Rainbow Trout Operational Welfare Indicator (OWI) Protocol: Ongrowing, Seawater

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Version 1.0

Centre for Aquaculture Progress

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An interactive version, as well additional materials such as scoring sheets and a field handbook can be found at https://hub.centreforaquacultureprogress.org/owi/trr/ong-sw/1.0/

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Contents

Executive summary

To understand, track, and compare fish welfare in commercial aquaculture production systems, the industry requires Operational Welfare Indicator (OWI) protocols that build on the full available scientific literature, provide transparency into the development of its indicators, and feature practical guidance on how to score fish welfare.

This protocol is the first such tool for assessing the welfare of farmed rainbow trout (*Oncorhynchus mykiss*) in seawater systems, including a scoring system based on a comprehensive species- and life-stage-specific literature review for every indicator.

This protocol serves farm personnel conducting routine welfare monitoring and other industry professionals, including certification bodies, auditors, and buyers. It is designed to align with the welfare monitoring and assessment requirements of key certifications, such as RSPCA Assured and Aquaculture Stewardship Council.

This protocol enables farm personnel to systematically monitor the welfare of their stock, identify potential issues early, and implement corrective actions. This proactive approach helps producers understand the relationship between welfare and production performance, resulting in reduced losses, improved regulatory compliance, strengthened consumer trust, and a stronger and more sustainable aquaculture industry.

Scope

Developed specifically for ongrowing rainbow trout in seawater, this protocol may be applied to juveniles or other production systems with careful interpretation.

While practical guidance on OWIs has been developed for other species, such as the Laksvel protocol for Atlantic salmon,[1] and valuable documentation on rainbow trout welfare has been published by Nofima[2] and APROMAR,[3] existing resources lack comprehensive, standardized, and threshold-based assessment across a wide range of indicators for rainbow trout producers. This protocol addresses that gap.

This protocol includes:

- **33 OWIs** across three categories:
  - 14 individual-based indicators
  - 5 group-based indicators
  - 14 environmental indicators
- **Scoring system** for each indicator, split into four tiers comprising levels 0–3
- **Reference images and scoring guidance** for practical field application

Stakeholder feedback

The Centre for Aquaculture Progress will regularly update this protocol as new scientific evidence emerges and operational practices evolve. We particularly welcome input from:

- Fish welfare researchers and academics
- Aquaculture producers
- Regulators and certification bodies

Stakeholder input will directly inform future updates and ensure the guidance continues to meet industry needs.

This protocol will be reviewed at least every two years, incorporating feedback from field implementation, updates to relevant certification standards, and advances in fish welfare science. Where the review identifies a need for revision, updated versions will be published with documented changes.

To submit feedback or questions, please fill out this form, or contact info@centreforaquacultureprogress.org.

Introduction

Rainbow trout, like other teleost fish, are animals capable of experiencing pain and stress. Ensuring their welfare during production requires systematic monitoring through Operational Welfare Indicators (OWIs). The increasing focus on OWI implementation reflects priorities shared across the aquaculture value chain — from producers, certification bodies, and seafood buyers, to consumers, governments, and NGOs — and aligns with broader international standards for health monitoring (for example, see Article 4.1.9(2)(e) of the Aquatic Animal Health Code published by the World Organisation for Animal Health). By improving welfare outcomes, producers simultaneously achieve better growth, survival, robustness, and production efficiency, while meeting regulatory requirements and evolving consumer expectations.

This protocol aims to provide a standardized, systematic framework for real-world commercial use, built upon the strongest available scientific evidence to support both on-farm management and compliance with key certification requirements. It is intended as a strong monitoring foundation that complements — rather than replaces — broader welfare requirements set by certification schemes, legislation, or other standards at system level.

These guidelines were primarily developed for ongrowing rainbow trout in seawater. Users can apply this protocol to other life stages or production systems, **though application in these contexts requires careful interpretation**. The indicators and scoring levels in this protocol describe welfare risk rather than welfare state. Because the internal experience of the fish cannot be directly observed, and because the relationship between any single indicator and actual welfare depends on the production context and individual fish history and physiology, scores should be understood as structured assessments of the likelihood of welfare compromise as opposed to definitive welfare judgements.

This document is a living document that will be updated and refined over time as rainbow trout welfare science matures.

Defining fish welfare and Operational Welfare Indicators (OWIs)

Fish welfare

Animal welfare is a complex and multifaceted topic, with many definitions and conceptual frameworks. Several widely recognized approaches include:

1. **Nature-based:** Animal welfare is achieved when animals can express natural behaviors and use evolved adaptations in environments that meet their species' behavioral and habitat needs.[4]
2. **Feeling-based:** Animal welfare depends on subjective emotional experiences, requiring freedom from negative states (e.g., pain, fear) while enabling positive experiences (e.g., comfort, pleasure).[5]
3. **Function-based:** Animal welfare is determined by proper biological functioning, including good health, normal growth and development, and the absence of disease, injury, or physiological dysfunction.[6]
4. **Coping-based:** Animal welfare reflects an individual's ability to cope with environmental challenges, where poor welfare occurs when adaptation mechanisms are overwhelmed or inadequate.[7]
5. **Affective balance:** Animal welfare represents the cumulative balance of positive and negative experiences over time, where good welfare occurs when positive experiences outweigh negative ones.[8]

These frameworks are recognized as complementary rather than competing perspectives, and overlap in places: the affective balance approach is a variant of the feeling-based approach, and the coping-based view combines the function- and feeling-based approaches. Modern integrated approaches, such as the Five Domains Model,[9] synthesize these perspectives by assessing welfare across multiple dimensions:

1. **Domain 1 (Nutrition):** Adequate food and water.
2. **Domain 2 (Physical Environment):** Appropriate thermal, atmospheric, and spatial conditions.
3. **Domain 3 (Health):** Absence of disease, injury, and functional impairment.
4. **Domain 4 (Behavioral Interactions):** Opportunities for species-typical behaviors, agency, and control.
5. **Domain 5 (Mental State):** Overall affective experience integrating domains 1–4.

This multidimensional framework acknowledges that welfare encompasses biological functioning, behavioral expression, and subjective experience, with each domain contributing to the animal's overall quality of life.

While acknowledging the complexity of the term 'welfare', this document adopts the definition used in the Laksvel protocol for Atlantic salmon: "quality of life as perceived by the fish itself."[10] This definition aligns with the feeling-based and affective balance perspectives by centering on the fish's subjective experience. However, because subjective experience cannot be directly measured, this protocol uses observable indicators across multiple welfare domains as proxies for internal states.

The protocol's indicators are organized to enable comprehensive assessment across these five welfare domains, acknowledging that no single indicator can capture the multifaceted nature of welfare.

Operational Welfare Indicators (OWIs)

Operational Welfare Indicators (OWIs) are standardized assessment tools designed for routine implementation in commercial aquaculture. Unlike some other proxy measures, such as plasma cortisol concentrations, that require laboratory analysis, OWIs enable practical on-farm assessment. These indicators are science-based, practically feasible, and provide actionable data for evidence-based management decisions.[11]

This document classifies OWIs into three categories:[12]

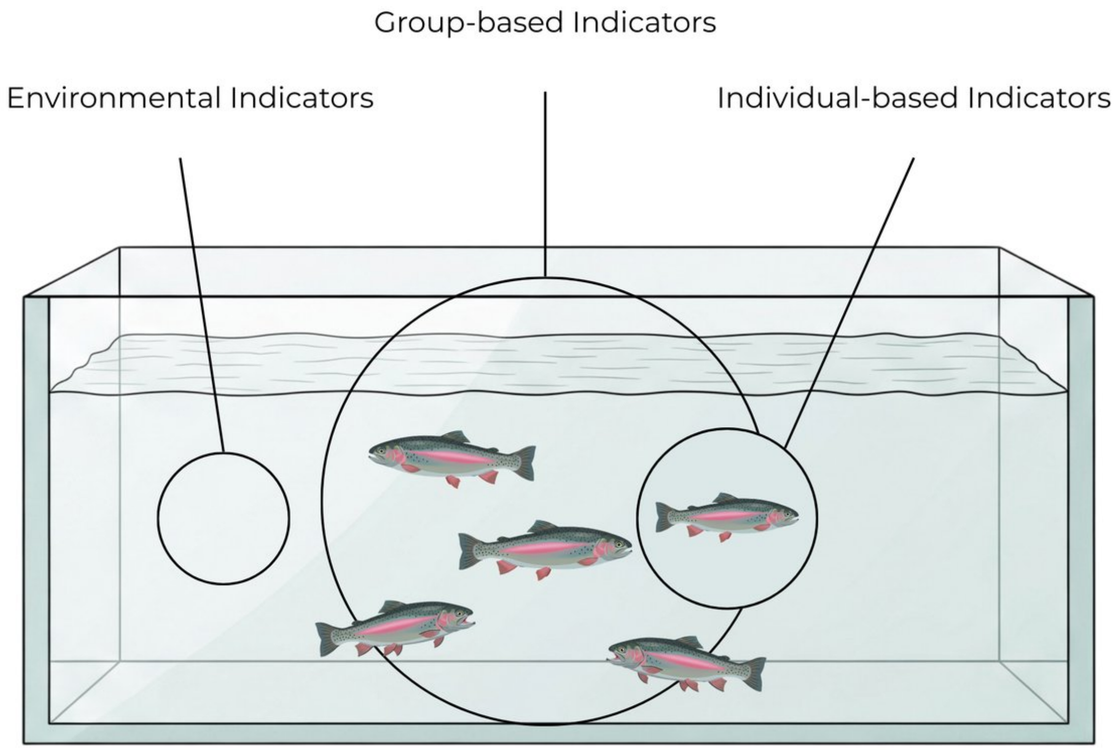
1. **Individual-based indicators** (animal-based outcome measures): These assess the physical condition of individual fish (e.g., fin and skin condition) through sampling or camera-based observations. Individual-based indicators directly reflect how fish have been affected by their environment and management, providing outcome measures of their welfare state.
2. **Group-based indicators** (outcome measures): These assess collective patterns at the population level, including behavior and mortality rates.
3. **Environmental indicators** (input measures): These assess the aquatic environment, including dissolved oxygen and temperature. Environmental indicators represent the conditions fish experience and are critical determinants of welfare, though they are inputs rather than direct outcomes.

Operational Welfare Indicators are most useful when considering multiple indicators from different categories together. Together, they should be individually interpreted as 'welfare risk indicators,' as no indicator can describe the internal welfare state of an animal, but will influence the likelihood of welfare compromise.

Relationship between OWI categories and welfare domains

The table below shows how the three OWI categories address the Five Domains of welfare:

Welfare domain	Individual-based indicators	Group-based indicators	Environmental indicators
1. Nutrition	Emaciation	Feeding behavior	N/A
2. Physical environment	Morphological damage related to the environment	Swimming behavior, mortality (e.g., due to poor water quality)	Water quality, stocking density
3. Health	Morphological damage, including those related to disease signs, deformities	Mortality, disease *(not in current protocol)*	Water quality (disease risk factors)
4. Behavioral interactions	Morphological damage from aggression (particularly fin damage)	Aberrant behavior, aggressive behavior, feeding behavior, and swimming behavior	Stocking density, water flow/velocity
5. Mental state	Pain and other negative affective states inferred from assessment of all individual-based indicators	Mental discomfort inferred from all group-based indicators	Mental discomfort inferred from all environmental indicators



Schematic representation of environmental, group-based, and individual-based indicators. Illustration created by the Centre for Aquaculture Progress.

Limitations

While OWIs provide valuable welfare assessment capabilities, users should be aware of several significant limitations:

1. **Evidence base variability:** The scientific foundation supporting individual indicators varies considerably. Some indicators are well-validated in ongrowing rainbow trout in seawater, while others rely on evidence from different salmonid species, alternative life stages, or laboratory studies that may not fully reflect commercial conditions. Users should interpret indicators with limited validation more cautiously. More information on specific indicators is provided in the section “Evidence quality” below.
2. **Indicator interpretation challenges:** Some indicators may interact with each other, producing compounding effects on welfare that an isolated assessment of individual indicators cannot capture.
3. **Sampling limitations:** Morphological assessments require the sampling of individuals, which may not accurately reflect the welfare status of the entire production group. In large-scale operations, localized welfare issues could be missed if sampling protocols are insufficient or if welfare problems are unevenly distributed throughout the population.
4. **Assessor subjectivity:** Many morphological indicators rely on visual scoring instead of detailed measurements. While this approach enables rapid assessment, it introduces inter-observer variability and requires standardized training protocols to ensure consistency across evaluators. In addition, because OWIs are developed and interpreted from a human perspective, there may be an inherent anthropocentric bias that may not fully reflect the fish’s subjective experience.
5. **Practical limitations:** OWI protocols must be pragmatic and flexible for implementation across different commercial settings, which inherently involves trade-offs with precision. Because most producers cannot monitor OWIs continuously, assessments are typically carried out at discrete time points, providing only snapshots of welfare. In addition, fish may physiologically adapt to chronic stress and appear outwardly normal, meaning that short-term observations can underestimate ongoing welfare issues.
6. **Equipment dependency:** Environmental monitoring capabilities depend on the availability of sensor technologies and their placement. Producers with limited monitoring equipment may have reduced capacity to track key environmental parameters, potentially missing welfare-relevant changes in water quality or environmental conditions. Some environmental parameters, like oxygen saturation, may also vary significantly in both space and time (at least in sea cages), with the most critical levels easily going undetected.
7. **Routine assessment only:** This protocol focuses on routine welfare assessment. Further, no concrete recommendations are provided for addressing identified welfare gaps. Producers should therefore ensure they have strong management plans in place that enable them to act upon identified welfare issues.

