5.5 Group based OWIs

The same group based OWIs for submerged sea cages can be used for snorkel sea cages, but with the addition of fish staying near the surface.

Fish staying near the surface are an important indicator for a risk of reduced welfare in snorkel sea cages. Ideally the fish should not be aggregating at the surface, or spend too much time in the snorkel.

5.6 Individual based OWIs

The individual based OWIs for surface cages can also be used for snorkel cages, with special attention to gill status.

Gill bleaching and Gill-status. Fish in snorkel sea cages can be more at risk of AGD than control sea cages at the same site [102]. However, the same authors also state that the effects of snorkel cages upon AGD levels in salmon is not currently known [102].

Scoring schemes for i) emaciation, ii) skin haemorrhages, iii) lesions/wounds, iv) scale loss, v) eye haemorrhages, vi) opercular damage, vii) snout damage, viii) vertebral deformities, ix) upper jaw deformity, x) lower jaw deformity, xi) sea lice infection, xii) active and healed fin damage, xiii) cataracts and xiv) the Speilberg scoring scheme for intra-abdominal lesions after the intraperitoneal vaccination of Atlantic salmon are provided at the end of this document.

5.7 Welfare management scenario: Managing an AGD outbreak in snorkel cages

AGD is a challenge for open cage production. This scenario summarises an experiment examining freshwater treatment in snorkel cages.

In this study, described in Wright et al., [102] a commercial snorkel cage (160 m in circumference, 25m deep, and snorkels of 90 m circumference and 10 m deep) was stocked in mid-summer with approximately 100,000 salmon (mean weight 55–84 g) and also cleaner fish (wrasse at 2.5%). Water was pumped into the snorkel from deeper areas in the water column that were below the snorkel edge using a LiftUp "air pump". AGD-gill scores were recorded weekly by farm staff (Figure 5.7-1) according to a 5-point scale [133]. When the AGD-scores increased in December, freshwater was pumped into the snorkel from a nearby river at a steady rate of 600–700 m^3 day^{-1} (450 l min^{-1}). This resulted in a rapid decline of the AGD scores (Figure 5.7-1) and the delivery of freshwater was therefore stopped in late January.

Pumping freshwater directly into the snorkel during an AGD outbreak may therefore be an effective tool to reduce the impacts and severity of AGD.

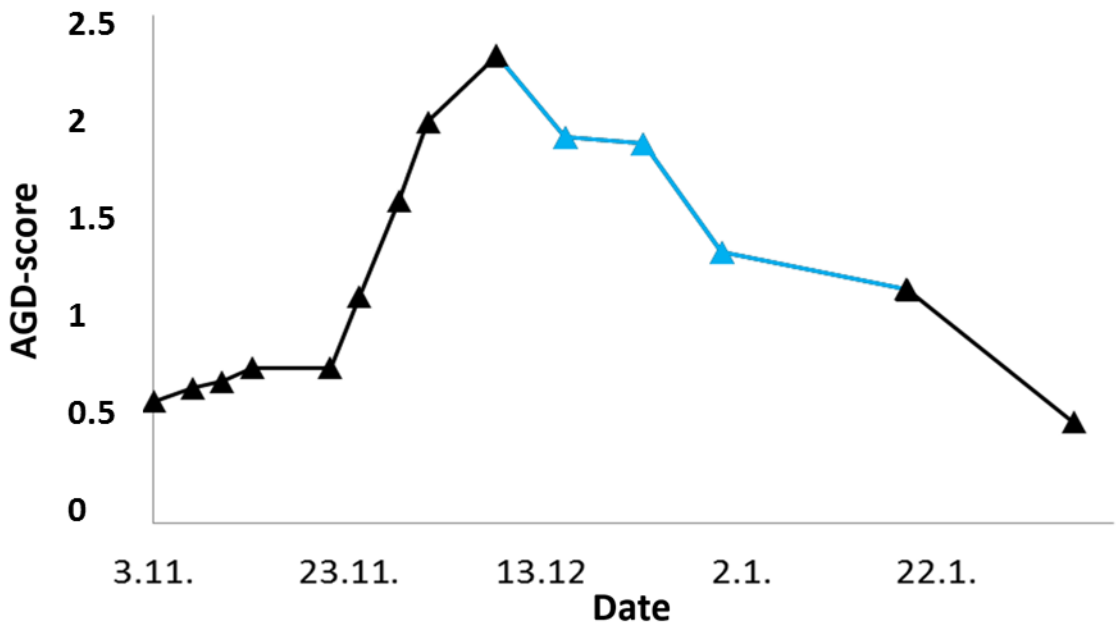


Figure 5.7.-1. *Mean AGD gill scores recorded on a weekly basis by fish farmers based on a 5-point scale. The blue line and symbols indicate the period when freshwater was supplied to the snorkel. Lars H. Stien, unpublished data.*

6 Semi-closed containment systems

6.1 Rearing Atlantic salmon in semi-closed containment systems

Semi-closed containment systems (S-CCS) in the sea are production systems that provide a dense or relatively dense physical barrier between the water environment in which the fish is reared and the surrounding environment [134]. The use of S-CCS can address a number of challenges the salmon industry faces today [135]. In S-CCS deeper water can be pumped into the system to avoid lice infestations and organic waste can be collected and removed from the system. Nilsen et al., [136] documented that the rearing of salmon in S-CCS provides an effective protection against sea lice infestation and the systems have no adverse effects on growth and survival rates. Other studies have also reported no lice infestations during post-smolt production in different types of S-CCS [135, 137]. In addition, the physical barrier reduces the risk of escapees and more stable temperature conditions within the rearing system can be beneficial in the early seawater grow-out phase [137].

In Norway, these systems are currently only allowed to produce post-smolts up to 1 kg but the potential of these systems for the full sea water grow-out phase is being considered by the aquaculture industry. A number of different S-CCS proto-types are currently being tested in Norway (Fig 6.1-1) but there is still little publicly available information about the performance and welfare of Atlantic salmon post-smolts under commercial production conditions in S-CCS.

6.2 Challenges to fish welfare

Environment:

- The transfer of salmon from hatcheries to the sea can be challenging, particularly if smolt quality is compromised and the first couple of weeks are often marked by poor appetite and mortalities.
- The presence of predators in the surrounding environment can have a negative effect on welfare as in other sea-based systems.
- Mass occurrences of jelly fish can severely compromise the operation of the system and cause poor welfare and mortalities (see section 6.7).

Pathogens:

- The intake water in S-CCS is not treated prior to entering the system which can allow pathogens to enter.
- The pumping of high volumes of water can upwell benthic sediments into the rearing system, introducing pathogenic species from marine sediments, such as *Vibrio sp.* and *Moritella viscosa* to the system [138].
- The microbial community of S-CCS can also be subjected to varying environmental conditions over a production cycle, due to e.g. seasonal changes and a high water turnover rate [138]. These parameters can include varying water temperature and light regimes or variable organic loads [138].
- Periodic blooms of e.g. bacteria or algae can also affect the health and welfare of post-smolts in S-CCS [138].

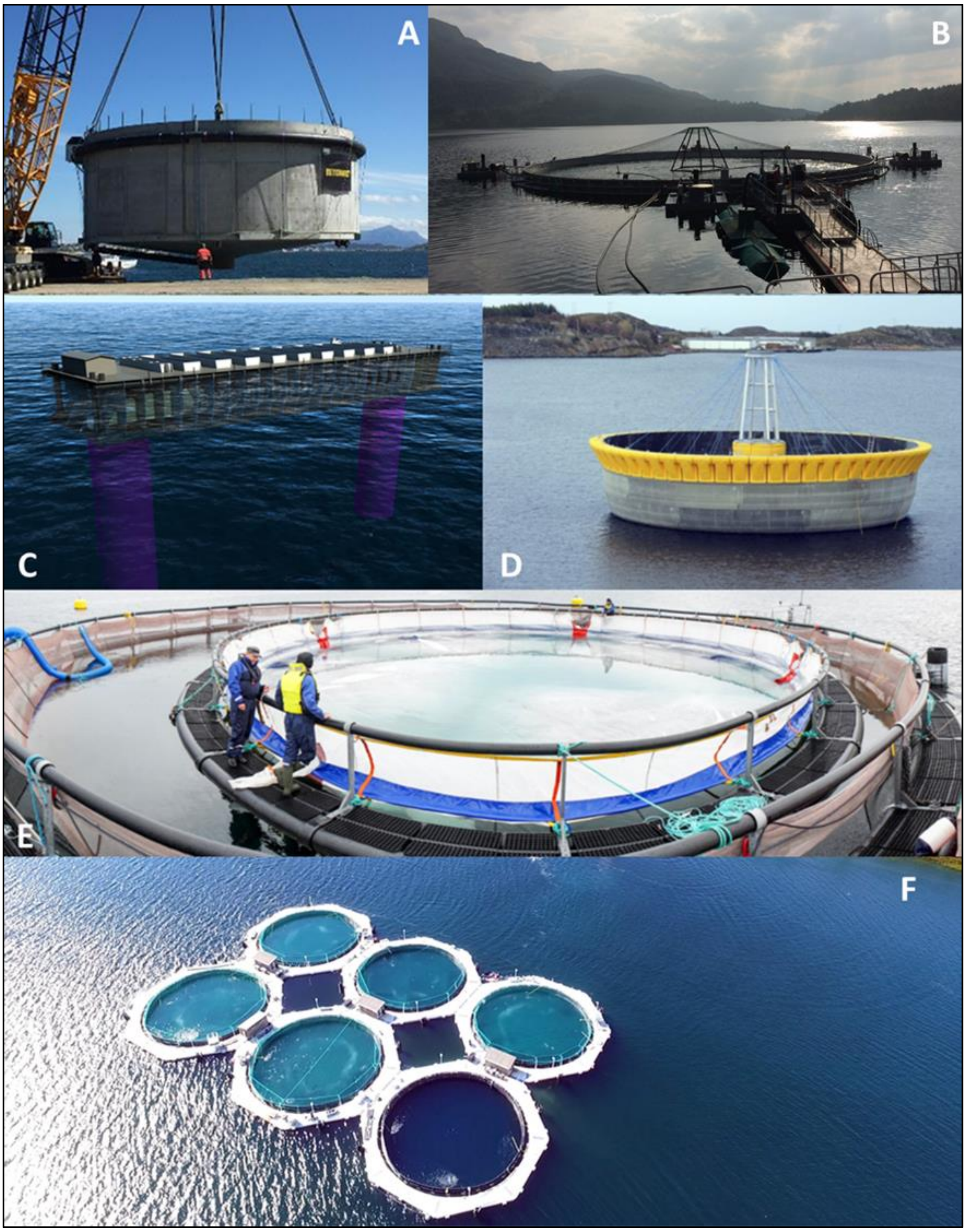


Figure 6.1-1. *Different type of semi-closed systems in the sea: A) "Salmon Home #1" concrete tank (photo: Jelena Kolarevic, Nofima); B) Neptun (photo: Aquafarm Equipment); C) Preline (photo: Lerøy); D) AgriMarine (photo: Per Gunnar Kvenseth); E) FlexiBag (photo: Nekton havbruk AS); F) AquaDesign (photo: AquaDesign AS). Photo C) is reproduced with permission from Klemet Steen, Lerøy, photos D) and E) are reproduced with permission from Maria Sørøy, Smøla Klekkeri og Settefiskanlegg AS and photo F) is reproduced with permission from Anders Næss, AquaDesign AS.*

6.3 Operational Welfare Indicators

Knowledge on appropriate OWIs for S-CCS is very limited. The aquaculture industry is still testing different prototypes of S-CCS with different designs, sizes and technical specifications and this can affect which OWIs are most appropriate for the different systems. The animal based OWIs that are appropriate for sea cages are also currently appropriate for welfare monitoring in S-CCS (Figure 6.3-1) while a wider range of environment based OWIs are necessary for S-CCS compared to sea cages.

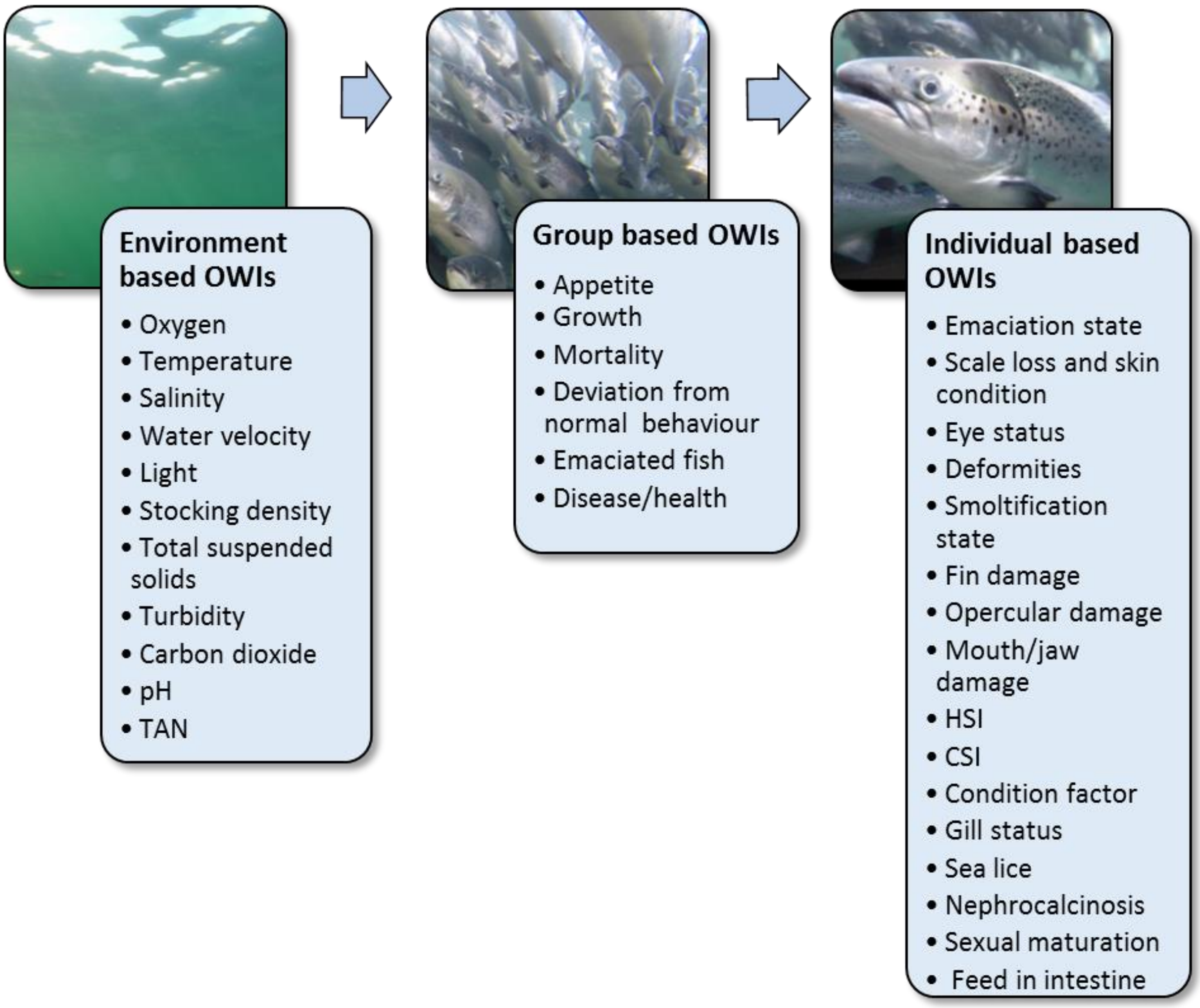


Figure 6.3-1. *Overview of fit for purpose OWIs for semi-closed containment systems in the sea. Environment based OWIs address the rearing environment, group based OWIs refer to the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration, Lars H. Stien.*

6.4 Environment based OWIs

The same environmental OWIs for sea-cages can be applied to S-CCS (please refer to Part B section 3.4 for full information) but attention also should be paid to a number of other environmental parameters. Water quality in the S-CCS is dependent on the quality of the pumped water, water exchange rate, system hydraulics, biomass and the feed load in the system.

Temperature. The same temperature thresholds and preferences that apply to post-smolts in sea cages also apply to post-smolts in S-CCS. However, water temperature in the S-CCS is dependent on the depth from which water is pumped and fish can be potentially subjected to higher winter and lower summer temperatures compared with the surrounding seawater; knowledge of seasonal changes in water temperature of the intake water would be valuable to predict growth, the biomass load to the system and potential periods with an increased risk of pathogen infestation.

Oxygen is added to S-CCS to maintain constant and optimal oxygen saturation in the system, but short-term oscillations in oxygen saturation are possible and can affect fish welfare [139].

Salinity in the SCC-S will be dependent on the salinity of the intake water.

Turbidity and Total suspended solids (TSS) can be higher in the S-CCS compared to the surrounding seawater [140] and suspended solids can accumulate in the system if water exchange rate is not adequate.

Water velocity is important for providing a training regime for salmon post-smolt that can promote higher muscle growth [141] and increase cardiac health and improve immune response [142, 143]. Specific water flow rate (L $min^{-1}kg^{-1}$) in flow-through S-CCS where oxygen is injected must be higher than 0.2-0.3 L $min^{-1}kg^{-1}$ to prevent excessive accumulation of CO_2 [14]. An adequate water flow rate is therefore important for the removal of metabolites and particles and for the establishment of the necessary water velocities.

Stocking density in the S-CCS must be > 25 kg m^{-3} in order to make this technology cost-effective [144]. Recent results have shown that it is possible to raise post-smolt Atlantic salmon at densities up to 75 kg m^{-3} without having a detrimental effect on a number of performance and welfare indicators [145]. Norwegian legislation has recently allowed for production of post-smolt in S-CCS up to 75 kg m^{-3} in certain cases related to R&D activities.

Light conditions for the optimal performance and welfare of post-smolts in S-CCS is unknown.

KNOWLEDGE GAP: The optimal light conditions (both light intensity and light quality) for post-smolt in S-CCS is unknown.

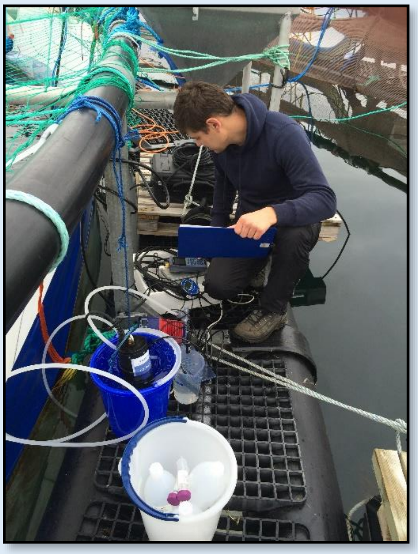
Carbon dioxide can accumulate in the S-CCS if water flow rate in the system is inadequate (reduced) or if the biological load to the system is not supported by the system design and dimensioning [139]. Carbon dioxide concentrations above 15 mg L^{-1} can cause nephrocalcinosis ([139]; Fig.6.4-1). Nilsen et al., [139] suggested CO_2 concentration should be < 8-10 mg L^{-1}.

pH. Reduced pH values are possible following increases in CO_2 and cause immediate changes in behaviour and appetite even when the values of two parameters are within acceptable ranges [139]. Nilsen et al., [139] suggested pH should be > 7.2-7.4.

Figure 6.4-1 *Nephrocalcinosis (photo by Veterinary Institute)*

Total ammonia nitrogen (TAN) can accumulate in the S-CCS over time as a product of fish metabolism [2] and should be regularly monitored. The suggested TAN concentration in S-CCS should be below 1-2 mg L^{-1} or below 5-10 µg L^{-1} NH_3-N [139].

How to measure water quality in S-CCS:



- Water quality in the S-CCS should be measured on a regular basis and at the same locations to ensure robust data collection.
- Temperature, oxygen, pH and CO_2 should be measured on a daily basis and in several locations within the S-CCS to cover the depth and width/length of the system.
- TAN, TSS and turbidity should be measured at least once a week and if any change in feeding regime or feed intake is planned or noted.
- Water velocity should be measured and adjusted to match changes in post-smolt growth and also if the water exchange rate is modified.
- Probes should be calibrated before water quality is measured and on-line probes should be properly maintained to prevent false readings due to biofouling.

6.5 Group based OWIs

The same group based OWIs for standard sea cages can be used for S-CCS, but with some amendments regarding fish behaviour.

Behaviour can vary in different S-CCS as shown in Fig.6.5-1. In the Preline semi-closed raceway system fish generally swim against the current without changing their position within the system, contrary to post-smolts in a standard cage that served as a reference group [141]. Behaviour in circular floating S-CCS can be more similar to the circular schooling behaviours seen in standard cages.

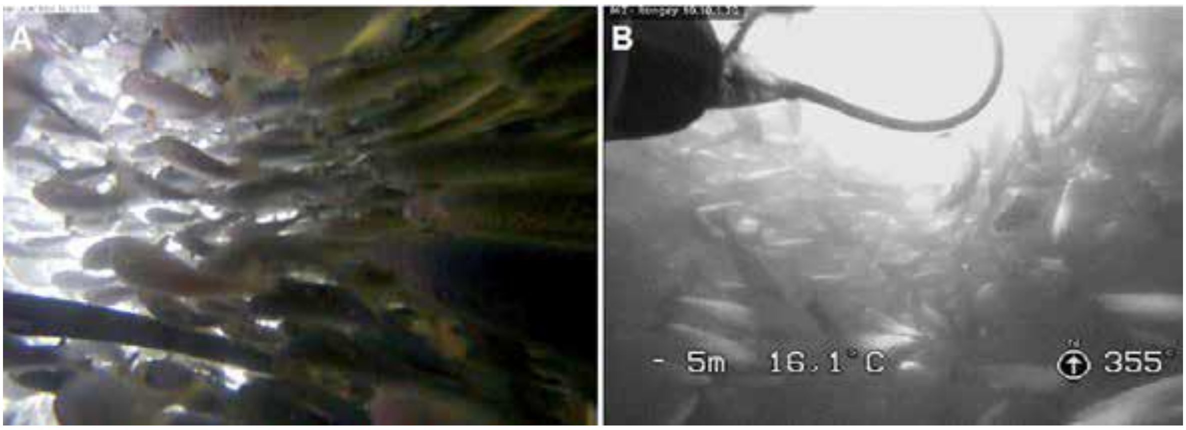


Figure 6.5-1 *Picture from underwater video recordings showing the general swimming pattern of fish in S-CCS Preline (A) and standard cage (B) facilities. Reproduced from Handeland et al.*, [141], *with permission from Å. Espmark.*

6.6 Individual based OWIs

The same individual based OWIs that apply to standard sea cages can also be applied to S-CCS. In addition, according to a commercial S-CCS study, nephrocalcinosis (Figure 6.4.1) is an additional OWI that is important to monitor [139].

Assessment of individual OWIs can be done at the same time as mandatory lice counting.

Morphological OWIs used for Atlantic salmon in sea cages can be applied to monitoring of welfare in S-CCS.

Scoring schemes for i) emaciation, ii) skin haemorrhages, iii) lesions/wounds, iv) scale loss, v) eye haemorrhages, vi) opercular damage, vii) snout damage, viii) vertebral deformities, ix) upper jaw deformity, x) lower jaw deformity, xi) sea lice infection, xii) active and healed fin damage, xiii) cataracts and xiv) the Speilberg scoring scheme for intra-abdominal lesions after the intraperitoneal vaccination of Atlantic salmon are provided at the end of this document.

Due to the risk of potential accumulation of CO_2 in the S-CCS, a scoring of **nephrocalcinosis** in kidneys on a regular basis would be advisable. An operational scoring scheme for morphological determination of nephrocalcinosis is currently being developed.

6.7 Welfare management scenario: External threat to S-CCS systems

This scenario demonstrates that although S-CCS systems protect the fish from many environmental and infectious threats it is still essential to monitor and respond to them. In order to avoid adverse effects the farm should monitor external water and the organisms within the water and carry out routine daily checks of intake filters.

In the scenario, Atlantic salmon smolts were transfer to a S-CCS at the end of May. During the first weeks in the S-CCS fish had poor appetite and number of fish developed wounds. The farmer identified inadequate smolt quality as one of the causes for this. Appetite improved at the end of June and fish with wounds were no longer observed. However, another instance of reduced appetite was noted (Day 1), followed by an increase in the number of moribund fish and an increase in mortality, with 300 mortalities (0.2%) on Day 3. The farmer at first assumed this was again due to poor smolt quality, but could not find fish with wounds amongst the fish that were sampled. On Day 4 the situation got dramatically worse. An increased number of moribund fish were noted and a large number of small jellyfish were also observed within the system (and also parts of larger jellyfish). A total of 660 fish (0.3%) died on Day 4. During the night and the next morning an additional 15,000 fish (7.8%) died (Day 5). The farmer noticed that water movement and water velocity in the system was very low, even though the pumps were running. Oxygen levels were normal (as oxygen is added to the system and is not dependent on the water flow in the system). When the pump intake screens were checked it was discovered that they were completely clogged by jellyfish and water could not be pumped into the system. Water quality improved rapidly after the screens were cleaned. Additional adjustments were also made to increase the water velocity and water exchange rate in the S-CCS. All measures led to an improved situation within the system by Day 6; mortality decreased, fish started to eat again, fish were more active and fewer moribund fish were observed. Mortality decreased over the following days and pump intake screens were regularly monitored especially when jellyfish were present in the seawater surrounding the S-CCS and in the system itself (Fig. 6.7-1).

Figure 6.7-1 *Occurrence of jellyfish in the S-CCS and in the surrounding seawater. Photo: J. Kolarevic*

7 Lice skirts

7.1 Rearing fish in sea cages with lice skirts

Salmon lice have evolved to situate themselves in the upper levels of the water column to improve their chances of encountering possible hosts [146, 147]. To circumvent this, lice skirts (usually made of tarpaulin or plankton sheeting) are wrapped around the upper 5-10 m of standard sea cages with the intent of directing potentially lice infested surface waters around the cages instead of into the cages (Figure 7.1-1). However, their effectiveness can depend on the environmental conditions at farm [148], the depth of the lice barriers [149] and if strong currents frequently deform the skirt making it less effective [150]. However, salmon lice do not tolerate low salinities, and in fjord systems with periods of brackish water at the surface this will force the lice down and thereby infect fish below shallow skirts (e.g. a 5 m deep skirt). At some farms, turbulence can occasionally shift the lice 20–30 m deeper in the water column [151], and strong currents can force the skirts upwards reducing their effectiveness [150].

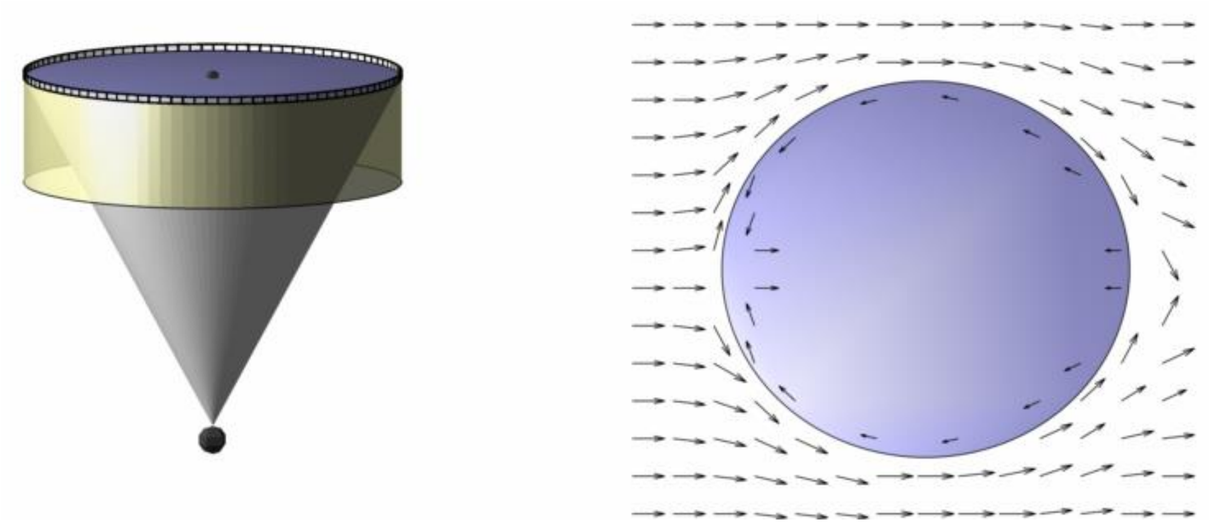


Figure 7.1-1*. Left: Diagram outlining the principle behind lice skirts wrapped around the upper meters of a cage. Right: Diagram showing water flow in and around a standard cage fitted with a 5 m deep lice skirt [152]. Illustrations: Lars H. Stien, reproduced with permission.*

7.2 Challenges to fish welfare

Lice skirts wrapped around a sea cage reduce water flow and water exchange substantially [153, 154] and may lead to reduced water quality and increase the aggregation of particles and pathogens within the skirts [155]. Studies on both 10 m deep lice skirts made of plankton sheeting and [154, 156] and 3 and 5 m deep lice skirts made of tarpaulin [153, 157] show periods of markedly reduced oxygen levels within the lice skirts. If this occurs, salmon can swim below the skirt if the cage is deep enough [152] but this is not always the case [157]. Increased turbidity increases the danger of gill damage and possibly AGD [155]. Another risk is that strong currents can potentially drag the skirts if they are not tensioned appropriately, leading to cage deformation and a reduction in the net volume [152]; this can potentially trap and damage the fish [e.g. 154].

7.3 Operational Welfare Indicators

Operational WIs for cages with lice skirts are generally the same as for standard sea cages, but extra attention should be paid to water quality within the skirts and also cage deformation (Figure 7.3-1). For group based OWIs it especially important to monitor whether the fish are gathering at surface or if they are avoiding the skirt volume. Amongst the individual based OWIs attention should also be paid to gill status.

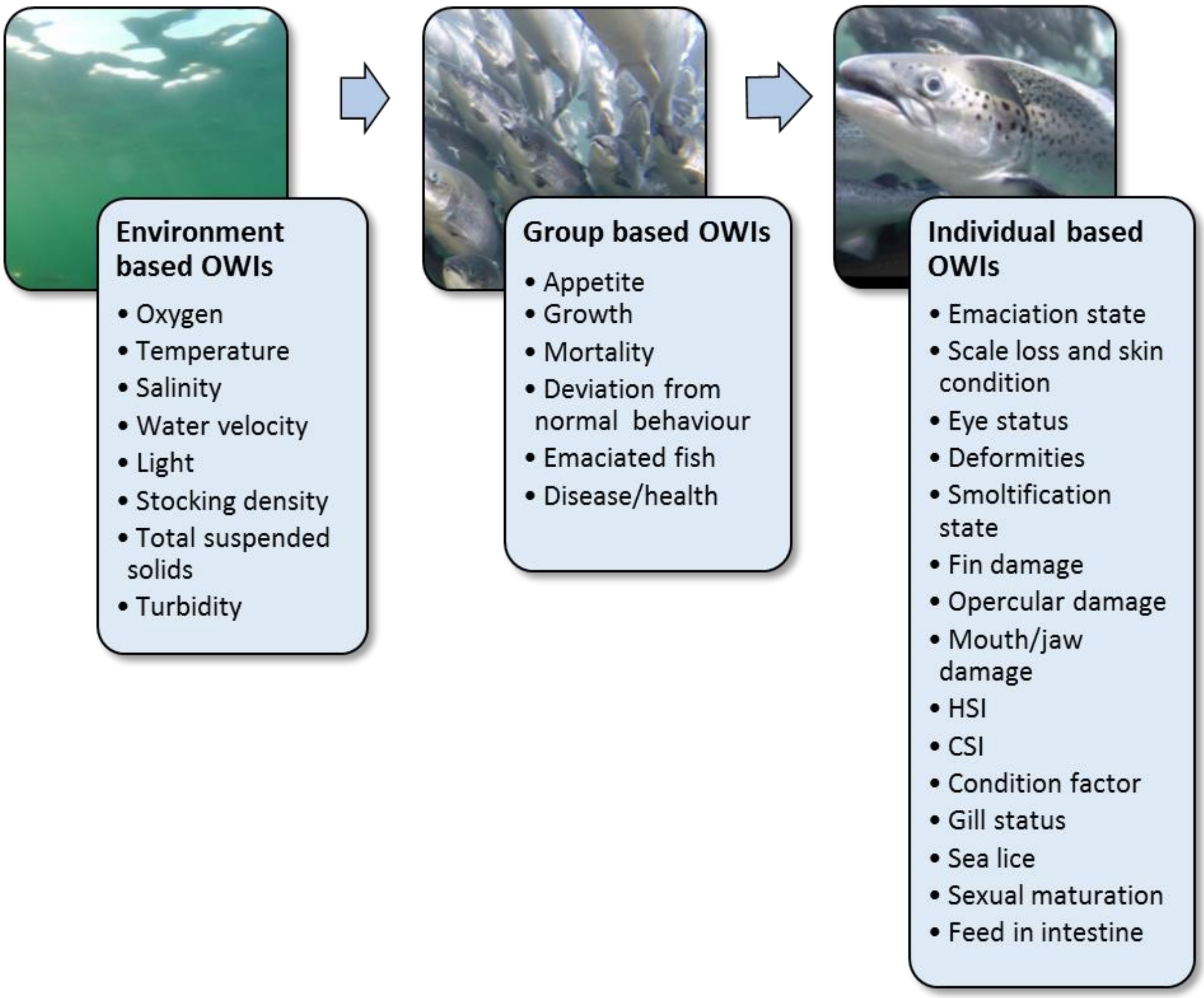


***Figure 3.3-2**. Overview of fit for purpose OWIs for sea cages fitted with lice skirts. Environment based OWIs address the rearing environment, group based OWIs refer to the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration, Lars H. Stien.*

7.4 Environment based OWIs

Oxygen and turbidity. The same environmental OWIs for sea-cages can be applied to sea-cages fitted with lice skirts, but extra attention should be paid to oxygen and turbidity levels within the skirt.

7.5 Group based OWIs

The same group based OWIs for standard sea cages can be used for cages fitted with lice skirts, but with some amendments regarding fish behaviour.

Fish at the surface "gasping for air" is an important indicator of reduced welfare [157]. It may also be self-reinforcing in that aggregation of fish may lead to depletion of oxygen levels, weakening the fish further.

Fish avoiding the enclosed volume may be a sign of decreased water quality.

7.6 Individual based OWIs

The same individual based OWIs for standard sea cages can be used for cages fitted with lice skirts, but additional attention should be paid to gill status.

Gill status. Fish in sea cages fitted with lice skirts may be at a higher risk of gill infection [155].

KNOWLEDGE GAP: The effects of skirts upon the levels of gill infection in caged salmon populations is still unknown.

Scoring schemes for i) emaciation, ii) skin haemorrhages, iii) lesions/wounds, iv) scale loss, v) eye haemorrhages, vi) opercular damage, vii) snout damage, viii) vertebral deformities, ix) upper jaw deformity, x) lower jaw deformity, xi) sea lice infection, xii) active and healed fin damage, xiii) cataracts and xiv) the Speilberg scoring scheme for intra-abdominal lesions after the intraperitoneal vaccination of Atlantic salmon are provided at the end of this document.

8 Morphological schemes for assessing fish welfare in different rearing systems

The following section is a summary of the scoring schemes used in this handbook.

This handbook suggests a unified scoring system (Tables 8.1-1, 8.1-2, 8.1-3) that is primarily aimed at farmers to help them assess welfare and rapidly detect potential welfare problems out on the farm. It is an amalgamation of the injury scoring schemes used in the Salmon Welfare Index Model (SWIM) [13], the injury scoring scheme developed by the Norwegian Veterinary Institute (NVI) [158, 159] and also from other schemes developed by J. F. Turnbull (University of Stirling) and J. Kolarevic and C. Noble (Nofima).

