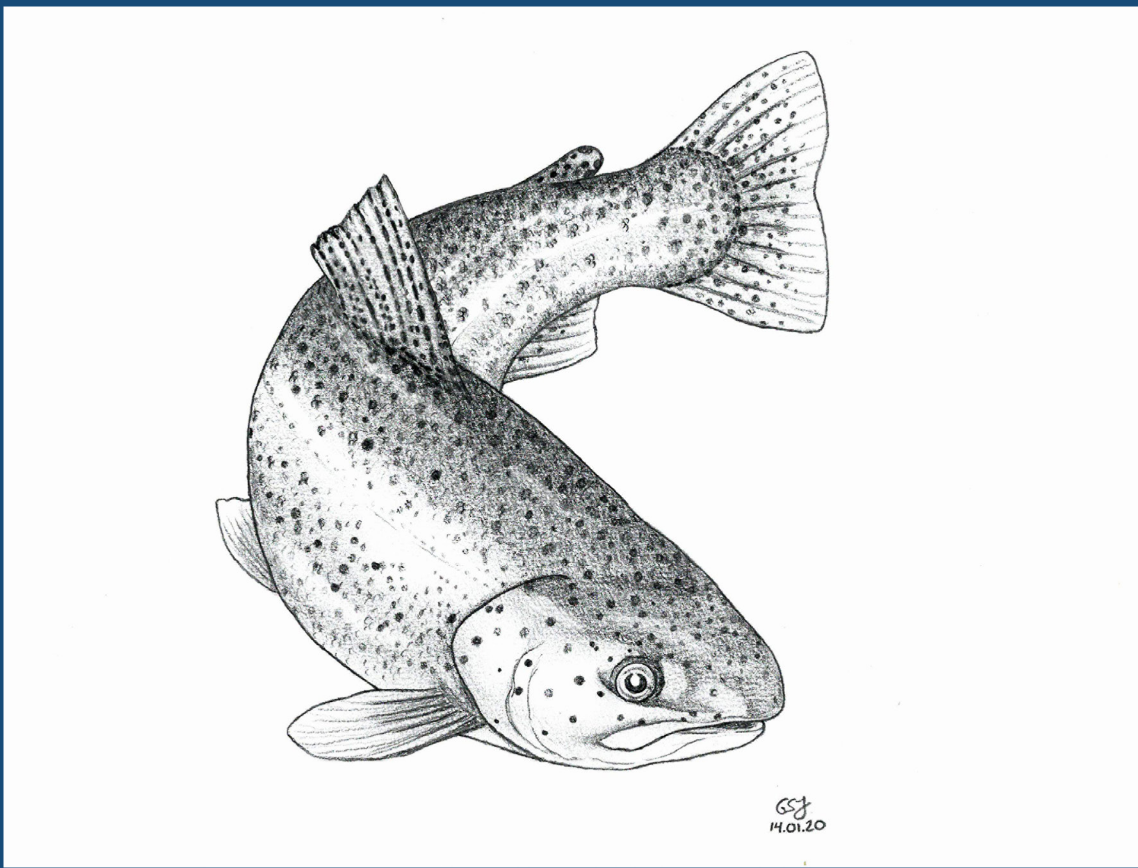
Welfare Indicators for farmed rainbow trout:

tools for assessing fish welfare



Edited by Chris Noble, Kristine Gismervik, Martin H. Iversen, Jelena Kolarevic, Jonatan Nilsson, Lars H. Stien and James F. Turnbull

Nofima An FHF-financed project, led by Nofima in partnership with:

INSTITUTE OF MARINE RESEARCH
Veterinærinstituttet Norwegian Veterinary Institute
NORD University
UNIVERSITY of STIRLING

FHF THE NORWEGIAN SEAFOOD RESEARCH FUND

Suggested citation: Noble, C., Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H. & Turnbull, J. F. (Eds.) (2020). Welfare Indicators for farmed rainbow trout: tools for assessing fish welfare 310 pp.

Editors are listed alphabetically after the first author

ISBN 978-82-8296-620-7

FHF financed project 901157 «FISHWELL: Kunnskapssammenstilling om fiskevelferd for laks og regnbueørret i oppdrett»

May 2020

https://nofima.no/fishwell/trout/

Authorship: Authors are credited for each chapter.

Front cover: Oddvar Dahl

Front cover illustration: © Gunhild Seljehaug Johansson

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 in?

- Flow through systems — B.1
- Sea cages — B.2

...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 for rainbow trout is an output of the Norwegian Seafood Research Fund (Fiskeri- og havbruksnæringens forskningsfinansiering, FHF) project «FISHWELL: Kunnskapssammenstilling om fiskevelferd for laks og regnbueørret i oppdrett». It utilizes the text and format of the earlier FISHWELL salmon handbook (Noble et al., 2018) as a basis for this work, updating the data and contents with literature based upon rainbow trout. 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 reference group of the FISHWELL project (Olai Einen, Cermaq; Solveig Gaasø, formerly of Marine Harvest Norway; Lene Høgset, formerly of STIM; 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 say a big thank you to Susanna Lybæk and her colleagues at Dyrevernalliansen for their very thorough and valuable comments and feedback on an earlier version of the salmon handbook.

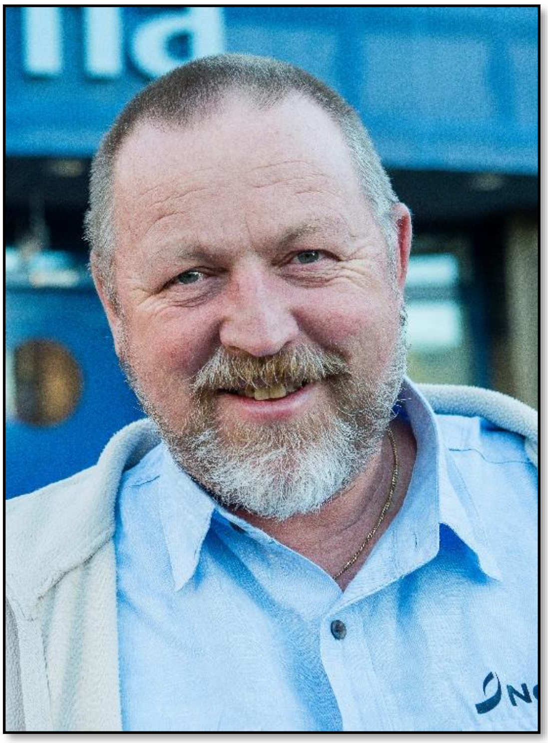
Many thanks also to Lars Speilberg of ScanVacc for kindly providing the pictures for the Speilberg Scale and Tim Ellis of CEFAS for permission to reproduce the table summarising the key factors affecting non-invasive methods of cortisol monitoring. Tony Wall of the Fish Vet Group also kindly gave permission to reproduce the morphological scheme for diagnosing and classifying eye cataracts. Many 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 rainbow trout. Many thanks also to the Humane Slaughter Association for their kind permission to reproduce their crowding intensity scale for rainbow trout. Many thanks also to Reidar Handegård of ILAB for inputs regarding TGP and nitrogen supersaturation for the salmon handbook, some of these suggestions have been also been adopted in this book. 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. Ola Sveen of Svanøy Havbruk also kindly provided pictures of one of their farms for use in the handbook.

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, comprehensive knowledge, counsel and expertise are deeply missed by all when knew him and is 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

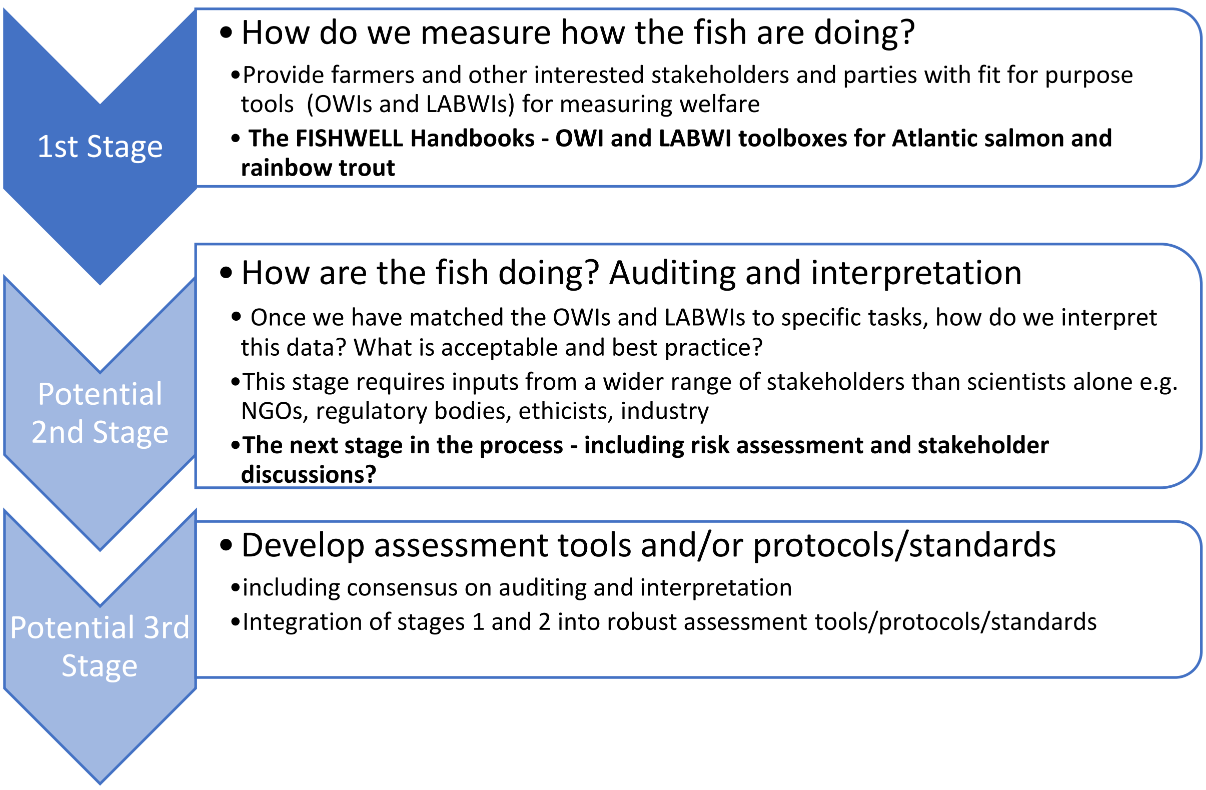
Thomas Torgersen

Objectives of the handbook

Our handbook has three key objectives:

1. Provide the user with an updated scientific summary of the welfare of rainbow trout 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 rainbow trout 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 rainbow trout 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.



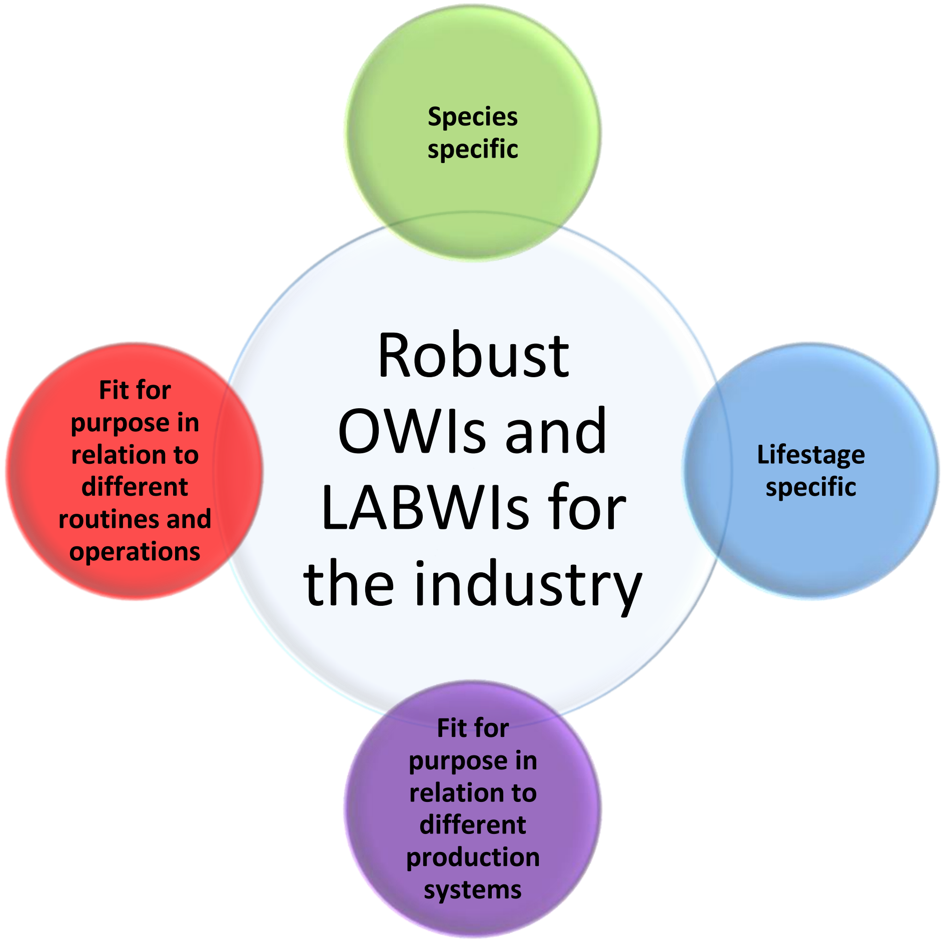
The OWIs and LABWIs have been evaluated in terms of their:

- **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 handbooks 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 rainbow trout – Part A. Knowledge and theoretical background

Jonatan Nilsson[1]*, Lars H. Stien[1]*, Martin H. Iversen[2]*, James F. Turnbull[3]*, Tore S. Kristiansen[1], Thomas Torgersen[1], Frode Oppedal[1], Ole Folkedal[1], Malthe Hvas[1], Kristine Gismervik[4], Kristian Ellingsen[4], Cecilie M. Mejdell[4], Jelena Kolarevic[5], David Izquierdo-Gomez[5], Bjørn-Steinar Sæther[5], Åsa M. Espmark[5], Kjell Ø. Midling[5] and Chris Noble[5]

*Joint first authors

1. Institute of Marine Research, P.O. Box 1870 Nordnes, No-5817 Bergen, Norway
2. Nord University, Faculty of Biosciences and Aquaculture, 8049 Bodø, Norway
3. University of Stirling, Institute of Aquaculture, School of Natural Sciences, Stirling, FK9 4LA, United Kingdom
4. Norwegian Veterinary Institute, P.O. Box 750 Sentrum, NO-0106 Oslo, Norway
5. Nofima, P.O. Box 6122 Langnes, NO-9291 Tromsø, Norway

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 and 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 processes 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 trout or play in juvenile mammals), ii) exploratory behaviours that improve 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 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 production units 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. Soutar (2015) has stated that the standards have helped put fish welfare in a central position in salmonid aquaculture. 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/trout).

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 the 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., 2019) and this standard also covers rainbow trout. 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. 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. British trout producers have incorporated this code into their own specific standard that includes both environmental and welfare based criteria for rainbow trout (Quality trout UK, 2019).

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 rainbow trout aquaculture in 2013, updated in 2019 (ASC, 2019). 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 2019 there were 142 ASC certified fish farms in Norway (https://www.barentswatch.no/ December, 2019). The Best Aquaculture Practices (BAP) Standards by the Global Aquaculture Alliance (BAP, 2016) has multi-species finfish and crustacean farm standards which apply to all types of production systems but have no specific standards for trout. 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) water flow, iv) low water oxygen content and v) excessive carbon dioxide content. Animal hazards included: i) aggression and ii) low/high stocking density. Husbandry hazards included: i) lack of staff training, ii) grading and iii) handling. Feeding hazards included: i) feed deprivation (long term) and ii) feeding to excess. Disease hazards included: i) rainbow trout fry syndrome, ii) eye lesions, iii) IPN and iv) proliferative kidney disease (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 environmental 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. These 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 an earlier version of the RSPCA welfare standards for farmed rainbow trout (RSPCA, 2014), 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 an earlier version of the RSPCA welfare standards for farmed rainbow trout (RSPCA, 2014), the Atlantic salmon welfare assessment protocol by The Norwegian Veterinary Institute, NVI (Grøntvedt et al., 2015; Gismervik et al., 2016, 2017) and in SWIM 1.1 for Atlantic salmon (Stien et al., 2013; Pettersen et al., 2014)*

Previously used RSPCA protocol for rainbow trout	NVI Protocol (Atlantic salmon)	SWIM 1.1 (Atlantic salmon)
Eye loss/damage	Eye damage	Eye status
Jaw deformity	Snout injury	Snout jaw wound
Operculum deformity	Cataract	Upper jaw deformity
Dorsal fin damage	Fin damage	Lower jaw deformity
Pectoral fin damage	Scale loss	Opercula status
Caudal fin damage	Skin haemorrhage	Fin condition
Pelvic 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
	Gill paleness	Gill status
		Condition factor
		Emaciation status
		Sexual maturity
		Smoltification state

2. Welfare needs of trout

Broadly speaking the welfare needs of salmonids can be categorised into needs directly linked to their 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).

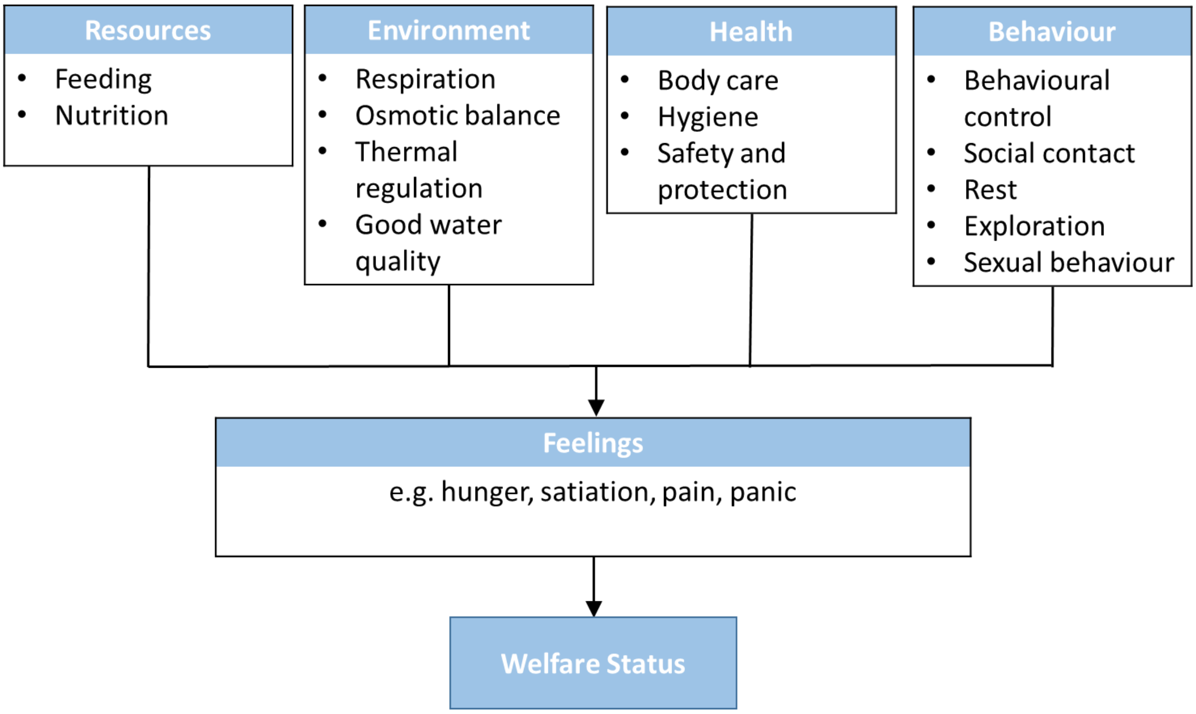


Fig. 2-1. *The welfare needs of salmonids can broadly be categorised into available resources, a suitable water environment, good health and freedom to express behaviours. The degree of fulfilment of these needs affects their mental state and thereby the welfare status of the animals. Adapted from "Mellor, 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 salmonids (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

Osmoregulation
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. 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. Rainbow trout exhibit highly energetic feeding behaviour and can be highly motivated and competitive around mealtimes (e.g. Brännäs and Alanärä, 1992; Noble et al., 2007a). They are also 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 preferences. Fish also show food aversion towards food associated with sickness (Manteifel and Karelina, 1996).

Feeding can be described as the process the animal uses to get food and when we apply it in terms of satisfying a need, the term appetite "*a natural desire to satisfy a bodily need, especially for food*" (OxfordDictionaries.com © Oxford University Press, 2018) may be a better fit. 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 terms of amount and content. 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 (Grove et al., 1978; Noble et al., 2005) and variability in appetite for a given life stage may not always be an indicator of poor welfare. Appetite and the motivation to feed may also 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).

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 (Wall, 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, Atlantic salmon), or iii) when technical or environmental conditions prevent the farmer feeding the fish to satiation within any given day. In juvenile rainbow trout, underfeeding leads to inequality in feed intake (McCarthy et al., 1992) potentially due to increased competition for feed. It can also increase size variation in the group (Jobling and Koskela, 1996) or increase fin damage (Moutou et al., 1998).

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 salmonid 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 trout. 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. Osmoregulation

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 (McCormick and Saunders, 1987) and to be able to survive in salt water the trout must be able to tolerate the hyper osmotic seawater. There is also a danger that the fish revert back to their freshwater physiology if they are kept in freshwater too long (McCormick and Saunders, 1987). Small fish are more sensitive to inappropriate salinities and small trout that are not fully adapted to seawater will suffer from dehydration and can potentially die if released too early into the sea.

2.4. Thermal regulation

Temperature is one of the most important environmental factors influencing salmonid biology. Salmonids 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, salmonids 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).

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, salmonids are confined to rearing units and optimal water quality conditions must be provided to avoid any potentially negative effects on their performance and welfare. 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 are 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 trout 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 also the case for salmonids. Trout have been shown to be aggressive in small groups (Laursen et al., 2013) and especially in pairs (Øverli et al., 1999). Comparative data on aggression in commercial farming conditions is somewhat scarce (Ellis et al., 2002) although it has been reported in cages (Phillips, 1985).

Anras and Lagardère (2004) reported that rainbow trout behaviour may be affected by stocking density when they are held in tanks and reported that fish under > 30 kg/m^3 densities mostly exhibited circular diurnal swimming patterns followed by reduced activity at night compared to fish at 136 kg/m^3 that exhibited unstructured diurnal swimming patterns that were also maintained at relatively high levels at night. Early work by Sutterlin et al. (1979) reported that rainbow trout did not exhibit any consistent circular swimming or rotational orientation (although this may have been due to the presence of staff during observation periods) when held in sea cages. Another study by Phillips, (1985) reported trout did exhibit this circular swimming activity when fish behaviour was monitored using underwater video. Phillips also reported that cage-held rainbow trout can aggregate near the surface, exhibit low activity at slack water and form polarized shoals and maintain station at higher water current speeds. They also reported frequent aggressive interactions in the form of chasing and charging.

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). 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). Little information exists on the basal resting mechanisms or 'sleep' in salmonids. However, anecdotal evidence indicates states of resting and rainbow trout are less active during the night compared to daytime (Anras and Lagardère, 2004).

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 behavioural control, 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 freshwater 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 salmonids have an inherent need to perform courtship and mate choice (Newcombe and Hartman, 1980) and also to spawn, and for anadromous fish this is preceded by migration back to and up their river (Robards and Quinn, 2002). This behaviour involves considerable risks such as injury and reduced growth (Fleming and Reynolds, 2004). Anadromous salmonids, including rainbow trout, often start the homeward migration and enter the river several months before spawning. The spring-spawning rainbow trout may enter the river before maturation in May to October (summer-run) or later (winter-run) as maturing fish in November to April (Robards and Quinn, 2002). The spawning behaviour consists of nest building by the females, where they utilise a tail-beating motion whilst on their side to dig a spawning pit for the eggs. Males perform courtship displays and will often be aggressive (Tautz and Groot, 1975). As with other salmonids, male rainbow trout may mature at the juvenile stage as precocious males (Taranger et al., 2010) and engage in spawning as sneak spawners.

.

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.*

			Environment				Health			Behaviour					Reso-urces	
		Welfare indicators / Needs	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		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		Seawater adaptation		×												
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 have been developed for salmon (Soares et al., 2011, 2013; Stien et al., 2016a) and a standard mortality curve for rainbow trout based on data from Norwegian rainbow trout farmers is presented here (Figure 3.1.1-1). 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.

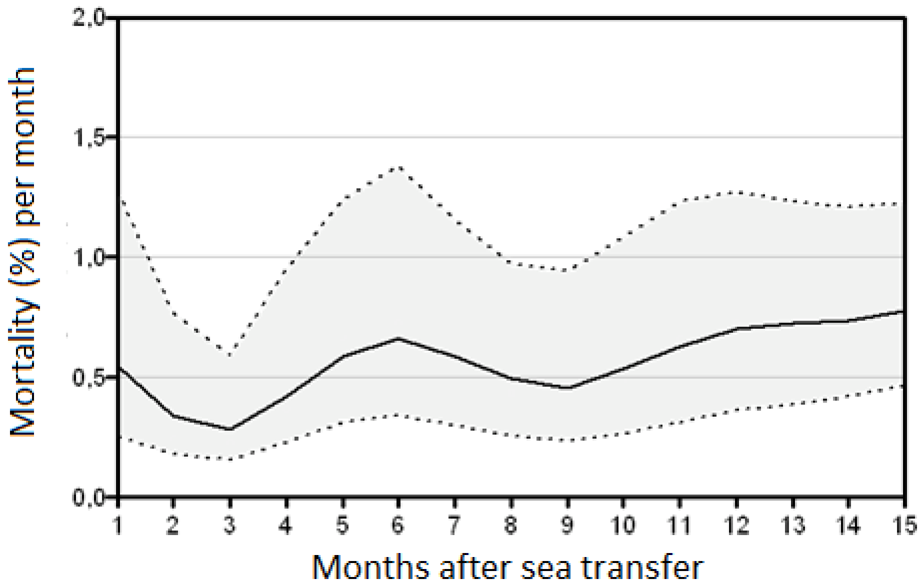


Fig. 3.1.1-1. *Standard mortality curve for the 15 first months of the on-growing of rainbow trout in sea cages based on reported data from all Norwegian rainbow trout farmers from 2009-2015. The curve gives the median monthly mortality rate, in addition to the 25- and 75-percentiles.*

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., (2017) used the distribution of total mortality after 15 months, based on reported monthly mortality data from all Norwegian trout 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. Kristiansen et al., (2014) showed that fish farms with high average mortality rates generally also had high variation in mortality between production cycles.

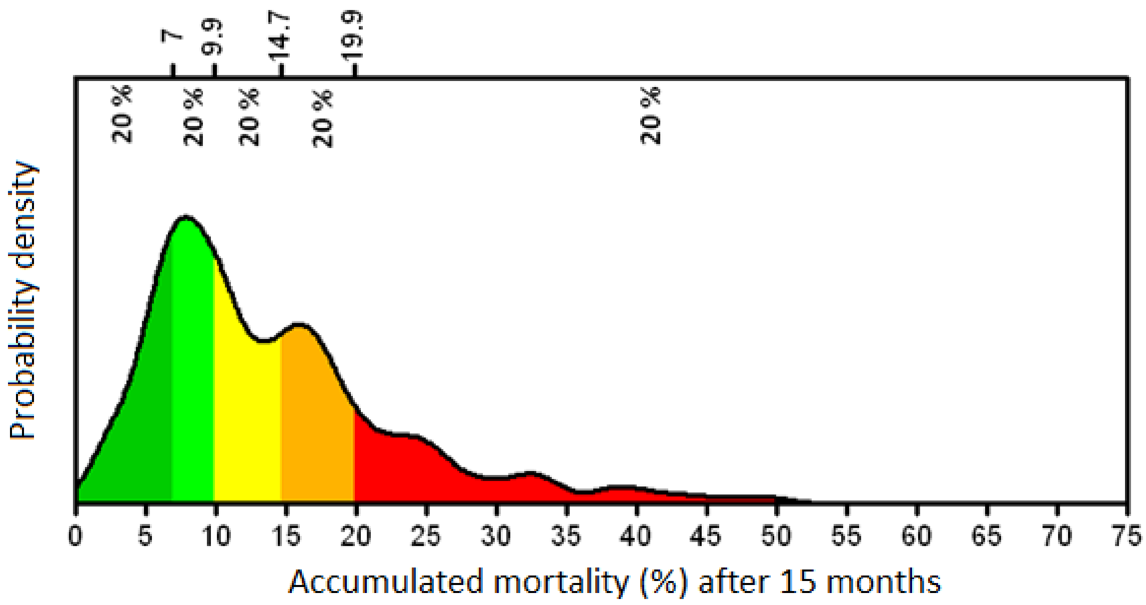


Fig. 3.1.1-2*. Mortality distribution after 15 months of on-growing of rainbow trout in sea cages. 0-7% (dark green, better than normal welfare), 7-9.9 % (green), 9.9-14.7 % (yellow), 14.7-19.9 % (orange), >19.9 % (red, worse than normal welfare).*

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 the 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 trout 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 statistics 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 without 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 rearing systems e.g. sea cages are equipped with underwater cameras. Behaviour can give an immediate indication of the state of the fish, indicators can be applied at both the group and individual level and behavioural measurements are usually non-invasive in most situations. 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). Various group level welfare indicators 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).

Rainbow trout behaviours that can be an indicator of a potential problem include:

- 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, rainbow trout; see also Sneddon et al., 2016 for more information).
- Reduced locomotor activity may also be a response to poor environmental conditions e.g. low oxygen levels (van Raaij et al., 1996), or low oxygen/high ammonia levels (Colson et al., 2019).
- Increased swimming activity and dispersed swimming can also be a response to a handling stressor such as crowding (Sadoul et al., 2015).
- Unstructured swimming at the bottom of the cage or tank can also be an indicator of acute stress (e.g. van Raaij et al., 1996; Anras and Lagardère, 2004).
- 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, 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 of trout (Ellis et al., 2002; Noble et al., 2007a).
- Body rocking behaviours and also the fish rubbing against surfaces has also been observed during nociception (Sneddon, 2006; Sneddon et al., 2016).

In an operational setting, behavioural indicators require careful interpretation and in any group of fish there will be a range of individual responses to any situation, with some fish acting more aggressively or taking more risks than others (Huntingford and Adams, 2005). Different fish may also react differently to a stressor e.g. some fish remain passive when exposed to low oxygen levels, whilst others exhibit pronounced avoidance and panic behaviours (van Raaij et al., 1996). Two similar types of behaviours may also represent different things. 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 feeding motivation, exploratory behaviour or feed anticipatory activity (all indicators 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 a situation where fish welfare may be reduced, such as fish competing for a potentially limited resource (e.g. in A. salmon, Noble et al., 2007b) and can indicate that the fish may e.g. 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 trout is mostly from their swim bladders, although this is dependent on the type of sonar used. A weak signal may therefore be that the fish have deflated swim bladders (Korsøen et al., 2009 in A. salmon). 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 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.

Qualitative Behavioural Assessment is used extensively in terrestrial species but is only just starting to be applied in aquaculture. 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, 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, 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. Appetite and feeding in rainbow trout can also be influenced by other factors including daylength, both natural (Landless, 1976a) and artificial (Sánchez-Vázquez & Tabata, 1998), oxygen saturation (Pedersen, 1987), the health status of the fish (Chin et al., 2004), ectoparastic level (Nagazawa, 2004), water chemistry including ammonia levels (Ortega et al., 2005) and being chronically stressed (Gregory and Wood, 1999).

Management practices such as handling can also impact upon appetite and feed intake in rainbow trout (e.g. Hoskonen and Pirhonen, 2006). 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 trout vary within and between days (Grove et al., 1978; Noble et al., 2005) even under stable environmental conditions, with minimal disturbance. If trout 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 (McCarthy et al., 1992), which can e.g. lead to increased injury (Moutou et al., 1998). Long-term feeding of maintenance rations to maintain fish size or limit growth rate can lead to a marked deterioration of welfare in salmonids, also including increased competition and injury (Cañon Jones et al., 2017, Atlantic salmon). 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 trout 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 trout and salmon 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 trout 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). 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 (Landless, 1976a; Austreng et al., 1987) 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 or their resilience. 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 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 (Dumas et al., 2007) and may be affected by several factors, such as ration size (Storebakken and Austreng, 1987) appetite (Linton et al., 1998), nutritional content of the feed (Kaushik et al., 1995), diseases, social interactions (Li and Brocksen, 1977), water quality parameters (Person-Le Ruyet et al., 2008) and can be indicative of a tertiary stress response (e.g. Ellis et al., 2002; Huntingford et al., 2006), several of which are indicators of reduced welfare. However, growth can be affected by factors that are not related to welfare, 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).

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. 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 (Jobling and Koskela, 1996). 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 (Jobling and Koskela, 1996).

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 can include: 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. Further, when using such technologies it is important to ensure a sample is taken that is a representative, e.g. by covering the entire depth range in the cage (Folkedal et al., 2012c; Nilsson et al., 2013).

Using growth rate as an OWI depends upon obtaining a good, representative sample of the fish and growth rate may be quantified as e.g. 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, diseases 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. Although effective vaccines are a clear benefit to the fish, vaccination may cause side effects such as abdominal adhesions, due to the adjuvant, which can be a significant welfare problem. 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, five of them in rainbow trout (Hjeltnes et al., 2017). 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 also viruses. 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 e.g. PRV-3 in rainbow trout (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, the 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 and they therefore have to be considered in context.

Table 3.1.5-1a. *Important infectious virus diseases in farmed rainbow trout 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 against the current (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). • In 2016 five outbreaks of SAV 3 in rainbow trout were reported in Norway, while marine SAV 2 outbreaks in rainbow trout in Norway have also been reported or suspected in recent years (Hjeltnes et. al., 2017). • Welfare impacts can be reduced by minimizing stress, euthanizing sick individuals (and those chronically affected) or 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). • Sleeping disease (SAV 2 FW) is seen in parts of Europe but has not yet been reported in Norway (NVI, 2017).
Infectious salmon anaemia (ISA)	Infectious salmon anaemia virus (ISAV)	(×)	×	• First detected in Norwegian rainbow trout in 2015, but without clinical disease or pathology and in connection with an ongoing outbreak of ISA in salmon (Hjeltnes et. al., 2016). The role that rainbow trout may have in the spread of infection is not known (NVI, 2017). • In salmon, the virus attacks the surface within all blood vessels and the heart, producing severe anaemia and circulatory disturbances that can be seen in gills, heart, liver, kidney, spleen etc. (Aamelfot et al., 2014). • In salmon mortality is often low with a chronic progression, daily mortality is typically 0.05-0.1% in affected cages, however high mortality has also been reported (OIE, 2015b). • Early detection of clinical ISA and rapid slaughtering of fish in net cages may prevent spread at the site. ISA is a notifiable disease and must be reported to the Norwegian authorities. 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)	×	×	• First reported in trout then later in salmon. • The virus attacks the pancreas, which is essential for digestion of food, and can also give necrotic enteritis. 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%. Fry are considered to be most susceptible (NVI, 2017). • A large proportion of fish develop a lifelong persistent infection, which can be activated during stress (EFSA, 2008a; NVI, 2017). • Stress can also increase mortality during outbreaks. Hence, in cases where the fish are very small, euthanizing the whole population may be the most welfare friendly strategy (EFSA, 2008a). Fish surviving IPN often have higher susceptibility to other diseases (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 rainbow trout 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-3, also referred to as PRV-om and virus y)	×	×	• First seen in rainbow trout in 2013 (Olsen et al., 2015) and the disease has not been diagnosed in rainbow trout since 2014 (Hjeltnes et. al., 2017). • PRV-3 is a variant of the PRV virus in salmon leading to an HSMI-like infection in the heart and skeletal musculature and also anaemia. Results in circulatory failure. • In laboratory trials, both rainbow trout and salmon can be infected by PRV-3, but salmon appear to be less susceptible to infection (Hauge et al., 2017). Experimental infection leads to heart inflammation (but has not resulted in clinical disease or death). • No primary outbreaks have yet been identified in rainbow trout held in seawater, but the spread of PRV-3 in seawater is likely (Hjeltnes et al., 2017). • No treatment or vaccine is available and the general advice regarding PRV-3 is to avoid handling infected fish.
Viral Haemorrhagic Septicaemia (VHS)	Viral haemorrhagic septicaemia virus (VHSV)			• Has not been identified in Norway since 2008 (Hjeltnes et. al., 2017). • This is a notifiable disease in Norway and an acute disease outbreak is characterized by high mortality, exophthalmus, haemorrhaging, anaemia and abnormal behaviour involving spiral swimming ("flashing" has also been observed). • Control is based on rapid eradication.

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

	Bacteria	FW	SW	Welfare impact
Yersinosis	*Yersinia ruckeri*	×	×	• In Norway, the disease is almost exclusively associated with farmed Atlantic salmon (Hjeltnes et. al., 2017) but is considered important for rainbow trout in other European countries. • Most common in the fresh water stage where acute septicaemia with high mortality can be seen in salmon fry (Poppe et al., 1999). • The name "redmouth disease" is derived from subcutaneous haemorrhaging of the mouth and throat of the fish in most but not all cases (EFSA, 2008b). • 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). • Yersinosis is not a notifiable disease.
Flavobacteriosis, Rainbow Trout Fry syndrome (RTFS)	*Flavo-bacterium psychrophilum*	×	(×)	• Rainbow trout is considered especially susceptible to flavobacteriosis and the disease has previously caused large losses in the freshwater phase in Norway (Hjeltnes et. al., 2017). • High mortality due to a systemic infection named rainbow trout fry syndrome (RTFS) can typically be seen 4-7 weeks after first feeding (Poppe et al., 1999). "Corkscrew" swimming can also be seen (NVI, 2017). • Often called "bacterial cold water disease" or "peduncle disease" as it usually occurs at colder water temperatures, 8-14°C (EFSA, 2008b). In addition, *F. psychrophilum* is associated with ulcers and fin erosion, which can have severe welfare impacts (EFSA, 2008b). • In recent years in Norway, the disease has mainly been detected in larger rainbow trout in brackish water fjord systems, where infection causes ulcers and bullae (Hjeltnes et. al., 2017). • In Norway there have been different strains affecting rainbow trout and salmon (NVI, 2017). • Systemic infection of *F. psycrophilum* in rainbow trout is a notifiable disease in Norway and four outbreaks were reported in 2016 (Hjeltnes et. al., 2017). Bacterial strains show reduced susceptibility to quinolone antibiotics (Hjeltnes et. al., 2017). • Outbreaks can be associated with a suboptimal environment and stress (NVI, 2017).
Winter ulcer	*Moritella viscosa, Tenaci-baculum* spp., *Aliivibrio (Vibrio) wodanis*		×	• Ulcers on the head, flanks and fins are typical welfare problems in autumn and winter and can lead to increased mortality and also a reduction in harvest quality (Bornø & Linaker, 2015). • *Moritella viscosa* is a major contributor. 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). • The main welfare aspects of winter ulcers are 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. • 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). • 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 3.1.5-2b. *Important infectious bacterial diseases in farmed rainbow trout in Norway and their welfare impact. FW = freshwater, SW = seawater.*

	Bacteria	FW	SW	Welfare impact
Bacterial Kidney Disease (BKD)	*Renibacterium salmoninarum*	**x**	**x**	• A notifiable disease in Norway. • Low yearly incidence in salmonids in Norway (Hjeltnes et. al., 2017). • It is usually a chronic disease often causing subclinical infections or low persistent mortalities that peak in the spring. • In fresh water, kidney damage causes osmoregulatory problems (NVI, 2017). • Kidney may be swollen with white nodular lesions (which may also occur in other organs). Fish may also have anaemia, protruding eyes and fluid accumulation in the abdominal cavity which may be indicative of circulatory disturbances (NVI, 2017). • The most important prophylactic measure is to keep the breeding population free from disease.
Cold water vibriosis	*Vibrio salmonicida (syn. Allivibrio salmonicida)*		**x**	• Mostly causes problems for Atlantic salmon but also seen in rainbow trout. • Typically associated with slowly increasing mortalities that can become severe if left untreated. • Incidence of the disease has decreased since the introduction of a vaccine. Monitoring of the vaccine side effects is considered important in relation to fish welfare (Hjeltnes et. al., 2017).

Table 3.1.5-3. *Important parasites and fungal diseases in farmed rainbow trout 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*		×	• Parvicapsulosis is a problem in salmon (mainly in the most northerly counties in Norway), where mortality may vary from low to severe (Bornø & Linaker, 2015). • Rainbow trout may be less susceptible as parvicapsulosis has not been diagnosed in rainbow trout by the Norwegian Veterinary Institute in the last decade. However, it is not a notifiable disease. • High parasite densities and significant pathological changes are observed in the pseudobranch (under the gill cover) of salmon. The psuedobranchs, which are involved in delivering oxygen to the eye and also the control of ion balance, can be completely degraded or be severely damaged (NVI, 2017). • Salmon with advanced parvicapsulosis are commonly thin, anaemic and have eye haemorrhages (Bornø & Linaker, 2015; NVI, 2017). • *P. psuedobranchicola* have a complex life cycle where polychaetes are the main host and fish are intermediate hosts. It has been found in wild sea trout and salmon (Bornø & Linaker, 2015).
Amoebic Gill Disease (AGD)	*Paramoeba perurans*		×	• AGD is an emerging serious disease affecting farmed salmon in Norway and is also seen in rainbow trout but since it is non-notifiable the number of outbreaks are unknown (Hjeltnes et al., 2017). • The amoebic parasites affect the gills, causing respiratory problems. Macroscopically visible gill changes including increased mucus production, which can be used for classification of the disease in a gill scoring system published for salmon (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 also freshwater itself have been factors limiting its use (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 fingerlings 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 at 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). 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.

3.2. 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 a systematic sample of fish is required (using it as an individual OWI). The same scenario is applicable to dorsal fin damage in juvenile trout. 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/ventilation rate

The gill beat (breathing) rate of fish increases when the need for oxygen supply increases. This can be due to challenging water quality conditions e.g. reduced oxygen levels in the water (Vigen, 2008), high nitrite levels (Aggergaard and Jensen, 2001) or a higher metabolic rate arising from higher activity levels or stress (Sneddon, 2003; Pounder, 2018; Altimiras & Larsen, 2000, 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 (Zhang et al., 2013). 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.

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 water state.

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 are 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 and 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.

Table 3.2.1-1. *The gill beat rate of rainbow trout before and during stress in various procedures.*

Fish size and life stage	Threshold level (if any) and reference	% change (calm to stress)	Reference
61 g ± 5 g in freshwater	52 beats/min (quiet) and 67 beats/min (after injection of noxious chemical to the lips)	22.4%	Sneddon, 2003
138 ± 6 g in freshwater	ca. 55 beats/min (undisturbed) and ca. 75 beats/min 30 minutes after handling	36.4%	Pounder et al., 2018
138 ± 6 g in freshwater	ca. 55 beats/min (undisturbed) and ca. 67-82 beats/min 30 minutes after removal from anaesthetic	21.8-49.1%	Pounder et al., 2018
200–300 g in freshwater	71 beats/min (quiet) - no significant differences in VR after Cortland saline injection	0%	Zhang et al., 2013
200–300 g in freshwater	71 beats/min (quiet) - 149.81 (stress after 140 mmol/L NH_4 HCO_3 injection)	111%	Zhang et al., 2013
250–380 g in freshwater	71±2 beats/min (quiet) and 77 ± 3 beats/min (after ammonia injection)	7.8%	Zhang & Wood, 2009
600-800 g in freshwater	77 beats/min (quiet) and 100 beats/min (swimming exercise)	33%	Stevens & Randall, 1967
357 ± 19	53.1±3.7 beats/min (quiet), 106.2±6.4 beats/min (stress) and recovery (20 min), 62±7.7 beats/min	50%	Altimiras & Larsen, 2000
441 ± 75 g freshwater	60 beats/min (calm) and 120-130 beats/min (stress)	53.8 %	Shabani et al., 2016

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 (VOR; 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 (VOR, 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; Pounder et al., 2018).

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

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

3.2.3. Sea lice

Rainbow trout are affected by sea lice, but the vast majority of the literature refers to Atlantic salmon. Although rainbow trout appear to be slightly more resistant to lice than Atlantic salmon (Jackson & Minchin, 1992; Jackson et al., 1997; Fast et al., 2002a; O'Donohoe et al., 2016), their responses to lice infection are quite similar (Fast et al., 2002a) and data from salmon may also be applicable to rainbow trout as well. Norwegian trout and salmon in the sea 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.*

In rainbow trout, a sea lice infestation involving pre-adult and adult lice can lead to primary stress responses including increased plasma cortisol levels (Fast et al., 2002a) and the area the lice attaches to can become inflamed (Nolan et al., 2000). In Atlantic salmon, 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). Trout can also respond in a similar way, with a more severe primary stress response to a stressor (Ruane et al., 2000) and changes to the skin and gills (Nolan et al., 2000). Rainbow trout infected with salmon lice are also more susceptible to pathogens (Mustafa et al., 2000). In Atlantic salmon, infections with larger numbers of sea 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).

As far as the authors are aware, there are no data on the limits at which lice infestation rates start to cause welfare problems in rainbow trout. In the absence of this data, and the suggestion by previous authors that trout responses to lice are similar to salmon (Fast et al., 2002a), we cautiously refer the reader to the published data on Atlantic salmon (also reported in Noble et al., 2018). While wild salmonids often have lice levels that can lead to welfare problems and mortality (Holst et al., 2003; Torrissen et al., 2013), lice levels are strictly controlled and regulated in commercial aquaculture and such levels are rarely if ever seen on farmed salmon (Folkedal et al., 2016). However, these levels may occur on some individuals, especially emaciated fish. Thus, for farmed salmon and trout, where lice levels are low, frequent handling and treatment associated with delousing may be a more serious welfare issue than the lice themselves.

The other sea lice species affecting Norwegian rainbow trout, *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 farms than *L. salmonis* and are smaller and less determined feeders. With regard to Atlantic salmon, McKinnon (1993) found little response by the immune system on A. 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 A. 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, either for salmon or trout.

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 fish, 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. 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. It is also likely that some lice will fall off during capture and will therefore not be recorded during counting.

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, ion exchange 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 and increasing the number of some potential infectious parasites in the water.

In the sea phase, gill disease is becoming increasingly prevalent and is certainly multifactorial but can result in high morbidity and mortality and can therefore also have a significant impact upon welfare. Amoebic gill disease (AGD) is triggered by the marine amoeba *Neoparamoeba perurans*. It is a serious emerging disease in Norway and also affects rainbow trout (Hjeltnes et al., 2017). High temperatures and salinity increase the risk of 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 (VKM, 2014). 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 provide 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 rainbow trout between condition factor and their total lipid content (Johansson et al., 2000). Rainbow trout condition factor may also vary throughout the year (e.g. Taylor et al., 2006). Very low condition factor may be an indication of emaciation and extremely high condition factor may be indicative of vertebral deformation (Choo et al., 1991). Rainbow trout can also accumulate large deposits of abdominal fat if overfed. The welfare implications of such obesity are not clear but it is a sign of poor feed management.

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. However, in long-term feed withdrawal studies on rainbow trout, values of < 1.0 have been reported in juvenile trout (ca. 55g mean weight) fasted for 4 months (Jørgensen et al., 2016). A fasting study on larger fish (ca. 280g mean weight) reported that *K* values dropped from an initial level of ca. 1.15 - 1.2 to ca. 1.05 after 1 month and ca. 0.9 after 4 months (Pottinger et al., 2003). We therefore suggest a *K* factor of ca. 1.0 or < 1.0 can be indicative of emaciation in farmed rainbow trout. 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. This can be the result of various health issues or theoretically lack of access to food. 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. Salmonids may become emaciated for various reasons, including disease (Stephen and Ribble 1995; Kent and Poppe 2002; Finstad et al., 2011; Hjeltnes et al., 2016), stress (Huntingford et al., 2006)and the behavioural environment the fish are exposed to (Adams et al., 2000).

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). Emaciated fish are more susceptible to disease and their tendency to stay in the surface water, which contains more pathogens and sea lice larvae in marine waters (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 of the cage. Such practices bias the sample in the opposite direction and fish with obvious welfare problems must be included in any welfare assessment. It is also necessary to take into account of the welfare of the individual emaciated fish. It can be difficult to catch them, but they should be removed and culled if possible.

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 (Folkedal et al., 2016).

Weakness of indicator

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

3.2.7. Sexual maturity state

Rainbow trout are naturally spring spawners, but maturation and spawning can be advanced or delayed with photoperiod manipulation (Bromage et al., 2001; Davies and Bromage, 2002; Wilkinson et al., 2010; Taylor et al., 2008). Salmonids like rainbow trout may mature both in the freshwater stage (precocious maturation) or after sea transfer (Fleming, 1998; Kause et al., 2003) and it can be a problem in rainbow trout aquaculture (Norberg et al., 2007). Precocious maturation only occurs in males, but early sea maturation predominantly occurs in males which mature earlier than females. In the wild, maturing salmonids in the sea migrate towards the river for spawning, but it is difficult to answer whether mature or maturing farmed rainbow trout also exhibit a behavioural need to undertake a spawning migration (cf. Huntingford et al., 2006). Salmonids start to physiologically adapt to a hypo osmotic environment during the maturation process (Persson et al., 1998; Makino et al., 2007) and maturing trout can experience high mortalities if they begin to mature in the sea cages (Albrektsen and Torrissen, 1988). 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). The reduced immune capacity and ability for osmoregulation, together with behavioural changes may lead to reduced welfare and increased mortality in sexually mature trout.

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.

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.

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).

3.2.8. Seawater adaptation

It has been widely reported that trout grow better in seawater than freshwater and the success of seawater adaptation is influenced by fish size, transfer conditions and the magnitude of change in salinity (Johnston and Cheverie, 1985; Le Bras et al., 2011). EFSA (2008b) state euryhalinity occurs in rainbow trout when the fish are greater than 50g and fish that are transferred at 70-100g have a good survival rate and are apparently able to cope with the transfer to sea outwith a specific smolting window. Fish raised in freshwater containing low Ca^{2+} may have problems adapting to sea water after transfer, but this can be remedied by feeding the fish specialist diets to encourage pre-adaptation to the marine environment (Perry et al., 2006). Even though some of the literature in this area is relatively old, it would indicate survival and performance are better with larger fish. With smaller fish, improvements are seen when there is a gradual introduction or the marine environment is not full strength sea water (Landless, 1976b; Jackson, 1981; Kiilerich et al., 2011). McKay and Gjerde (1985) have also reported that mortalities in fish that are newly transferred to seawater can be higher with higher salinities (32 ‰) and growth can also be reduced at salinities > 20 ‰. Survival can also be lower at higher temperatures, with one study finding better survival at 11 °C, compared with 17 °C, in small fish of 7 to 15 g (Johnsson and Clark, 1988). Wild type migratory rainbow trout undergo smoltification naturally or with photoperiod manipulation. This does not appear to be the case for at least some strains of domesticated rainbow trout. With regard to photoperiod manipulation, a recent paper by Morro et al., (2019) has tested the effects of different photoperiod regimes on rainbow trout seawater adaptation and reported that both the existing, well established constant light (LL) regime (18 weeks) and an Advanced Phase Photoperiod (APP) regime (6 weeks LD 12:12 and a further 12 weeks of LD 24:0) are suitable regimes for seawater adaptation and APP led to a longer adaptation window. However, the authors stated photoperiod does not appear to be a strong driver for seawater adaptation in trout and other potential environmental drivers, such as salinity or temperature should be examined (Morro et al., 2019). Finstad et al., (1988) also showed that low seawater temperature can affect osmoregulation in rainbow trout and care should be taken when transferring rainbow trout to sea in the autumn. Signs of lack of adaptation to the marine environment would be lack of growth and chronic low level mortalities.

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; Deschamps et al., 2008). Currently, one of the major constraints for the commercial production of rainbow trout (*Oncorhynchus mykiss*) is the incidence of skeletal deformities (Babaheydari et al., 2016).

A high incidence of vertebral deformities can significantly reduce the profitability of aquaculture production due to the downgrading of carcasses from "superior" to "ordinary" or even "production" grade in particularly severe cases (Branson and Turnbull, 2008). Vertebral deformities in rainbow trout may become apparent late in production, leading to increased costs associated with sorting (Witten et al., 2006). Other associated financial costs may result from decreased speed and efficiency of processing, reduced yields resulting from extra trimming and further waste associated with "visually undetectable" abnormalities (Boglione et al., 2001; Witten et al., 2006; Deschamps and Sire, 2010).

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 salmonids 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 salmonids, Witten et al., (2009) have developed a 20-type classification system for salmon 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). Currently, as in Atlantic salmon, a cogent system for the classification of vertebral deformities in rainbow trout has not yet been established. Instead, the longitudinal shortening of fish has often been described using the term "vertebral column compression syndrome" (VCCS; Aubin et al., 2005). Within this broad category, the two most commonly observed deformations in rainbow trout have been (a) "cyprinid conformation", due to antero-truncal vertebral fusion (Poynton, 1987), and (b) "short tail", due to trunco-caudal vertebral fusion (Aubin et al., 2005).

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., 2004; Fitzsimmons and Perutz, 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).

Relatively few studies have been conducted that have associated risk factors with vertebral deformities in rainbow trout specifically. However, some of those identified include low exchange recirculating aquaculture systems (RAS; Davidson et al., 2011), triploidy (Madsen et al., 2000), *Flavobacterium psychrophilum* infection (Madsen et al., 2001; Nematollahi et al., 2003), *Myxobolous cerebralis* infection (Baldwin et al., 2000), tryptophan deficiency (Akiyama et al., 1986), phosphorous deficiency (Shearer and Hardy, 1987; Sugiura et al., 2004), and vitamin C deficiency (Kitamura et al., 1965). In a study conducted by Fontagné et al. (2009) around 45% of rainbow trout fry fed a diet with low levels of calcium exhibited kyphosis that was externally discernible. Rainbow trout fry fed either a low calcium or low phosphorous diet also exhibited significantly modified skeletal ontogeny and vertebrae morphology. For rainbow trout broodstock it has been recommended that diets contain 200 IU/g of vitamin A (Fontagné, 2009) and levels of 20 UI/g that are common for commercial diets are not considered to be enough to fulfil the vitamin A requirements for this species and life stage. High vitamin A levels in the diet are beneficial for both early growth and reproduction in rainbow trout and do not result in skeletal deformities (Fontagné, 2009). The early life stages of rainbow trout are more susceptible than later life stages to dietary oxidative stress and an appropriate level of antioxidants, such as vitamins E and C, should be added to their feed in order to protect polyunsaturated fatty acids from lipid peroxidation (Fontagné, 2009). In addition, Fontagné (2009) pointed out the importance of dietary phospholipids for early growth and appropriate skeletal mineralization.

High egg incubation temperatures have previously been linked with a heightened incidence of vertebral deformities in Atlantic salmon (Ørnsrud et al., 2004; Fraser et al., 2015). Sub-optimal temperatures during egg incubation are a known risk factor for skeletal deformities in rainbow trout; however, more research is required in this area. Research shows that the egg incubation temperature for rainbow trout, both diploid and triploid, should be between 8-12 °C to minimize the occurrence of malformations, irrespective of the individual genetic strain and that 10 °C seems to be optimal for this species (Lein et al., 2009). In the same study, where eggs were exposed to three temperatures (6, 10 and 14 °C) the most common skeletal deformities were fused or compressed vertebrae (Lein et al., 2009). As per other salmonid species, vertebral deformities in rainbow trout are likely to be of multifactorial aetiology.

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 to detect minor deformations and when more accurate descriptions of the deformation is needed. The fish are typically radiographed with a portable X-ray apparatus, and from the digital images one can 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 (see Figure 3.2.9-1).

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.

Fig. 3.2.9-1. *Vertebral deformity in large rainbow trout. Photo: James F. Turnbull*

3.2.10. Fin damage and fin status

The fins of rainbow trout (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 risks also differ with life stage. Although biting plays an important role in fin damage, it does not appear to necessarily be simple aggression but biting for a variety of reasons. Many conditions can lead fish to start biting including higher stocking densities (Ellis et al., 2002 and references therein) and inequitable access to food or underfeeding (Moutou et al., 1998). Abrasion with the substrate or tank wall can also lead to damage to the pectoral and pelvic fins in trout (e.g. Bosakowski and Wagner, 1995). Ellis et al., (2002) also cover a number of water quality parameters and other factors that can also affect fin damage in trout.

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 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. There are many potential causes of damage including parasites, self-inflicted damage due to burrowing into the net, predators and faulty handling equipment. Any sign of superficial lesions should be thoroughly investigated.

Any superficial wound will rapidly become colonised with bacteria from the local environment, including *Vibrio* spp. in the marine environment and *Aeromonas* spp. in freshwater. The rapid colonisation of superficial lesions can make identification of the primary cause difficult. Bacteria may exacerbate an existing wound e.g. winter ulcer (Løvoll et al. 2009) or can initiate a lesion e.g. *Aeromonas salmonicida* and *Flavobacterium psychrophilum* (Bruno et al., 2013). 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 salmonids 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; a previous version of the RSPCA welfare standards for farmed rainbow trout (2014) state a sample of 100 fish should be taken during slaughter and if 10% have some damage then action should be taken. Handling trauma can also impact upon external (and internal) morphological indicators. For example, crush injuries from netting or fish being accidentally trapped in a pump valve can be diagnosed by the pattern of damage to the epidermis and underlying tissue.

3.2.12. Eye damage and eye status

Eyes can be damaged in numerous ways (Figure 3.2.12-1), with various aetiologies (Table 3.2.12-2) 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 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 the 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.

Fig. 3.2.12-1. *Exophthalmus in a young rainbow trout. Photo: James F. Turnbull*

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; Pettem et al., 2018). 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, rapid growth and a 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). Remø et al., (2017) compared cataractogenesis in both rainbow trout and Atlantic salmon raised at two temperatures (13 ^{o}C and 19^{o}C) in seawater. Cataract prevalence at the end of the study was nearly 100% in Atlantic salmon and ca. 50% in trout, regardless of water temperature. Cataract severity was also three times greater in salmon compared to trout (Remø et al., 2017). Metabolomics profiling showed differences in the metabolism and composition of the lens between the two species, potentially explaining the observed differences (Remø et al., 2017).

Table 3.2.12-2. *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. 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 or osmoregulatory disorders Trauma Gas bubble disease	Depending on aetiology, but always a sign that welfare is at risk. Risk of loss of sight and further damage	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. Loss of vision	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*.	Depends on severity and extent.	Avoid trauma, control parasites or infections.	Pettersen et al., 2014 Hjeltnes et al., 2016
Injuries-Irritants	Water quality Chemical Thermal Toxic UV-light	Pain and reduced sight	Depends on the cause, amongst others, overdosing of medicines	Hofer and Gatumu, 1994 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 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 deformities occurring after first feeding are more affected by culture conditions than genetic factors (Sadler et al., 2001). Ascorbic acid deficiencies can lead to shortened opercula in rainbow trout (Halver et al., 1969) and a diet that is deficient in phosphorus can lead to abnormally soft opercula in rainbow trout (Deschamps et al., 2016). 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 shortened opercula (Fig. 3.2.13-1) expose 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. Opercular erosion has been previously used as an OWI in rainbow trout (Noble et al., 2012c).

Fig. 3.2.13-1. *A rainbow trout with a shortened operculum. Photo: Chris Noble*

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 13 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) active fin damage, xiii) healed fin damage.

We have used pictures from the FISHWELL salmon handbook (Noble et al., 2018) in the following scoring system, as the conditions they describe are equally applicable to rainbow trout.

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 rainbow trout, 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 problem 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)*

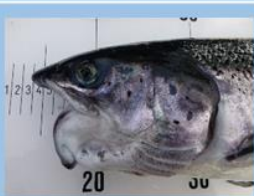
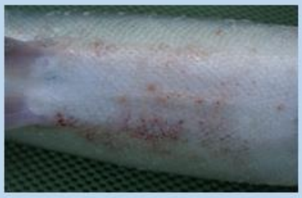
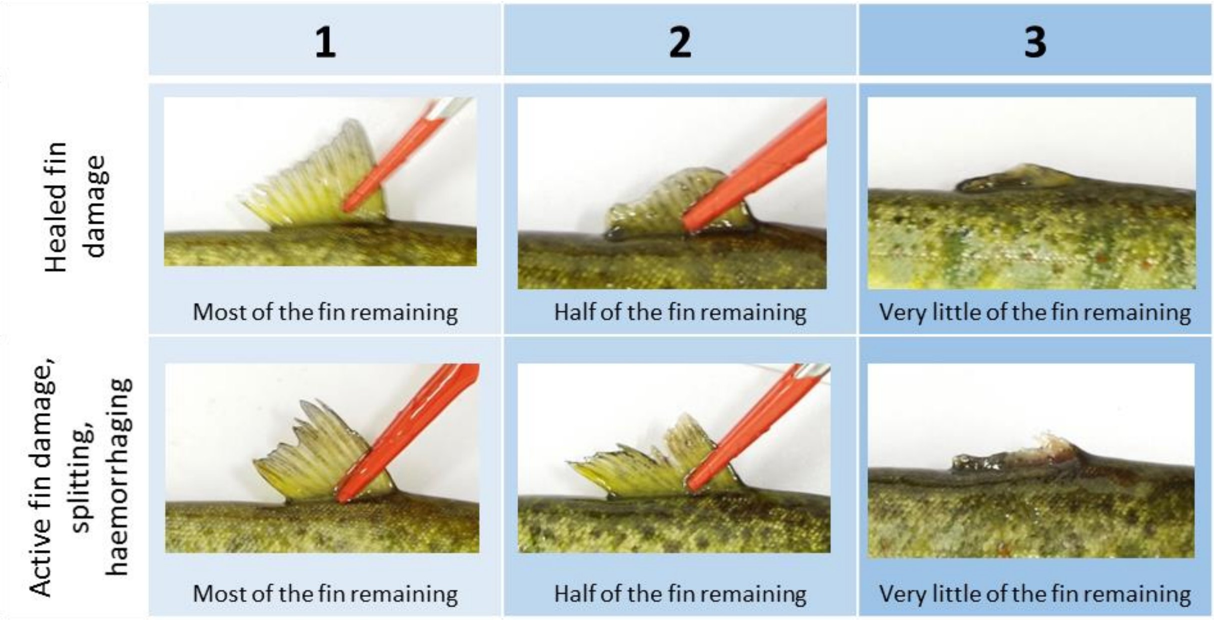
	1	2	3
Eye haemorrhage	 Minor haemorrhages	 Larger haemorrhages, or traumatic injury	 Large haemorrhages / traumatic injury. Eye may be ruptured
Exophthalmia	 Eye protruding a little	 Moderate eye protrusion	 Major eye protrusion
Opercular damage	 Operculum only partly covering gills	 Operculum absent 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)

[1] *For fingerlings "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)*



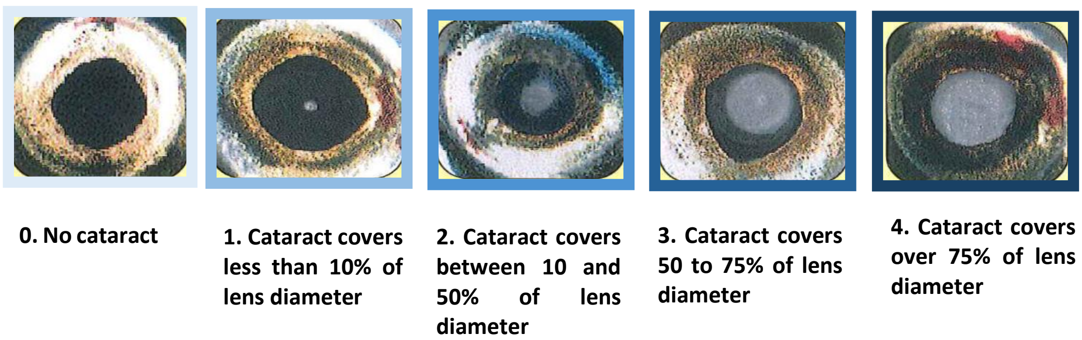


Fig. 3.2.13-3. *Morphological scheme for classifying eye cataracts in salmonids. Text reproduced from "Wall, T. & Bjerkås, E. 1999. A simplified method of scoring cataracts in fish. Bulletin of the European Association of Fish Pathologists 19(4), 162-165. Copyright, 1999" with permission from the European 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 trout 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. Blaufuss et al., 2019). 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). Rainbow trout suffer from a condition known as Rainbow Trout Gastro Enteritis, which behaves like an infectious condition and is most prevalent in high intensity production systems (Del-Pozo et al., 2010). Many diseases can affect the other abdominal organs causing a variety of gross appearances. Observations of any internal abnormality in more than one individual should be followed up by a thorough diagnostic investigation.

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 et al., 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). There is currently no obligation to vaccinate rainbow trout.

In Norway, the majority of Atlantic salmon and rainbow trout are vaccinated by injecting oil-based multivalent vaccines intraperitoneally (Brudeseth et al., 2013). 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; Raida and Buchmann, 2008), 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). NB: some of the above references are for Atlantic salmon, but the impacts can be applicable to rainbow trout.

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 fish 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 3.2.15-1 and Fig. 3.2.15-2). 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 Villumsen et al., (2015) for rainbow trout and Poppe and Breck (1997) for Atlantic salmon. In addition to the visible changes in the fish's abdominal cavity the side effects of vaccination in rainbow trout can include: reduced appetite (Rønsholdt and McLean, 1999; Vendrell et al., 1999), reduced growth (Rønsholdt and McLean, 1999) and vertebral deformities (Ellis et al., 1997). To minimise the potential side effects of vaccination it is important to monitor the side effects, work on the continuous improvement of vaccine formulation, search for alternative adjuvants (Villumsen et al., 2017) and the optimisation of vaccination routines.

Sampling and analytical considerations

The degree of vaccine side effects in individual fish are 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 Atlantic salmon aquaculture industry and is reproduced in Fig. 3.2.15-2 with kind permission from Lars Speilberg. The Speilberg scale has also been used in rainbow trout (Holten-Andersen et al., 2012; Chettri et al., 2015) 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

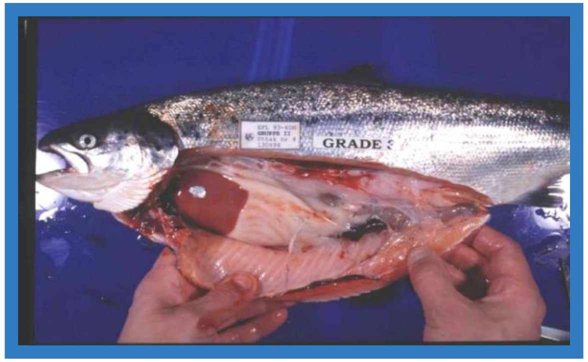
Fish needs to be sacrificed. It can be subjective (rather than objective) and requires adequate training to be reliable or comparable between sites. Different vaccine types may vary in efficacy and side effects, but the same vaccine may also vary in effects and side-effects (Poppe and Breck, 1997).

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. Scale originally developed for Atlantic salmon but has also been used in studies on rainbow trout (e.g. Holten-Andersen et al., 2012; Chettri et al., 2015).*

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. Although the pictures are from Atlantic salmon, they are equally applicable to rainbow trout. 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, "*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 even in the absence of stressful events (Bry, 1982). Therefore, a single cortisol measurement provides little if any information about fish welfare unless linked to other information.

Studies 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 (Bury et al., 2007; Choi et al., 2015; Khansari et al., 2019; Merkin et al., 2010; Taylor et al., 2007).

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-invasive 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. Salmonids such as rainbow trout are anadromous species that switch between hypo- and hyper-osmotic environments during their migration from fresh to seawater and back (McCormick, 2013). Table 3.2.17-1 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). Taylor et al., (2007) reported that typically osmolality for rainbow trout in freshwater was approximately 320 mOsm kg^{-1} in both diploids and triploids, while osmolality ranged from 320 to 370 mOsm kg^{-1} when exposed to seawater (which is below the 420 mOsm kg^{-1} lethal limit in rainbow trout, Alexis et al., 1984). Liebert and Schreck (2006) stated osmolality in trout held in freshwater was 250-280 mOsm kg^{-1} and 300-350 mOsm kg^{-1} in seawater. Rainbow trout can use up to 1 – 2 weeks to return to normal osmolality again (320 – 340 mOsm kg^{-1}) during the transition from fresh- to seawater (Liebert and Schreck, 2006; Taylor et al., 2006, 2007). Finstad et al., (1988) also showed that low seawater temperature can affect osmoregulation in rainbow trout and care should be taken when transferring rainbow trout to sea in the autumn.

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 or osmolarity to the closest mOsm kg^{-1} mOsm L^{-1} respectively. 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 rainbow trout, from a juvenile living in freshwater to a fish adapted to living in seawater includes various morphological, physiological, biochemical and behavioural changes (e.g. Morro et al., 2019).

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 rainbow trout can vary between ca. 140-155 mmol L^{-1} and chloride levels vary between 111-135 mmol L^{-1} (Liebert and Schreck, 2006). In seawater, ion levels increase slightly and vary from ca. 150-160 mmol L^{-1} (Na^+) to 130-140 mmol L^{-1} (Cl^-) (Liebert and Schreck, 2006). Rainbow trout also seem to take longer (up to 1-2 weeks) to stabilise ionic composition within the normal range during the transition from fresh- to seawater (Liebert & Schreck, 2006).

Most 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+}, Ca^{2+} and SO_4^{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 blood plasma magnesium concentrations do not exceed 2 mM in most cases, and in rainbow trout are normally less than 1 mM, regardless of the salinity (Liebert and Schreck, 2006). Changes in magnesium balance are a good indicator of acute stress (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; Stewart et al., 2016).