These limitations highlight the need for producers to routinely and systematically measure multiple indicators across different categories rather than focusing on single indicators or one-off assessments. In tandem, protocols such as this one must be regularly reviewed and updated to reflect emerging scientific evidence and evolving understanding of fish welfare.

Evidence quality

To address variability in the evidence base across indicators (for more information, see the "Limitations" section) and to ensure transparency, the table below summarizes the level of supporting evidence for each indicator. The table provides two considerations for each indicator:

- **Rainbow trout-specific evidence:** scored based on: (1) the quantity and quality of available studies, (2) appropriateness of study design for assessing welfare impacts (including sample size and duration), and (3) relevance to commercial seawater conditions. This column is about the strength of evidence that the metric is a valid indicator of welfare in rainbow trout.
  - Example scoring:
    - **High:** Multiple empirical studies (e.g., 4+) on rainbow trout, with some testing substantial welfare-relevant outcomes, usually with at least one testing rainbow trout in seawater (depending on how relevant it is for the indicator)
    - **Moderate:** Some empirical studies (e.g., 1–3) on rainbow trout testing substantial welfare-relevant outcomes
    - **Low:** Some rainbow trout-specific evidence, but substantial welfare-relevant outcomes are not tested (e.g., growth/production metrics only)
    - **Very low:** No empirical rainbow trout-specific evidence or the indicator is only mentioned incidentally, largely relying on assumptions, expert opinion, or extrapolation from other species
- **Threshold validation:** scored based on whether studies tested a wide range of realistic indicator levels that would be observed in commercial aquaculture against welfare parameters (such as mortality or cortisol), ideally within the same experiments.
  - This column is about the strength of evidence that the proposed thresholds correspond to different levels of welfare risk.
  - Example scoring:
    - **High:** A wide range of realistic indicator levels have been empirically tested against welfare parameters
    - **Moderate:** Multiple realistic indicator levels covering what would be observed in commercial aquaculture settings have been empirically tested against welfare parameters, but some specific thresholds may be partly interpolated or based on expert judgment
    - **Low:** Thresholds mainly rely on non-empirical data (e.g., regulatory or industry benchmarks or guidance), or testing against production metrics rather than welfare parameters
    - **Very low:** Thresholds are essentially untested, and reliant on expert judgment

Indicator	Rainbow trout-specific evidence	Threshold validation
Individual-based indicators		
Vertebral deformity	Moderate	Low (extrapolated from salmon)
Body condition	Moderate	Moderate
Scale loss	Very low	Moderate (extrapolated from salmon)
Skin hemorrhaging	Very low	Low (extrapolated from salmon)
Wounds	Low	Low (extrapolated from salmon)
Change of coloration	Low	Very low
Snout damage	Very low	Low (extrapolated from salmon)
Jaw deformity	Low	Low (extrapolated from salmon)
Eye opacity	Low	Low (extrapolated from salmon)
Eye injury	Very low	Low (extrapolated from salmon)
Operculum damage	Very low	Low (extrapolated from salmon)
Gill damage	Very low	Low (extrapolated from salmon)
Fin damage	High	Moderate
Sea lice	Low	Moderate (extrapolated from salmon)
Group-based indicators		
Mortality	Moderate	Low

Indicator	Rainbow trout-specific evidence	Threshold validation
Aberrant behavior	Low	Low
Aggressive behavior	Very low	Very low
Feeding behavior	Low	Low
Swimming behavior	Low	Low
Environmental indicators		
Stocking density	High	Moderate
Dissolved oxygen	High	High
Temperature	High	Moderate
Carbon dioxide	Low	Very low
pH	Moderate	Low
Turbidity	Low	Very low
Total suspended solids	Low	Very low
Ammonia	Low	Low
Nitrite	Very low	Very low
Nitrate	Very low	Very low
Metals	Low	Very low
Water velocity	Moderate	Low
Salinity	Very low	Very low
Hydrogen sulfide	Very low	Very low

While some indicators are more strongly validated than others, producers are encouraged to measure as many indicators as practicable and relevant to their production system. Additionally, tracking correlations between indicators over time can strengthen understanding of how these indicators reflect welfare within production systems. This table underscores the gaps in rainbow trout welfare research and should inform the priorities of research scientists.

For further details on the exact evidence relied on for the development of the scoring guidance for each indicator, please consult the evidence sections within each indicator.

Applicability of salmon research to rainbow trout

Where direct research on seawater rainbow trout is limited, evidence from Atlantic salmon can provide additional context. The two species belong to the same family (Salmonidae) and are reared as post-smolts in broadly similar environmental conditions. However, some key differences must be considered when applying evidence from Atlantic salmon to rainbow trout, including:

- The two species evolved in different parts of the world (Atlantic salmon in the North Atlantic Ocean and rainbow trout in the Pacific Ocean) and accordingly have adapted to different environmental conditions, e.g., different temperatures and salinities;
- Domestication: Atlantic salmon have only been domesticated for approximately the last 50 years, while rainbow trout have been domesticated for more than a century;
- Atlantic salmon is predominantly anadromous, with the vast majority of wild populations migrating to sea, while rainbow trout is facultatively anadromous.

Applicability of freshwater rainbow trout research to seawater rainbow trout

Before being transferred to seawater from freshwater, rainbow trout need to develop the capability to tolerate the new salinity conditions. Some key changes that occur during this smoltification process include:

- Change in gill chloride cells and pumps to adapt to new seawater conditions.[13]
- Increase in ion intake, e.g., through increased drinking and increased absorption of ions by the intestine.[14]
- Altered metabolism to sustain the increased energy demands of smoltification, including of proteins, lipids, and carbohydrates.[15]
- A change in physical appearance, including a more silver body color.

While key physiological changes occur as part of the smoltification process, rainbow trout reared in freshwater and rainbow trout reared in seawater are fundamentally the same animal, and as long as different rearing conditions (e.g., the chemistry of freshwater vs. seawater) and physiological differences are accounted for, findings based on freshwater rainbow trout studies can inform how we think about welfare in seawater rainbow trout.

Method

OWI selection process

This protocol selected OWIs through a comprehensive analysis of existing OWI frameworks and materials, a complete list of which is in the Appendix under 'OWI selection'.

The selection process involved:

- Systematic review of indicators used in established protocols (e.g., Laksvel for Atlantic salmon and Nofima's 'Welfare indicators for farmed rainbow trout: tools for assessing fish welfare').[16]
- Review of other materials considering operational welfare indicators, including those published by industry.
- Analysis of certification requirements from major schemes (e.g., ASC, RSPCA Assured).
- Consideration of commercial need for the indicator to be included, scientific validity, practical feasibility, and welfare relevance for rainbow trout.

Threshold development process

Scoring thresholds were aligned with Laksvel as many rainbow trout producers are already using the Laksvel system, making it easier for producers to adopt this protocol. Threshold recommendations for each OWI were developed through an indicator-specific systematic review of the current scientific literature, prioritizing evidence from ongrowing rainbow trout in commercial seawater conditions. Where such evidence was limited (which was often the case), the analysis incorporated evidence from different production systems, laboratory settings, alternative life stages, or other salmonids, as well as expert opinions.

Each paper was evaluated based on the following criteria:

- **Quality:** Study design, sample size, peer review status, etc.
- **Indicator relevance:** How directly the study investigated the impact of a specific indicator on welfare.
- **Context:** How closely the study context and findings apply to ongrowing rainbow trout in commercial seawater production, considering laboratory/commercial conditions, life stage, environmental conditions (e.g., stocking densities), and species.

Papers were qualitatively graded as high, moderate, low, or very low according to the quality and relevance of each study (considering factors such as study design, sample size, duration, and applicability to commercial seawater trout) with stronger and more relevant evidence given greater weight in setting the threshold recommendations. This approach ensures the recommendations are grounded in the best available science while providing practical guidance where specific knowledge gaps exist.

Scoring

Each OWI is scored on a scale from 0 to 3, describing the extent of injury or deviation. This scoring system is aligned with the Laksvel protocol for Atlantic salmon, and adapted for rainbow trout in seawater.

Level	Individual-based indicators	Group-based indicators	Environmental indicators
0	Free from injuries or deviations	Optimal for the specific population and production conditions	Within the optimal range
1	Indicates a minor injury or deviation that normally is assumed to have little impact on fish welfare, but still indicates that something is not optimal	Indicates a minor deviation that normally is assumed to have a minimal impact on fish welfare, but still indicates that something is not optimal	Edge of the optimal range
2	Indicates a clear injury or deviation	Indicates a clear deviation	Outside the optimal range
3	Indicates a severe injury or deviation that is assumed to have large consequences for the welfare of the fish and that increases the probability of early death	Indicates a severe deviation that is assumed to be caused by a severe impact on fish welfare, and that indicates an increased probability of mortality	Far outside the optimal range

Scores reflect the welfare state of the fish at the time of assessment for each indicator in isolation. A score describes the current extent of injury or deviation, and therefore the welfare risk at the time of assessment. For example, the early stages of a disease may present several indicators as a level 1 or 2 if the immediate welfare impact is not yet severe, even if the condition is likely to worsen. However, when scores are collected consistently over time, the pattern of change across assessments can indicate how welfare is evolving and inform early intervention.

Scoring levels are not linear in severity. Results from scoring should be presented as proportions of the assessed population at each level, not as average scores, as averages can obscure the distribution of welfare states within a group.

Where a fish has a level 3 score for one or more individual-based indicators, one could consider euthanasia.

Interpreting scores

Scores should be interpreted as **indicators of welfare risk**, not as direct measures of welfare state, as a fish's internal experience cannot be observed directly. A higher score indicates a greater likelihood of welfare compromise, but the relationship between a score and actual welfare impact depends on production conditions and the fish's individual history and physiology.

For some indicators, the relationship between the score and welfare compromise is relatively straightforward. For example, higher scores on mortality and wounds clearly represent poor welfare outcomes, while for other indicators, especially environmental indicators, the relationship between the score and welfare compromise is less direct. The tolerance of fish to a certain environmental condition may vary depending on other factors like the status of the fish, and a score that represents a welfare concern in one production context may be within normal variation in another with minimal impact on welfare.

For this reason, scores are most informative when multiple indicators are considered together and when tracked over time. The pattern of scores across indicators and over time provides a more complete picture of welfare risk than any individual score in isolation.

Further guidance on assessing OWIs

Indicator assessment frequency

This protocol includes over 30 indicators. While having more data can be beneficial, monitoring all indicators may not be practical in commercial farm settings.

While some recommendations for indicator assessment frequency are provided in this document, producers should decide which indicators to monitor at which frequencies, informed by their own site-specific experience, requirements from any certification bodies or regulators, and practical constraints. These indicators should work together to provide a balanced picture of welfare risk.

Routine assessment enables early problem detection. Research on early post-seawater transfer salmon found that welfare scores changed at intervals of 2–3 months, and concluded that this interval was too long to detect important changes, which suggests a higher frequency of monitoring is preferable.[17] Certification schemes also provide guidance: for morphological assessments, ASC requires monthly assessments; the Best Aquaculture Practices Farm Standard recommends weekly checks; Naturland advises at least every seven days during sensitive periods; and the RSPCA requires a minimum of four assessments in the freshwater stage and monthly in seawater.

At a minimum, behavioral indicators, mortality, dissolved oxygen, and temperature should be monitored **daily**. Depending on the production system, other water quality indicators may also need to be monitored **daily** (e.g., ammonia in RAS systems). Morphological indicators should be assessed ideally **weekly** and at a minimum **monthly**. Monthly assessment allows early detection of trends (e.g., rising deformity prevalence or declining condition factor) before they become serious welfare problems. If monthly sampling is not achievable due to practical constraints, the interval between assessments may be extended, but the sample size should be increased proportionally to compensate for the reduced frequency. For example, if sampling every two months instead of monthly, the sample size should be at least doubled. While a larger sample size will improve the reliability of that individual assessment, it does not compensate for the reduced ability to detect emerging problems in a timely manner.

Morphological OWI assessment should be **mandatory during any fish handling event** (e.g., grading, transfer, vaccination, harvest preparation). Fish are already being handled during these events, so the additional cost of scoring is minimal, and the sample obtained is often more representative than a dedicated sampling event. Post-mortem assessment of daily mortalities and at harvest is also strongly encouraged.

Producers should increase monitoring frequency of relevant indicators when specific risks are present (e.g., high temperatures, disease outbreaks, or after handling events) or when assessment results suggest emerging welfare problems.