Our suggested scheme standardises scoring for 14 different indicators to a 0-3 scoring system:

i) emaciation, ii) skin haemorrhages, iii) lesions/wounds, iv) scale loss, v) eye haemorrhages, vi) exophthalmia, vii) opercular damage, viii) snout damage, ix) vertebral deformities, x) upper jaw deformity, xi) lower jaw deformity, xii) sea lice infection, xiii) active fin damage, xiv) healed fin damage.

Pictures used in the system represent examples of each scoring category. We suggest dorsal, caudal and pectoral fins as the primary fins to monitor for fin damage. As a comprehensive system for the classification of vertebral deformities, similar to that in human medicine has not yet been developed for Atlantic salmon, we suggest a simplified scoring system similar to that used in the RSPCA welfare standards for farmed Atlantic salmon [160].

Cataract damage is classified using an existing and widely used 0-4 scoring scheme [161], see Fig 8.2. The scoring method records the cataract area in relation to the entire lens surface (looking through the pupil along the pupillary/optic disc axis). You can quickly assess large numbers of fish with minimal equipment to get an impression of the severity of the problem. If possible, a selected number of fish should be inspected under darkened conditions (also with better equipment) to give some indication of position, type, development and aetiology. However, it does not record the density of the cataract which can be important and should be annotated separately (T. Wall pers. comm.).

The degree of vaccine side effects in individual fish is often evaluated according to the “Speilberg scale” [162], see Table 8.3 and Fig. 8.4. The Speilberg Scale is widely used as a welfare indicator in the Norwegian aquaculture industry. The scale is based on a visual assessment of the extent and location of clinical changes within the abdominal cavity of the fish and it describes changes related to peritonitis; adhesions between organs, between organs and the abdominal wall and melanin deposits (see also [163] and references therein). A Speilberg score of 3 and above is generally regarded as undesirable.

Table 8.1-1. *Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. (Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. Nilsson. Photos: K. Gismervik, L. H. Stien, J. Nilsson, C. Noble, J. F. Turnbull, P. A. Sæther, I. K. Nerbøvik, I. Simion, B. Tørud, B. Klakegg, R. Andersen, C. Karlsen, K. J. Merok, F. Gregersen)*

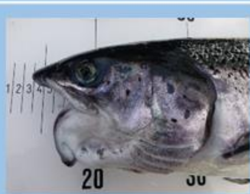
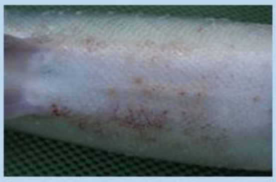
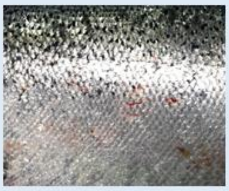
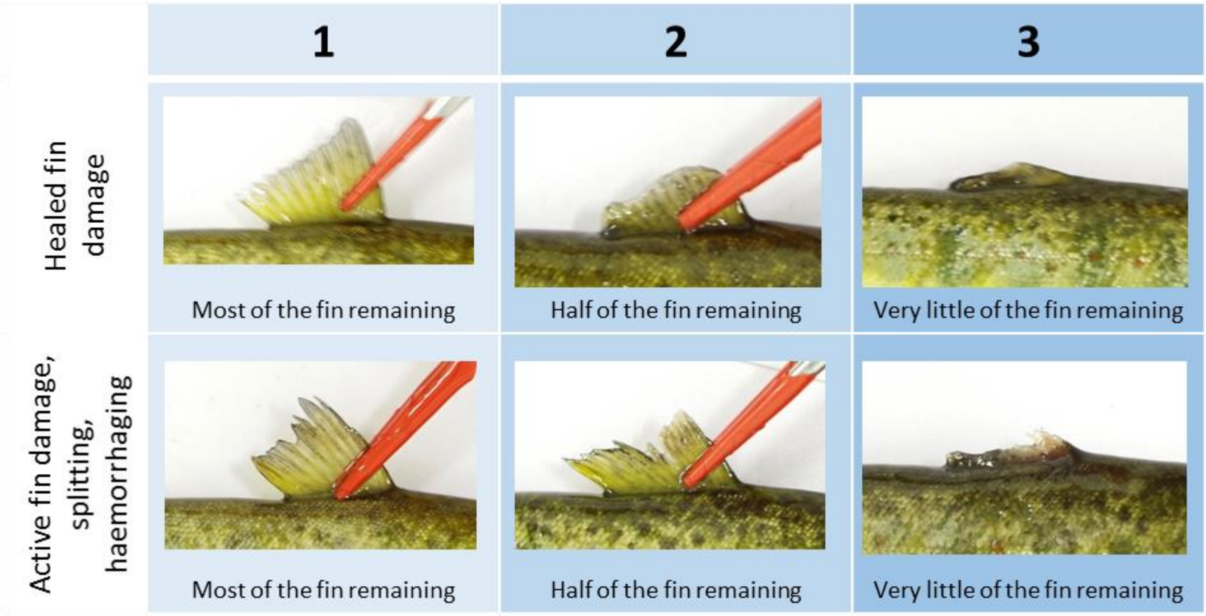
	1	2	3
Eye haemorrhage	 Minor haemorrhages	 Larger haemorrhages, or traumatic injury	 Large haemorrhages / traumatic injury. Eye may be ruptured
Exophthalmia	 Eye protruding a little	 Moderate eye protrusion	 Major eye protrusion
Opercular damage	 Operculum only partly covering gills	 Operculum absent on one of the gills (gill exposed)	 Both opercula absent (both gills exposed)
Snout damage	 Minor wound on snout (either jaw)	 Moderate wound and broken skin on snout	 Large deep and extensive wound. Can cover the whole head
Upper jaw deformity	 Suspected malformation	 Distinct malformation	 Major malformation, jaw pointing backwards
Lower jaw deformity	 Suspected malformation	 Distinct malformation	 Major malformation, jaw pointing backwards

***Table 8.1-2**. Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. (Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. Nilsson. Photos: K. Gismervik, L. H. Stien, J. Nilsson, C. Noble, J. F. Turnbull, P. A. Sæther, I. K. Nerbøvik, I. Simion, B. Tørud, B. Klakegg, R. Andersen, C. Karlsen, K. J. Merok, F. Gregersen)*

	1	2	3
Emaciation	 Potentially emaciated	 Emaciated	 Extremely emaciated
Vertebral deformity	 Signs of deformed spine	 Clearly visible spinal deformity (e.g. short tail)	 Extreme deformity
Skin haemorrhages	 Minor haemorrhaging, often on the belly of the fish	 Large area of haemorrhaging, often coupled with scale loss	 Significant bleeding, often with severe scale loss, wounds and skin edema
Lesions / wounds [1]	 One small wound (< 10 pence piece)[1], subcutaneous tissue intact (no muscle visible)	 Several small wounds	 Large, severe wounds, muscle often exposed (≥ 10 pence piece)
Scale loss	 Loss of individual scales	 Small areas of scale loss (< 10% of the fish)	 Large areas of scale loss (≥ 10% of the fish)
Sea lice infection	 Light infection	 0.05 - 0.08 pre-adult or adult lice cm^{-2} of fish skin	 ≥0.08 pre-adult or adult lice cm^{-2} of fish skin

[1] For pre-smolts "one small wound" should be < 1 cm. NB! Wounds that penetrate the abdominal cavity should be scored as a 3) irrespective of size

__Table 8.1-3__. Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. It is important to differentiate between healed lesions and active lesions. Active lesions indicate an ongoing problem that needs to be addressed (Figure: J. F. Turnbull, C. Noble, D. Izquierdo-Gomez, L. H. Stien, K. Gismervik, J. Nilsson. Photos: J. F. Turnbull)



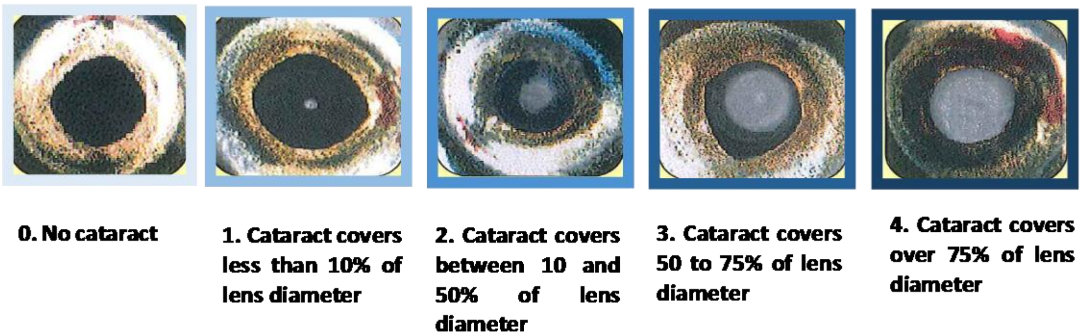


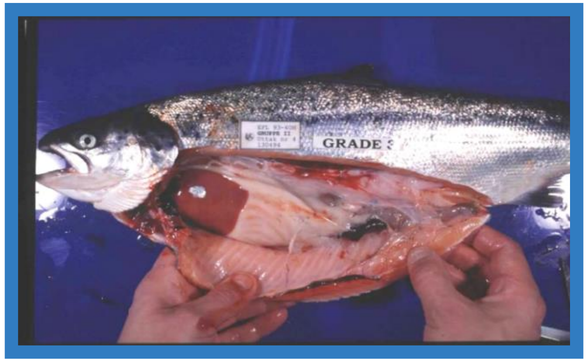
__Fig. 8.2.__ Morphological scheme for diagnosing and classifying eye cataracts in Atlantic salmon Text reproduced from "Wall, T. & Bjerkås, E. 1999. A simplified method of scoring cataracts in fish. Bulletin of the European Association of Fish Pathologists 19(4), 162-165. Copyright, 1999" [161] with permission from the European Association of Fish Pathologists. Figure: David Izquierdo-Gomez. Photos reproduced from "Bass, N. and T. Wall (Undated) A standard procedure for the field monitoring of cataracts in farmed Atlantic salmon and other species. BIM, Irish Sea Fisheries Board, Dun Laoghaire, Co. Dublin, Ireland, 2p." [164] with permission from T. Wall.

Table 8.3. *The Speilberg Scale, reproduced from "Midtlyng et al., 1996, Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (Salmo salar L.) against furunculosis. Fish & Shellfish Immunology 6, 335–350. Copyright 1996" with permission from Elsevier [162].*

Score	Visual appearance of abdominal cavity	Severity of lesions
0	No visible lesions	None
1	Very slight adhesions, most frequently localized close to the injection site. Unlikely to be noticed by laymen during evisceration	No or minor opaquity of peritoneum after evisceration
2	Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall. May be noticed by laymen during evisceration	Only opasicity of peritoneum remaining after manually disconnecting the adhesions
3	Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall. May be noticed by laymen during evisceration	Minor visible lesions after evisceration, which may be removed manually
4	Major adhesions with granuloma, extensively interconnecting internal organs, which thereby appear as one unit. Likely to be noticed by laymen during evisceration	Moderate lesions which may be hard to remove manually
5	Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may carry focal, prominent and/or heavily pigmented lesions or granulomas	Leaving visible damage to the carcass after evisceration and removal of lesions
6	Even more pronounced than 5, often with considerable amounts of melanin. Viscera unremovable without damage to fillet integrity	Leaving major damage to the carcass

1. Very slight adhesions, most frequently localized close to the injection site. Unlikely to be noticed by laymen during evisceration.

2. Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall. May be noticed by laymen during evisceration.



3. Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall. May be noticed by laymen during evisceration.

4. Major adhesions with granuloma, extensively interconnecting internal organs, which thereby appear as one unit. Likely to be noticed by laymen during evisceration

5. Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may carry focal, prominent and/or heavily pigmented lesions or granulomas

6. Even more pronounced than 5, often with considerable amounts of melanin. Viscera irremovable without damage to fillet integrity.

Fig. 8.4. *The Speilberg Scale for intra-abdominal lesions after intraperitoneal vaccination of Atlantic salmon. Photos provided and reproduced with kind permission from Lars Speilberg. Text reproduced from "Midtlyng et al., 1996, Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (Salmo salar L.) against furunculosis. Fish & Shellfish Immunology 6, 335–350. Copyright 1996" with permission from Elsevier [162].*

9 Summary table of which OWIs and LABWIs are fit for purpose for different rearing systems

***Table 9-1**. Where the reviewed welfare indicators are recommended for use in the production systems discussed in Part B of the handbook.*

	Usage area	Production systems						
	WI	Flow through system	Recirculating system	Sea cages	Sea cages with skirt	Submerged sea cages	Snorkel sea cages	Semi-closed system
Environment WIs	Temperature	×	×	×	×	×	×	×
	Salinity	×	×	×	×	×	×	×
	Oxygen	×	×	×	×	×	×	×
	• Total gas pressure		×					
	CO_2	×	×					×
	pH and alkalinity	×	×					×
	Total ammonia nitrogen		×					×
	Nitrite and Nitrate		×				×	
	Turbidity and susp. solids	×	×	×	×	×		×
	Feed Load		×					
	Water current speed	×	×	×	×	×	×	×
	Lighting	×	×	×	×	×	×	×
	Stocking density	×	×	×	×	×	×	×
Group WIs	Mortality rate	×	×	×	×	×	×	×
	Behaviour	×	×	×	×	×	×	×
	• Decreasing echo					×	×	
	Appetite	×	×	×	×	×	×	×
	• Growth	×	×	×	×	×	×	×
	Disease / health	×	×	×	×	×	×	×
	Emaciated fish	×	×	×	×	×	×	×
	Surface activity			×		×	×	
Individual WIs	Gill beat rate	×	×	×				
	Sea lice			×	×	×	×	×
	Gill bleaching and status			×	×	×	×	×
	Condition indices							
	• Condition factor		×	×	×	×	×	×
	• Hepato-somatic index	×	×	×	×	×	×	×
	• Cardio-somatic index	×	×	×	×	×	×	×
		×						
	Feed in intestine	×	×	×	×	×	×	×
	Emaciation state	×	×	×	×	×	×	×
	Sexual maturity state	×	×	×	×	×	×	×
	Smoltification state	×	×	×	×	×	×	×
	Vertebral deformation	×	×	×	×	×	×	×
	Fin damage	×	×	×	×	×	×	×
	Scale loss and skin condition	×	×	×	×	×	×	×
	Mouth jaw wound	×	×	×	×	×	×	×
	Eye haemorrhage and status	×	×	×	×	×	×	×
	Opercula deformation	×	×	×	×	×	×	×
	Mucus	×	×	×	×	×	×	×
	Lactate	×	×					

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Welfare Indicators for farmed Atlantic salmon: Part C – fit for purpose OWIs for different routines and operations

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Contents

1 How to monitor welfare in different routines and operations

The aim of this section of the handbook is to:

- Summarise and review the key scientific findings regarding fit for purpose OWIs for use during different routines and operations.
- Provide pragmatic and practical information on the optimal use of the OWIs, including knowledge based on practical experience.


Monitoring of crowding. Photo: Gismervik©

1.1 Crowding

Salmon are crowded repeatedly throughout the production cycle for various reasons such as vaccination, transport and slaughter. In tanks, draining is the normal method to reduce the water volume and crowd the fish (Figure 1.1-1). Unless the amount of inflowing water is reduced, the water exchange per biomass will not be changed. Still, with very high fish densities the water moves less freely in the tank and increases the risk for local areas of low oxygen. Stress can also increase the need for oxygen. In sea cages, fish are crowded using sweep nets or by forcing the fish into a smaller volume by lifting part or all of the cage. The water exchange per biomass is reduced during crowding in cages and the risk of low oxygen therefore increases unless oxygen is added to the water [1].

Figure 1.1-1. *Smolts crowded in a tank before pumping. Photo: J. Nilsson*

Challenges to fish welfare

- **Swimming and behavioural control**. Crowded fish are confined and restricted in their free swimming and behavioural control, which can lead to stress. Oxygen levels in the water may fall while the oxygen requirements of fish increase with activity levels. Mechanical contact with other individuals and the rearing unit may lead to damage to fins and skin, including scale loss [2].
- **Stress.** All these effects are potentially stressful, and crowding results in stress related physiological responses such as an increase in cortisol [2, 3], glucose [2], lactate [2, 4], and decreased pH in blood [2] and muscles [4].
- **Pre-rigor time and slaughter quality**. High stress levels and muscle activity during crowding may also be detrimental to flesh quality, leading to gaping in the fillet and texture softness [5]. It also reduces rigor mortis time and causes difficulties in the filleting process [4, 6].
- **Ulcers and mortality.** Smolts with skin damage resulting from crowding and pumping during the fresh water stage may develop ulcers and higher mortality after transfer to colder (5°C) sea water compared with warmer water (8°C) [2].
- **Current speed**. Crowding in cages at very low current speed increases the risk of low oxygen [1]. Strong currents may drag on the cage net and change the shape and volume of the cage. As behavioural control is reduced during crowding the fish may have a reduced ability to withstand high current speeds and may be crushed against the cage net.

How to minimise welfare challenges

- Stress levels and the time to recover from stress generally increase with the duration of crowding [2]. The crowding time should therefore be as short as possible. The RSPCA welfare standards for farmed Atlantic salmon [7] state crowding should be no longer than 2 hours.
- Crowding and other handling that may lead to skin damage should be avoided at low water temperatures to reduce the risk of developing winter ulcers and higher mortality [8].
- During crowding, the operation should be monitored closely and adjusted based on welfare indicators such as behaviour [9].
- To reduce the risk of low oxygen, water can be oxygenated during crowding.
- It is important to avoid "pockets" or shallow areas during crowding where fish can get stuck [10].

How to assess welfare during crowding

Physiological welfare indicators such as blood glucose and lactate have certain limitations as they are only detectable in the blood some time (minutes-hours) after the initiation of stress and the values are dependent on the condition/state of the fish in addition to the event itself (see Part A section 3). Measuring lactate and pH can give an indication of stress if the measurements are repeated during the crowding procedure [2] 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.

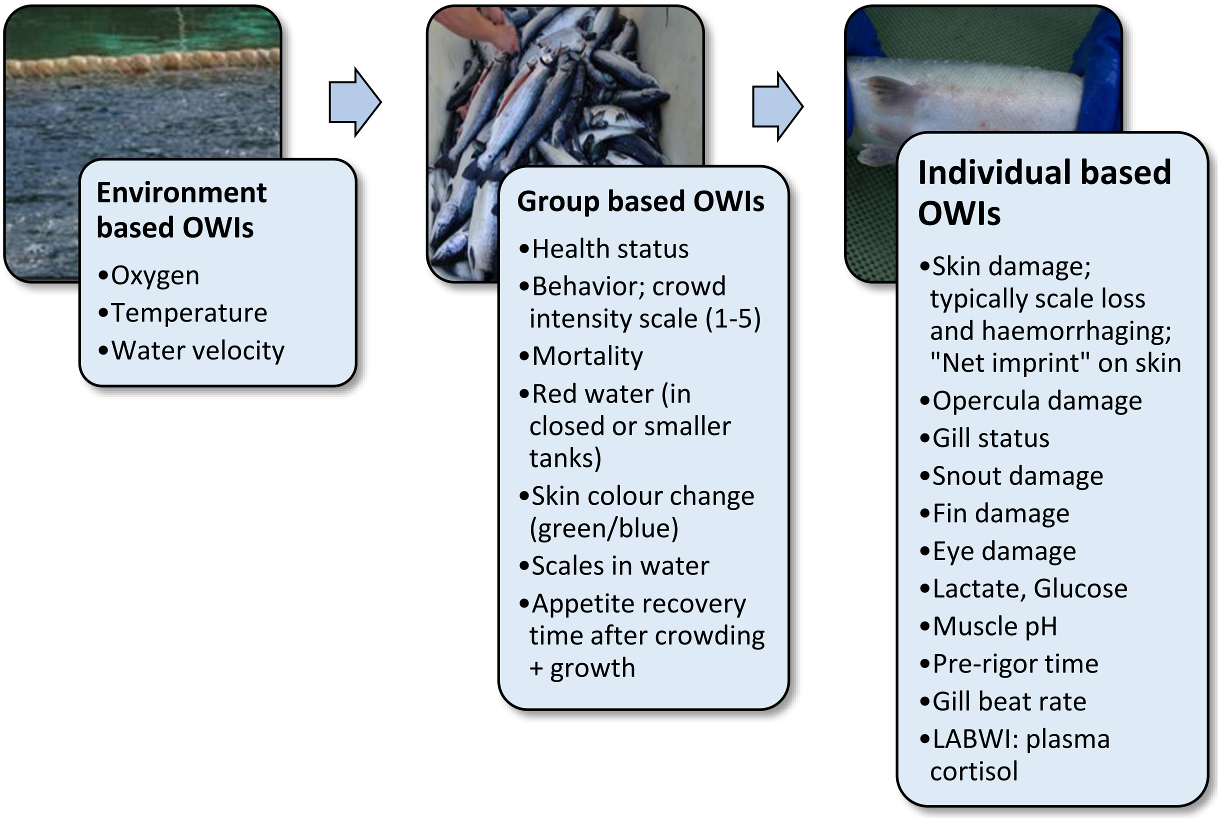


Figure 1.1-2. *Overview of fit for purpose OWIs for crowding. Environment based OWIs address the rearing environment, group based OWIs describe the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration: K. Gismervik.*

Environment based OWIs

Oxygen saturation. When fish density is increased and fish metabolism is elevated due to stress and increased activity during crowding, there is a risk for low oxygen conditions to occur. In sea cages located in stratified fjords low oxygen may arise without crowding or other stressful handling events if fish gather at high densities in preferred environments [11]. Crowding can increase the already high local densities resulting in a greater local oxygen demand. The oxygen requirement of salmon is temperature dependent. For parr and smolts, detailed data of the oxygen concentrations that maintain appetite and aerobic metabolism at different temperatures are not available, but experience does not suggest dramatically different oxygen requirements compared with that of post smolts (see Table 1.1-1). For instance, a limiting oxygen saturation (LOS) of 39% O_2 at 12.5°C has been found for parr [12]. For post-smolts, levels above the dissolved oxygen required for maximal feed intake (DO_{maxFI}) are always safe, while levels must never approach the routine limiting oxygen saturation (LOS) (Table 1.1-1.). As a general precautionary guideline oxygen saturation levels of >80% are often used [13] and the RSPCA welfare standards for farmed Atlantic salmon [7] recommend a minimum of 7mg L^{-1}.

Temperature	DO_{maxFI}	LOS
7	42%	24%
11	53%	33%
15	66%	34%
19	76%	40%

Table 1.1-1*. Showing lower limits for oxygen saturation with maximal feed intake (DO_{maxFI}) and limiting oxygen saturation (LOS) for Atlantic post-smolts of 300-500 g [14].*

Temperature. The metabolism of cold-blooded animals like fish is dependent on the ambient temperature. Every organism needs some energy to maintain body function and thus survive ("maintenance needs"). In addition to this, energy is required for other processes such as physical exertions, dealing with environmental changes, etc. The energy above maintenance needs is the "metabolic scope" and tells you how much "energy reserve" is left for other activities. The energy reserves of fish are highest at optimal temperatures, but decreases sharply when moving towards the lower and upper critical temperature ranges [15]. The optimal temperature for parr is 12-14°C [16], whilst post-smolt fish prefer temperatures around 17°C [17]. Parr can tolerate a wide temperature range from 0 to well above 20°C [18] while the critical temperatures for post-smolts are around 6 ° C and 18 ° C [11] (see Part A section 4.1.1 for more information). It is therefore more difficult for the fish to increase their metabolism when they are stressed near these temperature ranges. The solubility of oxygen also declines with increasing temperature, so that warmer water contains less oxygen than colder water with the same saturation. Low temperatures also increases the risk of winter ulcers. Damage from handling is often the initiating factor, leading to secondary infections with bacteria such as *Moritella viscosa* (see Table 3.1.5-2 in Part A for more information on winter ulcers) [19, 20].

Group based OWIs

Health Status. The health status of the fish must be known prior to crowding to ensure it can withstand the procedure.

Behaviour. There is little literature on salmon behaviour during crowding beyond that which is observable at the surface. However, behaviour is important and both the RSPCA welfare standards for farmed Atlantic salmon [7] and Mejdell et al., [9] employ a crowding intensity scale, based on surface observations (Figure 1.1-3). The goal is to have calm swimming behaviour with no dorsal fins breaking the surface of the water. Level 4 and 5 have been classified as unacceptable and not compliant with regulations by the Norwegian authorities [10]. In a study of a commercial crowding prior to slaughter, Erikson et al., [3] used a Remote Operated Vehicle to monitor behaviour below the surface and cameras in the cages and at the surface. They did not observe panic behaviour during crowding. They also concluded that blood based LABWIs, like cortisol and pH and the OWI lactate demonstrated an acute stress response that they did not detect from the behaviour of the fish. Elevated lactate levels in other studies [2] suggest high activity levels during crowding. Panic behaviour and burst swimming utilises the white muscles resulting in higher levels of lactate, and can also increase the risk of mechanical damage. Therefore, operators should be aware that even before panic behaviour is observed the fish may be stressed.

Mortality. Should be followed closely and on a regular basis following crowding to retrospectively assess problems or welfare threats associated with the procedure.

Return of appetite. The time it takes for appetite to return should be closely monitored after crowding. A reduction or loss of appetite can be caused by the initiation of a stress response [21]. The time it takes for appetite to return after e.g. handling can therefore, also be used as an OWI since it reflects how well the fish have dealt with the stressor. Appetite is easy to measure qualitatively by observing the fish when feed is offered.

Growth. Growth can be affected by short- or long-term stress. Acute changes in growth can be used as a warning system for potential problems, especially when the farmer has a robust system for monitoring growth.

Red water. According to practical experience, the crowding of post-smolts in closed and smaller containers can make it possible to detect bleeding as a colour change in water, so called "red water". This can also be observed during chilling of live fish in refrigerated seawater (RSW) tanks in slaughter houses. It is never a good sign and the cause should be investigated (see Part A section 3.1.6 for more information).

Scales in water. Indicates scale loss and damage to the mucus and the skin which can cause osmoregulatory problems and also secondary infections. All injuries during crowding indicate reduced welfare and should be investigated further. Rough handling or poorly maintained and managed equipment with protruding and rough edges may be a causal factor (see Part A section 3.1.6 for more information).

Skin colour change. The skin colour can change to green/blue during stressful crowding and changes in skin colour can therefore be qualitatively monitored from the start to the end of the crowding procedure [9].

Individual based OWIs

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.

Skin condition. Physical contact with other individuals, the rearing unit or other equipment may lead to various forms of skin damage, including e.g. "net imprinting" on the skin. Small haemorrhages in the skin can typically be seen ventrally. Scale loss may be observed both as free scales in the water and as areas on the fish where scales are missing. Stressful and long lasting crowding can lead to mucus loss and the skin colour can change to green/blue [9]. Since mucus and scales protect the fish from the environment and have a barrier function, the loss of these barriers can give rise to osmoregulation problems and infections. One should pay especially attention to the crowding of smolts, as experienced based knowledge has shown them to be especially vulnerable for scale loss during sea transfer [22]. Scales are more loosely embedded due to the smoltification process and the consequences of scale loss can be severe due to osmoregulation failure [22].

Opercular damage and gill status. Opercular damage includes broken or shortened or even the lack of opercula. It is important to distinguish between acute opercular injuries that may have occurred during crowding and other factors affecting the operculum, thus making the gills more vulnerable during crowding. To get a measure of gill status, an operator can score changes on the gill surface, visible as "white patches" (total gill score). If a case of AGD is suspected, it may also be relevant to crowding as long term diseases (such as AGD) increase the risk of mortality during treatment [23]. Gill bleeding should also be monitored in relation to mechanical injuries [24].

Snout damage. Can occur related to handling procedures, where the fish get forced against the net or the snout hits hard surfaces.

Eye damage. The eyes are especially vulnerable to mechanical trauma, or desiccation during handling, due to their position where they protrude slightly from the head and with no eyelids or self-lubrication for protection. Exophthalmus, also known as "pop eye" is recognized as an unspecific sign of disease that should be investigated further (see Part A, section 3.2.12). Exophthalmus increases the risk of mechanical damage.

Fin damage. Physical contact may also lead to damaged fins, especially fin splitting. As with other injuries it is important to differentiate between an active injury that occurred during crowding and old injuries.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Lactate. Struggling, panic and burst swimming increases anaerobic muscle activity, thus increasing lactate in the blood [2, 25, 26]. Lactate should stay below 6 mmol/l [2]. It is easily measured with handheld apparatus, but samples should be taken approximately one hour after muscle activity. However, Erikson et al., [3] did not find any significant correlation between crowding time and lactate levels.

Muscle pH. Increased stress/muscle activity produces more lactic acid which in turn reduces muscle pH [27]. A lowering in muscle pH that occurs gradually after death is desirable, as it contributes to increased shelf life.

Pre-rigor time. High or prolonged stress during crowding may lead to a shorter pre-rigor time [4]. Veiseth et al., [4] found that an active swimming period after the crowding procedure helped reduce stress and increased pre-rigor time. Reduced pre-rigor time is mostly used in connection with the slaughter process.

Glucose can be used as an OWI for crowding [28]. Elevation in plasma glucose is a relatively slow response to stress and peaks after around 3-6 hours in salmon [29], although the response is also dependent on the feeding status, diet type and other factors. Glucose levels should therefore be compared with pre-stress levels rather than any "standard stress levels". However, Gismervik et al., [24] did not find any changes in glucose from rest to crowding.

Gill beat rate. Naturally increases as the fishes' metabolism rises during activity and stress. Gill beat rate has been used as an OWI for crowding by Erikson et al., [3] where the authors found beat rates rising from 55-60 prior to crowding to around 80 beats per minute during crowding. This equated to an increase in gill beat rate of around 20-50% during crowding. Gill beat rate assessment is best carried out if the fish are swimming slowly or static. Qualitative changes in gill beat rate can be done from above the water, if visibility is good, or also using underwater cameras e.g. [3]. Changes in gill beat rate are difficult to quantify on the farm and usually must be assessed retrospectively e.g. from video footage. If the fish are relatively static, this can also be carried out manually by eye (e.g. with a stopwatch) but the results may be unreliable. Quantitative analysis of gill beat rate is therefore a LABWI. Changes in absolute gill beat rates can also be a problematic LABWI as different water states, velocities etc., can affect absolute values. We suggest using the percentage change in gill beat rate measured before, during and after a routine as a better LABWI as this goes some way towards circumventing these effects.

LABWI: Plasma cortisol is not an OWI, but a LABWI. We know that crowding stresses the fish and leads to a stress response [4]. Plasma cortisol measurements can be used to see how long the fish is affected by crowding stress and when it returns to resting state after the procedure [24] (see also Part A, section 3.2.16).

1. Goal: low stress, no vigorous activity

- ✓ Fish in the sides of the crowd swimming slowly
- ✓ Normal swimming behaviour, but not all in the same direction
- ✓ No dorsal fins on surface
- ✓ No white sides on surface

2. Acceptable: some fins on surface

- ✓ Normal swimming behaviour at suction point, low stress
- ✓ Few dorsal fins on surface
- ✓ No white sides on surface

3. Undesirable:

- Over-excited swimming behaviour (different directions)
- More than 20 dorsal fins on surface
- Some white sides constantly on surface

4. Unacceptable: overcrowding

- 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. Unacceptable: extreme overcrowding

- Whole crowd boiling
- Potential for large fish kill without rapid release
- *Panic in the population, the fish are exhausted*
- *Many fish floating on their side*

Figure 1.1-3. *Illustration of different levels of crowding intensity during surface monitoring of behaviour according to the RSPCA [7] and Mejdell et al., [9]. Classification terminology is in accordance with the RSPCA [7] and Mejdell et al., [9]. Level 1 is the goal and level 4 and 5 have been classified as unacceptable and not compliant with regulations by the Norwegian authorities [10]. Figure: D. Izquierdo-Gomez, C. Noble and K. Gismervik. Pictures and text (non-italics) reproduced with kind permission from Alistair Smart, Smart Aqua, Hazelwood Park, South Australia and the RSPCA [7]. Additional italic bullet points (for Level 5) reproduced with permission from Mejdell et al., [9].*

1.2 Pumping

Pumping is widely used during the transport and transfer of fish. In most situations pumping is performed in association with other handling procedures (e.g. crowding, grading, vaccination, some lice treatments) resulting in repeated handling stress [27, 30]. The pumping of both juvenile and adult fish is usually done with vacuum pumps. The fish are pumped under negative pressure ("vacuum") into a pipe whose dimensions should be adjusted in accordance with fish size. Swimming behaviour is restricted in the pipe and if the pumping stops the water quality in the pipe can rapidly deteriorate. The vacuum (0.3 – 0.7 bar for adult fish) continues until the fish are inside the pump chamber, from where they are pushed (1.5 – 2.0 bar for adult fish) out and into a pipe again. Pumping does not appear to harm the fish when performed correctly [25]. Most new technologies developed for treating or handling fish include pumping at some point and this should also be considered when assessing the welfare implications of new technologies [8, 31, 32].

Challenges to fish welfare

- **Pumping speed.** A correct pumping speed should guide fish smoothly through the pipe without the fish struggling. A pumping speed that is too low allows the fish to turn in the pipe and they may try to swim in the wrong direction or hold station within the pipe. A pumping speed that is too high may result in collisions and scale loss [2, 33]. Pumping speed should be above the critical swimming speed (U_{crit}) [34] (see Part A, section 4.2.1) to prevent fish holding station in the current and getting exhausted.
- **Height.** Experiments have failed to show negative effects of pumping heights [2, 25]. However, most farmers place the pumps close to the pump inlet, with good results.
- **Equipment.** Large discrepancies between pipe dimensions and fish size and also valves and bends in the pipe (Figure 1.2-1) may result in injuries to the opercula and fins. Bends may also result in external morphological damage as the fish collide with equipment and conspecifics.
- **Repeated pumping and handling** may increase the stress load on the fish [25, 27, 30].
- **Pumping of weak fish.** Pumping should only be done with fish that are healthy and robust and able to withstand the procedure.
- **Low pressure (vacuum).** Experiments where fish were pumped under low vacuum pressure did not show any negative effects or injuries to the fish [26]. However, blood (red water) can occasionally be observed in the pumping chambers and the authors (Espmark et al., [26]) concluded that this was not caused by the low pressure alone, but rather from mechanical injuries to the opercula and gills resulting from collisions. As the swim bladder expands when the surrounding pressure decreases in the vacuum pump, salmon release air from the bladder [26] which will negatively affect buoyancy until the fish have refilled the bladder.

***Figure 1.2-1**. Pipe bends may cause damage to the fish. Photo: Å. M. Espmark*

How to minimise welfare challenges

Most of the risk factors listed above may be reduced with a better knowledge and awareness of how pumping is best performed. The operator should ensure that i) the equipment has been updated and has undergone service, ii) the pipes are suitable for the size of fish, iii) there are no rough surfaces, bends and valves inside the pump or pipes that can harm the fish coming in at high speed, iv) the fish are not stuck inside the pump if the pumping is paused or stopped, and v) the operator can monitor and adjust pumping speed to ensure the fish are drifting easily forwards through the pump.