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). Similar results have been found in rainbow trout (Olsen et al., 2005). Pre-stress levels of the plasma glucose can be higher in fed (5.5-6 mmol L^{-1}) than fasted (1.5-2 mmol L^{-1}) rainbow trout in some studies (Farbridge and Leatherland, 1992) but not in others (Olsen et al., 2005). Rainbow trout fed a diet high in carbohydrates had higher plasma glucose levels (11 mmol L^{-1}) than trout fed a low carbohydrate diet (3 mmol L^{-1}), while glucose level was less affected by diet composition in salmon (Krogdahl et al., 2004). Plasma glucose levels can increase to 150 mg/100ml during exposure to a stressor (Pottinger and Carrick, 1999). It can also vary between 50-150 mg/100ml when trout are fasted from 3-9 days (Bermejo-Poza et al., 2017). 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. *Examples of plasma glucose levels in rainbow trout after various feeding regimes and before and after various stress treatments. Most glucose values are estimated from graphs, and some values are converted from other units.*

Stage	Feeding status	Treatment	Glucose (mmol L^{-1})	Reference
130 g	Fed	Pre-stress	5.8	Farbridge & Leatherland, 1992
130 g	Fasted	Pre-stress	1.7	Farbridge & Leatherland, 1992
Freshwater, 570 g	Fed low carb	Pre-stress	5	Krogdahl et al., 2004
Freshwater, 570 g	Fed high carb	Pre-stress	11	Krogdahl et al., 2004
100 g		Disturbance * 3	5.6	Barton & Schreck, 1987
Freshwater, 360 g	Fed	Crowding and chasing for 15 min	0.8	Olsen et al., 2005
Freshwater, 360 g	Fasted	Crowding and chasing for 15 min	0.6	Olsen et al., 2005
130 g	Fed	5 min chasing	7.2	Farbridge & Leatherland, 1992
130 g	Fasted	5 min chasing	4.4	Farbridge & Leatherland, 1992
Freshwater, 400 g	Fed	3 h confinement in 50 L tank	8.9	Pottinger & Carrick, 1999

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. However, lactate can also be produced under aerobic conditions (e.g. Brooks, 2018). 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 (Liebert and Schreck, 2006). The peak of plasma lactate during potential stressors such as seawater transfer, handling and fasting ranges from ca. 2-20 mmol L^{-1} (Olsen et al., 2005; Liebert and Schreck, 2006; López-Luna et al., 2013; Shabani et al., 2016), 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. *Some examples of plasma lactate levels in rainbow trout after various feeding regimes and before and after various stress treatments. Most lactate values are estimated from graphs, and some values are converted from other units.*

Stage	Feeding status	Treatment	Plasma lactate (mmol L^{-1})	Reference
Freshwater, 150-350 g	Fasted	Pre-stress	0.83	Milligan & Girard 1993
Freshwater, 360 g	Fed	Pre-stress	0.3	Olsen et al. 2005
Freshwater, 360 g	Fasted	Pre-stress	0.3	Olsen et al. 2005
100 g	Fed	Pre-stress	2.4	Barton and Schreck 1987
Freshwater, 360 g	Fed	Crowding and chasing for 15 min	1.8	Olsen et al. 2005
Freshwater, 360 g	Fasted	Crowding and chasing for 15 min	2.6	Olsen et al. 2005
Freshwater, 150-350 g	Fasted	5 min chasing	16.5	Milligan and Girard, 1993
Freshwater, 150-350 g	Fasted	Pre-stress	0.83	Milligan and Girard, 1993
Freshwater, 93 ± 7 g (mean ± SEM)	Fasted	Pre-stress	ca. 1.7-1.9	López-Patiño et al., 2014
Freshwater, 93 ± 7 g (mean ± SEM)	Fasted	15-45 minutes post 5 min handling stress	ca. 3.5-4.5	López-Patiño et al., 2014
Freshwater, 332 ± 34 g (mean ± SEM)	Fasted	3-9 days fasted	ca. 1.8-3.6	Bermejo-Poza et al., 2017
Freshwater, 215.0 ± 22.6 g (mean ± SEM)	Fasted	Fasting prior to slaughter	13-20	López-Luna et al., 2013
Seawater, ca. 60 g	Fed	Newly transferred to seawater (25 ‰)	ca. 5.5-9.0	Liebert and Schreck, 2006
Seawater, ca. 400 – 1000 g	Fasted	Resting ca. 7% U_{crit}	0.62	Thorarensen et al., 1996
Seawater, ca. 400 – 1000 g	Fasted for >24h	Critical swimming speed ca. 98% U_{crit}	1.95	Thorarensen et al., 1996

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.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 due to 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 rainbow trout and A. 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 salmonids will therefore be initiated from an already acidic muscle state and will progress faster in stressed rather than in unstressed salmonids (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 start 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.

3.2.22. 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 (Fast et al., 2002b; Castro and Tafalla, 2015). 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).

Fast et al., (2002b) reported that rainbow trout had a significantly thicker epidermis and higher mucous cell density than coho salmon and Atlantic salmon. Mucus viscosity can also be significantly higher in seawater than freshwater (Roberts and Powell, 2005). However, the size and density of mucous cells can 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 rainbow trout infected with sea lice (*Lepeophtheirus salmonis*) showed increased lysozyme activity (Fast et al., 2002b). Infestations with *Caligus rogercresseyi* (a sea lice affecting salmonid farming in Chile, González and Carvajal 2003) increases the quantity of mucus producing cells in the epidermis and gills in rainbow trout (Rojas et al., 2018). Another ectoparasite, *Neoparamoeba perurans*, that causes amoebic gill disease (AGD) has been shown to initiate a local gill response in rainbow trout (Roberts and Powell, 2005) but does not instigate a whole body response.

With regard to husbandry practices, routines such as feed withdrawal can affect the mucus layer and its composition in rainbow trout (Heming and Paleczny, 1987). In addition, nutritional components have been shown to alter mucosal parameters (e.g. Hoseinifar et al., 2015; Shakoori et al., 2019). Stressors such as transport can also increase epidermal mucus production and inhibit microbial gene expression in trout (Tacchi et al., 2015).

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 (De Mercado et al., 2018; 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 (De Mercado et al., 2018; 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) and another recent study (De Mercado et al., 2018) reported that cortisol, lactate and oxidative stress markers can be quantified from rainbow trout mucus.

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. With regard to rainbow trout, a review addressing the effects of water quality upon fish welfare (MacIntyre et al., 2008) stated that we are currently lacking robust scientific data on what water quality parameter levels are appropriate in operational farm situations and "*Water quality limits could be introduced for some parameters, but these would have to be ranges rather than single limits, and standardised protocols for measurement would need to be developed.*" 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 rainbow trout 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				×		×		×	×	×				
	Surface access					×	×		×		×	×			

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. However, literature from the 1970s, 1980s and more recent studies (e.g. Kluger et al., 1987; Boltaña et al., 2013, Rey et al., 2015) suggest that fish have the capacity and in some circumstances the need to control their temperature through selecting warmer or cooler water. Behavioural thermoregulations have also been demonstrated in salmonids (Oppedal et al., 2011a). 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 trout varies with different life stages and trout can adapt to temperatures between 0 and 22 °C (Ihssen, 1986). Kwain and McCauley, (1978) reported that the thermal preferences of rainbow trout decrease with age. FAO (http://www.fao.org/fishery/culturedspecies/Oncorhynchus_mykiss/en) recommend the preferred range of temperature 9-21 °C for rainbow trout culture and Jobling (1994) recommends 16-18 °C for optimal growth. Other authors suggest optimum temperature for growth is 13-19 °C under normoxic conditions, with fish expressing a preference for 16 °C (Schurmann et al., 1991), which is also the temperature interval preferred by rainbow trout from fingerlings to the adult stage (Coutant, 1977). Alanärä (1996) also reported that trout exhibit peak appetite at 15-16 °C. Trout can tolerate a rapid increase in temperature from 14°C to 19°C, while a corresponding drop from 14°C to 9°C increases plasma cortisol levels (Wagner et al., 1997). Kiessling et al., (2007) also state that the rapid chilling of rainbow trout to 0.5°C can cause a severe stress response; the stomach fills with water, leading to higher plasma osmolality. EFSA (2008b) state that due to differences in prior acclimation, the speed of temperature change, fish strain etc., it is not possible to provide clear thresholds for the effects of rapid changes in temperature on stress. However, we cover the potential effects of rapid, short-term increases in temperature upon fish welfare in relation to the warm water treatment of lice at the end of this section.

Eggs: Rainbow trout are naturally spring spawners and can tolerate slightly higher water temperatures than salmon. Eggs can be produced at < 15 °C and higher temperatures increase the risk of tissue damage and developmental disorders (EFSA, 2008b and references therein). The lower temperature range is somewhat unclear, but EFSA, (2008b) suggest a temperature as low as 0 °C is not detrimental to eggs. The RSPCA welfare standards for farmed rainbow trout (RSPCA, 2018b) recommend 1-10 °C for ova or alevins. Poppe et al., (2007) also state the optimal temperature for rainbow trout egg production is 10 °C, within a tolerance range 8-12 °C.

Fry and fingerlings: have a preferred optimal temperature range of 7-13 °C (Woynarovich et al., 2011) and the RSPCA welfare standards for farmed rainbow trout (RSPCA, 2018b) recommend 1-12 °C for fry.

Ongrowers: have a preferred temperature of around 16 °C within a range of 13-19 °C under normoxic conditions (Schurmann et al., 1991) although this preference and range varies under hypoxic conditions. Temperatures higher than 19 °C in marine or brackish waters can potentially lead to high mortalities (EFSA, 2008b). Sutterlin and Stevens (1992) reported that cage held rainbow trout with a mean weight of ca. 1.9kg had a temperature preference for ca. 13 °C within a range of 7-17 °C when held in stratified waters. The RSPCA welfare standards for farmed rainbow trout (RSPCA, 2018b) recommend 1-16 °C for ongrowers.

Warm water treatment: Bathing treatments that utilise warm water (29-34 °C) can be used for delicing trout. 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 rainbow trout at different life stages.*

	Range (°C)			References
Eggs	9	-	14	Roberts and Sheperd, 1974
	0	-	16	Jonsson and Finstad, 1995
		<	15	EFSA, 2008b
	8	-	12	Poppe et al., 2007
	1	-	10	RSPCA, 2018b
Fry/fingerlings	7	-	13	Woynarovich et al., 2011
	1	-	12	RSPCA, 2018b
Ongrowers	13	-	16	Schurmann et al., 1991
	7	-	17	Sutterlin and Stevens, 1992
	7	-	20	Woynarovich et al., 2011
	16	-	18	Jobling, 1994
		<	19	EFSA, 2008b
	1	-	16	RSPCA, 2018b

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 trout. It also affects other WIs like oxygen, diseases and parasites.

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

Salmonids are osmoregulators and maintain relatively constant blood ion levels at around 250-300 mOsm, or ~10 ppt (McCormick et al., 1989). Young trout are raised in freshwater, are hyperosmotic and have an active uptake of ions and excretion of water, while those moved to the sea for further ongrowing are hypo-osmotic and have to drink water and excrete ions. EFSA (2008b) state euryhalinity occurs in rainbow trout when the fish are greater than 50g and fish that are transferred at 70-100g have a good survival rate and are apparently able to cope with the transfer to sea outwith a specific smolting window. Fish raised in freshwater containing low Ca^{2+} may have problems adapting to sea water after transfer, but this can be remedied by feeding the fish specialist diets to encourage pre-adaptation to the marine environment (Perry et al., 2006). The literature in this area is relatively old, however, it would indicate survival and performance are better with larger fish. With smaller fish, improvements are seen when there is a gradual introduction or the marine environment is not full strength sea water (Landless, 1976b; Jackson, 1981; Kiilerich et al., 2011). McKay and Gjerde (1985) have also reported that mortalities in fish that are newly transferred to seawater can be higher with higher salinities (32 ‰) and growth can also be reduced at salinities > 20 ‰. Survival would also seem to be lower at higher temperatures, with one study finding better survival at 11 °C, compared with 17 °C, in small fish of 7 to 15 g (Johnsson and Clark, 1988). Wild type migratory rainbow trout undergo seawater adaptation naturally or with photoperiod manipulation. This does not appear to be the case for at least some strains of domesticated rainbow trout. Finstad et al., (1988) also showed that low seawater temperature can affect osmoregulation in rainbow trout and care should be taken when transferring rainbow trout to sea in the autumn. Signs of lack of adaptation to the marine environment would be lack of growth and chronic low level mortalities. Salinity also has an impact on broodstock survival with e.g. 100% mortalities in male broodstock reared in seawater (Albrektsen and Torrissen, 1988). The authors suggest brackish water (10-17 ‰) was best for survival of both broodstock and eggs (Albrektsen and Torrissen, 1988).

Sampling and analytical considerations

Although it appears that salinity has no significant effect upon the welfare of large trout, access to brackish water may be of benefit when transferring smaller fish and also for broodstock (Albrektsen and Torrissen, 1988). Fish infected with AGD and sea lice may also benefit from access to a layer of brackish water (Oldham et al., 2016, Atlantic salmon). 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., 2016). 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.

As far as the authors are aware, detailed data of the oxygen concentrations at which DO_{maxFI} are maintained, as described for Atlantic salmon by Remen et al., (2016) are not available for rainbow trout. However, Magnoni et al., (2018), Glencross (2009) and Pedersen (1987) have reported less detailed data on the effects of oxygen levels on appetite in trout (see below).

Shi et al., (2018) have reported the lowest oxygen saturation at which aerobic metabolism can be maintained (LOS) levels for a range of fasted diploid and triploid trout sizes ca. 15-130g and temperatures 13-25 °C (see Table 4.1.3.1 for further details). However they also stated that tolerance for low oxygen levels can be affected by feeding and that their data on oxygen tolerance of fasted trout may be lower than LOS data on fed fish as shown when they compared their LOS data on A. salmon with that of Remen et al., (2016).

Table 4.1.3-1. *The limiting oxygen saturation (LOS) for fasted diploid and triploid rainbow trout of ca. 15-130 g (DO levels in mg L^{-1}). Reprinted with permission from Springer Nature: Shi, K., Dong, S., Zhou, Y., Gao, Q., Li, L., Zhang, M. & Sun, D. (2018) Comparative Evaluation of Toleration to Heating and Hypoxia of Three Kinds of Salmonids. Journal of Ocean University of China 17(6), 1465-1472. [14] Copyright 2018.*

	LOS: diploid				LOS: triploid			
	Fish size				Fish size			
Temperature	16 g	40 g	79 g	131 g	16 g	39 g	79 g	130 g
13	4.7	4.4	4.2	3.2	4.1	3.9	3.6	3.1
17	5.0	5.1	4.9	3.8	4.3	4.2	4.0	3.4
21	5.4	5.3	5.2	4.5	4.8	4.7	4.2	3.6
25	5.9	5.6	5.3	4.8	5.0	4.8	4.5	4.0

Other data sources for minimum oxygen levels for the growth of rainbow trout vary a lot between 4 and 9 mg L^{-1} depending on the study (Davis, 1975; Pedersen et al., 1987; Ellis et al., 2002 and references therein). RSPCA (2018b) recommend > 70% saturation at a maximum temperature of 12 °C for fry at a maximum of 16 °C for ongrowers. Interesting, 70% saturation at 16 °C are very similar to the DO_{maxFI} for A. salmon at a similar temperature (Remen et al., 2016). EFSA, (2008b) recommend oxygen levels of the outflow water should be > 5 mg L^{-1}. Other work by Pedersen, (1987) on 100g fish at 15 °C reported that the critical oxygen level for food consumption was 6 mg L^{-1} and for feeding efficiency and growth rate it was 7 mg L^{-1}, corresponding to ~60% and ~70% of air saturation, respectively.

Exposing rainbow trout to supersaturation (130%) can lead to lower haematocrit and total red blood cell concentrations but does not affect feed conversion or growth in comparison to trout held at 100% or 65% saturations (Caldwell and Hinshaw, 1994). However, a supersaturation level of 150% did lead to greater mortalities in a *Yersinia ruckeri* challenge compared to fish exposed to 100% and 70% DO saturations (Caldwell and Hinshaw, 1995).

Eggs: Their oxygen requirements of trout 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 for salmonids (Crisp, 1996). It has previously been reported that rainbow trout egg survival is 100% when oxygen levels were 7.1 – 7.8 mg L^{-1} and water velocity past the eggs was > 95 cm h^{-1} (Crisp, 1996 and references therein). Oxygen levels that are too low during incubation can lead to premature hatching (Latham and Just, 1989) 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. RSPCA (2018b) recommend > 90% saturation in exit water for ova and alevins.

Fry and fingerlings: Detailed data of the LOS in fingerlings at different temperatures are reported in Table 4.1.3-1 (see columns on ca. 15 g and 40 g fish for both fasted diploid and triploid trout). As far as the authors are aware, oxygen concentrations where appetite is maintained at different temperatures is not available but experience does not suggest dramatically different oxygen requirements compared with that of ongrowers (see below). For example, Poulsen et al., (2011) reported that rainbow trout fingerlings (ca. 12 g) held at 17-19 °C spent significantly less time in water with DO saturations ≤ 80% when given the choice to spent time in 100% DO saturated water. Fish also significantly increased their swimming speed when in waters with DO levels of ≤ 40% and reduced the number of trips to waters with DO saturations of 30% (Poulsen et al., 2011). RSPCA welfare standards for farmed trout (RSPCA, 2018b) recommend > 70% saturation at maximum 12 °C for fry and fingerlings.

Ongrowers: The lowest oxygen saturation at which aerobic metabolism can be maintained (LOS) for fasted rainbow trout ongrowers at different temperatures are given in Table 4.1.3-1. Magnoni et al., (2018) have also reported that reducing DO levels from 7.9 to 4.5 mg L^{-1} in ca. 115 g trout held at 14 °C significantly reduced feed intake and growth rate. Glencross (2009) has also reported that appetite and growth rate was more than halved in ca. 55 g rainbow trout at 42% DO saturation compared to trout held at 87% saturation. Less detailed data for 100g rainbow trout at 15 °C (Pedersen, 1987) reported that the critical oxygen level for food consumption was 6 mg L^{-1} and for feeding efficiency and growth rate it was 7 mg L^{-1}, corresponding to ~60% and ~70% of air saturation, respectively. For comparison, 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 Atlantic salmon post-smolts at different temperatures are given in Table 4.1.3-2 for reference purposes. RSPCA welfare standards for farmed trout (RSPCA, 2018b) recommend > 70% saturation at maximum 16 °C for ongrowers.

Table 4.1.3-2. *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., 2016.*

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.

There is currently some debate about the value of measuring dissolved gasses by their partial pressure rather than mg L^{-1} or saturation, however, since the normal practice on farms is to measure in mg L^{-1} or saturation, we will not cover the debate here. This may be included in future editions.

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). CO_2 concentrations in aquaculture production facilities are far higher than those experienced by fish in the wild at present or even the levels predicted by the most pessimistic climate change models (Ellis et al., 2017). 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). Fish acclimate to elevated plasma CO_2 levels by increasing their plasma bicarbonate concentration, which leads to a reduced concentration of plasma chloride (Fivelstad, 2013). Levels of CO_2 also influence other water quality parameters. Increasing CO_2 levels results in reduced pH which can increase the toxicity of aluminium. Although in aquaculture CO_2 is often referred to in mg L^{-1} there are some reservations regarding the use of these units, which relate to partial pressure in a non-linear manner, affected by temperature and salinity (Ellis et al. 2017).

Response to CO_2 is highly variable with distinct intraspecific differences (Tucker et al., 2019) even within genetically identical stocks (Sadoul et al., 2017). However, the literature is limited for rainbow trout and we have provided additional extrapolated data from other salmonids, mostly Atlantic salmon where the majority of the work has been conducted.

With regard to rainbow trout, earlier work on trout weighing ca. 260 g by Danley et al., (2005) stated CO_2 levels of ~34 mg L^{-1} and ~49 mg L^{-1} had a significant detrimental effect upon growth and plasma chloride levels after 12 weeks of chronic exposure in comparison to fish held at CO_2 levels of ~22 mg L^{-1}. However, elevated CO_2 levels did not affect mortality (Danley et al., 2005). Other work carried out by Good et al., (2010) on rainbow trout held in RAS tanks from ca. 60 g to market size reported CO_2

levels of ~8 mg L^{-1} or ~24 mg L^{-1} for 6 months had no significant impacts upon growth or mortality. Nephrocalcinosis was also not observed in any sampled fish at either CO_2 level (Good et al., 2010). Hafs et al., (2012) reported that CO_2 levels ~49 mg L^{-1} resulted in lower growth in ongrowers (300-500g starting weight) in comparison to fish reared at ~30 mg L^{-1} and recommended CO_2 levels should be < 30 mg L^{-1} for rainbow trout. With regard to other recommendations for rainbow trout, RSPCA (2018b) recommend < 10 mg L^{-1} for ova, alevins and ongrowers.

With regard to Atlantic salmon, long-term exposure (weeks and months) to elevated CO_2 levels can have a negative effect on growth in Atlantic salmon parr (Fivelstad et al., 2007; Hosfeld et al., 2008). Atlantic 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}. Long-term exposure (weeks and months) to elevated CO_2 levels can also have a negative effect on growth in Atlantic salmon post-smolts (Fivelstad et al., 1998). For Atlantic 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.

Recommended maximum levels of CO_2 for rainbow trout:

- **< 10 mg L^{-1} (Wedemeyer, 1996; RSPCA, 2018b).**
- **< 30 mg L^{-1} (Hafs et al., 2012).**
- **Good et al., (2010) reported no differences in growth or survival between trout raised at CO_2 levels of ~8 mg L^{-1} or ~24 mg L^{-1} for 6 months and state "*Engineers designing WRAS can set water pumping rates to control CO_2 accumulation at 24 mg/L, which could reduce fixed and variable costs and improve a facility's profitability (compared to operating at 10 mg/L CO_2) without compromising overall fish performance.*"**

However, the adverse effects of carbon dioxide are dependent on other factors such as Dissolved Oxygen, pH and alkalinity (Noble and Summerfelt, 1996) and general safe levels are therefore difficult to define.

Sampling and analytical considerations

CO_2 should be measured on a regular basis during the freshwater phase or during land based production of rainbow trout 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, correlated with water hardness (dissolved calcium concentration). Acid water can have a wide range of negative effects on rainbow trout. These include: loss of calcium from the gills (Ye et al., 1991); ammonia excretion and toxicity (Wright and Wood, 1985; Randall and Wright 1989); blood acidosis (McDonald et al., 1980); carbon dioxide and oxygen transportation (Randall, 1991). It is also associated with increased problems with aluminium toxicity, although the relationship between aluminium toxicity and pH is complex (e.g. Havas and Rosseland, 1995).

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 freshwater stages (Henriksen et al., 1984). EFSA (2008b and references therein) state a pH of less than 4 can lead to significant mortalities in rainbow trout and a pH between 4.5 and 5.5 induces sublethal effects. Waters with low pH can decrease the swimming ability of rainbow trout (Ye and Randall, 1991). Stefansson et al., (2007) state trout only experience osmoregulatory problems when pH is less than 4.6.

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 gas pressure (TGP), oxygen and nitrogen supersaturation

Total gas pressure (TGP) is equal to the partial pressures of dissolved gases and the vapour pressure of water at a given temperature. It has been recommended that TGP is presented as the difference between TGP and atmospheric pressure (ΔP) (APHA/AWWA/WEF, 1992). However, the most common way of reporting TGP is as a percent of local atmospheric pressure (TGP%), according to Rogers (2005). In situations when the partial pressure of one or more gases dissolved in water exceeds their respective partial pressure in the atmosphere, supersaturation occurs (Shrimpton et al., 1990; Hjeltnes et al., 2012). Supersaturation can occur in lakes and rivers as a natural phenomenon, or due to heating of the water, photosynthesis, or it may have anthropological origins and be caused by e.g. thermal effluents and turbines in hydroelectric dams (Gültepe et al., 2011; Skov et al., 2013). Supersaturation can also happen in aquaculture systems due to sudden changes in barometric pressure, increased temperature or the excessive addition of oxygen (Colt, 1986; Hjeltnes et al., 2012).

Exposure to high TGP levels are considered to be a welfare risk for trout (RSPCA, 2018b). High TGP levels and nitrogen supersaturation have also been implicated in gas bubble disease (GBD) caused by the formation of gas bubbles in the vascular system leading to a number of pathologies and physiological changes (e.g. Weitkamp and Katz, 1980). Other work has also implicated oxygen supersaturation in GBD (Edsall and Smith, 1991; Machova et al., 2017).

The symptoms of GBD include i) haemorrhaging to the eye and the area around the eye and the base of the fins, ii) exophthalmia, iii) accumulation of gas bubbles in the lateral line (which is regarded as one of the first clinical sign of GBD in salmonids), iv) an increase in swim bladder pressure that can lead to rupture of the bladder, v) the formation of bubbles in the cardiovascular system, blocking blood flow and ultimately leading to mortality, vi) bubble formation in the gills or buccal cavity leading to blockage of water flow and death by asphyxiation, vii) subdermal emphysema along the surface of the body, and viii) reduced growth (reviewed in Gültepe et al., 2011 and Skov et al., 2013).

With regard to the effects of oxygen supersaturation on GBD, Machova et al., (2017) reported a case study where gas bubble disease was related to an oxygen supersaturation of up to 136% that led to rainbow trout mortalities. Exposing rainbow trout to oxygen pressures of 200% and 120% TGP while nitrogen pressure was kept at ca. 100% also led to GBD within 4 days of exposure and mortalities of 50% within 20 days (Edsall and Smith, 1991).

With regard to the effects of TGP on GBD, a study by Gültepe et al., (2011) reported that 200g rainbow trout exposed to 115% TGP compared to 104% TGP showed signs of GBD e.g. a darkened epidermis, exophthalmia, eye haemorrhaging, gas bubbles upon the operculum, significantly elevated i) partial pressures of O_2, ii) partial pressure of CO_2, iii) carboxyhaemoglobin levels, and iv) bicarbonate ion concentrations, increased swimming activity, panic episodes and reduced carbonic anhydrase enzyme activities in the eye lens. According to the Norwegian Food Safety Authority, TGP should not be higher than 100%.

With regard to nitrogen saturation 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%. Skov and colleagues (2013) looked at the effect of N_2 supersaturation on juvenile rainbow trout, both alone and in combination with increased TGP. They found that an exposure of up to 103% TGP in combination with nitrogen saturation between 104.5 and 107.6% negatively affected energy uptake and energy expenditure. However, N_2 supersaturation alone (102.4 - 105.2%) without TGP supersaturation (TGP ca. 100%) did not have the same effects. The effects observed at 103% TGP and supersaturated N2 were reversible within 25 days after end of exposure.

It is therefore important to monitor TGP, oxygen and nitrogen saturation and the relationships between these parameters, as these can have negative effects on trout's welfare. Since there is little data and a lot of uncertainty about s trout's tolerance to TGP, oxygen and nitrogen supersaturation, we recommend using the above values as guidelines and not as absolute limits.

4.1.7.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 body fluids of the fish, 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). In sea water the permeability of the gill epithelium for ammonium ions is significant, therefore NH_4^+ concentrations in the water may influence the toxicity of ammonia in seawater (Ip et al., 2001). Elevated water ammonia levels either reduce the excretion of ammonia from the fish or lead to a net uptake of ammonia from the surrounding environment (Randall and Tsui, 2002).

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).

The effects of exposure to increased ammonia concentrations will depend on stress levels, swimming activity and the feeding status of the exposed animals (Randall and Tsui, 2002). Ammonia levels in the white muscle of rainbow trout can increase after exercise (Mommsen and Hochachka, 1988) and a significant reduction (linear) in critical swimming velocity was observed for rainbow trout in association with increasing levels of water ammonia (Wicks et al., 2002). Increased internal ammonia concentrations caused by exercise increases the susceptibility of fish to acute ammonia toxicity. Acute toxicity tests showed that LC_{50} for resting rainbow trout was significantly higher compared to swimming trout (Randall and Tsui, 2002). Wicks and Randall (2002a) showed that fed trout are less sensitive than unfed fish with regard to external ammonia and that fasting exacerbates ammonia toxicity. A study by Bucking and Wood (2008) reported a transient postprandial increase in plasma ammonia that peaked threefold above resting values 12h after a meal and remained elevated after 24h. The same authors observed that the increase in plasma ammonia levels was correlated with the increased excretion of ammonia that was two to threefold higher 12h and 48h after feeding. The protective effects of feeding against ammonia toxicity in trout were attributed to the upregulated production and storage of glutamine in the muscles (Wicks and Randall, 2002b). Wood (2004) showed that a chronic exposure to sublethal levels of ammonia (PNH_3 ~23 µtorr) can stimulate growth, conversion efficiency and protein production, without a corresponding increase in feed consumption when juvenile rainbow trout were fed to satiation, but not when the trout were subjected to a restricted feed ration. Both the injection of cortisol and the stress caused by increased densities can exacerbate ammonia toxicity in rainbow trout (Randal and Tsui, 2002). Exposure to higher pH values (pH 10) reduces ammonia production in rainbow trout (Wilson et al., 1998) and exposure to sub-lethal levels of silver increases plasma ammonia concentrations (McGeer and Wood, 1998).

When the early life stages of rainbow trout were subjected to a chronic exposure of ammonia at pH 7.75 and a temperature of 11.4 ^{o}C, results showed that ammonia exposure did not affect either hatching success or the survival of sac fry, but had a significant and detrimental effect upon the growth, biomass and survival of swim-up fry at levels of 16.8 mg NH_3-N L^{-1} in comparison to 7.44 mg NH_3-N L^{-1} (Brinkman, 2009). The sac fry development to the swim-up stage was hindered by ammonia, but they seemed to recover if exposed to ammonia ≤ 7.44 NH_3-N L^{-1}. Chronic long-term exposure to sublethal levels of ammonia has an effect on morphological and physiological parameters in rainbow trout but the extent of the effect depends on the developmental stage, with larvae and adult stages particularly prone to exposure (Vosyliene and Kazlauskiene, 2004). The respiratory capacity of larvae was affected (and growth was consequently compromised) even at low NH_3 concentrations (0.006-0.18 mg L^{-1} NH_3), while exposure of rainbow trout adults to concentrations between 0.012-0.092mg L^{-1} NH_3 had no negative effect upon growth (Vosyliene and Kazlauskiene, 2004). In addition, ammonia toxicity had a negative effect on erythropoiesis (decrease in % of juvenile erythrocytes) of larvae and adults at levels ≥0.024mg L^{-1} NH_3 and also has negative effects on leukopoiesis in adult fish at levels of 0.024-0.09 mg L^{-1} NH_3 (Vosyliene and Kazlauskiene, 2004).

In a study on acute ammonia toxicity in hatchery-reared rainbow trout from 0.1 g to 2.6 kg (Thurston and Russo, 1983) the authors established a 96-hour medial lethal concentrations (96-hour LC_{50}) between 0.16 and 1.1 mg L^{-1} unionized ammonia (11-48 mg L^{-1} of TAN). The susceptibility to ammonia decreased from sac fry to juveniles and increased in adult stages and the toxicity decreased with a temperature increase from 12 ^{o}C to 19^{o}C (Thurston and Russo, 1983).

Both the frequency and duration of ammonia exposure will influence ammonia toxicity in rainbow trout (Milne et al., 2000). Trout can survive and recover from 24h long exposure to NH_3-N concentrations < 0.5 mg, while at higher concentrations fish could only recover if they were exposed for 1h (Milne et al., 2000). When rainbow trout were subjected to a combined exposure of 500 µM ammonia and 600 µM nitrite there was high mortality (Vedel et al., 1998) and although both toxins caused significant physiological damage, there were no observed interactive effects of nitrite and ammonia toxicity. A study by Becke et al., (2019) reported that unionized ammonia-N concentrations of up to 0.05 mg L^{-1} had only minor effects on rainbow trout physiology and gill health, and no negative effects on performance and fin condition. No relevant combined effects of increased ammonia and TSS on fish health and performance were observed in RAS (Becke et al., 2019).

Exposure to total ammonia-N levels of 700 µmol L^{-1} (under lab conditions) did not stop the formation of dominance hierarchies in rainbow trout but did lead to a decrease in aggression. At 1200 µmol L^{-1} aggression was markedly reduced and it was absent at 1500 µmol L^{-1}. Hierarchies also did not form during five days exposure at 1500 µmol L^{-1} (Grobler and Wood, 2018). However, trout did become acclimated to ammonia, as the observed behavioural and physiological changes disappeared over time and aggression and physiology decreased to control levels (Grobler and Wood, 2018).

Recommended maximum levels of UIA:

- **According to the European Food Safety Authority (2008b) the maximum recommended concentration of unionized ammonia for trout is 0.012 mg L^{-1}.**
- **According to Timmons and Ebeling (2007) the maximum recommended concentration of unionized ammonia for trout is 0.0125 mg L^{-1}.**
- **According to RSPCA welfare standards for farmed rainbow trout (RSPCA, 2018b), unionized ammonia levels should be kept < 0.025 mg L^{-1} for alevins, fry and ongrowers in recirculation aquaculture systems.**

However, Becke et al., (2019) have suggested that these levels are low as they did not note detrimental effects upon performance and welfare at mean levels of up to 0.03 mg L^{-1}.

Sampling and analytical considerations

While ammonia is more toxic in saltwater (mostly due to higher pH) the concentration can be higher in systems with low water turnover, more commonly seen in freshwater. 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 -20ºC 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, wastewater 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 rainbow trout, 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.

4.1.8. 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 toxicity depends on a number of factors such as fish species and size, water quality (pH, oxygen, temperature, cations and anions), exposure duration and the susceptibility of the individual fish (Kroupova et al., 2005). The single most important factor often mentioned is chloride concentration.

Rainbow trout are one of the more sensitive species to nitrite toxicity in freshwater due to its rapid uptake of chloride through gills (Svobodova et al., 2005). There can also be high individual variability in nitrite uptake and tolerance in rainbow trout (Stormer et al., 1996; Aggergaard and Jensen, 2001; Jensen, 2003; Svobodova et al., 2005) and fish can be classified as either sensitive or tolerant based upon this variability. Sensitive fish accumulate nitrite faster and exhibit more prominent physiological changes and die sooner than those that are more tolerant (Stormer et al., 1996; Aggergaard and Jensen, 2001; Jensen, 2003). This is related to a significantly elevated rate of nitrite influx via the gills in more sensitive individuals (Zachariasen, 2001).

Nitrite has a high affinity for the gill chloride uptake mechanism and if present in ambient water can bind to chloride/bicarbonate (Cl^-/HCO_3^-) gill transporters instead of chloride ions, allowing the uptake and accumulation of this anion (Jensen, 2003). Nitrite uptake via the gills leads to a build-up of nitrite in extracellular fluids and the severity of the build-up depends on the ratio of nitrite and ambient chloride concentrations (Jensen, 2003). Plasma nitrite levels in rainbow trout can reach millimolar concentrations if ambient nitrite and chloride are in the micromolar range. The same concentration of water nitrite can be tolerated if adequate concentrations of chloride are provided (Jensen 2003).

The review of nitrite by Jensen (2003) states the depletion in plasma chloride is bigger than the increase in plasma nitrite during nitrite exposure in rainbow trout. This can be due to increases in additional ions such as nitrate, lactate, bicarbonate and sometimes inorganic phosphase, which means the total amount of anions are unchanged (Jensen et al., 1987; Stormer et al., 1996). Nitrite exposure also affects potassium balance, particularly in more sensitive individuals, by stimulating a general loss of potassium from the skeletal muscles and red blood cells, but not the heart muscle (Stormer et al., 1996), causing significant elevations of extracellular potassium in the trout (Stormer et al., 1996).

The accumulation of nitrite in erythrocytes oxidises haemoglobin into methaemoglobin which cannot bind oxygen (Jensen, 2003). Therefore, when nitrite concentrations increase in the blood, the fraction of methaemoglobin also increases and the total oxygen-carrying capacity decreases (Lewis Jr. and Morris, 1986). Brown gills or blood are a good indicator of high levels of methaemoglobin (Lewis Jr. and Morris, 1986) which has a maximum absorption of around 635 nm in the optical spectrum (Kroupova et al., 2005). Methaemoglobin levels in the blood during nitrite exposure will be a balance between the creation of methaemoglobin and its transformation into haemoglobin by methaemoglobin reductase (Lewis Jr. and Morris, 1986). The accumulation of methaemoglobin is faster in nitrite sensitive rainbow trout and the speed of accumulation and other physiological changes are key to welfare threats such as early mortality (Stormer et al., 1996; Jensen, 2003). Methaemoglobin in different rainbow trout individuals can amount to 6-100% of total haemoglobin (Hofer and Gutumu, 1994). In nitrite sensitive rainbow trout with high levels of methaemoglobin the retina is severely affected, with effects ranging from necrosis of single retina neurons to the complete destruction of the retina (Hofer and Gatumu, 1994). Nitrite exposure also leads to increased ventilation rate and a fast and long lasting rise in heart rate that appears before an elevation in methaemoglobin or extracellular potassium in rainbow trout (Aggergaard and Jensen, 2001). The variability in heart rate also drops in nitrite sensitive individuals (Aggergaard and Jensen, 2001).

Exposure to nitrite can also increase the disease susceptibility of rainbow trout. For example, in a study that first exposed trout to 24h of 0.24 mg L^{-1} NO_2^- and then subsequently exposed the fish to *Saprolegnia parasitica,* this combination of factors led to a 100% increase in the proportion of infected fish in comparison to the control group (Carballo and Munoz, 1991; Carballo et al., 1995). The acute toxicity exposure of four different sized rainbow trout (2-235g) to nitrite reported 4 day median lethal concentrations (LC_{50}) of 0.19-0.39 mg L^{-1} NO_2–N (Russo et al., 1974). The subchronic exposure of 18.9g rainbow trout to levels between 0.01-3 mg L^{-1} NO_2^- over 28 days affected haematology, blood chemistry, growth, survival and gill histology and considerable physiological changes were visible at the lowest nitrite exposure concentrations (Kroupova at al., 2008). Estimated concentrations for no effects and the lowest observed effects were 0.01 and 0.2 mg L^{-1} NO_2^-, respectively.

Trout can detoxify nitrite by oxidizing it to non-toxic nitrate when extracellular nitrate concentrations increase to millimolar values (Jensen, 2003). Detoxification occurs partly in the liver where trout hepathocytes oxidize nitrite to nitrate and also in oxygenated trout red blood cells (Doblander and Lackner, 1997). Another way of preventing nitrite toxicity is the addition of chloride to freshwater. The recommended Cl^-: NO_2-N weight ratio for trout is > 20:1 (Timmons and Ebeling, 2007). Other results from a range of other fish species (Svobodova et al., 2005) call for the re-evaluation of the current recommendations. EFSA (2008b) and the Norwegian Food Safety Authority (Hjeltnes et al., 2012) recommends that nitrite levels are kept below 0.1 mg L^{-1} NO_2^- in rainbow trout production, with a maximum nitrite nitrogen (mg L^{-1}) range between 16 and 33 (Nordin and Pommen, 2009). A combined exposure of rainbow trout to nitrite (600 µM) and ammonia (500 µM) has been previously reported to lead to high mortalities, but interactive effects of these compounds on physiological parameters was not observed. However, each nitrogen compound did have multiple negative effects on blood physiology (Vedel et al., 1998).

Nitrate (NO_3^-) is the end product of nitrification and together with other ionic forms of inorganic nitrogen, it can be naturally found in water-based ecosystems due to e.g. runoff from surface and groundwaters, atmospheric deposition, biological degradation of organic matter, or be due to anthropological activities e.g. animal farming, industrial waste and sewage effluents (Camargo et al., 2005). Nitrate concentrations can reach 25 mg L^{-1} NO_3-N in surface waters and 100 mg L^{-1} NO_3-N in ground waters, while in recirculation aquaculture systems with good oxygenation NO_3-N can reach 500 mg L^{-1} (Camargo et al., 2005). Nitrate is less toxic than nitrite and ammonia partly due to low branchial permeability to nitrate (Camargo et al., 2005). The potential effects of nitrate on farmed fish have not been as extensively documented as for ammonia and nitrite. However, the use of recirculating aquaculture systems with low water exchange rates has driven interest in identifying safe concentration levels of nitrate in farmed fish. The primary potential toxic effect of nitrate is the conversion of haemoglobin into methaemoglobin, the form that does not carry oxygen (Camargo et al., 2005). Nitrate toxicity intensifies in line with increases in nitrate concentrations and also in line with the duration of exposure. Freshwater fish are more sensitive to nitrate toxicity than marine species (Camargo et al., 2005). Westin (1974) reported the 96-h LC_{50} value of nitrate for rainbow trout fingerlings is 1364 mg L^{-1} NO_3-N and recommended i) a maximum allowable chronic exposure level of 57 mg L^{-1} and ii) and exposure level of 5.7 mg L^{-1} NO_3-N for the best health and growth performance. Others have reported sublethal effects of nitrate on the eggs and fry of salmonids at levels < 25 mg L^{-1} and chronically toxic effects at levels < 200 mg L^{-1} (reviewed in Davidson et al., 2014). Rainbow trout fingerlings exposed to 14 mg L^{-1} NO_3-N for 8 days showed a passive intake of nitrate while maintaining unchanged plasma concentrations of this compound and no change in electrolyte balance or haematology (reviewed in Camargo et al., 2005). An overview of the nitrate toxicity for rainbow trout in freshwater is given in Table 4.1.8-1.

Table 4.1.8-1. *Freshwater nitrate toxicity concentrations for rainbow trout (Oncorhynchus mykiss). LOEC = the lowest observed effect concentration; NOEC = no observed effect concentration; NOAEL = no observed adverse effect level; h = hours; d = days. Table reproduced from "Camargo, J. A., Alonso, A. & Salamanca, A. (2005) Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 58(9), 1255-1267. Copyright 2005." With permission from Elsevier.*

Developmental stage	Concentration of nitrate nitrogen (mg NO_3-N L^{-1}) and the duration of exposure	References
Fingerlings	1355 (96-h LC_{50})	Westin, 1974
Eggs (anadromous)	1.1 (30 d LOEC)	Kincheloe et al., 1979
Fry (anadromous)	4.5 (30 d NOEC)	Kincheloe et al., 1979
Eggs (nonanadromous)	1.1 (30 d NOEC)	Kincheloe et al., 1979
Eggs (nonanadromous)	2.3 (30 d LOEC)	Kincheloe et al., 1979
Fry (nonanadromous)	1.1 (30 d NOEC)	Kincheloe et al., 1979
Fry (nonanadromous)	2.3 (30 d LOEC)	Kincheloe et al., 1979
Fingerlings	14.0 (8 d NOAEL)	Stormer et al., 1996

When evaluating the effect of temperature (5, 10 and 15 °C) on nitrate toxicity in rainbow trout, it was reported that nitrate was more toxic when an optimal metabolic temperature of 15°C was used (96-h LC_{50} of 1690 mg NO_3^- L^{-1}, Canadian Council of Ministers of the Environment, 2012a). Recently, Baker et al., (2017) evaluated nitrate toxicity in relation to water hardness. In acute toxicity tests, rainbow trout fry (0.3-0.6 g) were exposed to nitrate at water hardness levels between 11 mg L^{-1} (soft water) and 164 mg L^{-1} $CaCO_3$ (hard water). The 96h LC_{50} levels increased linearly from 808 mg L^{-1} NO_3-N at 11 mg L^{-1} $CaCO_3$ to 1913 mg L^{-1} NO_3-N at 164 mg L^{-1} $CaCO_3$. These data show that water hardness influences acute nitrate toxicity in rainbow trout.

Juvenile rainbow trout exposed to sublethal concentrations of NO_3-N (30 and 90 mg L^{-1} NO_3-N) in a recirculating aquaculture system showed significantly more side swimming behaviours at 90 mg L^{-1} NO_3-N compared to 30 mg L^{-1} NO_3-N (Davidson et al., 2014). The authors of the study concluded that concentrations of 80-100 mg L^{-1} NO_3-N had chronic welfare and health impacts on juvenile rainbow trout and have recommended a maximum NO_3-N limit of 75 mg L^{-1} for rainbow trout in RAS systems.

Recommended upper concentrations

- **Nitrite: EFSA (2008b) and the Norwegian Food Safety Authority (Hjeltnes et al., 2012) recommends that nitrite levels are kept below 0.1 mg L^{-1} NO_2^- in rainbow trout production. RSPCA (2018b) also recommends nitrite concentrations < 0.2 mg L^{-1} for all life stages (ova, alevins, fry and ongrowers) in RAS. No guidelines are given for recommended chloride levels in relation to nitrite exposure. Currently the guidelines for Cl^- requirements in relation to NO_2^- concentrations are also not specified by the Norwegian Food Safety Authority.**
- **Nitrate: 75 mg L^{-1} (Davidson et al., 2014). Current RSPCA (2018b) upper recommendations for trout in recirculating aquaculture systems are 50 mg L^{-1} for fry/fingerlings and ongrowers while limits for ova and alevins are not stated.**

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 salmonids. 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 salmonids 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 salmonids 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.

4.1.9.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. However, since suspended solids are often the primary cause of turbidity, those two parameters are often discussed together (Robertson-Bryan, Inc., 2006). Increased turbidity can hinder the observation of fish in tanks and cages. This makes observation of the fish difficult and can reduce the farmer's capacity to monitor the feeding response and assess fish health. The effects of turbidity are related to the nature of the substances implicated in reducing visibility. The optimal levels of turbidity for trout 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. The effect of suspended solids on fish are dependent on the species, temperature at the time of exposure, the type of suspended sediments (particle size and angularity), sediment contaminants, the duration and frequency of exposure and also its dose (reviewed in Kjelland et al., 2015). The effect of sediments on salmonids are grouped into lethal (mortalities), sublethal (tissue injury or changes in physiology) and behavioural (change in activity), as reviewed in Bash et al., (2001).

Rainbow trout have been classified as a species that is intolerant to sedimentation (Chapman et al., 2014) with the most sensitive life stage from fertilization to egg hardening (Scannell and Jacobs, 2001).The 48-d LC_{40} for rainbow trout eggs has been reported to be 7 mg L^{-1} TSS (Canadian Council of Ministers of the Environment, 2012b). It has also been reported that the feeding activity of rainbow trout drops sharply at turbidities > 70 Jackobs turbidity units (JTU) (reviewed in Kjelland et al., 2015). Increased turbidity also leads to decreased swimming performance (U_{crit}) in rainbow trout and changes in aerobic (elevated glucose) and anaerobic metabolism (reduced lactate) (Berli et al., 2014). As stated above, increased turbidity hampers visual observation of the fish by the farmers and may also affect the ability of the fish to see pellets. However, a study by Rowe et al., (2003) reported that the feeding rates of juvenile rainbow trout were not reduced by turbidity levels up to 160 NTU indicating that other, non-visual senses, such as the lateral line system, might be involved during feeding in turbid waters.

Fine suspended solids or solids with abrasive particles can have a negative effect on gill health and function, compromising oxygen transfer and providing a habitat for the growth of pathogens (Timmons and Ebeling, 2007). It has previously been reported that the exposure of rainbow trout to a mixture of inert solids (kaolin and diatomaceous earth) resulted in some mortalities at TSS values of 90 mg L^{-1} and a significant increase in mortality after continuous exposure to 270 mg L^{-1}. No mortalities were observed when rainbow trout were exposed to 553 mg L^{-1} gypsum for four weeks and after nine to ten months exposure to 200 mg L^{-1} of suspended solids from a coal washery. However, a turbidity of 25 NTU due to clay had a negative effect on the growth of juvenile rainbow trout (reviewed in Robertson-Bryan, Inc., 2006). A gradual increase in TSS up to 30 mg L^{-1} (average turbidity of 14.5 NTU) over four weeks in RAS had no effect on stress markers, haematological parameters (leukocyte count, haematocrit, RBC indices) and the gill health of rainbow trout (Becke et al., 2017). In addition, a long-term (18 weeks) exposure to the same TSS concentration had no effect on performance, health and physiology of rainbow trout (Becke et al., 2018). These results were further supported by a later study (Becke et al., 2019) who reported that TSS levels up to 70 mg L^{-1} for 13 weeks did not impact upon the welfare, health and growth performance of rainbow trout. However, it did lead to increased turbidity which impacted upon feeding behaviour and increased bacterial load (Becke et al., 2019).

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 for Atlantic salmon (Thorarensen and Farrell, 2011) and an upper limit of 25 mg L^{-1} has previously been suggested for rainbow trout. However, Becke et al., (2019) suggest this limit is too low (see above). RSPCA, (2018b) recommends a maximum concentration of non-spate suspended solids of < 25mg L^{-1} for all life stages of rainbow trout while recommendations for turbidity are not given separately. EFSA (2008b) concluded that the physical characteristics and the total amount of suspended solids in water are relevant to determine the possible negative effects on trout gills and skin but maximum concentrations of TSS are not given due to the effect that particle size and shape has on this parameter.

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° 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 °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. TSS can degrade water quality, clog equipment and can be damaging to fish gills and harbour pathogens. These parameters 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 with regard to 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 may result from an inadequate supply of water for the stocking density due to low current speed or reduced water exchange for other reasons such as fouled nets or slack water (e.g. Vigen, 2008). Current speed may also affect the volume of the cage by deformation, although this is related to the net and supporting structures 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 is only 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, high current velocities, even if they are well below U_{crit}, may have negative effects on growth with recommended current velocities for the optimal growth of rainbow trout between 0 and 1 body lengths s^{-1} (Farrell et al., 1991; Houlihan and Laurent, 1987). More recent work by Larsen et al., (2012) suggest current velocities of 0.9 body lengths s^{-1} promoted schooling and reduced the frequency of erratic behaviours in comparison to trout held in static water. McKenzie et al., (2012) also reported that current velocities of 0.9 body lengths s^{-1} improved recovery times after trout were subjected to an acute crowding stressor in comparison to trout held in static water. In other salmonids, current velocities that are too low may also lead to problems with fin biting and aggression (Solstorm et al., 2015, 2016) and maintaining active swimming in the population can improve growth and feed conversion since fish divert more energy to maintaining position and less to social interactions (e.g. Christiansen & Jobling, 1990). Anaerobic movement, which is often associated with especially aggressive social interactions is substantially less efficient in terms of energy utilisation (Marras et al., 2011).

There is a wide variation in recommended current velocities and even these relate to the experience of the fish in a complex manner (Taguchi and Liao, 2011). The same current speed in different systems will not have the same effect (Johansson et al., 2014). Therefore, strict current speed recommendations are not necessarily useful, and it is preferable to adjust current speed so that fish are actively swimming but not struggling to hold position or being actively washed backwards.

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 fish'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) in Atlantic salmon. In rainbow trout smoltification is less clear and seawater tolerance is more dependent on size (see section 3.2.8). Increased daylength has a positive effect on the growth of rainbow trout in the freshwater phase (Taranger et al., 2000; Taylor et al., 2005, 2007) and also increases seawater tolerance regardless of size (Wagner, 1974; Taranger et al., 2000), therefore reducing the duration of the freshwater stage. It has been reported that light intensities of 1600 lux can also improve growth in the freshwater phase compared to fish reared at 100 lux (Cho, 1992). A recent paper by Morro et al., (2019) has tested the effects of different photoperiod regimes on rainbow trout seawater adaptation and reported that both the existing, well established constant light (LL) regime (18 weeks) and an Advanced Phase Photoperiod (APP) regime (6 weeks LD 12:12 and a further 12 weeks of LD 24:0) are suitable regimes for seawater adaptation and APP led to a longer adaptation window. However, the authors stated photoperiod does not appear to be a strong driver for seawater adaptation in trout and other potential environmental drivers, such as salinity or temperature should be examined (Morro et al., 2019). Constant light has been found to have negative effects on the neurological development of salmon parr (Ebbesson et al., 2007). Sudden changes in light intensity can induce an acute stress response involving panic behaviour in rainbow trout, especially when lights are suddenly turned off (Mork and Gulbrandsen, 1994). This response can lead to increased oxygen consumption in Atlantic salmon (Folkedal et al., 2010) but the fish can habituate to this response within a week (Folkedal et al., 2010).

Seawater: Increased daylength has a positive effect on growth in the seawater phase (Taylor et al., 2006). Rainbow trout are natural spring spawners and extending daylength from midwinter through the spring results in earlier spawning than in controls (reviewed by Bromage et al., 2001). However, if this approach is adopted in 1 year old fish, it can prevent or delay spawning the following year (Davies and Bromage, 2002). In addition, the change in daylength appears far more important for maturation than daylength *per se* (Bromage et al., 2001). 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 influence of light conditions on the swimming behaviour of caged rainbow trout is much less studied. Light from the surface results in more daytime-like behaviour also at night (Oppedal, 1995) and their behavioural response to submerged lights is probably similar to that seen in salmon.

Sampling and analytical considerations

The fish's perception of daylength has an influence on hormonal development 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, the process is better understood for A. salmon where an appropriate intensity and spectrum must be used to avoid sexual maturation (Stien et al., 2014).

Strength of indicator

Light intensity and daylength 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}. Stocking density interacts with the welfare of the fish in a complex manner involving many interacting parameters including life stage, water quality, water velocity, social interactions, feed management, management practices and the choice of rearing system (e.g. Turnbull et al., 2008). The potential negative effects of high stocking density 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) associated with higher densities. The welfare needs that are directly or indirectly affected by stocking density include i) hygiene, ii) water quality, iii) behavioural control, iv) social contact and v) rest. For a fuller description of the potential effects of stocking density on rainbow trout welfare, please refer to the thorough review carried out by Ellis et al., (2002).

While there is clearly a risk of reduced welfare at either very high or very low stocking densities it is difficult to set minimum and maximum stocking density levels that will protect welfare. A given stocking density may result in good or bad welfare under different circumstances. A preferable approach is to monitor the behaviour and condition of the fish. Behaviour can be very difficult to assess or describe quantitatively under farmed conditions and depends on informed observation. The fish should preferably demonstrate a settled behaviour with little evidence of rapid chaotic movement or excessive reactivity to disturbance, feeding should be enthusiastic but not frantic. There should be minimal evidence of damage to fins, eyes and opercula (RSPCA, 2014). In terms of acceptable limits, based on literature and current practice the RSPCA (2018b) recommend that stocking density for first feeding and ongrowing in tanks should not exceed 60 kg m^{-3}. Generally stocking densities are maintained at lower levels for younger fish and increase towards the end of the production cycle. Previously published recommendations on stocking density for rainbow trout are incredibly variable even at the same life stage, most likely because the effects of stocking density upon welfare are complex and involve many interacting parameters (e.g. Turnbull et al., 2008). A good example of this is covered in Ellis et al., (2002), who have outlined some of these in relation to different types of rearing systems. The reported ranges were i) 4-55 kg m^{-3} for cages, ii) 40-267 kg m^{-3} for tanks and iii) 8-160 kg m^{-3} for raceways (see Ellis et al., 2002 and references therein).

Sampling and analytical considerations

Mean density in the aquaculture unit can be calculated as biomass (kg) / volume (m^3). However, often only an estimate of cage volume is available and the actual density experienced by the fish is also affected by uneven distribution in the rearing unit (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 good estimate of 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).

4.2.4.Surface access

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). 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). In Atlantic salmon, buoyancy is affected from the first day of submergence (Dempster et al., 2009), and is severely reduced after 3 weeks (Korsøen et al., 2009) and the first signs of reduced welfare appear (Korsøen et al., 2012a). After 6 weeks of submergence more severe signs such as compressed vertebrae may become evident in salmon (Korsøen et al., 2009). The submergence of rainbow trout has been studied in less detail but its effects may be similar to salmon (Fosseidengen et al., 1982). Rainbow trout of all stages should not be prevented from refilling their swim bladder for more than a week. In the rearing units currently used for trout production the natural surface will allow access to air. If cages are submerged, alternative routes to a surface must be available, such as a snorkel or air filled domes of sufficient size. Such alternative air access routes are under development for salmon (Stien et al., 2016b; Korsøen et al., 2012b) but have not yet been tested with rainbow trout.

Sampling and analytical considerations

In order to assess if air access during submergence has been sufficient, surface activity after re-surfacing may be estimated, with high activity indicating air access has been restricted. The number of jumps and rolls after the cage has resurfaced 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, for instance 2 hours. 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 has also been developed (Jovanović et al., 2016).

Strength of indicator

In open rearing units, trout will normally have access to the surface, which is easy to monitor.

Weakness of indicator

Securing sufficient access to air during submergence with air domes may be technically challenging due to the strong buoyancy of large air volumes. The requirements of trout, e.g. sufficient surface size, are not known. When estimating surface activity after re-surfacing, 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. With large group sizes and high activity levels it may also be difficult to keep track of the number of surface breaks.

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 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.

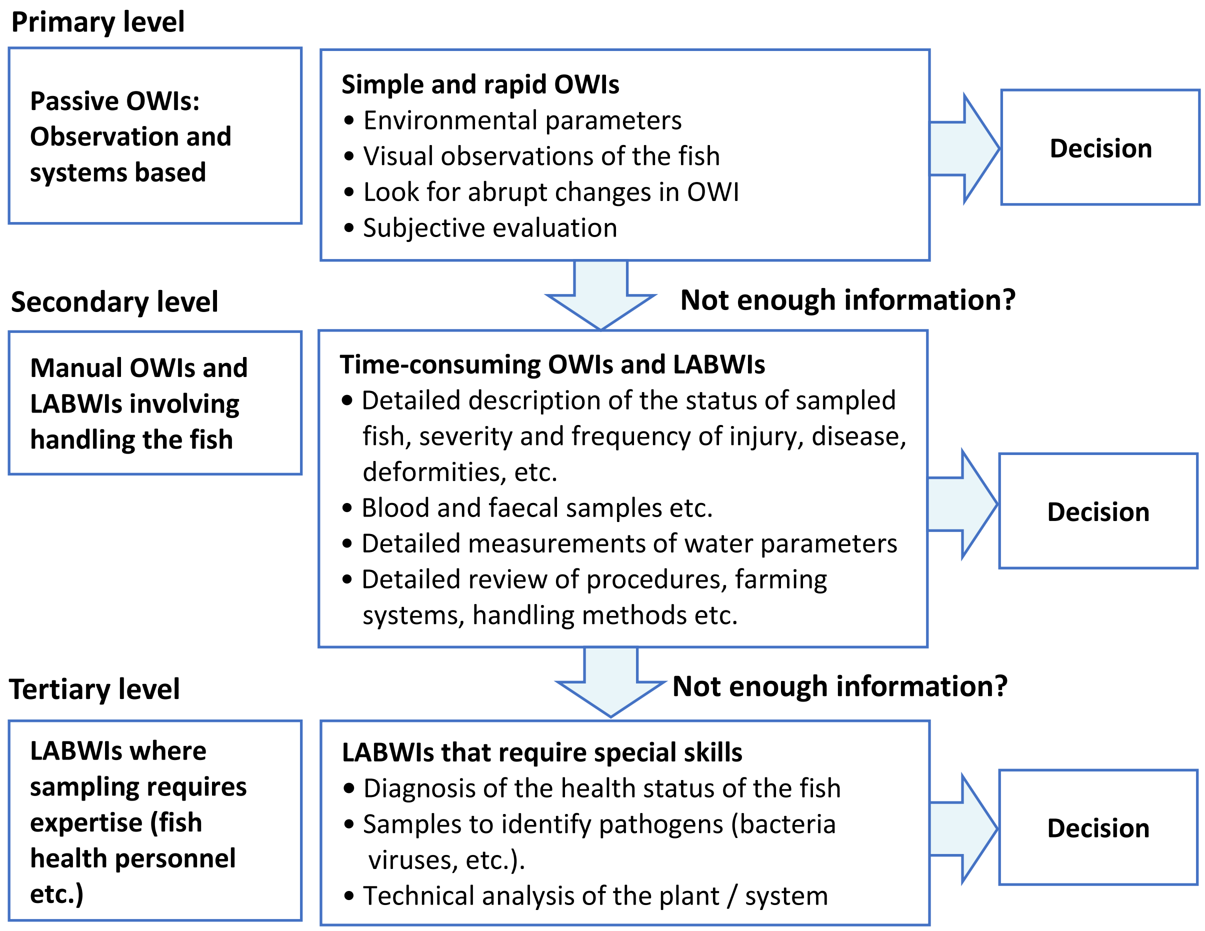


Fig. 5.1-1. *How the farmer can use OWIs and LABWIs as Early Warning Signals for compromised welfare (Figure: C. Noble, L. H. Stien and M. H. Iversen).*

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 environmental WIs, Table 5.2-2 the scoring of group based WIs and table 5.3-3 the scoring of 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 relatively 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	×		×
TGP and gas supersaturation	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.*

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

Most of the individual WIs are relatively easy to assess on the fish (Table 5.2-3). However, cardiovascular responses, nkaα1a and nkaα1b, 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 slaughterhouse.

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 (VOR)	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	×		×
• Seawater adaptation	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	×		×
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	×		×
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		×	
nkaα1a and nkaα1b	3.0		×	
Magnesium and sodium	3.0		×	
Chloride	3.0		×	
Osmolality	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. If this happens in the seawater rearing phase, questions that need to be asked could be a) was the fish fully adapted to sea water? 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 the severity of the problem, 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.

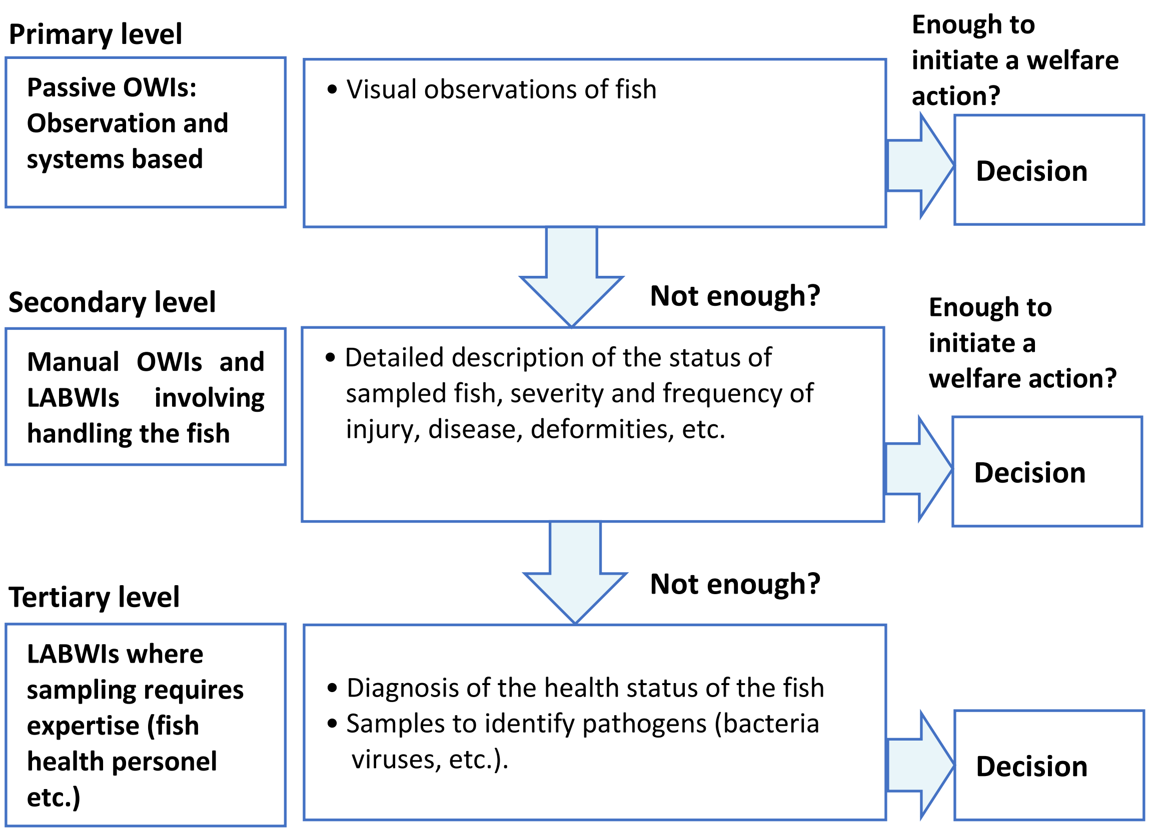


Fig. 5.3.1. *Application of OWIs and LABWIs at the farm as Early Warning Signals (figure: C. Noble and L. H. Stien)*

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 rainbow trout. 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 interpretation is not straight forward. 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.

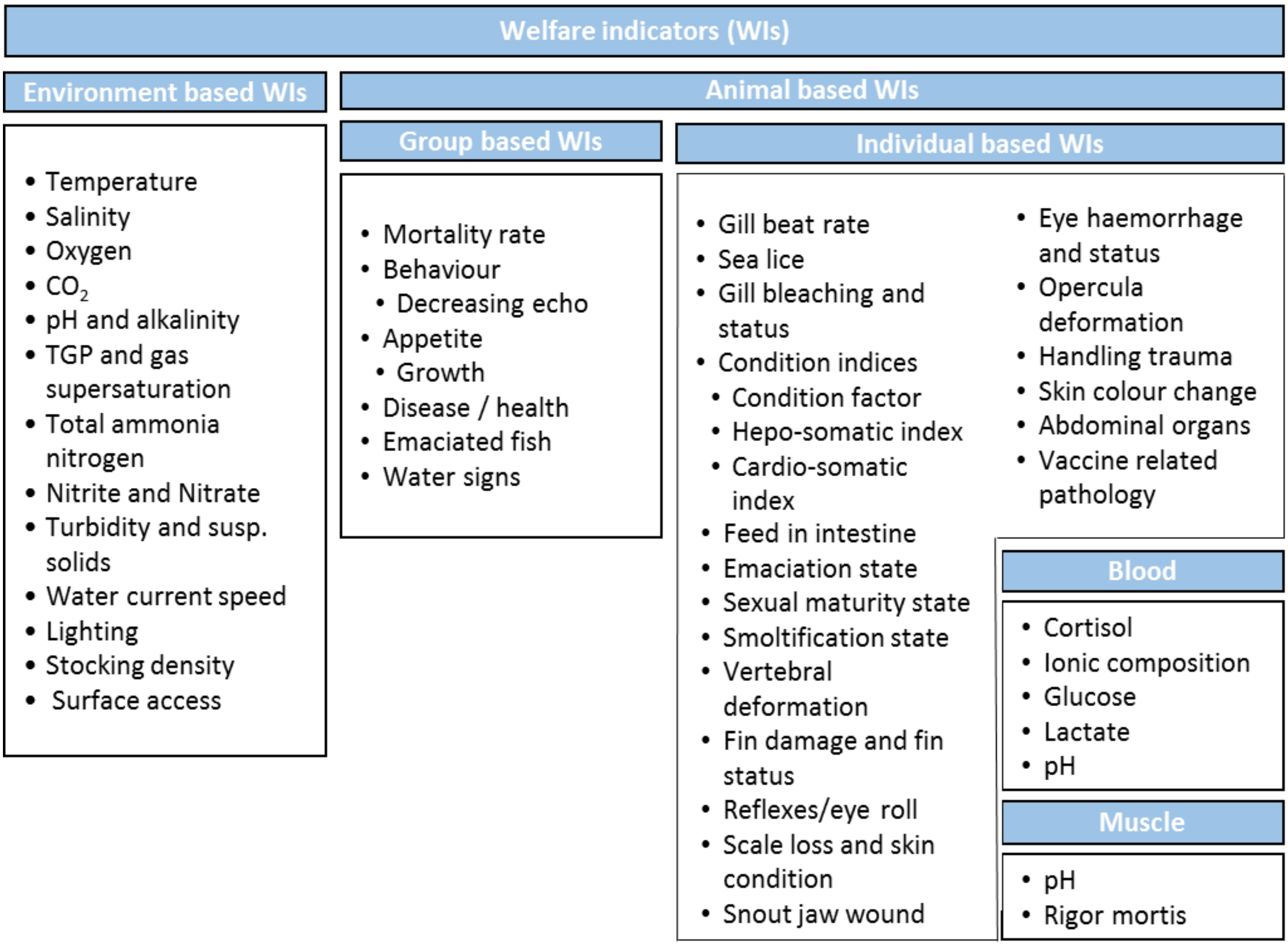


Fig. 5.5-1. *Summary of the WIs, OWIs and LABWIs covered in Part A of the handbook. Indicators are broken down into environment based and animal based WIs. Animal based WIs are further divided into group based and individual based WIs.*

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 13 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) active fin damage, xiii) healed fin damage.

We have used pictures from the FISHWELL salmon handbook (Noble et al., 2018) in the following scoring system, as the conditions they describe are equally applicable to rainbow trout.

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 rainbow trout, 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, primarily for salmon but it has also been used for trout. 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)*

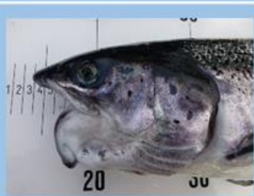
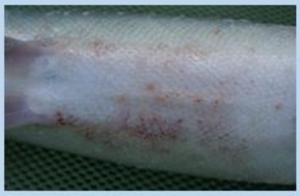
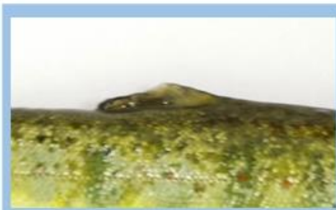
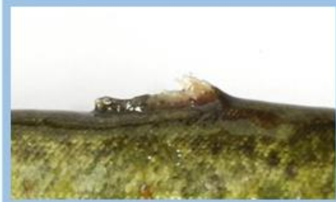
	1	2	3
Eye haemorrhage	 Minor haemorrhages	 Larger haemorrhages, or traumatic injury	 Large haemorrhages / traumatic injury. Eye may be ruptured
Exophthalmia	 Eye protruding a little	 Moderate eye protrusion	 Major eye protrusion
Opercular damage	 Operculum only partly covering gills	 Operculum absent 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

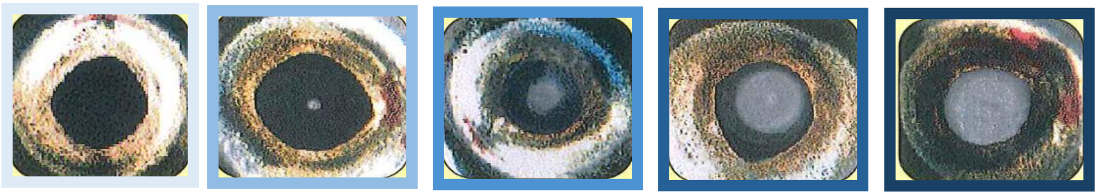
Table 6.1-2*. Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. (Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. 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)

[1] For fingerlings "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)*

	1	2	3
Healed fin damage	 Most of the fin remaining	 Half of the fin remaining	 Very little of the fin remaining
Active fin damage, splitting, haemorrhaging	 Most of the fin remaining	 Half of the fin remaining	 Very little of the fin remaining



0. No cataract

1. Cataract covers less than 10% of lens diameter

2. Cataract covers between 10 and 50% of lens diameter

3. Cataract covers 50 to 75% of lens diameter

4. Cataract covers over 75% of lens diameter

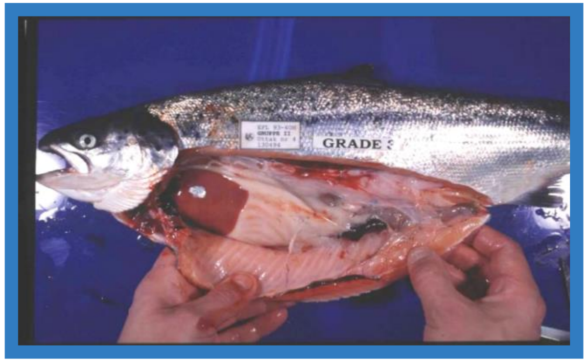
Fig. 6.2. *Morphological scheme for diagnosing and classifying eye cataracts in salmonids. Text reproduced from "Wall, T. & Bjerkås, E. 1999. A simplified method of scoring cataracts in fish. Bulletin of the European Association of Fish Pathologists 19(4), 162-165. Copyright, 1999" with permission from 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. Scale originally developed for Atlantic salmon but has also been used in studies on rainbow trout (e.g. Holten-Andersen et al., 2012; Chettri et al., 2015).*

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. Although the pictures are from Atlantic salmon, they are equally applicable to rainbow trout. 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.*

7.References

Abbott, J. C. & Dill, L. M. (1985) Patterns of aggressive attack in juvenile steelhead trout (Salmo gairdneri). *Canadian Journal of Fisheries and Aquatic Sciences* **42(11)**, 1702-1706.