Sampling individual-based indicators

Methodological notes

All indicators in this section are morphological individual-based outcome measures, meaning they require:

1. Sampling of individual fish from the population;
2. Scoring each sampled fish independently;
3. Calculating prevalence (percentage of fish falling into each category of ‘level 0’, ‘level 1’, ‘level 2’, and ‘level 3’); and
4. Using prevalence distributions to classify the population’s welfare status.

Sample size

While classical sampling theory suggests that a sample of 30 is often sufficient regardless of population size, practical aquaculture conditions often violate key statistical assumptions:

- Spatial heterogeneity (where fish are not uniformly distributed)
- Catchability bias (healthy vs. sick fish differ in capture probability)
- Disease clustering (infections often spatially aggregated)
- Behavioral segregation (subordinate/sick fish may occupy different zones)

This means that sampling 30 fish may not adequately represent the welfare status of the entire population, particularly in large-scale operations. To minimize bias in the sample, efforts should be made to sample fish that are representative, e.g., taking the sample right before feeding, using sweep nets (as opposed to netting individual fish at the surface or conducting stratified sampling).

We recommend scaling the sample size with the number of fish:

Number of fish per cage	Suggested sample (where practical)
< 20,000	30
20,000–100,000	50
≥ 100,000	100

The number of fish reasonably able to be sampled will depend on practical factors such as staff time, and achieving the above sample sizes may not always be possible. Further, a post-mortem analysis of morphological indicators is recommended for a substantial sample after harvest, as this does not impose further stress on the fish and can provide accurate prevalence data, informing future production.

We recommend sampling each cage at the site. The number of cages assessed may be reduced over time if consistent results and strong correlations are observed across cages, and cages are assessed on a rotating basis.

Automated assessment

The sampling process itself can be stressful for fish. Where possible, using observations from underwater camera systems (provided they have been validated against traditional sampling assessments) is recommended. Another benefit of using camera-based systems is a much higher sample size, though there is the limitation of often not being able to see the entire fish (e.g., only one lateral side may be visible for any given fish).

Validation of currently existing automated systems against expert human assessment is limited in the published literature. One key publicly available document is a report on the standardization of manual sea lice counts for validation of automatic camera systems by Bui et al. (2024),[18] which should be consulted when validating similar systems for individual-based indicators.

Sampling behavioral indicators

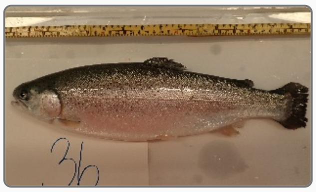
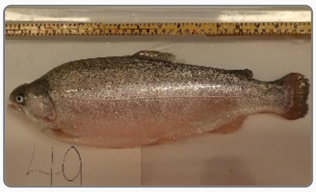
When assessing behavioral indicators, observations should be conducted using methods that do not themselves induce behavioral changes. This means avoiding actions that startle or disturb the fish, such as sudden movements near the cage, loud noises, or casting shadows over the water. Where practical, the use of underwater cameras is recommended as an alternative to direct observation, as cameras allow repeated and non-intrusive assessment (see also “Automated assessment” above).

Individual-based indicators

Indicators assessed on individual fish through visual examination or measurement.

Vertebral deformity

A well-formed spine is needed for efficient swimming, maintaining body position, and normal biological function. Deformity prevalence is a valuable indicator of prior and ongoing management practices, as deformities can arise from genetic factors, nutritional deficiencies, environmental stressors, or inappropriate rearing conditions. As deformities tend to arise from these developmental problems, they reflect longer-term, rather than acute, welfare issues. Deformities, including vertebral abnormalities, compromise essential biological functions and cause ongoing stress.

Level 0	Level 1	Level 2	Level 3
No visible vertebral deformity	Minor or suspected vertebral deformity, notable only with close inspection or running a hand over the fish	Clear vertebral deformity causing no functional deviation or injuries **AND** Fish is of normal size	Severe vertebral deformity causing functional deviation or injuries
			

Protocol application

Assessment requires handling the fish out of water. The fish should be viewed in profile and from above to evaluate the dorsal line for irregularities, subtle deviations, or kinks. Running a hand along the spine can help detect minor deformities not visible to the eye.

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- A fish with a straight dorsal profile and smooth body curvature is recorded as level 0.
- A minor vertebral deformity (level 1) does not have any clear, visible kinks, but will show some subtle irregularities or deviations.
- A level 2 vertebral deformity will include clear, visible deviations.
- A severe deformity (level 3) will include visible kinks or clear deviations or curves. The fish's swimming will likely be impacted.

- Vertebral deformities are chronic conditions and are not expected to improve over time.
- Studies show that many vertebral deformities are not visible externally. Due to the nature of OWIs, this protocol only enables assessment of externally visible deformities. This may underestimate the total prevalence of deformity, but it identifies fish with more severe welfare impacts.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

Key evidence

Source	Summary of source	Key result
Fjelldal et al., 2025	Experimental study investigating the progression of vertebral deformities and growth in 12000 female rainbow trout as they grew from 36 g to 5.5 kg in laboratory conditions over one year. Fish were initially raised in freshwater tanks and then transferred to sea cages. Between 80 and 100 fish were sampled at 4 different sampling points. Other validation groups were also assessed (different strains or fish raised in various conditions). Note: Deformities were detected using X-ray imaging, which identified internal vertebral deformities not visible by external inspection.	There was a dramatic increase in deformities, from 20% in fish at 36 g to 93% in fish at 5.5 kg. The average number of deformed vertebrae per affected fish also increased, from 4 to 12. Deformity severity was strongly negatively correlated with fish length and weight. Tail deformities correlated with caudal fin degradation, with the authors suggesting a bidirectional relationship. All validation groups showed a 74–83% deformity rate.
Davidson et al., 2011	Two experimental studies investigating the impacts of different freshwater RAS conditions on rainbow trout health and welfare. Experiment 1 compared very low water exchange and high water exchange in rainbow trout (151 g) over six months. Experiment 2 compared low water	Skeletal deformities increased substantially in near-zero exchange systems, reaching 38% in the most restricted system, compared with 0% in higher-exchange systems. Increased deformities correlated with increased mortality, but causation was not established. Poor water quality may have caused both increased deformities and mortality.

Source	Summary of source	Key result
	exchange to near-zero water exchange in rainbow trout (18 g).	
Aunsmo et al., 2008	Experimental study investigating how clinically scored vertebral deformity relates to growth and carcass downgrading in ongrowing Atlantic salmon raised in commercial environments, using a visual scale from 0–3 at slaughter.	Outcomes were not separated by score, so the results refer to all fish scoring between 1 and 3. All fish with vertebral deformity scores above 0 were downgraded from superior production quality, demonstrating the negative economic impact of detectable deformities. Deformed fish had a mean slaughter weight of approximately 2.8 kg (about 62% of the mean slaughter weight of 4.5 kg for superior-grade fish), indicating a significant and sustained growth deficit associated with visible deformities. Radiography showed compressed or fused vertebrae, mainly in the tail, and in approximately one-third of cases extending cranially. Increasing severity of vaccine-related adhesions and abdominal melanization increased the odds of spinal deformity, suggesting fish with severe internal lesions and deformity experience combined burdens of chronic pathology and reduced growth.

Body condition

Condition factor measures the relationship between a fish's weight and length. A healthy condition factor reflects adequate energy reserves and that a fish is feeding and growing well. Poor condition factor (appearing thin or emaciated) can indicate inadequate nutrition, poor feed management, chronic stress, disease, or suboptimal environmental conditions that impair growth and energy storage. It is a lagging indicator of welfare and can indicate sustained welfare compromise.

Condition factor

Level 0	Level 1	Level 2	Level 3
> 1.4	> 1.2 to ≤ 1.4	> 1 to ≤ 1.2	≤ 1

Emaciation

Level 0	Level 1	Level 2	Level 3
No signs of emaciation	Slightly lean	Clearly emaciated	Severely emaciated
			

Overall: Overall score is determined by the most severe subindicator

Protocol application

Assessment requires handling the fish out of water. If weighing and measuring individual fish is practical, use the condition factor scoring system. Whether the condition factor is measured or not, always conduct a visual assessment.

Condition factor (often denoted as 'K') is calculated as follows: **$K = 100 \times W / L^3$**, where W is the total body weight of the individual fish (g), and L is the fork length (cm).

In most cases, the condition factor will align with the fish's visual appearance. Where they do not match, visual assessment will take priority for scoring. For example, a fish might be calculated to have a level 0 K value of above 1.4 but still appear emaciated due to body shortening or deformity. In such cases, score the fish according to its visual category.

Scoring notes

- Decreasing condition factor over time is a sign of poor welfare, even if in absolute terms the condition factor remains in the level 0 or level 1 range. For example, a decrease in condition factor from 1.3 to 1.1 is a warning sign.
- Signs of emaciation include visible spine or ribs and reduced muscle mass.
- Very high condition factors may indicate deformity (e.g., when a spinal deformity has compressed the body length) or obesity and should be investigated, but are not scored as a welfare concern under this indicator. There is limited available data on how fish welfare is affected when fish reach extremely high body weights.
- Comparing the body condition of individual fish to their cohort can be a useful indicator when scoring. However, if the entire population is in poor condition, comparison to conspecifics alone may not identify the problem.
- **Validation:** The condition factor thresholds are based on the studies set out below. These mostly involved smaller freshwater rainbow trout, which tend to have lower condition factors than larger seawater rainbow trout. Further, it was not always clear how the condition factor was being measured (for example, whether the fork length or standard length was being used), meaning the results may not have been measured consistently. As rainbow trout grow out in seawater, their condition factor also increases. Based on expert opinion and the distribution of condition factors in Fjelldal et al. (2025), the condition factor thresholds for freshwater rainbow trout were adjusted up by 0.2 to account for seawater rainbow trout. The visual scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

Key evidence

Source	Summary of source	Key result
MacIntyre, 2008	Large-scale survey investigating welfare in rainbow trout conducted across 44 freshwater rainbow trout farms in the British Isles from July 2005 to April 2007. Each farm was visited twice, with up to four different systems (cages, ponds, raceways, tanks) sampled per visit. Both small (< 100 g) and large (> 100 g) fish were sampled, with 12 or 24 fish	Condition factor ranged from 0.84 to 2.20, with a mean of 1.31. The author considered this mean condition factor to be within the normal, healthy range. Increasing condition factor was associated with improved welfare up to 1.3, after which improvements plateaued, and large deviations were associated with worse welfare.

Source	Summary of source	Key result
	taken from each system, totaling 3699 fish sampled from 189 systems.	Condition factors below 1.0 were associated with increased cortisol.
Pottinger et al., 2003	Experimental study investigating biomarkers associated with fasting in 900 two-year-old ongrowing rainbow trout (282 g) in freshwater laboratory conditions over 17 weeks. Fish were divided into three groups: continuously fed (control), fasted and then refed (fasted for 9 weeks then fed for the remainder of the study period), and continuously fasted (fasted for the entire 17 weeks). At various intervals, 10 fish per tank were sampled.	Both fasted groups showed rapid, significant declines in body weight and condition factor. Refed fish quickly recovered weight and condition factor after feeding resumed, becoming indistinguishable from the control by the end of the study. Fasting did not consistently elevate cortisol levels. The baseline condition factor for the unfasted group was approximately 1.2. When fasted fish reached a condition factor of < 1.1 at week 11, growth hormone rose extremely high, and somatolactin levels showed clear, significant differences, with persistently high free fatty acids. After week 11, the level of free fatty acids dropped, indicating severe starvation.
Salem et al., 2007	Experimental study investigating the effect of starvation on global gene expression in 12 ongrowing rainbow trout (193 g) in laboratory conditions over 3 weeks. The control group was continuously fed.	Starved fish lost significant weight (172 g vs. 280 g in fed fish). In starved fish, 202 genes were down-regulated, compared with 27 up-regulated, indicating that the fish were entering a critical survival mode.
Gao, 2024	Fact sheet discussing the importance of measuring and calculating condition factor in farmed salmonid species.	Emaciation was defined as a condition factor < 0.9, while positive performance was defined as a condition factor > 1.1, and possible deformity was defined as > 1.6.

Scale loss

Scales form a protective barrier that, together with the skin, shields fish from infections and helps maintain osmoregulatory balance. Scales can be lost during handling events, crowding, pumping, netting, delousing operations, and interactions with other fish. Scale loss can lead to osmoregulatory problems, particularly when extensive.

Level 0	Level 1	Level 2	Level 3
No visible scale loss **OR** Only a few individual scales are missing	Loss of many individual scales **OR** One or two small areas of scale loss	Several small or larger areas of scale loss **OR** Individual scales are missing over the rib cage	Scale loss dominates the overall impression of the fish **OR** One or two small areas of scale loss over the rib cage
			

Protocol application

Assessment requires handling the fish for close inspection of the body surface.