How to assess welfare during pumping

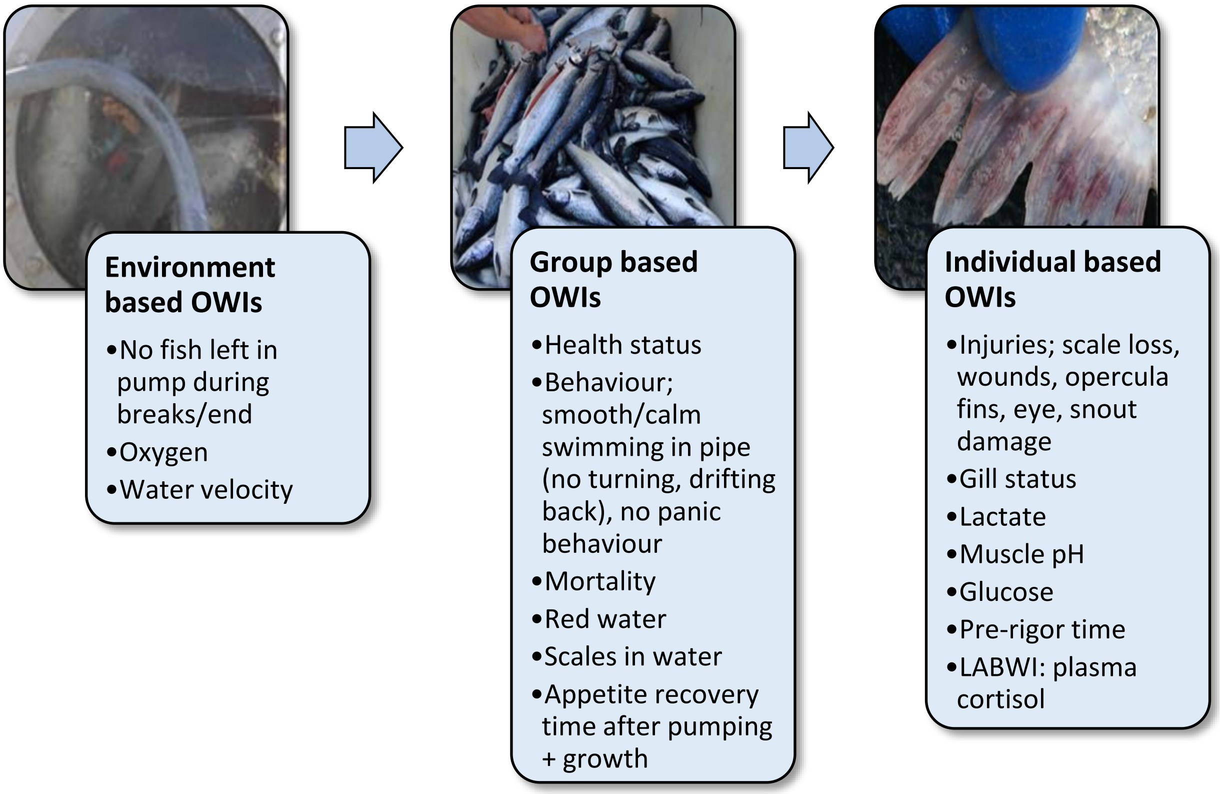


Figure 1.2-2. *Overview of fit for purpose OWIs for pumping. Environment based OWIs address the rearing environment, group based OWIs describe the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration: K. Gismervik. Photo of pump: Å. Espmark.*

Environment based OWIs

Oxygen. If the pumping stops, for any reason, the oxygen level will decrease inside the pipe and can rapidly reach levels that are harmful to the fish. One example where the pumping can be repeatedly stopped is around slaughter. For example, if the slaughter line is full the slaughter facility can stop the intake of fish. If communication between the slaughter line and the waiting cage is poor there can be a delay in reporting this stoppage, resulting in an accumulation of fish in the pipe. For parr and smolts, detailed data of the oxygen concentrations that maintain appetite and aerobic metabolism at different temperatures are not available, but experience does not suggest dramatically different oxygen requirements compared with that of post smolts (see Table 1.2-1.). For instance, a limiting oxygen saturation (LOS) of 39% O_2 at 12.5°C has been found for parr [12]. For post-smolts, levels above the dissolved oxygen required for maximal feed intake (DO_{maxFI}) are always safe, while levels must never approach the routine limiting oxygen saturation (LOS) [14] (Table 1.2-1.). As a general precautionary guideline, oxygen saturation levels of >80% are often used [13] and the RSPCA welfare standards for farmed Atlantic salmon [7] recommend a minimum of 7mg L^{-1}.

Temperature	DO_{maxFI}	LOS
7	42%	24%
11	53%	33%
15	66%	34%
19	76%	40%

Table 1.2-1. *Showing lower limits for oxygen saturation with maximal feed intake (DO_{maxFI}) and limiting oxygen saturation (LOS) for Atlantic post-smolts of 300-500 g [14].*

No fish left in pump during breaks/at the end of the procedure. The operator must ensure that fish are not stuck inside the pump if pumping is stopped.

Water velocity. The water velocity within the pump should be high enough to avoid fish swimming against the water until fatigued and should therefore be higher than the critical swimming speed [34] (U_{crit}, see Part A section 4.2.1). On the other hand, a water velocity that is too high may lead to fish damage. The upper limit for the speed depends on the equipment used, the sharpness of bends, the risk of hitting walls when exiting the pump etc. Measuring current velocity with a current meter inside the hose may be difficult, but by estimating the amount of water passing per second (time to fill up a known volume, flow rate in L s^{-1}), current velocity can be calculated as:

$$V = \frac{10 * Flow}{(3.14 * (\frac{Diameter}{200})^2)}$$

Where *V* is the current velocity in cm s^{-1}, *Flow* is flow rate in L s^{-1} and *Diameter* is the inner diameter of the hose in mm.

Group based OWIs

Health Status. The health status of the fish must be known prior to pumping to ensure it can withstand the procedure.

Mortality should be followed closely and on a regular basis following pumping to retrospectively assess problems or welfare threats associated with the procedure.

Return of appetite the time it takes for appetite to return should be closely monitored after pumping. A reduction or loss of appetite can be caused by the initiation of a stress response [21]. The time it takes for appetite to return after e.g. handling can therefore also be used as an OWI as it can reflect how well the fish have dealt with the stressor. Appetite is easy to measure qualitatively by observing the fish when feed is offered.

Growth can be affected by short-term or chronic stress. Acute changes in growth can be used as an early warning system for potential problems, particularly when the farmer has robust growth monitoring practices.

Red water. According to practical experience, blood (red water) can occasionally be observed in the pumping chambers, probably as a result of gill bleeding. Red water is never a good sign, and the cause should be investigated (see Part A section 3 and Part C section 1.12 for more information).

Scales in water. Indicates scale loss and damage to the mucus and the skin which can cause osmoregulatory problems and also secondary infections. All injuries during crowding indicates reduced welfare and should be investigated further. Rough handling and poorly maintained and managed equipment with protruding and rough edges may be a causal factor (see Part A section 3.1.6 for more information).

Behaviour. If the pipe is transparent, it is possible to observe the behaviour of the fish inside the pipe [26] (Fig 1.2-3). Swimming should be smooth and calm and the fish should not be very crowded in the pump. Undesirable behaviours include fish that remain in one place, are able to swim upstream against the flow, or drift backwards. Other signs of abnormal behaviour include fish swimming on their side or gasping behaviour. It is also possible to observe fish inside some pumps (e.g. Fig 1.2-4). Fish should not overtly struggle during pumping.

Figure 1.2-3. *The behaviour of smolts during pumping can be monitored through a transparent hose. Photo: Å. M. Espmark*

Figure 1.2-4. *The behaviour of fish inside the pump. There should not be too much panic activity in the pump and no red water should be seen. Photo: Å. M. Espmark*

Individual based OWIs

Skin condition. Salmon may lose scales and be wounded by high pumping speed and the incorrect use of equipment [2, 33] and handling trauma, such as cuts or crush injuries, can be caused by pumping [8, 32]. Small haemorrhages in the skin can typically be seen ventrally. Scale loss may be observed both as free scales in the water and as areas on the fish where scales are missing. Stressful and long lasting crowding can lead to mucus loss and the skin colour can change to green/blue [9]. Since mucus and scales protect the fish from the environment and have a barrier function, the loss of these barriers can give rise to osmoregulation problems and infections. Healing is dependent on temperature and environmental conditions, and wounds can take over 3 months to heal [22]. One should pay especially attention to the pumping of smolts, as experienced based knowledge has shown them to be especially vulnerable for scale loss during sea transfer [22]. Scales are more loosely embedded due to the smoltification process and the consequences of scale loss can be severe due to osmoregulation failure [22].

Opercular damage and gill status. Opercular damage includes broken, eroded or even the lack of opercula. It is important to distinguish between acute opercular injuries that may have occurred during pumping and other factors affecting the operculum, thus making the gills more vulnerable during the procedure. To get a measure of gill status, an operator can score changes on the gill surface, visible as "white patches" (total gill score). If a case of AGD is suspected, it may also be relevant to pumping as long term diseases (such as AGD) increase the risk of mortality during treatment [23]. Gill bleeding should also be monitored in relation to mechanical injuries [24].

Snout damage. Can occur related to handling procedures, where the fish get forced against the net or the snout hits hard surfaces.

Eye damage. The eyes are especially vulnerable to mechanical trauma, or desiccation during handling, due to their position where they protrude slightly from the head and with no eyelids or self-lubrication for protection. Exophthalmus, also known as "pop eye" is recognized as an unspecific sign of disease that should be investigated further (see Part A, section 3.2.12). Exophthalmus increases the risk of mechanical damage.

Fin damage. Physical contact may also lead to damaged fins, especially fin splitting. Fin damage has been recorded during pumping, and may be caused by collisions and the incorrect use of equipment. As with other injuries, it is important to differentiate between an active injury that occurred during pumping and old injuries.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Lactate. Struggling, panic and burst swimming increases anaerobic muscle activity, thus increasing lactate in the blood [2, 25, 26]. Lactate should stay below 6 mmol/l [2]. It is easily measured with handheld apparatus, but samples should be taken approximately one hour after muscle activity. Erikson et al., [3] found a significant relationship between lactate and pumping.

Muscle pH. Increased stress/muscle activity produces more lactic acid which in turn reduces muscle pH [27]. A lowering in muscle pH that occurs gradually after death is desirable, as it contributes to increased shelf life.

Pre-rigor time. Pumping prior to slaughter may shorten the pre-rigor time [26, 27].

Glucose can be used as an OWI for crowding [28] and may also be suitable for pumping. An elevation in plasma glucose is a relatively slow response to stress and peaks after around 3-6 hours in salmon [29], although the response is also dependent on feeding status, diet type and other factors. Glucose levels should therefore be compared with pre-stress levels rather than any "standard stress levels".

LABWI: Plasma cortisol is not an OWI, but a LABWI. We know that crowding stresses the fish and leads to a stress response [4] and it can therefore be a suitable indicator for pumping. Plasma cortisol measurements can be used to see how long the fish is affected by a stressor and when it returns to resting state after the procedure [24] (see also Part A, section 3.2.16).

1.3 Slaughter - stunning and killing in connection with slaughter

The fish must be unconscious during bleeding and remain unconscious until death. The purpose is to avoid the fish feeling pain and fear during bleeding and as they die. However, what happens to the fish during the time between the production cage and being stunned is also important, both for the sake of fish welfare and for product quality. Crowding, pumping, potentially low oxygen levels and air exposure causes stress to the fish and increases the risk of injuries. If the fish passes through sharp bends in the pipes at high speed it can cause injuries and haemorrhaging. Norwegian regulations require the equipment to be documented in terms of welfare. The stunning and killing equipment shall be operated, inspected and maintained by competent personnel with adequate training [9]. Fish welfare must be documented through control procedures. For Norwegian farmed salmonids, two different methods for stunning are used today; electrical stunning and percussive stunning. These methods differ in relation to risk factors for fish welfare. Electrical stunning uses electricity to "knock out" the brain activity, so the fish loses consciousness and thus sensibility (Figure 1.3-1). Electrical current is perceived by all animals as highly uncomfortable, and it is therefore important that the electricity is immediately passed through the brain and the fish is rendered insensible immediately [9]. Percussive stunning utilises a hard blow to the top of the skull that causes concussion and a loss of consciousness. A non-penetrating bolt is used for the percussive stunning of salmonids [9]. The energy of the blow is determined by the weight of the bolt and its speed. The fish will often die of brain damage. Manual clubbing with a club or "priest" should be available as a back-up for emergency use.

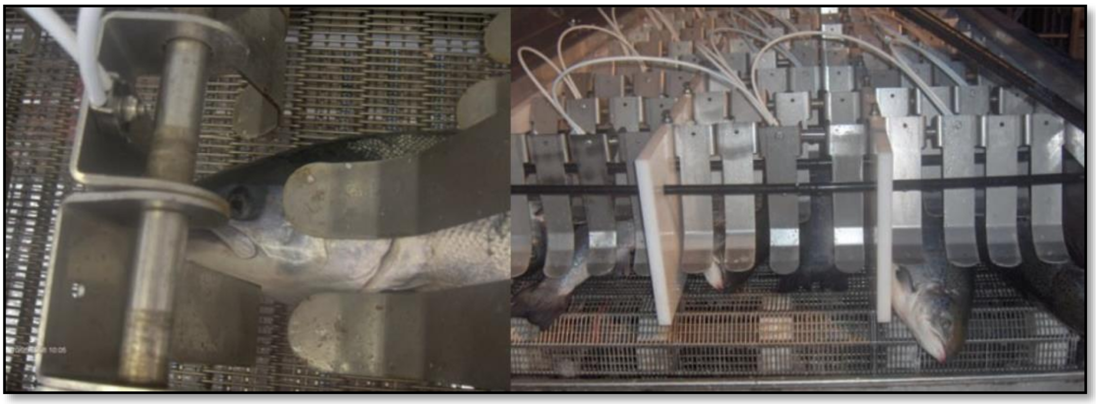


***Figure 1.3-1**. Illustrations of slaughter using electrical stunning [9]. Electricity passes from the metal plates, through the fish and to the surface. The picture on the left shows the plates touching the fish, and the picture on the right shows an example of where the fish is not correctly orientated in the machine, emerging tail first (this is not acceptable for welfare). Reproduced with permission from C. M. Mejdell.*

Challenges to fish welfare

- **General handling.** During slaughter, the fish can be injured during crowding and pumping (see Part C sections 1.1 and 1.2), particularly from sharp bends in the pipes or sharp edges on the equipment. See the later section on Individual based OWIs for how such injuries can be detected.

Electrical stunning

- In systems that handle the fish out of water the operator should make sure that the fish enters the stunner head first [35]. Air exposure after drainage and before euthanizing must be as short as possible [36]. The electricity must have sufficient power to cause the intended "knock out" immediately. There is a balance between the effects of stunning and potential damage to the flesh. Effective stunning is not only about voltage and current but also other parameters such as frequency (Hz) [10]. Electrostimulation of the muscles shortens pre-rigor time.
- Electrical stunning is, in principle, reversible and the fish can potentially wake up again within seconds or minutes. It is therefore important that the fish is bled properly and within a few seconds after stunning so that the fish die of blood loss before the effect of the stunning wears off [9, 36].
- In systems where electricity also passes through the heart of the fish it can cause heart rhythm deficits and cardiac arrest. Electrostimulation of the muscles shortens pre-rigor time (time to death). Electrical stunning can be combined with a percussive blow to ensure the duration of anaesthesia is long enough [9].
- There must be control and backup equipment for stunning and bleeding before transfer to the bleeding site.

Percussive stunning

- If the percussive blow is too weak or strikes the wrong part of the fish, it may not be rendered unconscious or may recover if it is not bled rapidly [9].
- The machine delivering the percussive blow must be adjusted according to fish size. Fish that are too large, sexually mature or too small must be sorted manually.
- The operator must ensure that fish enter the machine singly and with the correct orientation [9].
- Swim-in systems require that the fish are in good condition and not exhausted. A very long pre-rigor time can be achieved using this method, if the fish are treated gently [9].
- There must be control and backup equipment for stunning and bleeding before transfer to the bleeding site.

How to evaluate welfare during slaughter

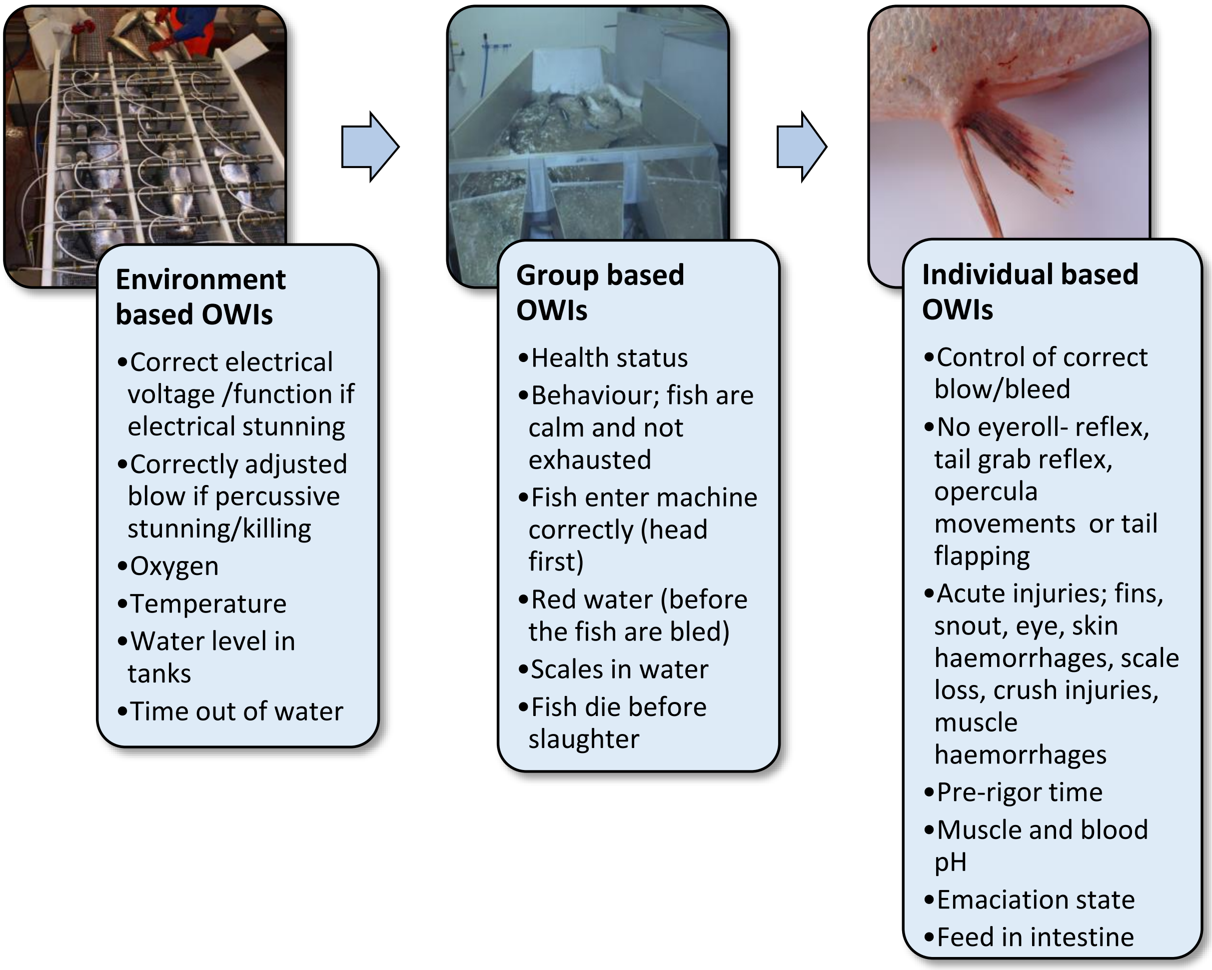


Figure 1.3-2. *Overview of fit for purpose OWIs for slaughter. Environment based OWIs address the stunning machines and environmental parameters in different holding tanks, group based OWIs apply to the group as a whole by observation of the slaughter process, while individual based OWIs are based on sampling individual fish. Photos and illustration: K. Gismervik. Group based OWI photo: C. M. Mejdell.*

Environment based OWIs

Correct electrical voltage/function if electrical stunning. Follow the manufacturer's manuals and update based on practical experience. See also Norwegian authorities guidance and interpretations of the slaughter regulation [10].

Correctly adjusted blow if percussive stunning/killing. Follow the manufacturer's manuals and update based on practical experience. Make sure the machine is adjusted to the size of the fish.

Oxygen saturation and temperature. The operator must ensure good water quality in the pipes and tanks, and routines for monitoring oxygen levels should be in place. Levels above the dissolved oxygen required for maximal feed intake (DO_{maxFI}) are always safe, while levels must never approach the limiting oxygen saturation (LOS) [14] (Table 1.1-1.). As a general precautionary guideline, oxygen saturation levels of >80% are often used [13] and the RSPCA welfare standards for farmed Atlantic salmon [7] recommend a minimum limit of 7mg L^{-1}. The solubility of oxygen decreases with increasing temperature, so that warmer water contains less oxygen than colder water with the same saturation rate. Salmon also react to acute changes in temperature such as increases in water temperature [37]. With regard to temperature drops, handling can cause more stress than the temperature drop itself [38]. The lower and upper critical temperature range is around 6°C and 18°C for post-smolts e.g. [11]. It is therefore extra challenging for the fish to increase metabolism during stress near these temperature ranges.

Water level in tanks must also be monitored to ensure the fish are covered in water and that the tanks for orienting the fish are working properly [35].

Time out of water. Air exposure should be minimised. The RSPCA welfare standards for farmed Atlantic salmon recommend a maximum exposure time of 15 seconds [7].

Group based OWIs

Health Status. The health status of the fish must be known before slaughter. This is to ensure that sick and injured fish are slaughtered as soon as possible [10]. It may also be appropriate to adjust the rate of slaughter in relation to health status.

Behaviour. Fish should be calm with no evidence of tail flapping or sudden movements, and the fish should not show signs of exhaustion or problems with balance when swimming. The fish should enter the machine correctly (head first during percussive/ electrical stunning in air). Tanks for orientation should not be too crowded, to avoid fish being pushed in the wrong direction by other individuals [35].

Red water. Poor crowding/pumping and other handling of the fish before slaughter can cause gill injuries or other wounds that bleed. One indicator for this can be a colour change in the water which can be observed during the chilling of live fish in refrigerated seawater (RSW) tanks in slaughter houses. It can be particularly obvious in tanks that are recycling the water. It is never a good sign and the cause should be investigated (see Part A section 3.1.6 for more information).

Scales in water. Indicates scale loss and damage to the mucus and the skin. Rough handling and poorly maintained and managed equipment with protruding and rough edges may be a causal factor (see Part A section 3.1.6 for more information).

Fish dying before slaughter. If you see dead or moribund fish in the process line before slaughtering try to find the cause e.g. the severity of the crowding process (see Part C, section 1.1). Moribund fish should be removed from the slaughter line as soon as possible and slaughtered manually as there is a danger that they will not enter the machines in the correct way.

Individual based OWIs

Control of correct blow/bleed. The percussive blow should be to the top of the head, in the middle and slightly behind the eyes. It should not fracture the skull as energy is partly absorbed instead of concentrating it on the brain for producing concussion with loss of consciousness. Haemorrhaging in the central parts of the brain are considered important for the desired effect and can also be seen macroscopically by opening the skull and brain and by visual inspection of the blow location [9, 10]. Cutting the aorta or the majority of gill arches on both sides is considered good practice during bleeding [39].

Control of unconsciousness. You should confirm that the salmon are unconscious or dead before they are bled or subjected to other slaughter processes. Simple reflex indicators such as eye roll and the ability to flip upright can easily be used as direct indicators of stress and can be evaluated individually or as an index [40]. The animal is classified as insensible if responses to these indicators are lacking [41, 42]. The vestibulo-ocular reflex (VER; the "eye roll") is the last reflex the fish loses during anaesthesia and is the first reflex to reappear after recovery [43], see Figure 1.3-3. Rhythmical opercula movements should also be absent in insensible fish. One occasional gasp sometimes occurs even in fish that are completely insensible, but if it happens in many fish or happens repeatedly on a single fish it may not be unconscious. Another reflex is the "tail-grab reflex" (i.e. grabbing the fish's tail and seeing if it attempts to escape [40]). The operator can also assess whether the fish responds to a needle puncture in the lip or skin and also if the fish attempts to adjust to normal position or make swimming movements if it is put into water. Reflex indices are simple, rapid and inexpensive and it is relatively easy to train people how to use them (e.g. at the slaughter facility).

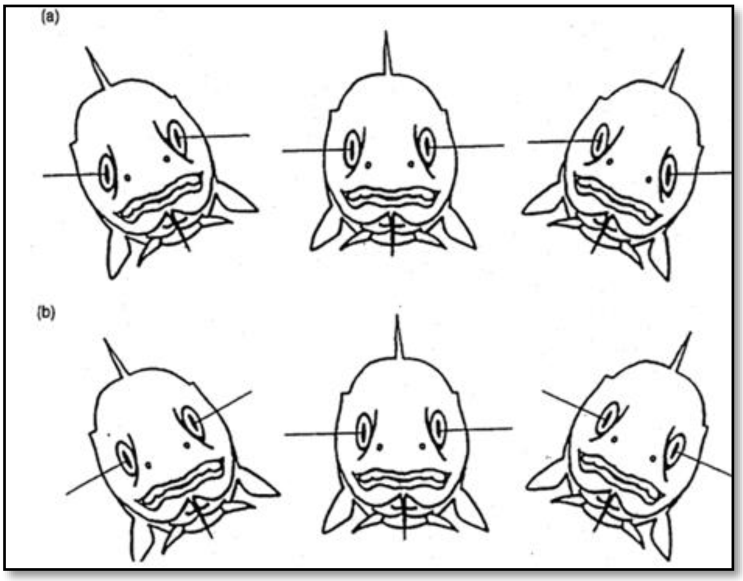


Figure 1.3-3*. Illustration of an eye roll reflex of a) living and b) dead cod. Reproduced from "Kestin, S.C., J.W. Van de Vis and D.H.F. Robb (2002) Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them. Veterinary Record. 150(10): p. 302-307. Copyright 2002", with permission from BMJ Publishing Group Limited [43]. If the fish is conscious it will try to keep the eyes in the horizontal plane if it is moved from side to side (A). If the fish is dead or insensible, the eyes do not move in relation to their changing position (B).*

Acute injuries. Equipment malfunction or hard handling may result in haemorrhages (red water such as in a live cooling tank), fin splitting, crush injuries, bleeding and snout injuries, and bruising under skin that can be visually checked after skin removal [9]. It is necessary to make the distinction between active damage and damage that occurred in the rearing pen.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Pre-rigor time. Either severe or long lasting stress can result in a shorter pre-rigor time than expected, resulting in problems during processing, e.g. during filleting. A short pre-rigor time should be investigated to detect any problems before or during slaughter [9].

Muscle and blood pH. Fish with high stress / muscle activity exhibit reduced pH in the muscle due to lactic acid. In cases of prolonged activity, the lactate may also affect the pH in the blood, but the blood has a good buffer capacity and a pH decrease will only be visible when the buffer capacity is exceeded [9]. If the fish has been stressed / exhausted before slaughter, it may have used up its energy reserves in the muscle, causing a rapid drop in muscle pH and strong rigor mortis. A lowering in muscle pH that occurs gradually after death is desirable, as it contributes to increased shelf life. It is not advisable to use muscle pH after slaughter as the only welfare indicator and it is very important to start monitoring it immediately to get a correct zero point [44] and to get a final pH. Mejdell et al., [9] reported that the pH in white muscles of rested salmon is in the range of pH 7.4-7.6. The lowest possible muscle pH of live salmon (in vivo) is pH 6.6-6.7. Blood is usually expected to have a pH of about 7.7-7.8 [9].

Emaciation state. During the slaughtering process, the proportion of emaciated fish can be assessed by looking at the size and shape of the fish, abdominal fat and also the fat around its organs. This may say something retrospectively about what the fish has experienced.

Feed in the intestine. Feed in the intestine often indicates that the fish has eaten during the last one to two days [45, 46], but this depends on the fish size and temperature. On slaughtered fish it is easy to check if there are feed residues in the stomach and intestines. Such a check can be used to evaluate whether the starvation time is sufficient to avoid contamination but is no longer than necessary for welfare reasons [47]. See also Part C, section 1.9 for more information.

Welfare checkpoints when using electrical and percussive stunning [9, 35]

Electrical stunning:

- ✓ Check that all electrical parameters are in accordance with the manufacturer's instructions.
- ✓ Check that the electricity passes through the head of the fish before any other part of its body.

Percussive stunning:

- ✓ Check that the fish enters the right way in (or out) of the stunning machine.
- ✓ Check that the blow from the bolt is in the right place over the brain.
- ✓ Record the number of fish that failed to be hit or if the blow is on the wrong spot.
- ✓ Check and adjust the machine, the behavioural conditions in the tanks, and / or use enough crew for correcting fish direction.

Both:

- ✓ Check that the fish are calm before stunning, lack an eye roll reflex and regular opercula movements (breathing) after stunning/percussive blow, before bleeding (if possible) and that it is properly bled before transfer to the bleeding tank.
- ✓ Remove 20 fish after the stunning/percussive blow and bleeding procedure and put them in a tank of water. Observe the fish for 10 minutes. If some show signs of temporary awakening in the form of eye roll reflex, regular opercula movements, balance recovery, or swimming it is an indicator of inadequate stunning or bleeding. Also check the bleed cut. For the percussive blow, the test may also be done with non-bled fish, to check that the stunning is irreversible.
- ✓ Make sure that the fish that come out of the bleeding tank are dead before entering further slaughter processes.
- ✓ Control and have adequate back-up systems / crew when needed for manual slaughter.

1.4 Euthanasia of individuals and groups on the farm

To prevent fish from excessive stress or suffering, it is sometimes necessary to euthanize them. It can be due to disease or injuries, after grading out weak/small individuals, to take blood samples or for the slaughter of brood stock. Close et al., [48] have listed 11 key criteria for the euthanasia of experimental animals (see Table 1.4-1.) and the same criteria are also important in commercial production, with the added challenge of large numbers of fish. The Farm Animal Welfare Committee [39] also state an animal "*must be rendered unconscious and insensible to pain instantaneously or unconsciousness must be induced without pain or distress*" prior to killing and that "*animals must not recover consciousness until death ensues*".

Table 1.4-1. *Criteria for euthanasia. The text has been adapted and reproduced from Close et al., [48], "Close, B., Banister, K., Baumans, V., Bernoth, E.M., Bromage, N., Bunyan, J., Erhardt, W., Flecknell, P., Gregory, N., Hackbarth, H., Morton, D. & Warwick, C. (1996). Recommendations for euthanasia of experimental animals: Part 1. Laboratory Animals, 30(4), p.293-316. Copyright 1996", with permission from SAGE Publications.*

Criteria for euthanasia according to Close et al., [48]
- Must be painless
- Achieve rapid unconsciousness and death
- Require minimum restraint
- Avoid excitement
- Appropriate for the life stage and species and health of the fish
- Minimize fear and psychological stress
- Reliable and reproducible
- Irreversible
- Simple to administer (in small doses if possible)
- Safe for the operator, and so far as possible also aesthetically acceptable for the operator
- Operators must be trained and have competence

Acceptable methods of euthanizing different life stages are listed below. There are older references regarding use of a waste disposal unit for fry <2 cm (see Close et al., [48]) but this cannot be considered good practice today without additional evidence. Maceration without prior stunning for euthanizing post-smolts is not acceptable for welfare [49]. However, maceration can be performed following electrical stunning or anaesthesia during emergency slaughter for disease control [36]. If the fish is not fit or healthy enough to be transported to the slaughter facility by well boat, there are designated boats for conducting emergency slaughter at a site. One challenge can be the availability of such boats, if for example, a severe disease affects a region. Electrical euthanasia can be the best choice in such boats [49]. For emergency euthanasia in fish that are not going for human consumption, more traditional pharmacological methods are also suitable, e.g. adding anaesthetics directly to the water in tanks [36].

Acceptable methods of euthanizing different life stages

- Fry – overdose of anaesthetic, blow to head if single fry, fish should be observed until death is confirmed if they are not killed individually
- Juveniles – overdose of anaesthetic, or blow to head behind the eyes and bleed/decapitation [50]
- Post-smolts – overdose of anaesthetic, or blow to head and bleeding. Slaughter boats can be used during emergency slaughter(Ex. electrical stunning + maceration, EFSA [36])
- Broodstock – anaesthetic and bleeding, or overdose anaesthetics

Challenges to fish welfare and how to minimize them

- If the stunning procedure is not carried out correctly there are risks of fish being conscious during the bleed. If a manual blow to the head is used (preferably using a priest), make sure it is hard enough, and the fish is hit correctly on head behind the eyes (not hitting the eyes). Bleeding should be carried out immediately after the blow to ensure the fish does not wake up again. Cutting the aorta or the majority of gill arches on both sides is considered good practice during the bleed [39].
- If using anaesthetics for euthanasia it is important to ensure adequate holding time and dose for the water temperature and size of the fish, especially during any potential emergency euthanasia of large numbers of individuals [39].
- Methods that are not acceptable for euthanasia are i) CO_2 saturated water, ii) live chilling + moderate CO_2 and iii) gill cutting whilst conscious (The Farm Animal Welfare Committee [39] state it can "*take 4.5-6 minutes to produce brain death*") [39].
- When removing mortalities from tanks or cages, confirm all the individuals are dead otherwise there are risks of fish suffocating in air.
- With regard to moribund fish, one of the greatest risks is actually capturing them to perform euthanasia. To capture them from big cages can be a challenge, especially when the farmer does not want to stress or injure other fish during the procedure. Small boats have been used within the cage to capture moribund fish during disease outbreaks. Still, better solutions for sorting out diseased individuals are urgently required.

How to assess welfare during euthanasia

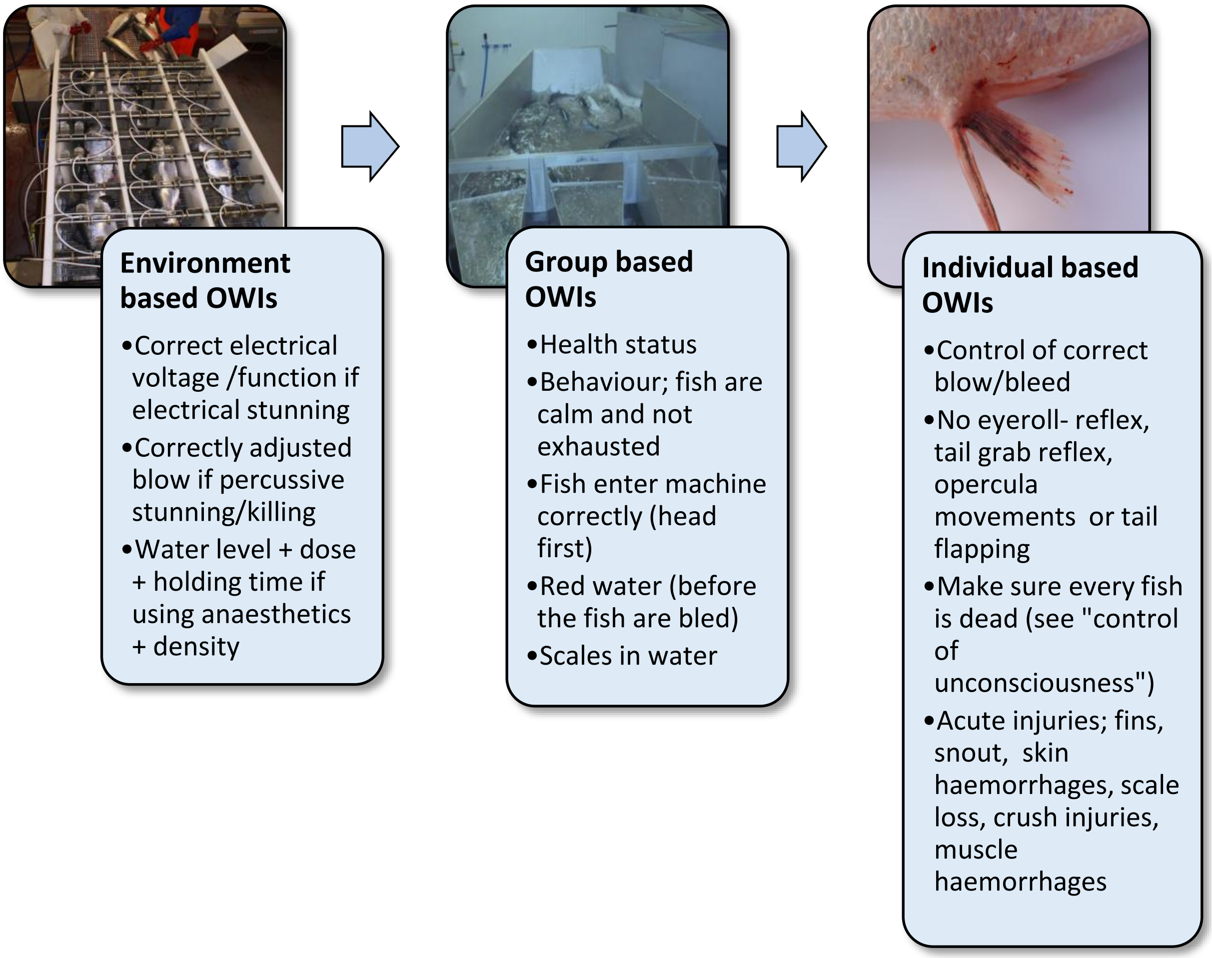


Figure 1.4-2. *Overview of fit for purpose OWIs for euthanizing fish. Environment based OWIs address the stunning machines or the bath with overdose anaesthetics, group based OWIs are what can be observed and checked during the euthanizing process, while individual based OWIs are based on sampling individual fish for close ups on missing reflexes and the correct blow/bleed where relevant. Photos and illustration K. Gismervik.*

Environment based OWIs

Correct electrical voltage/function if electrical stunning. Follow the manufacturer's manuals and update based on practical experience. See also Norwegian authorities guidance and interpretations of the slaughter regulation [10]

Correctly adjusted blow if percussive stunning/killing. Follow the manufacturer's manuals and update based on practical experience. Make sure the machine is adjusted to the size of the fish.