Adams, C., Huntingford, F., Turnbull, J., Arnott, S. & Bell, A. (2000) Size heterogeneity can reduce aggression and promote growth in Atlantic salmon parr. *Aquaculture International* **8**, 543–549.

Aggergaard, S. & Jensen, F. B. (2001) Cardiovascular changes and physiological response during nitrite exposure in rainbow trout. *Journal of Fish Biology* **59(1)**, 13-27.

Agius, C. & Roberts, R. J. (2003) Melano-macrophage centres and their role in fish pathology. *Journal of Fish Diseases* **26**, 499–509.

Akiyama, T., Mori, K. & Murai, T. (1986) Effects of temperature on the incidence of scoliosis and cataract in chum salmon fry caused by tryptophan deficiency. *Nippon Suisan Gakkaishi* **52(11)**.

Alanärä, A. (1996) The use of self-feeders in rainbow trout (*Oncorhynchus mykiss*) production. *Aquaculture* **145(1-4)**, 1-20.

Albrektsen, S. & Torrissen, O. J. (1988) Physiological changes in blood and seminal plasma during the spawning period of maturing rainbow trout held under different temperature and salinity regimes, and the effect on survival of the broodstock and the eyed eggs. *Inter. Council. Exp. Sea* C.M. 1988/ F:3 1-24.

Alexis, M. N., Papaparaskeva-Papoutsoglou, E. & Papoutsoglou, S. (1984) Influence of acclimation temperature on the osmotic regulation and survival of rainbow trout (*Salmo gairdneri*) rapidly transferred from fresh water to sea water. *Aquaculture* **40(4)**, 333-341.

Altimiras, J. & Larsen, E. (2000) Non-invasive recording of heart rate and ventilation rate in rainbow trout during rest and swimming. Fish go wireless!. *Journal of Fish Biology* **57(1)**, 197-209.

Andrews, M., Stormoen, M., Schmidt-Posthaus, H., Wahli, T. & Midtlyng, P. J. (2015) Rapid temperature-dependent wound closure following adipose fin clipping of Atlantic salmon *Salmo salar* L. *Journal of fish diseases* **38(6)**, 523-531.

Anil, M. H. (1991) Studies on the return of physical reflexes in pigs following electrical stunning. *Meat Science* **30(1)**, 13-21.

Anon. (2014) A Review of Farm Animal Welfare in the UK. Freedom Foods, Farm animal welfare: Past, present and future-Report, September 2014. https://www.rspcaassured.org.uk/media/1041/summary_report_aug26_low-res.pdf (Accessed 2016).

Anras, M. L. B. & Lagardère, J. P. (2004) Measuring cultured fish swimming behaviour: first results on rainbow trout using acoustic telemetry in tanks. *Aquaculture* **240(1-4)**, 175-186.

Arnesen, A. M., Johnsen, H. K., Mortensen, A. & Jobling, M. (1998) Acclimation of Atlantic salmon (*Salmo salar* L.) smolts to 'cold' sea water following direct transfer from fresh water. *Aquaculture,* **168**, 351-367.

APHA, AWWA, WEF (1992) Standard Methods for the Examination of Water and Wastewater, American Public Health Association Publication, 18th edition, USA.

ASC (2019) Aquaculture Stewardship Council (ASC). ASC Freshwater Trout Standard Version 1.2. https://www.asc-aqua.org/wp-content/uploads/2019/07/ASC-Freshwater-Trout-Standard_v1.2_final.pdf (Accessed 2019).

Ashley, P. J., Sneddon, L. U. & McCrohan, C. R. (2007) Nociception in fish: stimulus–response properties of receptors on the head of trout *Oncorhynchus mykiss. Brain research* **1166**, 47-54.

Aubin, J., Gatesoupe, F. J., Labbé, L. & Lebrun, L. (2005) Trial of probiotics to prevent the vertebral column compression syndrome in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquaculture Research* **36(8)**, 758-767.

Aunsmo, A., Larssen, R. B., Valle, P. S., Sandberg, M., Evensen, O., Midtlyng, P. J, Ostvik, A. & Skjerve, E. (2008a) Improved field trial methodology for quantifying vaccination side-effects in farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture* **284(1-4)**, 19-24.

Aunsmo, A., Guttvik, A., Midtlyng, P. J., Larssen, R. B., Evensen, O., & Skjerve, E. (2008b). Association of spinal deformity and vaccine-induced abdominal lesions in harvest-sized Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **31(7)**, 515-524.

Austreng, E., Storebakken, T. & Åsgård, T. (1987) Growth rate estimates for cultured Atlantic salmon and rainbow trout. *Aquaculture* **60**, 157-160.

Babaheydari, S. B., Keyvanshokooh, S., Dorafshan, S. & Johari, S. A. (2016) Proteomic analysis of skeletal deformity in diploid and triploid rainbow trout (*Oncorhynchus mykiss*) larvae. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics* **19**, 1-7.

Baker, J. A., Gilron, G., Chalmers, B. A. & Elphick, J. R. (2017) Evaluation of the effect of water type on the toxicity of nitrate to aquatic organisms. *Chemosphere* **168**, 435-440.

Balcombe, J. (2016). What a fish knows: The inner lives of our underwater cousins. Scientific American/Farrar, Straus and Giroux.

Baldwin, T. J., Vincent, E. R., Silflow, R. M. & Stanek, D. (2000) *Myxobolus cerebralis* infection in rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) exposed under natural stream conditions. *Journal of Veterinary Diagnostic Investigation* **12(4)**, 312-321.

BAP (2016). Best Aquaculture Practices (BAP). Standards and Guidelines for Salmon Farms. http://bap.gaalliance.org/bap-standards/ (Accessed 2016).

Barnes, R. K., King, H. & Carter, C. G. (2011) Hypoxia tolerance and oxygen regulation in Atlantic salmon, *Salmo salar* from a Tasmanian population. *Aquaculture* **318(3)**, 397-401.

Barton, B. A. (2002) Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology* **42**, 517-525

Barton B. A. & Iwama, G. K. (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteriods. *Annual Review of Fish Diseases* **1**, 3-26.

Barton, B. A. & Schreck, C. B. (1987) Metabolic cost of acute physical stress in juvenile steelhead. *Transactions of the American Fisheries Society* **116(2**), 257-263.

Barton, B. A. & Zitzow, R.E. (1995) Physiological-Responses of Juvenile Walleyes to Handling Stress with Recovery in Saline Water. *Progressive Fish-Culturist* **57**, 267-276.

Barton, B. A., Ribas, L., Acerete L. & Tort, L. (2005) Effects of chronic confinement on physiological responses of juvenile gilthead sea bream, *Sparus aurata* L., to acute handling. *Aquaculture Research* **36**, 172-179.

Bash, J., Berman, C. & Bolton, S. (2001) Effects of turbidity and suspended solids on salmonids. *Washington State Transportation Center (TRAC) Report No. WA-RD 526.1*, November 2001, Olympia, WA, 92 pp

Bass, N. & Wall, T. (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.

Beamish, F. W. H. (1978). Swimming capacity. In: *Fish Physiology Vol. 7 Locomotion.* Hoar, W. S., Randall, D. J. (Eds.). Academic Press Inc., New York, 101-187.

Becerra, J., Montes, G. S., Bexiga, S. R. R. & Junqueira, L. C. U. (1983) Structure of the tail fin in teleosts. *Cell and tissue research* **230(1)**, 127-137.

Becke, C., M., Steinhagen, D., Schumann, M. & Brinker, A. (2017) Physiological consequences for rainbow trout (*Oncorhynchus mykiss*) of short-term exposure to increased suspended solid load. *Aquacultural Engineering* **78**, 63-74.

Becke, C., M., Schumann, M., Steinhagen, D., Geist, J. & Brinker, A. (2018) Physiological consequences of chronic exposure of rainbow trout (*Oncorhynchus mykiss*) to suspended solid load in recirculating aquaculture systems. *Aquaculture* **484**, 228-241.

Becke, C., Schumann, M., Steinhagen, D., Rojas-Tirado, P., Geist, J. & Brinker, A. (2019) Effects of unionized ammonia and suspended solids on rainbow trout (*Oncorhynchus mykiss*) in recirculating aquaculture systems. *Aquaculture* **499**, 348-357.

Beitinger, T. L., Bennett, W. A. & McCauley, R. W. (2000) Temperature Tolerances of North American Freshwater Fishes Exposed to Dynamic Changes in Temperature. *Environmental Biology of Fishes* **58**, 237–275.

Bellgraph, B. J., McMichael, G. A., Mueller, R. P. & Monroe, J. L. (2010) Behavioural response of juvenile Chinook salmon *Oncorhynchus tshawytscha* during a sudden temperature increase and implications for survival. *Journal of Thermal Biology* **35**, 6–10.

Berg, A., Rødseth, O. M., Tangeras, A. & Hansen, T. J. (2006) Time of vaccination influences development of adherences, growth and spinal deformities in Atlantic salmon (*Salmo sala*r L). *Diseases of Aquatic Organisms* **69**, 239-248.

Berge, Å. I., Berg, A., Fyhn, H. J., Barnung, T., Hansen, T. & Stefansson, S. O. (1995) Development of salinity tolerance in underyearling smolts of Atlantic salmon (*Salmo salar* L.) reared under different photoperiods. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 243-251.

Berli, B. I., Gilbert, M. J., Ralph, A. L., Tierney, K. B. & Burkhardt-Holm, P. (2014) Acute exposure to a common suspended sediment affects the swimming performance and physiology of juvenile salmonids. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **176**, 1-10.

Bermejo-Poza, R., De la Fuente, J., Pérez, C., de Chavarri, E. G., Diaz, M. T., Torrent, F. & Villarroel, M. (2017) Determination of optimal degree days of fasting before slaughter in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **473**, 272-277.

Berntssen, M. H. G., Kroglund, F., Rosseland, B. O. & WendelarBonga, S. E. (1997) Responses of skin mucous cells to aluminium exposure at low pH in Atlantic salmon (*Salmo salar*) smolts. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1039–1045.

Birkeland, K. & Jakobsen, P. J. (1997) Salmon lice, *Lepeophtheirus salmonis*, infestation as a causal agent of premature return to rivers and estuaries by sea trout, *Salmo trutta*, juveniles. *Environmental Biology of Fishes* **49**, 129–137

Bito, M., Yamada, K., Mikumo, Y. & Amano, K. (1983) Difference in the mode of rigor mortis among some varieties of fish by modified cutting's method. *Bulletin-Tokai Regional Fisheries Research Laboratory* **109**, 89–93.

Bjerkås, E. & Sveier, H. (2004) The influence of nutritional and environmental factors on osmoregulation and cataracts in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **235**, 101–122.

Bjerkås, E., Bjørnestad, E., Breck, O. & Waagbø, R. (2001) Water temperature regimes affect cataract development in smolting Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **24**, 281–291.

Blackburn, J. & Clarke, W. C. (1989) Revised procedures for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. *Canadian Technical Report for Fisheries and Aquaculture,* **1515**.

Blaufuss, P. C., Gaylord, T. G., Sealey, W. M. & Powell, M. S. (2019) Effects of high-soy diet on S100 gene expression in liver and intestine of rainbow trout (*Oncorhynchus mykiss*). *Fish & shellfish immunology* **86**, 764-771.

Boglione, C., Gagliardi, F., Scardi, M. & Cataudella, S. (2001) Skeletal descriptors and quality assessment in larvae and post-larvae of wild-caught and hatchery-reared gilthead sea bream (*Sparus aurata* L. 1758). *Aquaculture* **192**, 1-22.

Bolger, T. & Conolly, P.L. (1989) The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology* **34**, 171–182.

Boltaña, S., Rey, S., Roher, N., Vargas, R., Huerta, M., Huntingford, F. A., Goetz, F. W., Moore, J., Garcia-Valtanen, P., Estepa, A. & MacKenzie, S. (2013) Behavioural fever is a synergic signal amplifying the innate immune response. *Proc. R. Soc. B* **280(1766**), 20131381.

Bornø, G. & Linaker, L. (2015) Fiskehelserapporten 2014, Harstad: Veterinærinstituttet 2015.

Bosakowski, T. & Wagner, E. J. (1995) Experimental use of cobble substrates in concrete raceways for improving fin condition of cutthroat (*Oncorhynchus clarki*) and rainbow trout (*O. mykiss*). *Aquaculture* **130(2-3)**, 159-165.

Boujard, T., Labbé, L. & Aupérin, B. (2002) Feeding behaviour, energy expenditure and growth of rainbow trout in relation to stocking density and food accessibility. *Aquaculture Research* **33(15)**, 1233-1242.

Boyd, C.E. (2000) Water Quality — an Introduction. Kluwer Academic Publishers, Norwell.

Braithwaite, V. A. & Huntingford, F. A. (2004) Fish and welfare: do fish have the capacity for pain perception and suffering? *Animal Welfare* **13**, 87-92.

Brännäs, E. & Alanärä, A. (1992) Feeding behaviour of the Arctic charr in comparison with the rainbow trout. *Aquaculture* **105(1)**, 53-59.

Branson, E. J. (2008) *Fish Welfare*. Blackwell Publishing Ltd, Oxford, U.K, 300 pp.

Branson, E.J. & Turnbull, T. (2008) Welfare and deformities in fish. In: *Fish welfare*. Branson E. J. (ed.), Blackwell Publishing Ltd, Oxford, pp. 202-216.

Bratland, A., Stien, L. H., Braithwaite, V. A., Juell, J. –E., Folkedal, O., Nilsson, J., Oppedal, F., Fosseidengen, J. E. & Kristiansen, T. S. (2010) From fright to anticipation: using aversive light stimuli to train reward conditioning in Atlantic salmon (*Salmo salar* L.). *Aquaculture International* **18**, 991-1001.

Breck O. & Sveier, H. (2001) Growth and cataract development in two groups of Atlantic salmon (*Salmo salar* L.) post smolt transferred to sea with a four week interval. *Bulletin of the European Association of Fish Pathologists* **21**, 91–103.

Breck, O., Bjerkås, E., Campbell, P., Arnesen, P., Haldorsen, P. & Waagbø, R. (2003) Cataract preventative role of mammalian blood meal, histidine, iron and zinc in diets for Atlantic salmon (*Salmo salar* L.) of different strains. *Aquaculture Nutrition* **9**, 341–350.

Breck, O., Bjerkås, E., Campbell, P., Rhodes, J. D., Sanderson, J. & Waagbø, R. (2005) Histidine nutrition and genotype affect cataract development in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **28**, 357–371.

Brett, J. R. (1964) The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Board of Canada* **21(5)**, 1183-1226.

Brett, J. R. (1965). The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). *Journal of the Fisheries Board of Canada* **22**, 1491-1501.

Brett, J. R. (1979) Environmental factors and growth. In *Fish Physiology, Vol. VIII*. Hoar, W. S., Randall, D. J. & Brett, J.R. (eds.). New York: Academic Press, 599-675.

Brett, J. R. & Groves, T. D. D. (1979) Physiological energetics. *Fish physiology* **8(6)**, 280-352.

Brinkman, S. F., Woodling, J. D., Vajda, A. M. & Norris, D. O. (2009) Chronic toxicity of ammonia to early life stage rainbow trout. *Transactions of the American Fisheries Society* **138(2)**, 433-440.

Bromage, N., Porter, M. & Randall, C. (2001) The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture* **197**, 63–98

Brooks, G. A. (2018) The science and translation of lactate shuttle theory. *Cell metabolism* **27(4)**, 757-785.

Broom, D. M. (1986) Indicators of poor welfare. *British veterinary journal* **142(6)**, 524-526.

Broom, D. M. (2016) Fish brains and behaviour indicate capacity for feeling pain. *Animal Sentience: An Interdisciplinary Journal on Animal Feeling* **1(3)**, 4.

Brown, C. (2015) Fish intelligence, sentience and ethics. *Animal Cognition* **18**, 1-17.

Brown, C., Laland, K. & Krause, J. (2011) Fish Cognition and Behaviour. John Wiley & Sons, Oxford, UK.

Brudeseth, B. E., Wiulsrød, R., Fredriksen, B. N., Lindmo, K., Løkling, K. E., Bordevik, M., Steine, N., Klevan, A. & Gravningen, K. (2013) Status and future perspectives of vaccines for industrialised fin-fish farming. *Fish & shellfish immunology* **35(6)**, 1759-1768.

Bruno, D. W., Noguera, P. A. & Poppe, T. T. (2013) A colour atlas of salmonid diseases (2nd Ed.). Springer Science & Business Media. 211pp

Bry, C. (1982) Daily variations in plasma cortisol levels of individual female rainbow trout *Salmo gairdneri*: evidence for a post-feeding peak in well-adapted fish. *General and comparative endocrinology* **48(4)**, 462-468.

Bucking, C. & Wood, C. M. (2008). The alkaline tide and ammonia excretion after voluntary feeding in freshwater rainbow trout. *Journal of Experimental Biology* **211(15)**, 2533-2541.

Bui, S., Oppedal, F., Korsøen, Ø. J., Sonny, D. & Dempster, T. (2013) Group behavioural responses of Atlantic salmon (*Salmo salar* L.) to light, infrasound and sound stimuli. *PLOS ONE* **8**, e63696

Bui, S., Dempster, T., Remen, M. & Oppedal, F. (2016) Effect of ectoparasite infestation density and life-history stages on the swimming performance of Atlantic salmon *Salmo salar*. *Aquaculture Environment Interactions* **8**, 387-395.

Burgetz, I. J., Rojas-Vargas, A. N. I. B. A. L., Hinch, S. G. & Randall, D. J. (1998) Initial recruitment of anaerobic metabolism during sub-maximal swimming in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* **201(19)**, 2711-2721.

Burnley, T. A., Stryhn, H., Burnley, H. J. & Hammell, K. L. (2010) Randomized clinical field trial of a bacterial kidney disease vaccine in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **33**, 545-557.

Bury, N. R. & Sturm, A. (2007) Evolution of the corticosteroid receptor signalling pathway in fish. *General and comparative endocrinology* **153(1-3)**, 47-56.

Cahu, C., Infante, J. Z. & Takeuchi, T. (2003) Nutritional components affecting skeletal development in fish larvae. *Aquaculture* **277**, 245-248.

Caldwell, C. A. & Hinshaw, J. (1994) Physiological and haematological responses in rainbow trout subjected to supplemental dissolved oxygen in fish culture. *Aquaculture* **126(1-2)**, 183-193.

Caldwell, C. A. & Hinshaw, J. M. (1995) Communications: Tolerance of Rainbow Trout to dissolved oxygen supplementation and a *Yersinia ruckeri* challenge. *Journal of Aquatic Animal Health* **7(2)**, 168-171.

Camargo, J. A., Alonso, A. & Salamanca, A. (2005) Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere* **58(9)**, 1255-1267.

Canadian Council of Ministers of the Environment (2012a) Canadian Water Quality Guidelines: Nitrate Ion. Scientific Criteria Document.

Canadian Council of Ministers of the Environment (2012b) Canadian Water Quality Guidelines: Total particulate matter. Scientific Criteria Document.

Cañon Jones, H. A., Noble, C., Damsgård, B. & Pearce, G. P. (2017) Evaluating the effects of a short-term feed restriction period on the behavior and welfare of Atlantic salmon, *Salmo salar*, parr using social network analysis and fin damage. *Journal of the World Aquaculture Society* **48**, 35-45.

Carballo, M. & Muñoz, M. J. (1991) Effect of sublethal concentrations of four chemicals on susceptibility of juvenile rainbow trout (*Oncorhynchus mykiss*) to *saprolegniosis*. *Applied and environmental microbiology* **57(6)**, 1813-1816.

Carballo, M., Munoz, M. J., Cuellar, M. & Tarazona, J. V. (1995) Effects of waterborne copper, cyanide, ammonia, and nitrite on stress parameters and changes in susceptibility to saprolegniosis in rainbow trout (*Oncorhynchus mykiss*). *Applied and Environmental Microbiology* **61(6)**, 2108-2112.

Carragher, J. F. & Sumpter, J. P. (1991) The mobilization of calcium from calcified tissues of rainbow trout (*Oncorhynchus mykiss*) induced to synthesize vitellogenin. *Comparative Biochemistry and Physiology* **99**, 169-172.

Castro, R. & Tafalla, C. (2015) Overview of fish immunity. In: *Mucosal health in Aquaculture.* Beck, B. H. & Peatman, E. (eds.). Academic Press, Oxford, UK. p. 3-55.

Chandroo, K. P., Duncan, I. J. H. & Moccia, R. D. (2004a) Can fish suffer?: perspectives on sentience, pain, fear and stress. *Applied Animal Behaviour Science* **86**, 225-250.

Chandroo, K. P., Yue, S. & Moccia, R. D. (2004b) An evaluation of current perspectives on consciousness and pain in fishes. *Fish and Fisheries* **5**, 281-295.

Chapman, J. M., Proulx, C. L., Veilleux, M. A., Levert, C., Bliss, S., André, M. È., Lapointe, N. W. & Cooke, S. J. (2014) Clear as mud: a meta-analysis on the effects of sedimentation on freshwater fish and the effectiveness of sediment-control measures. *Water research* **56**, 190-202.

Chervova, L. (1997) Pain sensitivity and behavior of fishes. *Journal of Ichthyology* **37**, 98-102.

Chettri, J. K., Skov, J., Jaafar, R. M., Krossøy, B., Kania, P. W., Dalsgaard, I. & Buchmann, K. (2015) Comparative evaluation of infection methods and environmental factors on challenge success: *Aeromonas salmonicida* infection in vaccinated rainbow trout. *Fish & shellfish immunology* **44(2)**, 485-495.

Chin, A., Guo, F. C., Bernier, N. J. & Woo, P. T. (2004) Effect of *Cryptobia salmositica*-induced anorexia on feeding behavior and immune response in juvenile rainbow trout *Oncorhynchus mykiss*. *Diseases of aquatic organisms* **58(1)**, 17-26.

Cho, C. Y. (1992) Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. *Aquaculture* **100(1-3)**, 107-123.

Choi, Y. J., Kim, N. N. & Choi, C. Y. (2015) Profiles of hypothalamus–pituitary–interrenal axis gene expression in the parr and smolt stages of rainbow trout, *Oncorhynchus mykiss*: Effects of recombinant aquaporin 3 and seawater acclimation. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **182**, 14-21.

Choo, P. S., Smith, T. K., Cho, C. Y. & Ferguson, H. W. (1991) Dietary excesses of leucine influence growth and body composition of rainbow trout. *The Journal of nutrition* **121(12)**, 1932-1939.

Christiansen, J. S. & Jobling, M. (1990) The behaviour and the relationship between food intake and growth of juvenile Arctic charr, *Salvelinus alpinus* L., subjected to sustained exercise. *Canadian Journal of Zoology* **68(10)**, 2185-2191.

Colson, V., Mure, A., Valotaire, C., Le Calvez, J. M., Goardon, L., Labbe, L., Leguen, I. & Prunet, P. (2019) A novel emotional and cognitive approach to welfare phenotyping in rainbow trout exposed to poor water quality. *Applied animal behaviour science* **210**, 103-112.

Colt, J. (1986) Gas supersaturation—impact on the design and operation of aquatic systems. *Aquacultural Engineering* **5(1)**, 49-85.

Coutant, C. C. (1977) Compilation of temperature preference data. *Journal of the Fisheries Board of Canada* **34(5)**, 739-745.

Coyne, R., Smith, P., Dalsgaard, I., Nilsen, H., Kongshaug, H., Bergh, Ø. & Samuelsen, O. (2006) Winter ulcer disease of post-smolt Atlantic salmon: An unsuitable case for treatment? *Aquaculture* **253(1)**, 171-178.

Crisp, D. T. (1996) Environmental requirements of common riverine European salmonid fish species in fresh water with particular reference to physical and chemical aspects. *Hydrobiologia* **323(3)**, 201-221.

Dabrowski, K., El-Fiky, N., Köck, G., Frigg, M. & Wieser, W. (1990) Requirement and utilization of ascorbic acid and ascorbic sulfate in juvenile rainbow trout. *Aquaculture* **91**, 317-337.

Danley, M. L., Kenney, P. B., Mazik, P. M., Kiser, R. & Hankins, J. A. (2005) Effects of carbon dioxide exposure on intensively cultured rainbow trout *Oncorhynchus mykiss*: physiological responses and fillet attributes. *Journal of the World Aquaculture Society* **36(3)**, 249-261.

Davidson, J., Good, C., Welsh, C. & Summerfelt, S. T. (2011) Abnormal swimming behavior and increased deformities in rainbow trout *Oncorhynchus mykiss* cultured in low exchange water recirculating aquaculture systems. *Aquacultural engineering* **45(3)**, 109-117.

Davidson, J., Good, C., Welsh, C. & Summerfelt, S. T. (2014) Comparing the effects of high vs. low nitrate on the health, performance, and welfare of juvenile rainbow trout *Oncorhynchus mykiss* within water recirculating aquaculture systems. *Aquacultural Engineering* **59**, 30-40.

Davies, B. & Bromage, N. (2002) The effects of fluctuating seasonal and constant water temperatures on the photoperiodic advancement of reproduction in female rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **205(1-2)**, 183-200.

Davis, J. C. (1975) Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. *Journal of the Fisheries Board of Canada* **32(12)**, 2295-2332.

Davis, K. B. (2006) Management of Physiological Stress in Finfish Aquaculture. *North American Journal of Aquaculture* **68**, 116–121.

Davis, M. W. (2010) Fish stress and mortality can be predicted using reflex impairment. *Fish and Fisheries* **11**, 1-11.

Dawkins, M. S. (1983) Battery hens name their price: Consumer demand theory and the measurement of ethological 'needs'. *Animal Behaviour* **31**, 1195-1205.

Dawkins, M. S. (1990) From an animal's point of view: Motivation, fitness, and animal welfare. *Behavioral and Brain Sciences* **13**, 1-9.

De Mercado, E., Larrán, A. M., Pinedo, J. & Tomás-Almenar, C. (2018) Skin mucous: a new approach to assess stress in rainbow trout. *Aquaculture* **484**, 90-97.

Del-Pozo, J., Crumlish, M., Turnbull, J. F. & Ferguson, H.W. (2010) Histopathology and ultrastructure of segmented filamentous bacteria-associated rainbow trout gastroenteritis. *Veterinary Pathology* **47**, 220-230.

Dempster, T., Korsøen, Ø., Folkedal, O., Juell, J. E., & Oppedal, F. (2009). Submergence of Atlantic salmon (*Salmo salar* L.) in commercial scale sea-cages: a potential short-term solution to poor surface conditions. *Aquaculture* **288(3)**, 254-263.

Deschamps, M. H. & Sire, J. Y. (2010) Histomorphometrical studies of vertebral bone condition in farmed rainbow trout, *Oncorhynchus mykiss*. *Journal of Applied Ichthyology* **26(2)**, 377-380.

Deschamps, M. H., Kacem, A., Ventura, R., Courty, G., Haffray, P., Meunier, F. J. & Sire, J. Y. (2008) Assessment of "discreet" vertebral abnormalities, bone mineralization and bone compactness in farmed rainbow trout. *Aquaculture* **279(1-4)**, 11-17.

Divanach, P., Papandroulakis, N., Anastasiadis, P., Koumoundouros, G. & Kentouri, M. (1997) Effect of water currents on the development of skeletal deformities in sea bass (*Dicentrarchus Zabrax* L.) with functional swimbladder during postlarval and nursery phase. *Aquaculture* **156**, 145-155.

Doblander, C. & Lackner, R. (1997) Oxidation of nitrite to nitrate in isolated erythrocytes: a possible mechanism for adaptation to environmental nitrite. *Canadian Journal of Fisheries and Aquatic Sciences* **54(1)**, 157-161.

Dumas, A., France, J. & Bureau, D. P. (2007) Evidence of three growth stanzas in rainbow trout (*Oncorhynchus mykiss*) across life stages and adaptation of the thermal-unit growth coefficient. *Aquaculture* **267(1-4)**, 139-146.

Duncan, I. J. H. (1993) Welfare is to do with what animals feel. *Journal of agricultural and environmental ethics* **6 (Suppl. 2)**, 8-14.

Duncan, I. J. H. (1996) Animal welfare defined in terms of feelings. *Acta Agriculturae Scandinavica. Section A. Animal Science. Supplementum* **27**, 29–35.

Duncan, I. J. H. (2005) Science-based assessment of animal welfare: farm animals. *Revue Scientifique et Technique (International Office of Epizootics)* **24(2)**, 483–92.

Easy, R. H. & Ross, N. W. (2009) Changes in Atlantic salmon (*Salmo salar*) epidermal mucus protein composition profiles following infection with sea lice (*Lepeophtheirus salmonis*). *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics* **4(3)**, 159-167.

Easy, R. H. & Ross, N. W. (2010) Changes in Atlantic salmon *Salmo salar* mucus components following short-and long-term handling stress. *Journal of fish biology* **77(7)**, 1616-1631.

Ebbesson, L. O., Ebbesson, S. O., Nilsen, T. O., Stefansson, S. O. & Holmqvist, B. (2007) Exposure to continuous light disrupts retinal innervation of the preoptic nucleus during parr–smolt transformation in Atlantic salmon. *Aquaculture* **273(2)**, 345-349.

Edsall, D. A. & Smith, C. E. (1991) Oxygen-induced gas bubble disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* **22(2)**, 135-140.

Edwards, S. L. & Marshall, W. S. (2013) Principles and patterns of osmoregulation and euryhalinity in fishes. In: McCormick, S. D., Farrell, A. P., Brauner, C. J. (Eds.), Euryhaline Fishes. Academic Press, USA, pp. 2-46.

EFSA (2008a) Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on Animal welfare aspects of husbandry systems for farmed Atlantic salmon. *The EFSA Journal* **736**, 1-31

EFSA (2008b) Scientific Opinion of the Panel on Animal Health and Animal Welfare on a request from the European Commission on the Animal welfare aspects of husbandry systems for farmed trout. *The EFSA Journal* **796**, 1-22.

EFSA (2009a) Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on welfare aspect of the main systems of stunning and killing of farmed Atlantic salmon. *The EFSA Journal* **1012**, 1-77.

EFSA (2009b) Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on Species-specific welfare aspects of the main systems of stunning and killing of farmed rainbow trout. *The EFSA Journal* **1013**, 1-55

Elliott, D. G. (2011) The skin. Functional Morphology of the Integumentary System in Fishes A2 - Farrell, Anthony P, Encyclopedia of Fish Physiology. Academic Press, San Diego, pp. 476-488.

Elliott, J. M. & Elliott, J. A. (1995) The effect of the rate of temperature increase on the critical thermal maximum for parr of Atlantic salmon and brown trout. *Journal of Fish Biology* **47(5)**, 917-919.

Ellis, A. (1997) Vaccines for farmed fish. In: *Veterinary vaccinology*. Pastoret, P. P., Blanco, J., Vanier P. & Verschueren, C. (eds.). Elsevier Press, Amsterdam, The Netherlands, 411-417.

Ellis, R. P., Urbina, M. A. & Wilson, R. W. (2017) Lessons from two high CO_2 worlds–future oceans and intensive aquaculture. *Global change biology* **23(6)**, 2141-2148.

Ellis, T., North, B., Scott, A. P., Bromage, N. R., Porter, M. & Gadd, D. (2002) The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology* **61(3)**, 493-531.

Ellis, T., Oidtmann, B., St-Hilaire, S., Turmbull, J. F., North, B. P., MacIntyre, C. M., Nikolaidis, J., Hoyle, I., Kestin, S. C. & Knowles, T. G. (2008) Fin erosion in farmed fish. In: *Fish welfare*. Branson E. J. (ed.). Blackwell Publishing. pp. 121–149.

Ellis, T., Berrill, I., Lines, J., Turnbull, J. F. & Knowles, T. G. (2012a) Mortality and fish welfare. *Fish physiology and biochemistry* **38(1)**, 189-199.

Ellis, T., Yildiz, H. Y., López-Olmeda, J., Spedicato, M. T., Tort, L., Øverli, Ø. & Martins, C. I. (2012b) Cortisol and finfish welfare. *Fish physiology and biochemistry* **38(1)**, 163-188.

Ellis, T., Sanders, M.B. & Scott, A.P. (2013) Non-invasive monitoring of steroids in fishes. *Wiener Tierarztliche Monatsschrift* **100**, 255-269.

Eriksen, M. S., Espmark, Å., Braastad, B. O., Salte, R. & Bakken. M., (2007) Long-term effects of maternal cortisol exposure and mild hyperthermia during embryogeny on survival, growth and morphological anomalies in farmed Atlantic salmon *Salmo salar* offspring. *Journal of Fish Biology* **70**, 462-473.

Erikson, U., Gansel, L., Frank, K., Svendsen, E. & Digre, H. (2016) Crowding of Atlantic salmon in net-pen before slaughter. *Aquaculture* **465**, 395-400.

Ersdal, C., Midtlyng, P.J. & Jarp, J. (2001) An epidemiological study of cataracts in seawater farmed Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* **45**, 229–236.

Evans, D. H. (2008) Teleost fish osmoregulation: what have we learned since August Krogh, Homer Smith, and Ancel Keys. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* **295**, R704-R713.

Evans, D. H. & Hyndman, K. A. (2006) Paracrine control of fish gill perfusion and epithellal transport. *Journal of Experimental Zoology Part a-Comparative Experimental Biology* **305A**, 125-125.

Evans, D. H., Piermarini, P. M. & Choe, K. P. (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews* **85**, 97-177.

Evans, D. H. C., Claiborne, J. B. & Currie, S. (2006) The physiology of fishes. CRC Taylor & Francis.

Evensen, O. (2009) Development in fish vaccinology with focus on delivery methodologies, adjuvants and formulations. *Options Mediterraneennes Serie A, Seminaires Mediterraneens* **86**, 177–186.

Farbridge, K. J. & Leatherland, J. F. (1992) Plasma growth hormone levels in fed and fasted rainbow trout (*Oncorhynchus mykiss*) are decreased following handling stress. *Fish Physiology and Biochemistry* **10(1)**, 67-73.

Farrell, A. P. (2007) Cardiorespiratory performance during prolonged swimming tests with salmonids: a perspective on temperature effects and potential analytical pitfalls. *Philosophical Transactions of the Royal Society B: Biological Sciences* **362(1487)**, 2017-2030.

Farrell, A. P., Johansen, J. A., Suarez, R. K. (1991) Effects of exercise-training on cardiac performance and muscle enzymes in rainbow trout, *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry* **9**, 303–312.

Fast, M. D., Ross, N. W., Mustafa, A., Sims, D. E., Johnson, S. C., Conboy, G. A., Speare, D. J., Johnson, G. R. & Burka, J. F. (2002a) Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*. *Diseases of aquatic organisms* **52(1)**, 57-68.

Fast, M. D., Sims, D. E., Burka, J. F., Mustafa, A. & Ross, N. W. (2002b) Skin morphology and humoral non-specific defence parameters of mucus and plasma in rainbow trout, coho and Atlantic salmon. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **132(3)**, 645-657.

Ferguson, H. W., Speare, D. J. (2006) Gills and Pseudobranchs. In: *Systemic Pathology of Fish: A Text and Atlas of Normal Tissues in Teleosts and their Responses in Disease*. Ferguson, H. W. (ed). Scotian Press, London. 25–63.

Fiess, J. C., Kunkel-Patterson, A., Mathias, L., Riley, L. G., Yancey, P. H., Hirano, T. & Grau, E. G. (2007) Effects of environmental salinity and temperature on osmoregulatory ability, organic osmolytes, and plasma hormone profiles in the Mozambique tilapia (*Oreochromis mossambicus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **146(2)**, 252-264.

Finstad, B., Staurnes, M. & Reite, O. B. (1988) Effect of low temperature on sea-water tolerance in rainbow trout, *Salmo gairdneri*. *Aquaculture* **72(3-4)**, 319-328.

Finstad, B., Bjørn, P. A., Todd, C. D., Whoriskey, F., Gargan, P. G., Forde, G. & Revie, C. W. (2011) The effect of sea lice on Atlantic salmon and other salmonid species. In: *Atlantic Salmon Ecology*. Aas, Ø., Einum, S., Klemetsen, A., Skurdal, J. (Eds.), Blackwell Publishing, Oxford. 253–276.

Fitzsimmons, S. D. & Perutz, M. (2006) Effects of egg incubation temperature on survival, prevalence and types of malformations in vertebral column of Atlantic Cod (*Gadus morhua*) larvae. *Bulletin-European Association of Fish Pathologists* **26**, 80-86.

Fivelstad, S. (2013) Long-term carbon dioxide experiments with salmonids. *Aquacultural Engineering* **53**, 40-48.

Fivelstad, S., Haavik, H., Løvik, G. & Olsen, A. B. (1998) Sublethal effects and safe levels of carbon dioxide in seawater for Atlantic salmon postsmolts (*Salmo salar* L.): ion regulation and growth. *Aquaculture* **160(3)**, 305-316.

Fivelstad, S., Olsen, A. B., Kløften, H., Ski, H. & Stefansson, S. (1999) Effects of carbon dioxide on Atlantic salmon (*Salmo salar* L.) smolts at constant pH in bicarbonate rich freshwater. *Aquaculture* **178(1)**, 171-187.

Fivelstad, S., Waagbø, R., Zeitz, S. F., Hosfeld, A. C. D., Olsen, A. B., & Stefansson, S. (2003) A major water quality problem in smolt farms: combined effects of carbon dioxide, reduced pH and aluminium on Atlantic salmon (*Salmo salar* L.) smolts: physiology and growth. *Aquaculture* **215(1)**, 339-357.

Fivelstad, S., Waagbø, R., Stefansson, S. & Olsen, A. B. (2007) Impacts of elevated water carbon dioxide partial pressure at two temperatures on Atlantic salmon (*Salmo salar* L.) parr growth and haematology. *Aquaculture* **269(1)**, 241-249.

Fjelldal, P. G. & Hansen, T. J. (2010) Vertebral deformities in triploid Atlantic salmon (*Salmo salar* L.) underyearling smolts. *Aquaculture* **309**, 131-136.

Fjelldal, P. G., Hansen, T., Breck, O., Sandvik, R., Waagbø, R., Berg, A. & Ørnsrud, R. (2009a) Supplementation of dietary minerals during the early seawater phase increase vertebral strength and reduce the prevalence of vertebral deformities in fast-growing under-yearling Atlantic salmon (*Salmo salar* L.) smolt. *Aquaculture Nutrition* **15(4)**, 366-378.

Fjelldal, P. G., van der Meeren, T., Jørstad, K.E. & Hansen, T.J. (2009b) A radiological study on vertebral deformities in cultured and wild Atlantic cod (*Gadus morhua*, L.). *Aquaculture* **289**, 6-12.

Fleming, I. A. (1998) Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. *Canadian journal of fisheries and aquatic science*, **55(S1)**, 59-76.

Fleming, I. A. & Reynolds, J. D. (2004) Salmonid breeding systems. In: *Evolution illuminated, Salmon and their relatives.* Hendry, A. P., Stearns, S. C. (Eds.). Oxford University Press, Oxford, UK. 264-294.

Folkedal, O., Torgersen, T., Nilsson, J. & Oppedal, F. (2010) Habituation rate and capacity of Atlantic salmon (*Salmo salar*) parr to sudden transitions from darkness to light. *Aquaculture* **307**, 170-172.

Folkedal, O., Torgersen, T., Olsen, R. E., Fernö, A., Nilsson, J., Oppedal, F., Stien, L. H. & Kristiansen, T. S. (2012a) Duration of effects of acute environmental changes on food anticipatory behaviour, feed intake, oxygen consumption, and cortisol release in Atlantic salmon parr. *Physiology & Behavior* **105**, 283–291.

Folkedal, O., Stien, L. H., Torgersen, T., Oppedal, F., Olsen, R. E., Fosseidengen, J. E., Braithwaite, V. A. & Kristiansen, T. S. (2012b) Food anticipatory behaviour as an indicator of stress response and recovery in Atlantic salmon post-smolt after exposure to acute temperature fluctuation. *Physiology & Behavior* **105**, 350-356.

Folkedal, O., Stien, L. H., Nilsson, J., Torgersen, T., Fosseidengen, J. E. & Oppedal, F. (2012c) Sea caged Atlantic salmon display size-dependent swimming depth. *Aquatic Living Resources* **25**, 143–149.

Folkedal, O., Pettersen, J. M., Bracke, M. B. M., Stien, L. H., Nilsson, J., Martins, C., Breck, O., Midtlyng, P. J. & Kristiansen, T. S. (2016) On-farm evaluation of the Salmon Welfare Index Model (SWIM 1.0) - Theoretical and practical considerations. *Animal Welfare* **25**, 135-149.

Fontagné, S. (2009) The impact of nutritional components of rainbow trout. In: *Control of malformations in fish aquaculture, Science and practice (FineFish)*. Baeverfjord, G., Helland, S. & Hough, C. (eds.). pp. 149.

Fontagné, S., Silva, N., Bazin, D., Ramos, A., Aguirre, P., Surget, A., Abrantes, A., Kaushik, S. J. & Power, D.M. (2009) Effects of dietary phosphorus and calcium level on growth and skeletal development in rainbow trout (*Oncorhynchus mykiss*) fry. *Aquaculture* **297**, 141-150.

Fosseidengen, J. K., Boge, E. & Huse, I. (1982) A survey with rainbow trout and salmon in submersible cages. *Norsk Fiskeoppdrett* **10**, 24-25.

Fraser, T. W. K., Fjeldall, P. G., Hansen, T. & Mayer, I. (2012) Welfare considerations in of triploid fish. *Reviews in Fisheries Science* **20**, 192-211.

Fraser, T. W. K., Hansen, T., Fleming, M. S. & Fjelldal, P. G. (2015) The prevalence of vertebral deformities is increased with higher egg incubation temperatures and triploidy in Atlantic salmon *Salmo salar* L. *Journal of Fish Diseases* **38**, 75-89.

Fry, F. E. J. (1971) The effect of environmental factors on the physiology of fish. *Fish Physiology* **6**, 1-98.

Furevik, D. M., Bjordal, Å., Huse, I. & Fernö, A. (1993) Surface activity of Atlantic salmon (*Salmo salar* L.) in net pens. *Aquaculture* **110**, 119-128.

Gansel, L. C., Rackebrandt, S., Oppedal, F. & McClimans, T. A. (2014) Flow fields inside stocked fish cages and the near environment. *Journal of Offshore Mechanics and Arctic Engineering* **136(3)**, 031201.

Gismervik, K., Østvik, A. & Viljugrein, H. (2016) Pilotflåte Helixir - dokumentasjon av fiskevelferd og effekt mot lus. Del 1 uten legemiddel. *Rapport 15, 2016. Veterinærinstituttets rapportserie*. Veterinærinstituttet, Oslo, Norge.

Gismervik, K., Nielsen, K. V., Lind, M. B. & Viljugrein, H. (2017) Mekanisk avlusing med FLS-avlusersystem- dokumentasjon av fiskevelferd og effekt mot lus. In: *Veterinærinstituttets rapportserie 6-2017*. Veterinærinstituttet, Oslo, pp. 41.

Glencross, B. D. (2009) Reduced water oxygen levels affect maximal feed intake, but not protein or energy utilization efficiency of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition* **15(1)**, 1-8.

GLOBALG.A.P. (2019) The GLOBALG.A.P. Aquaculture Standard. https://www.globalgap.org/uk_en/for-producers/globalg.a.p./integrated-farm-assurance-ifa/aquaculture/ (accessed 2019).

González, L. & Carvajal, J. (2003) Life cycle of *Caligus rogercresseyi*, (Copepoda: Caligidae) parasite of Chilean reared salmonids. *Aquaculture* **220(1-4)**, 101-117.

Good, C., Davidson, J., Welsh, C., Snekvik, K. & Summerfelt, S. (2010). The effects of carbon dioxide on performance and histopathology of rainbow trout *Oncorhynchus mykiss* in water recirculation aquaculture systems. *Aquacultural Engineering* **42(2)**, 51-56.

Gorman, K. F. & Breden, F. (2007) Teleosts as models for human vertebral stability and deformity. *Comparative Biochemistry & Physiology Part C* **145**, 28-38.

Gregory, T. R. & Wood, C. M. (1999) The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiological and Biochemical Zoology* **72(3)**, 286-295.

Grobler, J. M. & Wood, C. M. (2018) The effects of high environmental ammonia on the structure of rainbow trout hierarchies and the physiology of the individuals therein. *Aquatic Toxicology* **195**, 77-87.

Grove, D. J., Loizides, L. G. & Nott, J. (1978) Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. *Journal of Fish Biology* **12(5)**, 507-516.

Grøntvedt, R. N., Nerbøvik, I. -K. G, Viljugrein, H., Lillehaug, A., Nilsen, H. & Gjerve, A. –G. (2015) Termisk avlusing av laksefisk – dokumentasjon av fiskevelferd og effekt. *Rapport 13, 2015. Veterinærinstituttets rapportserie*. Veterinærinstituttet, Oslo, Norge.

Gültepe, N., Ateş, O., Hisar, O. & Beydemir, Ş. (2011) Carbonic anhydrase activities from the rainbow trout lens correspond to the development of acute gas bubble disease. *Journal of aquatic animal health* **23(3)**, 134-139.

Hafs, A. W., Mazik, P. M., Kenney, P. B. & Silverstein, J. T. (2012) Impact of carbon dioxide level, water velocity, strain, and feeding regimen on growth and fillet attributes of cultured rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **350**, 46-53.

Halver, J. E., Ashley, L. M. & Smith, R. R. (1969) Ascorbic acid requirements of coho salmon and rainbow trout. *Transactions of the American Fisheries Society* **98(4)**, 762-771.

Hammer, C. (1995) Fatigue and exercise tests with fish. *Comparative Biochemistry and Physiology Part A: Physiology* **112(1)**, 1-20.

Handeland, S. O., Berge, Å., Björnsson, B. T. & Stefansson, S. O. (1998) Effects of temperature and salinity on osmoregulation and growth of Atlantic salmon (*Salmo salar* L.) smolts in seawater. *Aquaculture* **168(1)**, 289-302.

Handeland, S. O., Berge, Å., Björnsson, B. T., Lie, Ø., Stefansson, S. O. (2000) Seawater adaptation by out-of-season Atlantic salmon (*Salmo salar* L.) smolts at different temperatures. *Aquaculture* **181**, 377-396.

Hansen, T., Stefansson, S. & Taranger, G. L. (1992) Growth and sexual maturation in Atlantic salmon, *Salmo salar* L., reared in sea cages at two different light regimes. *Aquaculture Research* **23(3)**, 275-280.

Hansen, T., Fjelldal, P. G., Yurtseva, A. & Berg, A. (2010) A possible relation between growth and number of deformed vertebrae in Atlantic salmon (*Salmo salar* L.). *Journal of Applied Ichthyology* **26(2)**, 355-359.

Hansen, T. J., Fjelldal, P. G., Folkedal, O., Vågseth, T. & Oppedal, F. (2017) Effects of light source and intensity on sexual maturation, growth and swimming behaviour of Atlantic salmon in sea cages. *Aquaculture Environment Interactions* **9**, 193-204.

Hauge, H., Vendramin, N., Taksdal, T., Olsen, A. B., Wessel, Ø., Mikkelsen, S. S., Alencar, A. L. F., Olesen, N. J. & Dahle, M. K. (2017) Infection experiments with novel Piscine orthoreovirus from rainbow trout (*Oncorhynchus mykiss*) in salmonids. *PloS one* **12(7)**, e0180293.

Havas, M. & Rosseland, B. O. (1995) Response of zooplankton, benthos, and fish to acidification: an overview. *Water, Air, & Soil Pollution* **85(1)**, 51-62.

Heming, T. A. & Paleczny, E. J. (1987) Compositional changes in skin mucus and blood serum during starvation of trout. *Aquaculture* **66(3-4)**, 265-273.

Henriksen, A., Skogheim, O. K. & Rosseland, B. O. (1984) Episodic changes in pH and aluminium-speciation kill fish in a Norwegian salmon river. *Vatten* **40**, 255-260.

Hevrøy, E. M., Boxaspen, K., Oppedal, F., Taranger, G. L. & Holm, J. C. (2003) The effect of artificial light treatment and depth on the infestation of the sea louse Lepeophtheirus salmonis on Atlantic salmon (*Salmo salar* L.) culture. *Aquaculture* **220(1)**, 1-14.

Hjeltnes, B., Bæverfjord, G., Erikson, U., Mortensen, S., Rosten, T. & Østergård, P. (2012) Risk assessment of recirculating systems in Salmonid hatcheries. Norwegian Scientific Committee for Food Safety (VKM), Doc, (09-808).

Hjeltnes, B., Walde, C., Bang Jensen, B. & Haukaas, A. (Eds) (2016). Fiskehelserapporten 2015. *Veterinærinstituttet*, p 74.

Hjeltnes, B., Bornø, G., Jansen, M. D., Haukaas, A., & Walde, C (Eds) (2017). Fiskehelserapporten 2016. Oslo*, Veterinærinstituttet,* p. 121.

Hofer, R., & Gatumu, E. (1994). Necrosis of trout retina (*Oncorhynchus mykiss*) after sublethal exposure to nitrite. *Archives of environmental contamination and toxicology* **26(1)**, 119-123.

Holmer, M. (2010) Environmental issues of fish farming in offshore waters: perspectives, concerns and research needs. *Aquaculture Environment Interactions* **1**, 57-70.

Holst, J. C., Jakobsen, P., Nilsen, F., Holm, M., Asplin, L. & Aure, J. (2003) Mortality of seaward-migrating post-smolts of Atlantic salmon due to salmon lice infection in Norwegian salmon stocks. In: *Salmon at the Edge.* Mills, D. (ed.). Blackwell Science, Oxford. 136–137.

Holten-Andersen, L., Dalsgaard, I., Nylén, J., Lorenzen, N. & Buchmann, K. (2012) Determining vaccination frequency in farmed rainbow trout using *Vibrio anguillarum* O1 specific serum antibody measurements. *PloS one* **7(11)**, e49672.

Hosfeld, C. D., Engevik, A., Mollan, T., Lunde, T. M., Waagbø, R., Olsen, A. B., Breck, O., Stefansson, S. & Fivelstad, S. (2008) Long-term separate and combined effects of environmental hypercapnia and hyperoxia in Atlantic salmon (*Salmo salar* L.) smolts. *Aquaculture* **280(1)**, 146-153.

Hosfeld, C. D., Hammer, J., Handeland, S. O., Fivelstad, S. & Stefansson, S. O. (2009) Effects of fish density on growth and smoltification in intensive production of Atlantic salmon (*Salmo salar* L.). *Aquaculture* **294(3)**, 236-241.

Hosfeld, C. D., Handeland, S. O., Fivelstad, S. & Stefansson, S. O. (2010) Physiological effects of normbaric environmental hyperoxia on Atlantic salmon (*Salmo salar* L.) presmolts. *Aquaculture* **308(1-2)**, 28-33.

Hoskonen, P. & Pirhonen, J. (2006) Effects of repeated handling, with or without anaesthesia, on feed intake and growth in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* **37(4)**, 409-415.

Houlihan, D. F. & Laurent, P. (1987) Effects of exercise training on the performance, growth, and protein turnover of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* **44(9)**, 1614-1621.

Hoseinifar, S. H., Mirvaghefi, A., Amoozegar, M. A., Sharifian, M. & Esteban, M. Á. (2015) Modulation of innate immune response, mucosal parameters and disease resistance in rainbow trout (*Oncorhynchus mykiss*) upon synbiotic feeding. *Fish & shellfish immunology* **45(1)**, 27-32.

Howes, G.B. (1894) On synostosis and curvature of the spine in fishes, with special reference to the sole. *Proceedings of the Zoological Society of London* 95-101.

Hulland, T. J. (1992) Muscles-II. General Reactions of Muscle. In: *Pathology of Domestic Animals: v.1 (Pathology of Domestic Animals Series)*. Jubb, K. V. F., Kennedy, P. C. & Palmer, N. (Eds). Academic Press Inc., London, UK, 190-191.

Huntingford, F. & Adams, C. (2005) Behavioural syndromes in farmed fish: implications for production and welfare. *Behaviour* **142(9-10)**, 1207-1221.

Huntingford, F. A., & Kadri, S. (2009) Taking account of fish welfare: lessons from aquaculture. *Journal of Fish Biology* **75(10)**, 2862-2867.

Huntingford, F. A., & Kadri, S. (2014) Defining, assessing and promoting the welfare of farmed fish. *Revue scientifique et technique (International Office of Epizootics)* **33(1)**, 233-244.

Huntingford, F. A., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandøe, P. & Turnbull, J. F. (2006). Current issues in fish welfare. *Journal of fish biology* **68(2)**, 332-372.

Ihssen, P. E. (1986) Selection of fingerling rainbow trout for high and low tolerance to high temperature. *Aquaculture* **57(1-4)**, 370.

Ip, Y. K., Chew, S. F. & Randall, D. J. (2001) Ammonia toxicity, tolerance and excretion. In: Wright, P.A., Anderson, P.M. (Eds.), Fish Physiology, vol. 20. Nitrogen excretion. Academic Press, San Diego, pp. 109–148.

Iversen, M. & Eliassen, R. A. (2009) The Effect of AQUI-S® Sedation on Primary, Secondary, and Tertiary Stress Responses during Salmon Smolt, *Salmo salar* L., Transport and Transfer to Sea. *Journal of the world aquaculture society* **40(2)**, 216-225.

Iversen, M. & Eliassen, R. (2012) Stressovervåkning av settefiskproduksjonen i Mainstream Norway AS 2009 - 2011. Stresskartlegging av laksesmolt (*Salmo salar* L.), og effekten av stressreduserende tiltak på stressnivå, dyrevelferd og produksjonsresultatet. *UiN-rapport nr 05/2012.* 54 pp.

Iversen, M. H. & Eliassen, R. A. (2014) The effect of allostatic load on hypothalamic-pituitary-interrenal (HPI) axis before and after secondary vaccination in Atlantic salmon postsmolts (*Salmo salar* L.). *Fish physiology and biochemistry* **40**, 527-538.

Iversen, M., Finstad, B., Nilssen, K. J. (1998) Recovery from loading and transport stress in Atlantic salmon (*Salmo salar* L.) smolts. *Aquaculture* **168**, 387-394.

Iversen, M., Eliassen, R. A. & Finstad, B. (2009) Potential benefit of clove oil sedation on animal welfare during salmon smolt, *Salmo salar* L. transport and transfer to sea. *Aquaculture Research* **40**, 233-241.

Iwata, M., Komatsu, S., Collie, N. L., Nishioka, R. S. & Bern, H. A. (1987) Ocular cataract and seawater adaptation in salmonids. *Aquaculture* **66**, 315–327.

Jackson, A. J. (1981) Osmotic regulation in rainbow trout (*Salmo gairdneri*) following transfer to sea water. *Aquaculture* **24**, 143-151.

Jackson, D. & Minchin, D. (1992) Aspects of the reproductive output of two caligid copepod species parasitic on cultivated salmon. *Invertebrate Reproduction & Development* **22**, 87-90.

Jackson, D., Deady, S., Leahy, Y. & Hassett, D. (1997) Variations in parasitic caligid infestations on farmed salmonids and implications for their management. *ICES Journal of Marine Science* **54(6)**, 1104-1112.

Jason, T., Quigley, J. T. & Hinch, S. G. (2006) Effects of rapid experimental temperature increases on acute physiological stress and behaviour of stream dwelling juvenile chinook salmon. *Journal of Thermal Biology* **31**, 429–441

Jensen, F. B. (2003) Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **135(1)**, 9-24.

Jensen, F. B., Andersen, N. A. & Heisler, N. (1987) Effects of nitrite exposure on blood respiratory properties, acid-base and electrolyte regulation in the carp (*Cyprinus carpio*). *Journal of Comparative Physiology B* **157(5)**, 533-541.

Jobling, M. (1994) Fish Bioenergetics. Chapman & Hall, London (1994) 309 pp.

Jobling, M. & Koskela, J. (1996) Interindividual variations in feeding and growth in rainbow trout during restricted feeding and in a subsequent period of compensatory growth. *Journal of Fish Biology* **49(4)**, 658-667.

Jobling, M., Alanärä, A., Noble, C., Sánches-Vázquez, J., Kadri, S. & Huntingford, F. (2012) Appetite and Feed Intake. In: *Aquaculture and Behavior.* Huntingford, F. A., Jobling, M., Kadri, S. (Eds.). Wiley-Blackwell, Oxford. ISBN: 978-1-4051-3089-9. 183-219.

Johansson, D., Laursen, F., Fernö, A., Fosseidengen, J. E., Klebert, P., Stien, L. H., Vågseth, T. & Oppedal, F. (2014) The interaction between water currents and salmon swimming behaviour in sea cages. *PloS one* **9(5)**, p.e97635.

Johansson, L., Kiessling, A., Kiessling, K. H. & Berglund, L. (2000) Effects of altered ration levels on sensory characteristics, lipid content and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). *Food Quality and Preference* **11(3)**, 247-254.

Johnsson, I. &.Clarke, W.C. (1988) Development of seawater adaptation in juvenile steelhead trout (Salmo gairdneri) and domesticated rainbow trout (*Salmo gairdneri*)—Effects of size, temperature and photoperiod. *Aquaculture* **71**, 247-263.

Johnston, C. E. & Cheverie, J. C. (1985) Comparative Analysis of lonoregulation in Rainbow Trout (*Salmo gairdneri*) of Different Sizes Following Rapid and Slow Salinity Adaptation. *Canadian Journal of Fisheries and Aquatic Sciences* **42(12)**, 1994-2003.

Jonsson, N. & Finstad, B. (1995) Sjøørret: økologi, fysiologi og atferd (Sea trout: ecology, physiology and behaviour). *NINA Fagrapport* 6 (1995), pp. 1–32 (In Norwegian with English summary)

Jovanović, V., Risojević, V., & Babić, Z. (2016) Splash detection in surveillance videos of offshore fish production plants. *2016 International Conference on Systems, Signals and Image Processing (IWSSIP)*, Bratislava, 23-25 May 2016

Jørgensen, E. H., Bernier, N. J., Maule, A. G. & Vijayan, M. M. (2016) Effect of long-term fasting and a subsequent meal on mRNA abundances of hypothalamic appetite regulators, central and peripheral leptin expression and plasma leptin levels in rainbow trout. *Peptides* **86**, 162-170.

Karlsbakk, E. (2015) Amøbisk gjellesykdom (AGD) – litt om den nye plagen. *Havforskningsrapporten* **2015**, 32-35.

Kause, A., Ritola, O., Paananen, T., Mäntysaari, E., & Eskelinen, U. (2003). Selection against early maturity in large rainbow trout *Oncorhynchus mykiss*: the quantitative genetics of sexual dimorphism and genotype-by-environment interactions. *Aquaculture* **228(1-4)**, 53-68.

Kaushik, S. J., Cravedi, J. P., Lalles, J. P., Sumpter, J., Fauconneau, B. & Laroche, M. (1995) Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **133(3)**, 257-274.

Kent, M. L., & Poppe, T. T. (2002). Infectious diseases of coldwater fish in marine and brackish water. In: *Diseases and disorders of finfish in cage culture*, (Eds: P. T. K. Woo, D. W. Bruno, and L. H. S. Lim. CABI Publishing, New York, 61-105.

Kent, M. L., Groff, J. M., Morrison, J. K., Yasutake, W. T. & Holt, R. A. (1989) Spiral swimming behavior due to cranial and vertebral lesions associated with *Cytophaga psychrophila* infections in salmonid fishes. *Diseases of Aquatic Organisms* **6**, 11-16.

Kestin, S. C. (1994) Pain and stress in fish. RSPCA, Horsham, West Sussex UK. 36 pp.

Kestin, S. C., Van de Vis, J. W. & Robb, D. H. F. (2002) Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them. *Veterinary Record* **150(10)**, 302-307.

Key, B. (2016) Why fish do not feel pain. *Animal Sentience* 2016.003

Khansari, A. R., Balasch, J. C., Vallejos-Vidal, E., Teles, M., Fierro-Castro, C., Tort, L. & Reyes-López, F. E. (2019) Comparative study of stress and immune-related transcript outcomes triggered by *Vibrio anguillarum* bacterin and air exposure stress in liver and spleen of gilthead seabream (*Sparus aurata*), zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology* **86**, 436-448.

Kiessling, A., Bjørnevik, M., Thomassen, M., Røra, M. B., Mørkøre, T., Roth, B., Erikson, U. & Jordheim, O. (2007) From Cage to Table. *Aquaculture Research: From Cage to Consumption*, 45-63.

Kiilerich, P., Milla, S., Sturm, A., Valotaire, C., Chevolleau, S., Giton, F., Terrien, X., Fiet, J., Fostier, A., Debrauwer, L. & Prunet, P. (2011) Implication of the mineralocorticoid axis in rainbow trout osmoregulation during salinity acclimation. *Journal of Endocrinology* **209**, 221-235.

Kincheloe, J. W., Wedemeyer, G. A. & Koch, D. L. (1979) Tolerance of developing salmonid eggs and fry to nitrate exposure. *Bulletin of Environmental Contamination and Toxicology* **23(1)**, 575-578.

Kitamura S., Ohara S., Suwa T. & Nakagawa K. (1965) Studies on vitamin requirements of rainbow trout. I. On the ascorbic acid. *Bulletin of the Japanese Society of Scientific Fisheries* **31**, 818-826.

Kjelland, M. E., Woodley, C. M., Swannack, T. M. & Smith, D. L. (2015) A review of the potential effects of suspended sediment on fishes: potential dredging-related physiological, behavioral, and transgenerational implications. *Environment Systems and Decisions* **35(3)**, 334-350.

Kluger, M. J., O'Reilly, B., Shope, T. R. & Vander, A. J. (1987) Further evidence that stress hyperthermia is a fever. *Physiology & behavior* **39(6)**, 763-766.

Knoph, M. B. (1996) Gill ventilation frequency and mortality of Atlantic salmon (*Salmo salar* L.) exposed to high ammonia levels in seawater. *Water Research* **30**, 837-842

Koppang, E. O., Kvellestad, A. & Fischer, U. (2015). Fish mucosal immunity: gill. In *Mucosal Health in Aquaculture.* Beck, B. H. & Peatman, E. (eds.). Academic Press, Oxford, UK. p. 93-133.

Korsøen, Ø. J., Dempster, T., Fjelldal, P. G., Oppedal, F. & Kristiansen, T. S. (2009) Long-term culture of Atlantic salmon (*Salmo salar* L.) in submerged cages during winter affects behaviour, growth and condition. *Aquaculture* **296**, 373–381.

Korsøen, Ø. J., Dempster, T., Oppedal, F. & Kristiansen, T. S. (2012a) Individual variation in swimming depth and growth in Atlantic salmon (*Salmo salar* L.) subjected to submergence in sea-cages. *Aquaculture* **334-337**, 142–151.

Korsøen, Ø. J., Fosseidengen, J. E., Kristiansen, T. S., Oppedal, F., Bui, S. & Dempster, T. (2012b) Atlantic salmon (*Salmo salar* L.) in a submerged sea-cage adapt rapidly to re-fill their swim bladders in an underwater air filled dome. *Aquacultural engineering* **51**, 1-6.

Kotrschal, K., Whitear, M. & Finger, T. E. (1993) Spinal and facial innervation of the skin in the gadid fish *Ciliata mustela* (Teleostei). *Journal of Comparative Neurology* **331(3)**, 407-417.

Koumoundouros, G., Oran, G., Divanach, P., Stefanakis, S. & Kentouri, M. (1997) The opercular complex deformity in intensive gilthead sea bream (*Sparus aurata* L.) larviculture. Moment of apparition and description. *Aquaculture* **156**, 165–177.

Kristensen, T., Åtland, Å., Rosten, T., Urke, H. A. & Rosseland, B. O. (2009) Important influent-water quality parameters at freshwater production sites in two salmon producing countries. *Aquacultural Engineering* **41**, 53–59.

Kristiansen, T. S., Stien, L. H., Fjelldal, P. G. & Hansen, T. (2014) Dyrevelferd i lakseoppdrett (eng: Animal welfare in salmon aquaculture). In: Taranger, G. L., Svåsand, T., Kvamme, B. O., Kristiansen, T. & Boxaspen, K. K., *Risikovurdering norsk fiskeoppdrett 2013, Fisken og havet* **særnr 2-2014**, 145-154.

Kristoffersen, S., Tobiassen, T., Steinsund, V. & Olsen, R. L. (2006) Slaughter stress, postmortem muscle pH and rigor development in farmed Atlantic cod (*Gadus morhua* L.). *International Journal of Food Science + Technology* **41**, 861-864

Krogdahl, Å., Sundby, A. & Olli, J.J. (2004) Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) digest and metabolize nutrients differently. Effects of water salinity and dietary starch level. *Aquaculture* **229**, 335–360.

Kroupova, H., Machova, J. & Svobodova, Z. (2005) Nitrite influence on fish: a review. *Vet. Med. – Czech* **50 (11)**, 461-471.

Kroupova, H., Machova, J., Piackova, V., Blahova, J., Dobsikova, R., Novotny, L. & Svobodova, Z. (2008) Effects of subchronic nitrite exposure on rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicology and environmental safety* **71(3)**, 813-820.

Kwain, W. H. & McCauley, R. W. (1978) Effects of age and overhead illumination on temperatures preferred by underyearling rainbow trout, *Salmo gairdneri*, in a vertical temperature gradient. *Journal of the Fisheries Board of Canada* **35(11**), 1430-1433.

Landeira-Dabarca, A., Álvarez, M. & Molist, P. (2014) Food deprivation causes rapid changes in the abundance and glucidic composition of the cutaneous mucous cells of Atlantic salmon *Salmo salar* L. *Journal of Fish Diseases* **37**, 899–909.

Landless, P. J. (1976a) Demand-feeding behaviour of rainbow trout. *Aquaculture* **7(1)**, 11-25.

Landless, P. J. (1976b) Acclimation of rainbow trout to sea water. *Aquaculture* **7(1)**, 173-179.

Larsen, B. K., Skov, P. V., McKenzie, D. J. & Jokumsen, A. (2012) The effects of stocking density and low level sustained exercise on the energetic efficiency of rainbow trout (*Oncorhynchus mykiss*) reared at 19 °C. *Aquaculture* **324**, 226-233.

Latham, K. E. & Just, J. J. (1989) Oxygen availability provides a signal for hatching in the rainbow trout (*Salmo gairdneri*) embryo. *Canadian Journal of Fisheries and Aquatic Sciences* **46(1)**, 55-58.

Laursen, D. C., Silva, P. I., Larsen, B. K. & Höglund, E. (2013) High oxygen consumption rates and scale loss indicate elevated aggressive behaviour at low rearing density, while elevated brain serotonergic activity suggests chronic stress at high rearing densities in farmed rainbow trout. *Physiology & behavior* **122**, 147-154.

Le Bras, Y., Dechamp, N., Krieg, F., Filangi, O., Guyomard, R., Boussaha, M., Bovenhuis, H., Pottinger, T. G., Prunet, P., Le Roy, P. & Quillet, E. (2011) Detection of QTL with effects on osmoregulation capacities in the rainbow trout (*Oncorhynchus mykiss*). *BMC genetics* **12(1)**, 46.

Leclercq, E., Taylor, J. F., Fison, D., Fjelldal, P. G., Diez-Padrisa, M., Hansen, T. & Migaud, H. (2011) Comparative seawater performance and deformity prevalence in out-of-season diploid and triploid Atlantic salmon (*Salmo salar*) post-smolts. *Comparative Biochemistry and Physiology, Part A* **158**, 116-125.

Ledy, K., Giamberini, L. & Pihan, J. C. (2003) Mucous cell responses in gill and skin of brown trout *Salmo trutta fario* in acidic, aluminium-containing stream water. *Diseases of aquatic organisms* **56(3)**, 235-240.

Lein. I., Helland, S., Hjelde, K. & Baeverfjord, G. (2009) Temperature effects on malformations in trout (*O. mykiss*). In: *Control of malformations in fish aquaculture, Science and practice (FineFish)*. Baeverfjord, G., Helland, S. & Hough, C. (eds.). pp. 149.

Lekang, O.-I., (2007) Aquaculture Engineering. Blackwell Publishing, Oxford, UK. 432 pp.

Lewis Jr, W. M. & Morris, D. P. (1986) Toxicity of nitrite to fish: a review. *Transactions of the American Fisheries Society* **115(2)**, 183-195.

Li, H. W. & Brocksen, R. W. (1977) Approaches to the analysis of energetic costs of intraspecific competition for space by rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology* **11(4)**, 329-341.

Liebert, A. M. & Schreck, C. B. (2006) Effects of acute stress on osmoregulation, feed intake, IGF-1, and cortisol in yearling steelhead trout (*Oncorhynchus mykiss*) during seawater adaptation. *General and Comparative Endocrinology* **148**, 195-202.

Ligon, F., Rich, A., Rynearson, G., Thornburgh, D. & Trush, W. (1999) Report of the scientific review panel on California forest practice rules and salmonid habitat. *Prepared for the Resources Agency of California and the National Marine Fisheries Service. Sacramento, CA, 2*.