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- Scale loss of 20% of the entire body can serve as a rough guide to distinguish between level 2 and level 3 scale loss.
- Scales floating in the water indicate that scale loss is a problem.
- Scale loss over the rib cage carries a high risk due to its proximity to vital organs and therefore warrants at least a level 2 score.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

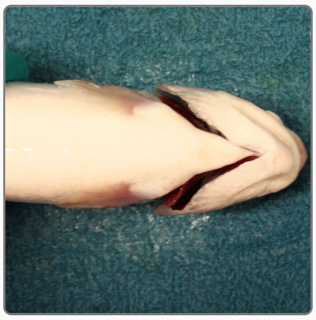
Key evidence

Source	Summary of source	Key result
Bouck and Smith, 1979	Experimental study investigating the effects of slime and scale removal on smolted coho salmon (~12cm fork length) in laboratory conditions over 10 days. After scale or slime removal, fish were held in either freshwater or seawater for 10 days, with at least 20 fish per treatment group. An additional experiment allowed fish to recover in freshwater for 1–5 days after 25% scale removal, then transferred to seawater. The control group experienced no scale or slime removal.	In the control group, approximately 32% of smolts died after direct transfer to seawater, establishing a baseline mortality. Mortality increased with descaling: 10% scale loss resulted in approximately 50% mortality, and 25% scale loss resulted in approximately 75% mortality after 10 days in seawater. Scale-removal treatments did not cause mortality in freshwater. In the recovery groups, smolts with 25% scale removal held in freshwater for 1 day before seawater transfer had 10% mortality, whereas those held for 5 days had 0% mortality. Scale removal over the rib cage caused the highest mortality, attributed to the proximity to internal body organs.
Nilsson and Madaro, 2025	Experimental study investigating the effect of controlled scale loss on physiology and growth in 480 Atlantic salmon (146 g) over 6 weeks in laboratory conditions. There were 4 treatment groups of 0%, 5%, 10% and 20% scale loss. Scales were removed from the left dorsal side using a blunt spatula. On the same day as descaling, fish in each treatment group were transferred from freshwater to either brackish water (25 ppt) or seawater (34 ppt) conditions. The temperature was controlled at 10°C. Fish were sampled on days 1, 7, and 42.	No negative welfare impacts of descaling were detected. There was zero mortality, and scale loss did not affect specific growth rate at either salinity. There was no relationship between cortisol, glucose, or lactate levels and descaling levels. No wounds were observed at any point, and by day 42, new scales had regrown to nearly their original size. The authors noted the same effects may not be observed under the more challenging conditions in commercial settings. For example, there were no temperature fluctuations, pathogen exposure, biological stressors, low oxygen levels, waves, or handling.
	Experimental study investigating the impacts of scale loss on survival after a	In the smolt experiment, fish were grouped as controls (never passed

Source	Summary of source	Key result
Kostecki et al., 1987	turbine passage in Atlantic salmon smolts in commercial conditions and presmolts (45–167 g) in laboratory conditions. Smolts were passed through one of two Ossberger turbines (450 or 850kW). The presmolts were divided into 5 groups with varying levels of scale removal: 0% (control), 10% in the caudal region, 10% in the abdominal region, 20% in the abdominal region, and 30% in the abdominal and dorsal regions. The fish were then held in freshwater for 8 days, followed by half-strength seawater for 4 days.	through the turbine), fish that died within 48 hours, and survivors beyond 48 hours. Average scale loss was 15.1% in controls, 24.2% in fish that died, and 15.9% in survivors. Fish that died had significantly more scale loss than survivors or controls. From the presmolts, only the group that had lost 30% of scales showed elevated mortality.

Skin hemorrhaging

The skin is the fish's largest organ and serves as an essential barrier against pathogens, parasites, and environmental stressors. Skin hemorrhaging prevalence can be strongly influenced by recent events, such as handling, crowding, or transport events. Damage to the skin compromises the skin's barrier function, leaving fish more susceptible to infection, osmotic stress, and secondary health problems. Minor hemorrhages will primarily reflect recent insults and will resolve in a matter of days or weeks. More serious skin hemorrhages can also indicate underlying disease.

Level 0	Level 1	Level 2	Level 3
No visible hemorrhaging	A few red spots **OR** Small fields with weak red color **OR** Healed hemorrhaging	Clear hemorrhaging (< 10% of the body surface)	Severe hemorrhaging (≥ 10% of the body surface)
			

Protocol application

Assessment requires handling the fish. Inspect both sides of the body, as well as the belly of the fish. Try to distinguish between fresh hemorrhages and healed hemorrhages. This indicator addresses visible skin hemorrhaging on the body. To avoid double-counting, hemorrhaging on the fins or at the fin base is scored under fin damage, and hemorrhaging on the snout under snout damage.

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- Distinguish between fresh and healed hemorrhages.
    - Fresh hemorrhages may have active bleeding, red or pink inflamed spots, potential swelling, or edema, and indicate recent trauma.
    - Healed hemorrhages show stable discoloration and/or scar tissue and no active bleeding, indicating the welfare issue has been largely resolved.
- Clear ulcerations, lesions, necrotic tissues, or wounds should be captured under the 'wounds' indicator.
- When scoring, note the location of any hemorrhaging (e.g., ventral/rib cage).
- When interpreting results, consider the timing of recent handling activities, which may have caused hemorrhages.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon. That scoring system reflects the size of the hemorrhage rather than its expected welfare impact.

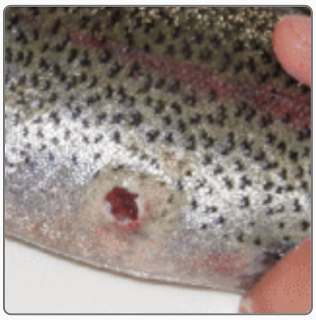
Key evidence

Source	Summary of source	Key result
Schill and Elle, 2000	Experimental study investigating the healing of electroshock-induced hemorrhages in 1210 juvenile rainbow trout (26–29cm in length) over 36–57 days in freshwater conditions. 502 fish were shocked with a continuous direct current of 375V and 7.5A, then kept in holding pens for 36 days (DC trial). 708 fish were shocked with a pulsed direct current of 325 V, 60 Hz, 25% duty cycle, 7.5A, and then kept in holding pens for 57 days (PDC trial). 50 fish per trial served as controls, handled but not shocked. Hemorrhages were counted and scored on a scale of 0–3. 0 = no hemorrhage; 1 = wound separate from the spine; 2 = wound on spine less than or equal to the width of two vertebrae; and 3 = wound on spine	One day after shocking, the DC trial group had 86.1% of fish with at least one hemorrhage, and a mean of 1.86 hemorrhages per fish, while the PDC trial group had 81.6% of fish with at least one hemorrhage and a mean of 1.45 hemorrhages per fish. In both groups, many hemorrhages were initially scored as level 2. By days 8–15, many of these had progressed to level 3. This was explained as the spreading of blood over time, making the lesions larger. Over time, the color changed from bright red to dull red or brown, and the edges faded. In the DC trial, the total number of visible hemorrhages per fish was reduced by 78% by day 36 compared to day 1. In the PDC trial, there was a 92.4% reduction by day 57. Towards the end, many of the remaining injuries were only faint grey areas.

Source	Summary of source	Key result
	greater than the width of two vertebrae.	
Noble et al., 2020	Literature review on many rainbow trout welfare indicators, including skin damage.	Hemorrhages primarily result from physical trauma, including handling, collisions, pumping, or crowding. Small hemorrhages are typically seen ventrally.

Wounds

Like skin hemorrhaging, wounds represent breaks in the skin, a key barrier against external dangers such as pathogens. Unlike skin hemorrhaging, wounds are characterized by superficial or deeper damage to the epidermis, and in some cases, exposure of the subcutaneous tissue and muscles. Wounds can result from handling, contact with equipment, aggressive interactions, or disease. Beyond causing pain and stress, open wounds in direct contact with water can quickly become infected, posing a welfare risk.

Level 0	Level 1	Level 2	Level 3
No wounds	Healed wounds **OR** 1 small superficial wound **OR** Erosion / scrapes only	Several small superficial wounds **OR** One moderate wound	Many small wounds **OR** Several moderate wounds **OR** Any large or extensive (e.g., exposing muscle) wounds **OR** Any infected wounds
			

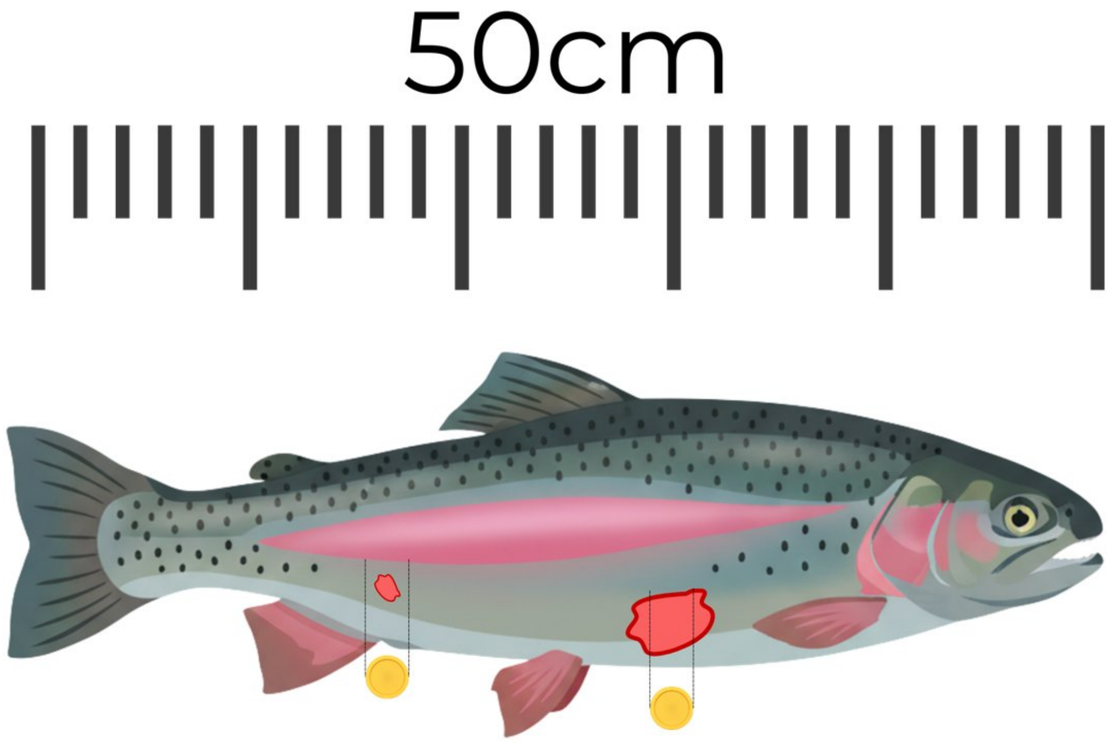
Protocol application

Scoring this indicator involves counting total wounds, determining wound size (small or large), and checking the depth and infection status. This indicator addresses wounds on the body. To avoid double-counting, wounds on the fins or at the fin base are scored under fin damage, and wounds on the snout under snout damage.

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- The status of injuries (fresh, healed, or infected) is critical to assess because fresh tissue damage is automatically more severe.
    - Fresh wounds may have active bleeding, red or pink inflamed spots, potential swelling, or edema, and indicate recent trauma.
    - Healed wounds have a stable size with scar tissue formed and no active bleeding, indicating the welfare issue has been largely resolved.
    - Infected wounds include wounds with white or grey fungal growth, excessive debris or mucus, visible discharge, redness extending further than 0.5 cm from the wound edge, or necrotic tissue.
- Wound depth is also important to assess, as the deeper the wound is, the more severe its impact on welfare.
- A superficial wound is one where the muscle is not exposed. This includes wounds exposing the dermis or hypodermis.
    - Wounds with any muscle exposed (or reaching the abdominal cavity) are automatically scored as level 3.
- Wound size classification is based on expert opinion, and inspired by guidance in FISHWELL.[19] Therefore:
    - Small wounds are visible only on close inspection.
    - Moderate wounds are < 2 cm in diameter.
    - large wounds are ≥ 2 cm in diameter.
    - For practical field reference, a UK one-penny coin, a 10-cent coin (Euro), or a US one-cent coin can be used to approximate this threshold.
    - For smaller fish, it may be appropriate to use stricter thresholds to differentiate between a small wound and a large wound.