Anaesthetic dosage, holding time, water level and density. During the use of anaesthetics, dosage or more correctly, over dosage levels, holding time, sufficient water level and fish density are important to efficiently kill all fish. See Part C section 1.6 for information on different anaesthetics.

Group based OWIs

Health status. Sick or injured fish must be handled at an appropriate speed and once the decision has been made to euthanize the fish, it should be carried out as soon as possible to prevent further suffering.

Behaviour. Fish should be calm with no evidence of tail flapping or sudden movements, and the fish should not show signs of exhaustion or problems with balance when swimming. The fish should enter the machine correctly (head first during percussive/ electrical stunning in air). Tanks for orientation should not be too crowded, to avoid fish being pushed in wrong direction by other individuals [10].

Red water in the euthanizing bath with lots of scales and other organic material is an indication that water quality is reduced, the fish has been damaged, or that the anaesthesia dosage has been consumed.

Individual based OWIs

Control of correct blow/bleed. The percussive blow should be to the top of the head, in the middle and slightly behind the eyes. It should not fracture the skull as energy is partly absorbed instead of concentrating it on the brain for producing concussion with loss of consciousness. Haemorrhaging in the central parts of the brain are considered important for the desired effect and can also be seen macroscopically by opening the skull and brain and by visual inspection of the blow location [9, 10]. Cutting the aorta or the majority of gill arches on both sides is considered good practice during bleeding [39].

Control of unconsciousness. You should confirm that the salmon are unconscious or dead before they are bled or subjected to other slaughter processes. Simple reflex indicators such as eye roll and the ability to flip upright can easily be used as direct indicators of stress and can be evaluated individually or as an index [40]. The animal is classified as insensible if responses to these indicators are lacking [41, 42]. The vestibulo-ocular reflex (VER; the "eye roll") is the last reflex the fish loses during anaesthesia and is the first reflex to reappear after recovery [43], see Figure 1.4-3. Rhythmical opercula movements should also be absent in insensible fish. One occasional gasp sometimes occurs even in fish that are completely insensible, but if it happens in many fish or happens repeatedly on a single fish it may not be unconscious. Another reflex is the "tail-grab reflex" (i.e. grabbing the fish's tail and seeing if it attempts to escape [40]). The operator can also assess whether the fish responds to a needle puncture in the lip or skin and also if the fish attempts to adjust to normal position or make swimming movements if it is put into water. Reflex indices are simple, rapid and inexpensive and it is relatively easy to train people how to use them (e.g. at the commercial production site).

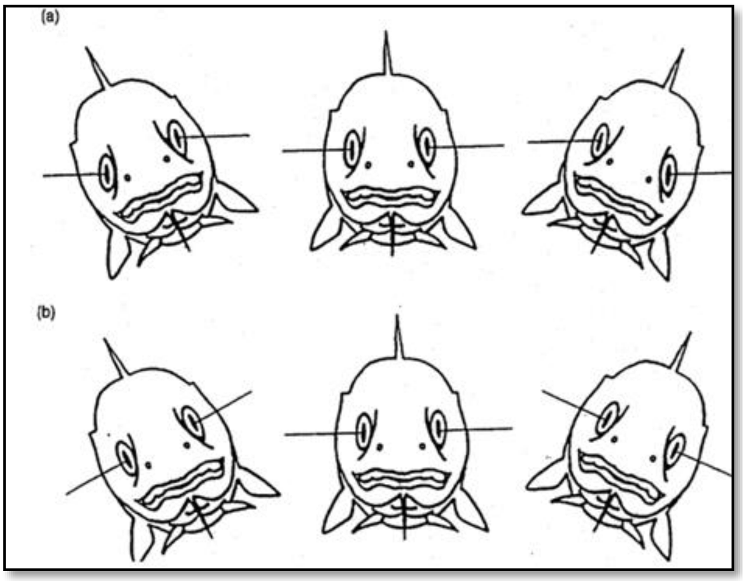


Figure 1.4-3*. Illustration of an eye roll reflex of a) living and b) dead cod. Reproduced from "Kestin, S.C., J.W. Van de Vis and D.H.F. Robb (2002) Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them. Veterinary Record. 150(10): p. 302-307. Copyright 2002", with permission from BMJ Publishing Group Limited [43]. If the fish is conscious it will try to keep the eyes in the horizontal plane if it is moved from side to side (A). If the fish is dead or insensible, the eyes do not move in relation to their changing position (B).*

Acute injuries. Equipment malfunction or hard handling may result in haemorrhages (red water such as in a live cooling tank), fin splitting, crush injuries, bleeding and snout injuries, and bruising under skin that can be visually checked after skin removal. It is important to handle the fish gently, even during the euthanizing process, and the assessment of acute injuries on individual fish can give an indication of this or if any equipment or procedure should be corrected.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

1.5 Bathing and medicinal treatments

Preventative health management is usually a better option for fish welfare than treatment with medicines. However, if the prevention is unsuccessful and the fish is infected with an infectious pathogen, treatment may be an appropriate alternative. This section describes medicinal treatments and their possible side effects and also includes fresh water bath treatments against sea lice and AGD. Sea lice can also be treated with non-medicinal methods and some of these methods are described in Part C section 2.2.1. For anaesthesia, see Part C section 1.6 and for vaccination see Part C section 1.7 of this handbook. The Norwegian Food Safety Authority has also made a separate guide to pharmaceuticals aimed at fish health professionals [51].

Medicinal treatments are utilised in Norwegian aquaculture, to varying extents and against different agents throughout the life of the fish. Welfare issues differ according to how the medicine is administered; bath treatments, in-feed treatments and injections. Little is known about the welfare challenges associated with in-feed treatments and injections are only performed to a very limited extent, with the exception of vaccination which is covered in Part C section 1.7. This current section therefore only deals with the welfare challenges associated with bathing.

Challenges to fish welfare

- In an aquaculture context, it is useful to distinguish between adverse reactions caused by the medicine and those caused by how the medicine is administered.
- The side effects of approved medicines (at the optimal dosage) are well documented through the approval scheme for medicinal products. Approved medicinal products are considered to be in tune with good welfare practice. Nevertheless, many individuals are often treated at the same time, in large units and there is therefore a high risk that different fish may receive different exposures to the treatment.
- Large production units also provide challenges associated with ensuring a consistent dose of medicine throughout the treatment volume. Some drugs can attach to, for example, the plastic wall of the tank or are absorbed or inactivated by organic matter in the water. If the distribution of the medicine becomes stratified, some individuals may avoid it.
- For some medicines, there is a relatively large difference between the dose that effects the pathogen and the dose that is harmful to the fish (large therapeutic margin), while for other medicines there is a smaller difference (small therapeutic margin). In general, there is an associated large risk with the use of medicines with small therapeutic margins in the aquaculture industry, due to the large numbers of fish involved.
- If a pathogen develops resistance to particular medicinal treatments, the response can be to use higher doses and / or a combination of multiple medicines. This is a practice that is insufficiently documented, and probably increases the risk of side effects and the risk of compromising fish welfare. In Norway, deviations in usage from the licenced recommendations, e.g. an increased dosage or its use in combination with other medicines, requires scientific documentation before approval is sought from the Norwegian Food Safety Authority [51].
- Prior to a bathing treatment, the fish will be crowded, mainly to minimise medicinal usage, reduce medicine costs and reduce environmental impact. This is done by lifting the net, by transferring the fish to a well boat or by reducing the water level in the fish tanks. Crowding along with possible pumping may adversely affect fish welfare through physiological side

effects, skin damage, loss of appetite and subsequent suboptimal growth [3, 25, 52]. See also Part C sections 1.1 and 1.2 on crowding and pumping in this handbook.

- Increased gill beat rate due to stress and or hypoxia may lead to increased absorption of the medicine and increase the risk of an overdose.
- The treatment of sea lice with hydrogen peroxide has led to episodes with high mortality rates, and thus poor fish welfare. Both water temperature and dose can affect the result [53]. Impaired sensitivity to the medicine is also an increasing problem [54]. Sensitivity tests have been developed for use on sea lice before treatment [55] in order to identify the optimal dose and reduce the risk to fish welfare from i) excessive doses or ii) of ineffective treatments, leading to poor control of the parasite or the necessity for repeated treatments. As hydrogen peroxide decomposes into oxygen and water, there may be a risk of oxygen supersaturation during treatment.
- Treatment against sea lice with freshwater has been reported to have a few negative effects on fish welfare [19, 56], but a relatively long treatment time of 6.5 - 8 hours to give the desired effect may increase the risk of injury during handling and crowding (see Part C, sections 1.1 and 1.2). In addition, the treatment operator must ensure the fish are supplied with enough freshwater of an appropriate water quality (see Figure 1.5-1).
- After treatment of AGD with freshwater, it has been shown that AGD levels can return to pre-treatment levels after few weeks [57, 58]. The amoeba can also show reduced sensitivity to freshwater after repeated treatments [59]. If treating advanced disease cases (measured by an AGD score) there is an increased risk of mortality during treatment [23].

Table 1.5-1. *Recommended levels of important water quality parameters for freshwater treatment of salmon for AGD and sea lice. Reproduced from "Powell, M.D., P. Reynolds, and T. Kristensen (2015) Freshwater treatment of amoebic gill disease and sea-lice in seawater salmon production: Considerations of water chemistry and fish welfare in Norway. Aquaculture. 448: p. 18-28. Copyright 2015", with permission from Elsevier [23].*

Water quality parameters	Before treatment (Recommended levels)	During treatment (Upper limits)
Conductivity (µS/cm)	<500	<1000
pH	6.0-6.7	6.0-6.8
ORP (mV)	40-100	<350
TOC/DOC (mg/L)	<3	If possible sampling for later analysis
Ca^{2+} (mg/L)	<10	If possible sampling for later analysis
Na^{+} (mg/L)	<10	
O_2 (%)	90-110	90-110
CO_2 (mg/L)	<5	<25
Salinity (‰)	<5	<5

How to minimize welfare challenges

- The Norwegian Animal Welfare Act §9 (https://www.regjeringen.no/en/dokumenter/animal-welfare-act/id571188/) states: "*Medical and surgical treatment shall be carried out taking into account the animal's welfare, and protect the animal's ability to function and its quality of life.*" The expected effect and utility of a treatment must be balanced against the risk of adverse effects on fish welfare. In some cases, euthanizing or slaughter may be a better option than treatment.
- An assessment of the necessity for a medicinal treatment should include:
  - ✓ Fish health status
    - Medical history
    - Gill Status
  - ✓ Water Quality
    - Water chemistry and temperature
    - The presence of algae, zoo plankton, jellyfish (sea water)
  - ✓ Sensitivity of the pathogen to the medicine
  - ✓ History of treatment - repeated treatment with the same active substance can potentially promote the development of resistance, increase the risk of the treatment failing and may also have adverse effects on the fish.
- When the decision is made to carry out a medicinal treatment, good preparation will increase the safety of the treatment in question. The operator should:
  - ✓ Have all relevant equipment that will be needed, of an appropriate quality and quantity
  - ✓ Use trained staff, preferably with prior experience
  - ✓ Have a treatment plan and procedures
  - ✓ Have instructions on how to use the product from the supplier and also from authorised animal health personnel
  - ✓ Carry out a trial treatment on a small portion of fish to make sure that the treatment does not have unexpected effects and to check its efficacy
  - ✓ Take water and gill samples (for retrospective investigation of any problems)
  - ✓ Adequately starve the fish prior to treatment
- An important measure to reduce any negative effects on fish welfare is to treat only one unit (tank or sea cage) on the first day of treatment. This treatment can then be evaluated with regard to fish welfare before the rest of the site is treated.
- A treatment log with all relevant data is required and will ensure an accurate start point for any retrospective evaluation of the treatment.
- If there are any signs of reduced welfare, the ongoing treatment should be discontinued. Any treatment procedure should therefore include clear criteria for when and how to discontinue treatment, including how quickly to dilute the treatment agent.

How to measure welfare during and after treatment

Bath treatments often involve both crowding and pumping of the fish and each of these procedures have their own welfare risks and ways to measure them (see Part C sections 1.1 and 1.2).

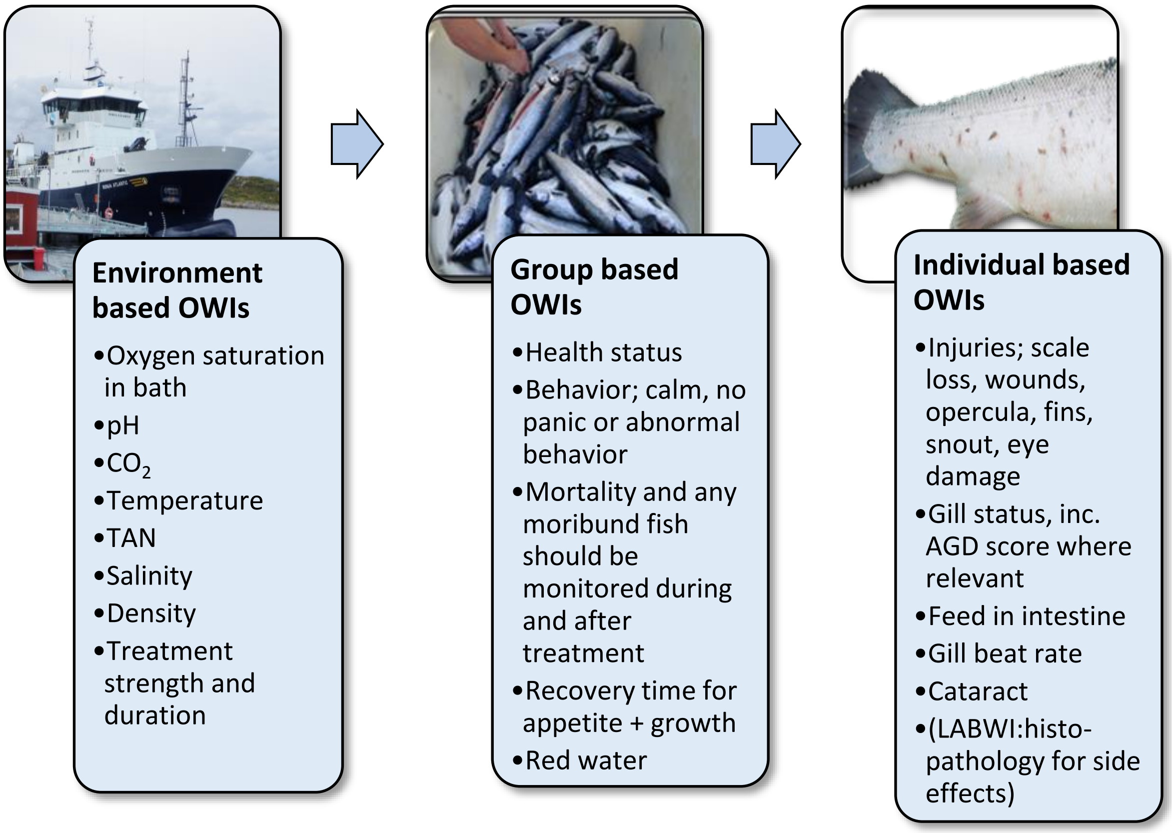


Figure 1.5-2. *Overview of fit for purpose OWIs during bathing and medicinal treatments. Environment based OWIs address the medicinal bath, group based OWIs are what can be observed and checked during the process, while individual based OWIs are based on sampling individual fish for close up examinations. Photos and illustration: K. Gismervik*

Environment based OWIs

Oxygen saturation and other water parameters. Bath treatments usually take place in a limited water volume without water exchange. It is therefore important to add additional oxygen and to monitor the oxygen levels in the bath during the treatment. This is to ensure that the fish are adequately oxygenated, but also to prevent an increased ventilation rate which may lead to increased medicinal uptake and increase the risk of poisoning. As a general precautionary guideline, oxygen saturation levels of >80% are often used [13] and the RSPCA welfare standards for farmed Atlantic salmon [7] recommend a minimum limit of 7mg L^{-1}. Modern well boats are commonly used for medicinal treatments and in addition to oxygen logging they also log CO_2, pH, temperature and total ammonium nitrogen (TAN). Properties such as temperature, pH and salinity can affect the NH_3: NH_4^+ ratio and thus the toxicity of ammonia. The maximum safe level of short-term exposure (4 hours) of NH_3-N is 0.1 mg L^{-1} according to Wedemeyer [60] (for further description see Part A, section 4.1.6). To limit the risk of TAN accumulation, the fish should be starved before treatment (see also Part C, section 1.9). It may also be appropriate to measure salinity in connection with freshwater treatments [23].

Temperature. For temperature recommendations, it is important to read the instructions from the supplier to see if there are limitations in relation to the medicines use or mixing strengths. In addition, ambient sea temperature may be relevant for retention times in relation to slaughter.

Treatment strength and duration. Direct measurements of active substance concentration may be possible with certain active substances, such as hydrogen peroxide. It is also important to know the acceptable treatment durations for each medicine and that this duration is observed and logged.

Density. A density that is too high during treatment can lead to injuries (see Part C Section 1.1, crowding) but the operator must also consider the amount of treatment agent used and its e.g. potential environmental impacts.

Group based OWIs

Health status. The health status of the fish must be known prior to the treatment to ensure it can withstand the procedure and the treatment dosage/duration. Veterinary or other fish health professionals should make this assessment.

Behaviour. It is important to observe the behaviour of the fish at the surface and in larger units also deeper in the cage/tank. Changes in behaviour or appearance may be indications of poisoning or injury sustained during treatment. Examples of changes in behaviour are balance problems, "gasping for air at the surface", panic behaviour or other abnormal swimming, vertical swimming, head shaking and clumping. It is also important to make sure the fish aren't too crowded (see Part C section 1.1).

Mortality. Increased mortality or the observation of moribund fish during a treatment is an indicator of severely compromised fish welfare and should result in the termination of the treatment. Elevated mortality after the procedure may be related to the treatment and should be further investigated by fish health professionals.

Return of appetite. The time it takes for appetite to return should be closely monitored after treatment. A reduction or loss of appetite can be caused by the initiation of a stress response [21]. The time it takes for appetite to return after a procedure can therefore also be used as an OWI as it can reflect how well the fish have dealt with the stressor. Appetite is easy to measure qualitatively by observing the fish when feed is offered.

Growth can be affected by short-term or chronic stress. Acute changes in growth can be used as an early warning system for potential problems, particularly when the farmer has robust growth monitoring practices.

Red water. Damaged gills or acute lesions such as bleeding can cause the water to turn red, especially when water is recycled. Red water is never a good sign and the cause should be investigated immediately (see Part A, Chapter 3 for more information).

Individual based OWIs

Injury and side effects. In addition to the stress and injuries that may occur during crowding and pumping (see Part C sections 1.1 and 1.2 of this handbook), it has been reported that some medicines may cause other types of injuries to the fish. Such damage may occur due to the uneven distribution of the medicine in the treatment volume. Hydrogen peroxide can cause damage to the skin, eyes and gills and may also affect the mucous cells [61, 62]. Cypermethrin and deltametrin can lead to altered pigmentation, and formalin can cause skin and gill damage [63]. In extreme cases, these changes can be recorded macroscopically, but in milder forms histopathology is required (LABWI).

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Gill status and AGD score. AGD scoring of the gills [64] is relevant for bathing treatments for AGD to assess the treatment effect and also because long term problems such as AGD increase the risk of mortality during the treatment [23]. To get a measure of gill status, the operator can score changes on the gill surface visible as "white patches" (total gill score).

Feed in the intestine often indicates that the fish has eaten during the last one to two days [45, 46] but this depends on the fish size and temperature. The stomach and intestines should be checked for feed residue. Such a check can be used to evaluate the starvation period before treatment or appetite after treatment (see also Part C, section 1.9).

Gill beat rate. Clear changes in gill beat rate (such as very fast opercular movements) may indicate that fish are under duress or exhausted and this, together with other indicators, can form a basis for deciding whether a treatment should be stopped.

Eye status and cataracts. Eyes may be affected by the bathing process, potentially leading to e.g. chemical burns, bleeding and desiccation during air exposure. Hydrogen peroxide can also be a risk factor for the formation of cataracts [65] and these should also be monitored after a treatment. The Norwegian Medicines Agency medicine database states white spots can appear in one or both eyes after hydrogen peroxide treatment, but that they usually disappear within 24 hours [66].

1.6 Anaesthesia

Fish handling almost always results in an increase in the fish's activity levels. All activity during the handling and capture of the fish has an effect on their physiology and behaviour, and fish often require immobilisation to reduce the risk of harm [67]. Commercial salmon producers do not sedate or anaesthetise the fish frequently. However, a typical production cycle involves numerous routines such as grading or vaccination, and these can be a potential stressor for the fish [68, 69, 70, 71, 72].

The sedation and anaesthesia of fish can be induced by the use of drugs, gases, hypothermia and electrical current [67, 73]. The choice of anaesthetics can depend on a) their availability (what is licensed for use), b) how cost effective they are, c) how easy they are to use, d) the nature of the investigation (relevant for research) and e) user health and safety [74].

Marking and Meyer [75] have listed the features of an ideal anaesthetic:

1. It's induction time should be < 15 minutes and preferably < 3 minutes
2. It's should have a short recovery time (< 5 minutes)
3. It should be non-toxic to the fish
4. It should not be harmful to those who administer it and it should also be straightforward to handle
5. It should have no lasting effect on the behaviour or physiology of the fish
6. It should be rapidly metabolised or excreted and leave no residues, and withdrawal time should be less than 1 hour in connection with slaughter
7. There should be no cumulative risks or effects associated with potential repeated exposure
8. It should be cost effective

In addition to these features:

9. An anaesthetic should alleviate stress, and reduce the risk for the fish in relation to additional potential stressors [76, 77, 78, 79, 80].

Commercial aquaculture in Europe primarily uses three anaesthetics: benzocaine, tricaine mesilate and iso-eugenol.

- **Benzocaine**. According to Ross and Ross [67] benzocaine is a "*crystalline ester of p-amino benzoic acid and ethanol*" (ethyl-4-aminobenzoate). The ingredient is closely to related tricaine, but is virtually insoluble in water (0.04 % W/v) as it lacks a sulphonyl side-group [67]. It must therefore be dissolved in acetone, ethanol or propylene glycol [67, 70, 73].
- **Tricaine mesilate (MS-222)** has been the most commonly used anaesthetic since its introduction in 1967 [81, 82]. A buffer (e.g. sodium bicarbonate) is required for use in fresh water to attain a neutral pH. Without buffering the pH can drop to damagingly low levels. It is much more water soluble (x 250) than its analogue, benzocaine.
- Both benzocaine and tricaine are local anaesthetic agents, blocking neuronal sodium cation channels and reducing the transference of nerve action potentials [83, 84].
- **Iso-eugenol** (2-methoxy-4-prop-1-enylphenol) is mixed with polysorbate 80, which acts as an emulsifier. Iso-eugenol has been tested on a wide variety of different fish species over the last couple of years and these species include rainbow trout and Atlantic salmon [85]. An additional positive effect of iso-eugenol was discovered by Iversen et al., [70], who showed that dosages above 20 mg L^{-1} (iso-eugenol) blocked a further surge in plasma cortisol.

- The only other anaesthetics that have shown similar effects on plasma cortisol are etomidate/ metomidate [70, 79, 86]. However, neither of these substances are approved for commercial aquaculture.
- **Benzocaine** and **tricaine mesilate** are both potent stressors that will elicit a stress response if fish are subjected to their respective anaesthetics [70].

Table 1.6-1 describes the different stage of anaesthesia according to Schoettger and Julin, [87]. Hikase et al., [88] also suggested the fish go through 5 stages of recovery from being anesthetized. These are i) the return of opercular activity, ii) limited return of equilibrium and swimming ability, iii) complete return of equilibrium, iv) fish reacts and potential avoids external stimuli, and v) complete return of normal behavioural repertoire and swimming activity.

Table 1.6-1. *Different stages of anaesthesia in fish (Schoettger and Julin, [87]). Reproduced from "Schoettger, R.A. og M. Julin (1967) Efficacy of MS-222 as an anesthetic on four salmonids. Invest. Fish Contr., U.S. Dept. Int. 13: p. 1-15. Copyright 1967", with permission from U.S. Geological Survey.*

Stage	Descriptor	Behavioural response
1	Light sedation	Partial loss of reaction to external stimuli.
2	Deep sedation	Partial loss of equilibrium, no reaction to external stimuli.
3a.	Total loss of equilibrium	Fish usually turns over but retain swimming ability.
3b.	Total loss of equilibrium	Swimming ability stops, but fish responds to pressure on the caudal peduncle.
4	Anaesthesia	Loss of reflex activity, no reaction to strong external stimuli.
5	Medullary collapse (death)	Respiratory movement ceases (death).

No further handling of the fish should occur before stage 3b or 4 as this could damage the skin and mucus layer of the fish. This is especially important for smolts and early post-smolts [67].

Challenges to fish welfare

- Improper use of anaesthetics may cause both an overdose and negative effects on fish welfare [67].
- Anaesthesia requires training and experience, and improper use can have fatal consequences for the fish.
- When sedating large units, there are challenges associated with getting a steady dose of anaesthetic throughout the treatment volume, especially when using iso-eugenol.
- Increased ventilation rate due to stress and or hypoxia may lead to increased absorption of the anaesthetic and increase the risk of an overdose.
- In the case of an overdose, the recovery time of the fish may be too long. This is especially important in large units, as anaesthetized fish may lay on the bottom of the tank and block the water outlet, affecting water circulation. In addition, the fish lying on the drain can damage their skin, a welfare threat in itself that can also increase the risk of secondary infections.

How to minimize welfare challenges

The Norwegian Animal Welfare Act §9 (https://www.regjeringen.no/en/dokumenter/animal-welfare-act/id571188/) states: "*Medical and surgical treatment shall be carried out taking into account the animal's welfare, and protect the animal's ability to function and its quality of life.*"

- Users must know the different chemical properties of the different types of anaesthetics they may utilise.
- The user should also identify the optimal anaesthetic dosage at different water temperatures so that induction time is less than 3 minutes and recovery time is as brief as possible [67, 75].
- Users should ensure that the anaesthetic procedure is carried out as smoothly as possible.
- Users should also ensure the anaesthetic bath is well oxygenated.
- To avoid an overdose, the user should try out the anaesthetic dose on a single fish or a small group of individuals, evaluate the results with regard to fish welfare and then carry out the procedure on the rest of the group.
- A recirculation pump can help ensure a steady dose of anaesthetic throughout the treatment volume. This may be particularly desirable for heavily soluble anaesthetics such as benzocaine and iso-eugenol.
- RSPCA welfare standards for farmed Atlantic salmon [7] state anaesthesia "*must only be administered by trained, competent personnel*". All anaesthetics should be used according to the manufacturer's instructions.
- If there are any signs of reduced welfare, the ongoing treatment should be discontinued. Any anaesthetic procedure should therefore include clear criteria for when and how to discontinue treatment, including how quickly to dilute the anaesthetic agent. These criteria could include a low gill beat rate, extended recovery time, damage to the fish and abnormal behaviour (see Figure 1.6-2).

How to measure welfare during and after anaesthesia

As stated before, an ideal anaesthetic should have an induction time of < 15 minutes (preferably < 3 minutes) to reach stage 3b/4, and recovery time should be as short as possible (5 minutes or less) [75].

- If it takes too long to reach stage 3b/4 - increase the dosage
- If stage 3b/4 is reached too rapidly - reduce the dosage.

It is essential that the recovery time is as rapid as possible, as anaesthetised fish will sink to the bottom of the tank, which could clog the outlet, reduce water circulation and can be potentially damaging to the epidermis of the fish.

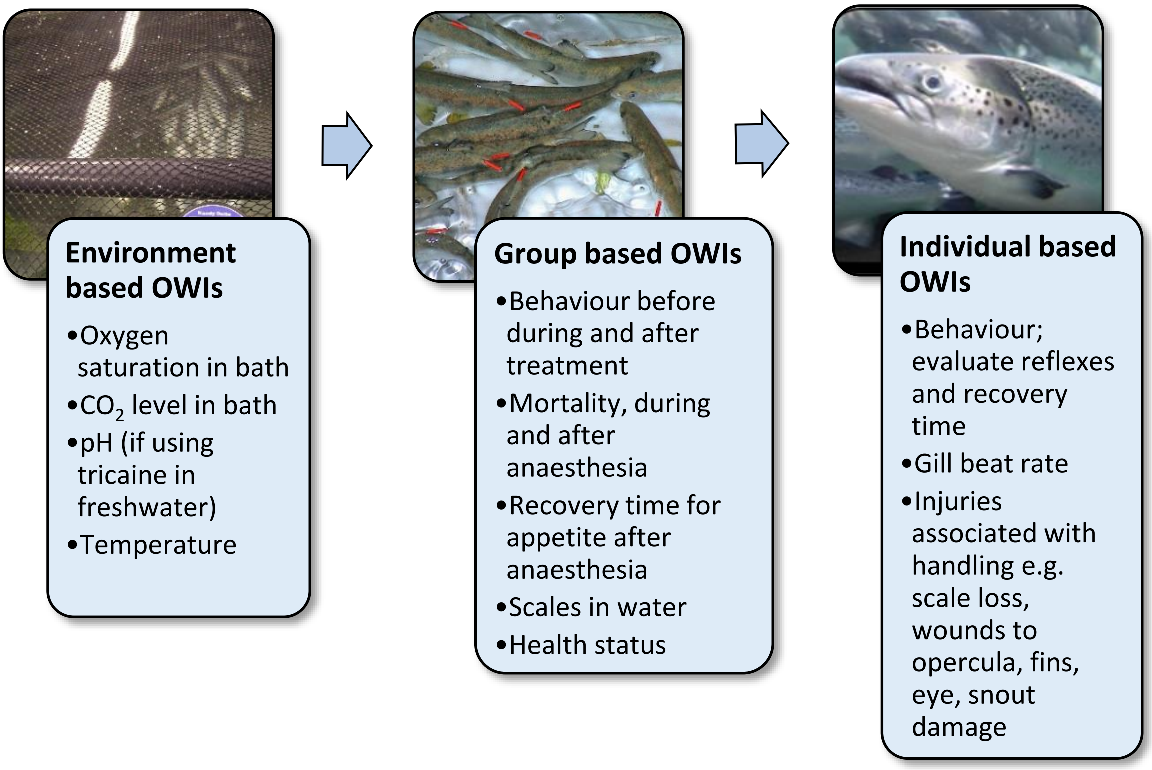


Figure 1.6-2. *Overview of fit for purpose OWIs for anaesthesia. Environment based OWIs specifically address the anaesthetic treatment, group based OWIs are what can be observed and checked during the anaesthesia process, while individual based OWIs are based on sampling individual fish for close up examinations. Illustration: M. H. Iversen and K. Gismervik. Photos: M. H. Iversen*

Environment based OWIs

Oxygen saturation. As a general precautionary principle, all anaesthesia baths must have an oxygen saturation of >80% [13] and be aerated if necessary. The RSPCA welfare standards for farmed Atlantic salmon [7] also recommend a minimum limit of 7mg L^{-1}. If sodium bicarbonate ($NaHCO_3$) is used to buffer tricaine, it is recommended that the bath is aerated for at least 15 minutes to reduce the accumulation of CO_2.

Carbon dioxide can accumulate in the anaesthetic bath if aeration is inadequate. Special care should be taken during tricaine anaesthesia combined with sodium bicarbonate ($NaHCO_3$). CO_2 concentration should ideally be below 15 mg L^{-1}.

pH must be monitored or taken into consideration while using tricaine in freshwater. The manufacturers recommend the addition of a buffer (like sodium bicarbonate) to prevent a drastic pH reduction that can harm the fish.

Water temperature must be measured during anaesthesia. At high temperatures above 10^{o}C, the fish must be monitored as the transition from stage 4 anaesthesia to stage 5 respiratory arrest may be relatively short at high doses (see Table 1.6-1).

Group based OWIs

Behaviour should be closely monitored both before, during and after anaesthesia. No additional handling of the fish should occur before the fish is in stage 4 – anaesthesia (see Table 1.6-1). This is especially important when the fish is going to be subjected to a potential painful procedure such as vaccination. Before stage 4 no true analgesic effect is obtained by the anaesthetic in question [67]. The anaesthesia dosage level can also be determined by monitoring behaviour (see Table 1.6-1).

Mortality. Should be followed closely both during and after anaesthesia to retrospectively assess problems or welfare threats associated with the procedure. An overdose with anaesthesia will lead to mortality.

Return of appetite. The time it takes for appetite to return should be closely monitored after anaesthesia. A reduction or loss of appetite can be caused by the initiation of a stress response [21]. The time it takes for appetite to return after a procedure can therefore also be used as an OWI as it can reflect on how well the fish have dealt with the stressor. Appetite is easy to measure qualitatively by observing the fish when feed is offered.

Scales in water. This indicates scale loss and damage to the skin which can cause osmoregulatory problems and also secondary infections.

Health status. Fish should be in good health prior to anaesthesia as fish in poor health are less tolerant of the procedure. This is especially important for fish with AGD and other diseases that affect the gill epithelium.

Individual based OWIs

Behaviour should be monitored when the fish is undergoing anaesthesia and also during recovery. Simple reflex indicators can be evaluated individually or as an index [40]. The vestibulo-ocular reflex (VER; the "eye roll") is the last reflex the fish loses during anaesthesia and is the first reflex to reappear after recovery (Kestin et al., [43], see also Part C Figure 1.3-3). Rhythmical opercula movements should also be absent in insensible fish. One occasional gasp sometimes occurs even in fish that are completely insensible, but if it happens in many fish or happens repeatedly on a single fish it may not be unconscious. Another reflex is the "tail-grab reflex" (i.e. grabbing the fish's tail and seeing if it attempts to escape [40]). The operator can also assess whether the fish attempts to adjust to its normal position or make swimming movements if it is put into water. Reflex indices are simple, rapid and inexpensive and it is relatively easy to train people how to use them (e.g. at the commercial production site).

Handling-related injuries. See Part C sections 1.1 and 1.2 for OWIs related to crowding and pumping. As a brief summary, the most common signs of problems with crowding and pumping are various injuries (such as scale loss, sores, opercular, eye, fin and snout damage) which can also lead to secondary infections.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Gill beat rate must be closely monitored during anaesthesia. Clear changes in gill beat rate (such as rapid and irregular opercular movements) may be a sign of an overdose and the fish must be transferred to oxygenated water immediately.