Linton, T. K., Morgan, I. J., Walsh, P. J. & Wood, C. M. (1998). Chronic exposure of rainbow trout (*Oncorhynchus mykiss*) to simulated climate warming and sublethal ammonia: a year-long study of their appetite, growth, and metabolism. *Canadian Journal of Fisheries and Aquatic Sciences* **55(3)**, 576-586.

López-Luna, J., Vásquez, L., Torrent, F. & Villarroel, M. (2013) Short-term fasting and welfare prior to slaughter in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **400**, 142-147.

López-Patiño, M. A., Hernández-Pérez, J., Gesto, M., Librán-Pérez, M., Míguez, J. M. & Soengas, J. L. (2014) Short-term time course of liver metabolic response to acute handling stress in rainbow trout, *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **168**, 40-49.

Lumsden, J. S. (2006) Gastrointestinal tract, swimbladder, pancreas and peritoneum. In: *Systemic Pathology of Fish: A Text and Atlas of Normal Tissues in Teleosts and their Responses in Disease*. Ferguson, H.W. (ed.). Scotian Press, London. 169–199.

Løvoll, M., Wiik-Nielsen, C. R., Tunsjø, H. S., Colquhoun, D., Lunder, T., Sørum, H. & Grove, S. (2009) Atlantic salmon bath challenged with *Moritella viscosa*–pathogen invasion and host response. *Fish & shellfish immunology* **26(6)**, 877-884.

Machova, J., Faina, R., Randak, T., Valentova, O., Steinbach, C., Kroupova, H. K. & Svobodova, Z. (2017) Fish death caused by gas bubble disease: a case report. *Veterinární medicína* **62(4)**, 231-237.

MacIntyre, C., Ellis, T., North, B. P. & Turnbull, J. F. (2008) The influences of water quality on the welfare of farmed trout: a Review. In: *Fish Welfare* (Ed: Branson, E.). Blackwells Scientific Publications, London, 150-178.

Madsen, L., Arnbjerg, J. & Dalsgaard, I. (2000) Spinal deformities in triploid all-female rainbow trout (*Oncorhynchus mykiss*). *Bulletin of the European Association of Fish Pathologists* **20**, 206-208.

Madsen, J., Arnbjerg, J. & Dalsgaard, I. (2001) Radiological examination of the spinal column in farmed rainbow trout *Oncorhynchus mykiss* (Walbaum): experiments with Flavobacterium psychrophilum and oxytetracycline. *Aquaculture Research* **32**, 235-241.

Magnoni, L. J., Eding, E., Leguen, I., Prunet, P., Geurden, I., Ozório, R. O. & Schrama, J. W. (2018) Hypoxia, but not an electrolyte-imbalanced diet, reduces feed intake, growth and oxygen consumption in rainbow trout (*Oncorhynchus mykiss*). *Scientific reports* **8(1)**, 4965.

Makino, K., Onuma, T. A., Kitahashi, T., Ando, H., Ban, M. & Urano, A. (2007) Expression of hormone genes and osmoregulation in homing chum salmon: a minireview. *General and comparative endocrinology* **152(2-3)**, 304-309.

Manteifel, Y.B. & Karelina, M. A. (1996) Conditioned food aversion in the goldfish, *Carassius auratus*. *Comparative Biochemistry and Physiology Part A: Physiology* **115**, 31-35.

Marras, S., Killen, S. S., Claireaux, G., Domenici, P., & McKenzie, D. J. (2011) Behavioural and kinematic components of the fast-start escape response in fish: individual variation and temporal repeatability. *Journal of Experimental Biology* **214**, 3102–3110.

Marschall, E .A., Quinn, T. P., Roff, D. A., Hutchings, J. A., Metcalfe, N. B., Bakke, T. A., Saunders, R. L. & Poff, N. L. (1998) A framework for understanding Atlantic salmon (*Salmo salar*) life history. *Canadian Journal of Fisheries and Aquatic Sciences* **55(S1)**, 48-58.

Martins, C. I. M., Galhardo, L., Noble, C., Damsgard, B., Spedicato, M. T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J. C., Carter, T., Planellas, S. R. & Kristiansen, T. (2012) Behavioural indicators of welfare in farmed fish. *Fish Physiology and Biochemistry* **38**, 17-41.

Maule, A. G., Tripp, R. A., Kaattari, S. L. & Schreck, C. B. (1989) Stress alters immune function and disease resistance in Chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Endocrinology* **120**, 135-142.

McCarthy, I. D., Carter, C. G. & Houlihan, D. F. (1992) The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Biology* **41(2)**, 257-263.

McCormick, S. D. (1994) Opercular membranes and skin. *Analytical Techniques* **3**, 231.

McCormick, S. D. (2013) Smolt Physiology and Endocrinology. In: *Euryhaline Fishes.* McCormick, S. D., Farrell, A. P. & Brauner, C. J. (eds.). Academic Press, USA, 199-251.

McCormick, S. D. & Saunders, R. L. (1987) Preparatory Physiological Adaptations for Marine Life of Salmonids: Osmoregulation, Growth, and Metabolism. *American Fisheries Society Symposium* **1**, 211-229.

McCormick, S. D., Saunders, R. L. & MacIntyre, A. D. (1989) The effect of salinity and ration level on growth rate and conversion efficiency of Atlantic salmon (*Salmo salar*) smolts. *Aquaculture* **82(1-4)**, 173-180.

McDonald, D. G., Hobe, H. & Wood, C.M. (1980) The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri*, to low environmental pH. *Journal of Experimental Biology* **88**, 109-131.

McDonald, G. & Milligan, L. (1997) Ionic, osmotic and acid-base regulation in stress. In: *Stress and Health in Aquaculture.* Iwama, G. K., Pickering, A. D., Sumpter, J. P. & Schreck, C. B. (eds.). Cambridge: Cambridge University Press. 119-145.

McGeer, J. C. & Wood, C. M. (1998) Protective effects of water Cl^- on physiological responses to waterborne silver in rainbow trout. *Canadian Journal of Fisheries and Aquatic Sciences* **55(11)**, 2447-2454.

McKay, L. R. & Gjerde, B. (1985) The effect of salinity on growth of rainbow trout. *Aquaculture* **49(3-4)**, 325-331.

McKenzie, D. J., Höglund, E., Dupont-Prinet, A., Larsen, B. K., Skov, P. V., Pedersen, P. B. & Jokumsen, A. (2012) Effects of stocking density and sustained aerobic exercise on growth, energetics and welfare of rainbow trout. *Aquaculture* **338**, 216-222.

McKinnon, B. M. (1993) Host response of Atlantic salmon (*Salmo salar*) to infection by sea lice (*Caligus elongatus*). *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 789-792.

McNeill, B., Perry, S. F. (2006) The interactive effects of hypoxia and nitric oxide on catecholamine secretion in rainbow trout (*Oncorhynchus mykiss*). *Journal of experimental biology* **209**, 4214–4223.

Mellor, D. J., Patterson-Kane, E. & Stafford, K. J. (2009) The Sciences of Animal Welfare. John Wiley & Sons Ltd, Oxford, UK, 212 pp.

Merker, B.H. (2016) The line drawn on pain still holds. *Animal Sentience* **2016.090**

Merkin, G. V., Roth, B., Gjerstad, C., Dahl-Paulsen, E. & Nortvedt, R. (2010) Effect of pre-slaughter procedures on stress responses and some quality parameters in sea-farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture **309**, 231–235.

Midtlyng, P. J. (1997) Vaccinated Fish Welfare: Protection Versus Side-Effects. In: *Fish Vaccinology*. Gudding, R., Lillehaug, A., Midtlyng, P. J. & Brown, F. (eds): Dev Biol Stand. Basel, Karger, vol 90, 371-379.

Midtlyng, P. J. & Lillehaug, A. (1998) Growth of Atlantic salmon *Salmo salar* after intraperitoneal administration of vaccines containing adjuvants. *Diseases of Aquatic Organisms* **32**, 91-97.

Midtlyng, P. J., Reitan, L. J. & Speilberg, L. (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.

Milligan, C. L. & Girard, S. S. (1993) Lactate metabolism in rainbow trout. *Journal of Experimental Biology* **180,** 175-193.

Millot, S., Nilsson, J., Fosseidengen, J. E., Bégout, M. –L., Fernö, A., Braithwaite, V. A. & Kristiansen, T. S. (2014) Innovative behaviour in fish: Atlantic cod can learn to use an external tag to manipulate a self-feeder. *Animal Cognition* **17**, 779-785.

Milne, I., Seager, J., Mallett, M. & Sims, I. (2000) Effects of short-term pulsed ammonia exposure on fish. *Environmental toxicology and chemistry* **19(12)**, 2929-2936.

Mommsen, T. P. & Hochachka, P. W. (1988) The purine nucleotide cycle as two temporally separated metabolic units: a study on trout muscle. *Metabolism-Clinical and Experimental* **37(6)**, 552-556.

Mommsen, T. P., Vijayan, M. M. & Moon, T. W. (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries* **9**, 211-268.

Moran, D., Tirsgård, B. & Steffensen, J. F. (2010) The accuracy and limitations of a new meter used to measure aqueous carbon dioxide. *Aquacultural engineering* **43(3)**, 101-107.

Mork, O. I. & Gulbrandsen, J. (1994) Vertical activity of four salmonid species in response to changes between darkness and two intensities of light. *Aquaculture* **127(4)**, 317-328.

Morro, B., Balseiro, P., Albalat, A., Pedrosa, C., Mackenzie, S., Nakamura, S., Shimizu, M., Nilsen, T. O., Sveier, H., Ebbesson, L. O. & Handeland, S. O. (2019) Effects of different photoperiod regimes on the smoltification and seawater adaptation of seawater-farmed rainbow trout (*Oncorhynchus mykiss*): Insights from Na+, K+–ATPase activity and transcription of osmoregulation and growth regulation genes. *Aquaculture* **507**, 282-292.

Moutou, K. A., McCarthy, I. D. & Houlihan, D. F. (1998) The effect of ration level and social rank on the development of fin damage in juvenile rainbow trout. *Journal of Fish Biology* **52(4)**, 756-770.

Mørkøre, T., Tahirovic, V. & Einen, O. (2008) Impact of starvation and handling stress on rigor development and quality of Atlantic salmon (*Salmon salar* L). *Aquaculture* **277(3)**, 231-238.

Munday, B. L., Foster, C. K., Roubal, F. R. & Lester, R. J. G. (1990) Paramoebic gill infection and associated pathology of Atlantic salmon, *Salmo salar* and rainbow trout, *Salmo gairdneri* in Tasmania. In *Pathology in marine science. Proceedings of the Third International Colloquium on Pathology in Marine Aquaculture, held in Gloucester Point, Virginia, USA, October 2-6, 1988.* (pp. 215-222). Academic Press Inc.

Murray, R. K. (1999) Muscle and the Cytoskeleton. In: *Harper's Biochemistry*. Murray R. K., Grannder D. K., Mayes, P. A. & Rodwell, V. W. (eds.). Appleton and Lange, Connecticut, USA, 715-736.

Murray, A. G. & Peeler, E. J. (2005) A framework for understanding the potential for emerging diseases in aquaculture. *Preventive veterinary medicine* **67(2-3)**, 223-235.

Mustafa, A., Speare, D. J., Daley, J., Conboy, G. A. & Burka, J. F. (2000) Enhanced susceptibility of seawater cultured rainbow trout, *Oncorhynchus mykiss* (Walbaum), to the microsporidian *Loma salmonae* during a primary infection with the sea louse, *Lepeophtheirus salmonis*. *Journal of fish diseases* **23(5)**, 337-341.

Nagasawa, K. (2004) Sea lice, *Lepeophtheirus salmonis* and *Caligus orientalis* (Copepoda: Caligidae), of wild and farmed fish in sea and brackish waters of Japan and adjacent regions: a review. *Zoological Studies* **43(2)**, 173-178.

Nash, R. D. M., Valencia, A. H. & Geffen, A. J. (2006) The origin of Fulton's condition factor-setting the record straight. *Fisheries* **31**, 236–238.

Neill, W. H. & Bryan, J. D. (1991) Responses of fish to temperature and oxygen, and response integration through metabolic scope. In: *Aquaculture and Water Quality, Advances in World Aquaculture.* Brune, D. E. & Tomasso, J. R. (eds.). Baton Rouge, LA: The World Aquaculture Society 30–57.

Nematollahi, A., Decostere, A. & Pasmans, F. (2003) *Flavobacterium psychrophilum* infections in salmonid fish. *Journal of Fish Diseases* **26**, 563-574.

Neves, K.J. & Brown, N.P. (2015) Effects of Dissolved Carbon Dioxide on Cataract Formation and Progression in Juvenile Atlantic Cod, *Gadus morhua* L. *Journal of the World Aquaculture Society* **46**, 1: doi: 10.1111/jwas.12166

Newcombe, C. P. & Hartman, G. F. (1980) Visual signals in the spawning behaviour of rainbow trout. *Canadian Journal of Zoology* **58(10)**, 1751-1757.

Nilsen, M., Amundsen, V. S. & Olsen, M. S. (2018) Swimming in a slurry of schemes: making sense of aquaculture standards and certification schemes. In: *Safety and Reliability-Safe Societies in a Changing World*. Haugen, S., Barros, A., van Gulijk, C., Kongsvik, T. & Vinnem J. E. (eds). Taylor & Francis Group, London, ISBN 978-0-8153-8682-7 3149-3156.

Nilsson, J., Kristiansen, T. S., Fosseidengen, J. E., Stien, L. H., Fernö, A., van den Bos, R. (2010) Learning and anticipatory behaviour in a "sit-and-wait" predator: The Atlantic halibut. *Behavioural Processes* **83**, 257–266

Nilsson, J., Folkedal, O., Fosseidengen, J. E., Stien, L. H. & Oppedal, F. (2013) PIT tagged individual Atlantic salmon registrered at static depth positions in a sea cage: Vertical size stratification and implications for fish sampling. *Aquacultural Engineering* **55**, 32– 36.

Noble, A. C. & Summerfelt, S. T. (1996) Diseases encountered in rainbow trout cultured in recirculating systems. *Annual Review of Fish Diseases* **6**, 65-92.

Noble, C., Mizusawa, K. & Tabata, M. (2005) Does light intensity affect self-feeding and food wastage in group-held rainbow trout and white-spotted charr? *Journal of Fish Biology* **66(5)**, 1387-1399.

Noble, C., Mizusawa, K., Suzuki, K., & Tabata, M. (2007a) The effect of differing self-feeding regimes on the growth, behaviour and fin damage of rainbow trout held in groups. *Aquaculture* **264(1)**, 214-222.

Noble, C., Kadri, S., Mitchell, D. F. & Huntingford, F. A. (2007b) Influence of feeding regime on intraspecific competition, fin damage and growth in 1+ Atlantic salmon parr (*Salmo salar* L.) held in freshwater production cages. *Aquaculture research* **38(11)**, 1137-1143.

Noble, C., Kadri, S., Mitchell, D. F. & Huntingford, F. A. (2007c) The impact of environmental variables on the feeding rhythms and daily feed intake of cage-held 1+ Atlantic salmon parr (*Salmo salar* L.). *Aquaculture* **269(1)**, 290-298.

Noble, C., Kadri, S., Mitchell, D. F. & Huntingford, F. A. (2008) Growth, production and fin damage in cage-held 0+ Atlantic salmon pre-smolts (*Salmo salar* L.) fed either a) on-demand, or b) to a fixed satiation–restriction regime: Data from a commercial farm. *Aquaculture* **257**, 163-168.

Noble, C., Berrill, I. K., Waller, B., Kankainen, M., Setälä, J., Honkanen, P., Mejdell, C. M., Turnbull, J. F., Damsgård, B., Schneider, O. & Toften, H. (2012a). A multi-disciplinary framework for bio-economic modeling in aquaculture: a welfare case study. *Aquaculture economics & management* **16(4)**, 297-314.

Noble, C., Cañon Jones, H. A., Damsgård, B., Flood, M. J., Midling, K. Ø., Roque, A., Sæther, B. -S. & Cottee, S. Y. (2012b) Injuries and deformities in fish: their potential impacts upon aquacultural production and welfare. *Fish physiology and biochemistry* **38(1)**, 61-83.

Noble, C., Flood, M. J. & Tabata, M. (2012c) Using rainbow trout *Oncorhynchus mykiss* as self-feeding actuators for white-spotted charr *Salvelinus leucomaenis*: Implications for production and welfare. *Applied Animal Behaviour Science* **138**, 125-131.

Noble, C., Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H. & Turnbull, J. F. (Eds.) (2018). Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare 351pp. ISBN 978-82-8296-556-9

Nolan, D. T., Reilly, P. & Bonga, S. E. W. (1999) Infection with low numbers of the sea louse, *Lepeophtheirus salmonis*, induces stress-related effects in post-smolt Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 947–959.

Nolan, D. T., Ruane, N. M., Van Der Heijden, Y., Quabius, E. S., Costelloe, J. & Bonga, S. E. (2000) Juvenile *Lepeophtheirus salmonis* (Krøyer) affect the skin and gills of rainbow trout *Oncorhynchus mykiss* (Walbaum) and the host response to a handling procedure. *Aquaculture Research* **31(11)**, 823-833.

Norberg, B., Taranger, G. L. & Tveiten, H. (2007) Reproductive Physiology in Cultured Cold-water Marine Fish. *From Cage to Consumption*, 66-79.

Nordin, R. N., Pommen, L. W. & Meays, C. L. (2009) Water Quality Guidelines for Nitrogen (Nitrate, Nitrite, and Ammonia). *Water Stewardship Division, Ministry of Environment, Province of British Columbia, Canada*, 1-29.

Nordgreen, J., Garner, J. P., Janczak, A. M., Ranheim, B., Muir, W. M. & Horsberg, T. E. (2009) Thermonociception in fish: effects of two different doses of morphine on thermal threshold and post-test behaviour in goldfish (*Carassius auratus*). *Applied Animal Behaviour Science* **119(1)**, 101- 107.

NVI (2017). Veterinærinstituttets faktabank. http://www.vetinst.no/sykdom-og-agens, accessed 04.05.17.

O'Byrne-Ring, N., Dowling, K., Cotter, D., Whelan, K. & MacEvilly, U. (2003) Changes in mucus cell numbers in the epidermis of the Atlantic salmon at the onset of smoltification. *Journal of fish biology* **63(6)**, 1625-1630.

O'Donohoe, P., Kane, F., McDermott, T. & Jackson, D. (2016) Sea reared rainbow trout *Oncorhynchus mykiss* need fewer sea lice treatments than farmed Atlantic salmon *Salmo salar*. *Bull. Eur. Ass. Fish Pathol* **36(5)**, 2016.

OIE (2015a) Aquatic Animal Health Code. http://www.oie.int/international-standard-setting/aquatic-code/ (accessed 2016)

OIE (2015b). Manual of Diagnostic Tests for Aquatic Animals. http://www.oie.int/international-standard-setting/aquatic-manual/access-online/ accessed 19.02.16

Oldham, T., Rodger, H. & Nowak, B. F. (2016) Incidence and distribution of amoebic gill disease (AGD)—an epidemiological review. *Aquaculture* **457**, 35-42.

Olsen, A. B., Birkbeck, T. H., Nilsen, H. K., MacPherson, H. L., Wangel, C., Myklebust, C., Laidler, L. A., Aarflot, L., Thoen, E., Nygård, S. & Thayumanavan, T. (2006) Vaccine-associated systemic *Rhodococcus erythropolis* infection in farmed Atlantic salmon *Salmo salar*. *Diseases of aquatic organisms* **72(1)**, 9-17.

Olsen, A. B., Hjortaas, M., Tengs, T., Hellberg, H. & Johansen, R. (2015) First Description of a new disease in rainbow trout (*Oncorhynchus mykiss* (Walbaum)) similar to heart and skeletal muscle inflammation (HSMI) and detection of a gene sequence related to piscine orthoreovirus (PRV). *PloS one* **10(7)**, e0131638.

Olsen, R. E., Sundell, K., Hansen, T., Hemre, G. -I., Myklebust, R., Mayhew, T. M. & Ringø, E. (2003) Acute stress alters the intestinal lining of Atlantic salmon, *Salmo salar* L.: An electron microscopical study. *Fish Physiology and Biochemistry* **26**, 211–221.

Olsen, R. E., Sundell, K., Mayhew, T. M., Myklebust, R. & Ringø, E. (2005) Acute stress alters intestinal function of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture* **205**, 480-495.

Olsen, Y. A., Einarsdottir, I. E. & Nilssen, K. J. (1995) Metomidate anaesthesia in Atlantic salmon, *Salmo salar*, prevents plasma cortisol increase during stress. *Aquaculture* **134**, 155-168

Oppedal, F. (1995) Growth, harvest quality, sexual maturation and behaviour of spring transferred rainbow trout (*Oncorhynchus mykiss*) given natural or continuous light in sea water. Cand scient. thesis, University of Bergen, Norway (in Norwegian).

Oppedal, F., Dempster, T. & Stien, L. H. (2011a) Environmental drivers of Atlantic salmon behaviour in sea-cages: a review. *Aquaculture* **311(1)**, 1-18.

Oppedal, F., Vågseth, T., Dempster, T., Juell, J.E. & Johansson, D. (2011b). Fluctuating sea-cage environments modify the effects of stocking densities on production and welfare parameters of Atlantic salmon (*Salmo salar* L.). *Aquaculture* **315**, 361–368.

Ortega, V. A., Renner, K. J. & Bernier, N. J. (2005) Appetite-suppressing effects of ammonia exposure in rainbow trout associated with regional and temporal activation of brain monoaminergic and CRF systems. *Journal of Experimental Biology* **208(10)**, 1855-1866.

Pagnotta, A. & Milligan, C. L. (1991) The role of blood glucose in the restoration of muscle glycogen during recovery from exhaustive exercise in rainbow trout (*Oncorhynchus mykiss*) and winter flounder (*Pseudopleuronectes americanus*). *Journal of Experimental Biology* **161**, 489–508.

Panksepp, J. (2005) Affective consciousness: Core emotional feelings in animals and humans. *Consciousness and Cognition* **14**, 30-80.

Panksepp, J. & Biven, L. (2012) The archaeology of mind: Neuroevolutionary origins of human emotions. WW Norton & Company, NY, 562 pp.

Peake, S. J. (2008) Swimming performance and behaviour of fish species endemic to Newfoundland and Labrador: A literature review for the purpose of establishing design and water velocity criteria for fishways and culverts*. Canadian Manuscript Report of Fisheries and Aquatic Sciences* **No. 2843**.

Pedersen, C. L. (1987) Energy budgets for juvenile rainbow trout at various oxygen concentrations. *Aquaculture* **62(3-4)**, 289-298.

Perry, S. F., Rivero-Lopez, L., McNeill, B. & Wilson, J. (2006) Fooling a freshwater fish: how dietary salt transforms the rainbow trout gill into a seawater gill phenotype. *Journal of Experimental Biology* **209(23)**, 4591-4596.

Person-Le Ruyet, J., Labbé, L., Le Bayon, N., Sévère, A., Le Roux, A., Le Delliou, H. & Quéméner, L. (2008) Combined effects of water quality and stocking density on welfare and growth of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Living Resources* **21(2)**, 185-195.

Pérez-Sánchez, J., Terova, G., Simó-Mirabet, P., Rimoldi, S., Folkedal, O., Calduch-Giner, J. A., Olsen, R. E. & Sitjà-Bobadilla, A. (2017) Skin mucus of gilthead sea bream (*Sparus aurata* L.). Protein mapping and regulation in chronically stressed fish. *Frontiers in physiology* **8**, 34.

Persson, P., Sundell, K. & Björnsson, B.T. (1994) Estradiol-17b-induced calcium uptake and resorption in juvenile rainbow trout *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry* **13**, 379-386.

Persson, P., Sundell, K., Björnsson, B. T. & Lundqvist, H. (1998) Calcium metabolism and osmoregulation during sexual maturation of river running Atlantic salmon. *Journal of Fish Biology* **52**, 334–349.

Peterson, R. H. & Anderson, J. M. (1969) Influence of Temperature Change on Spontaneous Locomotor Activity and Oxygen Consumption of Atlantic Salmon, *Salmo salar*, Acclimated to Two Temperatures. *Journal of the Fisheries Research Board of Canada* **26**, 93-109.

Peterson, T. S. (2015) Overview of mucosal structure and function in teleost fishes. In: *Mucosal health in Aquaculture*. Beck, B. H. & Peatman, E. (eds.). Academic Press, Oxford, UK. 55-67.

Pettem, C. M., Briens, J. M., Janz, D. M. & Weber, L. P. (2018) Cardiometabolic response of juvenile rainbow trout exposed to dietary selenomethionine. *Aquatic toxicology* **198**, 175-189.

Pettersen, J. M., Bracke, M. B. M, Midtlyng, P. J., Folkedal, O., Stien, L. H., Steffenak, H. & Kristiansen, T. S. (2014) Salmon welfare index model 2.0: an extended model for overall welfare assessment of caged Atlantic salmon, based on a review of selected welfare indicators and intended for fish health professionals. *Reviews in Aquaculture* **6**, 162–179.

Phillips, M. J. (1985) Behaviour of rainbow trout, *Salmo gairdneri* Richardson, in marine cages. *Aquaculture Research* **16(3)**, 223-232.

Pittman, K., Sourd, P., Ravnøy, B., Espeland, Ø., Fiksdal, I. U., Oen, T., Pittman, A., Redmond, K. & Sweetman, J. (2011) Novel method for quantifying salmonid mucous cells. *Journal of fish diseases* **34(12)**, 931-936.

Pittman, K., Pittman, A., Karlson, S., Cieplinska, T., Sourd, P., Redmond, K., Ravnøy, B. & Sweetman, E. (2013) Body site matters: an evaluation and application of a novel histological methodology on the quantification of mucous cells in the skin of Atlantic salmon, *Salmo salar* L. *Journal of fish diseases* **36(2)**, 115-127.

Poppe, T. (1999) Fiskehelse og fiskesykdommer. (Poppe T., ed.). Oslo: Universitetsforlaget, 411.

Poppe, T. T. (2000) Husbandry diseases in fish farming – an ethical challenge to the veterinary profession. *Norsk Veterinaer Tidsskrift* **112**, 91-96.

Poppe, T. T. & Breck, O. (1997) Pathology of Atlantic salmon *Salmo salar* intraperitoneally immunized with oil-adjuvanted vaccine. A case report. *Diseases of Aquatic Organisms* **29**, 219-226.

Poppe, T., Bergh, Ø., Espelid, S. & Nygaard, S. (1999) Fiskehelse og fiskesykdommer, Universitetsforlaget, Oslo.

Poppe, T. T., Bæverfjord, G. & Hansen, T. (2007) Effects of intensive production with emphasis on on-growing production: fast growth, deformities and production-related diseases. *Aquaculture Research: From Cage to Consumption*, 120-135.

Pörtner, H. O. (2010) Oxygen-and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology* **213(6)**, 881-893.

Pörtner, H. O. & Farrell, A. P. (2008) Physiology and climate change. *Science* **322(5902)**, 690-692.

Pottinger, T. G. & Carrick, T. R. (1999) A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress-responsiveness in female rainbow trout. *Aquaculture* **175(3-4)**, 351-363.

Pottinger, T. G., Rand-Weaver, M. & Sumpter, J. P. (2003) Overwinter fasting and re-feeding in rainbow trout: plasma growth hormone and cortisol levels in relation to energy mobilisation. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **136(3)**, 403-417.

Poulsen, S. B., Jensen, L. F., Nielsen, K. S., Malte, H., Aarestrup, K., & Svendsen, J. C. (2011). Behaviour of rainbow trout *Oncorhynchus mykiss* presented with a choice of normoxia and stepwise progressive hypoxia. *Journal of Fish Biology* **79(4)**, 969-979.

Powell, M. D., Jones, M. A. & Lijalad, M. (2009) Effects of skeletal deformities on swimming performance and recovery from exhaustive exercise in triploid Atlantic salmon. *Diseases of Aquatic Organisms* **85**, 59-66.

Pounder, K. C., Mitchell, J. L., Thomson, J. S., Pottinger, T. G. & Sneddon, L. U. (2018) Physiological and behavioural evaluation of common anaesthesia practices in the rainbow trout. *Applied Animal Behaviour Science* **199**, 94-102.

Poynton, S. L. (1987) Vertebral column abnormalities in brown trout, *Salmo trutta* L. *Journal of Fish Diseases* **10**, 53-57.

Provan, F., Jensen, L. B., Uleberg, K. E., Larssen, E., Rajalahti, T., Mullins, J. & Obach, A. (2013) Proteomic analysis of epidermal mucus from sea lice–infected Atlantic salmon, *Salmo salar* L. *Journal of fish diseases* **36(3)**, 311-321.

Quality trout UK (2019). http://www.qualitytrout.co.uk (Accessed 2019).

Raby, G. D., Clark, T. D., Farrell, A. P., Patterson, D. A., Bett, N. N., Wilson, S. M., Willmore, W. G., Suski, C. D., Hinch, S. G. & Cooke, S. J. (2015) Facing the river gauntlet: understanding the effects of fisheries capture and water temperature on the physiology of coho salmon. *PLoS One* **10(4)**, p.e0124023.

Raida, M. K. & Buchmann, K. (2008) Bath vaccination of rainbow trout (*Oncorhynchus mykiss* Walbaum) against *Yersinia ruckeri*: effects of temperature on protection and gene expression. *Vaccine* **26(8)**, 1050-1062.

Randall, D. J. (1991). The impact of variations in water pH on fish. In: *Aquaculture and Water Quality*. Brune, D. E. & Tomasso, J. R. (eds.). World Aquaculture Society. Baton Rouge. 90-104.

Randall, D. J. & Wright, P. A. (1989) The interaction between carbon dioxide and ammonia excretion and water pH in fish. *Canadian Journal of Zoology* **67**, 2936-2942.

Randall D. J. & Tsui T. K. N. (2002) Ammonia toxicity in fish. *Marine Pollution Bulletin* **45**, 17–23.

Redding, M. J. & Schreck, C. B. (1983) Influence of Ambient Salinity on Osmoregulation and Cortisol Concentration in Yearling Coho Salmon during Stress. *Transactions of the American Fisheries Society,* **112**, 800–807.

Reebs, S. G. (2002). Plasticity of diel and circadian activity rhythms in fishes. *Reviews in Fish Biology and Fisheries* **12(4)**, 349-371.

Reebs, S.G. (2008-2014) Sleep in fishes. Retrieved 24 July 2014. http://www.howfishbehave.ca/pdf/sleep%20in%20fishes.pdf

Remen, M. (2012) The oxygen requirement of Atlantic salmon (*Salmo salar* L.) in the on-growing phase in sea cages. *Doctoral thesis*, University of Bergen

Remen, M., Oppedal, F., Torgersen, T., Imsland, A. K. & Olsen, R. E. (2012) Effects of cyclic environmental hypoxia on physiology and feed intake of post-smolt Atlantic salmon: initial responses and acclimation. *Aquaculture* **326**, 148-155.

Remen, M., Oppedal, F., Imsland, A. K., Olsen, R. E. & Torgersen, T. (2013) Hypoxia tolerance thresholds for post-smolt Atlantic salmon: dependency of temperature and hypoxia acclimation. *Aquaculture* **416**, 41-47.

Remen, M., Sievers, M., Torgersen, T. & Oppedal, F. (2016) The oxygen threshold for maximal feed intake of Atlantic salmon post-smolts is highly temperature-dependent. *Aquaculture* **464**, 582-592.

Remø, S. C., Olsvik, P. A., Torstensen, B. E., Amlund, H., Breck, O. & Waagbø, R. (2011) Susceptibility of Atlantic salmon lenses to hydrogen peroxide oxidation ex vivo after being fed diets with vegetable oil and methylmercury. *Experimental Eye Research* **92**, 414–424.

Remø, S. C., Hevrøy, E. M., Breck, O., Olsvik, P. A. & Waagbø, R. (2017) Lens metabolomic profiling as a tool to understand cataractogenesis in Atlantic salmon and rainbow trout reared at optimum and high temperature. *PloS one* **12(4)**, e0175491.

Revie, C. W., Gettinby, G, Treasurer, J. W. & Rae, G. H. (2002) The epidemiology of the sea lice, *Caligus elongates* Nordmann, in marine aquaculture of Atlantic salmon, *Salmo salar* L., in Scotland. *Journal of Fish Diseases* **25**, 391-399.

Rey, S., Huntingford, F. A., Boltana, S., Vargas, R., Knowles, T. G. & Mackenzie, S. (2015) Fish can show emotional fever: stress-induced hyperthermia in zebrafish. *Proc. R. Soc. B* **282(1819)**, 20152266.

Rimstad, E., Dale, O. B., Dannevig, B. H. & Falk, K. (2011) Infectious Salmon Anaemia. In: *Fish diseases and disorders. Volume 3*. Woo, P. & Bruno, D. (eds.). Oxfordshire, UK: CAB International, 143-165.

Robards, M. D. & Quinn, T. P. (2002) The Migratory Timing of Adult Summer-Run Steelhead in the Columbia River over Six Decades of Environmental Change. *Transactions of the American Fisheries Society* **131(3)**, 523-536.

Robb, D. H. F. (2001) The Relationship Between Killing Methods and Quality. In: *Farmed Fish Quality.* Kestin, S. D. & Warris, P. D. (eds.). Fishing News Books. Cornwall, UK, 220-233.

Roberts, R. J. (2012) Fish pathology, 4th Edition. Wiley Blackwell, Hoboken, NJ. ISBN: 978-1-444-33282-7

Roberts, S. D. & Powell, M. D. (2005) The viscosity and glycoprotein biochemistry of salmonid mucus varies with species, salinity and the presence of amoebic gill disease. *Journal of Comparative Physiology B* **175(1)**, 1-11.

Roberts, R. J. & Rodger, H. D. (2012) The pathophysiology and systematic pathology of teleosts. In: *Fish Pathology.* Roberts, R. J. (ed.). Wiley Blackwell, Hoboken, NJ, 62–143.

Roberts, R. J. & Shepherd, C. J. (1974) Handbook of trout and salmon diseases. Fishing News (Books) Ltd.

Robertson-Bryan Inc. (2006) Suspended solids and turbidity requirements of freshwater aquatic life and example relationship between TSS (mg/L) and turbidity (NTUs) for a treated municipal effluent (Technical Memorandum), pp.23.

Rogers, G. (2005) Total Gas Saturation Considerations for Recirculating Aquatic Systems. *International Journal of Recirculating Aquaculture* **6**, 39-48.

Rojas, V., Sánchez, D., Gallardo, J. A. & Mercado, L. (2018) Histopathological changes induced by *Caligus rogercresseyi* in rainbow trout (*Oncorhynchus mykiss*). *Latin American journal of aquatic research* **46(4)**, 843-848.

Rønsholdt, B. & McLean, E. (1999) The effect of vaccination and vaccine components upon short-term growth and feed conversion efficiency in rainbow trout. *Aquaculture* **174(3-4)**, 213-221.

Rose, J. D. (2002) The neurobehavioral nature of fishes and the question of awareness and pain. *Reviews in Fisheries Science* **10**, 1-38.

Rosten, T., Åtland, Å., Kristensen, T., Rosseland, B.O. & Braathen, B.R. (2004) Vannkvalitet relatert til dyrevelferd. In: Mattilsynet (Ed.). KPMG Senter for havbruk og fiskeri, Trondheim, pp. 89.

Rowe, D. K., Dean, T. L., Williams, E. & Smith, J. P. (2003) Effects of turbidity on the ability of juvenile rainbow trout, *Oncorhynchus mykiss*, to feed on limnetic and benthic prey in laboratory tanks. *New Zealand Journal of Marine and Freshwater Research* **37(1)**, 45-52.

RSPCA (2014). RSPCA welfare standards for farmed rainbow trout. https://science.rspca.org.uk/sciencegroup/farmanimals/standards/trout (Accessed 2016)

RSPCA (2018a). RSPCA welfare standards for farmed Atlantic salmon. https://science.rspca.org.uk/sciencegroup/farmanimals/standards/salmon (Accessed 2018)

RSPCA (2018b). RSPCA welfare standards for farmed rainbow trout. https://science.rspca.org.uk/sciencegroup/farmanimals/standards/trout (Accessed 2018)

Ruane, N. M., Nolan, D. T., Rotllant, J., Costelloe, J. & Bonga, S. W. (2000) Experimental exposure of rainbow trout *Oncorhynchus mykiss* (Walbaum) to the infective stages of the sea louse *Lepeophtheirus salmonis* (Krøyer) influences the physiological response to an acute stressor. *Fish & Shellfish Immunology* **10(5)**, 451-463.

Russo, R. C., Smith, C. E. & Thurston, R. V. (1974) Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Board of Canada* **31(10)**, 1653-1655.

Sadler J., Pankhurst, P. M. & King, H. R. (2001) High prevalence of skeletal deformity and reduced gill surface area in triploid Atlantic salmon (*Salmo salar* L.). *Aquaculture* **198**, 369-386.

Sadoul, B., Leguen, I., Colson, V., Friggens, N. C. & Prunet, P. (2015) A multivariate analysis using physiology and behavior to characterize robustness in two isogenic lines of rainbow trout exposed to a confinement stress. *Physiology & behavior* **140**, 139-147.

Sadoul, B., Friggens, N. C., Valotaire, C., Labbé, L., Colson, V., Prunet, P. & Leguen, I. (2017) Physiological and behavioral flexibility to an acute CO_2 challenge, within and between genotypes in rainbow trout. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **209**, 25-33.

Salama, N. K. G., Murray, A. G., Christie, A. J. & Wallace, I. S. (2016) Using fish mortality data to assess reporting thresholds as a tool for detection of potential disease concerns in the Scottish farmed salmon industry. *Aquaculture* **450**, 283–288.

Salinas, I. & Parra, D. (2015) Fish mucosal immunity: intestine. In *Mucosal health in aquaculture.* Beck, B. H. & Peatman, E. (eds.). Academic Press, Oxford, UK. p. 136–70.

Sambraus, F., Glover, K. A., Hansen, T., Fraser, T. W. K., Solberg, M. F. & Fjelldal, P. G. (2014) Vertebra deformities in wild Atlantic salmon caught in the Figgjo River, southwest Norway. *Journal of Applied Ichthyology* **30**, 777-782.

Sambraus, F., Olsen, R. E., Remen, M., Hansen, T. J., Torgersen, T. & Fjelldal, P. G. (2017) Water temperature and oxygen: The effect of triploidy on performance and metabolism in farmed Atlantic salmon (*Salmo salar* L.) post-smolts. *Aquaculture* **473**, 1–12.

Samsing, F., Solsorm, D., Oppedal, F., Solstorm, F. & Dempster, T. (2015) Gone with the flow: current velocities mediate parasitic infestation of an aquatic host. *International Journal for Parasitology* **45**: 559–565

Sanahuja, I. & Ibarz, A. (2015) Skin mucus proteome of gilthead sea bream: a non-invasive method to screen for welfare indicators. *Fish & shellfish immunology* **46(2)**, 426-435.

Sánchez-Vázquez, F. J. & Tabata, M. (1998) Circadian rhythms of demand-feeding and locomotor activity in rainbow trout. *Journal of Fish Biology* **52(2)**, 255-267.

Sapolsky, R. M. (2000) Stress hormones: Good and bad. *Neurobiology of diseases* **7(5)**, 540-542.

Sauter, S. T., Crawshaw, L. I. & Maule, A. G. (2001) Behavioral Thermoregulation by Juvenile Spring and Fall Chinook Salmon, *Oncorhynchus Tshawytscha*, during Smoltification. *Environmental Biology of Fishes* **61**, 295–304.

Scannell, P. W. & Jacobs, L. L. (2001) Effects of total dissolved solids on aquatic organisms. In: *Alaska Department of Fish and Game: Division of habitat and restoration*. Technical Report No. 01-06.Technical Report.

Schmidt-Nielsen, K. (1997) Animal Physiology. Cambridge University Press, Cambridge, UK, pp- 405-424.

Schreck, C. B. (1981) Stress and compensation in telesotean fishes: responses to social and physical factors. In: *Stress and fish* Pickering, A. D. (ed.). Academic Press, London, 295– 321.

Schreck, C. B. (2010) Stress and fish reproduction: the roles of allostasis and hormesis. *General and comparative endocrinology* **165(3)**, 549-556.

Schreck, C. B., Whaley, R. A., Bass, M. L., Maughan, O. E., Solazzi, M. (1976) Physiological responses of rainbow trout (*Salmo gairdneri*) to electroshock. *Journal of the Fisheries Research Board of Canada* **33**, 76–8.

Schurmann, H., Steffensen, J. F., & Lomholt, J. P. (1991) The influence of hypoxia on the preferred temperature of rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology* **157(1)**, 75-86.

Scottish Salmon Producers Organisation (2016) Code of Good Practice for Scottish Finfish Aquaculture. http://thecodeofgoodpractice.co.uk/chapters/ (Accessed 2016)

Segner, H., Sundh, H., Buchmann, K., Douxfils, J., Sundell, K. S., Mathieu, C., Ruane, N., Jutfelt, F., Toften, H. & Vaughan, L. (2012) Health of farmed fish: its relation to fish welfare and its utility as welfare indicator. *Fish physiology and biochemistry* **38(1)**, 85-105.

Sfakianakis, D. G., Georgakopoulou, E., Papadakis, I. E., Divanach, P., Kentouri, M. & Koumoundouros, G. (2006) Environmental determinants of haemal lordosis in European sea bass *Dicentrarchus labrax* (Linnaeus. 1758). *Aquaculture* **254**, 54-64.

Shabani, F., Erikson, U., Beli, E. & Rexhepi, A. (2016) Live transport of rainbow trout (*Onchorhynchus mykiss*) and subsequent live storage in market: water quality, stress and welfare considerations. *Aquaculture* **453**, 110-115.

Shakoori, M., Hoseinifar, S. H., Paknejad, H., Jafari, V., Safari, R., Van Doan, H. & Mozanzadeh, M. T. (2019) Enrichment of rainbow trout (*Oncorhynchus mykiss*) fingerlings diet with microbial lysozyme: Effects on growth performance, serum and skin mucus immune parameters. *Fish & shellfish immunology* **86**, 480-485.

Shearer, K.D. & Hardy, R.W. (1987) Phosphorus deficiency in rainbow trout fed a diet containing deboned fillet scrap. *Progressive Fish-Culturist* **49**, 192-197.

Shephard, K. L. (1994) Functions for fish mucus. *Reviews in fish biology and fisheries* **4(4)**, 401-429.

Shi, K., Dong, S., Zhou, Y., Gao, Q., Li, L., Zhang, M. & Sun, D. (2018) Comparative evaluation of toleration to heating and hypoxia of three kinds of salmonids. *Journal of Ocean University of China* **17(6)**, 1465-1472.

Shrimpton, J. M., Randall, D. J. & Fidler, L. E. (1990) Factors affecting swim bladder volume in rainbow trout (*Oncorhynchus mykiss*) held in gas supersaturated water. *Canadian Journal of Zoology* **68(5)**, 962-968.

Skov, P. V., Pedersen, L. F. & Pedersen, P. B. (2013) Nutrient digestibility and growth in rainbow trout (*Oncorhynchus mykiss*) are impaired by short term exposure to moderate supersaturation in total gas pressure. *Aquaculture* **416**, 179-184.

Sneddon, L. U. (2003) The evidence for pain in fish: the use of morphine as an analgesic. *Applied Animal Behaviour Science* **83**, 153-162.

Sneddon, L.U. (2006) Ethics and welfare: Pain perception in fish. *Bulletin of the European Association of Fish Pathologists* **26**, 6-10

Sneddon, L. U. (2009) Pain Perception in Fish: Indicators and Endpoints. *Ilar Journal* **50**, 338-342.

Sneddon, L. U., Wolfenden, D. C. C. & Thomson, J. S. (2016) Stress managment and welfare. In: *Biology of stress in fish. Fish physiology volume 35.* Schreck, C. B., Tort, L., Farrell, A. P. & Brauner, C. J. (eds.). Academic Press, 464-521.

Soares, S., Green, D. M., Turnbull, J. F., Crumlish, M. & Murray, A. G. (2011). A baseline method for benchmarking mortality losses in Atlantic salmon (*Salmo salar*) production. *Aquaculture* **314**, 7–12.

Soares S., Murray A. G., Crumlish M., Turnbull J. F. & Green D. M. (2013) Factors affecting variation in mortality of marine Atlantic salmon *Salmo salar* in Scotland. *Diseases of Aquatic Organisms* **103**, 101–109.

Solstorm, F., Solstorm, D., Oppedal, F., Fernö, A., Fraser, T. W. K. & Olsen, R. E. (2015) Fast water currents reduce production performance of post-smolt Atlantic salmon *Salmo salar*. *Aquaculture Environment Interactions* **7**, 125–134.

Solstorm, F., Solstorm, D., Oppedal, F., Olsen, R. E., Stien, L. H. & Fernö, A. (2016) Not too slow, not too fast: water currents affect group structure, aggression and welfare in post-smolt Atlantic salmon *Salmo salar*. *Aquaculture Environment Interactions* **8**, 339-347.

Sommerset, I., Krossøy, B., Biering, E. & Frost, P. (2005) Vaccines for fish in aquaculture. *Expert review of vaccines* **4(1)**, 89-101.

Sopinka, N. M., Donaldson, M. R., O'Conner, C. M., Suski, C. D. & Cooke, S. J. (2016) Stress indicators in fish. In: *Biology of stress in fish. Fish physiology volume 35.* Schreck, C. B., Tort, L., Farrell, A. P. & Brauner, C. J. (eds.). Academic Press, 405-462.

Soutar, R. (2015) Vets and aquaculture: an evolving relationship. *Veterinary Record* **177(10)**, 252-255.

Spruijt, B. M., van den Bos, R, & Pijlman, F. T. (2001) A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. *Applied Animal Behaviour Science* **72(2)**, 145-171.

Standal, M. & Gjerde, B. (1987) Genetic variation in survival of Atlantic salmon during the sea-rearing period. *Aquaculture* **66**, 197–207.

Stefansson, S., Bæverfjord, G., Finn, R. N., Finstad, B., Fivelstad, S., Handeland, S., Kristensen, T., Kroglund, F., Rosseland, B. O., Rosten, T. & Salbu, B. (2007) Water quality–salmonids. Aquaculture research: from cage to consumption. *Norwegian Research Council*, Oslo, 101-119.

Stephen, C. & Ribble, C. S. (1995) An evaluation of surface moribund salmon as indicators of seapen disease status. *Aquaculture* **133(1)**, 1-8.

Stevens, E. D. & Randall, D. J. (1967) Changes in blood pressure, heart rate and breathing rate during moderate swimming activity in rainbow trout. *Journal of Experimental Biology* **46(2)**, 307-315.

Stewart, H. A., Noakes, D. L., Cogliati, K. M., Peterson, J. T., Iversen, M. H. & Schreck, C. B. (2016) Salinity effects on plasma ion levels, cortisol, and osmolality in Chinook salmon following lethal sampling. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **192**, 38-43.

Stien, L. H., Hirmas, E., Bjørnevik, M., Karlsen, Ø., Nortvedt, R., Rørå, A. M. B., Sunde, J. & Kiessling, A. (2005) The effects of stress and storage temperature on the colour and texture of pre-rigor filleted farmed cod (*Gadus morhua* L.). *Aquaculture research* **36(12)**, 1197-1206.

Stien, L., Amundsen, A., Mørkøre, T., Nortvedt, R. & Økland, S. N. (2006) Instrumental colour analysis of Atlantic salmon (*Salmo salar* L.) muscle. In: *Seafood research from fish to dish*. Luten, J. B., Jacobsen, C., Bekaert, K., Sæbø A. & Oehlenschlager, J. (eds.). Wageningen Academic Publishers, The Netherlands, 525-541.

Stien, L. H., Bracke, M., Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., Kittilsen, S., Midtlyng, P. J., Vindas, M. A., Øverli, Ø. & Kristiansen, T.S. (2013) Salmon Welfare Index Model (SWIM 1.0): a semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. *Reviews in Aquaculture* **5**, 33-57.

Stien, L. H., Fosseidengen, J. E., Malm, M. E., Dveier, H., Torgersen, T., Wright, D. W. & Oppedal, F. (2014) Low intensity light of different colours modifies Atlantic salmon depth use. *Aquacultural Engineering* **62**, 42–48.

Stien, L. H., Oppedal, F., Kristiansen, T. S. (2016a) Dødelighetsstatistikk for lakseproduksjon (eng: Mortality statistics for salmon production). In: *Risikovurdering norsk fiskeoppdrett 2016.* Svåsand, T., Karlsen, Ø., Kvamme, B. O., Stien, L. H., Taranger, G. L., Boxaspen, K.K. (eds.). Havforskningsinstituttet, Fisken og Havet, særnr; 2-2016, 129-134.

Stien, L. H., Dempster, T., Bui, S., Glaropoulos, A., Fosseidengen, J. E., Wright, D. W. & Oppedal, F. (2016b). 'Snorkel'sea lice barrier technology reduces sea lice loads on harvest-sized Atlantic salmon with minimal welfare impacts. *Aquaculture* **458**, 29-37.

Stien, L. H., Gismervik, K. & Kristiansen, T. S. (2017) Risiko for dødelighet og dårlig fiskevelferd i laks- og regnbueørretproduksjonen i sjø (Eng: Risk for increased mortality and poor fish welfare in production of salmon and rainbow trout in sea cages). In: Svåsand, T., Grefsrud, E. S., Karlsen, Ø., Kvamme, B. O., Glover, K., Husa, V., Kristiansen, T. S. (eds.) Havforskningsinstituttet, *Fisken og Havet* **særnr; 2-2017**, 117-123.

Storebakken, T. & Austreng, E. (1987) Ration level for salmonids: II. Growth, feed intake, protein digestibility, body composition, and feed conversion in rainbow trout weighing 0.5–1.0 kg. *Aquaculture* **60(3)**, 207-221.

Stormer, J., Jensen, F. B. & Rankin, J. C. (1996) Uptake of nitrite, nitrate, and bromide in rainbow trout, (*Oncorhynchus mykiss*): effects on ionic balance. *Canadian Journal of Fisheries and Aquatic Sciences* **53(9)**, 1943-1950.

Sugiura, S. H., Hardy, R. W. & Roberts, R. J. (2004) The pathology of phosphorous deficiency in fish – a review. *Journal of Fish Diseases* **27**, 255-265.

Summerfelt, S.T., Vinci, B. J. & Piedrahita, R. H. (2000) Oxygenation and carbon dioxide control in water reuse systems. *Aquacultural engineering* **22(1)**, 87-108.

Sutterlin, A. M. & Stevens, E. D. (1992) Thermal behaviour of rainbow trout and Arctic char in cages moored in stratified water. *Aquaculture* **102(1-2)**, 65-75.

Sutterlin, A. M., Jokola, K. J. & Holte, B. (1979) Swimming behavior of salmonid fish in ocean pens. *Journal of the Fisheries Board of Canada* **36(8)**, 948-954.

Sveen, L. R., Timmerhaus, G., Torgersen, J. S., Ytteborg, E., Jørgensen, S. M., Handeland, S., Stefansson, S. O., Nilsen, T. O., Calabrese, S., Ebbesson, L. & Terjesen, B.F. (2016) Impact of fish density and specific water flow on skin properties in Atlantic salmon (*Salmo salar* L.) post-smolts. *Aquaculture* **464**, 629-637.

Svobodova, Z., Machova, J., Poleszczuk, G., Hůda, J., Hamáčková, J. & Kroupova, H. (2005) Nitrite poisoning of fish in aquaculture facilities with water-recirculating systems. *Acta Veterinaria Brno* **74(1)**, 129-137.

Tacchi, L., Lowrey, L., Musharrafieh, R., Crossey, K., Larragoite, E. T. & Salinas, I. (2015) Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **435**, 120-127.

Taguchi, M. & Liao, J. C. (2011) Rainbow trout consume less oxygen in turbulence: the energetics of swimming behaviors at different speeds. *Journal of Experimental Biology* **214**, 1428-1436.

Tait, J. S. (1960) The first filling of the swim bladder in salmonids. *Canadian Journal of Zoology* **38**, 179-187.

Taranger, G. L., Arnesen, A. M., Bæverfjord, G., Handeland, S. O. & Åsgård, T. (2000) Økt kunnskap gir mer effektiv produksjon i oppdrett av laksefisk. *Fisken og havet* **særnummer 3**, 20-30.

Taranger, G. L, Carrillo, M., Schulz, R. V., Fontaine, P., Zanuy, S., Felip, A. Weltzien, F. -A., Dufour, S., Karlsen, Ø., Norberg, B., Andersson, E. & Hansen, T. (2010) Control of puberty in farmed fish. *General and Comparative Endocrinology* **165**, 483–515.

Tautz, A. F. & Groot, C. (1975) Spawning behavior of chum salmon (*Oncorhynchus keta*) and rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Board of Canada* **32(5)**, 633-642.

Taylor, J. F., Migaud, H., Porter, M. J. R. & Bromage, N. R. (2005) Photoperiod influences growth rate and plasma insulin-like growth factor-I levels in juvenile rainbow trout, *Oncorhynchus mykiss*. *General and comparative endocrinology* **142(1-2)**, 169-185.

Taylor, J. F., North, B. P., Porter, M. J. R., Bromage, N. R. & Migaud, H. (2006) Photoperiod can be used to enhance growth and improve feeding efficiency in farmed rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **256(1-4)**, 216-234.

Taylor, J. F., Needham, M. P., North, B. P., Morgan, A., Thompson, K. & Migaud, H. (2007) The influence of ploidy on saltwater adaptation, acute stress response and immune function following seawater transfer in non-smolting rainbow trout. *General and comparative endocrinology* **152(2-3)**, 314-325.

Taylor, J. F., Porter, M. J., Bromage, N. R. & Migaud, H. (2008) Relationships between environmental changes, maturity, growth rate and plasma insulin-like growth factor-I (IGF-I) in female rainbow trout. *General and comparative endocrinology* **155(2)**, 257-270.

Taylor R. S., Muller, W. J., Cook, M. T., Kube, P. D. & Elliott, N. G. (2009) Gill observations in Atlantic salmon (*Salmon salar*) during repeated amoebic gill disease (AGD) field exposure and survival challenge. *Aquaculture* **290**, 1-8.

Thorarensen, H. & Farrell, A. P. (2011) The biological requirements for post-smolt Atlantic salmon in closed-containment systems. *Aquaculture* **312(1)**, 1-14.

Thorarensen, H., Gallaugher, P. E., Kiessling, A. K. & Farrell, A. P. (1993) Intestinal blood flow in swimming chinook salmon *Oncorhynchus tshawytscha* and the effects of haematocrit on blood flow distribution. *Journal of Experimental Biology* **179**, 115-129.

Thorarensen, H., Gallaugher, P. & Farrell, A. P. (1996) Cardiac output in swimming rainbow trout, *Oncorhynchus mykiss*, acclimated to seawater. *Physiological zoology* **69(1)**, 139-153.

Thurston, R. V. & Russo, R. C. (1983) Acute toxicity of ammonia to rainbow trout. *Transactions of the American Fisheries Society* **112(5)**, 696-704.

Timmons, M. B. & Ebeling, J. M. (2007) Recirculating Systems. *Northeastern Regional Aquaculture Center*, Ithaca, NY.

Torgersen, T., Bracke, M. B. M. & Kristiansen, T. S. (2011) Reply to Diggles et al. (2011): Ecology and welfare of aquatic animals in wild capture fisheries. *Reviews in fish Biology and Fisheries* **21**, 767-769.

Tornberg, E., Wahlgren, M., Brøndum, J. & Engelsen, S. B. (2000) Pre-rigor conditions in beef under varying temperature-and pH-falls studied with rigometer, NMR and NIR. *Food Chemistry* **69(4)**, 407-418.

Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O. T., Nilsen, F., Horsberg, T. E. & Jackson, D. (2013) Salmon lice–impact on wild salmonids and salmon aquaculture. *Journal of fish diseases* **36(3)**, 171-194.

Tørud, B. & Håstein, T. (2008) Skin lesions in fish: causes and solutions. *Acta Veterinaria Scandinavica* **50**, 1.

Tröße, C., Waagbø, R., Breck, O., Stavrum, A. K., Petersen, K. & Olsvik, P. A. (2009) Genome-wide transcription analysis of histidine-related cataract in Atlantic salmon (*Salmo salar* L). *Molecular Vision* **15**, 1332-1350.

Tucker, E. K., Suski, C. D., Philipp, M. A., Jeffrey, J. D. & Hasler, C. T. (2019) Glucocorticoid and behavioral variation in relation to carbon dioxide avoidance across two experiments in freshwater teleost fishes. *Biological Invasions* **21(2)**, 505-517.

Turnbull, J. F., Richards, R. H. & Robertson, D. A. (1996) Gross, histological and scanning electron microscopic appearance of dorsal fin rot in farmed Atlantic salmon, *Salmo salar* L., parr. *Journal of Fish Diseases* **19(6)**, 415-427.

Turnbull, J. F., Bell, A., Adams, C., Bron, J. & Huntingford, F. (2005) Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis. *Aquaculture* **243(1)**, 121-132.

Turnbull, J. F., North, B. P., Ellis, T., Adams, C. E., Bron, J., MacIntyre, C. M. & Huntingford, F. A. (2008) Stocking Density and the Welfare of Farmed Salmonids. In: *Fish Welfare.* Branson, E. J. (ed.). Blackwell Publishing Ltd, Oxford, UK. doi: 10.1002/9780470697610.ch8

Vaagsholm, I. & Djupvik, H. O. (1998). Risk factors for skin lesions in Atlantic salmon, *Salmo salar* L. *Journal of fish diseases* **21(6)**, 449-454.

Valdenegro-Vega, V. A., Crosbie, P., Bridle, A., Leef, M., Wilson, R. & Nowak, B. F. (2014) Differentially expressed proteins in gill and skin mucus of Atlantic salmon (*Salmo salar*) affected by amoebic gill disease. *Fish & shellfish immunology* **40(1)**, 69-77.

van den Thillart, G. & van Waarde, A. (1985) Teleosts in hypoxia-aspects of anaerobic metabolism. *Molecular Physiology* **8**, 393-409.

van der Marel, M., Caspari, N., Neuhaus, H., Meyer, W., Enss, M. L. & Steinhagen, D. (2010) Changes in skin mucus of common carp, *Cyprinus carpio* L., after exposure to water with a high bacterial load. *Journal of fish diseases* **33(5)**, 431-439.

van Raaij, M. T., Pit, D. S., Balm, P. H., Steffens, A. B. & van den Thillart, G. E. (1996) Behavioral strategy and the physiological stress response in rainbow trout exposed to severe hypoxia. *Hormones and Behavior* **30(1)**, 85-92.

Varsamos, S., Nebel, C. & Charmantier, G. (2005) Ontogeny of osmoregulation in postembryonic fish: A review. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **141**, 401-429.

Varsamos, S., Flik, G., Pepin, J. F., Bonga, S. W. & Breuil, G. (2006) Husbandry stress during early life stages affects the stress response and health status of juvenile sea bass, *Dicentrarchus labrax*. *Fish & shellfish immunology* **20(1)**, 83-96.

Vatsos I. N., Kotzamanis Y., Henry M., Angelidis P. & Alexis M. N. (2010) Monitoring stress in fish by applying image analysis to their skin mucous cells. *European Journal of Histochemistry* **54**, 107–111.

Vedel, N. E., Korsgaard, B. & Jensen, F. B. (1998) Isolated and combined exposure to ammonia and nitrite in rainbow trout (*Oncorhynchus mykiss*): effects on electrolyte status, blood respiratory properties and brain glutamine/glutamate concentrations. *Aquatic Toxicology* **41(4)**, 325-342.

Veiseth, E., Fjæra, S. O., Bjerkeng, B. & Skjervold, P. O. (2006) Accelerated recovery of Atlantic salmon (*Salmo salar*) from effects of crowding by swimming. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **144(3)**, 351-358.

Vendrell, D., Balcázar, J. L., Ruiz-Zarzuela, I., de Blas, I., Gironés, O. & Múzquiz, J. L. (2007) Safety and efficacy of an inactivated vaccine against Lactococcus garvieae in rainbow trout (*Oncorhynchus mykiss*). *Preventive veterinary medicine* **80(2-3)**, 222-229.

Videler, J. J. (1993). Fish swimming (Vol. 10). Springer Science & Business Media.

Vigen, J. (2008) Oxygen variation within a seacage. Master Thesis, Department of Biology, University of Bergen, Norway, 73 p.

Vilhunen, S. & Hirvonen, H. (2003) Innate antipredator responses of Arctic charr (*Salvelinus alpinus*) depend on predator species and their diet. *Behavioral Ecology and Sociobiology* **55(1)**, 1-10.

Villumsen, K. R., Koppang, E. O. & Raida, M. K. (2015) Adverse and long-term protective effects following oil-adjuvanted vaccination against *Aeromonas salmonicida* in rainbow trout. *Fish & shellfish immunology* **42(1)**, 193-203.

Villumsen, K. R., Koppang, E. O., Christensen, D. & Bojesen, A. M. (2017) Alternatives to mineral oil adjuvants in vaccines against *Aeromonas salmonicida* subsp. *salmonicida* in rainbow trout offer reductions in adverse effects. *Scientific reports* **7(1)**, 5930.

Vindas, M. A., Johansen, I. B., Folkedal, O., Hôglund, E., Gorissen, M., Flik, G., Kristiansen, T. S. & Øverli, Ø. (2016) Brain serotonergic activation in growth-stunted farmed salmon: adaption versus pathology. *Royal Society open science* **3**, 160030.

VKM (2014) Panel on Animal Health and Welfare; Risk assessment of amoebic gill disease, VKM Report 2014: 11 [39 pp], ISBN nr. 978-82-8259-149-2, Oslo, Norway.

von Uexküll J (1921). Umwelt und Innenwelt der Tiere. 2. verm. u. verb. Aufl. Berlin: J. Springer.

Vosylienė, M. Z. & Kazlauskienė, N. (2004) Comparative studies of sublethal effects of ammonia on rainbow trout (*Oncorhynchus mykiss*) at different stages of its development. *Acta Zoologica Lituanica* **14(1)**, 13-18.

Waagbø, R., Hamre, K., Bjerkås, E., Berge, R., Wathne, E., Lie, Ø. & Torstensen, B. (2003) Cataract formation in Atlantic salmon, *Salmo salar* L., smolt relative to dietary pro-and antioxidants and lipid level. *Journal of fish diseases* **26(4)**, 213-229.

Waagbø, R., Tröße, C., Koppe, W., Fontanillas, R., Breck, O. (2010) Dietary histidine supplementation prevents cataract development in adult Atlantic salmon, *Salmo salar* L., in seawater. *British Journal of Nutrition* **104**, 1460–1470.

Wagner, H. H. (1974) Photoperiod and temperature regulation of smolting in steelhead trout (*Salmo gairdneri*). *Canadian Journal of Zoology* **52**, 219-234.

Wagner, E. J., Bosakowski, T. & Intelmann, S. (1997) Combined effects of temperature and high pH on mortality and the stress response of rainbow trout after stocking. *Transactions of the American Fisheries Society* **126(6)**, 985-998.

Wall, T. (2008). Disease and Medicines-the Welfare Implications. In: *Fish welfare*. Branson E. J. (ed.), Blackwell Publishing Ltd, Oxford, pp. 195-201.

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.

Warburton K. (2003) Learning of foraging skills by fish. *Fish and Fisheries* **4(3)**, 203-215.

Wargelius, A., Fjelldal, P. G., Grini, A., Gil-Martens, L., Kvamme, B. -O. & Hansen, T. (2010) MMP-13 (Matrix MetalloProteinase 13) expression might be an indicator for increased ECM remodeling and early signs of vertebral compression in farmed Atlantic salmon (*Salmo salar* L.). *Journal of Applied Ichthyology* **26**, 366–371.

Webb, P. W. (1975) Hydrodynamics and energetics of fish propulsion. *Bulletin of the Fisheries Research Board of Canada* **190**, 1-159.

Wedemeyer, G. A. (1996) Physiology of fish in Intensive culture systems. London, Chapman Hall.

Wedemeyer, G. A. (1997). Effect of rearing conditions on the health and physiological quality of fish in intensive culture. In: Iwama, G. K., Pickering, A. D., Sumpter, J. P., Schreck, C. B. (Eds.). Fish Stress and Heath in Aquaculture: 35-72.

Weitkamp, D. E. & Katz, M. (1980) A review of dissolved gas supersaturation literature. *Transactions of the American Fisheries Society* **109**, 659-702.

Wells, R. M. G. & Pankhurst, N. W. (1999) Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish. *Journal of the World Aquaculture Society* **2**, 276-284.

Wendelaar Bonga, S. E. W. (1997) The stress response in fish. *Physiological Reviews* **77**, 591-625.

Wendelaar Bonga, S.E.W. (2011) Hormonal responses to stress. In: *Encyclopaedia of Fish Physiology.* Anthony, P. F. (ed.). Academic Press, San Diego, USA. 1515-1523

Westin, D. T. (1974) Nitrate and nitrite toxicity to salmonoid fishes. *The Progressive Fish-Culturist* **36(2)**, 86-89.

Wicks, B. J., Joensen, R., Tang, Q. & Randall, D. J. (2002) Swimming and ammonia toxicity in salmonids: the effect of sub lethal ammonia exposure on the swimming performance of coho salmon and the acute toxicity of ammonia in swimming and resting rainbow trout. *Aquatic Toxicology* **59(1-2)**, 55-69.

Wicks B. J. & Randall D. J. (2002a) The effect of feeding and fasting on ammonia toxicity in juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquatic Toxicology* **59**, 71–82.

Wicks B. J. & Randall D. J. (2002b) The effect of sub-lethal ammonia exposure on fed and unfed rainbow trout: the role of glutamine in regulation of ammonia. *Comp Biochem Physiol A* **132**, 275–285.

Wilkinson, R. J., Longland, R., Woolcott, H. & Porter, M. J. (2010) Effect of elevated winter–spring water temperature on sexual maturation in photoperiod manipulated stocks of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **309(1-4)**, 236-244.

Wilson, J. M., Iwata, K., Iwama, G. K. & Randall, D. J. (1998) Inhibition of ammonia excretion and production in rainbow trout during severe alkaline exposure. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **121(1)**, 99-109.

Witten, P. E., Gil-Martens, L., Hall, B. K., Huysseune, A. & Obach, A. (2005) Compressed vertebrae in Atlantic salmon (*Salmo salar*): evidence for metaplastic chondrogenesis as a skeletogenic response late in ontogeny. *Diseases of aquatic organisms* **64(3)**, 237-246.

Witten, P. E., Obach, A., Huysseune, A. & Baeverfjord, G. (2006) Vertebrae fusion in Atlantic salmon (*Salmo salar*): development, aggravation and pathways of containment. *Aquaculture* **258**, 164-172.

Witten, P. E., Gil-Martens, L., Huysseune, A., Takle, H. & Hjelde, K. (2009) Towards a classification and an understanding of the developmental relationships of vertebral body malformations in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **295**, 6-14.

Wood, C. M. & Jackson, E. B. (1980) Blood acid-base regulation during environmental hyperoxia in the rainbow trout (*Salmo gairdneri*). *Respiration physiology* **42(3)**, 351-372.

Wood, C. M., Walsh, P. J., Thomas, S. & Perry, S. F. (1990) Control of red blood cell metabolism in rainbow trout after exhaustive exercise. *Journal of experimental biology* **154(1)**, 491-507.

Wood, C. M. (2004) Dogmas and controversies in the handling of nitrogenous wastes: is exogenous ammonia a growth stimulant in fish?. *Journal of Experimental Biology* **207(12)**, 2043-2054.

Woynarovich, A., Hoitsy, G. & Moth-Poulsen, T. (2011) Small-scale rainbow trout farming. *FAO Fisheries and Aquaculture Technical Paper* (**561**), I.

Wright, P. A. & Wood, C. M. (1985) An analysis of branchial ammonia excretion in the freshwater rainbow trout: Effects of environmental pH change and sodium uptake blockade. *Journal of Experimental Biology* **114**, 329-353.

Ye, X. & Randall, D. J. (1991) The effect of water pH on swimming performance in rainbow trout (*Salmo gairdneri*, Richardson). *Fish Physiology and Biochemistry* **9**, 15-21.

Ye, X., Randall, D. J. & He, X. (1991) The effect of acid water on oxygen consumption, circulating catecholamines and blood ionic and acid-base status in rainbow trout (*Salmo gairdneri*). *Fish Physiology and Biochemistry* **9**, 23-30.

Yue S., Moccia R. D. & Duncan I. J. H. (2004) Investigating fear in domestic rainbow trout, *Oncorhynchus mykiss*, using an avoidance learning task. *Applied Animal Behaviour Science* **87**, 343–354.

Zachariasen, F., (2001). Intraspecific differences in nitrite tolerance of rainbow trout. The role of gill and kidney. Cand. Scient. Thesis, Institute of Biology, University of Southern Denmark, Odense.

Zhang, L. & Wood, C. M. (2009) Ammonia as a stimulant to ventilation in rainbow trout *Oncorhynchus mykiss*. *Respiratory physiology & neurobiology* **168(3)**, 261-271.

Zhang, L., Nawata, C. M. & Wood, C. M. (2013) Sensitivity of ventilation and brain metabolism to ammonia exposure in rainbow trout, *Oncorhynchus mykiss*. *Journal of Experimental Biology* **216(21)**, 4025-4037.

Ørnsrud, R., Wargelius, A., Sæle, Ø., Pittman, K. & Waagbø, R., (2004) Influence of egg vitamin A status and egg incubation temperature on subsequent development of the early vertebral column in Atlantic salmon fry. *Journal of Fish Biology* **64**, 399-417.