- **Figure:** *Wound size reference on rainbow trout. The coin represents a 2cm diameter, approximating the threshold between a moderate wound (< 2cm) and a large wound (≥ 2cm).*
- When scoring, note the wound location to further identify trends (e.g., dorsal surface, lateral sides, ventral surface, head).
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

Key evidence

Source	Summary of source	Key result
Schmidt et al., 2016	Experimental study investigating the wound healing in 154 rainbow trout (112 g) in freshwater laboratory conditions over 1 year. Four circular wounds were made between the lateral line and dorsal fin on the left side of each fish. The wounds were spaced 1cm apart, 3mm deep, and penetrated the muscle.	Inflammatory and matrix-remodeling genes were strongly upregulated from day 1 to at least day 14, and remained elevated until day 100. Wound areas enlarged until day 14 due to swelling and then slowly contracted. By day 100, the epidermis had closed, but the dermis remained thickened and disorganized, and only

Source	Summary of source	Key result
	Fish were divided between two tanks, with half in each tank wounded. In one of the tanks, the fish were exposed to an immunomodulatory β-glucan product. The temperature in the first 100 days was around 8.5°C.	20% of wounds had fully contracted. No proper muscle regeneration was identified. After one year, scales had not fully regenerated, with some small, irregular scales around wound edges. At this point, the wound appeared cosmetically healed. The immunomodulatory β-glucan treatment had no effect on wound closure speed.
Ingerslev et al., 2010	Experimental study investigating the impacts of standardized skin and muscle injury on 80 rainbow trout (~43 g at time of injury, growing to ~84 g at the end of the experiment) in freshwater systems over 42 days. Each fish received approximately 50 punctures using a 25 needle array pressed twice in a 6mm x 6mm pattern to a 6 mm depth. The injury site was above the lateral line, on the left side, behind the dorsal fin.	On days 7, 14, and 21, clear bleeding was visible, with swollen skin on day 7 and signs of inflammation still present but nearly resolved by day 14. By day 28, bleeding was no longer visible, and external swelling had essentially resolved. Expression of IL-1β and IL-8 (pro-inflammatory cytokines) increased post-injury but was localized to the site of injury. Upregulation decreased as the injury healed and was about normal at day 28.

Change of coloration

Changes in coloration are a key visual indicator of stress, disease, and compromised welfare in farmed fish. In some species, subordinate fish develop darker coloration.[20] Changes in coloration can result from environmental challenges or physiological disturbances. Pale coloration can indicate anemia or severe stress, and patchy coloration can indicate skin disease or parasites. However, rainbow trout can have diverse natural colorations depending on genetics, environment, and age, so producers must first establish the normal population baseline before assessing deviations, while noting that the population baseline may shift seasonally.

Level 0	Level 1	Level 2	Level 3
Appropriate coloration for fish strain and environment (e.g., bright, normal patterns and color intensity)	Very mild color variations that are stable and not associated with signs of stressful conditions	Moderate darkening **OR** Loss of normal brightness **OR** Patchy areas are starting to form	Severe whole-body darkening **OR** Marked pale coloration **OR** Extensive patchy/ mottled appearance

Protocol application

Assessment requires handling the fish under consistent lighting conditions. Although patchy areas, subtle darkening, and loss of brightness can be distinguished in-water, light refraction at the water surface may alter perceived coloration or reduce photo/ video image quality. Furthermore, light refraction and turbidity may affect perceived coloration in automated underwater welfare scoring systems.

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- The population baseline should be used to assess this indicator rather than prescribed colors. However, assessing against a baseline can still be misleading if the whole population is stressed or diseased, so the assessor should be familiar with the expected appearance of a healthy trout for that specific site.
- Consider environmental factors (such as turbidity, lighting, and substrates) that can affect natural coloration.
- Change in coloration is a nonspecific indicator and must be interpreted alongside other indicators.

- **Validation:** This scoring system has not been validated for rainbow trout specifically and is based on expert opinion.

Key evidence

Source	Summary of source	Key result
Zhan et al., 2022	Experimental study investigating alterations to mucosal immune response following infectious hematopoietic necrosis virus (IHNV) in juvenile rainbow trout (6 g) in freshwater laboratory conditions over 30 days.	The study found darkening of the skin as one of the key clinical symptoms, appearing 4–7 days post-infection.
Clinton et al., 2021	Experimental study investigating gill responses to toxins in 128 juvenile (12 g) rainbow trout in freshwater laboratory conditions over 5 days.	The group with higher exposure to the toxin exhibited darker skin coloration. Only the rainbow trout with the most severe cases had a darker coloration.
Hofer and Gatumu 1994	Experimental study measuring retinal damage associated with nitrite exposure in 75 juvenile rainbow trout (5 g) in freshwater laboratory conditions over 20 days. The study used three treatment groups: one control group and two treatment groups with lower and higher nitrite levels.	In both nitrite treatment groups, approximately 15% of fish developed dark coloration. Fish that developed this were determined to be intolerant, as they also accumulated high nitrite in their blood, developed methemoglobinemia, showed retinal damage, and exhibited quiet behavior and reduced feeding.

Snout damage

The snout and mouth of fish are essential for feeding, respiration through the buccal-opercular pump, buoyancy regulation, and maintaining water flow over the gills. Snout injuries may result from collisions, handling, aggressive interactions, or infections, and can act as chronic stressors that impair performance and suppress immune function. In severe cases, wounds may become infected with bacteria such as *Tenacibaculum maritimum*, leading to “mouth rot,” a condition associated with rapid tissue deterioration and increased mortality risk. Snout wounds also increase metabolic costs related to tissue repair and osmoregulatory disturbance, while pain, impaired jaw movement, and reduced feeding ability may negatively affect growth, swimming performance, and recovery capacity. Damage severity likely determines functional impact, although this remains poorly validated. Snout injuries should also be distinguished from jaw deformities.

Level 0	Level 1	Level 2	Level 3
No visible snout damage	Minor snout damage (no open wounds)	Clear snout damage covering a large area **OR** Any open wounds	Severe snout damage **OR** Any wounds exposing the bone
			

Protocol application

Assessment requires handling the fish for close inspection of the snout and mouth area.

Injuries to the jaw that do not include the tip of the jaw should be recorded under skin hemorrhaging or wounds.

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- The status of injuries (fresh, healed, or infected) is critical to assess because fresh tissue damage is automatically more severe.
  - Fresh damage may have active bleeding, red or pink inflamed spots, potential swelling or edema, and may indicate recent trauma.
  - Healed damage will have a stable size with scar tissue formed and no active bleeding, indicating the welfare issue has been largely resolved.
  - Infected wounds can have white or grey fungal growth and excessive debris or mucus.
- Minor snout damage includes surface abrasions that affect only the epidermis or superficial dermis, with no exposed cartilage or bone, and no active bleeding.
- Clear snout damage includes any damage with any exposure of cartilage or bone, with active bleeding, tissue loss creating a depression in the snout profile, or signs of infection. Signs of infection include any white or grey fungal growth, or excessive debris or mucus, visible discharge, redness extending substantially from the wound edge, or necrotic tissue.
- Note whether the damage is bilateral (affecting both the upper and lower jaws) and the specific location (e.g., tip damage could impair precision feeding, rostral damage could affect the mouth opening or closing). Where the damage is bilateral, monitoring should be increased, and this may warrant preventative action.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

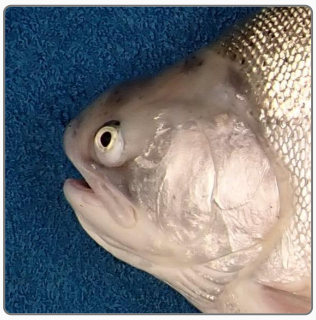
Key evidence

Source	Summary of source	Key result
Sneddon, 2003	Experimental study investigating behavioral and physiological responses consistent with pain perception in 25 rainbow trout (~61 g) in freshwater laboratory conditions. There were 5 treatment groups: control (only handled), saline-injected, acid-injected, acid- and-morphine-injected, and morphine-injected. The injection was in the trout's lips.	Acid-injected fish rested on the substrate and rocked from side to side on their pectoral fins. The authors interpreted this as possible stereotypic or comfort-seeking behavior. Fish also rubbed their lips against gravel or tank walls, which the authors interpreted as an attempt to relieve pain. These behaviors were nearly absent in controls and were significantly reduced by morphine.
	Experimental study investigating external injury outcomes after two	Fish in the warm water group displayed vigorous behavior, frantically

Source	Summary of source	Key result
Moltumyr et al., 2022	warm water exposures in 332 seawater-acclimated Atlantic salmon post-smolts (1380 g). Treatments were conducted at approximately 34°C (and 9°C for the control) for 30 seconds, administered twice, with 23–24 days between administrations. For the treatment, fish were dip-netted and placed in a plastic chamber for 30 seconds. Various external injuries were scored from 0 to 3 (increasing in severity). Final scoring was conducted 17–18 days after the second exposure.	trying to escape. Some fish displayed such vigorous escape behavior that they fainted after colliding with the walls. The fish in the control group behaved more calmly. Compared to the control group, treated fish showed a higher prevalence of scores 2 and 3 for scale loss, snout wounds, eye problems, and active fin injuries. The baseline prevalence for scale loss scores between 1 and 3 was very high for both the control and treatment groups, at 99–100%. The baseline prevalence for skin bleeding scores between 1 and 3 was 73–79%. Snout wounds had a prevalence of 7–12% before the treatment. At the end of the experiment, the prevalence was 99%, and wound severity was significantly higher in the warm water group.
Stien et al. (2025)	Review of injury-based welfare indicators of farmed salmon in the Laksvel protocol.	Snout wounds can affect either the upper or lower jaw, and do not need to be restricted only to the tip of the snout. When salmon are transported (e.g., through pumps or delousing systems), they move headfirst, increasing the risk of snout collision into equipment or other fish. Snout wounds can become infected, e.g., leading to mouth rot or yellow mouth.

Jaw deformity

A properly formed head and jaw are essential for effective feeding and respiration through the buccal pump. Most jaw deformities tend to occur during early life stages and, like vertebral deformities, are a valuable indicator of prior and ongoing management practices. Jaw deformities, such as jaw length discrepancies, curved jaws, or pugheadedness can prevent normal feeding and breathing. Jaw deformities must be differentiated from snout damage.

Level 0	Level 1	Level 2	Level 3
Normal jaw length and alignment	Minor jaw deformity or misalignment, visible only with close inspection	Clear jaw deformity or misalignment that will not affect the fish's ability to eat and grow normally	Severe jaw deformity or misalignment that may affect respiration and/or the fish's ability to eat
			

Protocol application

Assessment requires handling the fish for close inspection of jaw alignment and skull shape. Very minor deformities are only visible with close inspection and cannot be detected in-water.

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

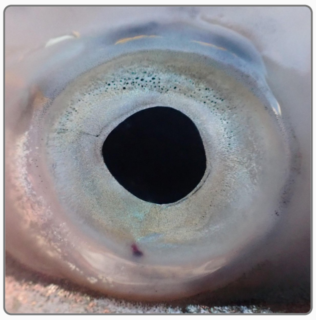
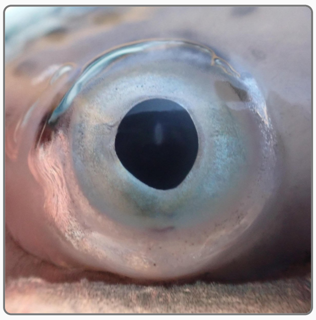
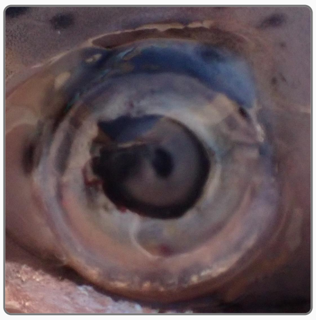
- Assess jaw shortening and pugheadedness separately for the most accurate results by checking for jaw length and alignment, and then checking for any compressed skull shape.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

Key evidence

Source	Summary of source	Key result
Godoy et al., 2023	Experimental study assessing jaw bone structure and osteogenic markers in normal and mandibular-deformed rainbow trout smolts (< 400 g) in freshwater commercial farms. 5 normal and 5 mandibular-deformed fish were compared.	Deformed jaws showed lower calcium and phosphorus content, indicating mechanically weak and/or painful jaws that limit function during feeding. Deformed fish showed poorer nutritional status, with lower plasma total protein and globulin levels and markedly reduced visceral fat, indicating a chronic energy deficit due to impaired feed intake. Deformed fish also had higher blood glucose and an altered mineral profile, suggesting physiological stress.
Näslund and Jawad, 2021	Literature review assessing the causes and impacts of pugheadedness in multiple species of fish, including rainbow trout.	Pugheadedness is linked with exophthalmos and is more likely in more deformed individuals. In severe cases, this can expose the anterior of the eyeball. Since the bones in the jaw may be severely deformed, this can restrict complete opening or closure of the mouth, potentially reducing feeding efficiency and, in turn, growth.

Eye opacity

A clear, healthy lens is essential for vision, enabling fish to feed, navigate their environment, and respond to threats or social cues. Eye opacity or cataracts can arise from nutritional, genetic, and environmental factors, as well as exposure to infections, parasites, or ultraviolet light. Cataracts cause the lens to cloud, leading to impaired vision. While eye opacity can negatively impact survival, growth, and performance, the welfare impact of eye opacity is context-dependent, influenced by factors such as feeding regime, competition level, stocking density, and environmental complexity. In commercial settings where feed is readily available and predation risk is absent, the functional consequences of impaired vision may be reduced, though the underlying condition still warrants monitoring and management.

Level 0	Level 1	Level 2	Level 3
No visible opacity	Minor opacity, ≤ 10% lens coverage	Clear opacity, > 10 to ≤ 50% lens coverage	Severe opacity, > 50% lens coverage
			

Protocol application

Assessment requires handling the fish for close visual inspection of the lens. In-water assessment is not reliable for scoring opacity levels.