Some general handling procedures regarding anaesthesia including recommendations from the RSPCA, for full details see RSPCA welfare standards for farmed Atlantic salmon [7]. *Reproduced with permission from the RSPCA.*

RSPCA welfare standards for farmed Atlantic salmon [7] state:

- Anaesthetics "*must be used according to manufacturers' instructions*".
- Anaesthesia "*must only be administered by trained, competent personnel*".
- "*There must be a team member with responsibility for monitoring oxygen levels in the anaesthetic bath and maintaining them at 7mg/litre*". (or 80% saturation [13])

Other recommendations:

- If sodium bicarbonate ($NaHCO_3$) is used to buffer tricaine, the baths should be

1.7 Vaccination

Atlantic salmon are vaccinated early in their production phase. Vaccination is an important procedure in modern aquaculture to protect and prevent disease outbreaks. In 2010, approximately 250 million Atlantic salmon smolts were vaccinated in Norway, with a cost of ca. 251 MNOK (ca. 45 million US$) [89]. The development of effective and efficient vaccines against a number of viruses and bacteria has drastically reduced the need of antibiotics since the 1990s [90, 91]. To ensure the health and welfare of Atlantic salmon after transfer to sea, all fish are individually vaccinated. However, the vaccination process can be a potential stressor and can have a long-term effect on plasma cortisol [92, 93, 94].

Challenges to fish welfare

- Fish are exposed to four potentially stressful routines during the vaccination process. These routines are crowding (see Part C section 1.1), loading/pumping (see Part C section 1.2), anaesthesia (see Part C section 1.6) and vaccination.
- Plasma cortisol levels are typically elevated for at least 72 hours and also up to two weeks after vaccination. This response is most likely due to the inflammatory reaction to oil-adjuvants in the vaccines.
- Earlier studies have shown that if stress hormones become elevated prior to vaccination they can have a negative impact on antibody production and the protective effects of the vaccine [92, 93, 94, 95, 96].
- In Norway, the most common method for vaccinating salmon is via intraperitoneally injected oil-based multivalent vaccines. The first oil-based vaccines came on the market in the early nineties. Each dose then had a volume of 0.2 ml. Recently, the volume of the doses in most vaccine types was reduced to 0.1 ml or 0.05 ml, mainly by reducing the volume of adjuvant. The oil-based adjuvant serves as a depot of the antigens and promotes an inflammatory reaction, thus increasing vaccine efficacy but with negative side effects for the fish.
- The changes in the vaccine formulations over the years are the result of a desire to balance the relationship between efficacy and adverse side effects [93, 97, 98, 99].
- Different vaccine types may differ in their efficacy and side effects, but the same vaccine may also vary in its protection and adverse effects [100].
- Factors known to influence the efficacy of a vaccination procedure include the vaccination technique, water temperature during vaccination [101], fish size at vaccination [101], hygiene, health status and individual fish differences [97, 98, 102].

How to assess welfare associated with vaccination

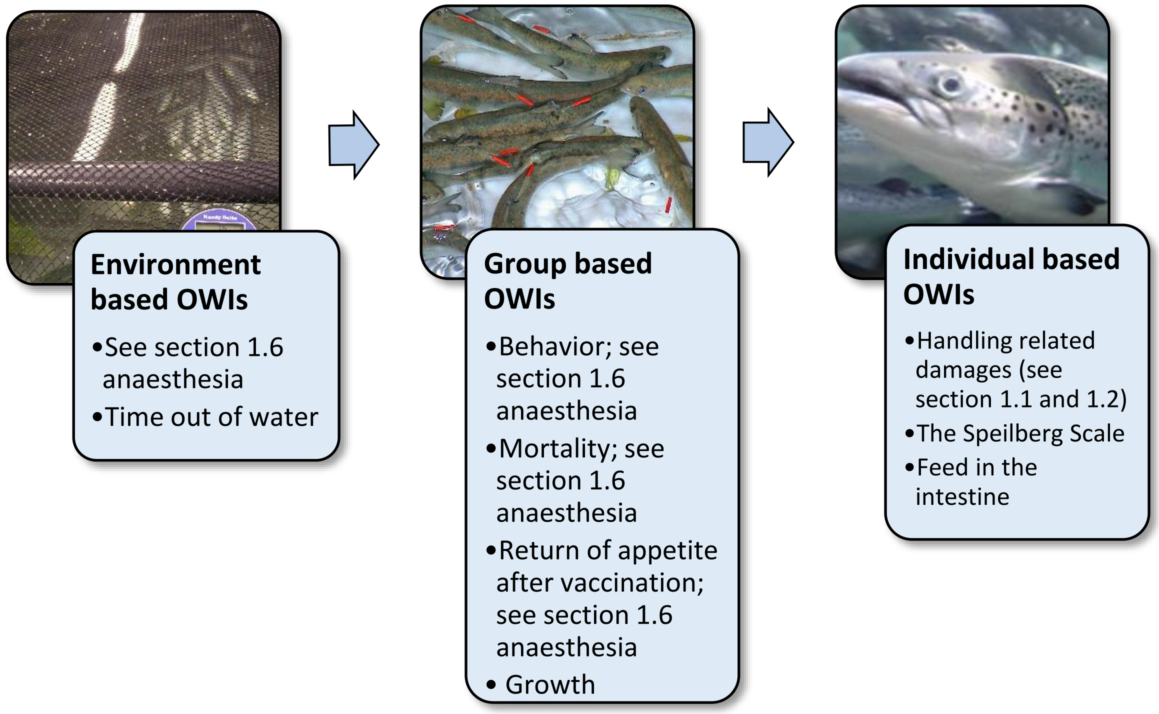


Figure 1.7-1. *Overview of fit for purpose OWIs for vaccination. Environment based OWIs specifically address the vaccination treatment, group based OWIs are what can be observed and checked during the vaccination process and afterwards, while individual based OWIs are based on sampling individual fish for close up examinations. Illustration: M. H. Iversen Photos: M. H. Iversen and L.H. Stien.*

Environment based OWIs

See section 1.6 anaesthesia for more details.

Time out of water. Air exposure should be minimised. The RSPCA welfare standards for farmed Atlantic salmon recommend a maximum exposure time of 15 seconds, unless anaesthetised [7]. The time the fish is exposed to air is particularly critical at high or low temperatures and when humidity is low.

Group based OWIs

Mortality. Should be followed closely and on a regular basis for the first 2 weeks after vaccination to monitor or retrospectively assess problems or welfare threats associated with the procedure.

Return of appetite. The time it takes for appetite to return should be closely monitored after vaccination. A reduction or loss of appetite can be caused by the initiation of a stress response [21]. It can therefore also be used as an OWI as it can reflect how well the fish have dealt with the stressor. Appetite is easy to measure qualitatively by observing the fish when feed is offered.

Growth can be affected by short-term or chronic stress. Acute changes in growth can be used as an early warning system for potential problems, particularly when the farmer has robust growth monitoring practices.

Behaviour. Abnormal behaviour could give an indication of a good or poorly executed vaccination, as e.g. stressed smolt and post-smolt salmon will typically aggregate in "clumps" at the bottom of the tank or sea cage. Highly stressed fish can also exhibit darting and flashing behaviours [103].

Individual based OWIs

Handling related damage. See Part C section 1.1 and 1.2 for OWIs related to crowding and pumping. In brief, the most common sign of problems associated with crowding and pumping in individual fish is initially damage, followed by the development of secondary infections.

Feed in the intestine. In order to evaluate the starvation period prior to vaccination or the feed intake after vaccination (indirect appetite), the salmon can be euthanised and the gastrointestinal tract can be checked for feed. It is particularly important that the fish are sufficiently starved before vaccination, as you want the best possible hygiene when injecting the abdominal cavity and you also avoid faecal contamination of the holding water. Feed in the intestine often indicates that the fish has eaten during the last one to two days [45, 46], but this depends on the fish size and temperature (see also Part C section 1.9).

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

The Speilberg Scale for scoring vaccine side effects is based on a visual assessment of the extent and location of clinical changes within the abdominal cavity of the fish [98]. It describes changes related to peritonitis; adhesions between the organs, between the organs and the abdominal wall and melanin deposits [98, and also Part A section 3.2.15 and references therein]. Generally, a Speilberg score of 3 and above is regarded as undesirable (see Table 1.7.2 and Figure 1.7.3 below).

Table 1.7.2. *The Speilberg Scale, reproduced from "Midtlyng, P.J., Reitan, L.J. and Speilberg, L. 1996 [98], Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (Salmo salar L.) against furunculosis. Fish & Shellfish Immunology 6, 335–350. Copyright 1996", with permission from Elsevier. Assessments are based upon the visual appearance of the abdominal cavity and the severity of lesions*

Score	Visual appearance of abdominal cavity	Severity of lesions
0	No visible lesions	None
1	Very slight adhesions, most frequently localized close to the injection site. Unlikely to be noticed by laymen during evisceration	No or minor opacity of peritoneum after evisceration
2	Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall. May be noticed by laymen during evisceration	Only opacity of peritoneum remaining after manually disconnecting the adhesions
3	Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall. May be noticed by laymen during evisceration	Minor visible lesions after evisceration, which may be removed manually
4	Major adhesions with granulomas, extensively interconnecting internal organs, which appear as one unit. Likely to be noticed by laymen during evisceration	Moderate lesions which may be hard to remove manually
5	Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may carry focal, prominent and/or heavily pigmented lesions or granulomas	Leaving visible damage to the carcass after evisceration and removal of lesions
6	Even more pronounced than 5, often with considerable amounts of melanin. Viscera cannot be removed without damage to fillet integrity	Leaving major damage to the carcass

1. Very slight adhesions, most frequently localized close to the injection site. Unlikely to be noticed by laymen during evisceration.

2. Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall. May be noticed by laymen during evisceration.

3. Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall. May be noticed by laymen during evisceration.

4. Major adhesions with granuloma, extensively interconnecting internal organs, which thereby appear as one unit. Likely to be noticed by laymen during evisceration

5. Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may carry focal, prominent and/or heavily pigmented lesions or granulomas

6. Even more pronounced than 5, often with considerable amounts of melanin. Viscera irremovable without damage to fillet integrity.

Figure 1.7-3. *The Speilberg Scale for intra-abdominal lesions after intraperitoneal vaccination of Atlantic salmon. Figure: D. Izquierdo-Gomez. Photos: Lars Speilberg, kindly reproduced with permission. Text reproduced from "Midtlyng, P.J., Reitan, L.J. and Speilberg, L. 1996 [98], Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (Salmo salar L.) against furunculosis. Fish & Shellfish Immunology 6, 335–350. Copyright 1996", with permission from Elsevier.*

1.8 Transport

Most live transport is done either on land by road transport (truck) or via sea by well boats. Fish can also be transported by helicopter, but this method will not be covered here. All life stages from juvenile, smolt to post-smolt are handled and transported during a commercial production cycle [104, 105, 106, 107, 108]. Fish are exposed to four potentially stressful routines during the transport process including crowding (see Part C section 1.1), loading/pumping (see Part C section 1.2) plus transport and unloading [71, 109, 110, 111] and several welfare risks can be linked with transport of live fish [108]. Life stage may also play a role in how stressful the transport process is, as experience suggests that Atlantic salmon smolts are more sensitive to stressors during transport compared to parr, most likely due to the natural increase in plasma cortisol during the smoltification process [112]. Handling procedures associated with loading, transport, and unloading have the potential to cause stress and physical injury, which can lead to long-term health issues. Water quality may also deteriorate during transport, which can jeopardise fish welfare even further. Smolts must also cope with an abrupt change in salinity when they are transferred from freshwater to seawater. Holding in transport tanks may also impact upon the ability of the fish to express their natural or normal behaviour [71, 103, 104, 108].

Challenges to fish welfare and how to minimize them

- **Transport – an important recovery phase**. Previous studies have shown that the actual stage where the fish are transported may be the least stressful component of the transport process when transferring fish from sea farms to the processing plants [71, 103, 113, 114, 115]. However, short transports may not provide adequate time for the fish to recover [30, 71] and if the fish do not get a sufficient opportunity to recover from the loading/unloading procedures (due to the short transport duration, poor weather or bad road/sea conditions) their ability to tolerate further stressors can be greatly reduced.
- **Weather and road/sea conditions during transport**. Bad weather or poor road/sea conditions could have a negative impact on fish welfare as fish may exhibit evidence of motion sickness (fish are commonly used to study motion sickness in vertebrates [116]). As the fish's lateral line system is highly sensitive [117], one may suspect that road transport could be potential stressor due to vibration, however, further studies are required to investigate this issue.
- **Water quality**. Another potential stressor that could negatively impact upon fish welfare during transport is poor water quality, e.g. when the well boat must close the vents and re-circulate water as the vessel passes through an area with restrictions due to diseases or unsuitable water conditions. Farrell [113] showed that a well boat with i) a live-hold volume of 650 m^3, ii) holding 62 tonnes of fish (average weight 5.13 ± 0.47 kg), iii) an initial DO saturation of 10 mg L^{-1}, and iv) a routine oxygen consumption of 3.1 mg O_2 min^{-1} kg^{-1}, would reach DO saturations of 5 mg L^{-1} in ca. 17 minutes. There is therefore a potentially short window before the fish must be given supplemental oxygen when they are subjected to closed, recirculating water conditions. This challenge may be exacerbated during summer when water temperatures are higher and the fish have a higher metabolic rate, meaning the time frame becomes even narrower [113]. However, during winter or if the fish are subjected to chilled holding water, this window can be extended (to ca. 30 minutes) [113]. With continual supplementation of oxygen, the live-holding tanks can stay closed. However, the build-up of ammonia and carbon dioxide in the holding water may become challenging at some point [103, 113, 114, 115, 118].

How to assess welfare associated with transport

Behaviour in a well-established welfare indicator in both terrestrial [119] and aquatic [13, 21, 37] animal production. However, quantifying the behaviour of fish in aquaculture can be difficult and a lot of work up to now has focused on stress physiology [103]. With regard to quantifying the effects of transport upon fish welfare, a lot of attention has been paid to physiological welfare indicators such as plasma cortisol, glucose and ions [69, 105, 107, 110, 120]. To assess welfare before transport see Part C sections 1.1 and 1.2 on crowding and pumping.

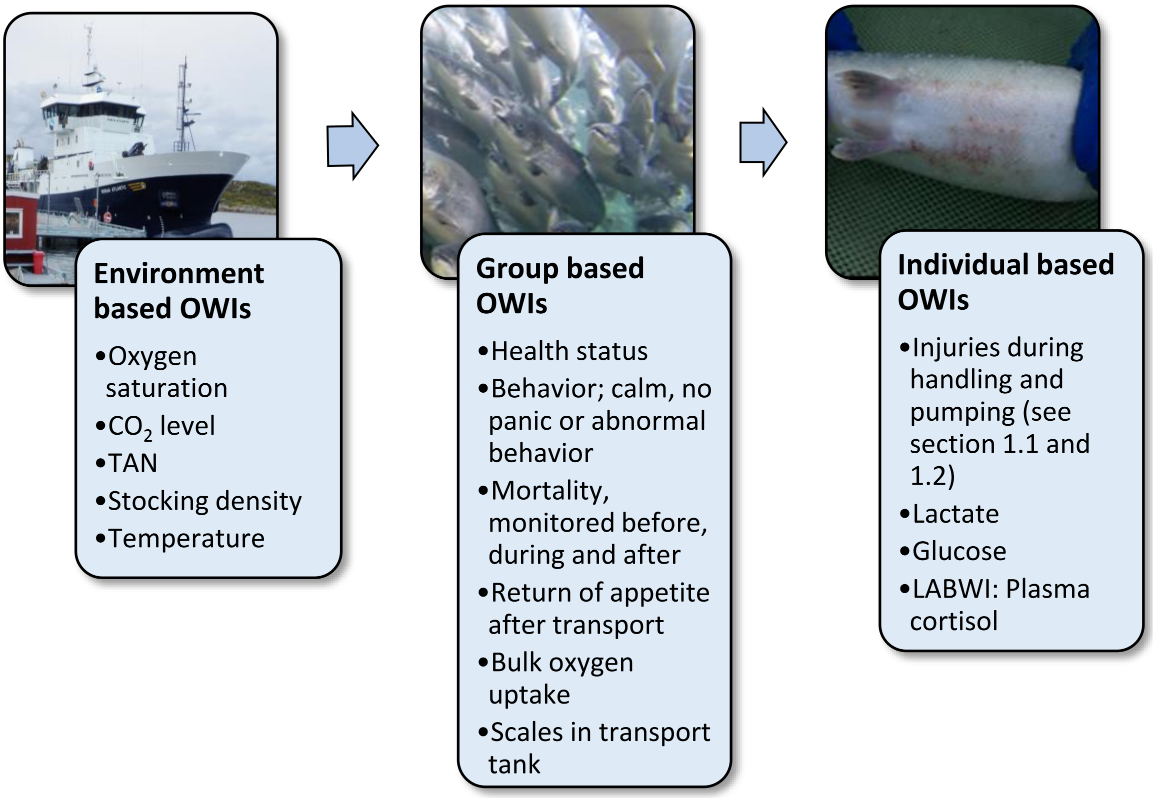


Figure 1.8-1. *Overview of fit for purpose OWIs for transport. Environment based OWIs specifically address the transport tank, group based OWIs address what can be observed and checked during the transport, while individual based OWIs are based on sampling individual fish. For key OWIs related to crowding and pumping see Figures 1.1.3-1 and 1.2.3-1. Photos and illustration: K. Gismervik. Group OWI photo: L. H. Stien*

Environment based OWIs

Oxygen saturation. As a general precautionary guideline, oxygen saturation levels of >80% are often used [13] and the RSPCA welfare standards for farmed Atlantic salmon [7] recommend a minimum limit of 7 mg L^{-1}. Fry can clump together on or near the bottom of the tank during transport and oxygen should therefore be supplied to the bottom of the tank [7]. Smolts and adults can also congregate at high densities during loading [103, 113].

Carbon dioxide can accumulate during transport (in closed tanks, or when the vents are closed in well boat transports) Tang et al., [115] showed that during closed-hold seawater transport PCO_2 could reach of 10 mmHg in 20 to 150 minutes depending on transport density and oxygen consumption rate.

LABWI: TAN. Properties such as temperature, pH and salinity can affect the NH_3: NH_4^+ ratio and thus the toxicity of ammonia. The maximum safe level of short-term exposure (4 hours) of NH_3-N is 0.1 mg L^{-1} according to Wedemeyer [60] (for further description see Part A, section 4.1.6). In order to reduce the risk of TAN accumulation, the fish should be starved before transport (see Part C, section 1.9). This is to ensure that the intestine is completely empty to reduce the risk of deteriorated water quality due to the build-up of faecal matter in the tanks.

Stocking density can be used as an indicator during transport. Norwegian legislation (Forskrift om transport av akvakulturdyr; FOR-2008-06-17-820) states that transport time and density should be adjusted to protect the welfare of the fish. Longer transports require greater attention to be paid to water quality, water temperature and stocking density. The RSPCA welfare standards for farmed Atlantic salmon [7] recommend that stocking density during road transport should not exceed 60-100 kg m^{-3} and in well boats should not exceed 40 -50 kg m^{-3} for smolts or i) 100 kg m^{-3} for 3.5 kg fish, ii) 110 kg m^{-3} for 4 kg fish, and iii) 125 kg m^{-3} for 5 kg fish. However, other factors have to be taken in consideration (such as initial water quality, size of the fish and transport time) when deciding appropriate stocking densities during a transport. Tang et al., [114] reported loading densities of 62-150 kg m^{-3} have no significant effect upon bulk oxygen uptake. Thus, stocking density is not a very sensitive OWI.

Temperature. The optimal temperature for parr is 12-14°C [16], whilst post-smolt fish prefer temperatures around 17°C [17]. Parr can tolerate a wide temperature range from 0 to well above 20°C [18] while the critical temperatures for post-smolts are around 6°C and 18°C [11] (see Part A section 4.1.1 for more information). The solubility of oxygen also declines with increasing temperature, so that warmer water contains less oxygen than colder water with the same saturation.

Group based OWIs (and WIs)

Health status. The health status of the fish must be known prior to transport to ensure it can withstand the procedure and also to minimise the risk of spreading disease.

Mortality should be followed closely during transport and on a regular basis for the first 4 weeks after transport to monitor and retrospectively assess problems or any welfare threats associated with the procedure.

Return of appetite. The time it takes for appetite to return should be closely monitored after transport. A reduction or loss of appetite can be caused by the initiation of a stress response [21]. The time it takes for appetite to return after a procedure can therefore also be used as an OWI as it can reflect how well the fish have dealt with the stressor. Appetite is easy to measure qualitatively by observing the fish when feed is offered.

Behavioural indicators. Schooling and abnormal behaviour could give an indication of a well or poorly executed transport, as typical stressed fry, smolt and post smolt salmon will aggregate in "clumps" at the bottom of the tank during transport and also in the tank or sea cage after unloading [7, 103, 113]. Highly stressed fish can also exhibit darting and flashing behaviours [103].

Scales in transport tank water. This indicates scale loss and damage to the skin which can cause osmoregulatory problems and also secondary infections.

Bulk oxygen uptake ($\dot{M}O_2$). Bulk $\dot{M}O_2$ represents the average rate of O_2 uptake in group-held fish and may be used as a welfare indicator for Atlantic salmon during transport. Tang et al., [114] concluded that $\dot{M}O_2$ is a valuable general measure of stress in groups of fish. Typical bulk $\dot{M}O_2$ values during transport ranged from 8 (after crowding and pumping stress) to 3 mg O_2 min^{-1} kg^{-1} (upon arrival after a 10 hour transport). Tang et al., [114] have stated that "*while bulk $\dot{M}O_2$ may not be a complete measure of stress or welfare, it does provide a relative measure of the cumulative response to the stressors encountered during live-haul, including the impact of loading densities*". However, water temperatures can influence bulk $\dot{M}O_2$ (increases with increasing temperature) [113, 114].

Individual based OWIs

Handling related injuries. See Part C sections 1.1 and 1.2 for a full description of the OWIs related to crowding and pumping prior to and after transport. In brief, the most common sign of problems associated with crowding and pumping in individual fish is different types of external injuries e.g. skin damage, followed by the development of superficial infections

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Lactate. Struggling and burst swimming increases anaerobic muscle activity, thus increasing lactate in the blood [2, 25, 26]. Lactate should stay below 6 mmol/l [2]. It is easily measured with handheld apparatus but samples should be taken approximately one hour after muscle activity. Samples should also be taken prior to (loading; pre-stress) and upon arrival at delivery point, since lactate should be close to pre-stress levels at the end of the transport [71].

Glucose can be used as an OWI for transport e.g. [71]. Elevation in plasma glucose is a relatively slow response to stress and peaks after around 3-6 hours in salmon [29] but the response is also dependent on the feeding status, diet type and other factors. Glucose levels should therefore be compared with pre-stress levels rather than any generic standard. Glucose should also be close to pre-stress levels at the end of the transport [71].

Plasma cortisol is not an OWI, but a LABWI. Figure 1.8.5-1 shows how plasma cortisol recovers when the fish arrive at the sea farm after a well-executed transport.

Physiological indicators such as plasma cortisol, lactate and glucose should have returned close to pre-stress levels by the end of the transport [121, 122].

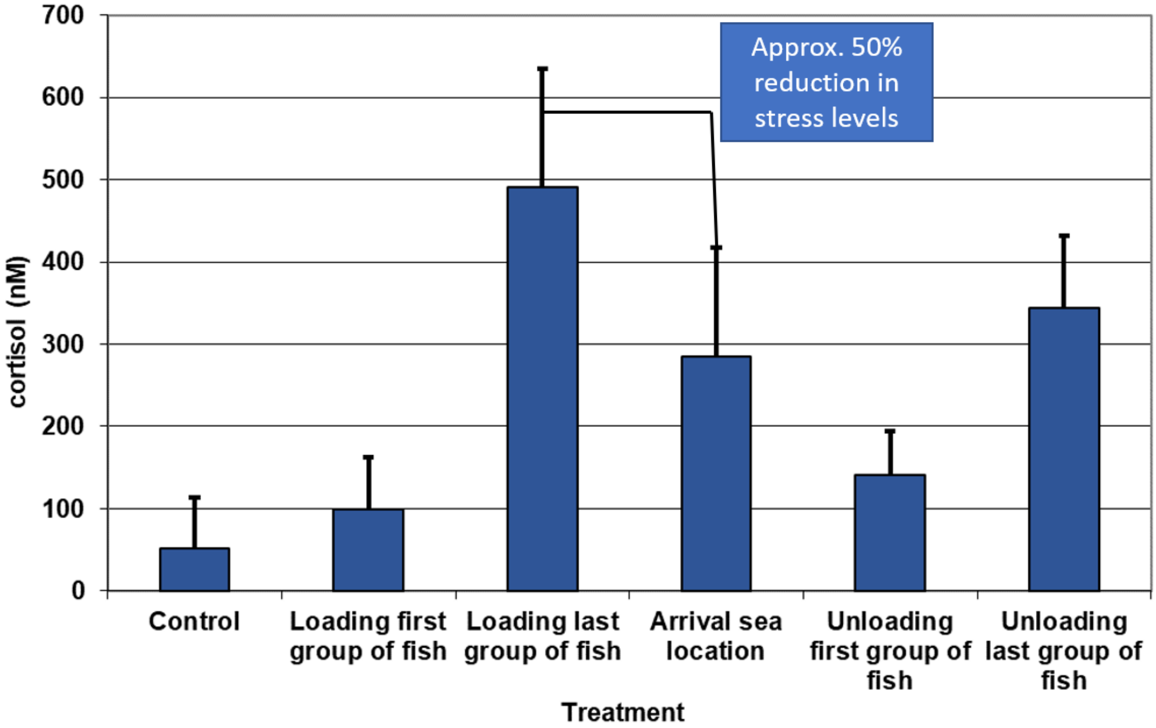


Figure 1.8-2. *Mean values of plasma cortisol in smolts before, during and after well boat transport. Note how plasma cortisol is reduced by ca. 50% after arrival at the sea site, giving the salmon ample time to recover between the two major stressors, loading and unloading. Figure reproduced from Iversen and Eliassen [121] with permission from M. H. Iversen.*

Some general advice regarding handling procedures during transport

The RSPCA welfare standards for farmed Atlantic salmon [7] have very robust guidelines in relation to different transport methods and life stages of salmon. Some brief pointers are highlighted here, but the authors suggest the reader refers to the RSPCA welfare standards for full details.

Some additional general handling procedures regarding fry transport (recommendations from the RSPCA, for full details see RSPCA welfare standards for farmed Atlantic salmon [7]). ***Reproduced with permission from the RSPCA.***

- The floor area of the transport tanks must be considered when calculating appropriate stocking densities for fry transport, as the fry can 'clump' together at the bottom of the tank.
- The location of the point of oxygen delivery must also consider this 'clumping' behavior of the fry.

Some additional general handling procedures regarding road transport (recommendations from the RSPCA, for full details see RSPCA welfare standards for farmed Atlantic salmon, [7]). ***Reproduced with permission from the RSPCA.***

- The transport tanks must be sufficiently insulated to ensure that the water temperature during transport remains relatively constant and does not fluctuate greater than ± 1.5 ^{o}C from the water temperature at the start of the journey.
- "*All unloading must be through valves which do not compromise the welfare of the fish, rather than netting fish from the tanks*".

Some general handling procedures regarding well boat transport (based on recommendations from the RSPCA [7], Iversen et. al. [71] and Iversen and Eliassen [121]. For full details see the above sources. ***Information from the RSPCA welfare standards for farmed Atlantic salmon reproduced with permission from the RSPCA.***

- *"The unloading of fish must not take place if adverse weather conditions are likely to compromise the welfare of the fish"*. [7]
- To make sure the fish have the opportunity to recovery from potential handling stressors during the transport process:
  - the transport route and its timing should be scheduled according to the weather and the expected water state, with the goal of avoiding waves >3m [71].
  - any transport < 4 hours long should wait a minimum of 4 further hours at the delivery site before unloading commences. This is to ensure the fish have a sufficient opportunity to recover from any potential loading stress [121].

1.9 Feed management, underfeeding and feed withdrawal

Feed management covers the choices a farmer has to make when they feed their fish. In the classical sense it refers specifically to how the farmer presents and distributes feed to the fish [123], not the choices of feed ingredients (which is feed nutrition). However, nutrition can impact upon feed management, for example, the energy content of feed can affect the length of time it takes for a fish to become satiated**.** Feed management covers six main factors: i) Ration size – how much feed to give the fish, ii) Frequency – how many times you feed the fish, both within and between days, iii) Temporal distribution of feed – when to feed the fish, iv) Spatial distribution – how to spread the feed, v) Feed rate – how fast do you feed the fish, and vi) the choice of feeding/feed waste monitoring technology to provide responsive rations.

Within feed management, we must also consider underfeeding (feed restriction) and fasting (feed withdrawal). Underfeeding is where the fish are fed, but at reduced amounts (below maximum feed intake or satiation and closer to, or below, the maintenance ration). Fasting is where feed is withheld from fish for a given number of days. This can be further classified as i) short-term fasting (7-10 days, [124]) or ii) long-term fasting (> 10 days).

Feed rate is also an important factor, many feed technologies give farmers good control of feed rate, allowing them to reduce competition and get as much feed to the fish when they need it.

Figure 1.9-1. *Feed delivery pipes going from the central feed barge to commercial rearing cages. Photo: C. Noble*

Challenges to fish welfare in daily feed management

- The primary welfare concerns of farmers and other stakeholders regarding the welfare impacts of feed management are mostly associated with **feed withdrawal** and **underfeeding**.
- Feed delivery rate can influence competition [123], but a study by Bailey et al., [125] reported delivery rate had no influence on growth rate.
- The predictability of the timing of feed delivery can also influence welfare in salmon parr; short-term unpredictability in feed delivery in fish that are accustomed to a specific feeding time can increase dorsal fin damage [126].
- A poor spatial distribution of feed can lead to size heterogeneity as fish which compete more effectively can potentially exclude poorer competitors from the feed resource (e.g. Thorpe et al., [127])
- The choice of feeding technology and feeding a fixed ration versus feeding in response to appetite can be detrimental to fish welfare [128, 129].

How to minimise welfare challenges in daily feed management

- Monitor appetite and feeding behaviour (e.g. via underwater cameras) and **feed a responsive ration in relation to changes in appetite.**
- Feed at a rate that does not lead to competition e.g. indicated by sustained increased swimming speeds during a meal [129], or competition between fish for individual pellets [129, 130].
- Distribute the feed widely over the cage surface.

Potential effects of fasting on welfare

- There is no clear and quantified relationship between the length of feed withdrawal and fish welfare [131, 132].
- Fish can tolerate short- and long-term periods of feed withdrawal and feed restriction [133].

Welfare risks of fasting (feed withdrawal)

- Fish may be subject to fasting for several husbandry reasons and some carry inherent welfare risks. This is dependent upon many factors including fish size, life stage, its condition, the size of its energy reserves and also other factors such as water temperature.
- In adult Atlantic salmon, 1 day of feed withdrawal can significantly decrease plasma glucose levels [124].
- Fasting can lead to decreased fish condition factor and emaciated fish [131, 134].

Welfare benefits of fasting (feed withdrawal)

- Fish may be subject to fasting for several husbandry reasons and some carry inherent welfare benefits. This is also dependent upon many factors such as those outlined above.
- If fish are subject to low oxygen levels or high water temperatures, feed may be withdrawn to lower metabolic rate and reduce oxygen demand. Any potential welfare costs related to this short-term period of fasting are a trade off against potentially fatal anoxia.
- Short-term fasting can also lessen the severity and impacts of certain fish diseases [135].
- Fasting prior to certain routines, e.g. lice treatments or to transport also reduces the metabolic rate of the fish and can reduce the rate of CO_2 and ammonia accumulation in transport water [136, 137].

- Long-term feed withdrawal (35 days of starvation prior to harvest) can increase a fish's tolerance to acute stress prior to slaughter [138].

Potential effects of underfeeding on welfare

- The opinion of the FAWC [139] is that the welfare risks of underfeeding, at least in the short-term are likely to be less than those for warm-blooded animals.
- However, for specific life stages such as parr and smolts, sudden periods of underfeeding (Turnbull, pers. comm.) or short- or longer-term underfeeding increases aggression and fin damage [140, 141] and the behavioural after-effects of this underfeeding can be persistent [141].
- Stephenson [142] stated "*CIWF and WSPA believe that starving farmed fish - that have previously been fed regularly - for prolonged periods is unacceptable in welfare terms.*"

Welfare risks of underfeeding (feed restriction)

- Fish may be subject to underfeeding for several husbandry reasons and some carry inherent welfare risks (see fasting section).
- Feed restriction can increase susceptibility to infection.
- In Atlantic salmon parr, both short (ca. 10 days) and long (ca. 30 days) periods of feed restriction can be detrimental fish welfare by increasing aggression and fin damage [140, 141].
- Underfeeding can lead to increased competition during feeding [129] and increased fin damage [128].
- The prolonged consequences of long term underfeeding can be depletion of energy reserves and nutritional status leading to reduced condition factor and even emaciated fish [131].

Welfare benefits of underfeeding (feed restriction)

- Feed restriction can also decrease susceptibility to infection [143].
- Balanced feed restriction or tightly controlled feeding with limited between-day variation in feed load may also help stabilise water quality in RAS or S-CCS systems [144, 145].

How to assess welfare associated with i) fasting, ii) underfeeding or iii) other feed management factors

To monitor the short- and longer-term impacts of i) underfeeding, ii) fasting and also iii) other feed management factors upon the fish, the farmer can use the following environment and animal-based OWIs. Although feeding and appetite is affected by a number of environment based OWIs we will only consider the most appropriate environmental indicators and focus on animal based indicators in relation to feed management.

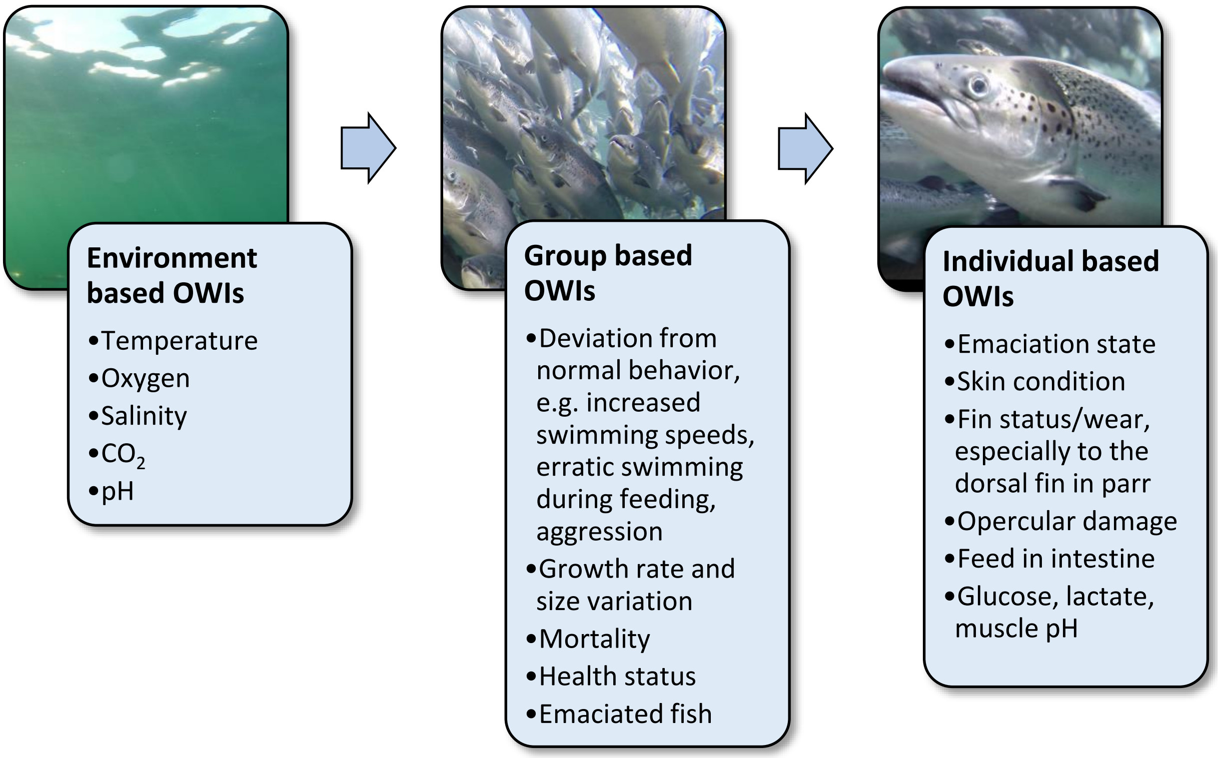


Figure 1.9-2. *Overview of fit for purpose OWIs for primarily fasting and underfeeding, but also other feed management factors. Environment based OWIs address the rearing environment, group based OWIs assess the group, while individual based OWIs are based on sampling individual fish for grading their external appearance. Illustration: C. Noble and L. H. Stien. Photos: L. H. Stien.*

Environment based OWIs

Temperature can affect both appetite and how the fish cope with feed restriction or feed withdrawal due to its effects upon metabolism. With regard to daily feed management, appetite decreases as fish approach their critical temperature ranges. Parr can tolerate a wide temperature range from 0 to well above 20°C [18] while the critical temperatures for post-smolts are around 6°C and 18°C [11] (see Part A section 4.1.1 for more information). The optimal temperature for parr is 12-14°C [16], whilst post-smolt fish prefer temperatures around 17°C [17]. Degree days should be considered for when assessing the implications of fasting periods [139, 142, 146].