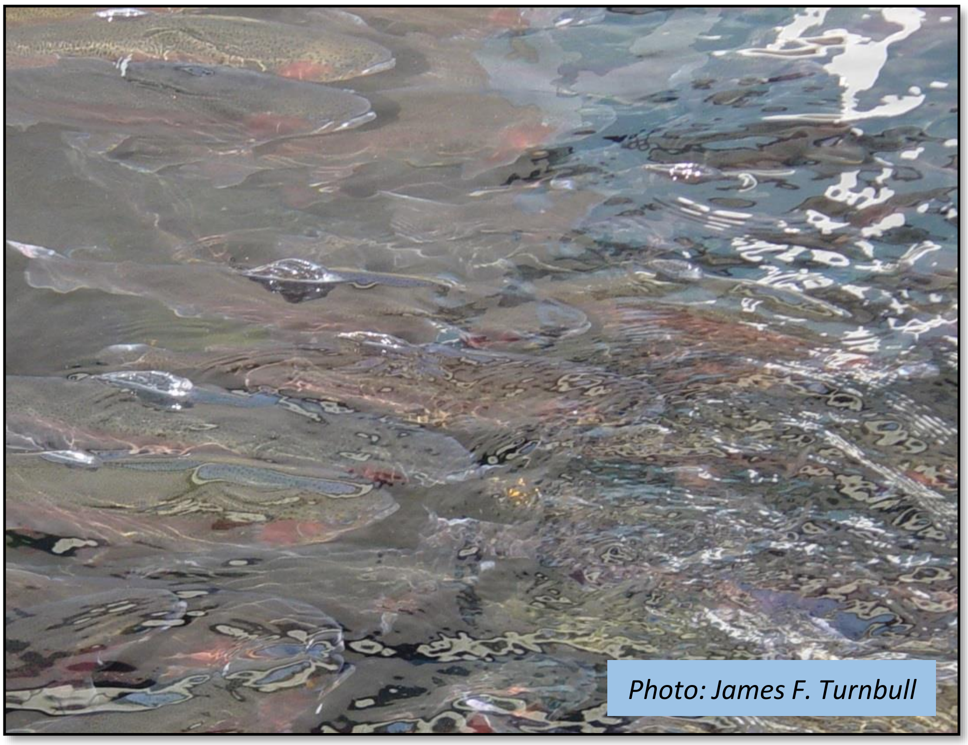
Øverli, Ø., Harris, C. A. & Winberg, S. (1999) Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain, Behavior and Evolution* **54(5)**, 263-275.

Welfare Indicators for farmed rainbow trout: Part B – Fit for Purpose OWIs for different production systems

Jelena Kolarevic[1]*, Chris Noble[1]*, James F. Turnbull[2]*, Lars H. Stien[3]*, Åsa M. Espmark[1], David Izquierdo-Gomez[1], Jonatan Nilsson[3], Frode Oppedal[3], Daniel W. Wright[3], Kristoffer V. Nielsen[4], Kristine Gismervik[4], Martin H. Iversen[5] and Bjørn-Steinar Sæther[1]

*Joint first authors

1. Nofima, P.O. Box 6122 Langnes, NO-9291 Tromsø, Norway
2. University of Stirling, Institute of Aquaculture, School of Natural Sciences, Stirling, FK9 4LA, United Kingdom
3. Institute of Marine Research, P.O. Box 1870 Nordnes, No-5817 Bergen, Norway
4. Norwegian Veterinary Institute, P.O. Box 750 Sentrum, NO-0106 Oslo, Norway
5. Nord University, Faculty of Biosciences and Aquaculture, 8049 Bodø, Norway



Contents

1 Flow-through aquaculture systems

1.1 Rainbow trout production 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 according to discharge consents before being released back to the aquatic environment. Additional oxygenation of the water is also used. The majority of rainbow trout life stages are produced in FT systems on land (from eggs to ongrowing), although some are grown in fresh water cages and some are moved to brackish or marine water cages for ongrowing.

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.** Flow-through systems are open systems with large volumes of water being passed through rearing tanks on daily basis. Although a certain level of intake water treatment can be used (e.g. UV or filters) this does not prevent the entrance of pathogens or fluctuations of potentially toxic water quality parameters in the rearing environment. FT systems are vulnerable and can be affected by changes in the surrounding environment. Some external threats can be monitored; others can be mitigated against but some such as sudden unpredicted toxic algal blooms in source water can be difficult to avoid or manage successfully. Water supply and quality in FT systems determines the biomass that can be produced while maintaining all critical water quality parameters. 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. Although oxygenation can increase the capacity of the FT system, it will reach a limit where accumulation of waste necessitates either filtering and recirculation or increased water exchange.
- **Inadequate oxygenation.** Oxygen is the primary water quality indicator that can limit the production of rainbow trout in FT systems. This is mainly due to the high oxygen demand and oxygen consumption of trout in the system, relatively low oxygen solubility in water and a limited supply of dissolved oxygen in the water [1]. In all modern aquaculture facilities, oxygen is added to support the intensity of biomass production. The addition of oxygen must increase with the biomass in the system. Failure to do so might create hypoxic conditions that in time can affect the trout'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 [2]. Mortality can occur after e.g. the failure of an oxygen supplementation system, or following a transfer of fish from a farm with high oxygen levels, or after 12-24h transport under high levels of DO [2] 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 [3] although background CO_2 levels in intake water can also play a role [4]. 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 [4] and references therein). For salmonid 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 Atantic salmon smolt production systems showed that 30% of the facilities had average CO_2 concentrations above recommended values [5]. The issue is particularly a problem in systems where water aeration (which can remove CO_2) is replaced by the injection of pure oxygen into the intake water. While oxygen injection is a much more effective way of maintaining optimal O_2 levels and enabling intensive production, it does not equilibrate other gases in the system. The lack of water degassing, low water exchange rates or background CO_2 concentrations in the intake water 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 [6]. 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 (see Noble et al., [4], case study on Atlantic salmon). CO_2 concentrations in aquaculture production facilities are far higher than those experienced by fish in the wild at present or even the levels predicted by the most pessimistic climate change models and we are just beginning to appreciate the consequences of some of those levels [7].
- **Water current speed** in tanks used for rearing juvenile rainbow trout is usually determined by the amount of water available for exchange [5], 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. It also has an impact on the behaviour of the fish including some undesirable behaviours such as fin biting [9, 10].
- **Metals,** particularly aluminium and iron, have been known to cause chronic or episodic toxicity problems. Aluminium is particularly problematic in low pH waters and affects mostly the gills and there is a lot of material available on the toxic effects of aluminium [11]. The toxicity of iron is dependent on the oxidation of Fe (II) to Fe (III), which is affected by temperature, pH and ionic strength [12]. Both metals can be toxic when fresh water with dissolved metals is mixed with seawater [6]. 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 [5].

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.
- Biosecurity (or keeping infections out) also intersects with hygiene practices (for preventing the spread of infections within and between facilities) and fish movements should be carried out under careful hygiene considerations.
- The water in the rearing facility is also a biosecurity risk and can be a vector for infectious agents via e.g. splashes. Infectious agents such as bacteria and viral agents can be spread this way [13] and fungal spores can also be transmitted through the air [e.g. 14].
- Each rearing facility should have its own set of equipment and little should be shared or transferred between facilities. If this is unavoidable, the user should follow good disinfection procedures (e.g. cleaning/disinfecting/drying the kit).

Rearing operations:

- **Monitoring of the environment** on a daily basis and recording and interpreting data is an essential part of effective management. The systematic monitoring of water quality is also a addressed in Norwegain aquaculture regulations § 22.Vannkvalitet og overvåking https://lovdata.no/dokument/SF/forskrift/2008-06-17-822. The necessity and nature of any monitoring depends on the nature of the system and surrounding environment. The more heavily loaded the system the wider the range of variables that need to be monitored and frequency may also need to be increased. Specific environments or times of year may be associated with specific risks such as a drop in pH associated with snow melt. It is important to know which environmental parameters can negatively affect the welfare of the trout in your system. The most important water quality parameters that are monitored are oxygen and temperature, while regular or periodical measurements of pH, nitrogenous compounds and CO_2 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. Fish should therefore be handled as little as possible and handling should be conducted in the least harmful and stressful manner. 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 (Figure 1.3-1).

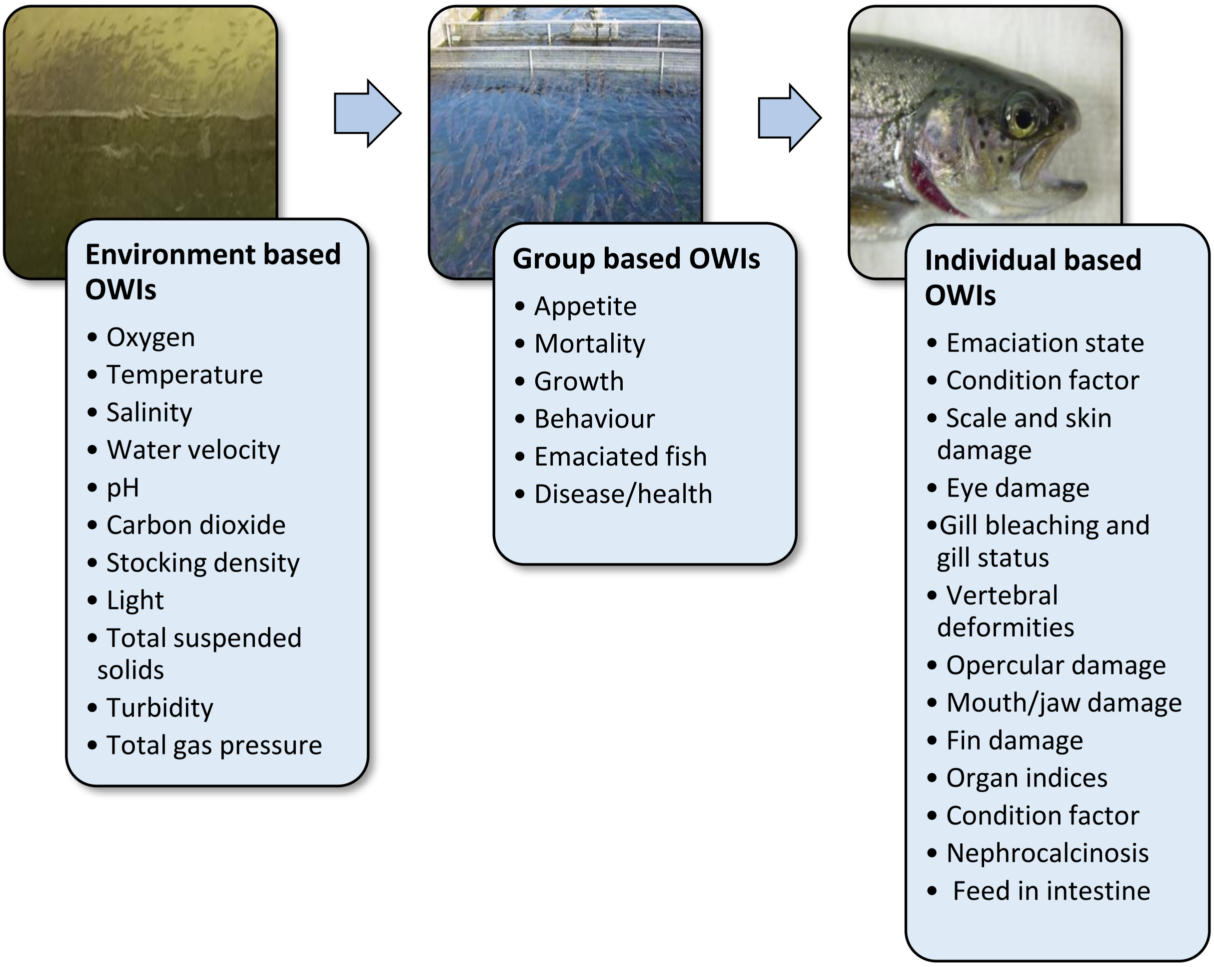


Figure 1.3-1. *Overview of OWIs suitable for flow-through land-based systems. Environment based OWIs address the rearing environment, group based OWIs address the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration Jelena Kolarevic, Chris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

1.4 Environment based OWIs

The recommended water quality parameters differ according to developmental stage with embryos and alevins being more susceptible. In the context of water quality there is relatively little literature on the potential interactions between water quality and the welfare of rainbow trout [15]. While literature refers to optimal levels for rainbow trout, most of this is based on limits at which gross negative production effects are observed.

Table 1.4-1 *Derived from RSPCA welfare standards for farmed rainbow trout [16] with permission from John Avizienius. These are the standards which have to be complied with when water is re-circulated but should be the target for flow through systems. Alternative sources are indicated.*

Water quality parameter	Ova / Alevins	Fry to Ongrowers
O_2 (mg L^{-1}) minimum	7.0	7.0
O_2 (% saturation) min. at exit	90	70
NH_3 ammonia (mg L^{-1})	< 0.025 (< 0.02*)	< 0.025 (< 0.02*)
CO_2 (mg L^{-1})	< 10 (< 2§)	< 10 (< 2§)
Temp (°C) Min-Max	1 – 10	1 – 12 (fry/fingerlings) 1 – 16 (ongrowers) (< 21§)
pH Min-Max	7 – 8 (6.5 - 8.5§)	7 – 8 (6.5 - 8.5§)
Turbidity (mg L^{-1})	< 25.0	< 25.0
Nitrite (mg L^{-1})	< 0.2	< 0.2
Nitrate (mg L^{-1})	N/A	< 50
Aluminium (mg L^{-1}) labile	0.075*	0.075*

*Wedemeyer, [17]

§FAO (http://www.fao.org/fishery/culturedspecies/Oncorhynchus_mykiss/en)

Oxygen is the most important water quality parameter that can limit production in FT systems. Oxygen requirements can differ between life stages but oxygen demand will increase with temperature as the metabolic rate of the fish effectively increases. The most important factors that will determine oxygen use are body size, temperature, stress, activity (swimming, feeding) and life stage. A recently published paper [18] outlines detailed data on the limiting oxygen saturations (LOS) of rainbow trout at different temperatures and at different sizes. (Table 1.4-2). LOS is the minimum level where the fish can maintain sufficient respiration and levels below this are therefore lethal. The LOS values in Table 1.4.-2 are measured on fasted fish, and a higher oxygen level may be required when fish are satiated [18] or during potentially stressful situations such as crowding. Oxygen levels should therefore always be well above the LOS levels. As a general precautionary guideline, oxygen saturation levels of > 80% are recommended, based upon data from Poulsen et al., [19] and the RSPCA welfare standards for farmed rainbow trout recommend a minimum of 70% / 7 mg L^{-1} for fry to ongrowers [16].

Table 1.4-2. *The limiting oxygen saturation (LOS) for fasted diploid and triploid rainbow trout of ca. 15-130 g (DO levels in mg L-1). Reprinted with permission from Springer Nature: Shi, K., Dong, S., Zhou, Y., Gao, Q., Li, L., Zhang, M. & Sun, D. (2018) Comparative Evaluation of Toleration to Heating and Hypoxia of Three Kinds of Salmonids. Journal of Ocean University of China 17(6), 1465-1472. [18] Copyright 2018.*

	LOS: diploid				LOS: triploid			
	Fish size				Fish size			
Temperature	16 g	40 g	79 g	131 g	16 g	39 g	79 g	130 g
13	4.7	4.4	4.2	3.2	4.1	3.9	3.6	3.1
17	5.0	5.1	4.9	3.8	4.3	4.2	4.0	3.4
21	5.4	5.3	5.2	4.5	4.8	4.7	4.2	3.6
25	5.9	5.6	5.3	4.8	5.0	4.8	4.5	4.0

Temperature. Trout can adapt to temperatures in the range of 0 – 22 °C [20] but temperature preferences in rainbow trout can vary with the life stage of the fish. Every effort should be made to maintain temperatures within the optimal range since by the time the critical or lethal temperatures (higher or lower) are reached the welfare of the fish will already have been compromised.

Eggs can be produced at < 15°C and higher temperatures increase the risk of tissue damage and developmental disorders [21 and references therein]. The lower temperature range is somewhat unclear, but EFSA, [21] suggest a temperature as low as 0 °C is not detrimental to eggs. The RSPCA welfare standards for farmed rainbow trout [16] recommend 1 – 10 °C for ova or alevins. Poppe et al., [22] also state the optimal temperature for rainbow trout egg production is 10 °C, within a tolerance range 8 – 12 °C. Sub-optimal temperatures during egg incubation are a known risk factor for skeletal deformities in rainbow trout; however, more research is required in this area. As per other salmonid species, vertebral deformities in rainbow trout are likely to be of multifactorial aetiology.

Fry and fingerlings have a preferred temperature range of 7 – 13 °C [23] and the RSPCA welfare standards for farmed rainbow trout [16] recommend 1 – 12 °C for fry.

It has been suggested that ongrowers have a preferred temperature of around 16 °C within a range of 13 – 19 °C under normoxic conditions [24]. The RSPCA welfare standards for farmed rainbow trout recommend 1 – 16 °C for ongrowers [16].

Changes in temperature should also be monitored and large or rapid changes avoided where possible. Boyd and Tucker [25] recommend the maximum rate of temperature change should be 0.5 °C min^{-1} for any temperature changes over 5 °C, or fish may suffer thermal shock.

Salinity is specific for life stages, with rainbow trout having the capacity to grow entirely in the fresh water environment or move to full strength salt water. According to EFSA [21] rainbow trout become euryhaline when the fish are greater than 50g and fish that are transferred at 70 – 100g have a good survival rate and are apparently able to cope with the transfer to sea outwith a specific smolting window. Fish raised in freshwater containing low Ca^{2+} may have problems adapting to sea water after transfer, but this can be remedied by feeding the fish specialist diets to encourage pre-adaptation to the marine environment [26]. Although literature is scarce, there is some evidence that salinity can affect appetite in rainbow trout. For example, a study by McKay and Gjerde [27], reported that salinities ≥ 10 ‰ significantly reduced appetite compared to fish raised at 0 ‰ in ca. 50 – 150g fish.

Carbon dioxide is a concern particularly for fresh-water life stages in FT systems and its solubility decreases with increasing temperature and salinity. 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 [28]). The negative effects of CO_2 on trout are summarized in Part A, section 4.1.4. In summary, earlier work on trout weighing ca. 260 g by Danley et al., [29] reported that CO_2 levels of ~34 mg L^{-1} and ~49 mg L^{-1} had a significant detrimental effect upon growth and plasma chloride levels after 12 weeks of chronic exposure in comparison to fish held at CO_2 levels of ~22 mg L^{-1}. However, elevated CO_2 levels did not affect mortality [29]. Other work carried out by Good et al., [3] on rainbow trout held in RAS tanks from ca. 60 g to market size reported no significant differences in growth and survival when fish were subjected to CO_2 levels of ~8 mg L^{-1} or ~24 mg L^{-1} for 6 months. Nephrocalcinosis was also not observed in any sampled fish at either CO_2 level [3]. Hafs et al., [30] reported that CO_2 levels ~49 mg L^{-1} resulted in lower growth in ongrowers (300 – 500g starting weight) in comparison to fish reared at ~30 mg L^{-1} and recommended CO_2 levels should be < 30 mg L^{-1} for rainbow trout. With regard to other recommendations for rainbow trout, RSPCA [16] recommend < 10 mg L^{-1} for ova, alevins and ongrowers and Wedemeyer [17] also recommends < 10 mg L^{-1}.

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 rainbow trout 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 [6]. 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 trout. 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. EFSA [21 and references therein] suggest trout should be reared in a pH range of 5.0 – 9.0, state a pH of less than 4 can lead to significant mortalities and a pH between 4.5 and 5.5 induces sublethal effects.

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 is well documented that water velocity that is either too high or too low can have a negative effect on health, welfare and performance, but there is no clear agreement in the literature regarding the ideal water velocity. Studies have found that rainbow trout swimming up to 3 body lengths per second fed to satiation had similar growth and feed conversion to those at lower velocities [31], whilst other studies recommend current velocities between 0 and 1 body lengths per second for optimal growth [32, 33]. More recent work by Larsen et al., [34] suggest current velocities of 0.9 body lengths s^{-1} promoted schooling and reduced the frequency of erratic behaviours in comparison to trout held in static water. McKenzie et al., [35] also reported that current velocities of 0.9 body lengths s^{-1} improved recovery times after trout were subjected to an acute crowding stressor in comparison to trout held in static water. Practical experience would suggest that the velocity should be high enough to encourage the fish to swim in a coordinated manner against the flow, rather than being washed backwards or milling about in an uncoordinated pattern. Such continuous coordinated swimming can be associated with lower levels of aggression and fin damage in salmonids e.g. [36] for Atlantic salmon, [37] for Arctic charr.

Light The optimal light quality (intensity and wavelength) for the optimal performance and welfare of rainbow trout reared in FT systems is still unclear. However, there is clear evidence that both maturation and growth are influenced by light and photoperiod [38]. Increased daylength has a positive effect on growth in the freshwater phase [39, 40] and also increases seawater tolerance regardless of size [41], therefore reducing the duration of the freshwater stage. Photoperiod manipulation can be used to promote seawater adaptation [Morro et al., 42] but the authors stated this factor does not appear to be the main driver for adaptation and other potential environmental drivers, such as salinity or temperature should be examined [42]. The RSPCA welfare standards for farmed rainbow trout state that tank covers should be removed from tanks at least 12 hours before seawater transfer so the fish can acclimate to the potential higher light intensities they will encounter in the cages, and that fish should not be subjected to rapid changes in light intensity [16].

KNOWLEDGE GAP: The optimal light conditions for rainbow trout (both light intensity and light quality) in land-based FT systems is unknown.

Stocking density is only indirectly related to welfare through access to food, water quality and social interactions. Therefore, stocking density should not be used as a sole indicator of good or bad welfare. However, the risk of poor welfare increases at higher stocking densities and at very low stocking densities where more territorial behaviour may be observed. The RSPCA welfare standards for farmed rainbow trout [16] state "*for first feeding and on-growing tanks, raceways and ponds, the maximum stocking density must not exceed 60 kg* m^{-3}". An earlier version of the RSPCA standards [43] monitored other individual based OWIs such as fins, eyes and opercular damage in relation to stocking density and stated the farmer should only maintain stocking densities near the highest level if evidence of such damage is observed in less than 10% of the population. In practice, farmers generally maintain lower stocking densities for younger fish. The effect of different stocking densities on differing welfare parameters is summarized in part A, chapter 4.2.3.

Turbidity is a measure of water clarity. Acceptable levels of turbidity are not available for trout as its potential effects depend on the temperature at the time of exposure, the type of suspended sediments (particle size and angularity), sediment contaminants, the duration and frequency of exposure and also its dose (reviewed in Kjelland et al., [44]). Turbidity has been reported to affect feeding activity, swimming performance, metabolism and the vision of rainbow trout (reviewed in Kjelland et al., [44], also see part A for more details). For example, it has been reported that feeding activity drops sharply at turbidities > 70 Jackobs turbidity units (JTU) (reviewed in Kjelland et al., [44]). However, it has also been reported by Rowe et al., [45] that levels of turbidity up to 160 Nephelometric Turbidity Units (NTU) did not affect the feeding rates of juvenile rainbow trout and other non-visual senses e.g. the lateral line system may play a role in feeding in turbid waters. Increased turbidity also prevents observation of fish in the tanks and can also effect water quality as water with high turbidity has less dissolved oxygen.

KNOWLEDGE GAP: optimal turbidity levels for rainbow trout are not specified (also dependent on the type of solids).

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 [46]. 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 a habitat for the growth of pathogens [46]. A definitive threshold value for an acceptable TSS level has not been agreed upon [46], but an upper limit of 15 mg L^{-1} has been suggested for Atlantic salmon [28] and RSPCA [16] recommends a maximum concentration of non-spate suspended solids of < 25mg L^{-1} for all life stages of rainbow trout (while the recommended TSS is not given separately). However, Becke et al., [47] suggest this limit is too low and reported that in certain circumstances (in RAS) TSS levels up to 70 mg L^{-1} did not affect the welfare, health and growth performance of rainbow trout but did increase turbidity which impacted upon feeding behaviour and increased bacterial load. It is important to keep in mind that the effect of TSS on the welfare of rainbow trout will be dependent upon the total amount and characteristics of suspended solids, making it difficult to set a definite maximum level of TSS that is acceptable for rainbow trout (see also EFSA [21]).

KNOWLEDGE GAP: The optimal TSS levels for rainbow trout are not specified.

Total gas pressure (TGP), oxygen and nitrogen supersaturation. According to Hjeltnes et al., [48] "*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*." Supersaturation is a welfare risk for trout [16]. 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 (GBD) [49]; however, the same authors also state TGP is more important than nitrogen saturation alone [49]. Oxygen supersaturation may also play a role in GBD in trout [50, 51].

It seems that fry are more vulnerable than adult fish when it comes to the effect of supersaturation. 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*" [48]. Their severity is closely linked to percentage supersaturation, the O_2: N_2 ratio and exposure time e.g. [48].

With regard to the effects of oxygen supersaturation on GBD, exposure to oxygen pressures of 200% and 120% TGP while nitrogen pressure was kept at ca. 100% led to GBD within 4 days of exposure and rainbow trout mortalities of 50% within 20 days [50]. Machova et al., [51] also reported a case study where gas bubble disease was related to an oxygen supersaturation of up to 136% that led to rainbow trout mortalities.

With regard to TGP, a study by Gültepe et al., [52] reported that 200g rainbow trout exposed to 115% TGP compared to 104% TGP showed signs of GBD e.g. darkened epidermis, eye hemorrhaging, exophthalmia, gas bubbles on the operculum, significantly elevated i) partial pressures of O_2, ii) partial pressure of CO_2, iii) carboxyhaemoglobin levels, and iv) bicarbonate ion concentrations, increased swimming activity, panic episodes and reduced carbonic anhydrase enzyme activities in the eye lens. According to the Norwegian Food Safety Authority, TGP should not be higher than 100%.

With regard to nitrogen supersaturation, negative effects have been observed on the fish at nitrogen saturations above 102% in Atlantic salmon and rainbow trout [53], and Lekang [53] recommended that N_2 is kept below 100.5%. Wedemeyer [54] also states that N_2 saturation in intensive production systems should be below 110%. Skov et al., [55] looked at the effect of N_2 supersaturation on juvenile rainbow trout, both alone and in combination with increased TGP. They found that an exposure of up to 103% TGP in combination with nitrogen saturation between 104.5 and 107.6% negatively affected energy uptake and energy expenditure. However, N_2 supersaturation alone (102.4 - 105.2%) without TGP supersaturation (TGP ca. 100%) did not have the same effects. The effects observed at 103% TGP and supersaturated N_2 were reversible within 25 days after the end of exposure.

Since there is a lot of uncertainty about trout'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 [49]. As there is still a lot of confusion regarding this, it is important to look at TGP, oxygen 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), oxygen and nitrogen supersaturation in rainbow trout and more knowledge is needed (also see Part A section 4.1.6 of this handbook).

Table 1.4.1-3 *Environment based OWIs appropriate for use in FT aquaculture systems.*

OWI	Relevant life stage
Temperature	Egg, fry, fingerlings and ongrowers. Especially critical during first feeding
Oxygen	Egg, fry, fingerlings and ongrowers.
Velocity	Egg, fry, fingerlings and ongrowers.
pH	Fry, fingerlings and ongrowers.
CO_2	Fry, fingerlings and ongrowers.
Stocking density	Fry, fingerlings and ongrowers.
Light	Fry, fingerlings and ongrowers.
Turbidity	Fry, fingerlings and ongrowers.
TSS	Fry, fingerlings and ongrowers.

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

Many of these group based OWIs are performance based indicators such as growth and survival, and while these have limitations, they can be useful tools if used correctly.

Appetite is a robust, passive OWI for tank rearing and can be an early warning signal for potential welfare problems [56]. 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 [57] and should be monitored continuously. Appetite can be suppressed by i) poor water quality [e.g. 58, 59], ii) environmental conditions including daylength, both natural [60] and artificial [61], iii) husbandry routines e.g handling [62], iv) outbreaks of disease [63], and v) stress [64] amongst a multitude of other factors. It can also vary widely within and between days [65, 66]. 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.

Mortality has to be recorded on a daily basis, see also [16]. 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 determined and recorded and dead fish are often preserved for further analysis and inspected by fish health personnel. Reduced survival is one of the most robust indications of deteriorating welfare and is also one of the most sensitive indicators of the early stages of disease outbreaks in the population, therefore recording monitoring and responding to changes in mortality rates is an essential aspect of health and welfare management. In aquaculture systems, improved survival is rarely if ever associated with a deterioration in welfare. While improved survival in isolation does not indicate good welfare, improvements in survival can be associated with improvements in many aspects of farm husbandry, environment and disease control. Therefore, improved survival can provide evidence of positive changes in welfare when combined with other indicators.

Growth may be affected by several factors, such as nutrition and diseases, social interactions [67, 68], water quality and chronic stress [e.g. 69] 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 well as overall growth rate, the variation in growth should be monitored, since a wider variation in growth may indicate inequitable access to food, undetected health issues or other problems. 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 [70, 71]. Behaviour is a general indicator and deviations may be caused by many different factors. Reduced locomotor activity may also be a response to poor environmental conditions e.g. low oxygen levels [72] or low oxygen/high ammonia levels [73]. Increased swimming activity and dispersed swimming can also be a response to a handling stressor such as crowding [74]. Unstructured swimming at the bottom of the cage or tank can also be an indicator of acute stress [e.g. 71, 72]. Swimming activity may also be affected by stocking density in tanks and Anras and Lagardère [71] reported fish under 30 kg/m^3

densities mostly exhibited circular diurnal swimming patterns followed by reduced activity at night compared to fish at 136 kg/m^3 that exhibited unstructured diurnal swimming patterns that were also maintained at relatively high levels at night. Aggression can be a problem in trout [75, 76, 77] and can be qualitatively or quantitatively monitored by visual observation of the fish. While dorsal fin damage is the most prevalent form of fin damage [78, 79] and whilst it may not always be associated with aggression, it is an indicator of damaging interactions that can be easily observed in FT systems with low turbidity. The problem can be quantifying the severity and prevalence of these lesions from surface observations, in many cases this is better done by examining of a sample of the fish (see Part A, 3.2.10 fin damage section).

Small scale experimental studies have shown that rainbow trout behaviour can be affected by feed management and McFarlane et al., [80] reported that activity levels are lowest when fish are fed to satiation, increase when fed to satiation but stressed by crowding twice weekly, and are at their highest when fish are subjected to a fasting/satiation feeding regime (where fish were fasted for a week and then fed to satiation for a week). This was especially apparent when fish were fasted and during the early phases of re-feeding in comparison to fish fed consistently to satiation or those under the satiation/stress regime [80]. Other studies have also shown that rainbow trout exhibit highly energetic feeding behaviour and can be highly competitive around meal times [e.g. 76, 81]. Swimming speeds can therefore be used as a possible OWI of increased competition for a feed resource.

Prevalence of emaciated fish. Emaciated fish are often found near the surface, isolated and often around the periphery of the group. 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 [82 for A. salmon, but equally applicable for rainbow trout]. The occurrence of these moribund or emaciated fish should be monitored [82] 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. An overview of disease characteristics for both fresh water and seawater stages of rainbow trout 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, fingerlings and ongrowers
Growth	Fry, fingerlings and ongrowers
Mortality	Fry, fingerlings and ongrowers
Behaviour (swimming, aggression)	Fry, fingerlings and ongrowers
Emaciated fish	Fingerlings and ongrowers
Disease / health status	Fry, fingerlings and ongrowers

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 rainbow trout 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. "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. Often this can indicate problems associated with handling events. 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. Wound healing is dependent on temperature and environmental conditions, in addition to the status of the wound e.g. wound depth [e.g. 83]. Sometimes wound healing can be relatively quick, but it has also been demonstrated that wounds can take over 3 months to heal [84]. Other studies on rainbow trout (where wound depths ranged from ca. 3 mm to the depth of the muscle layers), reported that scales did not regenerate, even after one year [83].

Eye damage. 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, including nutritional factors and parasitic infections. Obvious damage to the eyes may result from contact with equipment in or above the tank. 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 occur early in life but may not become apparent until later. These may be caused by nutritional problems, rearing conditions in the hatchery or genetic conditions e.g. [85, 86] amongst other factors. Fish with vertebral deformities may have impaired swimming and manoeuvrability making then less able to compete for food or more susceptible to injury. For more detailed information see Part A, section 3.2.9 of this handbook.

Opercular damage and gill status. 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 (see Part A section 3.2.13). This interferes with the respiratory efficiency of the opercular pump and can make the fish more susceptible to low oxygen saturation or times of high oxygen demand, through stress or exercise. While it would appear from practical experience that most opercular damage occurs early in life, it may become more easily detected as the fish grow. Opercular damage may make the the gills more vulnerable to damage during handling. Inspecting the gills can also give some indications of gill status of the fish.

Fin damage is an indication of some issues with the rearing environment. Dorsal fin damage is the most common form [78]. This may be associated with water velocity, feeding frequency or distribution and other factors [e.g. 9, 87, 88]. Other fins may also be damaged by interactions between fish or contact with the rearing tank or other structures. Fins have all the necessary neural apparatus to perceive damage and therefore injury to fins may cause pain, but also provide a portal of entry for infections and impede swimming performance and manoeuvrability [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 (based on photos from salmonids).

Feed in the intestine. Feed in the intestine is often an indicator that trout have eaten in the last 1-2 days [65] 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*). There are various ways to monitor condition factor from subjective assessment of the condition of the fish to calculations from weight and length. Condition factor (*K*) is calculated as 100 x body weight (g) x body length (cm)$^{-3}$. Even in a population with generally good condition factors there may be some thin or even emaciated fish which either have an underlying health issue or have failed to adapt to the feed provided. As condition factor (*K*) is variable and changes with both life stage and season it is difficult to define exact values that are indicative of reduced welfare [82]. However, in long-term feed withdrawal studies on rainbow trout, values of < 1.0 have been reported in juvenile trout (ca. 55g mean weight) fasted for 4 months [89]. A fasting study on larger fish (ca. 280g mean weight) reported that *K* values dropped from an initial level of ca. 1.15-1.2 to ca. 1.05 after 1 month and ca. 0.9 after 4 months [90]. We therefore suggest a *K* factor of ca. 1.0 or < 1.0 can be indicative of emaciation in farmed rainbow trout. Rainbow trout can also accumulate large deposits of abdominal fat if overfed. The welfare implications of such obesity are not clear but it is a sign of poor feed management.

Nephrocalcinosis is a pathology that has so far been related to high concentrations of dissolved CO_2 [91] 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. A scoring scheme for nephrocalcinosis is currently being validated.

Table 1.6-1 *Individual OWIs appropriate for use in flow-through aquaculture systems*

OWI	Relevant life stage
Fin, skin, eye, mouth, opercular, gill damage	Fry, fingerlings and ongrowers
Vertebral deformities	Fry, fingerlings and ongrowers
Emaciation state	Fingerlings and ongrowers
Feed in intestine	Fingerlings and ongrowers
Organ indexes	Fingerlings and ongrowers
Condition factor	Fingerlings and ongrowers
Nephrocalcinosis	Fingerlings and ongrowers
Feed in the intestine	Fry, fingerlings and ongrowers

2 Sea cages

2.1 Rearing trout in sea cages

In 2018 more than 17 million rainbow trout were transferred to Norwegian sea cage farm facilities (Norwegian Directorate of Fisheries). 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 [92, 93]. 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.

2.2 Challenges to fish welfare

Challenging water environment: Trout 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 [94]. Large losses can be experienced if the fish are sick, have been exposed to challenging transport conditions or if parts of the population are not physiologically ready to adapt to sea water. In Norway, trout 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 (> 19 °C, [21]). The location of the farm, in a fjord on the coast or offshore, also affects the challenges the trout face after transfer to the sea. The continuous flow of water through the cage means that the trout have to cope with seasonal changes, due to tidal currents, freshwater runoffs, storms, upwelling and blooms of phytoplankton or zooplankton (see Fig. 2.2-1). 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. 2.2-2). 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 [93]. 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. 2.2-1). Some phytoplankton or zooplankton can also damage the gills of the fish [95] and the influx of new water into the cage can expose the fish to other pathogens or harmful organisms such as poisonous algae, viruses, bacteria, parasites or stinging organisms such as jelly fish. In addition to bacteria and viruses, infectious stages of sea lice are also a component of the zooplankton and a welfare challenge to farmed trout [96]. 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 [97]. Another parasite that has become a major problem in Norway in recent years is the protozoan *Neoparamoeba perurans* that causes amoebic gill disease (AGD) and trout can also be affected [98].

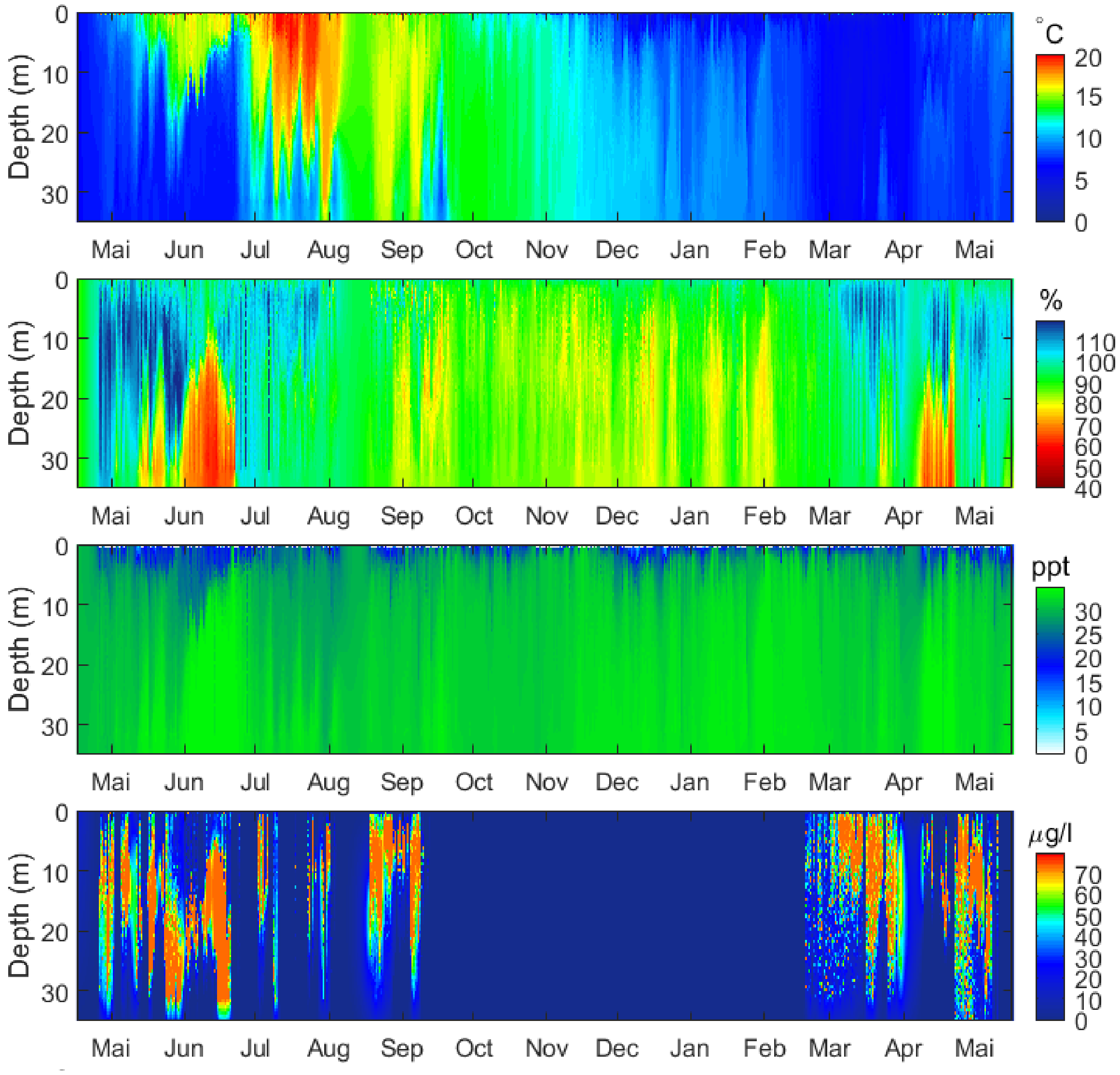


***Figure 2.2-1**. Temperature (°C), oxygen saturation (%), salinity (ppt) and fluorescence (µg L^{-1}) measured in a fjord in Western Norway. Upwelling occurred in June and also in April-May, creating sudden and long lasting poor oxygen conditions below 10 m. High concentrations of phytoplankton (measured 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.*

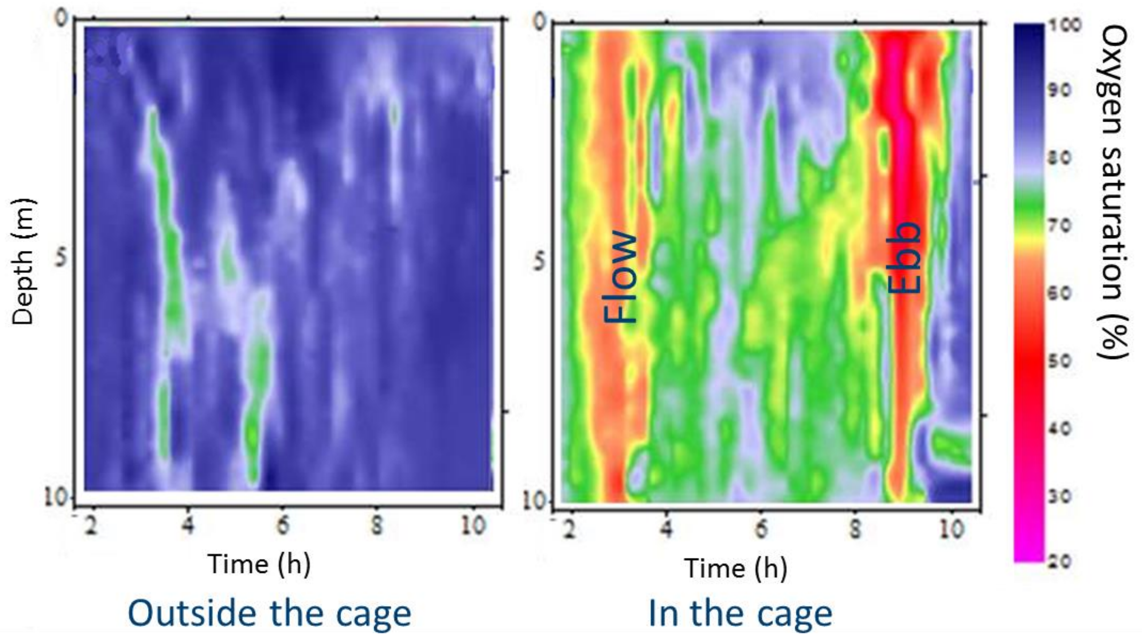


Figure 2.2-2. *Example of hypoxic conditions inside a sea cage at slack water. Illustration adapted from [99].*

Potentially dangerous environment: Farming out in a natural environment can mean the trout 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 environmental conditions. For example, wound healing is dependent on temperature, in addition to the status of the wound e.g. wound depth [e.g. 83]. Sometimes wound healing can be relatively quick, but it has also been demonstrated that wounds can take over 3 months to heal [83, 84]. Other studies on rainbow trout (where wound depths ranged from ca. 3 mm to the depth of the muscle layers), reported that scales did not regenerate, even after one year [83]. See Part C of this handbook for more information on fish welfare in relation to handling and other common husbandry operations.

2.3 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 2.3-1).

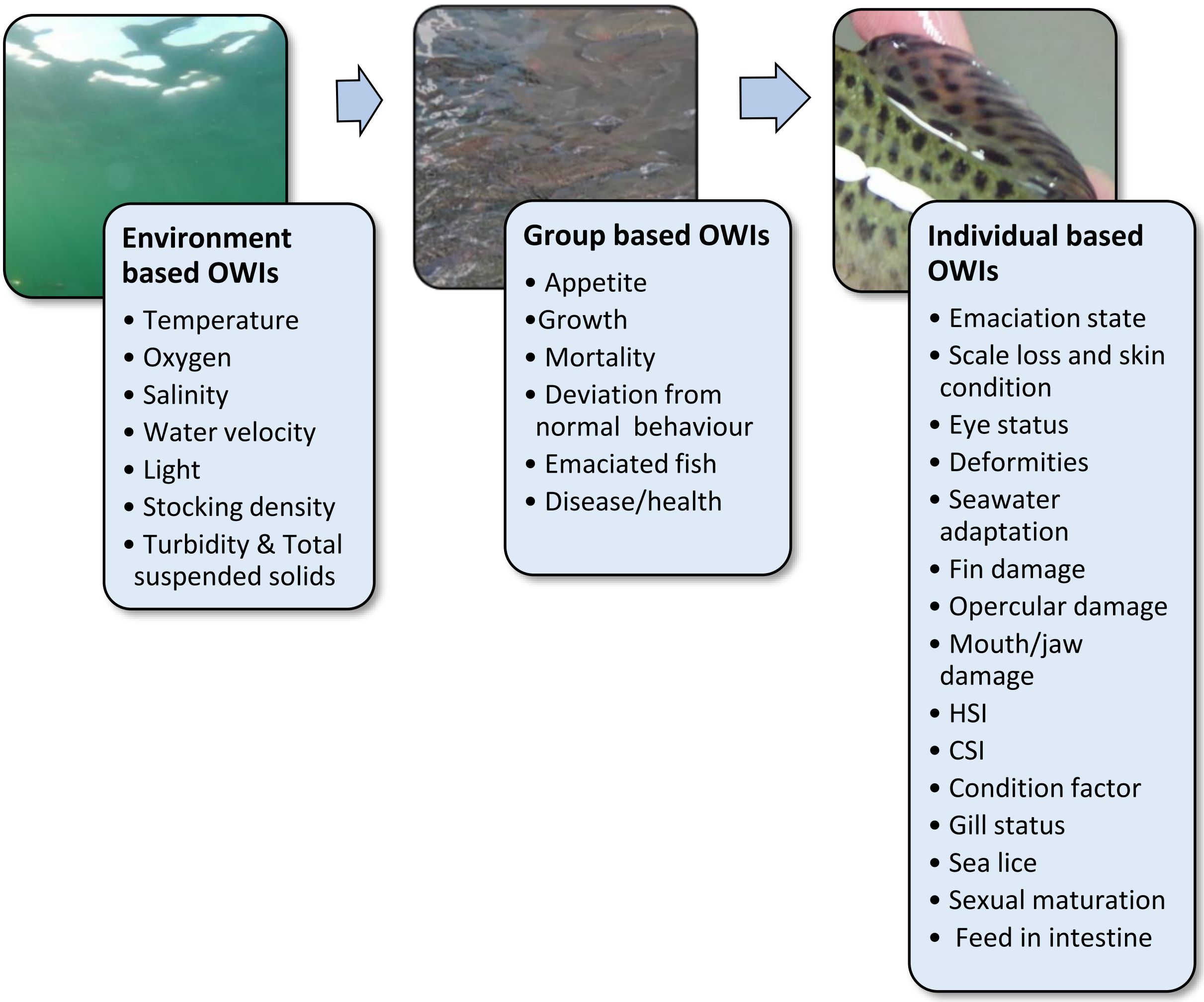


Figure 2.3-1*. Overview of fit for purpose OWIs for sea cages. Environment based OWIs address the rearing environment, group based OWIs refer to the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration, Lars H. Stien and Chris Noble.*

2.4 Environment based OWIs

Temperature is a major factor that influences the vertical distribution of trout held in sea cages [92]. Trout prefer temperatures around 16 °C within a range of 13-19 °C under normoxic conditions [24] although this preference and range varies under hypoxic conditions. Alanärä [100] has reported that trout exhibit peak appetite at 15-16 °C. Sutterlin and Stevens [92] also reported that cage held rainbow trout with a mean weight of ca. 1.9 kg had a temperature preference for ca. 13 °C within a range of 7-17 °C when held in stratified waters. Temperatures higher than 19 °C in marine or brackish waters can potentially lead to high mortalities [21] although trout can adapt to temperatures in the range of 0-22 °C [20] provided that the oxygen levels are sufficiently high and that there is a gradual transition in temperature e.g. [25].

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. Trout increase their metabolic activity with temperature and therefore need more oxygen at higher temperatures. Oxygen requirements can differ between life stages but oxygen demand will increase with temperature as the metabolic rate of the fish effectively increases. The most important factors that will determine oxygen use are body size, temperature, stress, activity (swimming, feeding) and life stage. A recently published paper [18] outlines detailed data on the limiting oxygen saturations (LOS) of rainbow trout at different temperatures and at different sizes. (Table 2.4.-2). LOS is the minimum level where the fish can maintain sufficient respiration and levels below this are therefore lethal. The LOS values in Table 2.4.-2 are measured on fasted fish, and a higher oxygen level may be required when fish are satiated [18] or during stressful situations such as crowding. Oxygen levels should therefore always be well above the LOS levels. As a general precautionary guideline, oxygen saturation levels of >80% are recommended, based upon data from Poulsen et al., [19] and the RSPCA welfare standards for farmed rainbow trout recommend a minimum of 70% / 7 mg L^{-1} for fry to ongrowers [16].

Table 2.4-2. *The limiting oxygen saturation (LOS) for fasted diploid and triploid rainbow trout of ca. 15-130 g (DO levels in mg L-1). Reprinted with permission from Springer Nature: Shi, K., Dong, S., Zhou, Y., Gao, Q., Li, L., Zhang, M. & Sun, D. (2018) Comparative Evaluation of Toleration to Heating and Hypoxia of Three Kinds of Salmonids. Journal of Ocean University of China 17(6), 1465-1472. [18] Copyright 2018.*

	LOS: diploid				LOS: triploid			
	Fish size				Fish size			
Temperature	16 g	40 g	79 g	131 g	16 g	39 g	79 g	130 g
13	4.7	4.4	4.2	3.2	4.1	3.9	3.6	3.1
17	5.0	5.1	4.9	3.8	4.3	4.2	4.0	3.4
21	5.4	5.3	5.2	4.5	4.8	4.7	4.2	3.6
25	5.9	5.6	5.3	4.8	5.0	4.8	4.5	4.0

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 2.2-1, [93 and references therein]). EFSA [21] state euryhalinity occurs in rainbow trout when the fish are greater than 50g and fish that are transferred at 70-100g have a good survival rate and are apparently able to cope with the transfer to sea outside a specific time window. Fish raised in freshwater containing low Ca^{2+} may have problems adapting to sea water after transfer but this can be remedied by feeding the fish specialist diets to encourage pre-adaptation to the marine environment [26]. With smaller fish improvements are seen when there is a gradual introduction or the marine environment is not full strength sea water [101, 102, 103]. Signs of lack of adaptation to the marine environment would be lack of growth and chronic low level mortalities. Sutterlin and Stevens [92] reported that cage-held rainbow trout reared in stratified waters had a preference for salinities levels < 25 ppt and temperatures > 10 ^{o}C; the fish actively avoided cooler deeper waters of higher salinity. McKay and Gjerde [27] also reported that salinities of >20 ‰ may be detrimental to production (growth, appetite ,mortality) in ca. 50-150g trout exposed to salinities ranging from 0-32 ‰ for 12 weeks.

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 2.2-1). 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 [95]. 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 [15]. It is well documented that water velocity that is either too high or too low can have a negative effect on health, welfare and performance, but there is no clear agreement in the literature regarding the ideal water velocity. 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 trout are able to maintain fast swimming primarily depends on their general fitness, water temperature and size. Studies have found that rainbow trout swimming up to 3 body lengths per second fed to satiation had similar growth and feed conversion to those at lower velocities [31]. Other studies recommend current velocities between 0 and 1 body lengths per second for optimal growth [32, 33]. More recent work by Larsen et al., [34] suggest current velocities of 0.9 body lengths s^{-1} promoted schooling and reduced the frequency of erratic behaviours in comparison to trout held in static water. McKenzie et al., [35] also reported that current velocities of 0.9 body lengths s^{-1} improved recovery times after trout were subjected to an acute crowding stressor in comparison to trout held in static water. In other salmonids, current velocities that are too low may also lead to problems with fin biting and aggression [104, 105] and maintaining active swimming in the population can improve growth and feed conversion since fish divert more energy to maintaining position and less to social interactions [e.g. 37].

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 [75]. However, there is little doubt that stocking densities that are either too low or too high can impair welfare in trout [35, 106]. The RSPCA welfare standards for farmed rainbow trout recommend stocking densities for cage held fish < 100 g should be < 10 kg m^{-3}, be < 15 kg m^{-3} at the farm overall, and < 17 kg m^{-3} per cage [16]. Densities below the Norwegian limit of 25 kg m^{-3} are not believed to markedly affect fish welfare in salmonids [82]. Stocking density in sea cages is therefore primarily an indirect welfare indicator as e.g. 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.

Light conditions in a sea cage vary with depth, time of day, weather and season. Increased daylength has a positive effect on growth in the seawater phase [107]. Rainbow trout are natural spring spawners and extending daylength from midwinter through the spring results in earlier spawning than in controls [reviewed by 108]. However, if this approach is adopted in 1 year old fish, it can prevent or delay spawning the following year [109]. In addition, the change in daylength appears to have a far more important effect on maturation than daylength *per se* [108]. The influence of light conditions on the swimming behaviour of caged rainbow trout is not as widely studied as in salmon. Trout 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 [110] and their behavioural response to submerged lights is probably similar to that seen in salmon. The RSPCA welfare standards for farmed rainbow trout state that tank covers should be removed from tanks at least 12 hours before seawater transfer so the fish can acclimate to the potential higher light intensities they will encounter in the cages, and the cages must be deep enough to make sure the fish arent damaged by UV radiation [16].

How to measure water quality in sea cages

- When measuring water quality in sea cages the goals are 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.
- Current speed can now be measured real-time online using commercially available technology in and around the farms.

2.5 Group based OWIs

Appetite or a fish's propensity or willingness to feed [111] is a robust, passive OWI for sea cages and can be an early warning signal for potential welfare problems [56]. 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 and feeding can be influenced by daylength [60], oxygen saturation [58], the health status of the fish [63], ectoparastic level [112] and stress [64]. It is well known that the appetite of trout can vary widely within and between days e.g [65]. 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).

Growth. Although growth rates in fish are flexible and may be affected by several factors, such as nutrition and diseases, social interactions [67, 68], water quality and chronic stress [e.g. 69], acute periods of poor growth below what is expected/normal (although this is very site specific) can be used as an OWI [56]. 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. Several standard mortality curves have been developed for salmon [97, 113, 114] and a standard mortality curve for Atlantic salmon based on data from Norwegian farmers has also been developed [94]. The mortality curves for rainbow trout in Fig. 2.5-1 are based upon the same principles and dataset parameters as Stien et al., [94]. The median daily mortality of rainbow trout was 0.02% and the total accumulated production mortality was 15% for rainbow trout transferred to sea between 2009-2015, showing that most production predominantly stays in the green area (Figure 2.5.1). 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.

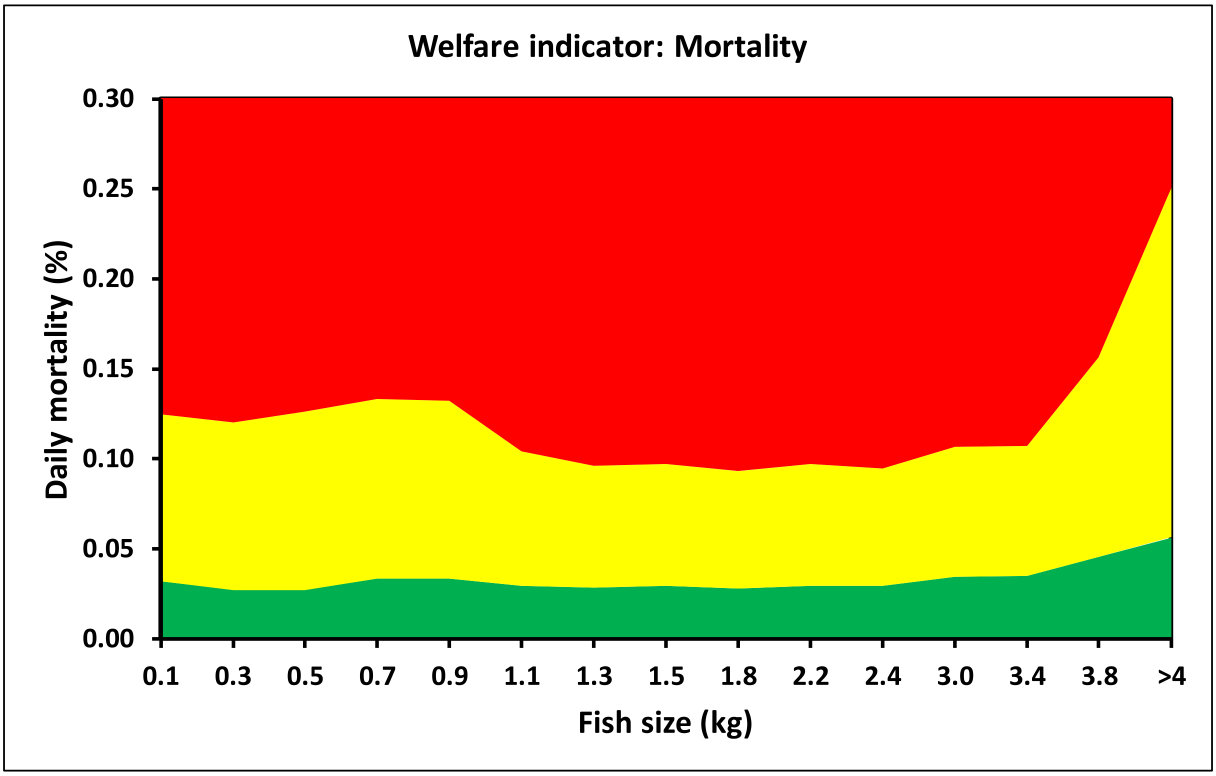


Figure 2.5-1. *Standard mortality curve for rainbow trout in sea cages in relation to fish size, based on data reported by the Norwegian industry for the year classes 2009-2015. 75 % of all observations are in the green area and can be categorized as "normal", while 5 % of the observations are in the red area and categorized as abnormal.*

Prevalence of emaciated fish. In all production systems some individuals may become thin or emaciated. Transfer to the sea involves exposing the fish to a completely new and fluctuating environment, which is stressful and may make individuals stop feeding. Emaciated fish are often found near the surface, isolated and often around the periphery of the group. In the marine phase, 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 as emaciation has been linked to parasitic load in rainbow trout, they can also be a vector for transmitting diseases to other healthier fish [115]. The occurrence of these moribund or emaciated fish should be monitored [82] 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. In comparison to Atlantic salmon, the behaviour of rainbow trout in sea cages has been less well studied.

The following is a summary of some of the reported rainbow trout behaviours observed in sea cages:

- In a study by Sutterlin and Stevens [92], adult trout (ca. 1.9kg) held in cages in waters with stratified temperatures and salinities had a distinct preference for salinities < 25 ppt and temperatures ca. 13 °C and actively avoided cooler deeper water of higher salinity. Trout also showed diel variations in temperature preference of up to 3-4 °C .
- Early work by Sutterlin et al., [116] reported that rainbow trout and Atlantic salmon behaviour in sea cages can be quite different, with A. salmon exhibiting a schooling type circular activity pattern in comparison to trout who did not exhibit any consistent circular swimming or rotational orientation (although this may have been due to the presence of staff during observation periods). Another study by Phillips, [117] reported circular swimming activity in trout when fish behaviour was monitored using underwater video. Phillips also reported that cage-held rainbow trout can aggregate near the surface, exhibit low activity at slack water and form polarized shoals and maintain station at higher water current speeds. They also reported frequent aggressive interactions in the form of chasing and charging. Feeding was also synchronised amongst some or all of the observed group if the feeding behaviour of one or more of the fish was rapid enough to elicit a response from the rest of their conspecifics. This was also noted in a study on cage-held rainbow trout by Brännäs and Alanärä [81] where all fish reacted when feed was introduced to the pen.
- Sutterlin et al., [116] also reported that cage held rainbow trout can be conditioned to the presence of farm staff and adapt their swimming behaviour in relation to feed expectation.
- Small scale experimental studies in tanks have shown that rainbow trout behaviour can be affected by feed management and McFarlane et al., [80] reported that activity levels are lowest when fish are fed to satiation, increase when fed to satiation but stressed by crowding twice weekly, and are at their highest when fish are subjected to a fasting/satiation feeding regime (where fish were fasted for a week and then fed to satiation for a week). This was especially apparent when fish were fasted and during the early phases of re-feeding in comparison to fish fed consistently to satiation or those under the satiation/stress regime [80]. This type of behaviour, although noted in tanks, may also be applicable in net cages. Other studies have also shown that rainbow trout exhibit highly energetic feeding behaviour and can be highly competitive around meal times [e.g. 81, 82 in cages and tanks, respectively]. Swimming speeds can therefore be used as a possible OWI of increased competition for a feed resource.

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 rainbow trout are given in Part A, section 3.1.5 of this handbook.

Measuring rainbow trout 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 [92] or differences in feeding regimes [80]. 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. Pinkiewicz et al., [119] have developed a system for quantifying the swimming speeds of cage-held Atlantic salmon, but as far as the authors are aware 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.

2.6 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 (§6 Forskrift om bekjempelse av lakselus i akvakulturanlegg, FOR-2012-12-05-1140 [120]). The lice count involves sampling fish from each cage, sedating each fish and carefully counting the lice on the fish and classifying them into different life stages. In Nordland, Troms and Finnmark 20 random fish must be sampled from each cage as of Monday in week 19 until (and including) Sunday in week 26, while it is enough to sample only 10 fish from each cage outside this period. South of Nordland the period when the farmer needs to sample 20 fish starts on Monday in week 14 and lasts until Sunday in week 21. 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 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 (based on photos from salmonids).

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. 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. Wound healing is dependent on temperature and environmental conditions, in addition to the status of the wound e.g. wound depth [83]. Sometimes wound healing can be relatively quick, but it has also been demonstrated that wounds can take over 3 months to heal [84]. Other studies on rainbow trout (where wound depths ranged from ca. 3 mm to the depth of the muscle layers), reported that scales did not regenerate, even after one year [83].

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 occur early in life but may not become apparent until later. These may be caused by nutritional problems, rearing conditions in the hatchery or genetic conditions e.g. [85, 86] amongst other factors. Fish with vertebral deformities may have impaired swimming and manoeuvrability making then less able to compete for food or more susceptible to injury. For more detailed information see Part A, section 3.2.9 of this handbook.

Opercular damage. 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. This interferes with the respiratory efficiency of the opercular pump and can make the fish more susceptible to low oxygen saturation or times of high oxygen demand, through stress or exercise. While it would appear from practical experience that most opercular damage occurs early in life, it may become more easily detected as the fish grow. Opercular damage may make the the gills more vulnerable to damage during handling.

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. It is an indication of some issues with the rearing environment. Dorsal fin damage is the most common form [78]. This may be associated with water velocity, feeding frequency or distribution and other factors [e.g. 9, 87, 88]. Other fins may also be damaged by interactions between fish or contact with the rearing tank or other structures. Fins have all the necessary neural apparatus to perceive damage and therefore injury to fins may cause pain. Fin damage may also provide a portal of entry for infections and impede swimming performance and manoeuvrability [9].

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*). There are various ways to monitor condition factor from subjective assessment of the condition of the fish to calculations from weight and length. Condition factor (*K*) is calculated as 100 x body weight (g) x body length $(cm)^{-3}$. Even in a population with generally good condition factors there may be some thin or even emaciated fish which either have an underlying health issue or have failed to adapt to the feed provided. As condition factor (*K*) is variable and changes with both life stage and season it is difficult to define exact values that are indicative of reduced welfare [82]. However, in long-term feed withdrawal studies on rainbow trout, values of < 1.0 have been reported in juvenile trout (ca. 55g mean weight) fasted for 4 months [89]. A fasting study on larger fish (ca. 280g mean weight) reported that *K* values dropped from an initial level of ca. 1.15-1.2 to ca. 1.05 after 1 month and ca. 0.9 after 4 months [90]. We therefore suggest a *K* factor of ca. 1.0 or < 1.0 can be indicative of emaciation in farmed rainbow trout. Rainbow trout can also accumulate large deposits of abdominal fat if overfed. The welfare implications of such obesity are not clear but it is a sign of poor feed management.

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. The RSPCA welfare standards for farmed rainbow trout state that the emaciation state of the fish should be monitored in relation to lice infestations, in addition to lesions/wounds/skin condition and appetite. In addition, any fish with severe physical injuries from lice should be euthanised [16].

Seawater adaptation is very important at seawater transfer. Fish that are not adapted for sea water rearing or only partly adapted will have problems with osmoregulation, growth and in the worst cases can die. EFSA [21] state euryhalinity occurs in rainbow trout when the fish are greater than 50g and fish that are transferred at 70-100g have a good survival rate and are apparently able to cope with the transfer to sea outwith a specific time window. Fish raised in freshwater containing low Ca^{2+} may have problems adapting to sea water after transfer, but this can be remedied by feeding the fish specialist diets to encourage pre-adaptation to the marine environment [26]. McKay and Gjerde [27] also reported that mortality levels in rainbow trout recently transferred to seawater were higher at near full salinity (32 ‰), and they also found that growth was reduced at salinities > 20 ‰. Signs of lack of adaptation to the marine environment would be lack of growth and chronic low level mortalities.

Sexual maturation. Salmonids like rainbow trout may mature both in the freshwater stage or after sea transfer [121, 122] and it can be a problem in rainbow trout aquaculture [123]. During maturation, the trout 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).

Feed in the intestine. Feed in the intestine is often an indicator that the trout have eaten in the last 1-2 days [65] 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 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 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 was initally developed for Atlantic salmon [124] and has been adapted for rainbow trout. It is an amalgamation of the injury scoring schemes used in the Salmon Welfare Index Model (SWIM) [82], the injury scoring scheme developed by the Norwegian Veterinary Institute (NVI) [125, 126] 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 13 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, xivii) healed fin damage.

We have used pictures from the salmon handbook in the following scoring system, as the conditions they describe are equally applicable to rainbow trout.

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 rainbow trout, we suggest a simplified scoring system similar to that used in the RSPCA welfare standards for farmed Atlantic salmon [127].