As it is not always practical to distinguish between opacity to the lens or the cornea, all opacity is included in this indicator.

Score based on the most affected eye, though recording both eyes is recommended to improve data quality.

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- While a level 1 opacity score indicates minimal welfare impact, minor cataracts can indicate parasitic infection, metabolic stress, or nutritional problems and should be actively managed to prevent progression.
- **Bilateral cases:** Where both eyes are scored as level 2 or level 3, the welfare risk is significantly higher than for unilateral cases. If observed, increase monitoring frequency and implement corrective action.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon. Eye anatomy is similar across salmonids, but species-specific validation is needed.

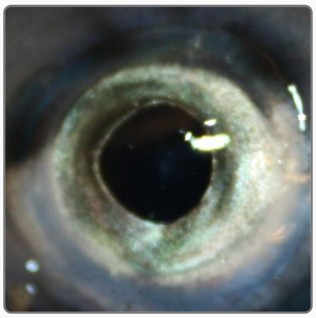
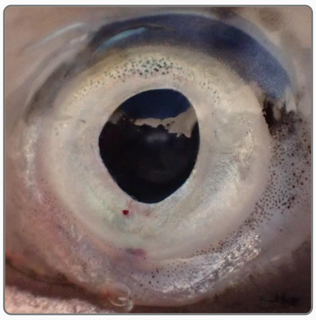
Key evidence

Source	Summary of source	Key result
Kuukka-Anttila et al., 2010	Experimental study investigating cataract growth in 969 rainbow trout with a natural *Diplostomum* infection in commercial settings across both freshwater and seawater sites over a three-year rearing period. A slit-lamp microscope was used to score cataracts on a scale of 0–5, with a maximum total score of 10 across both eyes. The fish were measured at three different times: after the first growing season (mean weight 50 g, freshwater), after the second growing season (mean weight 734 g, both freshwater and seawater), and after the third growing season (mean weight 2245 g, freshwater only).	All 969 individuals had cataracts with a mean individual score of 7.5 and a range from 4 to 10. The authors noted that *Diplostomum* load is directly related to cataract scores and that the findings suggest that genetic factors determine variation in tolerance. Initial body weight was not correlated with cataract score, but cataract severity measured in the second and third timepoints had a strongly negative relationship with body weight, indicating reduced growth and condition in fish with high cataract scores. In females, higher cataract scores were associated with later maturity. There was no clear relationship between cataract severity and mortality. The authors explained this could be because there were no predators and fish had unlimited access to feed.
Karvonen et al., 2004	Experimental study investigating cataract formation in response to eye fluke infection (*Diplostomum spathaceum*) in 160 rainbow trout (1	There was a strong relationship between higher parasite loads and more severe cataracts.

Source	Summary of source	Key result
	year old). Trout were exposed to natural infection in Lake Konnevesi for 112 days in cages or in a freshwater laboratory. Cataracts were scored on a scale from 0 to 4.	In the lake conditions, cataracts developed gradually, with 35% of eyes fully covered or opaque by day 112. In the laboratory conditions, cataracts developed more rapidly; 37% of eyes had a complete cataract, and 3% totally opaque by day 29. Cataract intensity remained stable after this time.
Remø et al., 2017	Experimental study investigating the formation of cataracts at different temperatures in 80 post-smolt rainbow trout (125 g) over 35 days in laboratory seawater conditions. Two temperatures were tested: 13°C and 19°C. Each lens was scored between 0 and 4. A score of 0 = no opacity, 1 = < 10% opacity, 2 = 10–50% opacity, 3 = 50–75% opacity, and 4 = 75–100% opacity.	At the start of the study, rainbow trout had a cataract prevalence of 10%, with a mean cataract score of 0.1. In the 13°C group, the prevalence was 53% and the mean score was 0.9, and in the 19°C group, the prevalence was 67% and the mean score was 1.3. The authors concluded that the trout's higher concentrations of N-acetyl-histidine and histidine made them less susceptible to cataracts than salmon.

Eye injury

Undamaged eyes are essential for vision, enabling fish to feed, navigate their environment, and respond to threats or social cues. The eyes of farmed fish are sensitive, and injuries can occur from physical trauma (e.g., handling, crowding, or contact with equipment), poor water quality, disease, parasites, or gas supersaturation (which can rupture blood vessels). Clinical signs of eye injury include bleeding and exophthalmos (protrusion of the eye). Like eye opacity, eye injuries can reduce visual capacity, compromising a fish's ability to navigate its environment properly. This can lead to increased behavioral and physiological stress.

Level 0	Level 1	Level 2	Level 3
No visible eye injury	Minor eye injury, visible only with close inspection	Clear eye injury with low likelihood of blindness	Severe eye injury with high likelihood of blindness
			

Protocol application

Assessment requires handling the fish for close visual inspection. Injury and parasite presence cannot be reliably assessed in-water.

Score based on the most affected eye, though recording both eyes is recommended to improve data quality.

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- Minor eye injuries (level 1) include minor hemorrhaging (e.g., small area at the lower edge of the eye) and minor protrusion.
- Clear eye injuries (level 2) include clear hemorrhaging covering a larger part of the eye and/or clear protrusion.

- Severe eye injuries (level 3) include severe hemorrhaging, severe protrusion, punctured eyes, visible parasites, and gas bubbles. A fish scoring level 3 is expected to have a high likelihood of being blind in that eye.
- **Bilateral cases:** Where both eyes are scored as level 2 or level 3, the welfare risk is significantly higher than for unilateral cases. If observed, increase monitoring frequency and implement corrective action.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

Key evidence

Source	Summary of source	Key result
Pushchina et al., 2020	Experimental study investigating the effects of mechanical eye injury on brain gene expression in 70 rainbow trout (370–455 g) in freshwater laboratory conditions. Injury was caused by a sterile needle, and fish were monitored for one week post-injury.	Fish experienced a 1.7-fold increase in cystathionine β-synthase concentration. The injury prompted the formation of specialized repair zones in the brain that were absent in the control. These phenomena were observed 1 week after eye injury, demonstrating an ongoing major response to significant stress.
Noble et al., 2020	Literature review covering a wide range of rainbow trout welfare indicators, including eye injury.	Visible eye conditions were associated with significant welfare concerns, with mechanical injuries being the most common cause of damage. Eye damage progresses from minor conditions to severe welfare impacts, including blindness, pain, and mortality.

Operculum damage

The operculum (gill cover) protects the gills and maintains efficient water flow for respiration. Operculum damage or shortening can result from developmental factors, aggressive interactions (e.g., nipping), handling events, or contact with equipment. When the operculum is shortened or damaged, gill filaments become exposed to physical injury, pathogens, and debris, increasing the risk of secondary infections. Operculum damage can also reduce a fish's capacity to pump water over the gills, compromising oxygen uptake and leading to respiratory stress.

Level 0	Level 1	Level 2	Level 3
The operculum fully covers the gills **AND** No folding	Minor shortening with limited gill tissue visible (< 10%) **OR** Minor folding, with gills fully covered	Clear shortening with some gill tissue visible (10–50%) **OR** Moderate folding, exposing part of the gills	Severe shortening, with gill tissue clearly visible (≥ 50%) **OR** Severe folding (cannot close)
			

Protocol application

Assessment requires handling the fish. The operculum must be observed when fully closed to estimate the percentage of gill tissue visible.

Score based on the most affected operculum, though recording both opercula is recommended to improve data quality.

Where opercular shortening is present, gills must be examined (see the following section on gill damage).

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- The operculum should be assessed when fully closed by estimating the percentage of gill tissue visible. This is important for consistent scoring.
- A folded operculum refers to the operculum being abnormally pressed, deformed, or collapsed against the fish's head, rather than lying flat and being able to move normally.
- **Bilateral cases:** Where both opercula are scored as level 2 or level 3, the welfare risk is significantly higher than for unilateral cases. If observed, increase monitoring frequency and implement corrective action.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

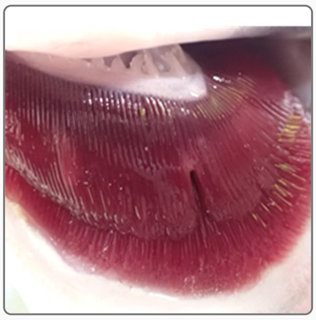
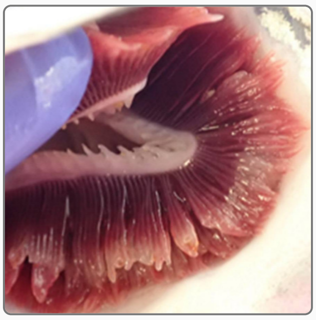
Key evidence

Source	Summary of source	Key result
Peters et al., 1984	Cross-sectional survey investigating environment–induced gill disease in 160 rainbow trout (58–405 g) from 8 German freshwater farms after at least 3 months on-site. Opercular shortening was classified as "slight" (< 30%) or "severe" (> 30%).	"Slight" operculum shortening occurred in all fish samples. Prevalence of any gill shortening ranged from ~15% to 80%. Only 3 of the 8 farms had any "severe" cases of operculum shortening.
Noble et al., 2020	Literature review on a number of rainbow trout welfare indicators, including opercular damage.	Damage to the opercula is associated with increased mortality rates and susceptibility to diseases. The authors note damage or deformities of the opercula can occur for many potential reasons, including suboptimal rearing conditions, dietary deficiencies, pollution, and traumatic injuries during competitive feeding.
Blaker and Ellis, 2022	Experimental study investigating the prevalence of opercular shortening on one cohort of Atlantic salmon (growing from < 1 g to ~300 g) in laboratory conditions over 14 months. Over the 14 months, there were 12 sampling instances, with at least 30 fish sampled each time. Fish were initially reared in freshwater, then	Opercular shortening was first recorded at the parr stage and increased in both prevalence and severity as fish grew. Short opercula were qualitatively associated with gill filament damage, with the extent of gill damage related to the area of operculum missing. Nipping was assessed as the primary

Source	Summary of source	Key result
	transferred to seawater after smoltification.	cause of short opercula in the experiment.

Gill damage

Gills are vital organs responsible for oxygen uptake, carbon dioxide release, and osmoregulation, making them essential for fish survival and performance. Because gills are in continuous contact with surrounding water, they are highly vulnerable to irritants, pathogens, and poor environmental conditions, and serve as an early indicator of suboptimal water quality or disease. Damage to gill tissue can reduce respiratory efficiency and impair the fish's ability to maintain ion and acid–base balance, leading to stress and compromised welfare.

Level 0	Level 1	Level 2	Level 3
Healthy red gills with no visible alteration	Minor alterations (affecting < 10% of gill area) **AND** No lesions	Clear alterations (affecting 10–50% of gill area) **OR** Any small lesion	Severe alterations (affecting ≥ 50% of gill area) **OR** Any large lesion
			

Protocol application

Score based on the most affected gill, though recording both gills is recommended to improve data quality.

Gill assessment requires direct handling to lift the operculum for visual inspection and cannot be completed with a camera.

Where gill damage is present, opercula must also be examined (see the previous operculum damage section).

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- Alterations include structural changes, color changes (pale areas, discoloration), mucus patches, hemorrhaging, and necrosis.