Oxygen levels can impact upon feed intake and appetite (e.g. Remen et al., [147] where appetite was suppressed in post-smolts at <70% oxygen saturation at 16°C). Oxygen solubility and therefore availability is affected by temperature and salinity, whilst oxygen demand is affected by e.g. life stage, feeding, levels of activity and temperature. For parr and smolts, detailed data of the oxygen concentrations that maintain appetite and aerobic metabolism at different temperatures are not available, but experience does not suggest dramatically different oxygen requirements compared with that of post smolts (see Table 1.2-1.). For instance, a limiting oxygen saturation (LOS) of 39% O_2 at 12.5°C has been found for parr [12]. For post-smolts, levels above the dissolved oxygen required for maximal feed intake (DO_{maxFI}) are always safe, while levels must never approach the routine limiting oxygen saturation (LOS) [14] (Table 1.2-1.). As a general precautionary guideline, oxygen saturation levels of >80% are often used [13] and the RSPCA welfare standards for farmed Atlantic salmon [7] recommend a minimum of 7mg L^{-1}.

Salinity can also affect appetite, especially following sea water transfer [148]. However, the potential effects of salinity upon appetite across various life stages are unclear. In principle, a salinity between 0-10 ‰ is recommended for fry and parr [149]. Newly transferred post-smolts show a preference for the halocline (<20 ‰ in the top layer of water column and >30 ‰ below; [11] and access to brackish water can be beneficial (28 ‰; [150]) in particular when water temperature is low (4°C). Stien et al., [13] state that salinity has little effect upon adult non-migratory salmon. Salinity should be <10 ‰ for broodstock.

CO_2 / pH. In freshwater farming, high levels of CO_2 can increase FCR in salmon parr [151]. Noble et al., [151] recorded a clear increase in FCR when salmon parr were kept at CO_2 levels of about 27 mg L^{-1} compared to CO_2 levels below 18 mg L^{-1}. Low pH can also cause decreased appetite in juvenile salmon. For example, Haya et al., [152] reported reduced appetite in parr exposed to a pH of 4.7 compared to fish maintained at a pH of 6.5.

Group based OWIs

Behaviour. Juvenile salmon show more aggression when hungry and underfed [131, 140, 141] and underfeeding has been suggested to lead to more fighting and injuries than no feeding at all [153]. Juvenile salmon also show increased and more erratic swimming speeds during feeding [129, 154] when fed a fixed ration versus a ration responsive to appetite. To the authors' knowledge there is no robust evidence of aggression in adult fish when fasted or underfed.

Growth can be negatively affected by underfeeding [128, 140, 141], as can size variation [140]. Growth can also be negatively affected by feed withdrawal [134, 155]. Acute changes in growth can be used as an early warning system for potential problems with regard to daily feed management, particularly when the farmer has robust growth monitoring practices.

Mortality can increase after feed deprivation, especially in fry and smaller fish [156] and also by underfeeding [157]. Underfeeding during the FW smolt phase can lead to increased mortality in the SW phase [158]. Mortality should therefore be followed closely and on a regular basis.

Health status can affect appetite. See, for example, Damsgård et al., [159].

Emaciated fish. The long-term consequences of underfeeding or starvation may be the depletion of energy reserves and reduced nutritional status. This again leads to reduced condition factor and emaciated fish [131].

Individual based OWIs

Fin damage. The most common sign of problems associated with underfeeding/fasting/poor feed management in parr and newly transferred post-smolts is initially morphological damage, primarily dorsal fin damage ([128, 129, 140, 141, 154] in parr/smolts and post-smolts). Abrupt changes in the frequency of grey dorsal fins (an indicator of increased aggression) for these life stages can also be used as a qualitative group OWI as it is observable without handling the fish.

Skin condition. Salmon may lose scales and get wounded during competition for feed. Skin condition can therefore also be used as an OWI.

Opercular damage includes broken or shortened opercula and can be affected by feeding [160].

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Emaciation state and condition factor. Reduced condition factor can result from underfeeding [128, 134, 140, 141] and prolonged feed withdrawal [161] can also lead to a reduced condition factor or emaciated fish.

Feed in the intestine often indicates that the fish has eaten during the last one to two days [45, 46], but this depends on the fish size and temperature. To evaluate daily feed intake or fasting periods, salmon may be euthanized and the intestines checked for feed residue, this also reflects appetite and access to food.

Glucose and Lactate. Glucose can be used as an OWI for poor feed management and 1 day of feed withdrawal can significantly decrease plasma glucose levels [124]. Lactate is also affected by poor feed management and should stay below 6 mmol/l [2].

Muscle pH. Increased stress/muscle activity produces more lactic acid which in turn reduces muscle pH [27].

Current advice regarding fasting

Current advice varies on the appropriate lengths of feed withdrawal in relation to fish welfare.

- RSPCA welfare standards for farmed Atlantic salmon [7] recommend starvation periods should be no longer than 72 hours in adult salmon, without the approval of a veterinary surgeon or senior management, but this threshold is not based upon the scientific literature and is currently under consultation. A 72 hour threshold is also recommended by HSA [162], Stephenson [142] and CIWF [163].
- RSPCA welfare standards for farmed Atlantic salmon [7] recommend starvation periods should be no longer than 48 hours for parr grading and smolt transportation.
- FAWC have proposed maximum limits of 48 hours [164].
- The Norwegian Food Safety Authority have no fixed limits on fasting due to limited knowledge.
- Other authors suggest fasting time should be limited to one week [165].
- Lines and Spence [166] suggest a feed withdrawal period of 1-5 days is unlikely to pose major welfare threats to salmon.
- López Luna et al., [146] have suggested fasting periods and their implications should be considered in degree days, as have Stephenson [142] and FAWC [139].

Knowledge gaps

Due to the mixed recommendations and insufficient scientific knowledge, solid, robust and quantitative data on the welfare effects of fasting at different life stages and in relation to differing routines (e.g. prior to slaughter) is urgently needed.

This approach should cover feed withdrawal periods of different durations and under different farming conditions, especially with regard to temperature. López-Luna et al., [146] have suggested degree days be accounted for when assessing the implications of fasting periods, as have Stephenson [142] and FAWC [139].

Until this data is available, we have outlined the potential OWIs that are suitable for assessing the effects of i) underfeeding, ii) fasting and iii) other feed management practices upon fish welfare at different life stages.

The farmers can then use these OWI tools to assess the impacts of each of the above procedures on the welfare of their fish.

The FAWC [139] also suggest "*it would be desirable to develop alternative approaches to the practice of feed restricting a whole pen when only some of the fish are to be moved, and to the use of feed restriction over long periods*"

1.10 System sanitation procedures e.g. tank and equipment washing

Cleaning and disinfection or sanitation of production units and equipment is essential for biosecurity and hygiene. It also plays a role in system maintenance, avoiding build-up of organic waste and therefore water quality issues. The primary process of sanitation is to clean before disinfecting since disinfectants will be less effective if potentially harmful organisms are protected by organic material. Drying and exposure to sunlight can also play an important role in sanitation. Water treatment in recirculating systems including ozonisation (Part B section 2) and net cleaning systems (Part C section 2.2.4) are covered in other sections.

Challenges to fish welfare

- Sanitation is primarily a benefit to fish welfare and is only a risk to welfare if it is conducted whilst the fish are in the system or if residues of potentially harmful substances remain in the water. The challenges in such cases are physical damage, stress associated with disturbance and the effects of toxic chemicals.

How to minimise welfare challenges

- Risks can be mitigated by good management processes, including equipment maintenance, staff training, supervision and monitoring of competence. There should be standard operating protocols and records of sanitation, including the safe and effective use of chemicals.
- There is some evidence that some regular disturbance is less harmful than either very rare or persistent disturbance [167], this may be a form of habituation or adaptation.
- If deviations from normal behaviour, appearance or production are observed this should be investigated.

How to assess welfare during sanitation

System sanitation should either be conducted when the fish are not in the system or organised to cause minimal disturbance.

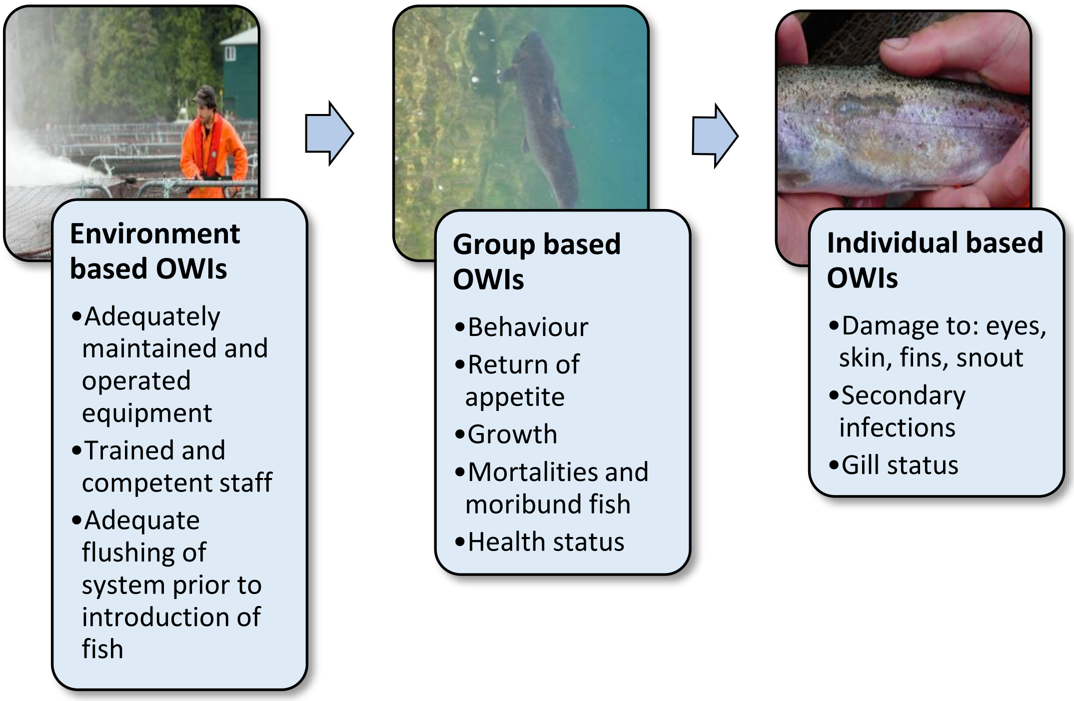


Figure 1.10-1. *Overview of fit for purpose OWIs during system sanitation. Environment based OWIs specifically address the environment, group based OWIs address what can be observed and checked during the operation, while individual based OWIs are based on sampling individual fish for close up examinations. Environment OWI photo: http://marineharvest.ca/about/blog-marine-harvest-canada/2012-container-blog/september-6-2012/. Group OWI photo: B. Glencross. Individual OWI photo: J. F. Turnbull.*

Environment based OWIs

Environmental OWIs relate to the appropriate procedures and operation during sanitation. The specific controls are dependent on the process and substances used but should follow manufacturer's instructions.

Group based OWIs

Abnormal behaviour including acute excessive responses to the process or chemical should be examined. Any persistent agitation or feeling/avoidance behaviour should be investigated.

Return to appetite. The time it takes for appetite to return should be closely monitored after system sanitation. A reduction or loss of appetite can be caused by the initiation of a stress response [21]. The time it takes for appetite to return can therefore also be used as an OWI as it can reflect how well the fish have dealt with the stressor. Appetite is easy to measure qualitatively by observing the fish when feed is offered.

Reduced growth this may be the result of reduced feed intake due to stress or an indication of problems such as effects of toxic substances.

Mortality and moribund fish should be followed closely and on a regular basis following system sanitation procedures to retrospectively assess problems or welfare threats associated with the procedure. This should be investigated by fish health specialists [168, 169, 170, 171].

Health status. The health status of the fish must be known prior to system sanitation to improve system sanitation in relation to infectious diseases (e.g. double disinfection with prolonged fallowing / drying).

Individual based OWIs

Morphological damage. Problems with the equipment or the procedure may lead to various forms of morphological damage, including damage to eyes, scale loss, snout damage and damage to fins.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Secondary infections. Depending on the system (fresh or salt water) a variety of secondary infections can result from initial damage during sanitation and in some cases, severe infections can result from relatively minor damage. Any signs of infection should be investigated by a health specialist.

Gill status. Following sanitation some chemicals may damage the gills. Abnormal behaviour may indicate a problem but it may also be necessary to investigate pathological changes on gross or post mortem examination.

1.11 Grading

Grading is conducted for a variety of reasons and can be essential for fish welfare and health. For example, grading can be used to ensure a uniform fish size before vaccination, for removing small or abnormal fish and also to select fish for harvest. Regardless of how carefully it is conducted it is a stressful and potentially harmful procedure for the fish. Therefore, fish should only be graded when essential and in general all handling of fish should be minimised.

Grading can be conducted in a variety of ways throughout the production cycle. It can be performed manually with small fish, by the use of grading machines, or passively with flexible net panels or similar. Grading is also conducted using well boats from sea cages.

Challenges to fish welfare

The risks associated with grading include those associated with feed withdrawal prior to grading (see Part C section 1.9), crowding (Part C section 1.1), pumping (Part C section 1.2) and transfer to a well boat (Part C section 1.8), potential for hypoxia due to air exposure or exposure to water with low dissolved oxygen and physical damage. The stress of the operation and the physical damage can increase the risk of secondary infections such as winter ulcers (*Moritella* spp.) in salt water (especially at lower temperatures) and fungal (*Saprolegnia* spp.) infections in fresh water.

The challenges associated with passive grading with nets or panels (Figure 1.11-1) with appropriate gaps are similar to those associated with crowding (Part C section 1.1), with the exception that fish nearing the size of the gaps may become stuck (covered below). Passive grading is potentially less harmful to welfare since feed is not normally withdrawn and the fish are not pumped or handled.

Figure 1.11-1. *Passive grading system. Photo reproduced with permission from Flexi-Panel by Grading Systems (UK) Ltd.*

How to minimise welfare challenges

Every effort should be made to reduce the need for grading. The reason for grading (or not) should be recorded to allow processes to be retrospectively evaluated. The number of times fish are graded can be reduced by robust planning of e.g. initial stocking densities. Staff should be adequately trained and grading should follow a detailed plan and standard operating procedures with adequate supervision. All equipment must be adequately maintained, monitored and appropriate for the task, e.g. with a minimal number of joins in fish pipes. There should be records of grading and these should be correlated with any subsequent problems.

Avoid:

- Protruding edges
- Sharp edges
- Rough surfaces
- Dry surfaces
- Abrupt changes of direction
- Long drops out of water

Water quality in any grading machines should be monitored and be of high quality. The time fish spend out of water should be minimised especially at high or low temperatures and when humidity is low. Where possible, grading should be avoided at low or high temperatures. The RSPCA welfare standards for farmed Atlantic salmon [7] recommend fish should be a minimum of 1.3 g in weight. They also outline density recommendations for grading by well boat based on fish size (see later in this section).

For planned routine grading, the fish should be health checked to ensure they are healthy enough to cope with the grading process. For example, gill pathology may make them vulnerable to low dissolved oxygen.

How to assess welfare during grading

Grading can be associated with a variety of handling procedures including a combination of feed withdrawal (Part C section 1.9), crowding (Part C section 1.1), pumping (Part C section 1.2) and transfer to a well boat (Part C section 1.8) and details of the risks, mitigation and specific welfare assessment relating to those processes can be found in the relevant sections.

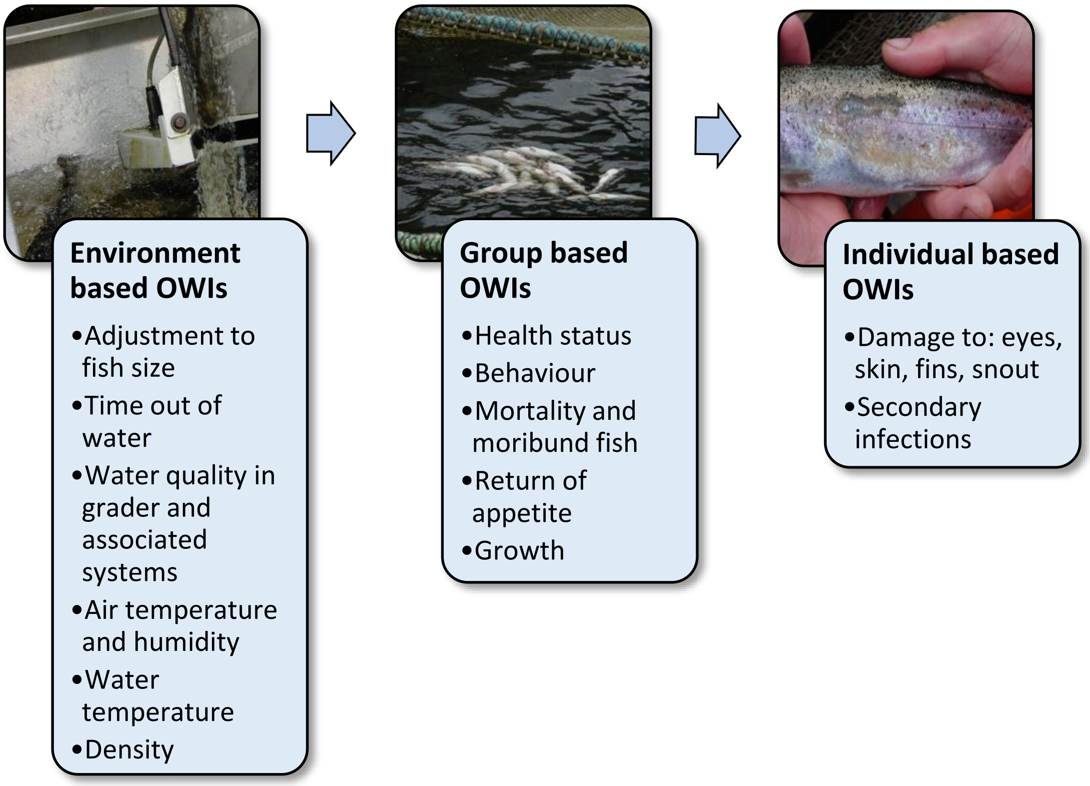


Figure 1.11-2. *Overview of fit for purpose OWIs for grading. Environment based OWIs specifically address the grading environment, group based OWIs what can be observed and checked during operation, while individual based OWIs are based on sampling individual fish for close up examinations. Figure: J. F. Turnbull and K. Gismervik, photos: J. F. Turnbull*

Environment based OWIs

Equipment adjusted to the size of fish. No fish should become trapped in the system.

Time out of the water should be minimised. RSPCA welfare standards for farmed Atlantic salmon [7] recommend a maximum air exposure time of 15 seconds.

Water quality including dissolved oxygen should be monitored in all the equipment or holding facilities associated with grading. As a general precautionary guideline oxygen saturation levels of >80% are often used [13] and the RSPCA welfare standards for farmed Atlantic salmon [7] recommend a minimum of 7mg L^{-1}.

Air temperature and humidity. With manual or machine grading, avoid excessively high or low temperatures and periods of low humidity.

Temperature. The optimal temperature for parr is 12-14°C [16], whilst post-smolt fish prefer temperatures around 17°C [17]. Parr can tolerate a wide temperature range from 0 to well above 20°C [18] while the critical temperature for post-smolt it is around 6 ° C and 18 ° C [11] (see Part A section 4.1.1 for more information). Low temperatures also increases the risk of winter ulcers. Damage from handling is often the initiating factor, leading to secondary infections with bacteria such as *Moritella viscosa* (see Table 3.1.5-2 in Part A for more information on winter ulcers) [19, 20].

Density. It is important to avoid densities that are too high during grading. The table below shows densities recommended by the RSPCA welfare standards for farmed Atlantic salmon [7].

Table 1.11-1. *Recommended maximum densities in the well when grading via well boat (RSPCA welfare standards for farmed Atlantic salmon [7]). Reproduced with permission from the RSPCA.*

Live weight of fish (kg)	Maximum stocking density (kg m^{-3}) in well boat when grading fish
5,0	125
4,0	110
3,5	100
3,0	90
2,0	75
1,0	60
0,1	45

Group based OWIs

After grading it is normal for the fish to take some time to settle down to their normal behaviour and this is system dependent. The group based OWIs are related to the persistence of the abnormality.

Health status. The health status of the fish must be known prior to grading to ensure it can withstand the procedure. It is important to check e.g. gill health.

Behaviour. Signs of abnormal behaviour such as persistent agitation, lethargy or abnormal shoaling and swimming after grading should be monitored.

Mortality and moribund fish should be followed closely and on a regular basis following grading procedures to retrospectively assess problems or welfare threats associated with the procedure. This should be investigated by fish health specialists [168, 169, 170, 171].

Return of appetite. Any persistent reduction in feeding may indicate damage or stress as a result of grading and should be carefully monitored [21]. Appetite is easy to measure qualitatively by observing the fish when feed is offered.

Growth. Some reduction in growth is normal if feed is withheld before grading but may be an indication of a problem if it is excessive or persistent.

Individual based OWIs

Morphological damage. Problems with the equipment or the procedure may lead to various forms of morphological damage, including damage to eyes, scale loss, snout damage and damage to fins.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Secondary infections. Depending on the system (fresh or salt water) a variety of secondary infections can result from initial damage during grading and in some cases, severe infections can result from relatively minor damage. Any signs of infection should be investigated by a health specialist.

1.12 Examination of live fish

Operations where fish are taken out of the units, inspected and returned alive

On numerous occasions it is necessary to sample live fish from the farm. This sampling can be for counting sea lice, assessing gill quality, assessing external injuries and deformities, weighing etc. Currently these examinations are mostly manual and they all have similar approaches. Future technology may be able to do part of these tests automatically and without removing the fish from the water.

Challenges to fish welfare

It is important to obtain a representative sample of fish for examination. In large units with many individuals, the fish may have to be crowded to ensure that the sample is reasonably representative. Crowding is a welfare risk (see Part C section 1.1 on crowding) and if many fish are crowded together it means that many more fish are prone to welfare risks than just the ones that a required for sampling.

After crowding, the fish are usually netted into an anaesthetic bath (see Part C section 1.6). When the fish is anesthetized, it is usually lifted out of the water and examined, before being introduced back to the rearing unit. Some systems are now available that allow the fish to be examined in water (e.g. for lice counting). Potential welfare risks regarding examination of live fish are listed in Table 1.12-1 below.

To the authors' knowledge, few studies have been conducted that directly address the welfare challenges associated with these types of operations, but all fish handling poses a risk of injury and stress. The salmon are adapted to life in water, are virtually weightless and have limited physical contact with any solid object. The skeleton and the skin is not adapted to the rigors of netting and other handling procedures, so this kind of operation can easily damage the fish [22]. The tolerance for handling varies with the life stage, size, water and air temperature, health, equipment and the handling process.

With regard to the welfare risks associated with air exposure, the scientific literature is somewhat scarce, but there has been some work done on catch and release in 300-500g rainbow trout (held in freshwater) which we will use as source material [172].

- Mortality in rainbow trout (after 12 hours) was reported for two differing situations, i) after exercise + 30 seconds air exposure: 38%, ii) after exercise + 60s air exposure: 72% [172].
- The RSPCA welfare standards for farmed Atlantic salmon recommend a maximum air exposure time of 15 seconds, unless the fish are anaesthetised [7].

Table 1.12-1. *Welfare risks of handling fish during live examinations. Table: K. V. Nielsen and K. Gismervik*

Operation	Risk factor	Increasing risk
Crowding	See Part C section 1.1 crowding	
Hand netting	External injuries: mucus layer, skin, scales, fins, eyes	Design of the dip net and adaption to fish size Too large mesh size Damaged net Too many fish netted at once
	Internal injuries	Too many fish netted at once
Sedation, see Part C section 1.6	Overdose of sedative - poisoning	Deviations from instructions for use / prescription (dose and / or holding time)
	Insufficient sedation may increase risk of injury	Deviations from instructions for use / prescription Use of force may be needed
	External injuries	Too little space in sedation tank and risk of losing fish
	Water quality	Recycling of anaesthetic bath High number of fish
Examination	External and internal injuries	Incorrect lifting technique Insufficiently anaesthetised Gloves have a rough surface
	Air exposure - Skin and gill damage (freezing / drying)	Low / high air temperature, low humidity and windy conditions Length of air exposure, max. 15 sec. unless anaesthetised (RSPCA, [7])
Return to rearing unit	External damage if thrown or netted	Collision with e.g. the bird net on the way to the water The design and condition of the dip net
In general	Stress	Temperatures near the lower and upper critical temperature range [15], which for post smolt is around 6°C and 18°C [11]
	Long term effects	Difficult to measure at the commercial scale

How to minimize welfare challenges

In general, the equipment used in the handling of live fish should be designed to ensure good fish welfare and the use of the equipment must ensure that the risk for the fish is minimized. Fish should not come into contact with sharp edges, rough or absorptive surfaces, knots (net), or be subjected to impact, pressure, strain (lifting by the tail), unnecessary crowding etc. As far as possible, the handling should be carried out in water. If fish welfare cannot be ensured during the examination, the fish should be euthanised after anaesthesia/stunning (and before examination).

How to assess welfare

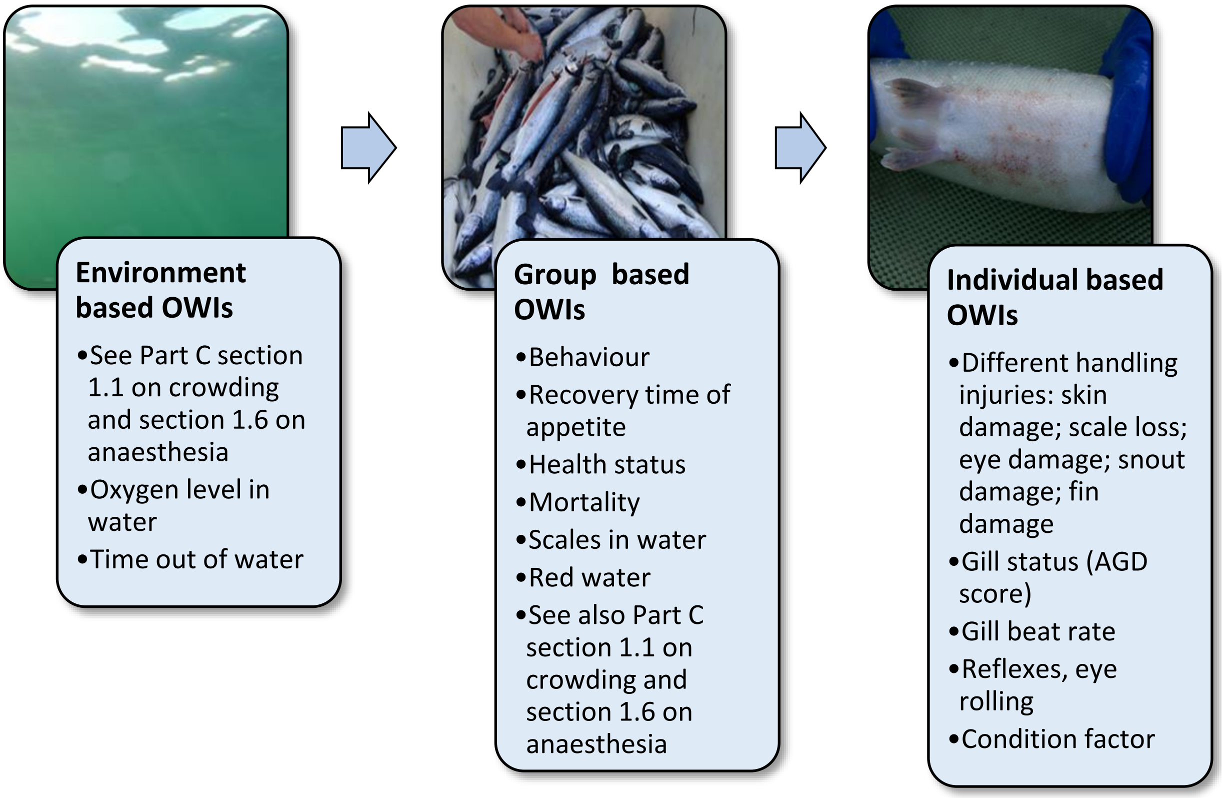


Figure 1.12-2. *Overview of fit for purpose OWIs for the examination of live fish. Environment based OWIs address the handling environment, group based OWIs address welfare at the group level, while individual based OWIs are based on sampling individual fish. Photos and illustration: K. Gismervik. Environment based OWI photo: L. H. Stien*

Environment based OWIs

Oxygen. It is necessary to monitor and ensure adequate oxygen levels for the fish during both crowding (see Part C section 1.1), during anaesthesia (Part C section 1.6) and during recovery. As a general precautionary guideline, oxygen saturation levels of >80% are often used [13] and the RSPCA welfare standards for farmed Atlantic salmon [7] also recommend a minimum of 7mg L^{-1}.

Duration of air exposure. Although the authors cannot find robust scientific data on the effects of air exposure on the welfare of Atlantic salmon, there is a paper on rainbow trout that reported mortality nearly doubled when air exposure increased from 30s to 60s in exercised fish [172]. The RSPCA welfare standards for farmed Atlantic salmon [7] adopt a cautionary approach and recommend a maximum air exposure time of 15 seconds, unless the fish are anaesthetised. Air exposure time is particularly critical at high or low temperatures and when humidity is low. If possible, live fish should be examined in water.

Group based OWIs

Since there are often relatively few fish sampled in relation to the total number in the aquaculture unit, it can be difficult to measure the long-term consequences of the procedure. If the number of sampled fish is high it may be necessary to look at all the factors listed below.

Return of appetite. The time it takes for appetite to return should be closely monitored after handling. A reduction or loss of appetite can be caused by the initiation of a stress response [21]. The time it takes for appetite to return after e.g. handling can therefore also be used as an OWI as it can reflect how well the fish have dealt with the stressor. Appetite is easy to measure qualitatively by observing the fish when feed is offered.

Behaviour. As with crowding and handling, the resumption of normal behaviour can be used as a qualitative OWI. Signs of abnormal behaviour such as persistent agitation, lethargy, abnormal shoaling and swimming e.g. side swimming or gasping at the surface should be monitored. During handling it is important to assess the behaviour of the fish during crowding (see Part C section 1.1) and the level of consciousness during anaesthesia (see Part C section 1.6).

Health status, mortality and clinical outbreaks. Examination of live fish is often carried out to assess health status. This may for example be related to gill health, lice counting, assessing external injuries and deformities, or to examine moribund fish swimming near the surface. Increased mortality may be the main reason for contacting veterinary or fish health personnel, and it is therefore important that mortality is monitored closely and regularly on a daily basis. Any fish that require euthanisation due to e.g. poor health should be examined by fish health professionals [168, 169, 170, 171]. When you release fish back into the rearing unit after anaesthesia and examination, there is a danger that the procedure may itself increase mortality. Mortality should be followed carefully and regularly after the examination of live fish to monitor and assess problems or welfare threats associated with the procedure. Fish that have been returned to the rearing unit but do not recover within a reasonable time should be taken up and euthanised as soon as possible. Or, if the fish is under anaesthesia too long or is severely injured during handling, it may be better that it is euthanised during the examination.

Scales in water. Indicates scale loss and damage to the mucus and the skin which can cause osmoregulatory problems and also secondary infections.

Red water. According to practical experience, the crowding of fish in closed and smaller containers can make it possible to detect bleeding as a colour change in water, so called “red water”. It has been seen in conjunction with anaesthesia in smaller and closed containers, and is best seen in light units. Although "red water" does not necessarily mean that the fish will die from treatment (Nilsson, pers. comm.), it is never a good sign and the cause should be investigated (see Part A section 3.1.6 for more information). There are examples of “red water” due to gill bleeding, seen during scoring fish in connection with mechanical de-licing [24], where immediate changes in the operation has been justified. Supplementary histopathological sampling (LABWI) can be considered for further investigation.

Individual based OWIs

External injuries. Physical contact with other individuals, or equipment, may lead to various forms of skin damage. It is therefore important to monitor the fish for external injuries, especially in view of acute changes in connection with this type of examination. Pay attention to the skin, scale loss, fins (e.g. active fin splitting or haemorrhaging), eyes, snout, opercula and gills.

Gill status and AGD score. In general, it may be relevant to score changes to the actual surface of the gills, visible as "white patches" (total gill score). AGD scoring of the gills can also be relevant. Gill bleeding should also be monitored in relation to mechanical injuries [24] and it is important that the gills are handled very carefully during the examination so that they are not damaged by the procedure itself.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document. External injuries can be assessed both qualitatively (change in observed status before and after) and quantitatively (if more information is required in the welfare audit)

Gill beat rate. Clear changes in gill beat rate (such as very fast opercular movements) may indicate that fish are under duress. This should be assessed throughout the procedure.

Reflexes, eye rolling. Simple reflex indicators such as eye roll and the ability to flip upright can easily be used as direct indicators of stress and can be evaluated individually or as an index [40]. The animal is classified as insensible if responses to these indicators are lacking [41, 42]. The vestibulo-ocular reflex (VER; the "eye roll") can also be used. It is the last reflex to be lost during anaesthesia and the first to appear after recovery [43], see also Part C Figure 1.3-3.

Condition factor is calculated from the weight and length of the fish (see Part A, section 3.2.5). A very low condition factor may be an indication of feed deprivation (see Part C section 1.9). An operator should also consider the appearance of the fish (shape, size) which may also be important e.g. fish with a very high condition factor may have vertebral deformation (see section A, chapter 3.2.5 for more information and references). If measurements of weight and length are performed on living fish, it is important to consider air exposure time (see time out of water).

Knowledge gap

A potential future OWI can be the evaluation of drying/freezing of epidermis associated with air exposure at low temperatures. The authors found no scientific literature on this, but its use as a potential OWI should be investigated.