Cataract damage is classified using an existing and widely used 0-4 scoring scheme [128], 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" [129], see Table 3.3 and Fig. 3.4. The Speilberg scale is widely used as a welfare indicator in the Norwegian aquaculture industry, primarily for salmon but it has also been used for trout. 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 [130] 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)*

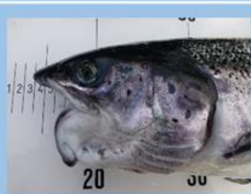
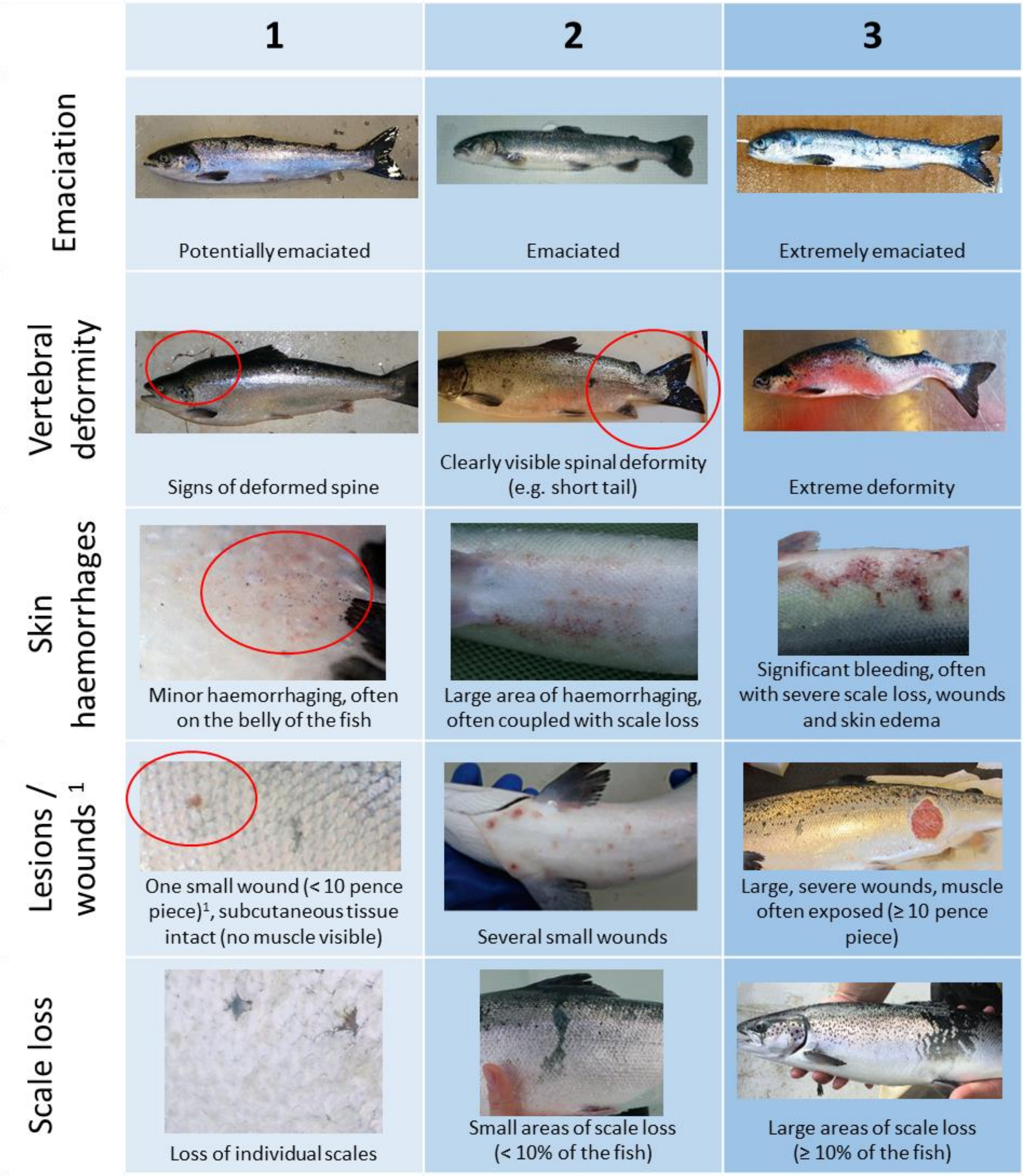
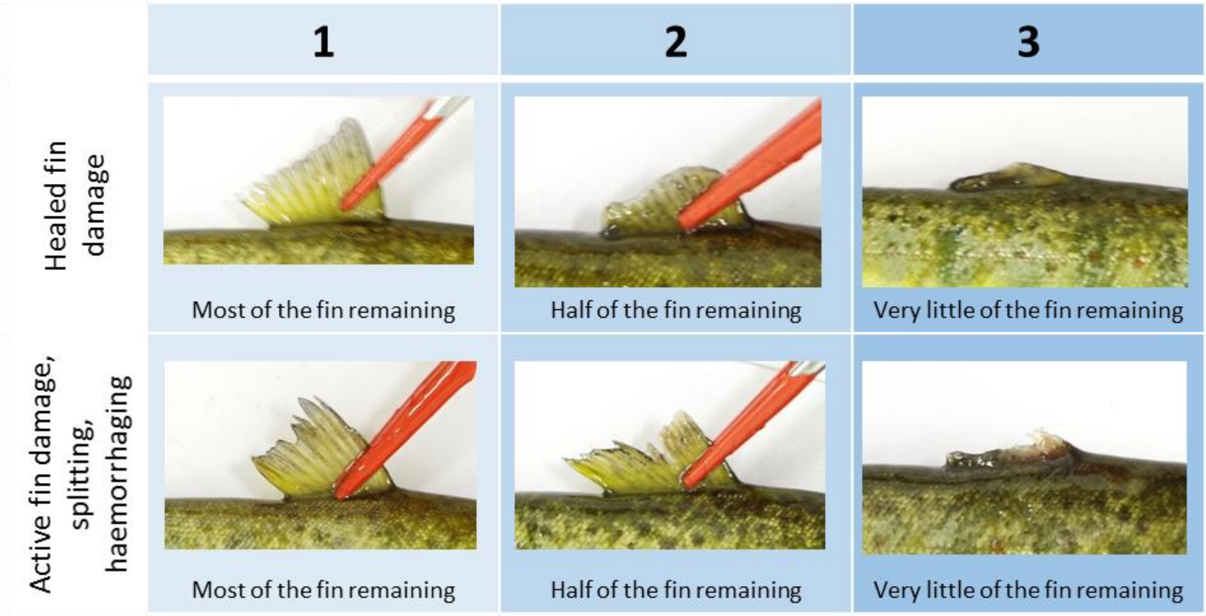
	1	2	3
Eye haemorrhage	 Minor haemorrhages	 Larger haemorrhages, or traumatic injury	 Large haemorrhages / traumatic injury. Eye may be ruptured
Exophthalmia	 Eye protruding a little	 Moderate eye protrusion	 Major eye protrusion
Opercular damage	 Operculum only partly covering gills	 Operculum absent 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

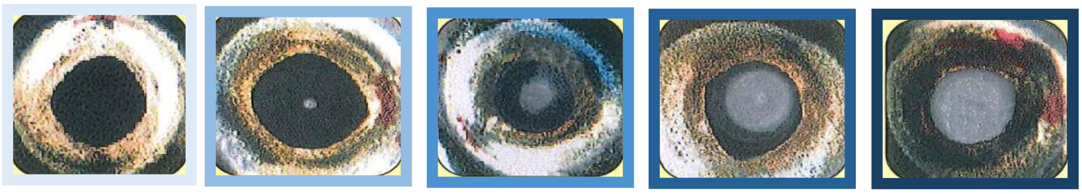
Table 3.1-2. *Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. (Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. 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] *For juveniles "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)*





0. No cataract

1. Cataract covers less than 10% of lens diameter

2. Cataract covers between 10 and 50% of lens diameter

3. Cataract covers 50 to 75% of lens diameter

4. Cataract covers over 75% of lens diameter

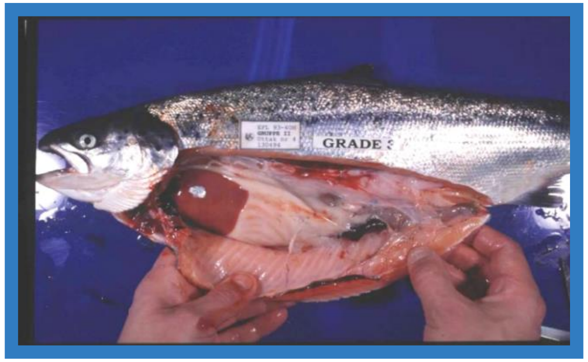
***Fig. 3.2.** Morphological scheme for diagnosing and classifying eye cataracts in salmonids. Text reproduced from "Wall, T. & Bjerkås, E. 1999. A simplified method of scoring cataracts in fish. Bulletin of the European Association of Fish Pathologists 19(4), 162-165. Copyright, 1999" [128] with permission 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." [131] 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" with permission from Elsevier [129]. Scale originally developed for Atlantic salmon but has also been used in studies on rainbow trout [e.g. 132, 133].*

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. Although the pictures are from Atlantic salmon, they are equally applicable to rainbow trout. 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 [129].*

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

***Table 4-1**. Where the reviewed welfare indicators are recommended for use in the production systems discussed in Part B of the handbook.*

		Production systems	
	WI / Usage area	Flow through systems	Sea Cages
Environment WIs	Temperature	×	×
	Salinity	×	×
	Oxygen	×	×
	CO_2	×	
	pH and alkalinity	×	
	Total gas pressure	×	
	Turbidity and susp. solids	×	×
	Water current speed	×	×
	Lighting	×	×
	Stocking density	×	×
Group WIs	Mortality rate	×	×
	Behaviour	×	×
	Appetite	×	×
	• Growth	×	×
	Disease / health	×	×
	Emaciated fish	×	×
Individual WIs	Gill beat rate	×	
	Sea lice		×
	Gill bleaching and status	×	×
	Condition indices		
	• Condition factor	×	×
	• Hepo-somatic index	×	×
	• Cardio-somatic index	×	×
	Emaciation state	×	×
	Sexual maturity state	×	×
	Seawater adaptation	×	×
	Vertebral deformation	×	×
	Fin damage and fin status	×	×
	Scale loss and skin condition	×	×
	Mouth/jaw wound	×	×
	Eye damage	×	×
	Opercular damage	×	×
	Nephrocalcinosis	×	
	Feed in the intestine	×	×

5 References

[1] Smart, G. R. (1981) Aspects of water quality producing stress in intensive fish culture. In: *Stress and Fish* (Ed: Pickering, A. D.) Academic Press, London. 277-293.

[2] Colt, J. (2006) Water quality requirements for reuse systems. *Aquacultural Engineering* **34**, 143-156.

[3] Good, C., Davidson J., Welsh, C., Snekvik, K. & Summerfelt, S. (2010) The effects of carbon dioxide on performance and histopathology of rainbow trout *Oncorhynchus mykiss* in water recirculation aquaculture systems. *Aquacultural Engineering* **42**, 51-56.

[4] Noble, C., Kankainen, M., Setälä, J., Berrill, I. K., Ruohonen, K., Damsgård, B. & Toften, H. (2012) The bio-economic costs and benefits of improving productivity and fish welfare in aquaculture: Utilizing CO_2 stripping technology in Norwegian Atlantic salmon smolt production. *Aquaculture Economics & Management* **16(4)**, 414-428.

[5] Kristensen, T., Åtland, Å., Rosten, T., Urke, H. A. & Rosseland, B. O. (2009) Important influent-water quality parameters at freshwater production sites in two salmon producing countries. *Aquacultural Engineering* **41**, 53–59.

[6] Rosten, T., Åtland, Å., Kristensen, T., Rosseland, B. O. & Braathen, B. (2004) Mattilsynet / Vannkvalitet / Repport 09.08.2004, p.85.

[7] Ellis, R. P., Urbina, M. A. & Wilson, R. W. (2017) Lessons from two high CO_2 worlds–future oceans and intensive aquaculture. *Global change biology* **23(6)**, 2141-2148.

[8] Rosten, T., Urke, H. A., Åtland, Å., Kristensen, T. & Rosseland, B. O. (2007) Sentrale driftsog vannkvalitetsdata fra VK Laks – undersøkelsene fra 1999 til 2006 (Water Quality Monitoring Program, WQ). Norwegian Institute for Water Research (NIVA) pp. 16.

[9] Latremouille, D. N. (2003) Fin erosion in aquaculture and natural environments. *Reviews in Fisheries Science* **11(4)**, 315-335.

[10] Turnbull, J. F., Richards, R. H. & Robertson, D. A. (1996) Gross, histological and scanning electron microscopic appearance of dorsal fin rot in farmed Atlantic salmon, *Salmo salar* L., parr. *Journal of Fish Diseases* **19(6)**, 415-427.

[11] Wang, Z., Meador, J. P. & Leung, K. M. (2016) Metal toxicity to freshwater organisms as a function of pH: A meta-analysis. *Chemosphere* **144**, 1544-1552.

[12] Teien, H.C., Garmo, Ø. A., Åtland, Å. & Salbu, B. (2008) Transformation of iron species in mixing zones and accumulation on fish gills. *Environmental Science & Technology* **42 (5)**, 1780–1786.

[13] Wooster, G. A. & Bowse, P. R. (1996) The aerobiological pathway of a fish pathogen: survival and dissemination of *Aeromonas salmonicida* in aerosols and its implications in fish health management. *Journal of the World Aquaculture Society* **27**, 7-14.

[14] Ross, A. J., Yasutake, W.T. & Leek, S. (1975) *Phoma herbarum*, a fungal plant saprophyte, as a fish pathogen. *Journal of the Fisheries Research Board of Canada* **32**, 1648-1652.

[15] MacIntyre, C. M., Ellis, T., North, B. P. & Turnbull, J. F. (2008) The influences of water quality on the welfare of farmed rainbow trout: a review. In Branson, E.J. (Ed), Fish Welfare. Blackwell Publishing, 150-168.

[16] RSPCA (2018). RSPCA welfare standards for farmed rainbow trout. https://science.rspca.org.uk/sciencegroup/farmanimals/standards/trout (Accessed April 2019).

[17] Wedemeyer, G. A. (1996) Physiology of fish in Intensive culture systems. London, Chapman Hall.

[18] Shi, K., Dong, S., Zhou, Y., Gao, Q., Li, L., Zhang, M. & Sun, D. (2018) Comparative Evaluation of Toleration to Heating and Hypoxia of Three Kinds of Salmonids. *Journal of Ocean University of China* **17(6)**, 1465-1472.
[19] Poulsen, S. B., Jensen, L. F., Nielsen, K. S., Malte, H., Aarestrup, K. & Svendsen, J. C. (2011) Behaviour of rainbow trout *Oncorhynchus mykiss* presented with a choice of normoxia and stepwise progressive hypoxia. *Journal of Fish Biology* **79(4)**, 969-979.
[20] Ihssen, P. E. (1986) Selection of fingerling rainbow trout for high and low tolerance to high temperature. *Aquaculture* **57(1-4)**, 370.
[21] EFSA (2008) Scientific Opinion of the Panel on Animal Health and Animal Welfare on a request from the European Commission on the Animal welfare aspects of husbandry systems for farmed trout. *The EFSA Journal* **796**, 1-22.
[22] Poppe, T. T., Bæverfjord, G. & Hansen, T. (2007) Effects of intensive production with emphasis on on-growing production: fast growth, deformities and production-related diseases. *Aquaculture Research: From Cage to Consumption*, 120-135.
[23] Woynarovich, A., Hoitsy, G. & Moth-Poulsen, T. (2011) Small-scale rainbow trout farming. *FAO Fisheries and Aquaculture Technical Paper* (**561**), I.
[24] Schurmann, H., Steffensen, J. F. & Lomholt, J. P. (1991) The influence of hypoxia on the preferred temperature of rainbow trout Oncorhynchus mykiss. *Journal of Experimental Biology* **157(1)**, 75-86.
[25] Boyd, C. E., & Tucker, C. S. (1998). Water quality requirements. In: Pond Aquaculture Water Quality Management (pp. 87-153). Springer, Boston, MA.
[26] Perry, S. F., Rivero-Lopez, L., McNeill, B. & Wilson, J. (2006) Fooling a freshwater fish: how dietary salt transforms the rainbow trout gill into a seawater gill phenotype. *Journal of Experimental Biology* **209(23)**, 4591-4596.
[27] McKay, L. R. & Gjerde, B. (1985) The effect of salinity on growth of rainbow trout. *Aquaculture* **49(3-4)**, 325-331.
[28] Thorarensen, H. & Farrell, A. (2011) The biological requirements for post-smolt Atlantic salmon in closed-containment systems. *Aquaculture* **312**, 1-14.
[29] Danley, M. L., Kenney, P. B., Mazik, P. M., Kiser, R. & Hankins, J. A. (2005) Effects of carbon dioxide exposure on intensively cultured rainbow trout *Oncorhynchus mykiss*: physiological responses and fillet attributes. *Journal of the World Aquaculture Society* **36(3)**, 249-261.
[30] Hafs, A. W., Mazik, P. M., Kenney, P. B. & Silverstein, J. T. (2012) Impact of carbon dioxide level, water velocity, strain, and feeding regimen on growth and fillet attributes of cultured rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **350**, 46-53.
[31] Parker, T. M. & Barnes, M. E. (2015) Effects of different water velocities on the hatchery rearing performance and recovery from transportation of Rainbow Trout fed two different rations. *Transactions of the American Fisheries Society* **144(5)**, 882-890.
[32] Farrell, A. P., Johansen, J. A., Suarez, R. K. (1991) Effects of exercise-training on cardiac performance and muscle enzymes in rainbow trout, *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry* **9**, 303–312.
[33] Houlihan, D. F. & Laurent, P. (1987) Effects of exercise training on the performance, growth, and protein turnover of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* **44(9)**, 1614-1621.
[34] Larsen, B. K., Skov, P. V., McKenzie, D. J. & Jokumsen, A. (2012) The effects of stocking density and low level sustained exercise on the energetic efficiency of rainbow trout (*Oncorhynchus mykiss*) reared at 19 ^{o}C. *Aquaculture* **324**, 226-233.

[35] McKenzie, D. J., Höglund, E., Dupont-Prinet, A., Larsen, B. K., Skov, P. V., Pedersen, P. B. & Jokumsen, A. (2012) Effects of stocking density and sustained aerobic exercise on growth, energetics and welfare of rainbow trout. *Aquaculture* **338**, 216-222.
[36] Adams, C., Huntingford, F., Turnbull, J., Arnott, S. & Bell, A. (2000) Size heterogeneity can reduce aggression and promote growth in Atlantic salmon parr. *Aquaculture International* **8**, 543–549.
[37] Christiansen, J. S. & Jobling, M. (1990) The behaviour and the relationship between food intake and growth of juvenile Arctic charr, *Salvelinus alpinus* L., subjected to sustained exercise. *Canadian Journal of Zoology* **68(10)**, 2185-2191.
[38] Taylor, J. & Migaud, H. (2008) Shining The Light On Trout: Where we are now? *Finfish News* **1 (5)**, 11-17.
[39] Taylor, J. F., Migaud, H., Porter, M. J. R. & Bromage, N. R. (2005) Photoperiod influences growth rate and plasma insulin-like growth factor-I levels in juvenile rainbow trout, *Oncorhynchus mykiss*. *General and comparative endocrinology* **142(1-2)**, 169-185.
[40] Taylor, J. F., Needham, M. P., North, B. P., Morgan, A., Thompson, K. & Migaud, H. (2007) The influence of ploidy on saltwater adaptation, acute stress response and immune function following seawater transfer in non-smolting rainbow trout. *General and comparative endocrinology* **152(2-3)**, 314-325.
[41] Wagner, H. H. (1974) Photoperiod and temperature regulation of smolting in steelhead trout (*Salmo gairdneri*). *Canadian Journal of Zoology* **52**, 219-234.
[42] Morro, B., Balseiro, P., Albalat, A., Pedrosa, C., Mackenzie, S., Nakamura, S., Shimizu, M., Nilsen, T. O., Sveier, H., Ebbesson, L. O. & Handeland, S. O. (2019) Effects of different photoperiod regimes on the smoltification and seawater adaptation of seawater-farmed rainbow trout (*Oncorhynchus mykiss*): Insights from Na+, K+–ATPase activity and transcription of osmoregulation and growth regulation genes. *Aquaculture* **507**, 282-292.
[43] RSPCA (2014). RSPCA welfare standards for farmed rainbow trout. https://science.rspca.org.uk/sciencegroup/farmanimals/standards/trout (Accessed 2016)
[44] Kjelland, M. E., Woodley, C. M., Swannack, T. M. & Smith, D. L. (2015) A review of the potential effects of suspended sediment on fishes: potential dredging-related physiological, behavioral, and transgenerational implications. *Environment Systems and Decisions* **35(3)**, 334-350.
[45] Rowe, D. K., Dean, T. L., Williams, E. & Smith, J. P. (2003) Effects of turbidity on the ability of juvenile rainbow trout, *Oncorhynchus mykiss*, to feed on limnetic and benthic prey in laboratory tanks. *New Zealand Journal of Marine and Freshwater Research* **37(1)**, 45-52.
[46] Timmons, M. & Ebeling, J. (2007) Recirculating Aquaculture. Cayuga Aqua Ventures, Ithaca, NY.
[47] Becke, C., Schumann, M., Steinhagen, D., Rojas-Tirado, P., Geist, J. & Brinker, A. (2019) Effects of unionized ammonia and suspended solids on rainbow trout (*Oncorhynchus mykiss*) in recirculating aquaculture systems. *Aquaculture* **499**, 348-357.
[48] Hjeltnes, B., Bæverfjord, G., Erikson, U., Mortensen, S., Rosten, T. & Østergård, P. (2012) Risk assessment of recirculating systems in Salmonid hatcheries. Norwegian Scientific Committee for Food Safety (VKM), Doc. (09-808).
[49] Weitkamp, D. E. & Katz, M. (1980) A review of dissolved gas supersaturation literature. *Transactions of the American Fisheries Society* **109**, 659-702.
[50] Edsall, D. A. & Smith, C. E. (1991) Oxygen-induced gas bubble disease in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* **22(2)**, 135-140.
[51] Machova, J., Faina, R., Randak, T., Valentova, O., Steinbach, C., Kroupova, H. K. & Svobodova, Z. (2017) Fish death caused by gas bubble disease: a case report. *Veterinární medicína* **62(4)**, 231-237.

[52] Gültepe, N., Ateş, O., Hisar, O. & Beydemir, Ş. (2011) Carbonic anhydrase activities from the rainbow trout lens correspond to the development of acute gas bubble disease. *Journal of aquatic animal health* **23(3)**, 134-139.

[53] Lekang, O.-I., (2007) Aquaculture Engineering. Blackwell Publishing, Oxford, UK. 432 pp.

[54] Wedemeyer, G. A. (1997). Effect of rearing conditions on the health and physiological quality of fish in intensive culture. In: Iwama, G. K., Pickering, A. D., Sumpter, J. P., Schreck, C. B. (Eds.). Fish Stress and Heath in Aquaculture: 35-72.

[55] Skov, P. V., Pedersen, L. F. & Pedersen, P. B. (2013) Nutrient digestibility and growth in rainbow trout (*Oncorhynchus mykiss*) are impaired by short term exposure to moderate supersaturation in total gas pressure. *Aquaculture* **416**, 179-184.

[56] Huntingford, F. A., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandøe, P. & Turnbull, J. F. (2006) Current issues in fish welfare. *Journal of Fish Biology* **70**, 1311-1316.

[57] Helland S. J., Helland B. G. & Nerland S. (1996) A simple method for the measurement of daily feed intake of groups of fish in tanks. *Aquaculture* **139**, 157-163.

[58] Pedersen, C. L. (1987) Energy budgets for juvenile rainbow trout at various oxygen concentrations. *Aquaculture* **62(3-4)**, 289-298.

[59] Ortega, V. A., Renner, K. J. & Bernier, N. J. (2005) Appetite-suppressing effects of ammonia exposure in rainbow trout associated with regional and temporal activation of brain monoaminergic and CRF systems. *Journal of Experimental Biology* **208(10)**, 1855-1866.

[60] Landless, P. J. (1976) Demand-feeding behaviour of rainbow trout. *Aquaculture* **7(1)**, 11-25.

[61] Sánchez-Vázquez, F. J. & Tabata, M. (1998) Circadian rhythms of demand-feeding and locomotor activity in rainbow trout. *Journal of Fish Biology* **52(2)**, 255-267.

[62] Hoskonen, P. & Pirhonen, J. (2006) Effects of repeated handling, with or without anaesthesia, on feed intake and growth in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* **37(4)**, 409-415.

[63] Chin, A., Guo, F. C., Bernier, N. J. & Woo, P. T. (2004) Effect of *Cryptobia salmositica*-induced anorexia on feeding behavior and immune response in juvenile rainbow trout *Oncorhynchus mykiss*. *Diseases of aquatic organisms* **58(1)**, 17-26.

[64] Gregory, T. R. & Wood, C. M. (1999) The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiological and Biochemical Zoology* **72(3)**, 286-295.

[65] Grove, D. J., Loizides, L. G. & Nott, J. (1978) Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. *Journal of Fish Biology* **12(5)**, 507-516.

[66] Noble, C., Mizusawa, K. & Tabata, M. (2005) Does light intensity affect self-feeding and food wastage in group-held rainbow trout and white-spotted charr? *Journal of Fish Biology* **66(5)**, 1387-1399.

[67] Kaushik, S. J., Cravedi, J. P., Lalles, J. P., Sumpter, J., Fauconneau, B. & Laroche, M. (1995) Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **133(3)**, 257-274.

[68] Li, H. W. & Brocksen, R. W. (1977) Approaches to the analysis of energetic costs of intraspecific competition for space by rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology* **11(4)**, 329-341.

[69] Person-Le Ruyet, J., Labbé, L., Le Bayon, N., Sévère, A., Le Roux, A., Le Delliou, H. & Quéméner, L. (2008) Combined effects of water quality and stocking density on welfare and growth of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Living Resources* **21(2)**, 185-195.

[70] Peakall, D. (1994) Animal Biomarkers as Pollution Indicators. London: Chapman and hall, Chapter 7.

[71] Anras, M. L. B. & Lagardère, J. P. (2004) Measuring cultured fish swimming behaviour: first results on rainbow trout using acoustic telemetry in tanks. *Aquaculture* **240(1-4)**, 175-186.

[72] van Raaij, M. T., Pit, D. S., Balm, P. H., Steffens, A. B. & van den Thillart, G. E. (1996) Behavioral strategy and the physiological stress response in rainbow trout exposed to severe hypoxia. *Hormones and Behavior* **30(1)**, 85-92.

[73] Colson, V., Mure, A., Valotaire, C., Le Calvez, J. M., Goardon, L., Labbe, L., Leguen, I. & Prunet, P. (2019) A novel emotional and cognitive approach to welfare phenotyping in rainbow trout exposed to poor water quality. *Applied animal behaviour science* **210**, 103-112.

[74] Sadoul, B., Leguen, I., Colson, V., Friggens, N. C. & Prunet, P. (2015) A multivariate analysis using physiology and behavior to characterize robustness in two isogenic lines of rainbow trout exposed to a confinement stress. *Physiology & behavior* **140**, 139-147.

[75] Ellis, T., North, B., Scott, A. P., Bromage, N. R., Porter, M. & Gadd, D. (2002) The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology* **61(3)**, 493-531.

[76] Noble, C., Mizusawa, K., Suzuki, K. & Tabata, M. (2007) The effect of differing self-feeding regimes on the growth, behaviour and fin damage of rainbow trout held in groups. *Aquaculture* **264(1-4)**, 214-222.

[77] Damsgård, B. & Huntingford, F. (2012) Fighting and Aggression. In: Aquaculture and Behavior (Eds: Huntingford, F., Jobling, M. & Kadri, S.) Wiley-Blackwell. 248-285.

[78] St-Hilaire, S., Ellis, T., Cooke, A., North, B. P., Turnbull, J. F., Knowles, T. & Kestin, S. (2006) Fin erosion on rainbow trout on commercial trout farms in the United Kingdom. *Veterinary Record* **159**, 446-450.

[79] Ellis, T., Hoyle, I., Oidtmann, B., Turnbull, J. F., Jacklin, T. E. & Knowles, T. G. (2009) Further development of the “Fin Index” method for quantifying fin erosion in rainbow trout. *Aquaculture* **289(3-4)**, 283-288.

[80] McFarlane, W. J., Cubitt, K. F., Williams, H., Rowsell, D., Moccia, R., Gosine, R. & McKinley, R. S. (2004) Can feeding status and stress level be assessed by analyzing patterns of muscle activity in free swimming rainbow trout (*Oncorhynchus mykiss* Walbaum)?. *Aquaculture* **239(1-4)**, 467-484.

[81] Brännäs, E. & Alanärä, A. (1992) Feeding behaviour of the Arctic charr in comparison with the rainbow trout. *Aquaculture* **105(1)**, 53-59.

[82] Stien, L. H., Bracke, M. B. M, Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., Kittilsen, S., Midtlyng, P. J., Vindas, M. A., Øverli, Ø. & Kristiansen, T. S. (2013) Salmon Welfare Index Model (SWIM 1.0): a semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. *Reviews in Aquaculture* **5**, 33-57.

[83] Schmidt, J. G., Andersen, E. W., Ersbøll, B. K. & Nielsen, M. E. (2016) Muscle wound healing in rainbow trout (*Oncorhynchus mykiss*). *Fish & shellfish immunology* **48**, 273-284.

[84] Takle, H. R., Ytteborg, E., Nielsen, K. V., Karlsen, C. R., Nilsen, H. K., Sveen, L., Colquhoun, D. J., Olsen, A. B., Sørum, H. & Nilsen, A. (2015) Sårproblematikk og hudhelse i laks-og regnbueørrettoppdrett. Nofima Rapportnr: 05/2015. ISBN: 978-82-8296-260-5.

[85] Shearer, K.D. & Hardy, R.W. (1987) Phosphorus deficiency in rainbow trout fed a diet containing deboned fillet scrap. *Progressive Fish-Culturist* **49**, 192-197.

[86] Lein. I., Helland, S., Hjelde, K. & Baeverfjord, G. (2009) Temperature effects on malformations in trout (*O. mykiss*). In: Control of malformations in fish aquaculture, Science and practice (FineFish). (Eds: Baeverfjord, G., Helland, S. & Hough, C.) pp. 149.

[87] Bosakowski, T. & Wagner, E. J. (1995) Experimental use of cobble substrates in concrete raceways for improving fin condition of cutthroat (*Oncorhynchus clarki*) and rainbow trout (*O. mykiss*). *Aquaculture* **130(2-3)**, 159-165.

[88] Ross, R. M., Watten, B. J., Krise, W. F., DiLauro, M. N. & Soderberg, R. W. (1995) Influence of tank design and hydraulic loading on the behavior, growth, and metabolism of rainbow trout (*Oncorhynchus mykiss*). *Aquacultural Engineering* **14(1)**, 29-47.

[89] Jørgensen, E. H., Bernier, N. J., Maule, A. G. & Vijayan, M. M. (2016) Effect of long-term fasting and a subsequent meal on mRNA abundances of hypothalamic appetite regulators, central and peripheral leptin expression and plasma leptin levels in rainbow trout. *Peptides* **86**, 162-170.

[90] Pottinger, T. G., Rand-Weaver, M. & Sumpter, J. P. (2003) Overwinter fasting and re-feeding in rainbow trout: plasma growth hormone and cortisol levels in relation to energy mobilisation. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **136(3)**, 403-417.

[91] Harrison, J. G. & Richards, R. H. (1979) The pathology and histopathology of nephrocalcinosis in rainbow trout *Salmo gairdneri* Richardson in fresh water. *Journal of Fish Diseases* **2(1)**, 1-12.

[92] Sutterlin, A. M. & Stevens, E. D. (1992) Thermal behaviour of rainbow trout and Arctic char in cages moored in stratified water. *Aquaculture* **102(1-2)**, 65-75.

[93] Oppedal, F., Dempster, T. & Stien, L. H. (2011) Environmental drivers of Atlantic salmon behaviour in sea-cages: A review. *Aquaculture* **311**, 1–18.

[94] Stien, L. H., Gismervik, K. & Kristiansen, T. S. (2017) Risiko for dødelighet og dårlig fiskevelferd i laks- og regnbueørretproduksjonen i sjø (Eng: Risk for increased mortality and poor fish welfare in production of salmon and rainbow trout in sea cages). In: Svåsand, T., Grefsrud, E. S., Karlsen, Ø., Kvamme, B. O., Glover, K., Husa, V., Kristiansen, T. S. (eds.) Havforskningsinstituttet, *Fisken og Havet* **særnr; 2-2017**, 117-123.

[95] Rodger, H., Henry, L. & Mitchell, S. (2011) Non-infectious gill disorders of marine salmonid fish. *Reviews in Fish Biology and Fisheries* **21**, 423–440.

[96] Fast, M. D., Ross, N. W., Mustafa, A., Sims, D. E., Johnson, S. C., Conboy, G. A., Speare, D. J., Johnson, G. R. & Burka, J. F. (2002) Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*. *Diseases of aquatic organisms* **52(1)**, 57-68.

[97] Stien, L. H., Oppedal, F. & Kristiansen, T. S. (2016) Dødelighetsstatistikk for lakseproduksjon (eng: Mortality statistics for salmon production). In: Svåsand, T., Karlsen, Ø., Kvamme, B. O., Stien, L. H., Taranger, G. L. & Boxaspen, K. K. (eds). *Risikovurdering norsk fiskeoppdrett 2016. Havforskningsinstituttet, Fisken og Havet, særnr; 2-2016*, 129-134.

[98] Powell, M. D., Reynolds, P. & Kristensen, T. (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**, 18-28.

[99] Vigen, J. (2008) Oxygen variation within a sea cage. Master thesis. Department of Biology, University of Bergen, Bergen, 73 pp.

[100] Alanärä, A. (1996) The use of self-feeders in rainbow trout (*Oncorhynchus mykiss*) production. *Aquaculture* **145(1-4)**, 1-20.

[101] Landless, P. J. (1976) Acclimation of rainbow trout to sea water. *Aquaculture* **7(1)**, 173-179.

[102] Jackson, A. J. (1981) Osmotic regulation in rainbow trout (*Salmo gairdneri*) following transfer to sea water. *Aquaculture* **24**, 143-151.

[103] Kiilerich, P., Milla, S., Sturm, A., Valotaire, C., Chevolleau, S., Giton, F., Terrien, X., Fiet, J., Fostier, A., Debrauwer, L. & Prunet, P. (2011) Implication of the mineralocorticoid axis in rainbow trout osmoregulation during salinity acclimation. *Journal of Endocrinology* **209**, 221-235.

[104] Solstorm, F., Solstorm, D., Oppedal, F., Fernö, A., Fraser, T. W. K. & Olsen, R. E. (2015) Fast water currents reduce production performance of post-smolt Atlantic salmon *Salmo salar*. *Aquaculture Environment Interactions* **7**, 125–134.

[105] Solstorm, F., Solstorm, D., Oppedal, F., Olsen, R. E., Stien, L. H. & Fernö, A. (2016) Not too slow, not too fast: water currents affect group structure, aggression and welfare in post-smolt Atlantic salmon *Salmo salar*. *Aquaculture Environment Interactions* **8**, 339-347.
[106] North, B. P., Turnbull, J. F., Ellis, T., Porter, M. J., Migaud, H., Bron, J. & Bromage, N. R. (2006) The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **255(1-4)**, 466-479.
[107] Taylor, J. F., North, B. P., Porter, M. J. R., Bromage, N. R. & Migaud, H. (2006) Photoperiod can be used to enhance growth and improve feeding efficiency in farmed rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **256(1-4)**, 216-234.
[108] Bromage, N., Porter, M. & Randall, C. (2001) The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture* **197**, 63–98.
[109] Davies, B. & Bromage, N. (2002) The effects of fluctuating seasonal and constant water temperatures on the photoperiodic advancement of reproduction in female rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **205(1-2)**, 183-200.
[110] Oppedal, F. (1995) Growth, harvest quality, sexual maturation and behaviour of spring transferred rainbow trout (*Oncorhynchus mykiss*) given natural or continuous light in sea water. Cand scient. thesis, University of Bergen, Norway (in Norwegian).
[111] Jobling, M., Alanärä, A., Noble, C., Sánchez-Vázquez, J., Kadri, S. & Huntingford, F. (2012) Appetite and feed intake. In: Aquaculture and behaviour (Huntingford, F., Jobling, M. & Kadri, S.). Wiley-Blackwell, West Sussex, UK, p. 183–219.
[112] Nagasawa, K. (2004) Sea lice, Lepeophtheirus salmonis and Caligus orientalis (Copepoda: Caligidae), of wild and farmed fish in sea and brackish waters of Japan and adjacent regions: a review. Zoological Studies 43(2), 173-178.
[113] Soares, S., Green, D. M., Turnbull, J. F., Crumlish, M. & Murray, A. G. (2011) A baseline method for benchmarking mortality losses in Atlantic salmon (*Salmo salar*) production. *Aquaculture* **314**, 7–12.
[114] Soares S., Murray A. G., Crumlish M., Turnbull J. F. & Green D. M. (2013) Factors affecting variation in mortality of marine Atlantic salmon *Salmo salar* in Scotland. *Diseases of Aquatic Organisms* **103**, 101–109.
[115] Muzzall, P. M. (1984). Parasites of trout from four lotic localities in Michigan. *Proceedings of the Helminthological Society of Washington* **51**, 261-266.
[116] Sutterlin, A. M., Jokola, K. J. & Holte, B. (1979) Swimming behavior of salmonid fish in ocean pens. *Journal of the Fisheries Board of Canada* **36(8)**, 948-954.
[117] Phillips, M. J. (1985) Behaviour of rainbow trout, *Salmo gairdneri* Richardson, in marine cages. *Aquaculture Research* **16(3)**, 223-232.
[118] Martins, C. I., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M. T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J. C., Carter, T. & Planellas, S. R. (2012) Behavioural indicators of welfare in farmed fish. *Fish Physiology and Biochemistry* **38(1)**, pp.17-41.
[119] Pinkiewicz, T. H., Purser, G. J. & Williams, R. N. (2011) A computer vision system to analyse the swimming behaviour of farmed fish in commercial aquaculture facilities: A case study using cage-held Atlantic salmon. *Aquacultural Engineering* **45**, 20–27.
[120] Forskrift om bekjempelse av lakselus i akvakulturanlegg, FOR -2012-12-05-1140.
[121] Fleming, I. A. (1998) Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. *Canadian journal of fisheries and aquatic science*, **55(S1)**, 59-76.

[122] Kause, A., Ritola, O., Paananen, T., Mäntysaari, E., & Eskelinen, U. (2003). Selection against early maturity in large rainbow trout *Oncorhynchus mykiss*: the quantitative genetics of sexual dimorphism and genotype-by-environment interactions. *Aquaculture* **228(1-4)**, 53-68.

[123] Norberg, B., Taranger, G. L. & Tveiten, H. (2007) Reproductive Physiology in Cultured Cold-water Marine Fish. *From Cage to Consumption*, 66-79.

[124] Noble, C., Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H. & Turnbull, J. F. (Eds.) (2018). Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare 351pp. ISBN 978-82-8296-556-9

[125] Gismervik, K., Østvik, A. & Viljugrein, H. (2016) Pilotflåte Helixir - dokumentasjon av fiskevelferd og effekt mot lus. Del 1 uten legemiddel. *Rapport 15, 2016. Veterinærinstituttets rapportserie.* Veterinærinstituttet, Oslo, Norge.

[126] Grøntvedt, R. N., Nerbøvik, I. -K. G, Viljugrein, H., Lillehaug, A., Nilsen, H. & Gjerve, A. –G. (2015) Termisk avlusing av laksefisk – dokumentasjon av fiskevelferd og effekt. *Rapport 13, 2015. Veterinærinstituttets rapportserie.* Veterinærinstituttet, Oslo, Norge.

[127] RSPCA (2018). RSPCA welfare standards for farmed Atlantic salmon. https://science.rspca.org.uk/sciencegroup/farmanimals/standards/salmon (Accessed October 2018).

[128] 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.

[129] Midtlyng, P. J., Reitan, L. J. & Speilberg, L. (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.

[130] Pettersen, J. M., Bracke, M. B. M, Midtlyng, P. J., Folkedal, O., Stien, L. H., Steffenak, H. & Kristiansen, T. S. (2014) Salmon welfare index model 2.0: an extended model for overall welfare assessment of caged Atlantic salmon, based on a review of selected welfare indicators and intended for fish health professionals. *Reviews in Aquaculture* **6**, 162–179.

[131] Bass, N. & Wall, T. (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.

[132] Chettri, J. K., Skov, J., Jaafar, R. M., Krossøy, B., Kania, P. W., Dalsgaard, I. & Buchmann, K. (2015) Comparative evaluation of infection methods and environmental factors on challenge success: *Aeromonas salmonicida* infection in vaccinated rainbow trout. *Fish & shellfish immunology* **44(2)**, 485-495.

[133] Holten-Andersen, L., Dalsgaard, I., Nylén, J., Lorenzen, N. & Buchmann, K. (2012) Determining vaccination frequency in farmed rainbow trout using *Vibrio anguillarum* O1 specific serum antibody measurements. *PloS one* **7(11)**, e49672.

Welfare Indicators for farmed rainbow trout: Part C – fit for purpose OWIs for different routines and operations

Kristine Gismervik[1*], Chris Noble[2*], James F. Turnbull[3*], Kristoffer Vale Nielsen[1], Jonatan Nilsson[4], Åsa M. Espmark[2], Cecilie M. Mejdell[1], Bjørn-Steinar Sæther[2], Lars H. Stien[4], David Izquierdo-Gomez[2], Jelena Kolarevic[2], Kjell Ø. Midling[2], Kristian Ellingsen[1] and Martin H. Iversen[5]

* Joint first authors

1. Norwegian Veterinary Institute, P.O. Box 750 Sentrum, NO-0106 Oslo, Norway
2. Nofima, P.O. Box 6122 Langnes, NO-9291 Tromsø, Norway
3. University of Stirling, Institute of Aquaculture, School of Natural Sciences, Stirling, FK9 4LA, United Kingdom
4. Institute of Marine Research, P.O. Box 1870 Nordnes, NO-5817 Bergen, Norway
5. Nord University, Faculty of Biosciences and Aquaculture, 8049 Bodø, Norway

Photo: Gismervik©

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.
- Highlight knowledge gaps. In general, information regarding validated welfare indicators in rainbow trout under Norwegian farming conditions is somewhat scarce. If this is the case, general knowledge from Atlantic salmon is used where appropriate.


Monitoring of crowding. Photo: Gismervik©

1.1 Crowding

Trout 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. 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].

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 in both salmon [2] and trout [3].
- **Stress.** All these effects are potentially stressful, and crowding results in stress related physiological responses such as an increase in cortisol, glucose and lactate in trout [4, 5], and decreased pH in muscles and blood [6].
- **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 [7]. It also reduces pre-rigor mortis time and causes difficulties in the filleting process [4].
- **Ulcers and mortality.** Physical damage resulting from crowding can result in skin damage, fin damage (e.g. [3]) and even death. Damage to the skin and fins can lead to secondary infections or the stress of crowding may precipitate sub-clinical disease into a full outbreak. Crowding also facilitates the transmission of pathogens.
- **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 the fish experience reduced behavioural control during crowding they 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 increases with the duration of crowding [3]. The crowding time should therefore be as short as possible. The RSPCA welfare standards for farmed rainbow trout state crowding must be no longer than 2 hours and the same group of fish must not be crowded greater than i) twice a week or ii) three times a month unless this is required for fish welfare reasons by the designated vet [8]. CIWF also state that 24-48 hours should be left between crowding procedures if repeated crowding is unavoidable [9].
- 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 [10].
- Fish should be crowded gradually [9, 11] and both the fish and the operation should be monitored closely. The operation should also be monitored and adjusted based on welfare indicators such as behaviour [12].
- 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 [13].

- When crowding in sea cages or using sweep nets, nets should be clean to avoid any potential water quality problems [8] and the area of the crowd should be narrow and deep rather than wide and shallow [9, 11], as this can increase potential abrasion with the net, expose the fish to higher light intensities and may lead to high activity levels in the crowd [11].

How to assess welfare during crowding

Physiological parameters such as blood glucose and lactate have certain limitations as welfare indicators 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 3.2.16-3.2.20). Measuring lactate and pH can give an indication of stress if the measurements are repeated during the crowding procedure [4], or carried out before, during and after it. Although physiological parameters may provide information to guide best practice for future crowding events, they are not good "stop signals" concerning welfare during ongoing operations.

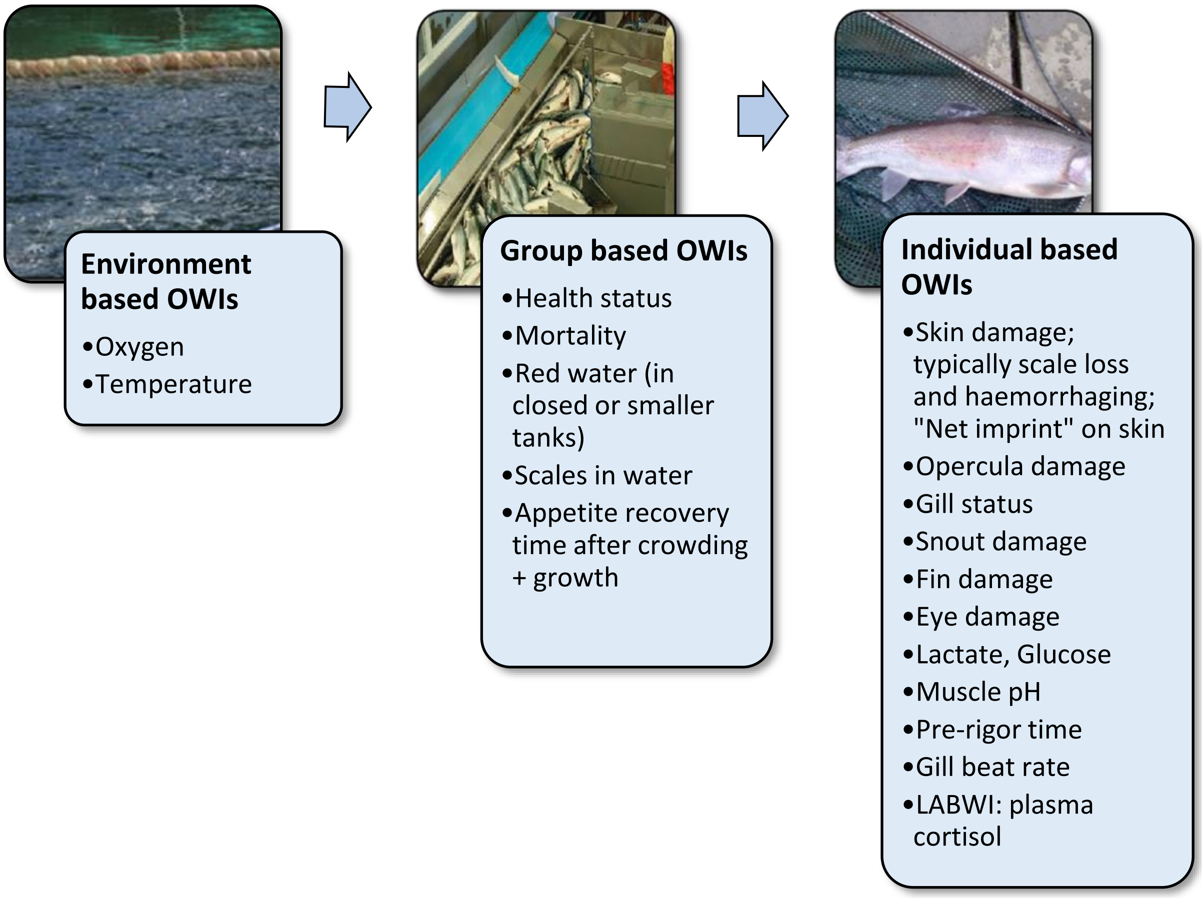


Figure 1.1-1. *Overview of fit for purpose OWIs for crowding. Environment based OWIs address the rearing environment, group based OWIs describe the population as a whole, while individual based OWIs are based on sampling individual fish. Photos and illustration K. Gismervik, J. F. Turnbull.*

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. A recently published paper [14] outlines detailed data on the limiting oxygen saturations (LOS) of rainbow trout at different temperatures and at different sizes (Table 1.1-2). LOS is the minimum level where the fish can maintain sufficient respiration and levels below this are therefore lethal. The LOS values in Table 1.1-2 are measured on fasted fish, and a higher oxygen level may be required when fish are satiated [14] or during stressful situations such as crowding. Oxygen levels should therefore always be well above the LOS levels. As a general precautionary guideline, oxygen saturation levels of >80% are recommended, based upon data from Poulsen et al., [15] and the RSPCA welfare standards for farmed rainbow trout recommend a minimum of 7 mg L^{-1} [8].

Table 1.1-2. *The limiting oxygen saturation (LOS) for fasted diploid and triploid rainbow trout of ca. 15-130 g (LOS levels in mg L^{-1}). Reprinted with permission from Springer Nature: Shi, K., Dong, S., Zhou, Y., Gao, Q., Li, L., Zhang, M. & Sun, D. (2018) Comparative Evaluation of Toleration to Heating and Hypoxia of Three Kinds of Salmonids. Journal of Ocean University of China 17(6), 1465-1472. [14] Copyright 2018.*

Temperature (° C)	LOS: diploid				LOS: triploid			
	Fish size				Fish size			
	16 g	40 g	79 g	131 g	16 g	39 g	79 g	130 g
13	4.7	4.4	4.2	3.2	4.1	3.9	3.6	3.1
17	5.0	5.1	4.9	3.8	4.3	4.2	4.0	3.4
21	5.4	5.3	5.2	4.5	4.8	4.7	4.2	3.6
25	5.9	5.6	5.3	4.8	5.0	4.8	4.5	4.0

Temperature. Trout can adapt to temperatures in the range of 0-22 °C [16] but temperature preferences in rainbow trout can vary with the life stage of the fish. Every effort should be made to maintain temperatures within the optimal range since by the time the critical or lethal temperatures (higher or lower) are reached the welfare of the fish will already have been compromised. Fry and fingerlings have a preferred temperature range of 7-13 °C [17] and the RSPCA welfare standards for farmed rainbow trout [8] recommend 1-12 °C for fry. Recommended temperatures for rainbow trout ongrowers held in sea cages are around 7-17 °C [18]. Other authors suggest ongrowers have a preferred temperature of around 16 °C within a range of 13-19 °C under normoxic conditions [19]. The RSPCA welfare standards for farmed rainbow trout recommend 1-16 °C for ongrowers [8].

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 decrease sharply when moving towards the lower and upper critical temperature ranges [20]. It is therefore more difficult for the fish to deal with stress by increasing their metabolism at low or high temperatures. 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 increase the risk of winter ulcers. Damage from handling is often the initiating factor, leading to secondary infections with bacteria in winter time [21].

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 the behaviour of rainbow trout during crowding. However, behaviour is a key OWI and both Compassion in World Farming: Food business [9] and the Humane Slaughter Association [11] suggest using a crowding intensity scale, based on surface observations (Table 1.1-3). EFSA [3] have also included behaviour as one of their key monitoring points during the crowding of rainbow trout and state there should be "*no excessive swimming activity, fight and flight behaviour*". The goal is to have calm swimming behaviour and for rainbow trout, the dorsal fins can break the surface in some systems during normal swimming with no evidence of adverse effects, so the given situation must be taken into account, and this is addressed in the crowding intensity scale below ([11], Table 1.1-3, see also figure 1.1-4).

Table 1.1-3. *A crowding intensity behavioural scale, developed by the Humane Slaughter Association [11] that has been suggested for use with rainbow trout [9, 11]. Text reproduced from "HSA (2016) Humane Harvesting of Fish. Humane Slaughter Association. https://www.hsa.org.uk/downloads/publications/harvestingfishdownload-updated-with-2016-logo.pdf" copyright 2016 [11] with kind permission from the Humane Slaughter Association. Both HSA and CIWF state the operator should always aim for the crowding intensity to be level 1 and that levels 3, 4 and 5 are unacceptable [9, 11].*

Level	Crowding behaviour
1	No vigorous activity, occasional fins breaking the surface of the water.
2	Fins and part of the fish above the water over the whole surface of the crowd.
3	Fins and part of the fish above the water over the whole surface of the crowd. Some burrowing, gasping and vigorous activity in parts of the crowd.
4	The whole surface of the crowd vigorously burrowing, gasping and splashing.
5	The whole surface of the crowd boiling with violent splashing.

However, monitoring behaviour from the surface may give the observer a limited overview of behaviour of the group, especially in low lighting or poor visibility conditions. In a study of a commercial crowding situation in Atlantic salmon prior to slaughter, Erikson et al., [22] 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 [4] 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.

Figure 1.1-4. *Rainbow trout in a raceway with protruding dorsal fins but no evidence of adverse welfare. Photo: J. F. Turnbull.*

Mortality should be routinely monitored and any changes during or following crowding may be used 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 [23]. 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. 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 with Atlantic salmon (but equally applicable to trout), crowding in closed and smaller containers can make it possible to detect bleeding as a colour change in water, so called "red water". It is always a bad sign, and the cause should be investigated.

Scales in water. The loss of scales is inevitably preceded by loss or damage to the mucous and epithelial layer which results in osmoregulatory problems and may lead to secondary infections (see Part A 3.1.6 skin condition). Any damage during crowding is an indication of poor welfare and should be thoroughly investigated. It may result from rough handling or damaged equipment e.g. protruding or rough edges or abrasion with the crowding net (See Part A section 3.1.6 skin condition for more information).

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. scale loss and "net imprinting" on the skin. Small haemorrhages in the skin can typically be seen ventrally. 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. Wound healing is dependent on temperature and environmental conditions, in addition to the status of the wound e.g. wound depth [24, 25]. Sometimes wound healing can be relatively quick, but it has also been demonstrated that wounds can take over 3 months to heal [25, 26]. Other studies on rainbow trout (where wound depths ranged from ca. 3 mm to the depth of the muscle layers), reported that scales did not regenerate, even after one year [24].

Opercular damage and gill status. Opercular damage includes broken, shortened or even the lack of opercula. It is important to differentiate between acute damage that may have occurred during crowding and other factors affecting the operculum, thus making the gills more vulnerable during crowding. Inspecting the gills can also give some indications of gill status e.g. haemorrhages in relation to mechanical injuries [27] or also reveal poor gill health.

Snout damage. Can occur related to handling procedures, where the fish get forced against the tank wall, net or other structures.

Eye damage and status. 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 (based on photos from salmonids). 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).

Lactate. Struggling and burst swimming increases anaerobic muscle activity, thus increasing lactate in the blood [4, 5].

Muscle pH. Increased stress/muscle activity produces more lactate acid which in turn reduces muscle pH [6].

Glucose. 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]. Similar results have been found in rainbow trout [5]. Glucose levels are however also dependent on diet type, feeding status and other factors and should therefore be compared with pre-stress levels rather than any "standard stress levels".

Pre-rigor time. High or prolonged stress during crowding may lead to a shorter pre-rigor time in both trout and salmon [4, 30]. Veiseth et al., [30] found that an active swimming period after the crowding procedure helped reduce stress and increased pre-rigor time in A. salmon. Reduced pre-rigor time is mostly used in connection with the slaughter process.

Gill beat rate ("breathing") naturally increases as the fish's metabolism rises during activity and stress. Gill beat rate has been used as an OWI for crowding in Atlantic salmon [22] and also for the transport of rainbow trout (involving crowding, handling and transport, [31]) where the authors found an increase in gill beat rate during exposure to stressors. Gill beat rate assessment is best carried out if the fish are swimming slowly or static and is not easy to assess when crowding fish. Qualitative changes in gill beat rate can be done from above the water, if visibility is good, or also using underwater cameras e.g. [22]. Changes in gill beat rate are difficult to quantify on the farm and usually must be assessed 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 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 and when it returns to resting state after the procedure (see also Part A, section 3.2.16).

1.2 Pumping

Pumping is widely used during the transport and transfer of fish. Pumping is mostly performed in association with other handling procedures (e.g. crowding, grading, vaccination, some lice treatments) resulting in repeated handling stress [4]. 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 salmonids when performed correctly [32], however other studies have reported that both crowding and pumping are a stressor for rainbow trout e.g. [4] and that crowding and pumping are major welfare hazards [3]. 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 [10, 33, 34].

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, 35]. Pumping speed should be above the critical swimming speed (U_{crit}) [36] (see Part A, section 4.2.1) to prevent fish holding station in the current and getting exhausted.
- **Height.** Literature relating pumping height to welfare in salmonids is scarce. However, in Atlantic salmon experiments have failed to show negative effects of pumping heights [2, 32]. Most farmers place the pumps close to the pump inlet, with good welfare 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 fish e.g. to the opercula and fins. Bends may also result in other external damage as the fish collide with equipment and conspecifics [3].
- **Repeated pumping and handling** may increase the stress load on the fish [4, 32, 37].
- **Pumping of weak fish.** Pumping should only be done with fish that are healthy and robust and able to withstand the procedure. Sick, previously injured or stressed fish should not be pumped.
- **Low pressure (vacuum).** Literature relating pumping pressure to welfare in salmonids is scarce. Experiments where A. salmon were pumped under low vacuum pressure did not show any negative effects or injuries to the salmon [38]. Blood (red water) was occasionally observed in the pumping chambers and the authors (Espmark et al., [38]) concluded that this was not caused by the low pressure alone, but rather from mechanical injuries to the opercula and gills resulting from high speed and collisions. As the swim bladder expands when the surrounding pressure decreases in the vacuum pump, salmonids release air from the bladder [38] which will negatively affect buoyancy until the fish have refilled the bladder. Therefore, they should be given the opportunity to easily reach the water surface after pumping. EFSA [3] state fish may be injured in the vacuum pressure valve. Care should be taken to ensure this does not occur.

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

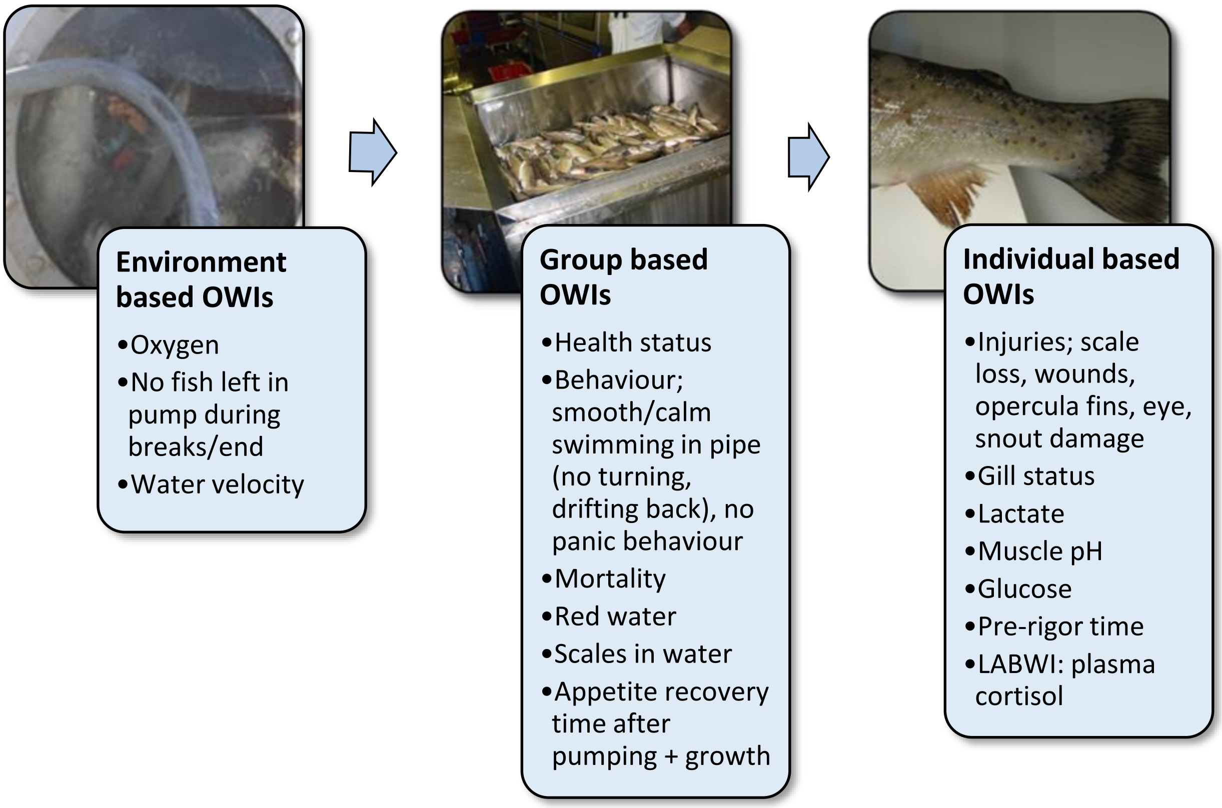


Figure 1.2-2. *Overview of fit for purpose OWIs for pumping. Environment based OWIs address the rearing environment, group based OWIs describe the group as a whole, while individual based OWIs are based on sampling individual fish. Illustration: K. Gismervik, photo of pump: Å. Espmark, other photos J. F. Turnbull*

Environment based OWIs

Oxygen. If the pumping stops, for any reason, the oxygen level will decrease inside the pipe and can rapidly drop to levels that are harmful to the fish. One example where the pumping can be repeatedly stopped is around slaughter [3]. 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. A recently published paper [14] outlines detailed data on the limiting oxygen saturations (LOS) of rainbow trout at different temperatures and at different sizes (Table 1.1-2). LOS is the minimum level where the fish can maintain sufficient respiration and levels below this are therefore lethal. The LOS values in Table 1.1-2 are measured on fasted fish, and a higher oxygen level may be required when fish are satiated [14] or during stressful situations. Oxygen levels should therefore always be well above the LOS levels. As a general precautionary guideline, oxygen saturation levels of >80% are recommended, based upon data from Poulsen et al., [15] and the RSPCA welfare standards for farmed rainbow trout recommend a minimum of 7 mg L^{-1} [8].

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, as this can lead to e.g. oxygen depletion and even the fish drying out.

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 [36] (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, such as 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 [23]. 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 3.1.6 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 pumping indicate 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 [3] (see also 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 [38] (Fig. 1.2-3). Swimming should be smooth and calm. Undesirable behaviours include fish that remain in one place or can swim upstream against the flow, or drift backwards. Other signs of abnormal behaviour include fish swimming on their side or gasping behaviour. The fish should not be very crowded in pipes or in the pump. 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 fish 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. Fish may lose scales and be wounded by high pumping speed and the incorrect use of equipment [2, 35]. Handling trauma, such as cuts or crush injuries, can be caused by pumping [3, 10, 34]. 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. 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. Any damage in connection with pumping is an indicator of poor welfare and should be investigated. Wound healing is dependent on temperature and environmental conditions, in addition to the status of the wound e.g. wound depth [24, 25]. Sometimes wound healing can be relatively quick, but it has also been demonstrated that wounds can take over 3 months to heal [25, 26]. Other studies on rainbow trout (where wound depths ranged from ca. 3 mm to the depth of the muscle layers) reported that scales did not regenerate, even after one year [24].

Opercular damage and gill status. Opercular damage includes broken, eroded or even the lack of opercula (with the latter two also being potential artefacts of earlier damage). It is therefore 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. Inspecting the gills can also give some indications of gill status e.g. haemorrhages in relation to mechanical injuries [27] or also reveal poor gill health.

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

Eye damage and status. 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 lead injuries [3] including fin damage, especially fin splitting. Fin damage has been recorded during pumping of A. salmon and may be caused by collisions and the incorrect use of equipment [2]. 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 (based on photos from salmonids). 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).

Lactate. Struggling, panic and burst swimming increases anaerobic muscle activity, thus increasing lactate in the blood [4, 5]. It is easily measured with handheld apparatus, but samples should be taken approximately one hour after muscle activity. Merkin et al., [4] found no significant relationship between lactate and pumping after both short- and long-term crowding in rainbow trout and suggested this may because the fish had already reached high/maximal levels during crowding.

Muscle pH. Increased stress/muscle activity produces more lactic acid which in turn reduces muscle pH, as shown after pumping and crowding in A. salmon [37]. 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 [4].

Glucose. Glucose can be used as an OWI for crowding [28] and may also be suitable for pumping. Elevation in plasma glucose is a relatively slow response to stress and peaks after around 3-6 hours in salmon [29]. Similar results have been found in rainbow trout [5]. Glucose levels are however also dependent on feeding status, diet type and other factors and 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 pumping 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 a stressor and when it returns to resting state after the procedure (see also Part A, section 3.2.16). Merkin et al., [4] found no significant relationship between cortisol and pumping after both short- and long-term crowding in rainbow trout and suggested this may because the fish had already reached high/maximal levels during crowding.

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 and found suitable for practical use. The stunning and killing equipment shall be operated, inspected and maintained by competent personnel with adequate training [12]. 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 [12]. Percussive stunning utilises a hard blow to the top of the skull that causes concussion, a loss of consciousness and bleeding in vital brain areas. A non-penetrating bolt is used for the percussive stunning of salmonids [12]. 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.

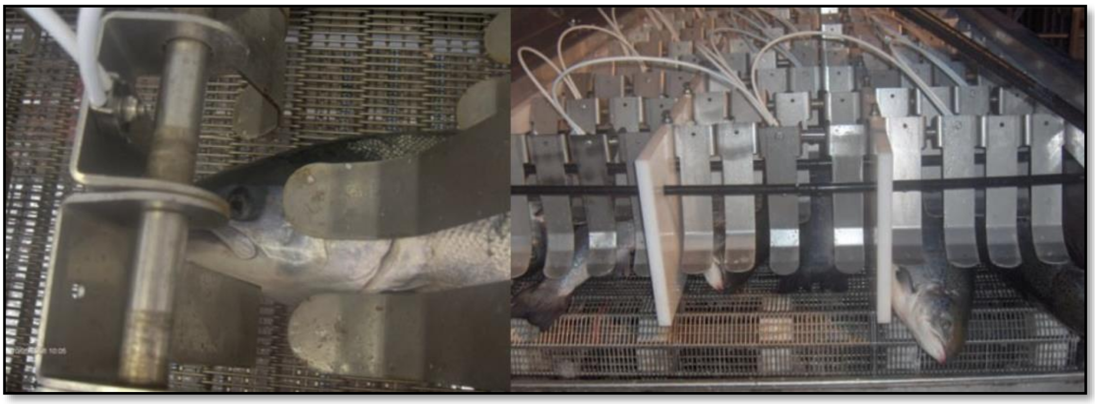


Figure 1.3-1. *Illustrating the slaughter of A. salmon using electrical stunning [12]. Electricity passes from the metal plates, through the fish and to the surface. The picture on the left shows the plates touching the fish, and the picture on the right shows an example of where the fish is not correctly orientated in the machine, emerging tail first (this is not good enough 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 headfirst [39]. Air exposure after drainage and before euthanizing must be as short as possible [3]. 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) [13]. 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 [3, 12, 40].
- In systems where electricity also passes through the heart of the fish it can cause heart rhythm deficits and cardiac arrest. Electrical stunning can be combined with a percussive blow to ensure the duration of anaesthesia is long enough [12].
- 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 [12].
- 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 [12].
- 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 [12].
- There must be control and backup equipment for stunning and bleeding before transfer to the bleeding site.

How to evaluate welfare during slaughter

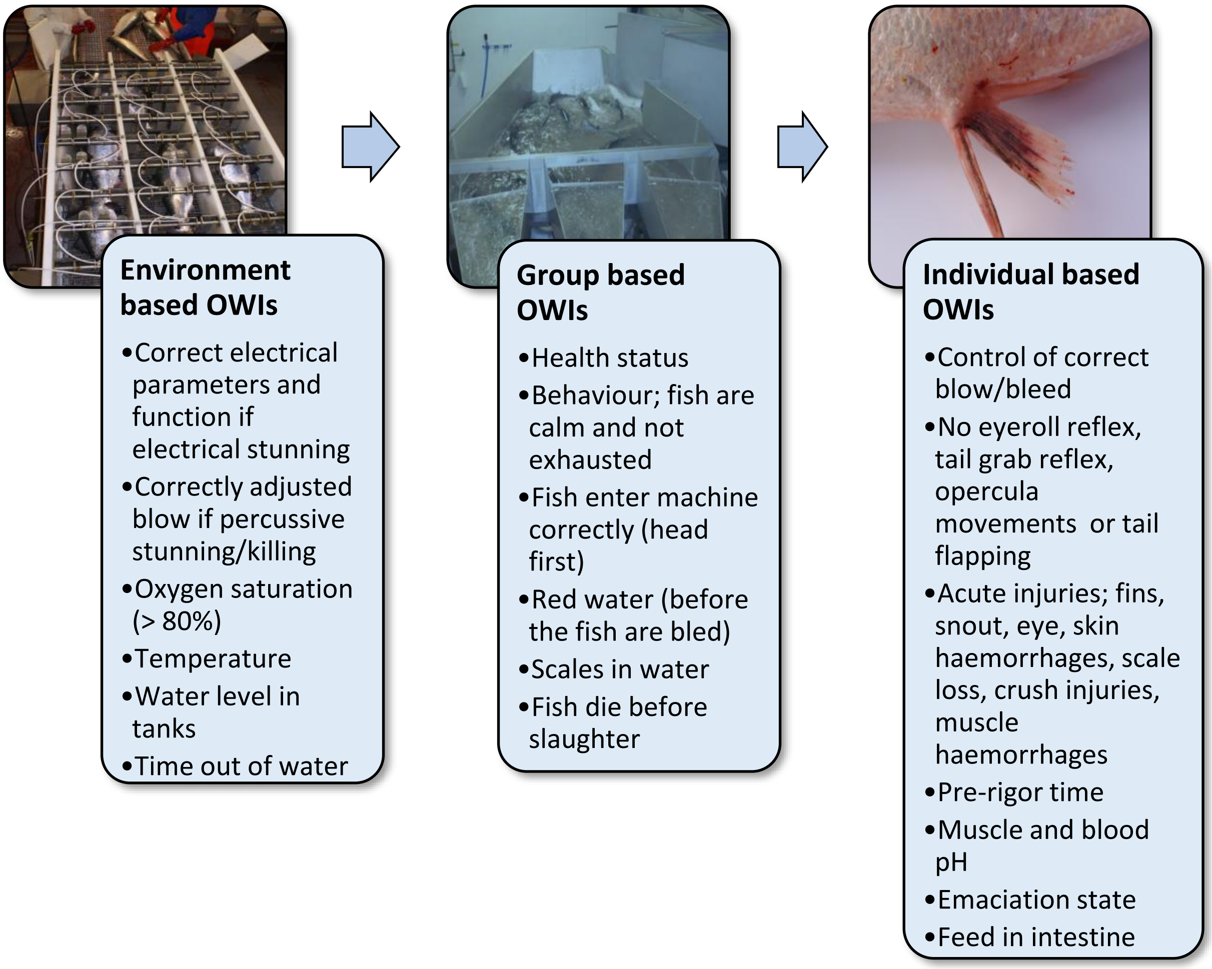


Figure 1.3-2. *Overview of fit for purpose OWIs for slaughter. Environment based OWIs address the stunning machines and environmental parameters in different holding tanks, group based OWIs apply to the group as a whole by observation of the slaughter process, while individual based OWIs are based on sampling individual fish. Photos and illustration: K. Gismervik. Group based OWI photo: C. M. Mejdell.*

Environment based OWIs

Correct electrical parameters and function if electrical stunning. Follow the manufacturer's manuals and update based on practical experience, provided the impact of any changes are monitored for effects on fish welfare. See also Norwegian authorities guidance and interpretations of the slaughter regulation [13].

Correctly adjusted blow if percussive stunning/killing. Follow the manufacturer's manuals and update based on practical experience, provided the impact of any changes are monitored for effects on fish welfare. 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. A recently published paper [14] outlines detailed data on the limiting oxygen saturations (LOS) of rainbow trout at different temperatures and at different sizes (Table 1.1-2). LOS is the minimum level where the fish can maintain sufficient respiration and levels below this are therefore lethal. The LOS values in Table 1.1-2 are measured on fasted fish, and a higher oxygen level may be required when fish are satiated [14] or during stressful events. Oxygen levels should therefore always be well above the LOS levels. As a general precautionary guideline, oxygen saturation levels of >80% are recommended, based upon data from Poulsen et al., [15] and the RSPCA welfare standards for farmed rainbow trout recommend a minimum of 7 mg L^{-1} [8]. The solubility of oxygen decreases with increasing temperature, so that warmer water contains less oxygen than colder water with the same saturation rate. Trout can adapt to temperatures in the range of 0-22 °C [16] but temperature preferences in rainbow trout can vary with the life stage of the fish. Every effort should be made to maintain temperatures within the optimal range since by the time the critical or lethal temperatures (higher or lower) are reached the welfare of the fish will already have been compromised. Recommended temperatures for rainbow trout ongrowers held in sea cages are around 7-17 °C [18]. Other authors suggest ongrowers have a preferred temperature of around 16 °C within a range of 13-19 °C under normoxic conditions [19]. The RSPCA welfare standards for farmed rainbow trout recommend 1-16 °C for ongrowers [8]. Trout can also react to acute changes in temperature such as increases in water temperature [41] or decreases in water temperature [42] by e.g. increasing gill beat rate.

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 [39].

Time out of water. Air exposure should be minimised as prolonged air exposure can damage the gill lamellae [43].The RSPCA welfare standards for farmed rainbow trout recommend a maximum exposure time of 15 seconds [8] and EFSA recommends air exposure should be limited to 10 seconds [3]. There is also a study that reported mortality nearly doubled in rainbow trout when air exposure increased from 30 seconds to 60 seconds in exercised fish [44].

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 [13]. 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 (headfirst 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 [39] and fish should not be left for too long in the tank. Fish should be calm with no evidence of conscious movements after stunning.

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 slaughterhouses. 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 which may result in osmoregulatory problems and may lead to secondary infections. Any damage during the slaughtering process before euthanizing is an indication of poor welfare and should be thoroughly investigated

Rough handling and poorly maintained and managed equipment with protruding and rough edges may be a causal factor [3] (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 [12, 13]. Cutting the aorta or the majority of gill arches on both sides is considered good practice during bleeding [45].

Control of unconsciousness. You should confirm that the trout 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 [46]. The animal is classified as insensible if responses to these indicators are lacking [47, 48]. The vestibulo-ocular reflex (VOR or the "eye roll") is the last reflex the fish loses during anaesthesia and is the first reflex to reappear after recovery [49] (see Fig. 1.3-3). Be aware that live-chilled fish may have a very slow VOR reflex. 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 [46]) or nipping the fin between the nails of your thumb and forefinger. 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).