- For assessors familiar with 5-level clinical gill scoring systems (e.g., Tassal's gill score guide for amoebic gill disease),[21] the approximate mapping is: score 0 = level 0, scores 1–2 = level 1, scores 3–4 = level 2, score 5 = level 3.
- A combination of gill damage and a shortened or folded operculum signals a problem requiring urgent intervention.
- Gill hyperplasia can cause overgrown gill tissue to extend beyond the operculum. This may resemble operculum shortening, but it is a distinct condition and it does not mean the operculum itself is damaged or shortened. Assessors should closely inspect the operculum edge when scoring to determine whether the operculum is normal but the gill tissue is protruding, or that the operculum is truly shortened or damaged.
- **Bilateral cases:** Where both gills are scored as level 2 or level 3, the welfare risk is significantly higher than for unilateral cases. If observed, increase monitoring frequency and implement corrective action.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

Key evidence

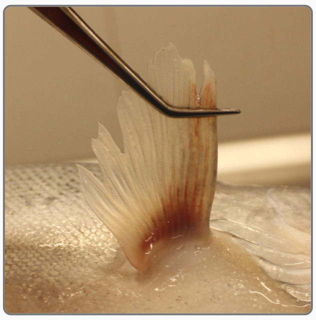
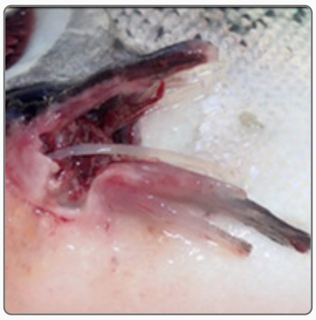
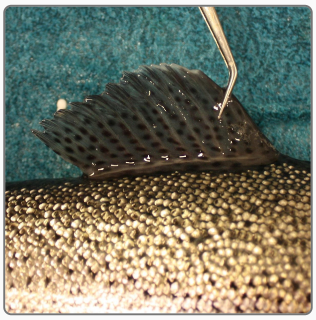
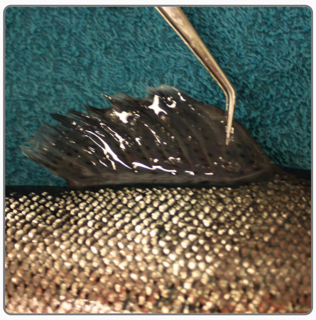
Source	Summary of source	Key result
Strzyżewska-Worotyńska et al., 2017	Cross-sectional study investigating the environmental impacts on gills of ongrowing rainbow trout (500–850 g) in 6 different commercial freshwater farms (3 flow-through systems and 3 RAS systems) in either spring or autumn. In each season, 6 fish were sampled per farm, resulting in a total of 72 trout. Gills were classified as stage I (mild, reversible changes that did not impair function), stage II (intermediate lesions with functional impact), and stage III (terminal lesions with irreversible organ damage).	Most sampled fish had normal gills. When lesions were present, they were mostly mild and/or reversible. Stage I consisted mainly of epithelial lifting and mild hyperplasia. However, the authors considered that pronounced epithelial hyperplasia with clear lamella fusion impacted function. Stage I lesions made up 73–79% of all lesions. Stage II included telangiectasia, aneurysms, and hemorrhages in the gills, which were larger and more numerous in the fish sampled from RAS systems. These intermediate lesions accounted for about 10% of the lesions. Stage III included fibrosis and necrosis. These damages made up to 7% of all lesions and were about 5 times more common in RAS systems.
Zhan et al., 2022		

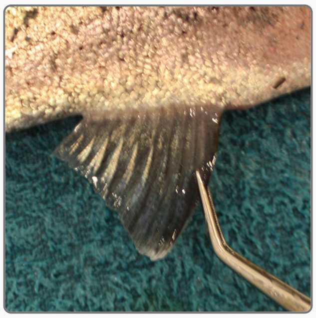
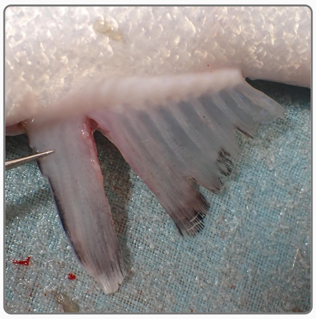
Source	Summary of source	Key result
	Experimental study investigating alterations to the mucosal immune system in response to infectious hematopoietic necrosis virus (IHNV) in juvenile rainbow trout (5.66 g) in freshwater laboratory conditions over 30 days.	Fish infected with the virus showed pale gills. Within 10 days, mortality reached approximately 30%.
Noble et al., 2020	Literature review covering a wide range of rainbow trout welfare indicators, including gill damage.	Gill damage can make fish more susceptible to low oxygen levels, stress, or exercise. In freshwater systems, poor water quality can make the gills vulnerable to parasites. In seawater systems, gill disease can result in high morbidity and mortality.

Fin damage

Fins are critical for swimming efficiency, maneuverability, balance, maintaining position in water currents, and natural behaviors. Fin damage can reduce swimming performance, feeding efficiency, and the ability to avoid aggression or predators, impacting fish welfare and survival. Fin damage may arise from aggressive fin-nipping, poor water quality, overcrowding, rough handling, or contact with abrasive surfaces, making it a valuable indicator of welfare.

- Different fins serve different purposes, and the impact of damage varies, for example:
    - Caudal fin: damage can reduce swimming efficiency.
    - Dorsal and anal fins: damage can affect balance and maneuverability.
    - Pectoral fins: damage can impair steering, braking, and precise movements.
    - Pelvic fins: damage affects stability, but is least critical for survival.

Level 0	Level 1	Level 2	Level 3
No visible damage to the fin	Minor alteration of the fin	Clear alteration of the fin	Severe alteration of the fin **OR** Any necrotic lesions or ulcerations
			
			

Level 0	Level 1	Level 2	Level 3
			
			

Protocol application

Assessment requires handling the fish. All fins should be examined, which requires turning the fish to inspect both sides.

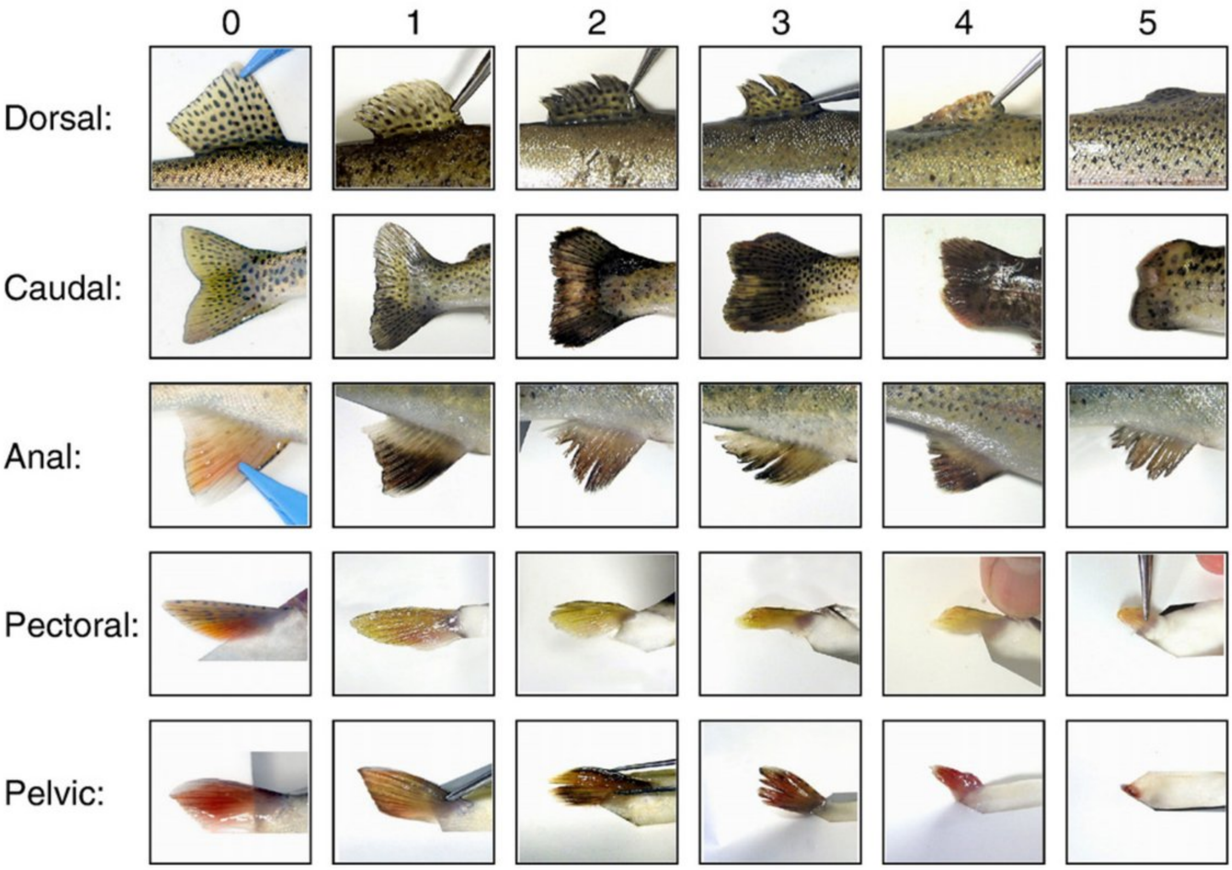
Score based on the most damaged fin, though recording all fins is recommended to improve data quality.

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- In line with the Laksvel scoring system for Atlantic salmon:
  - Level 1 damage includes slight fraying, minor erosion, splitting that is < 50% of the fin length, and minor hemorrhaging.
  - Level 2 damage includes moderate erosion, clear hemorrhaging, splitting which is ≥ 50% of the fin length, as well as moderate tissue or ray loss.
  - Level 3 damage includes severe erosion, extensive splitting, large areas of hemorrhaging, and severe shortening.
- Healed fin injuries that would originally be scored as level 3 are scored as level 2. Healed fin injuries that would originally be scored as level 2 are scored as level 1.
- Damage across multiple fins can indicate systemic issues (chronic aggression, poor water quality, overcrowding, etc.).

- **Bilateral cases:** Where both fins in a pair (i.e., pectoral or pelvic fins) are scored as level 2 or level 3, the welfare risk is significantly higher than for unilateral cases. If observed, increase monitoring frequency and implement corrective action.
- **Validation:** Scoring fin damage has been validated specifically for rainbow trout. A macroscopic scoring key was developed and validated by Hoyle et al., (2007) for farmed rainbow trout (below), and fin damage has been well-studied in rainbow trout.



- **Figure:** *Protocol for scoring fin damage in juvenile rainbow trout. Reproduced from Hoyle et al., (2007). 0 corresponds to level 0, 1 to level 1, 2–4 to level 2, and 5 to level 3.*

Key evidence

Source	Summary of source	Key result
Weirup, 2022	Cross-sectional study investigating chronic stress in ongrowing rainbow trout across 8 commercial freshwater systems in Germany. 10 adult female rainbow trout (~18 months old) were sampled per system. Fish were examined for	Chronic stress (estimated through scale cortisol) correlated strongly with total fin injury across all farms. Fin injuries were common and varied significantly between farms, while other types of morphological damage were rare.

Source	Summary of source	Key result
	external morphological damage, including fin damage, and sampled for scale cortisol using advanced laboratory techniques.	Limited water supply and exchange, poor water quality, high stocking densities, and high feeding frequencies increased stress and fin damage.
Cvetkovikj et al., 2015	Questionnaire-based survey investigating the relationship between husbandry practices and fin damage that was conducted across 7 commercial trout farms in Macedonia.	Lower levels of daily feed ration, increased number of meals, and grading frequency were the strongest predictors of fin damage across different fins. Fin damage reflects aggression and competition during feeding and suboptimal husbandry practices.
Person-Le Ruyet et al., 2008	Experimental study investigating the impacts of different water quality levels and stocking densities on ongrowing rainbow trout (111.5 g) in freshwater conditions over 84 days. Three stocking densities were tested: 25, 75, and 120 kg/m^3. Two water quality levels were tested: high (with oxygen concentration of 12 mg/L and total ammonia nitrogen of 0.3 mg/L) and low (with oxygen concentration of 6 mg/L and total ammonia nitrogen of 0.6 mg/L).	Higher stocking density and better water quality both correlated with increased fin damage over time. The authors hypothesized that higher fin damage under better water quality conditions resulted from higher fish metabolic activity and greater activity during feeding compared to the low water quality group, which led to more physical contact. Pectoral fins deteriorated faster than dorsal fins under poorer conditions.

Sea lice

Sea lice are a significant problem in open seawater salmonid aquaculture. Sea lice attach to fish skin and feed on mucus, skin tissue, and blood, causing significant welfare problems. Different sea lice species pose varying levels of risk to fish. Heavy infestations cause physical damage, including skin lesions, scale loss, and open wounds, which create gateways for infection.

Regulations have different maximum levels of sea lice. These levels are not set based on farmed fish welfare, but on impacts to wild salmonid populations. Examples:

- **Scotland:** 2.0 adult female lice per fish on average results in enhanced regulatory oversight.[22]
- **Norway:** maximum of 0.5 adult female lice per fish, or below 0.2 in the period of salmonid out-migration.[23]
- **Ireland:** must reduce sea lice levels at 0.5 ovigerous female lice per fish during the sensitive period, or below 2.0 adult female lice per fish.[24]
- **Canada:** must take delousing actions when reaching an average of 3.0 motile sea lice per fish.[25]

It is difficult to provide a scoring method appropriate for rainbow trout production, as the welfare impact of sea lice depends on a number of variables, including:

- The absolute lice count (number of lice per fish)
- Relative intensity of the infestation (e.g., the number of lice per gram of body weight, or the number of lice per cm^2 of body surface area)
- The lice developmental stage (e.g., mobile preadult/adult stages cause more damage than the sessile chalimus stage)

Given the multi-factorial nature of the welfare impact of sea lice, paired with the practical difficulties of counting sea lice in the field (especially for earlier life stages), the thresholds for this indicator are based on damage attributable to sea lice only.

Level 0	Level 1	Level 2	Level 3
No skin damage	Associated small areas of dryness	Associated moderate skin damage (lesions, sores, scale loss, skin loss, patches of dryness)	Associated severe skin damage (lesions, sores, scale loss, skin loss, patches of dryness)

Protocol application

Assessment requires handling individual fish for reliable counting.

This indicator captures damage caused by sea lice infestation (e.g., damage located under attachment sites). Such damage may also appear under 'wounds' or 'skin hemorrhaging' indicators; this overlap is not a problem as the indicators will be interpreted together.

Calculate the distribution of scores across the thresholds within the sample.

Scoring notes

- Damage should be interpreted together with recorded lice numbers, recent delousing, and rising damage/lice count prevalence.
- **Validation:** The damage thresholds are based on expert opinion.