1.13 Summary tables of which OWIs and LABWIs are fit for purpose for different routines and operations

Table 1.13-1. *Summary of the environment based OWIs and LABWIs that are fit for purpose for different handling operations*

	Usage area	Handling operation											
	WI	Crowding	Pumping	Slaughter	Euthanizing	Bath & Medical treatments	Anaesthesia	Vaccination	Transport	Feed management & withdrawal	System sanitation	Grading	Examination of live fish
Environment WIs	Temperature	×		×		×	×	×	×	×		×	×
	Salinity					×				×			
	Oxygen	×	×	×		×	×	×	×	×		×	×
	CO_2					×	×	×	×	×			×
	pH and alkalinity					×	×	×					×
	Total ammonia nitrogen					×			×				
	Water current speed	×	×										
	Stocking density				×	×		×	×			×	
	Time out of water			×				×				×	×
	Holding time					×							

Table 1.13-2. *Summary of the group and individual based OWIs and LABWIs that are fit for purpose for different handling operations*

	WI / Usage area	Handling operation: Crowding	Pumping	Slaughter	Euthanizing	Bath & Medical treatments	Anaesthesia	Vaccination	Transport	Feed management & withdrawal	System sanitation	Grading	Examination of live fish
Group WIs	Mortality rate - acute	×	×	×		×	×	×	×		×	×	×
Group WIs	• Longer-term	×	×			×	×	×	×	×	×	×	×
Group WIs	Behaviour	×	×	×	×	×	×	×	×	×	×	×	×
Group WIs	• Bellies showing	×	×		×	×	×	×	×		×	×	×
Group WIs	• Equilibrium loss					×	×	×	×		×	×	×
Group WIs	• Abnormal swimming	×	×		×	×	×	×	×	×	×	×	×
Group WIs	• Crowding Scale	×	×			×		×	×			×	×
Group WIs	• Gasping at the surface	×	×		×	×	×	×	×		×		×
Group WIs	• Vertical swimming	×				×		×					
Group WIs	• Head shaking					×	×		×				
Group WIs	• Clumping	×				×		×	×		×	×	
Group WIs	• Aggression									×			
Group WIs	Appetite	×	×			×	×	×	×	×	×	×	×
Group WIs	• Growth	×	×			×		×		×	×	×	
Group WIs	Disease and health status	×	×	×	×	×	×	×	×	×	×	×	×
Group WIs	Emaciated fish									×			×
Group WIs	Scales or blood in water	×	×	×	×	×	×	×	×				×
Group WIs	Bulk oxygen uptake								×				
Group WIs	Change in skin colour – blue to green	×			×	×							
Individual WIs	Handling trauma	×	×	×	×	×	×	×	×		×	×	×
Individual WIs	• Scale loss and skin condition	×	×	×	×	×	×	×	×	×	×	×	×
Individual WIs	• Mouth jaw wound	×	×	×	×	×			×		×	×	×
Individual WIs	• Fin damage and fin status	×	×	×	×	×	×	×	×	×	×	×	×
Individual WIs	• Eye haemorrhage and status	×	×	×	×	×			×	×	×	×	×
Individual WIs	• Haemorrhaging		×	×									
Individual WIs	Cataract					×							
Individual WIs	Reflex, eye rolling			×	×		×	×					×
Individual WIs	AGD score	×	×			×							×
Individual WIs	Gill bleaching and status	×	×			×		×	×				×
Individual WIs	Gill beat rate	×		×	×	×	×	×	×				×
Individual WIs	Opercula damage	×	×							×	×		×
Individual WIs	Condition factor									×			×
Individual WIs	Moribund fish			×		×			×		×	×	×
Individual WIs	Emaciation state			×						×			
Individual WIs	Correctly adjusted blow if percussive stunning/killing			×	×								
Individual WIs	Vaccine related pathology (Speilberg score)							×					
Individual WIs	Feed in the intestine			×		×		×		×			
Individual WIs	Muscle pH	×	×	×						×			
Individual WIs	Pre-rigor time	×	×	×									
Individual WIs – Blood	Cortisol	×	×						×				
Individual WIs – Blood	Glucose	×	×						×	×			
Individual WIs – Blood	Lactate	×	×						×	×			
Individual WIs – Blood	pH			×									

2 How to monitor welfare during the development of new technology

The aim of this section of the handbook is to summarise and review the key scientific findings regarding potential fit for purpose OWIs for use during the documentation of new technology in relation to fish handling/operations.

2.1 First considerations and an OWI/LABWI toolbox for new technology

The aquaculture industry is constantly developing new technology with the goal of improving production and the handling of fish. In particular there have been rapid developments and innovations concerning de-licing technology over the last few years. Norwegian legislation makes it clear that both the technology supplier and the farmer have a responsibility to ensure the equipment is welfare friendly. Technological innovations need to take the biology of the fish into consideration at all steps of their development, and the "3 Rs" (Replace, Reduce and Refine) approach should be considered during stepwise welfare documentation (Figure 2.1-1 below). According to Norwegian legislation a new technology must be tested and evaluated as being suitable for fish welfare before it is used commercially. This approach often require applications for permission according to relevant welfare legislation.

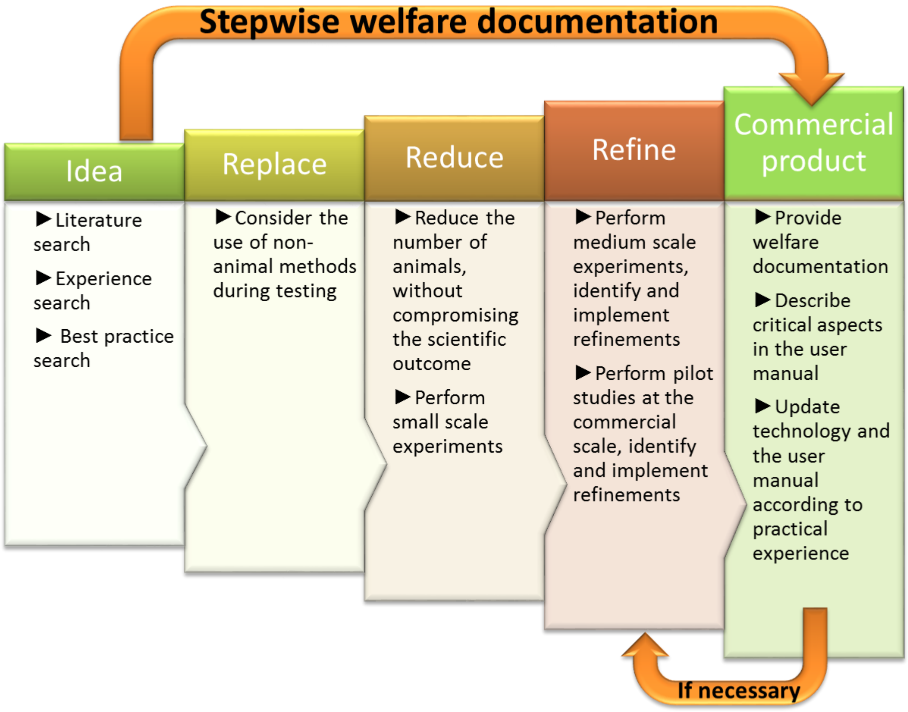


Figure 2.1-1 *Suggested stepwise welfare documentation from the concept to the commercial product with implementation of the «3 Rs» (Replace, Reduce and Refine) during development of new technology (180). According to Norwegian legislation a new technology must be tested and evaluated as being suitable for fish welfare before it is used commercially. Illustration reproduced from Gismervik et al., [173] with permission from K. Gismervik.*

Points that the farmer should consider

Before purchasing any new technology, check the following:

- ✓ Is there any welfare documentation available for the technology?
  - If no: such documentation is required according to Norwegian law and regulations [174] (see Figure 2.1-1)
  - If yes:
- ✓ Check if relevant OWIs and LABWIs for ensuring the welfare needs of the fish are documented. The following link can provide a checklist: http://www.imr.no/filarkiv/2015/06/skjema_for_velferdsvurdering_av_ny_teknologi_i_oppdrett_v1_0.pdf/nn-no.
- ✓ Refer to this handbook for a list of potentially fit-for-purpose OWIs and LABWIs (see e.g. Part C section 1.13).
- ✓ Check if the documentation is given by someone impartial, with competence in fish welfare.
- ✓ Check if there are user manuals available describing how to ensure fish welfare throughout the process, outlining limitations of use due to fish size, health status, etc.

Before you use new technology, check the following:

- ✓ Are potential risks identified and appropriate welfare actions implemented?
- ✓ Are there routines to ensure fish welfare is accounted for before, during and after the use of the technology?
- ✓ Are there criteria describing when to stop or cancel the operation as a result of welfare concerns?

During use, check the following:

- ✓ Is fish welfare documented during and after use?
- ✓ Is there documentation for optimizing the procedures during use and preventing poor welfare?

First considerations in the evaluation of new technology:

To avoid handling related damage to the fish see the OWIs listed in Part C sections 1.1 and 1.2 on crowding and pumping. For example, it is important to inspect and check that there are no severe angles in pipes or dewatering systems or other abrupt changes of direction that may lead to the fish being damaged. Also check for and avoid sharp or protruding edges, rough surfaces, dry surfaces or drops that may harm the fish. Also avoid spaces where fish can be crushed, trapped or damaged. It is important to minimise time out of water. As a general rule, time out of water is more harmful at both low and high temperatures and low humidity.

For basic documentation, the more novel the technology, the more extensive the testing should be. The goal is to use the most relevant OWIs and LABWIs from the toolbox. Thresholds/limits for some OWIs can be hard to define as they may be affected by temperature, genetics, environment, life stage, and uncertainty in measurements [175]. However, changes from before/during/after treatment or handling can be used as a baseline. Morphological scoring systems for quantifying different injuries are described in more detail in Part C, section 3.

2.2 Description of new technologies and appropriate OWIs for monitoring and scoring

2.2.1 Mechanical and thermal de-licing

Various technologies for mechanical and thermal de-licing (without using chemicals) have been developed over the last decade, and many are still under development. These de-licers can be classified by their lice removal technique, either by:

- Temperature adjusted seawater (e.g. Thermolicer and Optilicer)
- Seawater flushing and turbulence (e.g. Flatsetsund de-licer and Hydrolicer)
- Soft brushes and seawater flushing (e.g. Skamik)

It is important to evaluate their de-licing efficiency against their impact on fish welfare (see the following challenges to fish welfare section for specific risks). However, many factors affect fish welfare, among them crowding, the health status of the fish, water temperature and technical adjustments [24]. Technologies using seawater flushing and temperature adjusted water have previously been reported as acceptable in relation to fish welfare during initial testing [31, 176]. However, in 2016 and 2017, mechanical and thermal de-licing was reported to have major negative impacts on fish welfare when compared with medicinal treatments [19, 177]. It is potentially a problem that not all welfare documentation is widely available for scientific evaluation and that the main documentation that exists relates to the developmental stages of the technology [31, 32, 56]. An overview of the available welfare documentation on mechanical and thermal de-licing procedures and associated OWIs used are given in Table 2.2.1-3.

Figure 2.2.1-1. *Mechanical de-licing may be challenging for fish welfare at low temperatures, as potential injuries will heal more slowly and there is an increased risk of developing winter ulcers (Photo: K. Gismervik).*

Challenges to fish welfare

- A common feature of all mechanical and thermal de-licers is that the fish have to be handled, firstly by crowding (see Part C section 1.1) then by pumping through different pipes (see Part C section 1.2) with different kinds of water drainage, temperatures of water baths or water flushing systems, or in combination with brushes. Crowding and pumping have been suggested as welfare risk factors during mechanical and thermal de-licing [31, 32]. Crowding was also found to be a major risk factor during mechanical or thermal de-licing in a survey by Gismervik et al., [173].
- All this handling can cause direct injuries to the fish, stress during and after the operation, a reduction/loss of mucus, secondary infections and can also lead to increased mortality rates [24, 31, 177, 178]. The gills, eyes and snout are especially vulnerable. Eyes and snout are also rich in nociceptors, which are receptors perceiving noxious tissue-damaging stimulus and are associated with feeling pain [179, 180]. At lower temperatures there will be an increased risk of developing winter ulcers [19] (see Part A Table 3.1.5-2 for more information).
- In 2017, head injuries including brain haemorrhaging, bleeding in the palate and eye haemorrhaging were reported after thermal delicing, which may be related to panic behaviour that has been observed during and after exposure to the treatment bath [181].
- It is important to evaluate the general health status of the fish before the operation, as diseased fish have reduced tolerance to handling. In a survey by Gismervik et al., [173] the fish's health status was also found to be one of the main risk factors.
- In general, many fish health professionals have reported increased acute mortality after the thermal de-licing [19, 181] and this is also supported by mortality figures reported to authorities [182]. In addition, high mortality has been observed following thermal de-licing especially when fish were diagnosed with AGD and/or gill irritation [31].
- Water quality in the temperature adjusted water chamber can be another risk factor for fish welfare during thermal de-licing. High ammonia and turbidity values have been recorded and this is assumed to be stressful for the fish, although more information on this is required [31]. Gas supersaturation has also been registered in the treatment bath [181].
- Bleeding from the gills and scale loss have also been identified as risk factors for poor welfare associated with mechanical de-licing [24] and the correct adjustment of the equipment is important. It is also important to know what size of fish the technology is suitable for [8, 24].
- If cleaner fish are stocked with the salmon, their welfare should also be considered during mechanical and thermal de-licing, especially with regard to e.g. their capture and removal before they enter the dewatering/ de-licing procedure [56, 181].

Table 2.2.1-2. *Svåsand et al., [183] identified these risks factors and potential consequences for fish welfare when using mechanical delicing. Table is translated and adapted from Svåsand et al., [183] with permission from L. H. Stien.*

Risk factor	Source	Consequence
Reduced tolerance	Compromised fish health	Increased mortality
Crowding	Lifting of the net and pumping	Stress, increased oxygen demand, crush injuries, fin damage and wounds. Secondary infections
Physical trauma	Irregularities in the pumping system e.g. sharp edges and bends	Impact injuries, fin damage, gill damage and wounds. Secondary infections
Physical trauma	Dewatering	Injuries and wounds. Secondary infections
Overheating	Fish are held too long in heated water	Thermal stress and mortality

How to minimize welfare challenges

- Fish should be in good health before the operation. During disease outbreaks, other options should be considered (e.g. in cage treatments, postponing the treatment, biological de-licing, possibilities of slaughter etc.). However, postponing lice treatment for too long may not be an option, due to regulations and the fact that high lice levels can have a severe welfare impact (see Part A section 3.2.3). Technological solutions for preventing lice from attaching to the fish can be important tools to reduce the welfare impact of de-licing [177].
- Monitor water pressure and flow, the density of fish in the treatment unit (weight or number per minute/hour), water temperature in the treatment chambers and operation speed. Have clear guidelines for acceptable fish size, health, temperatures, starvations periods etc., [24, 31, 32, 56]. Ensure that fish do not get caught in the system during low-intensity periods or during breaks [24, 56].
- Optimize crowding and pumping (see Part C section 1.1 and 1.2).
- Ensure that there are periods during the de-licing operation where OWIs are actively used to assess welfare (Figure 2.2.1-2). Gismervik et al., [24] found that the scoring of external acute injuries during mechanical de-licing can help ensure that the equipment is properly adjusted. It was recommended to take regular sampling before, during and after the procedure, monitoring e.g. gill haemorrhaging, scale loss and epidermal haemorrhaging (amongst others) while checking de-licing efficacy.
- Ensure that the technology has effective lice collection procedures, as neither heated water nor flushing will kill lice [24, 32, 56]. The collection of lice via filtration of the treatment water is important in order to avoid rapid re-infection, which can mean the fish need to be de-liced again in the near future [24].
- Having camera surveillance in the cage that the fish are returned to can help detect abnormal behaviour and possible mortalities as early as possible [56].
- Conduct the operation when the ambient sea water temperatures are appropriate, e.g. do not perform in the winter, due to risks of developing winter ulcers.

- Ensuring optimal water quality and water exchange in the temperature adjusted treatment chambers in thermal de-licing. High ammonia and turbidity values have been recorded [31]. Gas supersaturation has also been registered in the treatment bath [181].
- For thermal de-licing you must also ensure the correct temperature and exposure time [31, 32] and this may vary with the ambient sea temperature [32].
- The welfare of cleaner fish must also be considered if they are stocked with the salmon.

How to assess welfare associated with mechanical and thermal de-licers

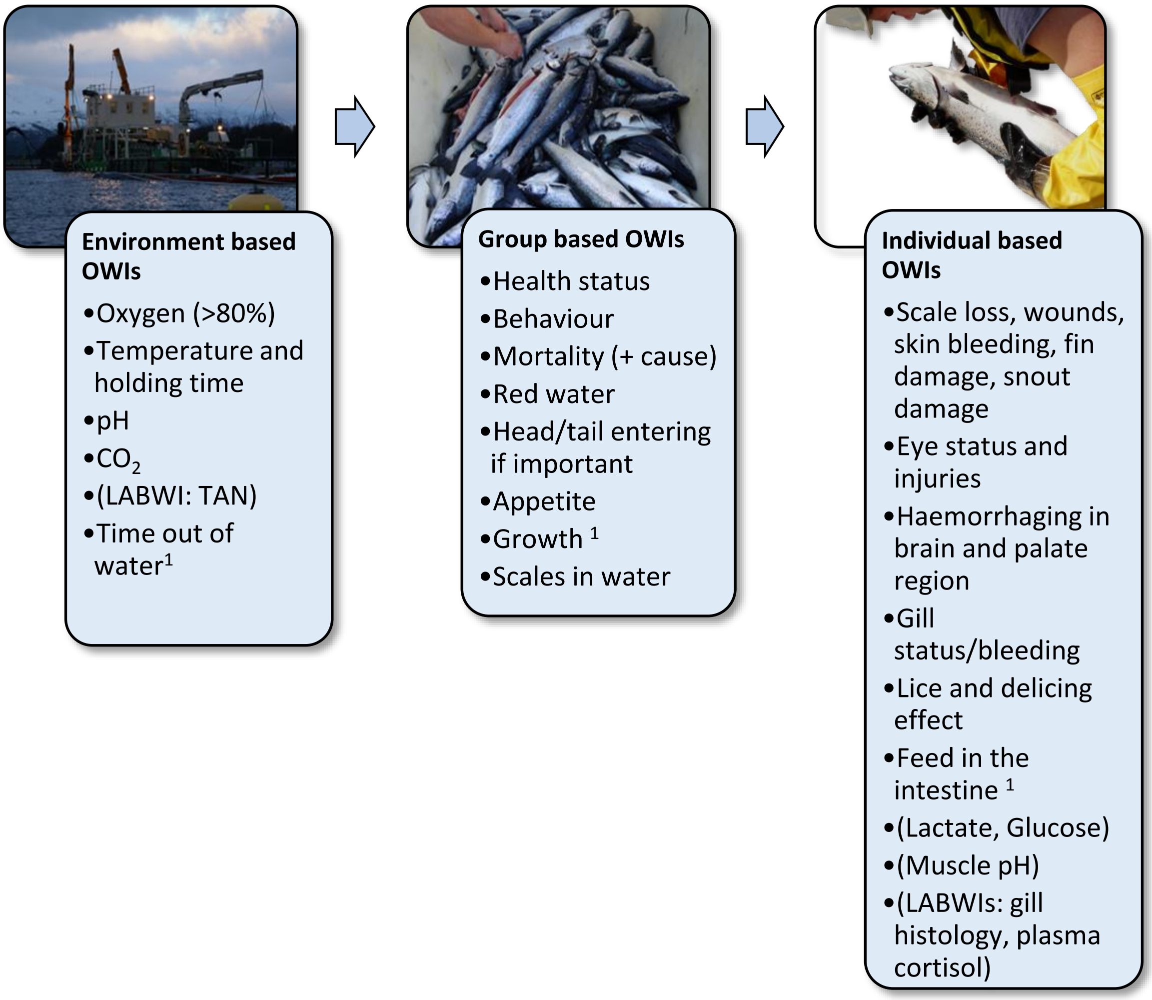


***Figure 2.2.1-2**. Overview of OWIs and LABWIs that may be suitable for mechanical and thermal de-licing. Environment based OWIs address the rearing environment, group based OWIs address the group, while individual based OWIs are based on sampling individual fish. [1]Based on general knowledge and not described in the welfare documentation available. OWIs in brackets are most relevant during the development phase or during sampling. Photos and illustration: K. Gismervik.*

Environment based OWIs

Oxygen saturation. The respiratory effects of differing oxygen saturation levels vary with temperature. Levels must never approach the limiting oxygen saturation (LOS) (Part C Table 1.1-1.). As a general precautionary guideline oxygen saturation levels of >80% are often used [13] and the RSPCA welfare standards for farmed Atlantic salmon [7] recommend a minimum of 7mg L^{-1}. During mechanical and thermal de-licing, oxygen levels during crowding (especially during summer time) and in the temperature adjusted treatment chambers (thermal de-licing) can be important.

Temperature. Measurements of holding time, temperature and water quality parameters in temperature adjusted water chambers are important. Excessively high temperatures and keeping fish too long in the warm water can impact upon welfare [183] and lead to mortalities. The upper temperature limits for use should be stated by the supplier. Low temperatures increase the risk for development of ulcers. Damage from handling is often the initiating factor, leading to secondary infections with bacteria like *Moritella viscosa* and *Vibrio* spp. in winter time (see Part A, Table 3.1.5-2 for more information on winter ulcers) [19, 20].

Carbon dioxide can accumulate in treatment chambers if the water flow rate in the system is inadequate or if biological load to the system is not supported by the system design (Nilsen et al., 2014). It is be important to test this during the development phase [32]. **pH** must also be monitored.

LABWI: TAN. Properties such as temperature, pH and salinity can affect the NH_3: NH_4^+ ratio and thus the toxicity of ammonia. The maximum safe level of short-term exposure (4 hours) of NH_3-N is 0.1 mg L^{-1} according to Wedemeyer [60] (for further description see Part A, section 4.1.6). In order to reduce the risk of TAN accumulation, the fish should be starved before treatment (see Part C, section 1.9). This is to ensure that the intestine is completely empty to reduce the risk of deteriorated water quality due to the build-up of faecal matter in the tanks.

Time out of water. Air exposure should be minimized. The RSPCA welfare standards for farmed Atlantic salmon recommend a maximum exposure time of 15 seconds [7]. The time the fish is exposed to air is particularly critical at high or low temperatures and when humidity is low.

Group based OWIs

Health status should be known before the treatment, as it is well known that operations like mechanical de-licing can result in high mortality in diseased or weak fish [31].

Mortality should be followed closely and on a regular basis following de-licing to retrospectively assess problems or welfare threats associated with the procedure. It is important to find the reasons for mortality, so the technology can be adjusted if necessary, or routines adjusted during use.

Behaviour. For behavioural OWIs linked to crowding and pumping please see Part C sections 1.1 and 1.2. Swimming should be smooth and calm. Fish should not struggle and there should not be red water inside the pump. Panic behaviour and fast swimming also increases the risk for mechanical damage as the fish enter and exit the treatment chambers. Some behaviour can also be seen with cameras inside the hose/treatment chamber. As with crowding and handling, the resumption of normal behaviour can be used as a qualitative OWI after the procedure.

Red water. According to practical experience, the crowding of post-smolts in closed and smaller containers can make it possible to detect bleeding as a colour change in water, so called “red water” [24]. Red water is never a good sign, and cause should be investigated (see Part A section 3 and Part C section 1.12 for more information).

Head/tail entering (if important- technology dependent). Some of the de-licers are designed to accept the fish in a certain way (head or tail first) to minimise damage. If so, the directions can be observed and counted with the use of cameras or by staff.

Return of appetite. The time it takes for appetite to return should be closely monitored after mechanical de-licing. A reduction or loss of appetite can be caused by the initiation of a stress response [21]. The time it takes for appetite to return after the procedure can therefore also be used as an OWI as it can reflect how well the fish have dealt with the stressor. Appetite is easy to measure qualitatively by observing the fish when feed is offered.

Growth can be affected by short-term or chronic stress. Acute changes in growth can be used as an early warning system for potential problems, particularly when the farmer has robust growth monitoring practices.

Scales in water/filter. Indicates scale loss and damage to the mucus and the skin which can cause osmoregulatory problems and also secondary infections.

Individual based OWIs

Injuries are one of the most common signs of poor welfare with these technologies. Injuries should be monitored before, during and after operations so actions can be undertaken. No fish should be left in the de-licer during breaks or at the end of the process.

Skin condition. Physical contact with other individuals, pipes or other equipment may lead to various forms of skin damage. Small haemorrhages in the skin can typically be seen ventrally. Scale loss may be observed both as free scales in the water and as areas on the fish where scales are missing. Poor handling can lead to mucus loss. Since mucus and scales protect the fish from the environment and are functioning as barriers, losses can give rise to osmoregulation problems and infections. Sharp edges may result in wounds/cuts.

Opercular damage and gill status. Includes broken or shortened or even the lack of opercula. It is important to distinguish between acute injuries that occur during the procedure and other factors that make the gills more vulnerable during de-licing. To get a measure of gill status, an operator can score changes on the gill surface, visible as “white patches” (total gill score). If a case of AGD is suspected, it may also be relevant to perform AGD scoring. A severe outbreak of AGD can increase the risk of mortality during treatment [23]. Gill bleeding should also be monitored in relation to mechanical injuries [24].

Snout damage can occur when fish are pressed against the net or hit hard surfaces.

Fin damage. Physical contact may also lead to damaged fins, especially fin splitting. As with other injuries it is important to distinguish between acute injuries that occur during the procedure and older injuries.

Eye status. Eyes are vulnerable to mechanical trauma and there can be a risk of haemorrhaging and desiccation if fish are handled out of water. Exophthalmus, also known as "pop eye" is recognized as an unspecific sign of disease that should be investigated further (see Part A, section 3.2.12). Exophthalmus increases the risk of mechanical damage.

Haemorrhaging in the brain or palate region. In 2017, haemorrhages to the brain, palate region and eyes were detected on Atlantic salmon in connection with thermal de-licing [181]. Fish health services observed the problem during autopsies on mortalities involving apparently healthy large fish and also as a clinical symptom in moribund fish collected after the procedure. Panic behaviour has been observed during and following exposure to the treatment bath and it has been discussed whether this could have contributed to the damage [181]. Haemorrhaging to the brain and palate region can be investigated by the autopsy of daily mortalities, moribund fish and possibly a random sample to gain more knowledge on how widespread the problem may be.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Lice and de-licing effect. As the purpose is to remove lice, the effect should be monitored by counting lice on the fish before, during and after the operation. The effect must be good enough to avoid rapidly repeated treatments but this has to be balanced against any potential adverse effects on the fish.

Feed in the intestine. To evaluate the feed withdrawal period before de-licing and also feed intake afterwards (as an indirect indicator of appetite) fish can be euthanised and the stomach and intestine should be checked for feed residue. Feed in the intestine often indicates that the fish has eaten during the last one to two days [45, 46] but this depends on the fish size and temperature (see also Part C, section 1.9).

Lactate. Struggling and burst swimming increases anaerobic muscle activity, thus increasing lactate in the blood [2, 25, 26] and should stay below 6 mmol/l [2]. It is easily measured with handheld apparatus, but samples should be taken approximately one hour after muscle activity. However, Gismervik et al., [24] found it challenging to get a good lactate sample because of this time lag in potential lactate increase during mechanical de-licing. Erikson et al., [3] did not find any significant correlation with crowding time and lactate level and Gismervik et al., [24] found no differences in lactate values from before and during crowding in their study.

Glucose can be used as an OWI for crowding (e.g. Skjervold et al., [28]). Elevation in plasma glucose is a relatively slow response to stress and peaks after around 3-6 hours in salmon [29], although the response is also dependent on the feeding status, diet type and other factors. Glucose levels should therefore be compared with pre-stress levels rather than any "standard stress levels". Indicators like glucose and lactate can also help direct future best practice procedures, but are not a good "stop signal" concerning welfare during ongoing operations.

Muscle pH. Increased stress/muscle activity produces more lactic acid which in turn reduces muscle pH [27] e.g. due to crowding and pumping [27].

LABWIs: Plasma cortisol and gill histology. We know that handling stresses the fish and leads to a stress response [4]. Plasma cortisol measurements can be used to see how long the fish is affected by handling stress and when it returns to its resting state after the procedure [24] (see also Part A, section

3.2.16). Gill histology may be relevant for the assessment of mechanical damage in addition to gill status (see also Part A section 3.2.4).

Table 2.2.1-3. Existing welfare documentation for mechanical and thermal de-licers and their associated OWIs and LABWIs

Reference	Technology	Principle	No. cages / localities / temperature	No. fish (+size)	Follow up time after de-licing	OWIs and LABWIs used	De-licing effect (%) M=motiles F=Mature Females C=Chalimus
Grøntvedt et al., [31]	Thermolicer	30-34°C (25-30 sec)	4 cages (closely monitored) / 4 localities	217,234 salmon at <2.2 kg 50,694 rainbow trout at ca. 2.5 kg	3 weeks	**Environment based:** ammonia, nitrite, nitrate, pH, turbidity **Group based:** mortality and appetite **Individual based:** gills, scale loss, snout-, eye-, fin damage, wounds, skin haemorrhaging, AGD score, total gill score, cataract, lice **LABWI:** gill histology	M (75-100%) C (0%)
Roth [32]	Optilicer	28-34°C (20-30 sec)	Several	Several	4 weeks (mortality)	**Environment based:** CO_2, O_2, TOC, ammonia **Group based:** mortality **Individual based:** gills, scale loss, snout-, eye-, fin damage, wounds, ventral haemorrhaging, blood pH, haematocrit, lactate, glucose **LABWI:** gill histology, Na^+, K^+, Ca^{2+}	M (58-100%) C (0%)
Gismervik et al., [24]	Flatsetsund de-licer	Seawater flushing 0.2-0.8 bar (0.2-0.3 bar docu-mented in study)	3 cages (closely monitored) / 3 localities Ca. 8-14 °C	118,534 salmon at ca. 4.6 kg 291,380 salmon at ca. 2 kg	3 weeks	**Environment based:** O_2, temperature **Group based:** mortality, appetite (behaviour) **Individual based:** gills, scale loss, snout-, eye-, fin damage, wounds, skin haemorrhaging, total gill score, cataract, lice, glucose, lactate **LABWI:** cortisol, gill histology	M (81-100%) F (76-91%)
Nilsen et al., [176]	Flatsetsund de-licer	Seawater flushing 0.27-0.37 bar	1/1 (repeated experiment) 4-5 °C	31,950 fish at ca. 2.9 kg	1 week (mortality monitored for 2 weeks)	**Environment based:** TOC **Group based:** mortality, behaviour, flight response, **Individual based:** scale loss, snout-, eye-, fin damage, wounds, skin haemorrhaging, lice, muscle pH	M (57-68%) with pre-flush M (2-27% without)
None available	Hydrolicer	Seawater flushing					
None available	Skamik	Brushes and seawater flushing					

Knowledge gaps

- Knowledge on the accumulation of additive stress, handling and environmental factors during multiple de-licing events is lacking. If problems occur with these technologies it can negatively affect welfare [19]. This knowledge gap also applies to cleaner fish.
- Basic references for the upper limits and duration of temperature adjusted water treatment and their effects upon fish welfare are inadequate for post-smolts, and must also be related to ambient water temperatures [16, 181, 184, 185].
- There is a knowledge gap concerning high turbidity and ammonia values, as well as gas supersaturation in temperature adjusted water treatments with a short residence time (<1 minute) [31, 35, 181].
- In 2017, haemorrhages to the brain, palate region and eyes were detected on Atlantic salmon in connection with thermal de-licing [181]. The extent of the problem and whether there are differences between different thermal de-licers or the equipment settings is unclear. The risk of brain haemorrhaging in relation to other types of mechanical de-licing systems is not documented.

2.2.2 Treatment barge (bathing treatment)

The treatment barge "HeliXiR" (and similar technologies) are new technologies that are designed to provide controlled bathing treatments. The fish passes through a treatment tank with a pre dosed chemical by means of a "screw", which provides a controlled treatment time (between 15-45 minutes). Lice that fall in the bath are filtered off (150 µm). The recycling of water and chemicals can lead to reduced chemical use and water quality is maintained by aeration and oxygenation. An operation will consist of crowding, pumping, dewatering, medicinal treatment in the bath, dewatering and piping the fish out to the cage again. At the time of publication of this handbook, testing of the technology with chemicals was not complete, and the report describing the welfare implications of the new technology was conducted using only salt water in the tank [8]. The technology is included in this manual as an example of how welfare indicators can be used in the development of new technologies.

Challenges to fish welfare

- Crowding (see Part C section 1.1).
- Pumping (see Part C section 1.2), with new technology adjustment of the pumps has been especially important since fish damage related to pumping has been observed [8].
- Dewatering should be adjusted in relation to fish size. Fish can become stuck between bars if they are too small (has occurred with fish < 1kg). Water velocity and pipe angles have to be considered in relation to the flow of the fish through the machine. The operator should make sure fish are not crowded in the bottom of the dewatering box before entering the treatment tank as this may cause eye injuries [8].
- When delivering the fish into the tank, the process should be monitored to ensure that the fish do not hit the screw, screw wing or wall.
- Bathing treatments with chemicals (see Part C section 1.5).
- Cleaner fish may have different tolerance limits for medication. The technology aims to remove cleaner fish from before the treatment after they are pumped on board.

How to minimize welfare challenges

The technology is still new and there are no data on its full operation. As with other new technologies, an approach that incrementally documents fish welfare throughout the course of its development is advised.

- In general, fish should be in good health before the operation.
- Fish density in the tanks (number per chamber) should be monitored, with clear guidelines for acceptable use according to fish size, health, temperatures, starvations periods, etc.
- Water quality should be monitored and the tank automatically logs O_2, CO_2, pH and temperature. Check loggers work and are calibrated frequently. The treatment process aims to ensure homogeneous water quality and a consistent dose of medicine throughout the treatment volume, which may be challenging in other treatments utilising tarpaulins / well boat [186]. It is also possible to take random water samples for LABWIs such as ammonia and turbidity.
- Crowding and pumping should be optimised to minimise physical trauma (see Part C sections 1.1 and 1.2).
- Only carry out the procedure when ambient water temperatures are appropriate. Low temperatures increase the risk for development of ulcers. Damage from handling is often the

initiating factor, leading to secondary infections with bacteria like *Moritella viscosa* and others in winter time (see Part A, table 3.1.5-2 for more information on winter ulcers) [19, 20].

- It is important to carry out stepwise testing with regard to the chosen medicines, using an appropriate suite of OWIs (and LABWIs) both before, during and after the operation.

How to assess welfare on a treatment barge (bathing treatment)

In addition to the potential OWIs and LABWIs for the actual treatment, one must also customize OWIs and LABWIs based on the medicinal treatment (further described in Part C section 1.5). See also Part C sections 1.1 and 1.2 for information on crowding and pumping.

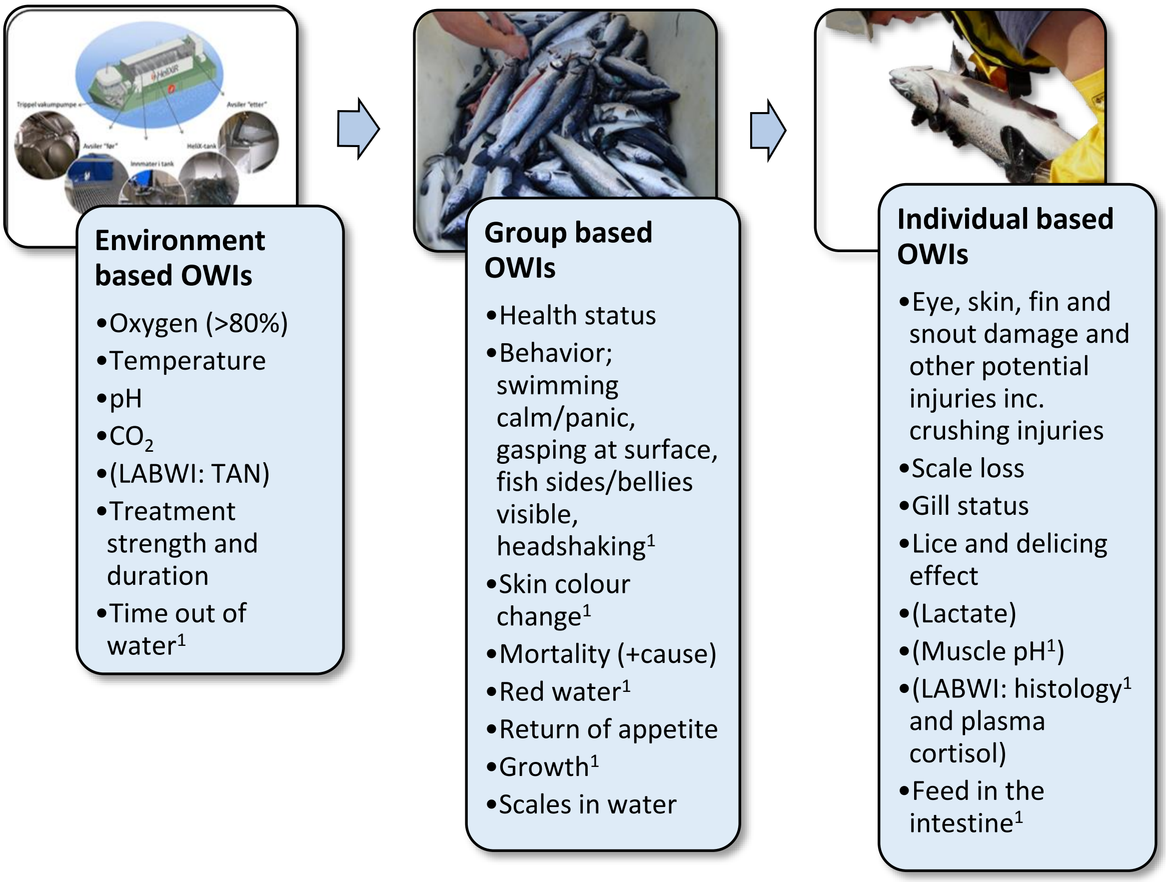


Figure 2.2.2-1. *Overview of OWIs and LABWIs that may be suitable for treatment barges. Environment based OWIs address the rearing environment, group based OWIs address the group, while individual based OWIs are based on sampling individual fish. [1]Based on general knowledge and not described in the welfare documentation available. OWIs in brackets are most relevant during the development phase or during sampling. Photos and illustration: K. Gismervik.*

Environment based OWIs

Oxygen saturation. The respiratory effects of differing oxygen saturation levels vary with temperature. Levels must never approach the limiting oxygen saturation (LOS) (Part C Table 1.1-1.). As a general precautionary guideline oxygen saturation levels of >80% are often used [13] and the RSPCA welfare standards for farmed Atlantic salmon [7] recommend a minimum of 7mg L^{-1}. Saturation should not go above 100%. In addition to measuring oxygen in the treatment tanks, it can be important to measure oxygen levels during crowding (especially during summer time).