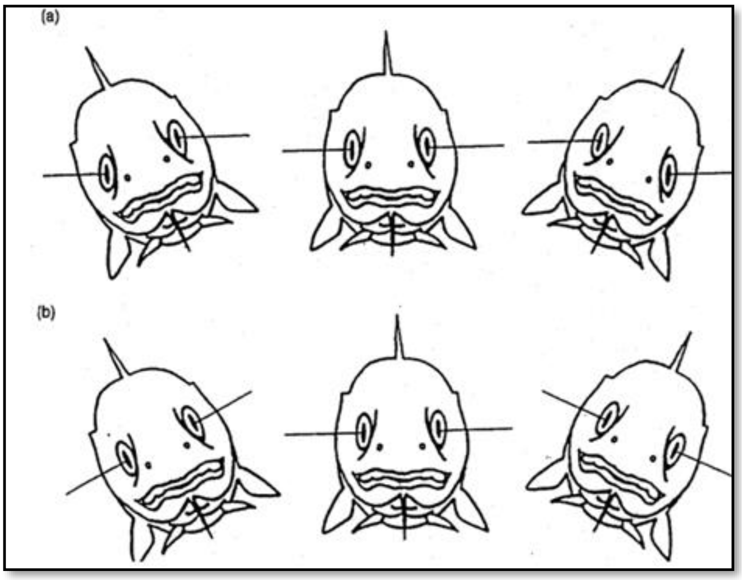


***Figure 1.3-3**. Illustration of an eye roll reflex of a) living and b) dead cod. Reproduced from "Kestin, S.C., J.W. Van de Vis and D.H.F. Robb (2002) Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them. Veterinary Record. 150(10): p. 302-307. Copyright 2002", with permission from BMJ Publishing Group Limited [49]. 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, snout injuries, eye damage and bruising under skin that can be visually checked after skin removal [12].

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 (based on photos from salmonids). 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).

Pre-rigor time. Either severe or long lasting stress can result in a shorter pre-rigor time [4] 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 [12, 50, 51, 52].

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 [12]. 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 [53] and to get a final pH.

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 [54] 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 [55]. See also Part C, section 1.9 for more information.

Welfare checkpoints when using electrical and percussive stunning [12, 39]

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 broodstock. Close et al., [56] 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 [45] 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*". After euthanizing, you must ensure that the animal is dead. This is stated in the Norwegian Animal Welfare Act [57].

Table 1.4-1. *Criteria for euthanasia. The text has been adapted and reproduced from Close et al., [56], "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., [56],
- 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., [56]) but this cannot be considered good practice today without additional evidence. Maceration without prior stunning for euthanizing is not acceptable for welfare [58]. However, maceration can be performed following electrical stunning or anaesthesia during emergency slaughter for disease control [40]. 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 [58]. 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 [3].

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
- Fingerlings – overdose of anaesthetic, or blow to head behind the eyes and bleed/decapitation [59]
- Ongrowers – overdose of anaesthetic or blow to head and bleeding. Slaughter boats can be used during emergency slaughter(Ex. electrical stunning + maceration, EFSA [3])
- 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 [45].
- 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 [45].
- 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 state it can "*take 4.5-6 minutes to produce brain death*") [45].
- 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.

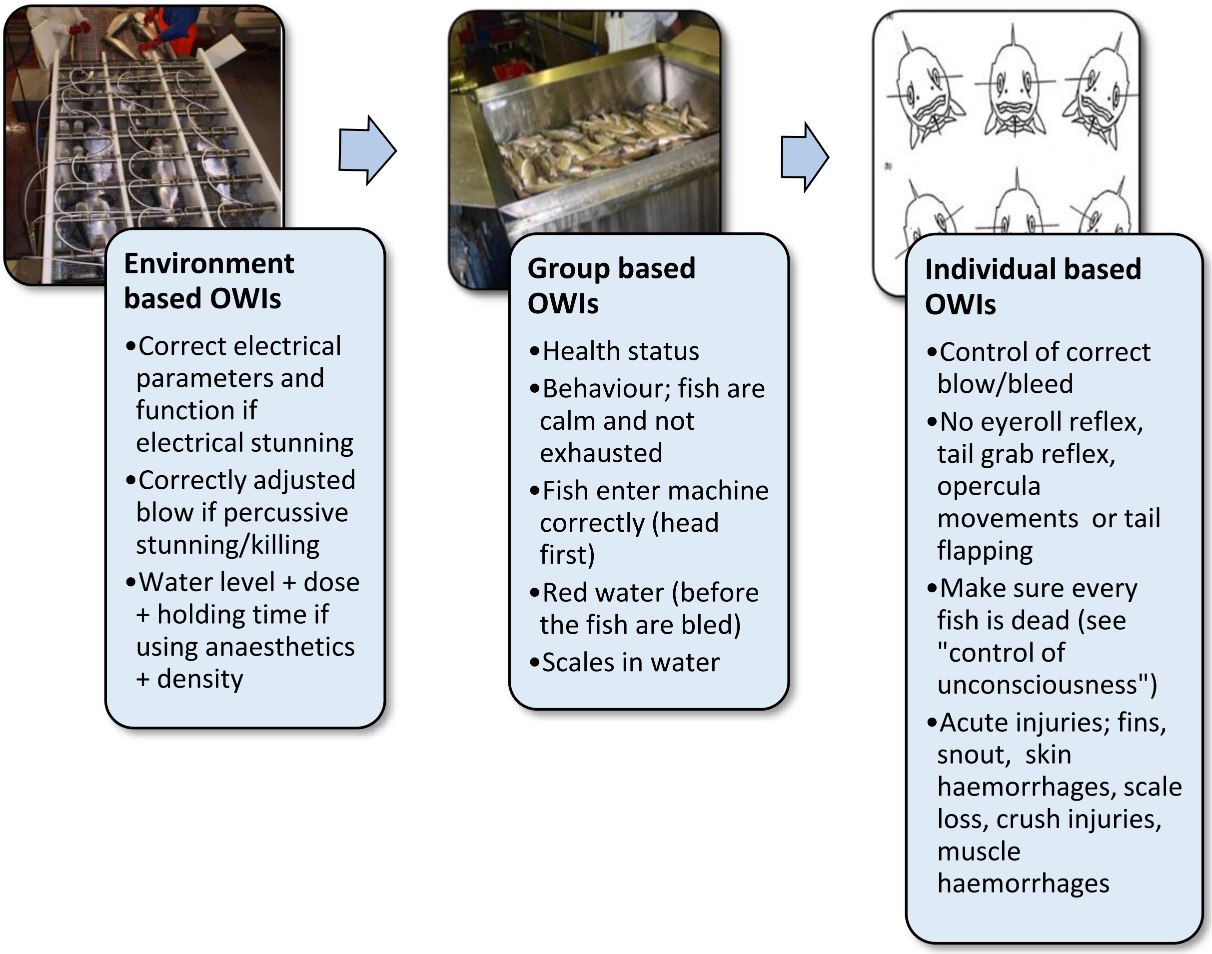


Figure 1.4-2. *Overview of fit for purpose OWIs for euthanizing fish. Environment based OWIs address the stunning machines or the bath with overdose anaesthetics, group based OWIs are what can be observed and checked during the euthanizing process, while individual based OWIs are based on sampling individual fish for close ups on missing reflexes and the correct blow/bleed where relevant. Illustration and environmental OWI photo: K. Gismervik. Photo group based OWI: J. F. Turnbull. Illustration individual based OWI: 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 [49].*

Environment based OWIs

Correct electrical voltage /function if electrical stunning. Follow the manufacturer's manuals and update based on practical experience, provided the impact of any changes are monitored for effects on fish welfare. See also Norwegian authorities guidance and interpretations of the slaughter regulation [13].

Correctly adjusted blow if percussive stunning/killing. Follow the manufacturer's manuals and update based on practical experience, provided the impact of any changes are monitored for effects on fish welfare. Make sure the machine is adjusted to the size of the fish.

Anaesthetic dosage, water level and density. During the use of anaesthetics, dosage or more correctly, over dosage levels, 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 (headfirst 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 [39] or allowing fish to remain in the tank for a protracted period.

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 is 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 [12, 13]. Cutting the aorta or the majority of gill arches on both sides is considered good practice during bleeding [45].

Control of unconsciousness. You should confirm that the trout are unconscious or dead before they are bled or subjected to euthanasia. 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 [46]. The animal is classified as insensible if responses to these indicators are lacking [47, 48]. The vestibulo-ocular reflex (VOR or the "eye roll") is the last reflex the fish loses during anaesthesia and is the first reflex to reappear after recovery [49], 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 [46]) or nipping the fin between the nails of your thumb and forefinger. 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.

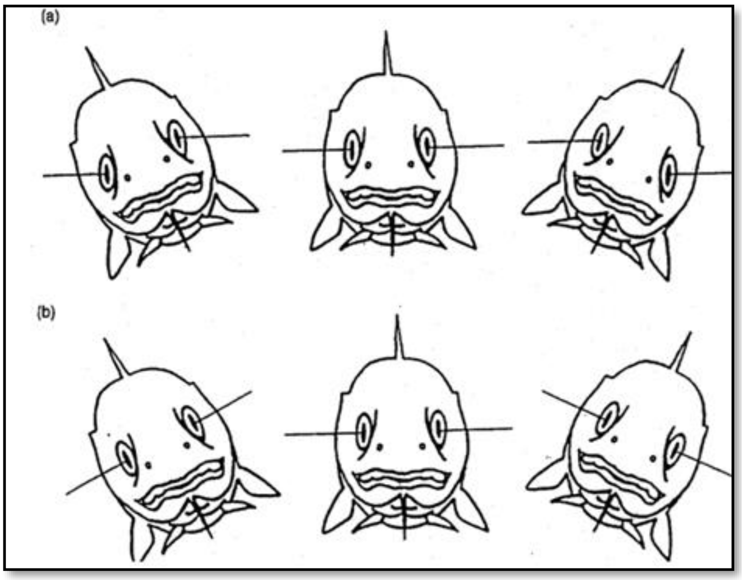


Figure 1.4-3. *Illustration of an eye roll reflex of a) living and b) dead cod. Reproduced from "Kestin, S.C., J.W. Van de Vis and D.H.F. Robb (2002) Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them. Veterinary Record. 150(10): p. 302-307. Copyright 2002", with permission from BMJ Publishing Group Limited [49]. 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 [12]. 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 (based on photos from salmonids). 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).

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 outlines OWIs for conducting medicinal treatments and their possible side effects. 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 [60].

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

Medicinal side effects include adverse drug reactions (ADR's) which are defined by WHO as "a response to a drug that is noxious and unintended and which occurs in doses normally used for the treatment, prophylaxis, or diagnosis of disease, or the modification of physiological function" [61].

- 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 for justification. The Norwegian Food Safety Authority can be contacted for more information [60].
- 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, muscle and skeletal damage [3, 4]. 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.

How to minimize welfare challenges

- The Norwegian Animal Welfare Act § 9 [57] 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).

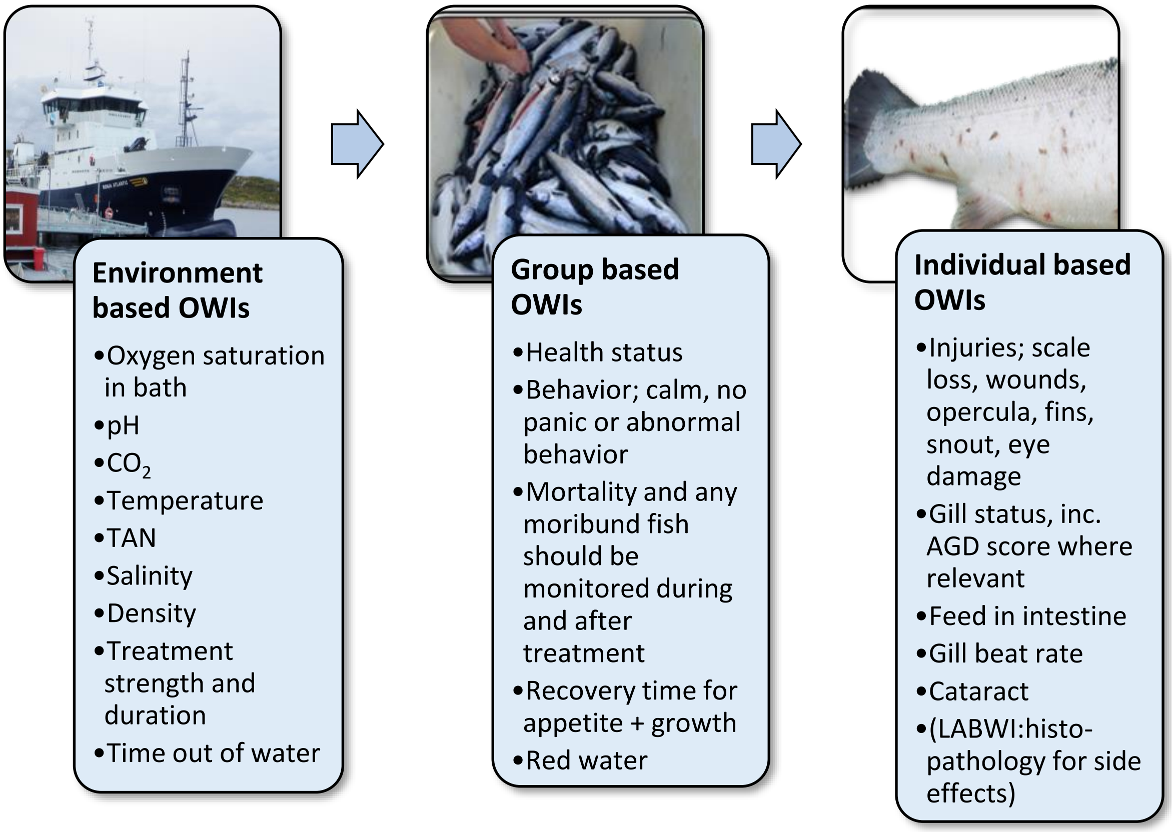


Figure 1.5-1. *Overview of fit for purpose OWIs during bathing and medicinal treatments. Environment based OWIs address the medicinal bath, group based OWIs are what can be observed and checked during the process, while individual based OWIs are based on sampling individual fish for close up examinations. 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 [15] and the RSPCA welfare standards for farmed rainbow trout [8] recommend a minimum limit of 7 mg 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. Rainbow trout can tolerate acute exposures (< 24 h) of NH_3-N concentrations of < 0.5 mg L^{-1} according to Milne et al., [62] (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 e.g. freshwater treatments [63].

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. 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.

Time out of water. Air exposure should be minimised as prolonged air exposure can damage the gill lamellae [43]. The RSPCA welfare standards for farmed rainbow trout recommend a maximum exposure time of 15 seconds [8] and EFSA recommends air exposure should be limited to 10 seconds [3]. There is also a study that reported mortality nearly doubled in rainbow trout when air exposure increased from 30 seconds to 60 seconds in exercised fish [44].

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 [23]. 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, section 3.1.6 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. In extreme cases, these changes can be recorded macroscopically e.g. damage to the gills, eyes and skin, but in milder forms histopathology is required (LABWI).

Gill status and AGD score. AGD scoring of the gills as developed for salmon [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 [63]. 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 [54] 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, and it may also be relevant to monitor cataracts.

Scoring schemes for e.g. skin haemorrhages, lesions/wounds, scale loss, eye haemorrhages, opercular damage, snout damage, active fin damage and cataracts are provided at the end of this document (based on photos from salmonids). 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).

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 influences their physiology and behaviour and fish often require immobilisation to reduce the risk of harm [65]. Commercial trout producers do not sedate or anaesthetise the fish frequently. However, a typical production cycle involves numerous routines that can be potential stressors for the fish e.g. vaccination, grading, handling, transport, and differing treatments for parasites or disease [65, 66, 67, 68, 69, 70].

The sedation and anaesthesia of fish can be induced by the use of drugs, gases, hypothermia and electrical current [65, 71]. 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 [72].

Marking and Meyer [73] have listed the features of an ideal anaesthetic:

1. Its induction time should be < 15 minutes and preferably < 3 minutes
2. It 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. 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 [74, 75, 76, 77, 78, 79].

Commercial aquaculture in Europe primarily uses three anaesthetics: benzocaine, tricaine mesilate and iso-eugenol.

- **Benzocaine**. According to Ross and Ross [65] 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 [65]. It must therefore be dissolved in acetone, ethanol or propylene glycol [65, 71, 76].
- **Tricaine mesilate (MS-222, Finquel Vet)** has been the most commonly used anaesthetic since its introduction in 1967 [80, 81]. 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 [82, 83].
- **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 [80, 84, 85]. An additional positive effect of iso-eugenol was discovered by Iversen et al., [76], who showed that dosages above 20 mg L^{-1} (iso-eugenol) blocked a further surge in plasma cortisol in A. salmon.

- The only other anaesthetics that have shown similar effects on plasma cortisol are etomidate/ metomidate [74, 86]. However, neither of these substances are approved for commercial aquaculture.
- Some anaesthetics e.g. tricaine mesilate are potent stressors that will elicit a stress response in trout [67, 80].

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.

Challenges to fish welfare

- Improper use of anaesthetics may cause both an overdose and negative effects on fish welfare [65].
- 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 [57] 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 [65, 73].
- 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 rainbow trout [8] state "*The medication must only be administered to fish by suitably trained staff*". 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) [73].

- 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.

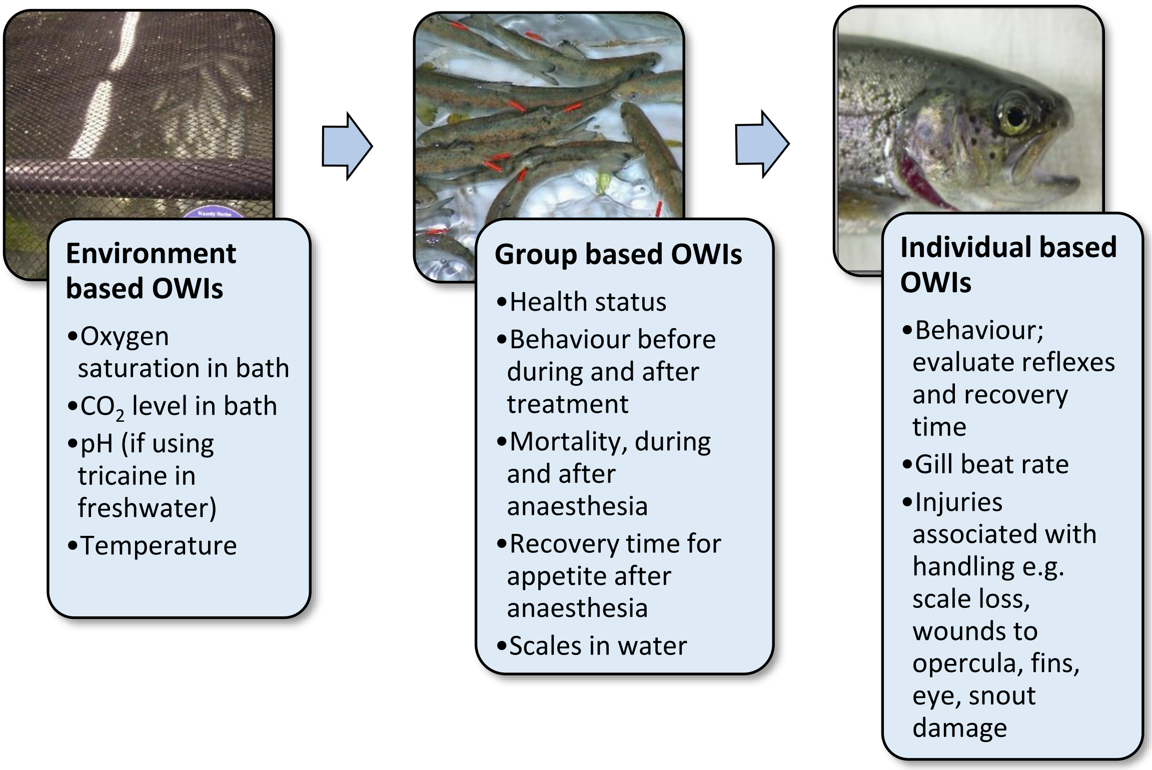


Figure 1.6-2. *Overview of fit for purpose OWIs for anaesthesia. Environment based OWIs specifically address the anaesthetic treatment, group based OWIs are what can be observed and checked during the anaesthesia process, while individual based OWIs are based on sampling individual fish for close up examinations. Illustration: M. H. Iversen and K. Gismervik. Photos: M. H. Iversen and C. Noble.*

Environment based OWIs

Oxygen saturation. As a general precautionary principle, all anaesthesia baths must have an oxygen saturation of >80% [15] and be aerated if necessary. The RSPCA welfare standards for farmed rainbow trout [8] also recommend a minimum limit of 7mg L^{-1}. If sodium bicarbonate ($NaHCO_3$) is used to buffer Finquel Vet, 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 Finquel Vet anaesthesia combined with sodium bicarbonate ($NaHCO_3$). The negative effects of CO_2 on trout are summarized in Part A, section 4.1.4. In summary, Hafs et al., [89] recommend CO_2 levels should be < 30 mg L^{-1}, RSPCA [8] recommend < 10 mg L^{-1} and Wedemeyer [90] also recommends < 10 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. EFSA [91 and references therein] suggest trout should be reared in a pH range of 5.0 – 9.0, state a pH of less than 4 can lead to significant mortalities and a pH between 4.5 and 5.5 induces sub lethal effects.

Water temperature must be measured during anaesthesia. At temperatures above 10 °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 [92] (see Table 1.6-1).

Group based OWIs

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.

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 [65, 93]. 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 [23]. 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.

Individual based OWIs

Behaviour should be monitored when the fish is undergoing anaesthesia and also during recovery. 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 [46]. The animal is classified as insensible if responses to these indicators are lacking [47, 48]. The vestibulo-ocular reflex (VOR or the "eye roll") is the last reflex the fish loses during anaesthesia and is the first reflex to reappear after recovery [49], see also 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 [46]) or nipping the fin between the nails of your thumb and forefinger. 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).

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 (based on photos from salmonids). 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 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 rainbow trout [8]. ***Reproduced with permission from the RSPCA.***

RSPCA welfare standards for farmed rainbow trout [8] state:

- Anaesthetics "*must be used according to the manufacturer's data sheet, unless otherwise specified by a vet*".
- Anaesthesia "*must only be administered to fish by suitably trained staff*".
- *Oxygen levels in the recovery tank must be: a) monitored regularly b) maintained at a minimum of 7mg/litre*".

Other recommendations:

- Maintain oxygen levels at >80% saturation [15].
- If sodium bicarbonate ($NaHCO_3$) is used to buffer Finquel vet, the baths should be aerated for at least 15 minutes to reduce the build-up of CO_2 prior to introducing fish.

1.7 Vaccination

Atlantic salmon and rainbow trout are vaccinated early in their production phase. Vaccination is an important procedure in modern aquaculture to protect and prevent disease outbreaks. The development of effective and efficient vaccines against a number of viruses and bacteria has drastically reduced the need of antibiotics since the 1990s [94, 95]. To ensure the health and welfare of salmonids after transfer to sea, all fish are individually vaccinated. However, the vaccination process can be a potential stressor [70].

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 in salmonids. This response is most likely due to the inflammatory reaction to oil-adjuvants in the vaccines [96].
- 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 [e.g. 97].
- In Norway, the most common method for vaccinating trout 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 [95].
- Different vaccine types may differ in their efficacy and side effects, but the same vaccine may also vary in its protection and adverse effects [e.g. 98 in A. salmon].
- Factors known to influence the efficacy of a vaccination procedure in salmonids include the vaccination technique, water temperature during vaccination [99], fish size at vaccination [99], hygiene, health status and individual fish differences [100, 101, 102].

How to assess welfare associated with vaccination

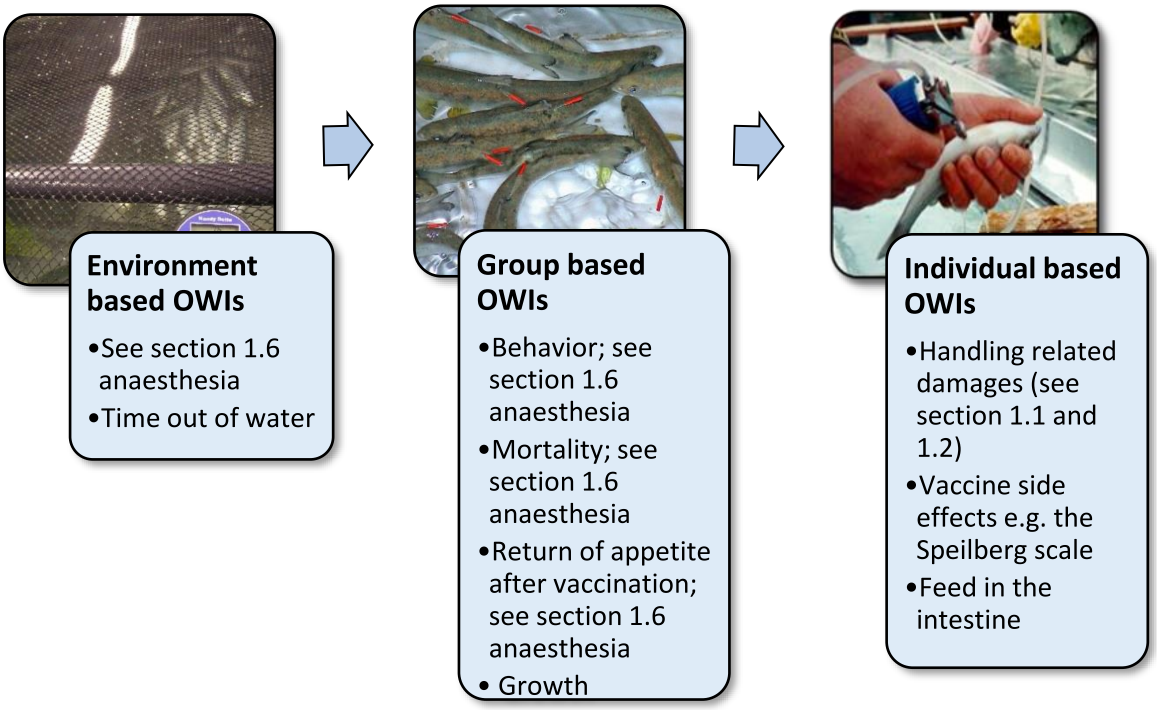


Figure 1.7-1. *Overview of fit for purpose OWIs for vaccination. Environment based OWIs specifically address the vaccination treatment, group based OWIs are what can be observed and checked during the vaccination process and afterwards, while individual based OWIs are based on sampling individual fish for close up examinations. Illustration: M. H. Iversen Photos: M. H. Iversen and A. Lillehaug*

Environment based OWIs

See section 1.6 anaesthesia for more details.

Time out of water. Air exposure should be minimised as prolonged air exposure can damage the gill lamellae [43].The RSPCA welfare standards for farmed rainbow trout recommend a maximum exposure time of 15 seconds [8] and EFSA recommends air exposure should be limited to 10 seconds [3]. There is also a study that reported mortality nearly doubled in rainbow trout when air exposure increased from 30 seconds to 60 seconds in exercised fish [44].

Group based OWIs

Behaviour. Abnormal behaviour could be an indication of a poorly executed vaccination, as e.g. stressed fish will typically aggregate in “clumps” [e.g. 103] at the bottom of the tank or sea cage. Highly stressed fish can also exhibit fleeing and flashing type behaviours [e.g. 103].

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 [23]. 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.

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 [54], 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 (based on photos from salmonids). 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).

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 [101]. The Speilberg scale is widely used as a welfare indicator in the Norwegian Atlantic salmon aquaculture industry and is reproduced in Fig. 1.7.3 with kind permission from Lars Speilberg. The Speilberg scale has also been used in rainbow trout [104, 105]. It describes changes related to peritonitis; adhesions between the organs, between the organs and the abdominal wall and melanin deposits [101], 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 [101], 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. Scale originally developed for Atlantic salmon but has also been used in studies on rainbow trout [e.g. 104, 105].*

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. Although the pictures are from Atlantic salmon, they are also applicable to rainbow trout. Figure: D. Izquierdo-Gomez. Photos: Lars Speilberg, kindly reproduced with permission. Text reproduced from "Midtlyng, P.J., Reitan, L.J. and Speilberg, L. 1996 [101], 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 eggs to ongrowers are handled and transported during a commercial production cycle. 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 and several welfare risks can be linked with the transport of live fish [106]. 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. Seawater adapted trout 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.

Challenges to fish welfare and how to minimize them

- **Transport – an important recovery phase**. Previous studies in salmonids 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 [e.g. 107, 108]. However, short transports may not provide adequate time for the fish to recover [4] 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 [109]). As the fish's lateral line system is highly sensitive [110], 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. 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 [111]. However, during winter or if the fish are subjected to chilled holding water, this window can be extended [111]. 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 [e.g. 112].

How to assess welfare associated with transport

Behaviour is a well-established welfare indicator in both terrestrial [113] and aquatic [23, 114] animal production. However, quantifying the behaviour of fish in aquaculture can be difficult. 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 [e.g. 31, 115]. To assess welfare before transport, see Part C sections 1.1 and 1.2 on crowding and pumping.

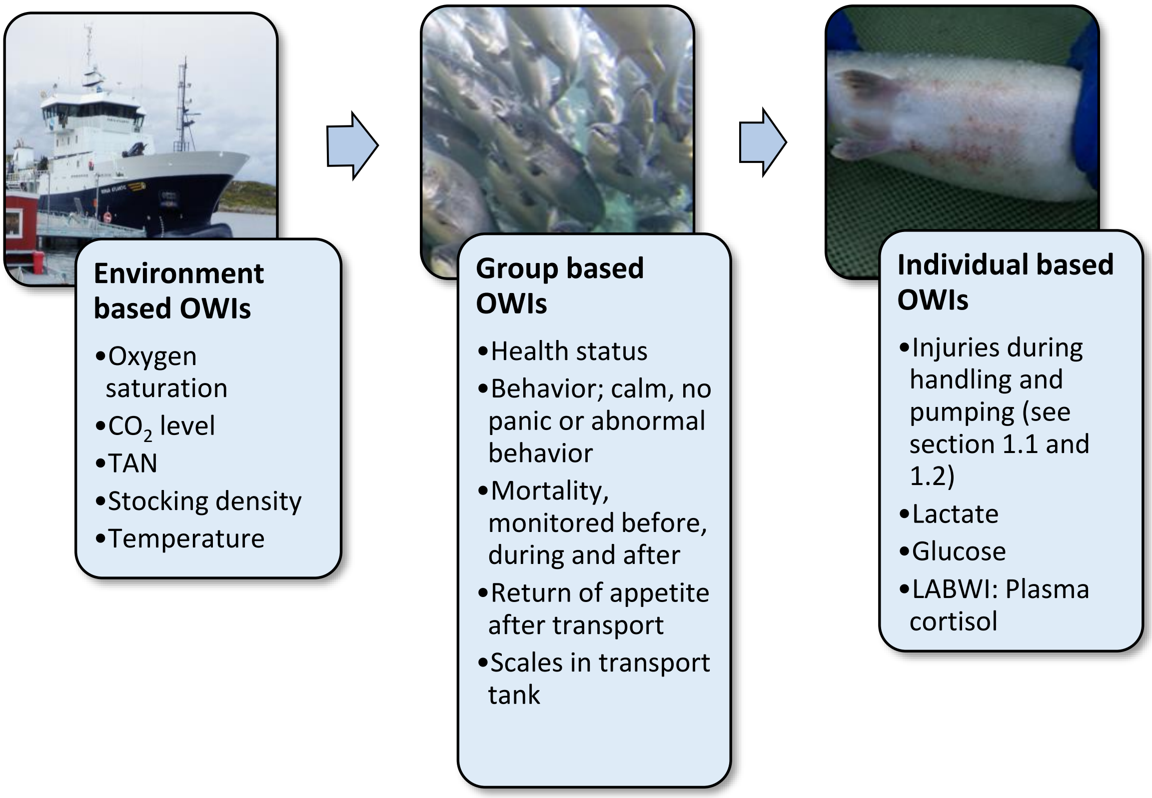


Figure 1.8-1. *Overview of fit for purpose OWIs for transport. Environment based OWIs specifically address the transport tank, group based OWIs address what can be observed and checked during the transport, while individual based OWIs are based on sampling individual fish. For key OWIs related to crowding 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 [15] and the RSPCA welfare standards for farmed rainbow trout [8] recommend a minimum limit of 7 mg L^{-1}.

Carbon dioxide can accumulate during transport (in closed tanks, or when the vents are closed in well boat transports). The negative effects of CO_2 on trout are summarized in Part A, section 4.1.4. In summary, Hafs et al., [89] recommend CO_2 levels should be < 30 mg L^{-1}, RSPCA [8] recommend < 10 mg L^{-1} when water is recycled and Wedemeyer [90] also recommends < 10 mg L^{-1}.

LABWI: TAN. Properties such as temperature, pH and salinity can affect the NH_3: NH_4^+ ratio and thus the toxicity of ammonia. Rainbow trout can tolerate acute exposures (< 24 h) of NH_3-N concentrations of < 0.5 mg L^{-1} according to Milne et al., [62] (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). 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 rainbow trout [8] state stocking density during road transport should not exceed 160 kg m^{-3} dependent on fish size (see Table 1.8-2).

Table 1.8-2. *Maximal stocking densities of different fish sizes during road transport according to the RSPCA welfare standards for farmed rainbow trout, RSPCA [8]. Reproduced with kind permission from the RSPCA.*

Fish size (grams)	Maximum stocking density (kg m^{-3})
1 – 4	40
5 – 19	85
20 – 49	95
50 – 99	110
100 – 224	130
225 – 449	140
450 – 999	160
1000 +	150

Temperature. Trout can adapt to temperatures in the range of 0-22 °C [16] but temperature preferences in rainbow trout can vary with the life stage of the fish. Every effort should be made to maintain temperatures within the optimal range since by the time the critical or lethal temperatures (higher or lower) are reached the welfare of the fish will already have been compromised. Fry and fingerlings have a preferred temperature range of 7-13 °C [17] and the RSPCA welfare standards for farmed rainbow trout [8] recommend 1-12 °C for fry. Recommended temperatures for rainbow trout ongrowers held in sea cages are around 7-17 °C [18]. Other authors suggest ongrowers have a preferred temperature of around 16 °C within a range of 13-19 °C under normoxic conditions [19]. The RSPCA welfare standards for farmed rainbow trout recommend 1-16 °C for ongrowers [8] (see also 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 [23]. 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. Abnormal behaviour could be an indication of a poorly executed transport, as e.g. stressed fish will typically aggregate in "clumps" [e.g. 103] at the bottom of the tank or sea cage. Highly stressed fish can also exhibit fleeing and flashing type behaviours [e.g. 103].

Scales in transport tank water. This indicates scale loss and damage to the skin which can cause osmoregulatory problems and also secondary infections.

Individual based OWIs *to use after transport*

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 (based on photos from salmonids). 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).

Lactate. Struggling, panic and burst swimming increases anaerobic muscle activity, thus increasing lactate in the blood [4, 5]. 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 [31].

Glucose can be used as an OWI for transport e.g. [112]. Elevation in plasma glucose is a relatively slow response to stress and peaks after around 3-6 hours in trout [116] 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 [112].

Plasma cortisol is not an OWI, but a LABWI. We know that transport stresses the fish and leads to elevated plasma cortisol levels in trout [31]. 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 (see also Part A, section 3.2.16).

Some general advice regarding handling procedures during transport

The RSPCA welfare standards for farmed rainbow trout [8] have robust guidelines in relation to different transport methods and life stages. 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 juvenile transport (recommendations from the RSPCA, for full details see RSPCA welfare standards for farmed rainbow trout [8]). ***Reproduced with permission from the RSPCA.***

- *"To minimise thermal shock and to avoid the inhibition of oxygen release into the water, the water temperature used for transportation must be as close as possible to that from which the fish came. As a guide, a difference of more than 3 or 4 °C would not be expected. Where the difference is greater, transport water should be mixed with receiving water in order to acclimatize the fish."*

Some additional general handling procedures regarding road transport (recommendations from the RSPCA, for full details see RSPCA welfare standards for farmed rainbow trout, [8]). ***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 °C from the water temperature at the start of the journey.
- "*Fish must be allowed to settle before departure*".

Some general handling procedures regarding well boat transport of salmonids (based on recommendations from Iversen et al., [67] and Iversen and Eliassen [117]. For full details see the above sources.

- 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 [67].
  - 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 [117].

1.9 Feed management, underfeeding and feed withdrawal

In this section we will cover the effects of feed management upon the welfare of rainbow trout. We will address species specific evidence when outlining fit for purpose OWIs and LABWIs for trout and also supplement this with evidence from other salmonids (mostly Atlantic salmon) where appropriate.

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 [118], 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, [119]) 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 trout rearing cages. Photo kindly provided by Ola Sveen, Svanøy Havbruk AS.*

Challenges to fish welfare in daily feed management

- Rainbow trout exhibit highly energetic feeding behaviour and can be highly competitive around mealtimes [e.g. 120, 121].
- 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 [118] and if the rate is too slow fish may not receive enough feed to grow at the best rate [122].
- Feeding frequency can also influence welfare in rainbow trout, but optimal frequency depends on the size of the fish. For example, it has been suggested that trout fry should be fed often, and this frequency should decrease as the fish grow [91]. However, this feeding frequency should not go too low as the fish get bigger. For example, limiting daily feeding to a single 3 hour feeding window can increase aggression and hinder the recovery from dorsal fin damage in comparison to fish fed 3 times per day or given free access to self-feeders during daylight hours, even when fish are fed to satiation e.g. in 90g trout [121]. Gélineau et al., [123] also reported that giving trout time limited access to self-feeders increased size variation. Another study suggested feeding hourly fixed rations (compared to every 10 minutes or continuously) can increased mortality and hinder growth rate [124]. However, feeding at a very high frequency (32 times per day over 18 hours compared to 8 times per day during 2 x 2 hours) in 20g rainbow trout was detrimental to growth [125] and the authors suggested this was due in part to the high frequency of competition around the higher number of meal times. In other salmonids, such as Atlantic salmon, 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., [126]). However, rainbow trout can exhibit similar high energy feeding behaviour irrespective of whether feed is distributed over a narrow or wide area [120].
- The choice of feeding technology and feeding a fixed ration versus feeding in response to appetite can be detrimental to fin damage [127]. However, another study [123] reported better growth in fed to satiation by hand rather than by self-feeding.

How to minimise welfare challenges in daily feed management

- Trout can be highly competitive (and potentially aggressive) around a meal.
- A farmer should monitor appetite and feeding behaviour (e.g. via underwater cameras) and **feed a responsive ration in relation to changes in appetite for every meal.**
- Feed at a rate that does not lead to competition and be careful when choosing feeding frequency; frequencies that are either too low or too high can be detrimental to welfare. Depending on the life stage, 2-8 meals per day should suffice [e.g. 121, 125] and perhaps more when feeding fry [91].
- Distribute the feed widely over the water surface.

Potential effects of fasting on welfare

- It is difficult to find information on a clear and quantified relationship between the length of feed withdrawal and fish welfare [see 128, 129].
- Fish can tolerate short- and long-term periods of feed withdrawal and feed restriction [130] and rainbow trout can adapt their metabolic rate as a reaction to feed withdrawal [131].

Welfare risks of fasting (feed withdrawal)

- Fish may be subject to fasting for several husbandry reasons and some carry inherent welfare risks. Risks are 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.
- Feed withdrawal can lead to utilization of reserves of body fat and other operative tissues [3, 91]. The length of fasting period can affect the stress response of trout; fish fasted for 9 days had a higher stress response than those fasted for 2 days [132]. The same authors suggested the effects of pre-slaughter fasting could be mediated by feeding the fish once every two days in the month prior to fasting instead of daily.
- Fasting can lead to decreased fish condition factor and emaciated fish [129].
- Stevenson [133] stated "*CIWF and WSPA believe that starving farmed fish - that have previously been fed regularly - for prolonged periods is unacceptable in welfare terms.*"

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 [134].
- Fasting prior to certain routines, e.g. bathing 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 [e.g. 91, 135].

Potential effects of underfeeding on welfare

- The opinion of the FAWC [136] 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 various life stages of rainbow trout, sudden periods of underfeeding or short- or longer-term underfeeding can be detrimental to welfare and lead to e.g. fin damage [137].

Welfare risks of underfeeding (feed restriction)

- Fish may be subject to underfeeding for several husbandry reasons and some carry inherent welfare risks.
- In rainbow trout weighing < 50g, underfeeding leads to inequality in feed intake [138] potentially due to increased competition for feed.
- In rainbow trout weighing < 230g, underfeeding increases size variation in the group [139].
- It can also increase fin damage in trout weighing ca. 25 g [137].

- The prolonged consequences of long-term underfeeding can be the depletion of energy reserves and nutritional status leading to reduced condition factor and even emaciated fish [129].

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.

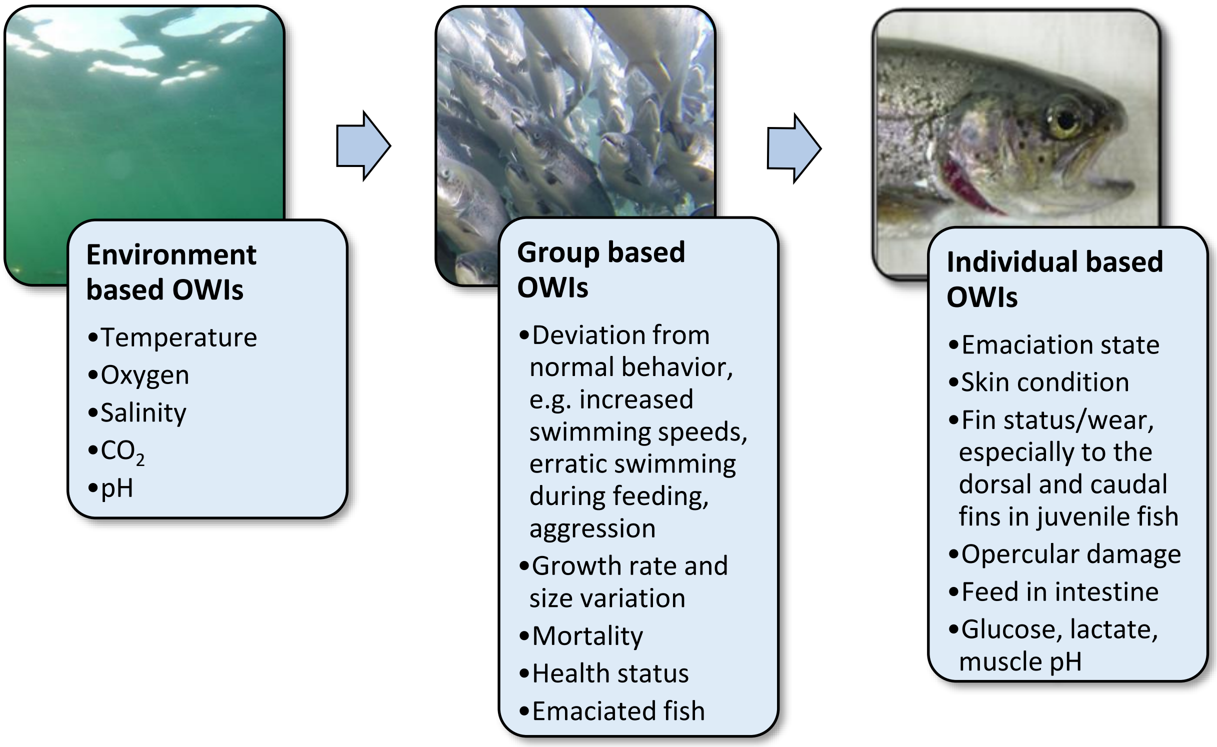


Figure 1.9-2. *Overview of fit for purpose OWIs for primarily fasting and underfeeding, but also other feed management factors. Environment based OWIs address the rearing environment, group based OWIs assess the group, while individual based OWIs are based on sampling individual fish for grading their external appearance. Illustration: C. Noble and L. H. Stien. Photos: L. H. Stien and Chris Noble.*

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. Trout can adapt to temperatures in the range of 0-22 °C [16] but temperature preferences in rainbow trout can vary with the life stage of the fish. Every effort should be made to maintain temperatures within the optimal range since by the time the critical or lethal temperatures (higher or lower) are reached the welfare of the fish will already have been compromised. Fry and fingerlings have a preferred temperature range of 7-13 °C [17] and the RSPCA welfare standards for farmed rainbow trout [8] recommend 1-12 °C for fry. Recommended temperatures for rainbow trout ongrowers held in sea cages are around 7-17 °C [18]. Other authors suggest ongrowers have a preferred temperature of around 16 °C within a range of 13-19 °C under normoxic conditions [19]. The RSPCA welfare standards for farmed rainbow trout recommend 1-16 °C for ongrowers [8].

Oxygen levels can impact upon feed intake and appetite in rainbow trout (e.g. EFSA [91]) and feeding itself can also reduce oxygen saturation levels [140]. 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. A recently published paper [14] outlines detailed data on the limiting oxygen saturations (LOS) of rainbow trout at different temperatures and at different sizes (Table 1.1-2). LOS is the minimum level where the fish can maintain sufficient respiration and levels below this are therefore lethal. The LOS values in Table 1.1-2 are measured on fasted fish, and a higher oxygen level may be required when fish are satiated [14] or during stressful situations such as crowding. Oxygen levels should therefore always be well above the LOS levels. As a general precautionary guideline, oxygen saturation levels of >80% are recommended, based upon data from Poulsen et al., [15] and the RSPCA welfare standards for farmed rainbow trout recommend a minimum of 7 mg L^{-1} [8].

Salinity is specific for life stages, with rainbow trout having the capacity to grow entirely in the freshwater environment or move to full strength saltwater. EFSA [91] state euryhalinity occurs in rainbow trout when the fish are greater than 50g and fish that are transferred at 70-100g have a good survival rate and are apparently able to cope with the transfer to sea out with a specific smolting window. Although literature is scarce, there is some evidence that salinity can affect appetite in rainbow trout. For example, a study by McKay and Gjerde [141], reported that salinities ≥ 10 ‰ significantly reduced appetite compared to fish raised at 0 ‰ in ca. 50 – 150g fish.

CO_2 / pH. Good et al., [142] did not report reduced growth or feed intake in trout reared at CO_2 levels of 24 mg L^{-1}. EFSA [91] suggest trout should be reared in a pH range of 5.0 – 9.0 and lower pH values within this range (a sub lethal pH of 5.2 in comparison to pH 6.3) may even stimulate appetite in some situations [143].

Group based OWIs

Behaviour. Aggression can occur in both juvenile [144] and adult trout [145] and it has been suggested that aggression increases when fish are underfed, either by a corresponding increase in fin damage [137] or by increased inequality in fed intake [138].

Growth can be negatively affected by underfeeding [e.g. 146] as can size variation [139]. Growth can also be negatively affected by feed withdrawal [147]. 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 [148] and is also affected by feeding regime [124] so should therefore be followed closely and on a regular basis.

Health status can affect appetite. See, for example, Chin et al., [149].

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 [129].

Individual based OWIs

Fin damage. The most common sign of problems associated with underfeeding/fasting/poor feed management is initially morphological damage, primarily dorsal fin damage in juvenile rainbow trout [e.g. 137]. 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. Trout 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 in A. salmon [150]. It has also been hypothesized that the opercula can suffer from traumatic injuries during highly competitive feeding in trout and has been used as an OWI for trout in previous studies [151].

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 (based on photos of salmonids). 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).

Emaciation state and condition factor. Reduced condition factor can result from underfeeding [152] and prolonged feed withdrawal can also lead to a reduced condition factor or emaciated fish [153]. As condition factor (*K*) is variable and changes with both life stage and season it is difficult to define exact values that are indicative of reduced welfare [114]. However, in long-term feed withdrawal studies on rainbow trout, values of < 1.0 have been reported in juvenile trout (ca. 55g mean weight) fasted for 4 months [154]. A fasting study on larger fish (ca. 280g mean weight) reported that *K* values dropped from an initial level of ca. 1.15-1.2 to ca. 1.05 after 1 month and ca. 0.9 after 4 months [153]. We therefore suggest a *K* factor of ca. 1.0 or < 1.0 can be indicative of emaciation in farmed rainbow trout. Rainbow trout can also accumulate large deposits of abdominal fat if overfed. The welfare implications of obesity are not clear, but it is a sign of poor feed management.

Feed in the intestine often indicates that the fish has eaten during the last one to two days [54] but this depends on the fish size and temperature. To evaluate daily feed intake or fasting periods, trout 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 [155]. Elevation in plasma glucose is a relatively slow response to stress and can peak around 6 hours after fasting in trout and then decreases [155], 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, glucose levels are reduced when trout are subject to prolonged feed withdrawal in comparison to fed controls [153]. Lactate is also affected by fasting, with a short term reduction 6h after fasting, but in general there is no difference between 1 and 3 days fasting [155].

Muscle pH. Is not affected by feed withdrawal periods up to 3 days prior to slaughter [156].

Current advice regarding fasting

Current advice varies on the appropriate lengths of feed withdrawal in relation to fish welfare.

- RSPCA welfare standards for farmed rainbow trout [8] recommend starvation periods should be no longer than 54 degree days in rainbow trout, without the approval of a veterinary surgeon or senior management and a welfare risk assessment must also be undertaken. The standards also state that "*After any period of fasting, food must be reintroduced in a way that: a) encourages the fish to resume feeding b) minimises waste c) can be demonstrated not to compromise fish welfare*" RSPCA [8].
- A 72-hour threshold is recommended by Stephenson [133] and CIWF [157].
- FAWC and HSA have proposed maximum limits of 48 hours [158, 159].
- The Norwegian Food Safety Authority have no fixed limits on fasting due to limited knowledge but state it should be as short as possible. (Akvakulturforskriften § 27: Fôring says with regard to fasting: «*Fisk skal ikke fôres når fôringen er uheldig ut fra hensynet til fiskens velferd, hygiene eller kvalitet. Perioden uten fôring skal være så kort som mulig*.») https://lovdata.no/dokument/SF/forskrift/2008-06-17-822.
- Lines and Spence [160] suggest a feed withdrawal period of 1-5 days is unlikely to pose major welfare threats to numerous fish species.
- López-Luna et al., [161] have suggested degree days be accounted for when assessing the implications of fasting periods, as have Stephenson [133] and FAWC [136]. López-Luna et al., [161] suggested a fasting period of 68 degree days (72 hours of fasting) did not affect the welfare of trout at slaughter and that water temperature alone (22.7 degree days) had a greater impact. EFSA [3] suggest a fasting limit of 50 degree days, and Bermejo-Poza et al., [131] suggest a fasting period of ca. 17 - 23 degree days (< 96 hours of fasting) to reduce the stress response of trout at slaughter.
- Bermejo-Poza et al., [132] also suggested that reducing feeding frequency to once every two days in the month prior to slaughter can improve their stress response during the final 2 days or fasting prior to slaughter.
- Another paper by Bermejo-Poza et al., [162] reported 5 days of fasting (107 degree days) did not significantly affect weight, condition factor or HSI in comparison to controls. They also reported that liver glycogen and some liver colour parameters changed after 5 days of fasting, indicating that energy reserves were being mobilized.

Knowledge gaps

- Although the literature on fasting in rainbow trout is more widespread and detailed than in Atlantic salmon [e.g. 131, 132, 154, 156, 162], there are still a number of mixed recommendations. The suite of available data still needs to be built upon in relation to different life stages and routines.
- This approach should cover feed withdrawal periods of different durations and under different farming conditions, especially with regard to temperature (see López-Luna et al., [161]).
- 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 [136] 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. 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 in trout [163], 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.

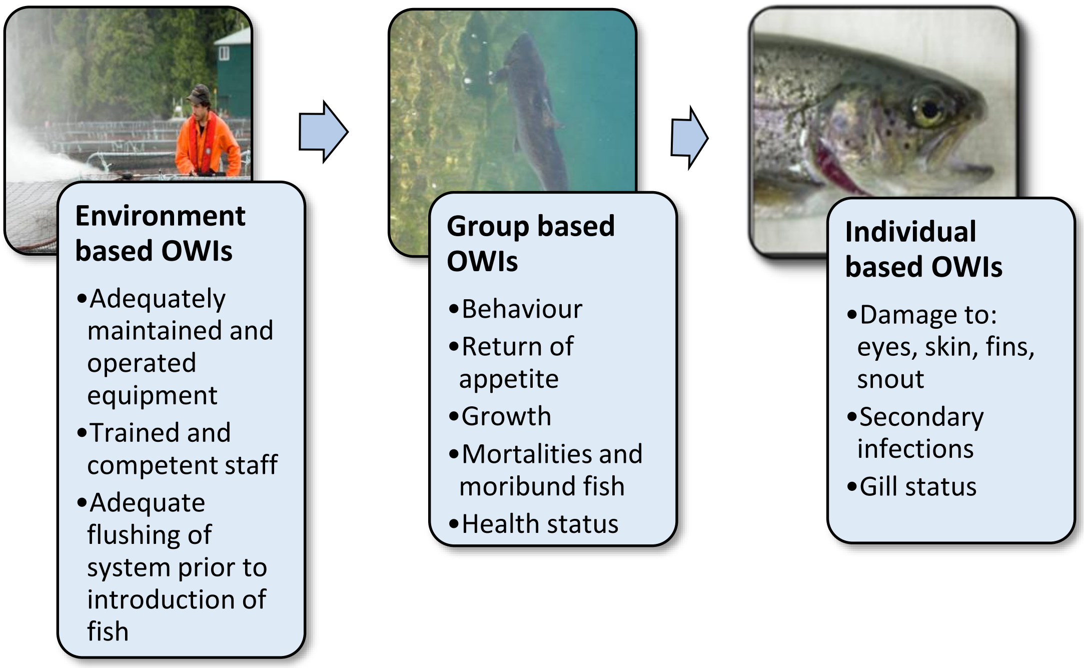


Figure 1.10-1. *Overview of fit for purpose OWIs during system sanitation. Environment based OWIs specifically address the environment, group based OWIs address what can be observed and checked during the operation, while individual based OWIs are based on sampling individual fish for close up examinations. 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: C. Noble.*

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 fleeing/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 [23]. 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 [e.g. 164].

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 (based on photos from salmonids). 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).

Secondary infections. Depending on the system (fresh or saltwater) 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), and the potential for hypoxia due to air exposure or exposure to water with low dissolved oxygen and physical damage. Earlier work by Flos et al., [165] has reported that grading had a significant impact on stress levels of trout for up to 10 hours after the event. The stress of the operation and the physical damage can increase the risk of secondary infections such as winter ulcers (*Moritella* spp.) in saltwater (especially at lower temperatures) and fungal (*Saprolegnia* spp.) infections in freshwater.

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 rainbow trout [8] recommend at least 90% of fish should be a minimum of 1.3 g in weight.

For planned routine grading, the fish should be health checked to ensure they are healthy enough to cope with the grading process (see also RSPCA welfare standards for farmed rainbow trout [8]). 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 suitable OWIs relating to those processes can be found in the relevant sections.

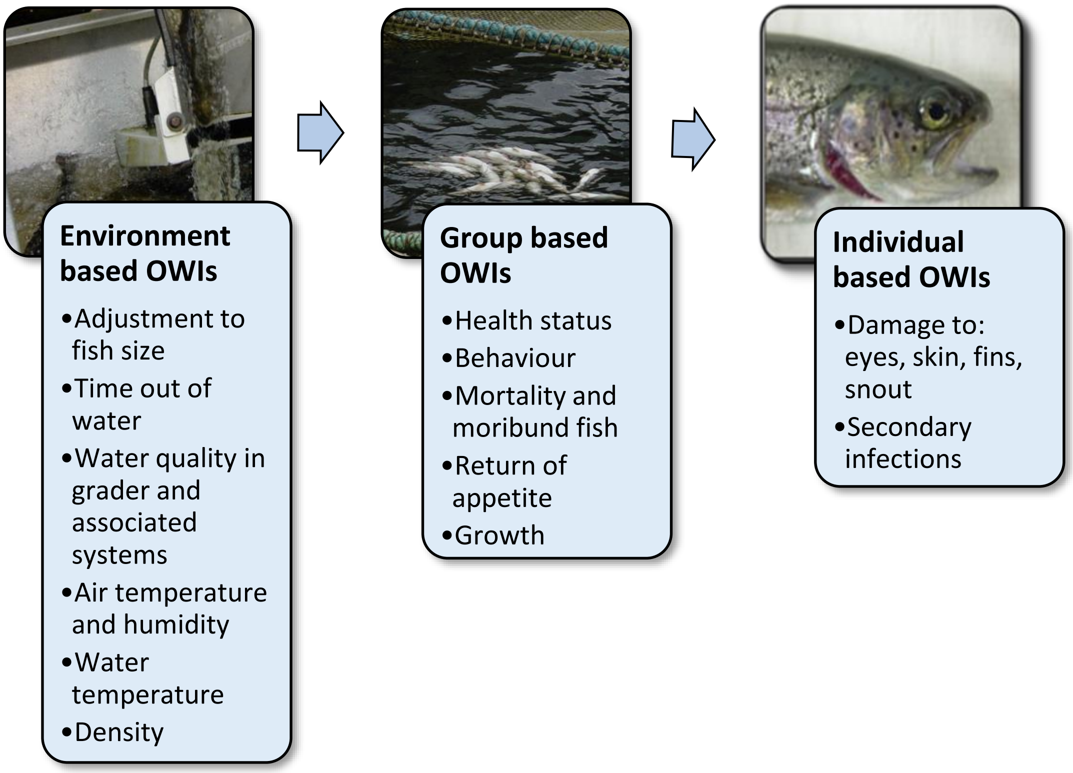


Figure 1.11-2. *Overview of fit for purpose OWIs for grading. Environment based OWIs specifically address the grading environment, group based OWIs address what can be observed and checked during operation, while individual based OWIs are based on sampling individual fish for close up examinations. Figure: 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. Air exposure should be minimised as prolonged air exposure can damage the gill lamellae [43]. The RSPCA welfare standards for farmed rainbow trout recommend a maximum exposure time of 15 seconds [8] and EFSA recommends air exposure should be limited to 10 seconds [3]. There is also a study that reported mortality nearly doubled in rainbow trout when air exposure increased from 30 seconds to 60 seconds in exercised fish [44].

Water quality including dissolved oxygen should be monitored in all the equipment or holding facilities associated with grading. A recently published paper [14] outlines detailed data on the limiting oxygen saturations (LOS) of rainbow trout at different temperatures and at different sizes (Table 1.1-2). LOS is the minimum level where the fish can maintain sufficient respiration and levels below this are therefore lethal. The LOS values in Table 1.1-2 are measured on fasted fish and a higher oxygen level may be required when fish are satiated [14] or during stressful situations such as crowding. Oxygen levels should therefore always be well above the LOS levels. As a general precautionary guideline, oxygen saturation levels of >80% are recommended, based upon data from Poulsen et al., [15] and the RSPCA welfare standards for farmed rainbow trout recommend a minimum of 7 mg L^{-1} [8].

Air temperature and humidity. With manual or machine grading, avoid excessively high or low temperatures and periods of low humidity.

Temperature. Trout can adapt to temperatures in the range of 0-22 °C [16] but temperature preferences in rainbow trout can vary with the life stage of the fish. Every effort should be made to maintain temperatures within the optimal range since by the time the critical or lethal temperatures (higher or lower) are reached the welfare of the fish will already have been compromised. Fry and fingerlings have a preferred temperature range of 7-13 °C [17] and the RSPCA welfare standards for farmed rainbow trout [8] recommend 1-12 °C for fry. Recommended temperatures for rainbow trout ongrowers held in sea cages are around 7-17 °C [18]. Other authors suggest ongrowers have a preferred temperature of around 16 °C within a range of 13-19 °C under normoxic conditions [19]. The RSPCA welfare standards for farmed rainbow trout recommend 1-16 °C for ongrowers [8]. See also Part A section 4.1.1 for more information.

Density. It is important to avoid densities that are too high during grading.

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 [e.g. 164].

Return of appetite. Any persistent reduction in feeding may indicate damage or stress as a result of grading and should be carefully monitored [23]. 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 (based on pictures from salmonids). 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).

Secondary infections. Depending on the system (fresh or saltwater) 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 anaesthetized, 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.

Numerous studies on rainbow trout have shown that fish handling poses a risk of injury and stress [e.g. 165, 166, 167]. Salmonids are adapted to life in water, are virtually weightless and have limited physical contact with any solid object. The skeleton and the skin are not adapted to the rigors of netting and other handling procedures, so this kind of operation can easily damage the fish [26]. 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. However, air exposure should be minimised as prolonged air exposure can damage the gill lamellae [43]. The RSPCA welfare standards for farmed rainbow trout recommend a maximum exposure time of 15 seconds [8] and EFSA recommends air exposure should be limited to 10 seconds [3]. There is also a study that reported mortality nearly doubled in rainbow trout when air exposure increased from 30 seconds to 60 seconds in exercised fish [44].

***Table 1.12-1**. Welfare risks of handling fish during live examinations. Table: K. V. Nielsen and K. Gismervik*

Operation	Risk	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 A risk of losing the fish
	External injuries	Too little space in sedation tank, increases the risk of injury
	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), hypoxia	Low / high air temperature, low humidity and windy conditions Length of air exposure, max. 15 sec. unless anaesthetised (RSPCA, [8])
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
	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

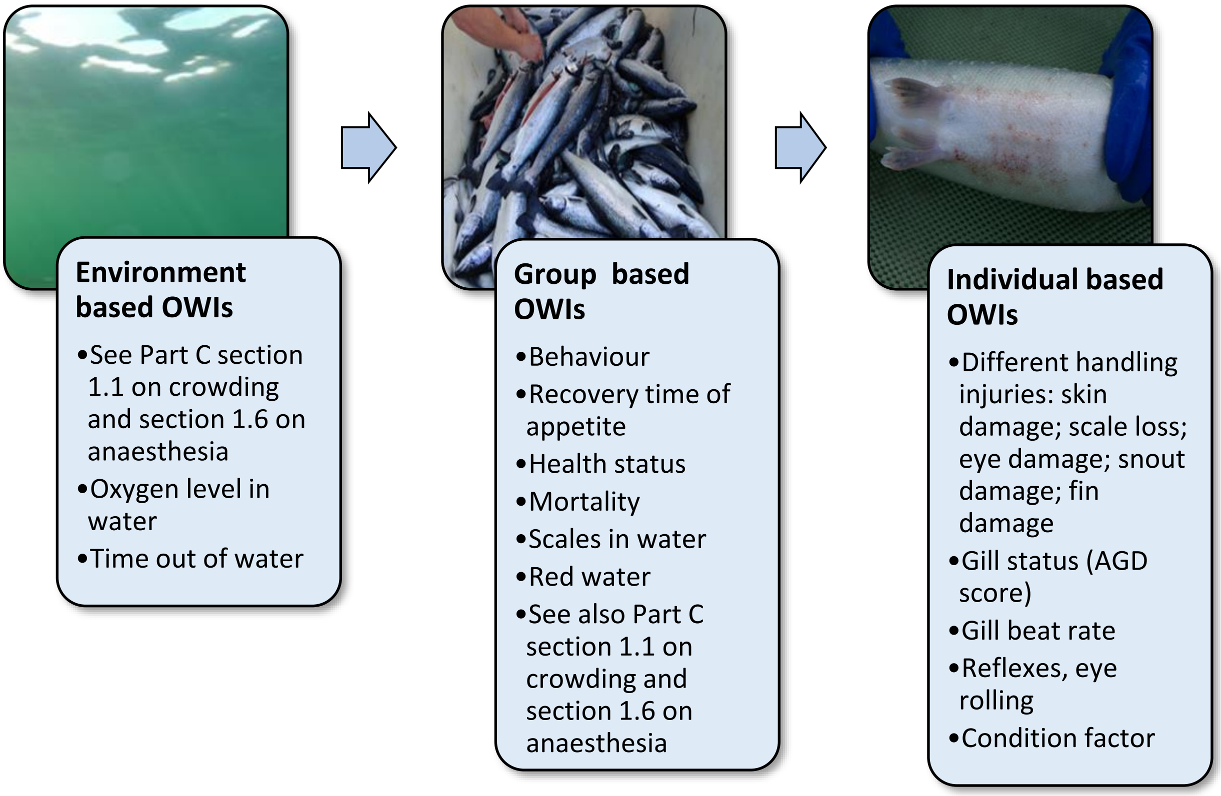


Figure 1.12-2. *Overview of fit for purpose OWIs for the examination of live fish. Environment based OWIs address the handling environment, group based OWIs address welfare at the group level, while individual based OWIs are based on sampling individual fish. Photos and illustration: K. Gismervik. Environment 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 [15] and the RSPCA welfare standards for farmed rainbow trout [8] also recommend a minimum of 7mg L^{-1}.

Time out of the water. Air exposure should be minimised as prolonged air exposure can damage the gill lamellae [43]. The RSPCA welfare standards for farmed rainbow trout recommend a maximum exposure time of 15 seconds [8] and EFSA recommends air exposure should be limited to 10 seconds [3]. There is also a study that reported mortality nearly doubled in rainbow trout when air exposure increased from 30 seconds to 60 seconds in exercised fish [44]. 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 [23]. 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 [e.g. 164]. 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 with salmon, 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 lighter coloured 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 [27] 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 [27] 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 (based on photos from salmonids). 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.

Control of unconsciousness. 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 [46]. The animal is classified as insensible if responses to these indicators are lacking [47, 48]. The vestibulo-ocular reflex (VOR or the “eye roll”) is the last reflex the fish loses during anaesthesia and is the first reflex to reappear after recovery [49], see 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 [46]) or nipping the fin between the nails of your thumb and forefinger. 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.

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) and other factors such as health problems. 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*

Usage area		WI	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	×	×	×		×	×	×	×		×	×	×
		• Longer-term	×	×			×	×	×	×	×	×	×	×
		Behaviour	×	×	×	×	×	×	×	×	×	×	×	×
		• Bellies showing	×	×		×	×	×	×	×		×	×	×
		• Equilibrium loss					×	×	×	×		×	×	×
		• Abnormal swimming	×	×		×	×	×	×	×	×	×	×	×
		• Crowding Scale	×	×			×		×	×			×	×
		• Gasping at the surface	×	×		×	×	×	×	×		×		×
		• Vertical swimming	×				×		×					
		• Head shaking					×	×		×				
		• Clumping	×				×		×	×		×	×	
		• Aggression									×			
		Appetite	×	×			×	×	×	×	×	×	×	×
		• Growth	×	×			×		×		×	×	×	
		Disease and health status	×	×	×	×	×	×	×	×	×	×	×	×
		Emaciated fish									×			×
		Scales or blood in water	×	×	×	×	×	×	×	×				×
Individual WIs		Handling trauma	×	×	×	×	×	×	×	×		×	×	×
		• Scale loss and skin condition	×	×	×	×	×	×	×	×	×	×	×	×
		• Mouth jaw wound	×	×	×	×	×			×		×	×	×
		• Fin damage and fin status	×	×	×	×	×	×	×	×	×	×	×	×
		• Eye haemorrhage and status	×	×	×	×	×			×	×	×	×	×
		• Skin Haemorrhaging		×	×									
		Cataract					×							
		Reflex, eye rolling			×	×		×	×					×
		AGD score	×	×			×							×
		Gill bleaching and status	×	×			×		×	×				×
		Gill beat rate	×		×	×	×	×	×	×				×
		Opercula damage	×	×							×	×		×
		Condition factor									×			×
		Moribund fish			×		×			×		×	×	×
		Emaciation state			×						×			
		Correctly adjusted blow if percussive stunning/killing			×	×								
		Vaccine related pathology (Speilberg score)							×					
		Feed in the intestine			×		×		×		×			
		Muscle pH	×	×	×						×			
		Pre-rigor time	×	×	×									
	Blood	Cortisol	×	×						×				
		Glucose	×	×						×	×			
		Lactate	×	×						×	×			
		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 requires applications for permission according to relevant welfare legislation.

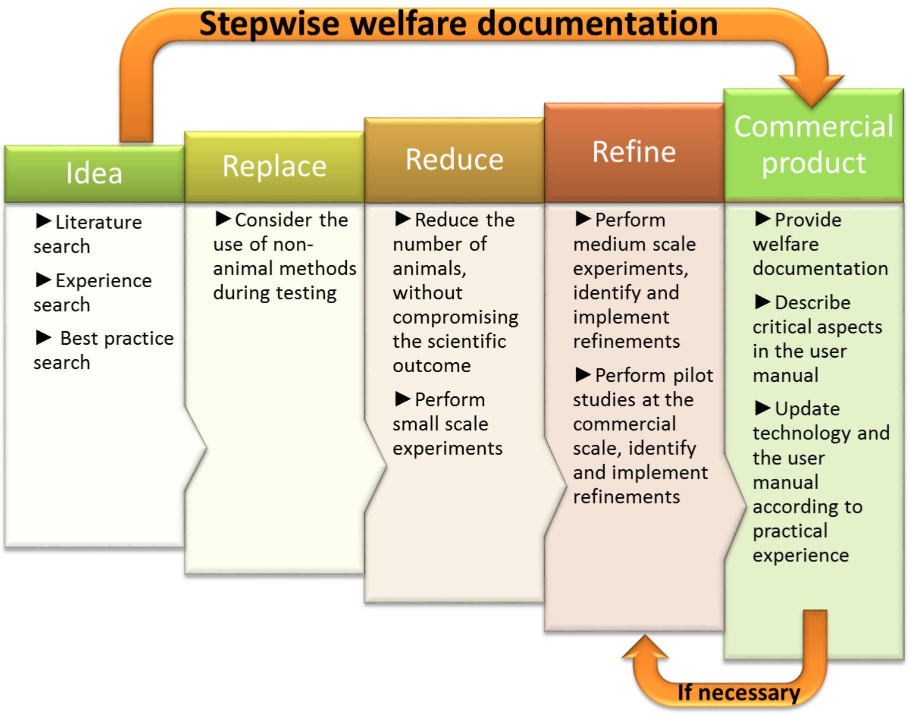


***Figure 2.1-1** Suggested stepwise welfare documentation from the concept to the commercial product with implementation of the «3 Rs» (Replace, Reduce and Refine; from laboratory animal science), during development of new technology. According to Norwegian legislation a new technology must be tested and evaluated as being suitable for fish welfare before it is used commercially. Illustration reproduced from Gismervik et al., [168] 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 [169] (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.
- ✓ Where relevant, check if the documentation addresses any issues associated with potential fish pain.