Temperature. The optimal temperature for post-smolt fish is around 17°C [17] and the critical temperatures for post-smolts are around 6°C and 18°C [11] (see Part A section 4.1.1 for more information). The solubility of oxygen also declines with increasing temperature, so that warmer water contains less oxygen than colder water with the same saturation. It is also important to be aware that salmon reacts to acute changes in temperature, particularly increases in water temperature [37]. Low temperatures increase the risk for development of ulcers. Damage from handling is often the initiating factor, leading to secondary infections with bacteria like *Moritella viscosa* and *Vibrio* spp. in winter time (see Part A, Table 3.1.5-2 for more information on winter ulcers) [19, 20].

Carbon dioxide can accumulate in treatment chambers if the water flow rate in the system is inadequate or if biological load to the system is not supported by the system design. It is important to test this during the development phase [32]. **pH** must also be monitored.

LABWI: TAN. Properties such as temperature, pH and salinity can affect the NH_3: NH_4^+ ratio and thus the toxicity of ammonia. The maximum safe level of short-term exposure (4 hours) of NH_3-N is 0.1 mg L^{-1} according to Wedemeyer [60] (for further description see Part A, section 4.1.6). In order to reduce the risk of TAN accumulation, the fish should be starved before treatment (see Part C, section 1.9). This is to ensure that the intestine is completely empty to reduce the risk of deteriorated water quality due to the build-up of faecal matter in the tanks.

Treatment strength and duration. Direct measurements of an active substance concentration may be possible with certain active substances, such as hydrogen peroxide. It is also important to know the acceptable treatment durations for each medicine, and that this is complied with and logged.

Time out of water. Air exposure should be minimized. The RSPCA welfare standards for farmed Atlantic salmon recommend a maximum exposure time of 15 seconds [7]. The time the fish is exposed to air is particularly critical at high or low temperatures and when humidity is low.

Group based OWIs

Health status should be known before the treatment, to ensure the fish can withstand the procedure.

Mortality should be followed closely and on a regular basis during and after the treatment to retrospectively assess problems or welfare threats associated with the procedure. Autopsies should be performed on mortalities or moribund fish to identify the cause of the problem. To discover whether e.g. impacts are the likely cause of death, it may be appropriate to check for brain haemorrhaging or haemorrhaging in the palate region [181].

Behaviour For behavioural OWIs linked to crowding and pumping please see Part C section 1.1 and 1.2. Suitable indicators for monitoring behaviour in the treatment tank include fish gasping at the surface, visible fish sides and abdomen, swimming (calm or panic) and head shaking. Some behaviour can also be seen with cameras inside the hose/treatment chamber [8]. As with crowding and handling, the resumption of normal behaviour can be used as a qualitative OWI after the procedure.

Skin colour change. Skin colour can change to green/blue during stressful crowding [9] and changes in skin colour can therefore be qualitatively monitored from the start to the end of the bathing treatment.

Red water. According to practical experience, the crowding of post-smolts in closed and smaller containers can make it possible to detect bleeding as a colour change in water, so called "red water". Red water is never a good sign, and the cause should be investigated (see Part A section 3 and Part C section 1.12 for more information).

Return of appetite. The time it takes for appetite to return should be closely monitored after treatment. A reduction or loss of appetite can be caused by the initiation of a stress response [21]. The time it takes for appetite to return after a procedure can therefore also be used as an OWI as it can reflect how well the fish have dealt with the stressor. Appetite is easy to measure qualitatively by observing the fish when feed is offered.

Growth can be affected by short-term or chronic stress. Acute changes in growth can be used as an early warning system for potential problems, particularly when the farmer has robust growth monitoring practices.

Scales in water/filter. Indicates scale loss and damage to the mucus and the skin which can cause osmoregulatory problems and also secondary infections.

Individual based OWIs

Injuries should be monitored before, during and after operations so actions can be undertaken. Treatment tanks should also be checked during breaks or at the end of the operation to make sure they contain no fish. In addition to external injuries that can be easily detected, the operator should also ensure that the technology does not cause other less visible damage, such as that observed with thermal de-licing in 2017 [181].

Skin condition. Physical contact with other individuals, pipes or other equipment may lead to various forms of skin damage. Small haemorrhages in the skin can typically be seen ventrally. Scale loss may be observed both as free scales in the water and as areas on the fish where scales are missing. Poor handling can lead to mucus loss. Since mucus and scales protect the fish from the environment and function as barriers, losses can give rise to osmoregulation problems and infections. Sharp edges may result in wounds/cuts.

Opercular damage and gill status. Includes broken or shortened or even the lack of opercula. It is important to distinguish between acute injuries that occur during the procedure and older injuries that may make the gills more vulnerable during treatment. To get a measure of gill status, the operator can score changes on the gill surface, visible as "white patches" (total gill score). If a case of AGD is suspected, it may also be relevant to perform AGD scoring. A severe outbreak of AGD can increase the risk of mortality during treatment [23]. Gill bleeding should also be monitored in relation to mechanical injuries [24] or medicinal side effects.

Snout damage can occur when fish are pressed against the net or hit hard surfaces.

Fin damage. Physical contact may also lead to damaged fins, especially fin splitting. As with other injuries it is important to distinguish between acute injuries that occur during the procedure and older injuries.

Eye status and cataracts. Eyes may be affected by the procedure, potentially leading to e.g. chemical burns, bleeding and desiccation during air exposure. Exophthalmus, also known as "pop eye" is recognized as an unspecific sign of disease that should be investigated further (see Part A, section 3.2.12). Exophthalmus increases the risk of mechanical damage.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Lice and de-licing effect. If the purpose of the treatment is to remove lice, the effect should be monitored by counting lice on the fish before and after the operation. The effect must be good enough to avoid rapidly repeated treatments but this has to be balanced against any potential adverse effects on the fish.

Feed in the intestine. To evaluate the feed withdrawal period before treatment and also feed intake afterwards (indirect indicator of appetite) fish can be euthanised and the stomach and intestines should be checked for feed residue. Feed in the intestine often indicates that the fish has eaten during the last one to two days [45, 46], but this depends on the fish size and temperature (see also Part C, section 1.9).

Lactate. Struggling and burst swimming increases anaerobic muscle activity, thus increasing lactate in the blood [2, 25, 26] and should stay below 6 mmol/l [2]. This is easily measured with handheld apparatus, but samples should be taken approximately one hour after muscle activity. Erikson et al., [3] did not find any significant correlation with crowding time and lactate level and Gismervik et al., [8] found no differences in lactate values in their study on treatment barges.

Muscle pH. Increased stress/muscle activity produces more lactic acid which in turn reduces muscle pH [27] e.g. due to crowding and pumping [27].

LABWIs: Plasma cortisol and gill histology. We know that handling stresses the fish and leads to a stress response [4]. Plasma cortisol measurements can be used to see how long the fish is affected by handling stress and when it returns to resting state after the procedure [24] (see also Part A, section 3.2.16). Gill histology may be relevant for the assessment of potential injuries in relation to the treatment or medicinal usage, in addition to gill status (see also Part A section 3.2.4).

Table 2.2.2-2. *Existing welfare documentation for treatment barges (without the use of medicines) and their associated OWIs and LABWIs*

Reference	Technology	Principle	No. cages / localities / temperature	No. fish (+size)	Follow up time after de-licing	OWIs and LABWIs used	De-licing effect (%) M=motiles F=Mature Females C=Chalimus
Gismervik et al., [8]	Treatment barge "HeliXiR"	Fish are passed through a water tank (133 m^3) for medical treatment. This testing was done with NO medicines, only seawater in tank	3 cages / 3 localities ca 5-15 °C	182,108 salmon at 1.2-4.7 kg	Up to 2 weeks	**Environment based:** O_2, CO_2, pH, temperature, ammonia, pH, turbidity, salinity **Group based:** Behaviour, mortality (+ cause) and appetite **Individual based:** Gill bleaching, scale loss, snout-, eye-, fin damage, wounds, skin haemorrhaging, total gill score, cataract, lice, lactate, glucose, cortisol	Limited effect C (0%)

Knowledge gap

The technology has not been tested with medicinal treatments [8].

2.2.3 Optical de-licing (laser)

This technology uses camera vison and lasers for continuously shooting any potential lice on the salmon in the cages. Some of the potential benefits of this passive, in-cage de-licing technology are that the fish do not require handling or feed withdrawal periods. According to the producer, there have been no reported wounds or losses since the technology was commercialised in 2014, see http://en.stingray.no/. They also state that behaviour was checked during earlier stages of the technology development, and that lasers have no negative effects on the vision of the salmon. However, open welfare documentation (reports/papers) were not available when this handbook was published. For more information on the technology, see the producer's webpage.

How to assess welfare with the use of optical de-licers

As no scientific documentation is available, general advice is summed up in Figure 2.2.3.-1.

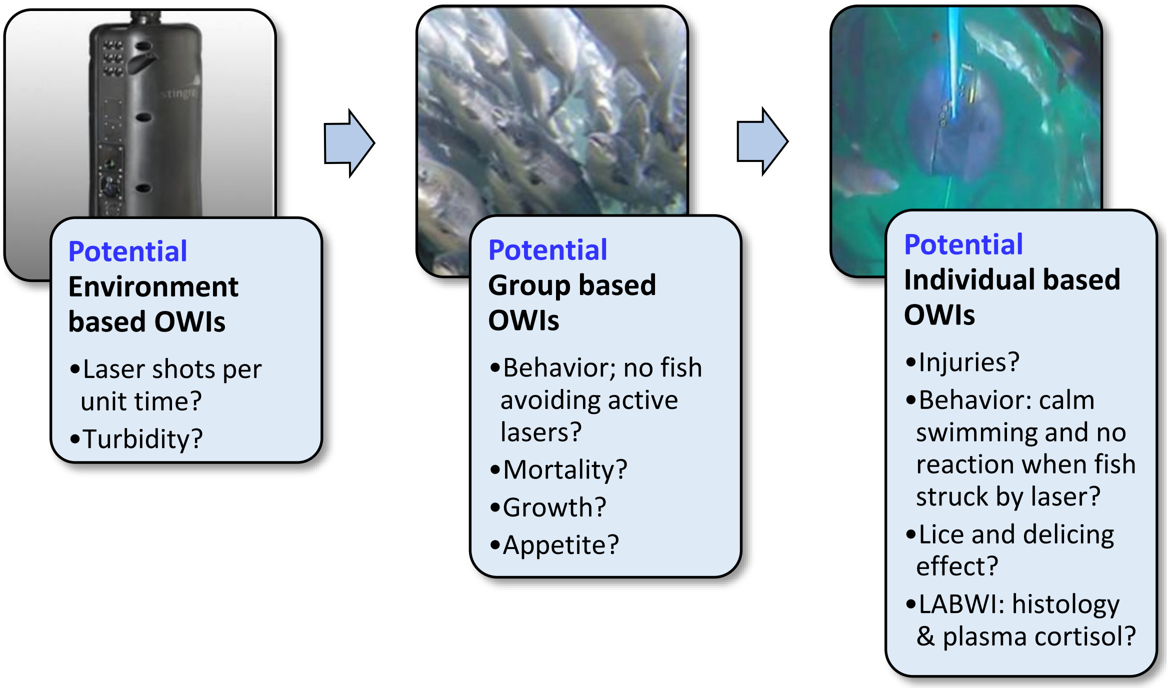


Figure 2.2.3-1. Overview of the potential OWIs and LABWIs that may be suitable for the laser treatment of lice. Based on general advice in the absence of documentation. Environment based OWIs address the rearing environment, group based OWIs address the group, while individual based OWIs are based on sampling individual fish. Illustration: K. Gismervik, group OWI photo: L. H. Stien. Other photos reproduced with permission: www.stingray.no

Potential Environment based OWIs

Laser shots per unit time and turbidity? Are described in more detail in the knowledge gaps section**.**

Potential Group based OWIs

Behaviour? Check that fish are not avoiding the laser area, cameras can give information of the density.

Mortality? As with all new technologies, mortality should be monitored and causes investigated.

Growth? Can be affected by short-term or chronic stress. Acute changes in growth can be used as an early warning system for potential problems, particularly when the farmer has robust growth monitoring practices.

Appetite? Acute loss of appetite is a general welfare indicator, and it may be worth checking technical equipment if there are no other obvious reasons.

Potential Individual based OWIs

Injuries? Checking individual fish for potential injuries to e.g. the skin and eye in tandem with lice counts or other operations (see Part C section 1.12) can be used to document that the technology is not harming the fish at the macro level.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Behaviour? Cameras can be used to ensure that the technology is not affecting fish behaviour. One should observe calm swimming and no reaction when a laser shot hits the fish.

Lice and delicing effect? Lice levels should be monitored to check the technology is working as intended, and action should be taken if the numbers are rising.

LABWIs: Plasma Cortisol and gill histology? Plasma cortisol can be used for measuring stress in controlled trials. LABWIs such as skin and eye histology can be used to check for less visible injuries.

Knowledge gaps

- At the time of publication the authors are aware of no documentation regarding the welfare effects of laser de-licing treatments.
- The technology gives information on how many shots it delivers per unit time. Whether this information can be used to check that the equipment is functioning properly is unclear. High turbidity may also impede the technologies efficacy when shooting lice? The thresholds for potential impacts are unknown.
- The technology produces bright light during use. There is no open documentation on whether this may scare / stress the fish, except that the manufacturer states that normal behaviour is observed.
- Laser technology is known to cause eye damage to humans [187]. As we have found no documentation on its potential effects on the eye and body of salmon, it should be audited as a potential risk during welfare assessments.

2.2.4 Net cleaning equipment

The accumulation of organisms and debris occurs on any surface in the aquatic environment. The rate and nature of settlement is dependent upon the time of year, light levels and the location. Growth of organisms upon salmon net pens can have many negative consequences. They can result in reduced water exchange through the net and therefore reduced dissolved oxygen [188, 189] and increased resistance to water flow which may increase distortion of the nets or the strain on the physical structure and moorings [190]. Organisms growing on the net use and thereby further reduce available dissolved oxygen [191, 192], release waste products into the water and can be a reservoir for infections [193, 194, 195]. Growth on the nets may also serve as a source of natural feed for cleaner fish, reducing their consumption of lice [196].

Since antifouling systems on marine nets have limited efficacy, nets must be cleaned to avoid the adverse effects described above. A common solution is net cleaning rigs or systems (Figure 2.2.4-1), which can be of various sizes from two head rigs which are easily operated by one person to larger systems requiring cranes or ROVs. These systems use hydrostatic pressure from jets to force the cleaning heads against the net and then remove fouling with rotating discs which clean with high pressure water jets (Figure 2.2.4-2). In areas and times of year with high levels of fouling, nets may have to be cleaned as often as once a week. A limited number of farms still practice swimming fish to a new pen and changing or drying the fouled net. This is potentially less harmful to fish but is practically impossible in most cases.

Figure 2.2.4-1. *Example of a net cleaning rig from AKVA with 4 cleaning heads. Photograph courtesy of N. Ribeiro, with permission.*

Figure 2.2.4-2. *Example of a net cleaning rig from AKVA in action on a net with relatively low levels of fouling. Photograph courtesy of N. Ribeiro, with permission.*

Challenges to fish welfare

- Failing to clean nets when necessary has many adverse consequences as described above. However, cleaning nets may also result in challenges to fish welfare.
- The nature of these challenges is related to the amount and nature of the fouling on the nets and the direction and velocity of the water flow.
- Often when cleaning nets fish can be observed swimming, apparently undisturbed, through the debris washed off the net. At other times they appear agitated by the debris and may try to actively avoid it.
- There is the suspicion that some organisms washed off the nets may be potentially harmful to fish gills. Organisms containing stinging cells or nematocysts such as hydroids are thought to be the greatest risk. Although there are on-going research projects there is very little published information available on this topic [197, 198, 199]. However, recent work by Bloecher et al., [199] has reported that the stinging cells of the hydroid *Ectopleura larynx* can remain active in the debris washed off the net and can irritate the gills of the fish.

How to minimise welfare challenges

Since net cleaning is a necessity on the majority of net pen sea farms at present, the only option is to try to minimise the potential adverse effects.

- This may be achieved by cleaning at a time when the water flow is slow enough to allow cleaning but fast enough to remove the debris, with minimal contamination of the pen being cleaned and other pens in on the farm.
- In practice this is not always possible, given that many farms have to clean on a continual basis.
- Regular cleaning has the advantage of reducing the amount of fouling organisms on the cage and therefore the amount of debris released into the water. Preventing build up is potentially more important if there is settlement of Cnidaria on the net, however, to the authors' knowledge this practical experience is not yet supported by scientific data.
- Risks can be further mitigated by good management processes, such as good equipment maintenance, staff training supervision and monitoring of competence. There should be standard operating protocols and records of justification for cleaning or not cleaning nets.
- Any indication of adverse effects should be investigated including the pathological assessment of the gills of the fish.

How to assess welfare during net cleaning

Assessment of fish welfare during net cleaning is based on observations at the time from the surface or with camera systems and the subsequent evaluation of group and individual welfare indicators. This can identify any issues and provide the opportunity to avoid or militate against them in the future.

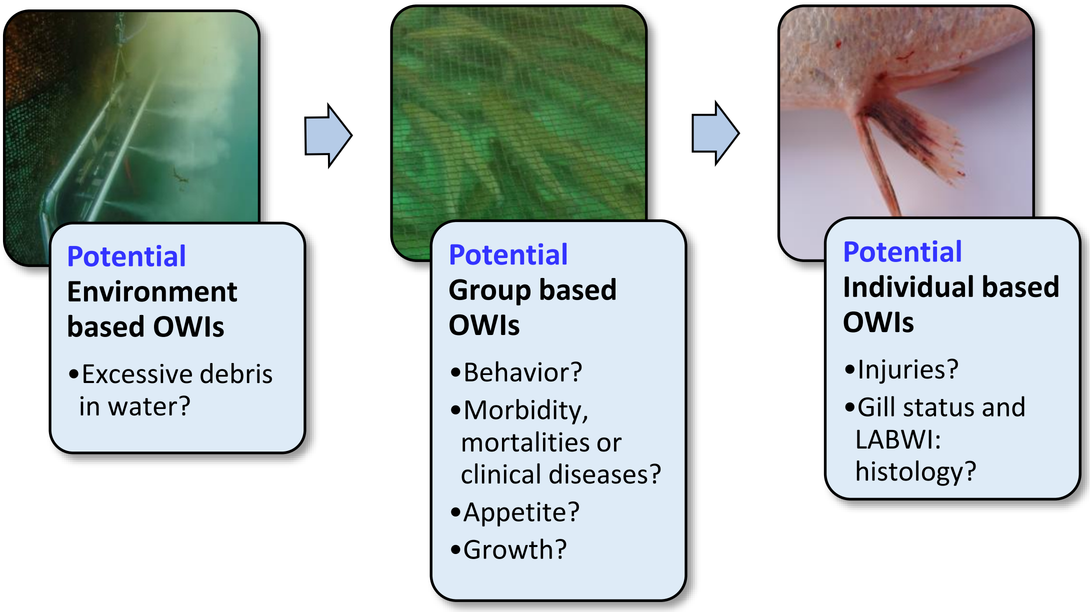


Figure 2.2.4-3. *Overview of potential OWIs and LABWIs that may be suitable for net cleaning. Based on general advice in the absence of documentation. Environment based OWIs specifically address the environment, group based OWIs can be observed and checked during the operation, while individual based OWIs are based on sampling individual fish. Figure: J. F. Turnbull and K. Gismervik. Photos: N. Ribeiro, J. F. Turnbull & K. Gismervik.*

Potential Environment based OWIs

Excessive concentrations of debris? Although large or dense clouds of debris moving towards or surrounding the fish may be an indication of a potential problem, the risk from debris is not only dependent on its density but also its composition.

Potential Group based OWIs

Abnormal behaviour? Agitated fish or fish persistently moving away from the debris washed off the net may indicate irritating material in the debris.

Appetite? Any reduction in feeding may indicate damage or stress as a result of the cleaning process and should be carefully monitored [21]. Practical farm experience reported in [199] suggests the cleaning process can lead to a loss of appetite in some cases. However, to the authors' knowledge this practical experience is not yet supported by scientific data.

Growth? A reduction in growth may be the result of reduced feed intake due to stress or an indication of more serious problems such as clinically significant gill damage [200].

Clinical diseases, morbidities or mortalities? In severe cases fish may become sick and die or have to be removed from the pen. This should be investigated by fish health specialists [168, 169, 170, 171].

Potential Individual based OWIs

Injuries? If the fish are driven to excessive escape or avoidance behaviour, damage may occur due to physical contact with other individuals, the wall of the pen or other equipment. Damage may lead to various forms of skin damage, including scale loss, snout damage and damage to fins.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active and healed fin damage are provided at the end of this document.

Gill status and LABWI: histology? Following net cleaning, fish may show increased signs of gill pathology including behaviour indications and pathological changes on gross or post mortem examination (this may be macroscopic, by direct microscopy or by histology to check for less visible injuries) [198, 199]

Knowledge gaps concerning net cleaning robots

- As far as the authors are aware, at the time of publication there are no publications available on potential adverse effects of net cleaning robots upon fish welfare, only limited publications regarding the potential effects of net cleaning [197, 198, 199].

3 Morphological schemes for assessing fish welfare in different routines and operations

The following section is a summary of the scoring schemes used in this handbook.

This handbook suggests a unified scoring system (Tables 3.1-1, 3.1-2, 3.1-3) that is primarily aimed at farmers to help them assess welfare and rapidly detect potential welfare problems out on the farm. It is an amalgamation of the injury scoring schemes used in the Salmon Welfare Index Model (SWIM) [13], the injury scoring scheme developed by the Norwegian Veterinary Institute (NVI) [8, 31] and also from other schemes developed by J. F. Turnbull (University of Stirling) and J. Kolarevic and C. Noble (Nofima).

Our suggested scheme standardises scoring for 14 different indicators to a 0-3 scoring system:

i) emaciation, ii) skin haemorrhages, iii) lesions/wounds, iv) scale loss, v) eye haemorrhages, vi) exophthalmia, vii) opercular damage, viii) snout damage, ix) vertebral deformities, x) upper jaw deformity, xi) lower jaw deformity, xii) sea lice infection, xiii) active fin damage, xiv) healed fin damage.

Pictures used in the system represent examples of each scoring category. We suggest dorsal, caudal and pectoral fins as the primary fins to monitor for fin damage. As a comprehensive system for the classification of vertebral deformities, similar to that in human medicine has not yet been developed for Atlantic salmon, we suggest a simplified scoring system similar to that used in the RSPCA welfare standards for farmed Atlantic salmon [201].

Cataract damage is classified using an existing and widely used 0-4 scoring scheme [202], see Fig 3.2. The scoring method records the cataract area in relation to the entire lens surface (looking through the pupil along the pupillary/optic disc axis). You can quickly assess large numbers of fish with minimal equipment to get an impression of the severity of the problem. If possible, a selected number of fish should be inspected under darkened conditions (also with better equipment) to give some indication of position, type, development and aetiology. However, it does not record the density of the cataract which can be important and should be annotated separately (T. Wall pers. comm.).

The degree of vaccine side effects in individual fish is often evaluated according to the "Speilberg scale" [98], see Table 3.3 and Fig. 3.4. The Speilberg Scale is widely used as a welfare indicator in the Norwegian aquaculture industry. The scale is based on a visual assessment of the extent and location of clinical changes within the abdominal cavity of the fish and it describes changes related to peritonitis; adhesions between organs, between organs and the abdominal wall and melanin deposits ([98] see also [203] and references therein). A Speilberg score of 3 and above is generally regarded as undesirable.

Table 3.1-1. *Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. (Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. Nilsson. Photos: K. Gismervik, L. H. Stien, J. Nilsson, C. Noble, J. F. Turnbull, P. A. Sæther, I. K. Nerbøvik, I. Simion, B. Tørud, B. Klakegg, R. Andersen, C. Karlsen, K. J. Merok, F. Gregersen)*

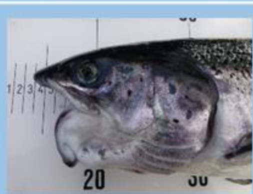
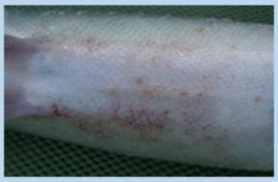
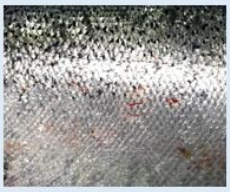
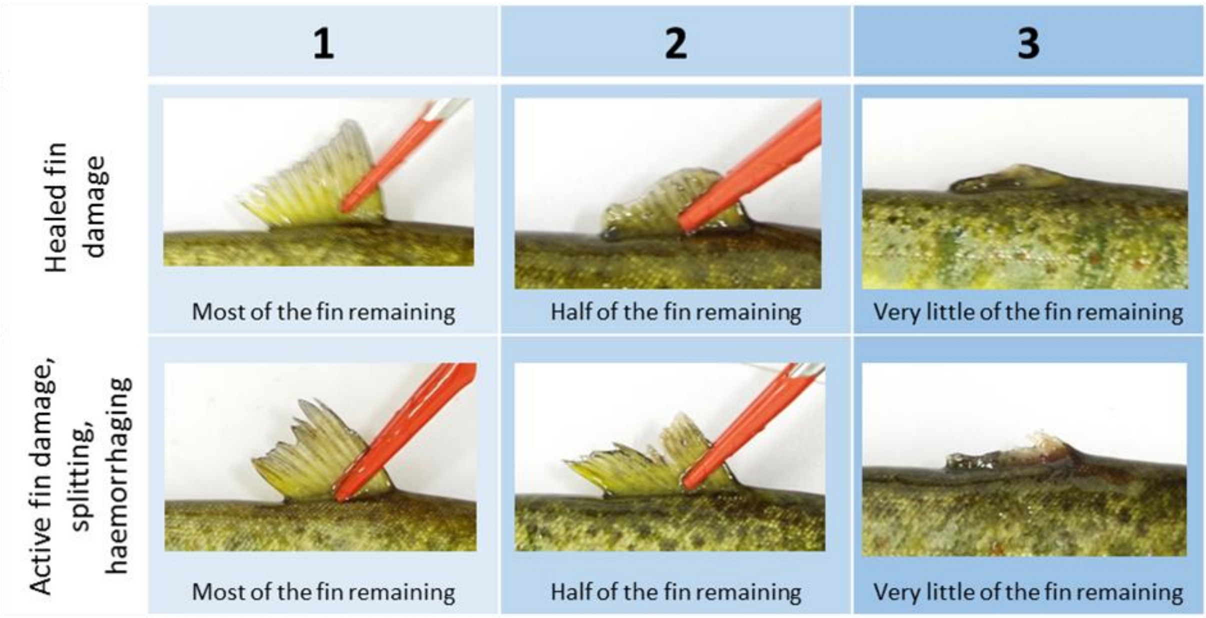
	1	2	3
Eye haemorrhage	 Minor haemorrhages	 Larger haemorrhages, or traumatic injury	 Large haemorrhages / traumatic injury. Eye may be ruptured
Exophthalmia	 Eye protruding a little	 Moderate eye protrusion	 Major eye protrusion
Opercular damage	 Operculum only partly covering gills	 Operculum absent on one of the gills (gill exposed)	 Both opercula absent (both gills exposed)
Snout damage	 Minor wound on snout (either jaw)	 Moderate wound and broken skin on snout	 Large deep and extensive wound. Can cover the whole head
Upper jaw deformity	 Suspected malformation	 Distinct malformation	 Major malformation, jaw pointing backwards
Lower jaw deformity	 Suspected malformation	 Distinct malformation	 Major malformation, jaw pointing backwards

Table 3.1-2. *Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. (Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. Nilsson. Photos: K. Gismervik, L. H. Stien, J. Nilsson, C. Noble, J. F. Turnbull, P. A. Sæther, I. K. Nerbøvik, I. Simion, B. Tørud, B. Klakegg, R. Andersen, C. Karlsen, K. J. Merok, F. Gregersen)*

	1	2	3
Emaciation	 Potentially emaciated	 Emaciated	 Extremely emaciated
Vertebral deformity	 Signs of deformed spine	 Clearly visible spinal deformity (e.g. short tail)	 Extreme deformity
Skin haemorrhages	 Minor haemorrhaging, often on the belly of the fish	 Large area of haemorrhaging, often coupled with scale loss	 Significant bleeding, often with severe scale loss, wounds and skin edema
Lesions / wounds [1]	 One small wound (< 10 pence piece)[1], subcutaneous tissue intact (no muscle visible)	 Several small wounds	 Large, severe wounds, muscle often exposed (≥ 10 pence piece)
Scale loss	 Loss of individual scales	 Small areas of scale loss (< 10% of the fish)	 Large areas of scale loss (≥ 10% of the fish)
Sea lice infection	 Light infection	 0.05 - 0.08 pre-adult or adult lice cm^{-2} of fish skin	 ≥0.08 pre-adult or adult lice cm^{-2} of fish skin

[1] *For pre-smolts "one small wound" should be < 1 cm. NB! Wounds that penetrate the abdominal cavity should be scored as a 3) irrespective of size*

Table 3.1-3. *Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. It is important to differentiate between healed lesions and active lesions. Active lesions indicate an ongoing problem that needs to be addressed (Figure: J. F. Turnbull, C. Noble, D. Izquierdo-Gomez, L. H. Stien, K. Gismervik, J. Nilsson. Photos: J. F. Turnbull)*



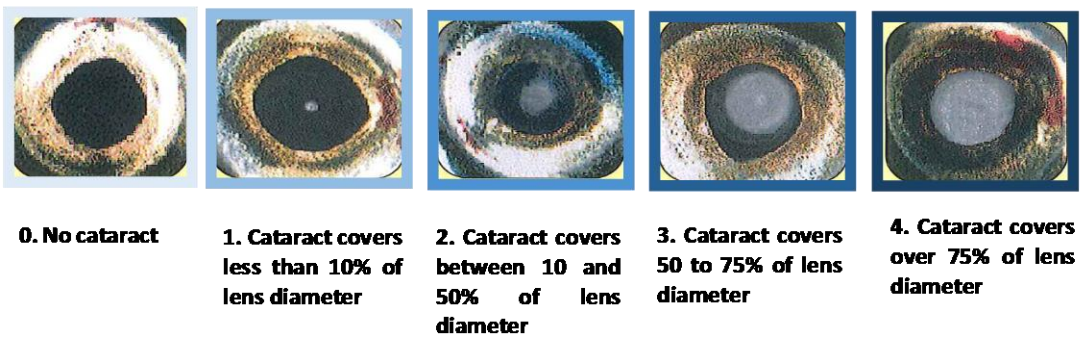


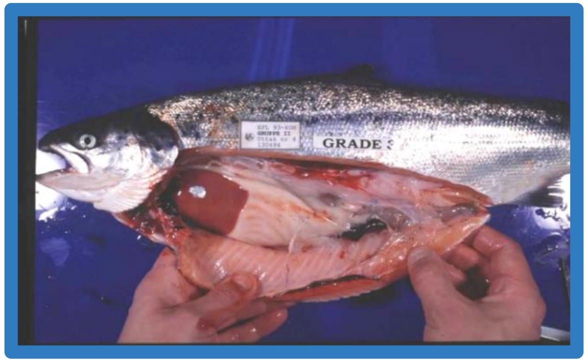
Fig. 3.2. *Morphological scheme for diagnosing and classifying eye cataracts in Atlantic salmon Text reproduced from "Wall, T. & Bjerkås, E. 1999, A simplified method of scoring cataracts in fish. Bulletin of the European Association of Fish Pathologists 19(4), 162-165. Copyright, 1999" [202] with permission from the European Association of Fish Pathologists. Figure: David Izquierdo-Gomez. Photos reproduced from "Bass, N. and T. Wall (Undated) A standard procedure for the field monitoring of cataracts in farmed Atlantic salmon and other species. BIM, Irish Sea Fisheries Board, Dun Laoghaire, Co. Dublin, Ireland, 2p." [204] with permission from T. Wall.*

Table 3.3. *The Speilberg Scale, reproduced from "Midtlyng et al., 1996, Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (Salmo salar L.) against furunculosis. Fish & Shellfish Immunology 6, 335–350. Copyright 1996" [98] with permission from Elsevier.*

Score	Visual appearance of abdominal cavity	Severity of lesions
0	No visible lesions	None
1	Very slight adhesions, most frequently localized close to the injection site. Unlikely to be noticed by laymen during evisceration	No or minor opaquity of peritoneum after evisceration
2	Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall. May be noticed by laymen during evisceration	Only opasicity of peritoneum remaining after manually disconnecting the adhesions
3	Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall. May be noticed by laymen during evisceration	Minor visible lesions after evisceration, which may be removed manually
4	Major adhesions with granuloma, extensively interconnecting internal organs, which thereby appear as one unit. Likely to be noticed by laymen during evisceration	Moderate lesions which may be hard to remove manually
5	Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may carry focal, prominent and/or heavily pigmented lesions or granulomas	Leaving visible damage to the carcass after evisceration and removal of lesions
6	Even more pronounced than 5, often with considerable amounts of melanin. Viscera unremovable without damage to fillet integrity	Leaving major damage to the carcass

1. Very slight adhesions, most frequently localized close to the injection site. Unlikely to be noticed by laymen during evisceration.

2. Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall. May be noticed by laymen during evisceration.



3. Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall. May be noticed by laymen during evisceration.

4. Major adhesions with granuloma, extensively interconnecting internal organs, which thereby appear as one unit. Likely to be noticed by laymen during evisceration

5. Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may carry focal, prominent and/or heavily pigmented lesions or granulomas

6. Even more pronounced than 5, often with considerable amounts of melanin. Viscera irremovable without damage to fillet integrity.

Fig. 3.4*. The Speilberg Scale for intra-abdominal lesions after intraperitoneal vaccination of Atlantic salmon. Photos provided and reproduced with kind permission from Lars Speilberg. Text reproduced from "Midtlyng et al., 1996, Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (Salmo salar L.) against furunculosis. Fish & Shellfish Immunology 6, 335–350. Copyright 1996" [98] with permission from Elsevier.*

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