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 [170]. 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. **One of the main risks during handling is injury to the fish, poor water quality or the stress of the procedure itself.**

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 [27]. Technologies using seawater flushing and temperature adjusted water have previously been reported as acceptable in relation to fish welfare during initial testing [33, 34]. 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 [21, 171]. It has also been reported that rainbow trout have nociceptors (receptors for harmful stimuli) that respond to e.g. heat, pressure and chemical stimulation [172, 173].

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 [33, 34, 174].

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.

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 [21, 33, 34]. Crowding was also found to be a major risk factor during mechanical or thermal de-licing in a survey by Gismervik et al., [168].
- 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 [27, 33, 171, 175]. 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 [173, 176]. At lower temperatures there will be an increased risk of developing winter ulcers [21] (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 of salmonids, which may be related to panic behaviour that has been observed during and after exposure to the treatment bath [177].
- It is important to evaluate the general health status of the fish before the operation, as diseased fish have reduced tolerance to handling [175]. In a survey by Gismervik et al., [168] 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 thermal de-licing [21, 177] and this is also supported by mortality figures reported to authorities [175, 178]. In addition, high mortality has been observed following thermal de-licing especially when fish were diagnosed with AGD and/or gill irritation [33].
- 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 [33]. Gas supersaturation has also been registered in the treatment bath [177].
- Gill bleeding and scale loss have also been identified as risk factors for poor welfare associated with mechanical de-licing [27] and the correct adjustment of the equipment is important. It is also important to know what size of fish the technology is suitable for [10, 27].
- If cleaner fish are stocked with the rainbow trout, 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 [174, 177].

Table 2.2.1-1. *Svåsand et al., [179] identified these risks factors and potential consequences for fish welfare when using mechanical delicing. Table is translated and adapted from Svåsand et al., [179] 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 [171].
- 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. [27, 33, 34, 174]. Ensure that fish do not get caught in the system during low-intensity periods or during breaks [27, 174].
- 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., [27] found that the scoring of external acute injuries during mechanical de-licing in A. salmon 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 [27, 34, 174]. 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 [27].
- Having camera surveillance in the cage that the fish are returned to can help detect abnormal behaviour and possible mortalities as early as possible [174].
- 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.
- Ensure optimal water quality and water exchange in the temperature adjusted treatment chambers in thermal de-licing. High ammonia and turbidity values have been recorded [33]. Gas supersaturation has also been registered in the treatment bath [177].

- For thermal de-licing you must also ensure the correct temperature and exposure time [33, 34] and this may vary with the ambient sea temperature [34, 177]. Critical temperatures should also be paid attention to with regard to potential nociception, panic reactions and pain [177].
- The welfare of cleaner fish must also be considered if they are stocked with the rainbow trout.

How to assess welfare associated with mechanical and thermal de-licers

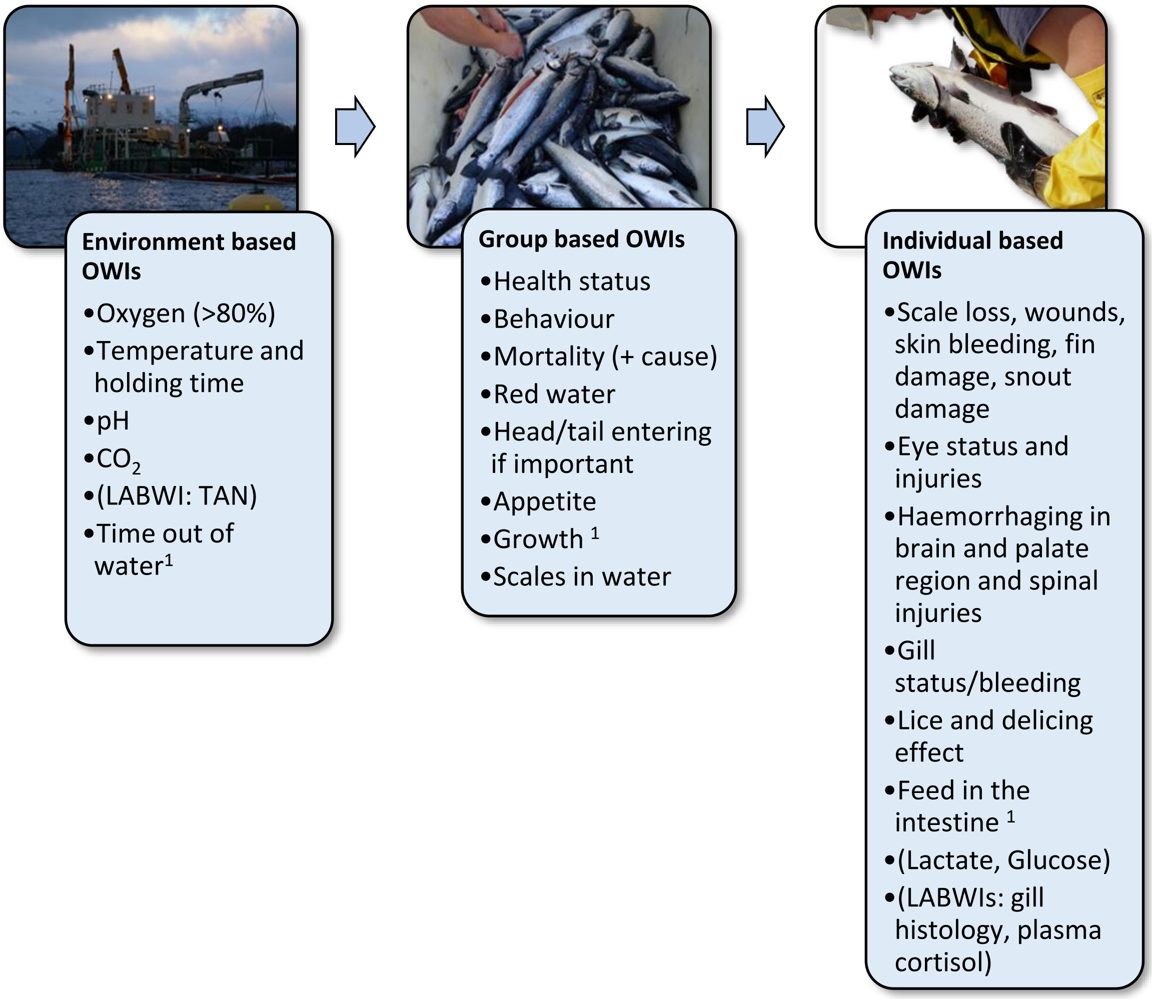


***Figure 2.2.1-2**. Overview of OWIs and LABWIs that may be suitable for mechanical and thermal de-licing. Environment based OWIs address the rearing environment, group based OWIs address the group, while individual based OWIs are based on sampling individual fish. [1]Based on general knowledge and not described in the welfare documentation available in salmonids. 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. A recently published paper [14] outlines detailed data on the limiting oxygen saturations (LOS) of rainbow trout at different temperatures and at different sizes (Part C Table 1.1-2). LOS is the minimum level where the fish can maintain sufficient respiration and levels below this are therefore lethal. The LOS values in Table 1.1-2 are measured on fasted fish, and a higher oxygen level may be required when fish are satiated [14] or during stressful situations. Oxygen levels should therefore always be well above the LOS levels. As a general precautionary guideline, oxygen saturation levels of >80% are recommended, based upon data from Poulsen et al., [15]. The RSPCA welfare standards for farmed rainbow trout recommend a minimum of 7 mg L^{-1} [8]. Levels must never approach the limiting oxygen saturation (LOS) (Part C Table 1.1-2.). During mechanical and thermal de-licing, oxygen levels during crowding (especially during summertime) 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 [179] and lead to mortalities. The upper temperature limits for use should be stated by the supplier. Low temperatures increase the risk for the development of ulcers. Damage from handling is often the initiating factor, leading to secondary infections with bacteria such as *Moritella viscosa* and *Vibrio* spp. in wintertime (see Part A, Table 3.1.5-2 for more information on winter ulcers) [21, 180].

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 [34]. The negative effects of CO_2 on trout are summarized in Part A, section 4.1.4. In summary, Hafs et al., [89] recommend CO_2 levels should be < 30 mg L^{-1}, RSPCA [8] recommend < 10 mg L^{-1} when water is recycled and Wedemeyer [90] also recommends < 10 mg L^{-1}.

pH must also be monitored. EFSA [91 and references therein] suggest trout should be reared in a pH range of 5.0 – 9.0, state a pH of less than 4 can lead to significant mortalities and a pH between 4.5 and 5.5 induces sub lethal effects.

LABWI: TAN. Properties such as temperature, pH and salinity can affect the NH_3: NH_4^+ ratio and thus the toxicity of ammonia. Rainbow trout can tolerate acute exposures (< 24 h) of NH_3-N levels of < 0.5 mg L^{-1} according to Milne et al., [62] (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 the water. Air exposure should be minimised as prolonged air exposure can damage the gill lamellae [43]. The RSPCA welfare standards for farmed rainbow trout recommend a maximum exposure time of 15 seconds [8] and EFSA recommends air exposure should be limited to 10 seconds [3]. There is also a study that reported mortality nearly doubled in rainbow trout when air exposure increased from 30 seconds to 60 seconds in exercised fish [44]. Air exposure time 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 thermal and mechanical de-licing can result in high mortality in diseased or weak fish [33, 175].

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 salmon post-smolts in closed and smaller containers can make it possible to detect bleeding as a colour change in water, so called "red water" [27]. Red water is never a good sign, and its 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 [23]. 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 [63]. Gill bleeding should also be monitored in relation to mechanical injuries [27].

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 [177]. 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 [177]. Haemorrhaging to the brain and palate region (and also spinal injuries/haemorrhaging) 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 (based on photos from salmonids). 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).

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 [54] 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 [4, 5].

Glucose. 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]. Similar results have been found in rainbow trout [5]. Glucose levels are however also dependent on diet type, feeding status and other factors and 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.

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 [166] (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 thermal de-licers in rainbow trout 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., [33]	Thermolicer	30-34ºC (25-30 sec)	1 cage (closely monitored) /1 locality	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%) * *salmon included C (0%)
Roth et al., [34]	Optilicer	28-34ºC (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, **LABWI:** gill histology	M (58-100%) C (0%)

Knowledge gaps concerning mechanical and thermal de-licing

- Mechanical and thermal de-licing technologies are relatively new and their use is increasing rapidly.
- 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 and have serious consequences for the fish [21]. 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 rainbow trout and must also be related to ambient water temperatures [177, 178, 181, 182].
- 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) [34, 39, 40].
- In 2017, haemorrhages to the brain, palate region and eyes were detected on Atlantic salmon in connection with thermal de-licing [177]. 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 scarce [177].
- Available documentation on the welfare aspects of the mechanical de-licing of rainbow trout is missing [27, 183].

2.2.2 Optical de-licing (laser)

This technology uses camera vision and lasers for continuously shooting any potential lice on the salmon and trout in sea cages. Some of the potential benefits of this passive, in-cage de-licing technology are that the fish do not require crowding, 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 2.2.2.-1.

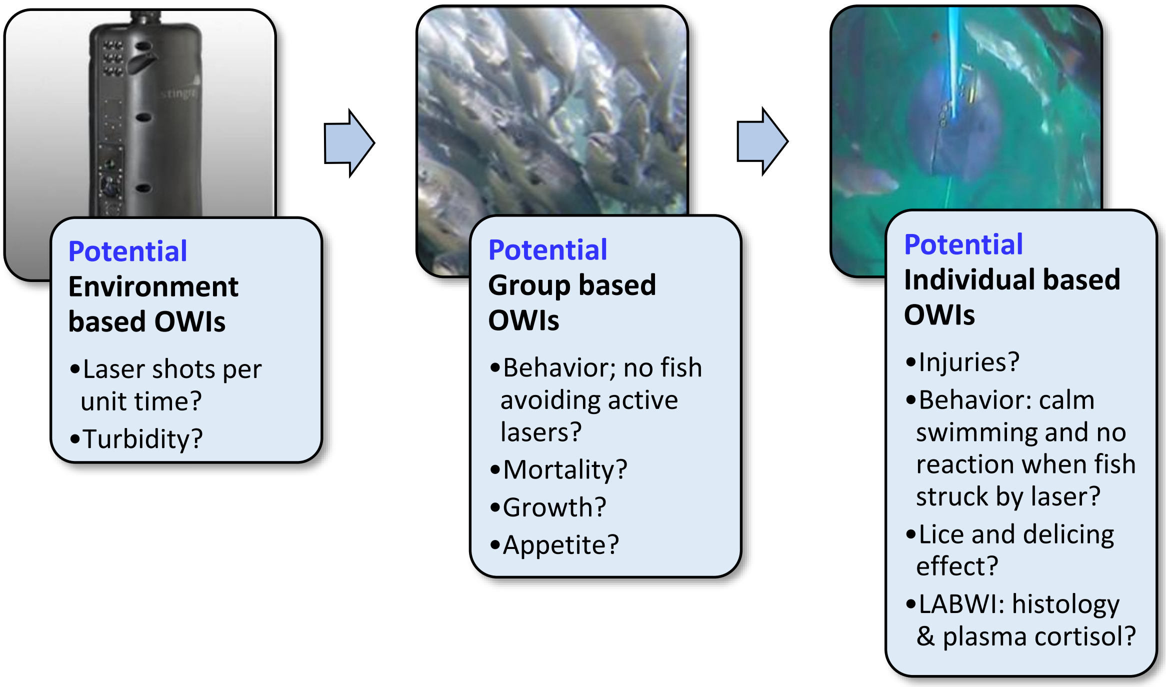


Figure 2.2.2-1. *Overview of the potential OWIs and LABWIs that may be suitable for the laser treatment of lice. Based on general advice in the absence of documentation. Environment based OWIs address the rearing environment, group based OWIs address the group, while individual based OWIs are based on sampling individual fish. Illustration: K. Gismervik, group OWI photo: L. H. Stien. Other photos reproduced with kind permission from Stingray 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, potential 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 (based on photos of salmonids). 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).

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 concerning optical de-licing

- At the time of writing 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 [184]. As we have found no documentation on its potential effects on the eye and body of trout, it should be audited as a potential risk during welfare assessments.

2.2.3 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 trout net cages can have many negative consequences. They can result in reduced water exchange through the net and therefore reduced dissolved oxygen [185, 186] and increased resistance to water flow which may increase distortion of the nets or the strain on the physical structure and moorings [187]. Organisms growing on the net use and thereby further reduce available dissolved oxygen [188, 189], release waste products into the water and can be a reservoir for infections [190, 191, 192]. Growth on the nets may also serve as a source of natural feed for cleaner fish, reducing their consumption of lice [193].

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 2.2.3-1), 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 2.2.3-2). 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 cage and changing or drying the fouled net. This is potentially less harmful to fish but is practically impossible in most cases.

Figure 2.2.3-1. *Example of a net cleaning rig from AKVA with 4 cleaning heads. Photograph courtesy of N. Ribeiro, with permission.*

Figure 2.2.3-2. *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 [194, 195, 196]. However, recent work by Bloecher et al., [196] 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 cage 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 cage being cleaned and other cages 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.

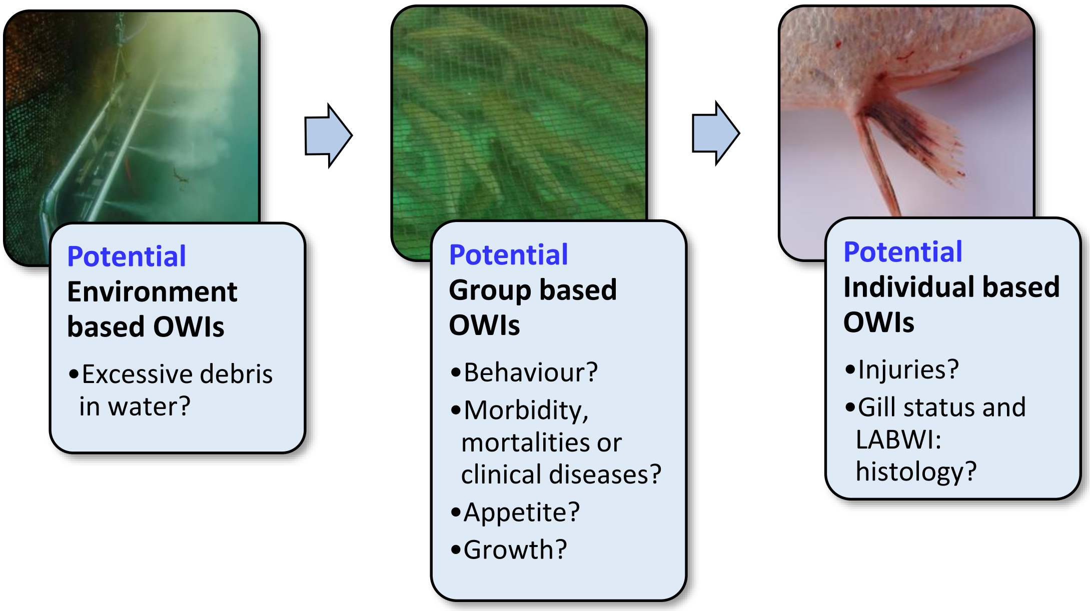


Figure 2.2.3-3. *Overview of potential OWIs and LABWIs that may be suitable for net cleaning. Based on general advice in the absence of documentation. Environment based OWIs specifically address the environment, group based OWIs can be observed and checked during the operation, while individual based 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 [23]. Practical farm experience reported in [196] 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 [197].

Clinical diseases, morbidities or mortalities? In severe cases fish may become sick and die or have to be removed from the cage. This should be investigated by fish health specialists [e.g. 164].

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 cage 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 (based on photos from salmonids). 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 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) [195, 196].

Knowledge gaps concerning net cleaning robots

- As far as the authors are aware, at the time of writing 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 [194, 195, 196].

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) [114], the injury scoring scheme developed by the Norwegian Veterinary Institute (NVI) [10, 33] 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 13 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) active fin damage, xiii) healed fin damage.

We have used pictures from the FISHWELL salmon handbook in the following scoring system, as the conditions they describe are applicable to rainbow trout.

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 rainbow trout, we suggest a simplified scoring system similar to that used in the RSPCA welfare standards for farmed Atlantic salmon [198].

Cataract damage is classified using an existing and widely used 0-4 scoring scheme [199], 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” [101], see Table 3.3 and Fig. 3.4. The Speilberg scale is widely used as a welfare indicator in the Norwegian aquaculture industry, primarily for salmon but it has also been used for trout. 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 ([101] see also [200] 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)*

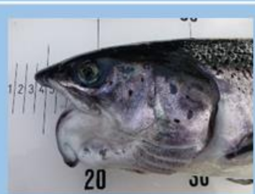
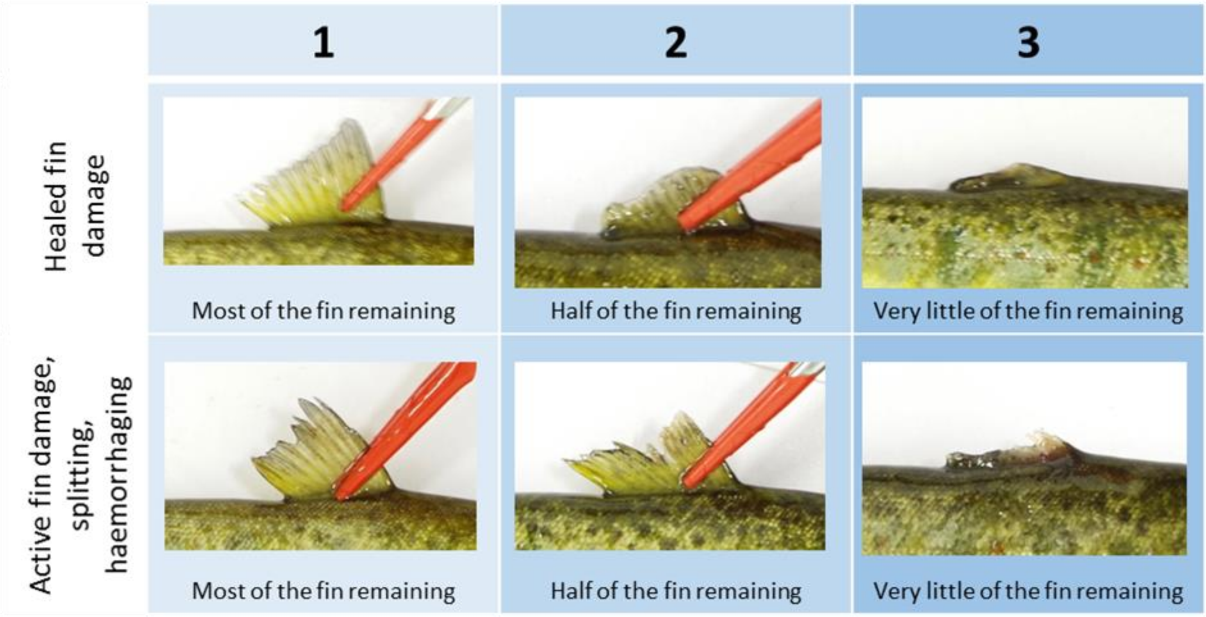
	1	2	3
Eye haemorrhage	 Minor haemorrhages	 Larger haemorrhages, or traumatic injury	 Large haemorrhages / traumatic injury. Eye may be ruptured
Exophthalmia	 Eye protruding a little	 Moderate eye protrusion	 Major eye protrusion
Opercular damage	 Operculum only partly covering gills	 Operculum absent 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

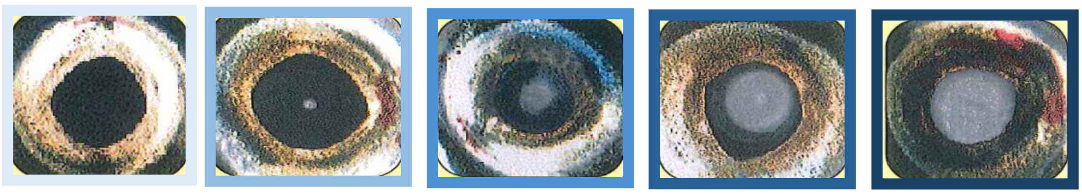
Table 3.1-2. *Morphological scheme for diagnosing and classifying key external injuries. Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated). Level 1, minor to Level 3, clear evidence of the OWI. (Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. 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)

[1] For fingerlings "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)*





0. No cataract

1. Cataract covers less than 10% of lens diameter

2. Cataract covers between 10 and 50% of lens diameter

3. Cataract covers 50 to 75% of lens diameter

4. Cataract covers over 75% of lens diameter

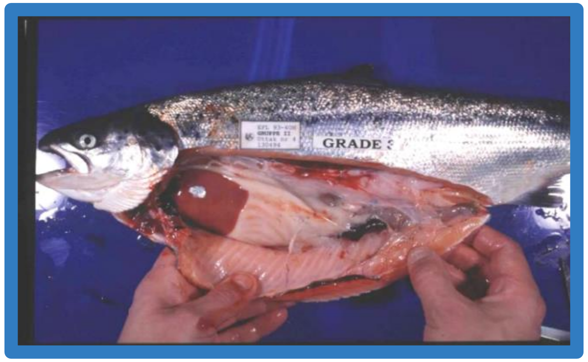
Fig. 3.2. *Morphological scheme for diagnosing and classifying eye cataracts in salmon and other species. Text reproduced from "Wall, T. & Bjerkås, E. 1999, A simplified method of scoring cataracts in fish. Bulletin of the European Association of Fish Pathologists 19(4), 162-165. Copyright, 1999" [199] 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." [201] 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" [101] with permission from Elsevier. Scale originally developed for Atlantic salmon but has also been used in studies on rainbow trout [e.g. 104, 105].*

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. Although the pictures are from Atlantic salmon, they are also applicable to rainbow trout. 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" [101] with permission from Elsevier.*

4 References

[1] Vigen, J. (2008) Oxygen variation within a sea cage. Master thesis. Department of Biology, University of Bergen, Bergen, 73 pp.

[2] Espmark, Å. M., Kolarevic, J., Hansen, Ø. A. & Nilsson, J. (2015) Pumping og håndtering av smolt. Nofima rapport nr. 6/2015.

[3] EFSA (2009) Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on Species-specific welfare aspects of the main systems of stunning and killing of farmed rainbow trout. *The EFSA Journal* **1013**, 1-55.

[4] Merkin, G. V., Roth, B., Gjerstad, C., Dahl-Paulsen, E. & Nortvedt, R. (2010) Effect of pre-slaughter procedures on stress responses and some quality parameters in sea-farmed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **309**, 231–235.

[5] Olsen, R. E., Sundell, K., Mayhew, T. M., Myklebust, R. & Ringø, E. (2005) Acute stress alters intestinal function of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture* **205**, 480-495.

[6] Milligan, C. L. & Wood, C. M. (1986) Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. *Journal of Experimental Biology* **123(1)**, 123-144.

[7] Lefèvre, F., Bugeon, J., Aupérin, B. & Aubin, J. (2008) Rearing oxygen level and slaughter stress effects on rainbow trout flesh quality. *Aquaculture* **284(1-4)**, 81-89.

[8] RSPCA (2018). RSPCA welfare standards for farmed rainbow trout. https://science.rspca.org.uk/sciencegroup/farmanimals/standards/trout (Accessed July 2019)

[9] CIWF: Food business (2018). Humane Slaughter: Rainbow trout. https://www.compassioninfoodbusiness.com/media/7434844/humane-slaughter-rainbow-trout.pdf (Accessed July 2019).

[10] Gismervik, K., Østvik, A. & Viljugrein, H. (2016) Pilotflåte HeliXiR - dokumentasjon av fiskevelferd og effekt mot lus. Del 1 uten legemiddel. *Rapport 15, 2016. Veterinærinstituttets rapportserie*. Veterinærinstituttet, Oslo, Norge.

[11] HSA (2016) Humane Harvesting of Fish. Humane Slaughter Association. https://www.hsa.org.uk/downloads/publications/harvestingfishdownload-updated-with-2016-logo.pdf (Accessed July 2019).

[12] Mejdell, C. M., Midling, K. Ø., Erikson, U., Evensen, T. H. & Slinde, E. (2009) Evaluering av slaktesystemer for laksefisk i 2008–fiskevelferd og kvalitet. *Veterinaerinstituttets rapportserie, 01-2009.* Veterinærinstituttet, Oslo, Norge.

[13] Mattilsynet (2014). Veiledning om krav til god fiskevelferd ved slakteri for akvakulturdyr, Mattilsynet 2014, www.mattilsynet.no

[14] Shi, K., Dong, S., Zhou, Y., Gao, Q., Li, L., Zhang, M. & Sun, D. (2018) Comparative Evaluation of Toleration to Heating and Hypoxia of Three Kinds of Salmonids. *Journal of Ocean University of China* **17(6)**, 1465-1472.

[15] Poulsen, S. B., Jensen, L. F., Nielsen, K. S., Malte, H., Aarestrup, K. & Svendsen, J. C. (2011) Behaviour of rainbow trout *Oncorhynchus mykiss* presented with a choice of normoxia and stepwise progressive hypoxia. *Journal of Fish Biology* **79(4)**, 969-979.

[16] Ihssen, P. E. (1986) Selection of fingerling rainbow trout for high and low tolerance to high temperature. *Aquaculture* **57(1-4)**, 370.

[17] Woynarovich, A., Hoitsy, G. & Moth-Poulsen, T. (2011) Small-scale rainbow trout farming. *FAO Fisheries and Aquaculture Technical Paper* **(561)**, I.

[18] Sutterlin, A. M. & Stevens, E. D. (1992) Thermal behaviour of rainbow trout and Arctic char in cages moored in stratified water. *Aquaculture* **102(1-2)**, 65-75.

[19] Schurmann, H., Steffensen, J. F. & Lomholt, J. P. (1991) The influence of hypoxia on the preferred temperature of rainbow trout Oncorhynchus mykiss. *Journal of Experimental Biology* **157(1)**, 75-86.

[20] Farrell, A. P. & Richards, J. G. (2009) Defining hypoxia: an integrative synthesis of the responses of fish to hypoxia. *Fish physiology* **27**, 487-503.

[21] Hjeltnes, B., Bornø, G., Jansen, M. D., Haukaas, A., & Walde, C (Eds) (2017). Fiskehelserapporten 2016. Oslo*, Veterinærinstituttet,* p. 121.

[22] Erikson, U., Gansel, L., Frank, K., Svendsen, E. & Digre, H. (2016) Crowding of Atlantic salmon in net-pen before slaughter. *Aquaculture* **465**, 395-400.

[23] Huntingford, F. A., & Kadri, S. (2014) Defining, assessing and promoting the welfare of farmed fish. *Revue scientifique et technique (International Office of Epizootics)* **33(1)**, 233-244.

[24] Schmidt, J. G., Andersen, E. W., Ersbøll, B. K. & Nielsen, M. E. (2016) Muscle wound healing in rainbow trout (*Oncorhynchus mykiss*). *Fish & shellfish immunology* **48**, 273-284.

[25] Schmidt, J. G., Nielsen, M. E. & Ersbøll, B. K. (2013) Wound healing in rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*): with a focus on gene expression and wound imaging. Technical University of Denmark Danmarks Tekniske Universitet, Department of Informatics and Mathematical ModelingInstitut for Informatik og Matematisk Modellering. 194 pp.

[26] Takle, H. R., Ytteborg, E., Nielsen, K. V., Karlsen, C. R., Nilsen, H. K., Sveen, L., Colquhoun, D. J., Olsen, A. B., Sørum, H. & Nilsen, A. (2015). Sårproblematikk og hudhelse i laks-og regnbueørrettoppdrett. *Nofima rapport 5/2015.* p. 108.

[27] Gismervik, K., Nielsen, K. V., Lind, M. B. & Viljugrein, H. (2017) Mekanisk avlusing med FLS-avlusersystem- dokumentasjon av fiskevelferd og effekt mot lus. In: *Veterinærinstituttets rapportserie 6-2017*. Veterinærinstituttet, Oslo*,* pp. 41.

[28] Kubilay, A. & Uluköy, G. (2002) The effects of acute stress on rainbow trout (*Oncorhynchus mykiss*). *Turkish Journal of Zoology* **26(2),** 249-254.

[29] Olsen, R. E., Sundell, K., Hansen, T., Hemre, G. -I., Myklebust, R., Mayhew, T. M. & Ringø, E. (2003) Acute stress alters the intestinal lining of Atlantic salmon, *Salmo salar* L.: An electron microscopical study. *Fish Physiology and Biochemistry* **26**, 211–221.

[30] Veiseth, E., Fjæra, S. O., Bjerkeng, B. & Skjervold, P. O. (2006) Accelerated recovery of Atlantic salmon (*Salmo salar*) from effects of crowding by swimming. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **144(3)**, 351-358.

[31] Shabani, F., Erikson, U., Beli, E. & Rexhepi, A. (2016) Live transport of rainbow trout (*Oncorhynchus mykiss*) and subsequent live storage in market: water quality, stress and welfare considerations. *Aquaculture* **453**, 110-115.

[32] Espmark, Å. M. O., Midling, K. Ø., Nilsson, J. & Humborstad, O. B. (2016) Effects of pumping height and repeated pumping in Atlantic salmon *Salmo salar*. *Natural Resources* **7(06)**, 377-383.

[33] Grøntvedt, R. N., Nerbøvik, I. -K. G, Viljugrein, H., Lillehaug, A., Nilsen, H. & Gjerve, A. –G. (2015) Termisk avlusing av laksefisk – dokumentasjon av fiskevelferd og effekt. *Rapport 13, 2015. Veterinærinstituttets rapportserie*. Veterinærinstituttet, Oslo, Norge.

[34] Roth, B. (2016) Avlusing av laksefisk med Optilice: Effekt på avlusing og fiskevelferd. *Nofima rapport nr. 59-2016*. Nofima. Tromsø. pp. 41.

[35] Grizzle, J. M. & Lovshin, L. L. (1994) Effect of pump speed on injuries to channel catfish (*Ictalurus punctatus*) during harvest with a turbine pump. *Aquacultural engineering* **13(2)**, 109-114.

[36] Hammer, C. (1995) Fatigue and exercise tests with fish. *Comparative Biochemistry and Physiology Part A: Physiology* **112(1)**, 1-20.

[37] Roth, B., Grimsbø, E., Slinde, E., Foss, A., Stien, L. H. & Nortvedt, R. (2012) Crowding, pumping and stunning of Atlantic salmon, the subsequent effect on pH and rigor mortis. *Aquaculture* **326**, 178-180.

[38] Espmark, A.M., Humborstad, O. B. & K. Ø., Midling (2012) Pumping av torsk og laks, faktorer som påvirker velferd og kvalitet. *Nofimarapport nr.6-2012*. Nofima. Tromsø.

[39] Mejdell, C. & Gismervik, K. (2009) Dokumentasjon av metode for retningsorientering av laksefisk før slaktebedøving, in *Veterinærinstituttets rapportserie 15-2009*, Veterinærinstituttet: Oslo. pp. 14.

[40] EFSA (2009) Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on welfare aspect of the main systems of stunning and killing of farmed Atlantic salmon. *The EFSA Journal* **1012**, 1-77.

[41] Hughes, G. M. & Roberts, J. L. (1970) A study of the effect of temperature changes on the respiratory pumps of the rainbow trout. *Journal of Experimental Biology* **52(1)**, 177-192

[42] Black, M. C., Millsap, D. S. & McCarthy, J. F. (1991) Effects of acute temperature change on respiration and toxicant uptake by rainbow trout, *Salmo gairdneri* (Richardson). *Physiological Zoology* **64(1)**, 145-168.

[43] Cook, K. V., Lennox, R. J., Hinch, S. G. & Cooke, S. J. (2015) Fish out of water: how much air is too much? *Fisheries* **40(9)**, 452-461.

[44] Ferguson, R. A. & Tufts, B. L. (1992) Physiological effects of brief air exposure in exhaustively exercised rainbow trout (*Oncorhynchus mykiss*): implications for" catch and release" fisheries. *Canadian journal of fisheries and aquatic sciences* **49(6)**, 1157-1162.

[45] FAWC (2014) Opinion on the welfare of farmed fish at the time of killing, in *FAWC- Farm Animal Welfare Committee*: London, UK.

[46] Davis, M. W. (2010) Fish stress and mortality can be predicted using reflex impairment. *Fish and Fisheries* **11**, 1-11.

[47] Robb, D. H. F., Wotton, S. B., McKinstry, J. L., Sorensen, N. K. & Kestin, S. C. (2000) Commercial slaughter methods used on Atlantis salmon: determination of the onset of brain failure by electroencephalography. *Veterinary Record* **147(11)**, 298-303.

[48] Mejdell, C. M., Erikson, U., Slinde, E. & Midling, K. Ø. (2010) Bedøvingsmetoder ved slakting av laksefisk. *Norsk veterinærtidsskrift* **2**, 83-90.

[49] Kestin, S. C., Van de Vis, J. W. & Robb, D. H. F. (2002) Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them. *Veterinary Record* **150(10)**, 302-307.

[50] Roth, B., Slinde, E. & Arildsen, J. (2006) Pre or post mortem muscle activity in Atlantic salmon (*Salmo salar*). The effect on rigor mortis and the physical properties of flesh. *Aquaculture* **257(1-4)**, 504-510.

[51] Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T. S. & Seland, A. (1997) Handling stress and storage temperature affect meat quality of farmed-raised Atlantic salmon (*Salmo salar*). *Journal of Food Science* **62(4)**, 898-905.

[52] Duran, A., Erdemli, U., Karakaya, M. & Tyilmaz, M. (2008) Effects of slaughter methods on physical, biochemical and microbiological quality of rainbow trout *Oncorhynchus mykiss* and mirror carp *Cyprinus carpio* filleted in pre-, in-or post-rigor periods. *Fisheries Science* **74(5)**, 1146-1156.

[53] Kristoffersen, S., Tobiassen, T., Steinsund, V. & Olsen, R. L. (2006) Slaughter stress, postmortem muscle pH and rigor development in farmed Atlantic cod (*Gadus morhua* L.). *International Journal of Food Science + Technology* **41**, 861-864

[54] Grove, D. J., Loizides, L. G. & Nott, J. (1978) Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. *Journal of Fish Biology* **12(5)**, 507-516.

[55] Southgate, P. & Wall, T. (2001) Welfare of farmed fish at slaughter. *In Practice* **23(5)**, 277-284.

[56] Close, B., Banister, K., Baumans, V., Bernoth, E. M., Bromage, N., Bunyan, J., Erhardt, W., Flecknell, P., Gregory, N., Hackbarth, H. & Morton, D. (1996) Recommendations for euthanasia of experimental animals: Part 1. *Laboratory Animals* **30(4)**, 293-316.

[57] Lov om dyrevelferd, LOV-2009-06-19-97, www.lovdata.no

[58] Tobiassen, T., Mejdell, C. M., Midling, K. Ø. & Akse, L. (2010) Sanitetsslakting på merdkanten, in *Nofima rapport 48-2010*, Nofima. Tromsø. pp. 24.

[59] Brattelid, T. & Smith, A. (2011) Compendium in laboratory animal science for fish researchers. *Norwegian School of Veterinary Science and Norecopa: Oslo*. pp. 190.

[60] Mattilsynet (12.09.2016 (versjon 2)) Veileder-forsvarlig forskrivning og bruk av legemidler: www.mattilsynet.no

[61] Ferner, R. E. & Butt, T. F. (2008) Adverse drug reactions. *Medicine* **36(7)**, 364-368.[62] Milne, I., Seager, J., Mallett, M. & Sims, I. (2000) Effects of short-term pulsed ammonia exposure on fish. *Environmental toxicology and chemistry* **19(12)**, 2929-2936.

[63] Powell, M. D., Reynolds, P. & Kristensen, T. (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**, 18-28.

[64] Taylor, R. S., Muller, W. J., Cook, M. T., Kube, P. D. & Elliott, N. G. (2009) Gill observations in Atlantic salmon (*Salmo salar*, L.) during repeated amoebic gill disease (AGD) field exposure and survival challenge. *Aquaculture* **290(1-2)**, 1-8.

[65] Ross, L. G. & Ross, B. (2008) Anaesthetic & sedative techniques for aquatic animals. Third edition, Blackwell Science, UK, London. pp. 222.

[66] Anderson, W. G., McKinley, R. S. & Colavecchia, M. (1997) The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. *North American Journal of Fisheries Management* **17(2)**, 301-307.

[67] Pirhonen, J. & Schreck, C. B. (2003) Effects of anaesthesia with MS-222, clove oil and CO_2 on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*). *Aquaculture* **220(1-4)**, 507-514.

[68] Wendelaar Bonga, S. E. W. (1997) The stress response in fish. *Physiological Reviews* **77**, 591-625.

[69] Øverli, Ø., Sørensen, C., Kiessling, A., Pottinger, T. G. & Gjøen, H. M. (2006) Selection for improved stress tolerance in rainbow trout (*Oncorhynchus mykiss*) leads to reduced feed waste. *Aquaculture* **261(2)**, 776-781.

[70] Khansari, A. R., Balasch, J. C., Vallejos-Vidal, E., Teles, M., Fierro-Castro, C., Tort, L. & Reyes-López, F. E. (2019) Comparative study of stress and immune-related transcript outcomes triggered by *Vibrio anguillarum* bacterin and air exposure stress in liver and spleen of gilthead seabream (*Sparus aurata*), zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*). *Fish & shellfish immunology* **86**, 436-448.

[71] Burka, J. F., Hammell, K. L., Horsberg, T. E., Johnson, G. R., Rainnie, D. J. & Speare, D. J. (1997) Drugs in salmonid aquaculture–a review. *Journal of veterinary pharmacology and therapeutics* **20(5)**, 333-349.

[72] Cho, G. K. & Heath, D. D. (2000) Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile chinook salmon *Oncorhynchus tshawytscha* (Walbaum). *Aquaculture research* **31(6)**, 537-546.

[73] Marking, L. L. & Meyer, F. P. (1985) Are better anesthetics needed in fisheries? *Fisheries* **10(6)**, 2-5.

[74] Olsen, Y. A., Einarsdottir, I. E. & Nilssen, K. J. (1995) Metomidate anaesthesia in Atlantic salmon, *Salmo salar*, prevents plasma cortisol increase during stress. *Aquaculture* **134(1-2)**, 155-168.

[75] Keene, J. L., Noakes, D. L. G., Moccia, R. D. & Soto, C. G. (1998) The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* **29(2)**, 89-101.

[76] Iversen, M., Finstad, B., McKinley, R. S. & Eliassen, R. A. (2003) The efficacy of metomidate, clove oil, Aqui-S (TM) and Benzoak (R) as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. *Aquaculture* **221**, 549-566.

[77] Davis, K. B. & Griffin, B. R. (2004) Physiological responses of hybrid striped bass under sedation by several anesthetics. *Aquaculture* **233(1-4)**, 531-548.

[78] Small, B. C. (2003) Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish *Ictalurus punctatus*. *Aquaculture* **218(1-4)**, 177-185.

[79] Small, B. C. (2004) Effect of isoeugenol sedation on plasma cortisol, glucose, and lactate dynamics in channel catfish *Ictalurus punctatus* exposed to three stressors. *Aquaculture* **238(1-4)**, 469-481.

[80] Holloway, A. C., Keene, J. L., Noakes, D. G. & Moccia, R. D. (2004) Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. *Aquaculture research* **35(11)**, 1025-1030.

[81] Ortuno, J., Esteban, M. A. & Meseguer, J. (2002) Effects of four anaesthetics on the innate immune response of gilthead seabream (*Sparus aurata* L.). *Fish & shellfish immunology* **12(1)**, 49-59.

[82] Kiessling, A., Johansson, D., Zahl, I. H. & Samuelsen, O. B. (2009) Pharmacokinetics, plasma cortisol and effectiveness of benzocaine, MS-222 and isoeugenol measured in individual dorsal aorta-cannulated Atlantic salmon (*Salmo salar*) following bath administration. *Aquaculture* **286(3-4)**, 301-308.

[83] Zahl, I. H., Kiessling, A., Samuelsen, O. B. & Olsen, R. E. (2010) Anesthesia induces stress in Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*). *Fish physiology and Biochemistry* **36(3)**, 719-730.

[84] Auperin, B., Goardon, L., Quemeneur, A., Thomas, J. L., Aubin, J., Valotaire, C., Rouger, Y. & Maisse, G. (1998) Preliminary study on the use of Aqui'S (R) as anesthetic for handling and sampling of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). *Bulletin Français de la Pêche et de la Pisciculture* **350-51**, 291-301. (in French with English abstract).

[85] Sanderson, T. B. & Hubert, W. A. (2007) Assessment of gaseous CO_2 and AQUI-S as anesthetics when surgically implanting radio transmitters into cutthroat trout. *North American Journal of Fisheries Management* **27(4)**, 1053-1057.

[86] Pickering, A. D. (1992). Rainbow trout husbandry: management of the stress response. *Aquaculture* **100(1-3)**, 125-139.

[87] Schoettger, R. A. & Julin, A. M. (1967) Efficacy of MS-222 as an anesthetic on four salmonids (No. 13). *Invest. Fish Contr., U.S. Dept. Int.* **13**, 1-15.

[88] Hikasa, Y., Takase, K., Ogasawara, T. & Ogasawara, S. (1986) Anesthesia and recovery with tricaine methanesulfonate, eugenol and thiopemal sodium in the carp, *Cyprinus carpio*. *Japanese Journal of Veterinary Science* **48**, 341-351.

[89] Hafs, A. W., Mazik, P. M., Kenney, P. B. & Silverstein, J. T. (2012) Impact of carbon dioxide level, water velocity, strain, and feeding regimen on growth and fillet attributes of cultured rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **350**, 46-53.

[90] Wedemeyer, G. A. (1997). Effect of rearing conditions on the health and physiological quality of fish in intensive culture. In: Iwama, G. K., Pickering, A. D., Sumpter, J. P., Schreck, C. B. (Eds.). Fish Stress and Heath in Aquaculture: 35-72.

[91] EFSA (2008) Scientific Opinion of the Panel on Animal Health and Animal Welfare on a request from the European Commission on the Animal welfare aspects of husbandry systems for farmed trout. *The EFSA Journal* **796**, 1-22.

[92] Soivio, A., Nyholm, K. & Huhti, M. (1977) Effects of anaesthesia with MS 222, neutralized MS 222 and benzocaine on the blood constituents of rainbow trout, *Salmo gairdneri*. *Journal of Fish Biology* **10**, 91-101.

[93] Gilderhus, P. A. & Marking, L. L. (1987) Comparative efficacy of 16 anesthetic chemicals on rainbow trout. *North American Journal of Fisheries Management* **7(2)**, 288-292.

[94] Muktar, Y., Tesfaye, S. & Tesfaye, B. (2016) Present status and future prospects of fish vaccination: a review. *J Veterinar Sci Technol* **7(2)**.

[95] Marana, M. H., Sepúlveda, D., Chen, D., Al-Jubury, A., Jaafar, R. M., Kania, P. W., Henriksen, N. H., Krossøy, B., Dalsgaard, I., Lorenzen, N. & Buchmann, K. (2019) A pentavalent vaccine for rainbow trout in Danish aquaculture. *Fish & shellfish immunology* **88**, 344-351.

[96] Iversen, M. H. & Eliassen, R. A. (2014) The effect of allostatic load on hypothalamic-pituitary-interrenal (HPI) axis before and after secondary vaccination in Atlantic salmon post smolts (*Salmo salar* L.). *Fish physiology and biochemistry* **40**, 527-538.

[97] Lovy, J., Speare, D. J., Stryhn, H. & Wright, G. M. (2008) Effects of dexamethasone on host innate and adaptive immune responses and parasite development in rainbow trout *Oncorhynchus mykiss* infected with *Loma salmonae*. *Fish & shellfish immunology* **24(5)**, 649-658.

[98] Poppe, T. T. & Breck, O. (1997) Pathology of Atlantic salmon *Salmo salar* intraperitoneally immunized with oil-adjuvanted vaccine. A case report. *Diseases of Aquatic Organisms* **29(3)**, 219-226.

[99] Berg, A., Bergh, Ø., Fjelldal, P. G., Hansen, T., Juell, J. E. & Nerland, A. (2006) Animal welfare and fish vaccination—effects and side-effects (in Norwegian). *Fisken og havet 9-2006*.

[100] Midtlyng, P. J. (1996) A field study on intraperitoneal vaccination of Atlantic salmon (*Salmo salar* L.) against furunculosis. *Fish & Shellfish Immunology* **6**, 553-565.

[101] Midtlyng, P. J., Reitan, L. J. & Speilberg, L. (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.

[102] Midtlyng, P. J. & Lillehaug, A. (1998) Growth of Atlantic salmon *Salmo salar* after intraperitoneal administration of vaccines containing adjuvants. *Diseases of Aquatic Organisms* **32(2)**, 91-97.

[103] Sneddon, L. U., Wolfenden, D. C. C. & Thomson, J. S. (2016) Stress management and welfare. In: *Biology of stress in fish. Fish physiology volume 35*. Schreck, C. B., Tort, L., Farrell, A. P. & Brauner, C. J. (eds.). Academic Press, 464-521.

[104] Holten-Andersen, L., Dalsgaard, I., Nylén, J., Lorenzen, N. & Buchmann, K. (2012) Determining vaccination frequency in farmed rainbow trout using Vibrio anguillarum O1 specific serum antibody measurements. *PloS one* **7(11)**, e49672.

[105] Chettri, J. K., Skov, J., Jaafar, R. M., Krossøy, B., Kania, P. W., Dalsgaard, I. & Buchmann, K. (2015) Comparative evaluation of infection methods and environmental factors on challenge success: *Aeromonas salmonicida* infection in vaccinated rainbow trout. *Fish & shellfish immunology* **44(2)**, 485-495.

[106] Southgate, P. J. (2008) Welfare of fish during transport. In: *Fish Welfare*. Branson, E. J. (ed.). Blackwell Publishing. 185-194.

[107] Nomura, M., Sloman, K. A., Von Keyserlingk, M. A. G. & Farrell, A. P. (2009) Physiology and behaviour of Atlantic salmon (*Salmo salar*) smolts during commercial land and sea transport. *Physiology & behavior* **96(2)**, 233-243.

[108] Iversen, M. & Eliassen, R. A. (2005) Salmon smolt (*Salmo salar* L) production and stress reducing measures. The effects of AQUI-S sedation during vaccination, transport and transfer to sea on survivability, appetite, growth, immunological capacity, primary, secondary and tertiary stress response. *Nordland Research Institute–NF report no 5/2005*, 1-55.

[109] Hilbig, R., Anken, R. H., Bauerle, A. & Rahmann, H. (2002) Susceptibility to motion sickness in fish: a parabolic aircraft flight study. *Journal of gravitational physiology: a journal of the International Society for Gravitational Physiology* **9(1)**, P29-30.

[110] Bone, Q., Marshall, N. & Blaxter, J. (1982) Biology of Fishes. Glasgow: Blackie and Son.

[111] Farrell, A. P. T. (2006) Bulk oxygen uptake measured with over 60,000 kg of adult salmon during live-haul transportation at sea. *Aquaculture* **254(1-4)**, 646-652.

[112] Tacchi, L., Lowrey, L., Musharrafieh, R., Crossey, K., Larragoite, E. T. & Salinas, I. (2015) Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **435**, 120-127.

[113] Fraser, A. F. & Broom, D. M. (1990) Farm animal behaviour and welfare. 3rd ed. London. Bailliere Tindall. pp. 437.

[114] Stien, L. H., Bracke, M. B. M, Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., Kittilsen, S., Midtlyng, P. J., Vindas, M. A., Øverli, Ø. & Kristiansen, T. S. (2013) Salmon Welfare Index Model (SWIM 1.0): a semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. *Reviews in Aquaculture* **5**, 33-57.

[115] Schreck, C. B., Solazzi, M. F., Johnson, S. L. & Nickelson, T. E. (1989) Transportation stress affects performance of coho salmon, *Oncorhynchus kisutch. Aquaculture* **82(1-4)**, 15-20.

[116] Van Heeswijk, J. C. F., Vianen, G. J. & Van den Thillart, G. E. E. J. M. (2006) The adrenergic control of hepatic glucose and FFA metabolism in rainbow trout (*Oncorhynchus mykiss*): increased sensitivity to adrenergic stimulation with fasting. *General and comparative endocrinology* **145(1)**, 51-61.

[117] Iversen, M. & Eliassen, R. (2012) Stressovervåkning av settefiskproduksjonen i Mainstream Norway AS 2009 - 2011. Stresskartlegging av laksesmolt (*Salmo salar* L.), og effekten av stressreduserende tiltak på stressnivå, dyrevelferd og produksjonsresultatet. *UiN-rapport nr 05/2012.* pp. 54.

[118] Talbot, C., Corneillie, S. & Korsøen, Ø. (1999) Pattern of feed intake in four species of fish under commercial farming conditions: implications for feeding management. *Aquaculture Research* **30(7)**, 509-518.

[119] Soengas, J. L., Strong, E. F., Fuentes, J., Veira, J. A. R. & Andrés, M. D. (1996) Food deprivation and refeeding in Atlantic salmon, *Salmo salar*: effects on brain and liver carbohydrate and ketone bodies metabolism. *Fish Physiology and Biochemistry* **15(6)**, 491-511.

[120] Brännäs, E. & Alanärä, A. (1992) Feeding behaviour of the Arctic charr in comparison with the rainbow trout. *Aquaculture* **105(1)**, 53-59.

[121] Noble, C., Mizusawa, K., Suzuki, K. & Tabata, M. (2007) The effect of differing self-feeding regimes on the growth, behaviour and fin damage of rainbow trout held in groups. *Aquaculture* **264(1)**, 214-222.

[122] Bailey, J. & Alanärä, A. (2001) A test of a feed budget model for rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* **32(6)**, 465-469.

[123] Gélineau, A., Corraze, G. & Boujard, T. (1998) Effects of restricted ration, time-restricted access and reward level on voluntary food intake, growth and growth heterogeneity of rainbow trout (*Oncorhynchus mykiss*) fed on demand with self-feeders. *Aquaculture* **167(3)**, 247-258.

[124] Holm, J. C., Refstie, T. & Bø, S. (1990) The effect of fish density and feeding regimes on individual growth rate and mortality in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **89(3)**, 225-232.

[125] Linnér, J. & Brännäs, E. (2001) Growth in Arctic charr and rainbow trout fed temporally concentrated or spaced daily meals. *Aquaculture International* **9(1)**, 35-44.
[126] Thorpe, J. E., Talbot, C., Miles, M. S., Rawlings, C. & Keay, D. S. (1990) Food consumption in 24 hours by Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture* **90(1)**, 41-47.
[127] Suzuki, K., Mizusawa, K., Noble, C. & Tabata, M. (2008) The growth, feed conversion ratio and fin damage of rainbow trout *Oncorhynchus mykiss* under self-feeding and hand-feeding regimes. *Fisheries science* **74(4)**, 941-943.
[128] Robb, D.H.F. (2008) Welfare of fish at harvest. In: *Fish welfare*. Branson, E. J. (ed.). London: Blackwell Publishing. 217-242.
[129] Jobling, M., Alanärä, A., Noble, C., Sánches-Vázquez, J., Kadri, S. & Huntingford, F. (2012) Appetite and Feed Intake. In: *Aquaculture and Behavior.* Huntingford, F. A., Jobling, M., Kadri, S. (Eds.). Wiley-Blackwell, Oxford. ISBN: 978-1-4051-3089-9. 183-219.
[130] Huntingford, F. A., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandøe, P. & Turnbull, J. F. (2006). Current issues in fish welfare. *Journal of fish biology* **68(2)**, 332-372.
[131] Bermejo-Poza, R., De la Fuente, J., Pérez, C., de Chavarri, E. G., Diaz, M. T., Torrent, F. & Villarroel, M. (2017) Determination of optimal degree days of fasting before slaughter in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **473**, 272-277.
[132] Bermejo-Poza, R., De la Fuente, J., Pérez, C., Lauzurica, S., González de Chávarri, E., Diaz, M. T. & Villarroel, M. (2016) Reducing the effect of pre-slaughter fasting on the stress response of rainbow trout (*Oncorhynchus mykiss*). *Animal Welfare* **25(3)**, 339-346.
[133] Stevenson, P. (2007) Closed Waters: The welfare of farmed Atlantic salmon, rainbow trout, Atlantic cod, and Atlantic halibut (UK: Compassion in World Farming).
[134] Wall, T. (2008) Disease and medicines- the welfare implications. In: *Fish welfare*. Branson, E. J. (ed.). London: Blackwell Publishing. 195-201.
[135] Ashley, P. J. (2007) Fish welfare: Current issues in aquaculture. *Applied Animal Behaviour Science* **104(3-4)**, 199-235.
[136] FAWC (2014) Farm Animal Welfare Council, Opinions published by the Farm Animal Welfare Committee. Welfare of Farmed Fish. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/319323/Opinion_on_the_welfare_of_farmed_fish.pdf accessed 15th October 2016.
[137] Moutou, K. A., McCarthy, I. D. & Houlihan, D. F. (1998) The effect of ration level and social rank on the development of fin damage in juvenile rainbow trout. *Journal of Fish Biology* **52(4)**, 756-770.
[138] McCarthy, I. D., Carter, C. G. & Houlihan, D. F. (1992) The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Biology* **41(2)**, 257-263.
[139] Jobling, M. & Koskela, J. (1996) Interindividual variations in feeding and growth in rainbow trout during restricted feeding and in a subsequent period of compensatory growth. *Journal of Fish Biology* **49(4)**, 658-667.
[140] Wagner, E. I., Miller, S. A. & Bosakowski, T. (1995) Ammonia excretion by rainbow trout over a 24-hour period at two densities during oxygen injection. *The Progressive Fish-Culturist* **57(3)**, 199-205.
[141] McKay, L. R. & Gjerde, B. (1985) The effect of salinity on growth of rainbow trout. *Aquaculture* **49(3-4)**, 325-331.
[142] Good, C., Davidson, J., Welsh, C., Snekvik, K. & Summerfelt, S. (2010) The effects of carbon dioxide on performance and histopathology of rainbow trout *Oncorhynchus mykiss* in water recirculation aquaculture systems. *Aquacultural Engineering* **42(2)**, 51-56.

[143] Dockray, J. J., Reid, S. D. & Wood, C. M. (1996) Effects of elevated summer temperatures and reduced pH on metabolism and growth of juvenile rainbow trout (*Oncorhynchus mykiss*) on unlimited ration. *Canadian Journal of Fisheries and Aquatic Sciences* **53(12)**, 2752-2763.

[144] Abbott, J. C. & Dill, L. M. (1985) Patterns of aggressive attack in juvenile steelhead trout (*Salmo gairdneri*). *Canadian journal of fisheries and aquatic sciences* **42(11)**, 1702-1706.

[145] de Lourdes Ruiz-Gomez, M., Kittilsen, S., Höglund, E., Huntingford, F. A., Sørensen, C., Pottinger, T. G., Bakken, M., Winberg, S., Korzan, W. J. & Øverli, Ø. (2008) Behavioral plasticity in rainbow trout (*Oncorhynchus mykiss*) with divergent coping styles: when doves become hawks. *Hormones and behavior* **54(4)**, 534-538.

[146] Storebakken, T. & Austreng, E. (1987) Ration level for salmonids: II. Growth, feed intake, protein digestibility, body composition, and feed conversion in rainbow trout weighing 0.5–1.0 kg. *Aquaculture* **60(3)**, 207-221.

[147] Quinton, J. C. & Blake, R. W. (1990) The effect of feed cycling and ration level on the compensatory growth response in rainbow trout, *Oncorhynchus mykiss*. *Journal of Fish Biology* **37(1)**, 33-41.

[148] Jürss, K., Bittorf, T. & Vökler, T. (1986) Influence of salinity and food deprivation on growth, RNA/DNA ratio and certain enzyme activities in rainbow trout (*Salmo gairdneri* Richardson). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **83(2)**, 425-433.

[149] Chin, A., Guo, F. C., Bernier, N. J. & Woo, P. T. (2004) Effect of *Cryptobia salmositica*-induced anorexia on feeding behavior and immune response in juvenile rainbow trout *Oncorhynchus mykiss*. *Diseases of aquatic organisms* **58(1)**, 17-26.

[150] Kolarevic, J., Bæverfjord, G., Takle, H., Ytteborg, E., Reiten, B. K. M., Nergård, S. & Terjesen, B. F. (2014) Performance and welfare of Atlantic salmon smolt reared in recirculating or flow through aquaculture systems. *Aquaculture* **432**, 15-25.

[151] Noble, C., Flood, M. J. & Tabata, M. (2012) Using rainbow trout *Oncorhynchus mykiss* as self-feeding actuators for white-spotted charr *Salvelinus leucomaenis*: Implications for production and welfare. *Applied animal behaviour science* **138(1)**, 125-131.

[152] Gregory, T. R. & Wood, C. M. (1999) Interactions between individual feeding behaviour, growth, and swimming performance in juvenile rainbow trout (*Oncorhynchus mykiss*) fed different rations. *Canadian Journal of Fisheries and Aquatic Sciences* **56(3)**, 479-486.

[153] Pottinger, T. G., Rand-Weaver, M. & Sumpter, J. P. (2003) Overwinter fasting and re-feeding in rainbow trout: plasma growth hormone and cortisol levels in relation to energy mobilisation. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **136(3)**, 403-417.

[154] Jørgensen, E. H., Bernier, N. J., Maule, A. G. & Vijayan, M. M. (2016) Effect of long-term fasting and a subsequent meal on mRNA abundances of hypothalamic appetite regulators, central and peripheral leptin expression and plasma leptin levels in rainbow trout. *Peptides* **86**, 162-170.

[155] Hoseini, S. M., Yousefi, M., Rajabiesterabadi, H. & Paktinat, M. (2014) Effect of short-term (0–72 h) fasting on serum biochemical characteristics in rainbow trout *Oncorhynchus mykiss*. *Journal of applied ichthyology* **30(3)**, 569-573.

[156] López-Luna, J., Torrent, F. & Villarroel, M. (2014) Fasting up to 34° C days in rainbow trout, *Oncorhynchus mykiss*, has little effect on flesh quality. *Aquaculture* **420**, 63-70.

[157] CIWF (2009) Compassion in world farming. The welfare of farmed fish. Available from: http://www.ciwf.org.uk/resources/publications/fish_farming/default.aspx Accessed 15th October 2016.

[158] FAWC (1996) Farmed Animal Welfare Council. Report on the welfare of farmed fish. FAWC Report 2765 https://www.gov.uk/government/publications/fawc-report-on-the-welfare-of-farmed-fish Accessed on 1st November 2016.

[159] HSA (2005) Humane Slaughter Association. Humane Harvesting of Salmon and Trout. Guidance Notes no. 5 (Wheathampstead, UK).
[160] Lines, J. A. & Spence, J. (2012) Safeguarding the welfare of farmed fish at harvest. *Fish physiology and biochemistry* **38(1)**, 153-162.
[161] López-Luna, J., Bermejo-Poza, R., Bravo, F. T. & Villarroel, M. (2016) Effect of degree-days of fasting stress on rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **462**, 109-114.
[162] Bermejo-Poza, R., Fernández-Muela, M., De la Fuente, J., Pérez, C., de Chavarri, E. G., Díaz, M. T., Torrent, F. & Villarroel, M. (2019) Physio-metabolic response of rainbow trout during prolonged food deprivation before slaughter. *Fish physiology and biochemistry* **45(1)**, 253-265.
[163] Jentoft, S., Aastveit, A. H., Torjesen, P. A. & Andersen, Ø. (2005) Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **141(3)**, 353-358.
[164] Ellis, T., Berrill, I., Lines, J., Turnbull, J. F. & Knowles, T. G. (2012) Mortality and fish welfare. *Fish physiology and biochemistry* **38(1)**, 189-199.
[165] Flos, R., Reig, L., Torres, P. & Tort, L. (1988) Primary and secondary stress responses to grading and hauling in rainbow trout, *Salmo gairdneri*. *Aquaculture* **71(1-2)**, 99-106.
[166] Barton, B. A., Peter, R. E. & Paulencu, C. R. (1980) Plasma cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. *Canadian Journal of Fisheries and Aquatic Sciences* **37(5)**, 805-811.
[167] Vijayan, M. M. & Moon, T. W. (1992) Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* **49(11)**, 2260-2266.
[168] Gismervik, K., Nilsen, A., Nielsen, K. V. & Mejdell, C. M. (2017) Kapittel 3. Fiskevelferd, in *Fiskehelserapporten 2016*, B. Hjeltnes, et al., Editors., Veterinærinstituttet: Oslo. pp. 21-33.
[169] Akvakulturdriftsforskriften. Forskrift om drift av akvakulturanlegg, FOR-2008-06-17-822, www.lovdata.no
[170] Martínez-Porchas, M., Martinez-Cordova, L. R. & Ramos-Enriquez, R. (2009) Cortisol and glucose: reliable indicators of fish stress. *Pan-American Journal of Aquatic Sciences* **4(2)**, 158-178.
[171] Svåsand T., Grefsrud E. S., Karlsen Ø., Kvamme B. O., Glover, K. S, Husa, V. & Kristiansen, T. S. (2017) Risikorapport norsk fiskeoppdrett 2017. *Fisken og havet, særnr.* 2-2017.
[172] Ashley, P. J., Sneddon, L. U. & McCrohan, C. R. (2007) Nociception in fish: stimulus–response properties of receptors on the head of trout *Oncorhynchus mykiss*. *Brain research* **1166**, 47-54.
[173] Sneddon, L. U., Braithwaite, V. A. & Gentle, M. J. (2003) Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proceedings of the Royal Society of London B: Biological Sciences* **270(1520)**, 1115-1121.
[174] Holan, A. B., Roth, B., Breiland, M. S. W., Kolarevic, J., Hansen, Ø. J., Iversen, A., Hermansen, Ø., Gjerde, B., Hatlen, B., Mortensen, A. & Lein, I., Johansen, L. –H., Noble, C., Gismervik, K. & Espmark, A. M. (2017) Beste praksis for medikamentfrie metoder for lakseluskontroll (MEDFRI). *Nofima rapport 10/2017.* Nofima. Tromsø. pp. 124.
[175] Overton, K., Dempster, T., Oppedal, F., Kristiansen, T. S., Gismervik, K. & Stien, L. H. (2018) Salmon lice treatments and salmon mortality in Norwegian aquaculture: a review. *Reviews in Aquaculture* 1-20.
[176] Sneddon, L. U. (2009) Pain Perception in Fish: Indicators and Endpoints. *Ilar Journal* **50**, 338-342.
[177] Hjeltnes, B., Bang-Jensen, B., Bornø, G., Haukaas, A., Walde, C. S. (2018) Fiskehelserapporten 2017, Veterinærinstituttet: Oslo. pp. 106.

[178] Grefsrud, E. S., Glover, K., Grøsvik, B. E., Husa, V., Karlsen, Ø., Kristiansen. T., Kvamme, B. O., Mortensen, S., Samuelsen, O. B., Stien, L. H. & Svåsand, T. (2018). Risikorapport norsk fiskeoppdrett 2018. *Fisken og havet, særnr.* 1-2018.

[179] Svåsand T., Karlsen Ø., Kvamme B. O., Stien, L. H., Taranger, G. L. Boxaspen, K. K., (2016) Risikovurdering av norsk fiskeoppdrett 2016. *Fisken og havet, særnr.* 2-2016. pp. 190.

[180] Tørud, B. & Håstein, T. (2008) Skin lesions in fish: causes and solutions. *Acta Veterinaria Scandinavica* **50**, 1.

[181] Elliott, A. (2010). A comparison of thermal polygons for British freshwater teleosts. In: *Freshwater Forum*, (Vol. 5, No. 3).

[182] Beitinger, T. L., Bennett, W. A. & McCauley, R. W. (2000) Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental biology of fishes* **58(3)**, 237-275.

[183] Nilsen, A., Erikson, U., Aunsmo, A., Østvik, A. & Heuch, P. A. (2010) Mekanisk fjerning av lakselus" FLS avlusersystem"-test av ejektorpumpe fra Flatsetsund Engineering AS. *Veterinærinstituttets rapportserie 11-2010*.

[184] Barkana, Y., & Belkin, M. (2000) Laser eye injuries. *Surv Ophthalmol*, **44(6)**, 459-78.

[185] Phillippi, A. L., O'Connor, N. J., Lewis, A. F. & Kim, Y. K. (2001) Surface flocking as a possible anti-biofoulant. *Aquaculture* **195(3-4)**, 225-238.

[186] Madin, J., Chong, V. C. & Hartstein, N. D. (2010) Effects of water flow velocity and fish culture on net biofouling in fish cages. *Aquaculture research* **41(10)**, 602-617.

[187] Beveridge, M., (2004) Cage Aquaculture. Third Edition ed., Oxford: Blackwell Publishing.

[188] Wildish, D. J., Keizer, P. D., Wilson, A. J. & Martin, J. L. (1993) Seasonal changes of dissolved oxygen and plant nutrients in seawater near salmonid net pens in the macrotidal Bay of Fundy. *Canadian Journal of Fisheries and Aquatic Sciences* **50(2)**, 303-311.

[189] Cronin, E. R., Cheshire, A. C., Clarke, S. M. & Melville, A. J. (1999) An investigation into the composition, biomass and oxygen budget of the fouling community on a tuna aquaculture farm. *Biofouling* **13(4)**, 279-299.

[190] Andersen, R. J., Luu, H. A., Chen, D. Z., Holmes, C. F., Kent, M. L., Le Blanc, M. & Williams, D. E. (1993) Chemical and biological evidence links microcystins to salmon 'netpen liver disease'. *Toxicon* **31(10)**, 1315-1323.

[191] Cribb, T. H., Adlard, R. D., Hayward, C. J., Bott, N. J., Ellis, D., Evans, D. & Nowak, B. F. (2011) The life cycle of *Cardicola forsteri* (Trematoda: Aporocotylidae), a pathogen of ranched southern bluefin tuna, *Thunnus maccoyi*. *International Journal for Parasitology* **41(8)**, 861-870.

[192] Tan, C. K., Nowak, B. F. & Hodson, S. L. (2002) Biofouling as a reservoir of *Neoparamoeba pemaquidensis* (Page, 1970), the causative agent of amoebic gill disease in Atlantic salmon. *Aquaculture* **210(1-4)**, 49-58.

[193] Kvenseth, P. (1996) Large-scale use of wrasse to control sea lice and net fouling in salmon farms in Norway. In: *Wrasse: Biology and Use in Aquaculture*. Sayer, M. D. J., Treasurer, J. W., & Costello, M. J. (eds.). Wiley-Blackwell: Cambridge. 196-203.

[194] Baxter, E. J., Sturt, M. M., Ruane, N. M., Doyle, T. K., McAllen, R. & Rodger, H. D. (2012) Biofouling of the hydroid *Ectopleura larynx* on aquaculture nets in Ireland: Implications for finfish health. *Fish Veterinary Journal* **13**, 18-30.

[195] Floerl, O., Sunde, L. M. & Bloecher, N. (2016) Potential environmental risks associated with biofouling management in salmon aquaculture. *Aquaculture Environment Interactions* **8**, 407-417.

[196] Bloecher, N., Powell, M., Hytterød, S., Gjessing, M., Wiik-Nielsen, J., Mohammad, S. N., Johansen, J., Hansen, H., Floerl, O. & Gjevre, A. G. (2018) Effects of cnidarian biofouling on salmon gill health and development of amoebic gill disease. *PloS one* **13(7)**, p.e0199842.

[197] Neill, W. H., Brandes, T. S., Burke, B. J., Craig, S. R., Dimichele, L. V., Duchon, K., Edwards, R. E., Fontaine, L. P., Gatlin, D. M., III., Hutchings, C., Miller, J. M., Ponwith, B. J., Stahl, C. J., Tomasso, J. R. & Vega, R. R. (2004) Ecophys.Fish: A simulation model of fish growth in time-varying environmental regimes. *Reviews in Fisheries Science* **12**, 233-288.

[198] RSPCA (2018). RSPCA welfare standards for farmed Atlantic salmon. https://science.rspca.org.uk/sciencegroup/farmanimals/standards/salmon (Accessed June 2019).

[199] 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.

[200] Pettersen, J. M., Bracke, M. B. M, Midtlyng, P. J., Folkedal, O., Stien, L. H., Steffenak, H. & Kristiansen, T. S. (2014) Salmon welfare index model 2.0: an extended model for overall welfare assessment of caged Atlantic salmon, based on a review of selected welfare indicators and intended for fish health professionals. *Reviews in Aquaculture* **6**, 162–179.

[201] Bass, N. & Wall, T. (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.