Key evidence

Source	Summary of source	Key result
Dalvin et al., 2020	Experimental study investigating histopathological changes associated with initial infestation of 80 copepodids in 112 ongrowing rainbow trout (330 g) in seawater laboratory conditions for up to 41 days post-infestation. Fish were sampled at multiple time points post-infestation.	At the early stages of infection, only minor epidermal erosions were observed. At 16 days post infection, at a mean of 32 preadult lice per fish, there was more evident tissue damage. At 41 days post infection, a mean of 11 adult lice per fish was recorded. At this time, the adult female lice were fully mature and carrying their first set of egg strings. Up to this point, shallow ulcerations were detected where the louse mouth tube had contacted the skin. Other changes observed were a local depletion of mucus-producing goblet cells and immune cell infiltration, as well as moderate epidermal hyperplasia and edema. No gross lesions with scale loss or hemorrhages were observed.
		Even low subclinical infection levels (e.g., 0.04 lice/g or 10 preadult/adult

Source	Summary of source	Key result
Wagner et al., 2008	Review article on the welfare impacts of sea lice infection on salmonids.	lice of both sexes per fish (citing Nolan et al., 1999)) were found to induce a stress response in Atlantic salmon. Higher levels, such as 0.2 lice/g, can lead to changes in host mucus, physical damage, and compromised immune function. Infection levels of 0.08 lice/g were also found to show altered cardiac and swimming performance. It is well recognized that high mobile sea lice loads (> 0.75 lice/g) can cause morbidity and death.

Group-based indicators

Indicators assessed at the population or pen level, reflecting group-level welfare.

Mortality

Mortality serves as a crude but important welfare indicator. While high mortality can reasonably be assumed to indicate poor welfare, fish in aquaculture systems can have poor welfare even with low mortality levels.

The Norwegian Food Safety Authority has stated that the average mortality rate in Norway is too high and unacceptable.[26] In 2024, 2.4 million rainbow trout died during the marine phase in Norway, representing an estimated annual cumulative mortality rate of 15.0%.[27] In 2021, approximately 5% of all Norwegian locations for Atlantic salmon and rainbow trout achieved mortality rates below 5% in the sea phase.[28] Individual farmers, scientific institutions, and others have argued that this is a feasible mortality rate to achieve.

Beyond recording mortality rates, producers should also record causes of mortality to build an understanding of trends over time. This is crucial for enabling appropriate actions to avoid and prevent further mortalities.

Mortality per day

Level 0	Level 1	Level 2	Level 3
≤ 0.014 %/day	> 0.014 to ≤ 0.029 %/day	> 0.029 to ≤ 0.061 %/day	> 0.061 %/day

Mortality per annum

Level 0	Level 1	Level 2	Level 3
≤ 5 %/year	> 5 to ≤ 10 %/year	> 10 to ≤ 20 %/year	> 20 %/year

Overall: Overall score is determined by the most severe subindicator

Protocol application

The figures above refer to both annualized and daily mortality rates (with daily mortality rates extrapolated from annual rates, rounded for simplicity). Annual rates should be the primary metric for overall assessment, with daily rates necessary for operational tracking and management decisions.

Scoring notes

- If daily mortality ≥ 0.029%, immediate investigation should be initiated.

- Along with mortality rates, it is critical to track and record causes of mortality. Understanding why fish are dying is essential for identifying patterns, implementing preventive measures, and distinguishing normal background mortality from emerging problems. Where many mortalities (e.g., > 20% of recorded mortalities) cannot be explained, monitoring should increase, and management practices should be reassessed.
- Raw mortality numbers are not the only important metric. Increasing mortality rates, even if absolute rates remain in the level 0 or level 1 categories, are still cause for concern.
- If mortality rates increase or spike, this should be thoroughly investigated, and management practices should be reassessed.
- Being aware of seasonal trends is also essential for making improved management decisions.
- **Validation:** These thresholds are based on Norwegian industry aspirations, the Laksvel protocol, and expert opinion.

Aberrant behavior

Aberrant behavior includes fish that are unusually lethargic or frantic, swimming deep or on their side, body rocking, surface gasping, burrowing, or clumping. This includes stereotypic behavior: repetitive behavior that lacks a clear purpose, often observed in captive animals with limited ability to engage in natural behaviors. It is generally accepted that these types of behaviors are a response to a poor environment. These behaviors may also indicate the influence of stressors, disease, or deformities.

Producers should understand normal swimming behaviors in their production to better identify abnormal behaviors.

Minor aberrant behavior

Level 0	Level 1	Level 2	Level 3
No minor aberrant behaviors	Low prevalence (< 2%) of minor aberrant behaviors	Noticeable prevalence (2–5%) of minor aberrant behaviors	High prevalence (> 5%) of minor aberrant behaviors

Major aberrant behavior

Level 0	Level 1	Level 2	Level 3
No major aberrant behaviors	Isolated individuals (< 0.1%) expressing major aberrant behaviors	Low prevalence (0.1–2%) of major aberrant behaviors	Noticeable prevalence (> 2%) of major aberrant behaviors

Overall: Overall score is determined by the most severe subindicator

Protocol application

Use a consistent method for observing and assessing behavior to ensure data are standardized, including the location and time of observation. An example protocol could be: observe behavior for five minutes before feeding, five minutes during feeding, and then five minutes at another time in the day. For consistency, conduct observations at the same time each day. When in doubt, assess conservatively and use the more severe threshold level.

Prevalence percentage estimates are based on the visible portion of the population, acknowledging that using camera systems or surface observation will be unable to capture the behavior of all individuals in a given enclosure.

Using non-intrusive methods is preferred (e.g., underwater camera) as this avoids altering fish behavior through human presence.

Scoring notes

- Fish can behave aberrantly in many ways, and not all aberrant behaviors indicate the same severity of welfare issues. For example:
  - **Minor aberrant behaviors** can include:
    - Slight lethargy (reduced activity, but activity is still present).
    - Minor positioning changes (slightly deeper or higher than usual).
    - Reduced schooling participation.
    - Slightly increased ventilatory activity.
    - Some increased thigmotaxis.
  - **Major aberrant behaviors** can include:
    - Surface gasping (indicating respiratory distress).
    - Body rocking or stereotypies.
    - Side swimming.
    - Severe lethargy (minimal movement, poor responsiveness).
    - Frantic swimming.
    - Loss of balance or equilibrium.
    - Erratic movements.
    - Significantly increased ventilatory activity.
    - Significantly increased thigmotaxis.
- What behaviors are considered aberrant also depends on the baseline trends in the production system.
- Acute and chronic aberrant behavior have different welfare implications. Acute behavior is linked to a specific stressor (e.g., a handling event) over a short period. Chronic behavior persists over weeks, indicating a systemic welfare problem.
  - Where aberrant behaviors can be linked to an acute stressor, note this for future reference.
- To facilitate consistency between observers, it is recommended to conduct periodic calibration sessions (scoring the same cage simultaneously and then discussing any points of disagreement).
- **Validation:** The prevalence thresholds are derived from Pettersen et al. (2013) with a higher threshold applied to minor aberrant behaviors as the study did not distinguish between minor and major aberrant behaviors. The distinction between minor and major aberrant behaviors is based on expert opinion and should be adapted to the baseline behavior patterns of each production system.

Key evidence

Source	Summary of source	Key result
Davidson et al., 2011	Two experimental studies investigating the impacts of different freshwater RAS conditions on rainbow trout health and welfare. Experiment 1 compared very low water exchange and high water exchange in rainbow trout (151 g) over six months. Experiment 2 compared low water exchange to near-zero water exchange in rainbow trout (18 g).	In both studies, trout in lower-exchange systems swam significantly faster than those in higher-exchange systems. In study 1, high-exchange fish swam at approximately 0.7 BL/s, and low-exchange fish swam at approximately 1.4 BL/s. In study 2, the very low-exchange trout swam at approximately 2.1 BL/s, and the near-zero exchange trout swam at approximately 3.4 BL/s. The authors hypothesized that this was a physiological flight response caused by the chronically stressful water quality conditions. Approximately 4 times more side swimming was observed in the low-exchange system in study 1. The authors hypothesized this could be due to musculature imbalance, skeletal deformities, or swim bladder deviation. In study 2, the near-zero exchange group displayed more abnormal behaviors, including erratic swimming, swimming at oblique angles, surface swimming, and yawning/gulping. These behaviors became more severe over time. In the near-zero system, the prevalence of skeletal deformity reached up to 38%. The authors hypothesized that abnormal swimming may contribute to more deformities. Mortality was also higher in this group.
Sneddon, 2003	Experimental study investigating behavioral and physiological responses consistent with pain perception in 25 rainbow trout (~61 g) in freshwater laboratory conditions.	Acid-injected fish rested on the substrate and rocked from side to side on their pectoral fins. The authors interpreted this as possible stereotypic or comfort-seeking behavior.

Source	Summary of source	Key result
	There were 5 treatment groups: control (only handled), saline-injected, acid-injected, acid- and morphine-injected, and morphine-injected. The injection was in the trout's lips.	Fish also rubbed their lips against gravel or tank walls, which the authors interpreted as an attempt to relieve pain. These behaviors were nearly absent in controls and were significantly reduced by morphine.
Pettersen et al., 2013	Review paper presenting a welfare index to assess the welfare of Atlantic salmon in sea cages. Behavior was included as an indicator.	The authors concluded that aberrant behavior was a sign of severe welfare issues. They suggested that 0% was ideal, < 0.1% acceptable, 0.1–2% poor, and > 2% critical for welfare. They categorized evidence of aggression as potentially serious and similar in severity to abnormal behavior. They suggested that surface gasping and lethargy could indicate severe gill disease.

Aggressive behavior

Aggressive behavior has been defined as "behavior that actually or potentially causes harm to another animal," excluding killing other animals for food or attacking a potential predator.[29] Aggressive behaviors, including nipping and increased chasing, can be evidenced by physical injuries such as bite marks. Aggression can lead to the formation of a hierarchy, in which subordinate fish are at higher risk of stress, e.g., due to an inability to compete for food. Aggressive behavior can also indicate insufficient resources or space.

Aggressive behavior during feeding time

Level 0	Level 1	Level 2	Level 3
No aggressive behavior **OR** Normal aggressive behavior with no harmful outcomes	Moderate aggressive behavior with minimal harm	Elevated aggressive behavior with visible harm	Severe aggressive behavior causing significant harm **OR** Fish are visibly excluded from the feeding area

Aggressive behavior outside feeding

Level 0	Level 1	Level 2	Level 3
No aggressive behavior **OR** Rare isolated aggressive interactions with no harmful outcomes only	< 5% of fish involved in aggressive interactions **OR** Any prevalence of aggressive interactions causing minor harm only (fin nipping, brief chasing)	5–20% of fish involved in aggressive interactions **OR** Any prevalence of aggressive interactions causing visible harm (bite marks, moderate fin damage)	> 20% of fish involved in aggressive interactions **OR** Any prevalence causing severe harm (open wounds, severe fin damage) **OR** Clear spatial exclusion or subordination tendencies

Overall: Overall score is determined by the most severe subindicator

Protocol application

Use a consistent method for observing and assessing behavior to ensure data are standardized, including the location and time of observation. An example protocol could be: observe behavior for five minutes before feeding, five minutes during feeding, and then five minutes at another time in the day. Feeding behavior should also be scored during this time. For consistency, conduct observations at the same time each day.

Prevalence percentage estimates are based on the visible portion of the population, acknowledging that using camera systems or surface observation will be unable to capture the behavior of all individuals in a given enclosure.

Using non-intrusive methods is preferred (e.g., underwater camera) as this avoids altering fish behavior through human presence.

Count and note aggressive acts during the observation period, then use a combination of prevalence and severity to determine the score.

When in doubt, assess conservatively and use the more severe threshold level.

Scoring notes

- **Normal aggressive behavior** can involve brief chasing, establishment of a feeding hierarchy, threat postures, brief confrontations, or positioning disputes during feeding. These behaviors typically do not result in physical harm.
- **Moderate aggressive behavior** can involve minor fin nipping, extensive chasing, or positioning disputes outside of feeding.
- **Elevated aggressive behavior** can involve acts occurring more frequently and resulting in visible harm (such as bite marks).
- **Severe aggressive behavior** can involve persistent harassment, causing severe harm such as open wounds or severe fin damage.
- **Prevalence outside feeding:** Some aggressive behavior during feeding is normal (hierarchy establishment, competition for food). Aggressive behavior outside of feeding time is a stronger indicator of welfare problems, and the prevalence thresholds in the non-feeding subindicator reflect this: < 5% is likely individual personalities with some potential for emerging issues, 5–20% indicates systemic problems (environmental stressors, resource competition), and > 20% represents a population-level welfare problem requiring immediate intervention.
- Visible harms resulting from aggressive behavior include wounds or tissue punctures from biting, fin damage as a result of nipping, and chasing wounds (e.g., scrapes or scale loss).
- Aggressive behavior during feeding should be interpreted alongside the feeding behavior indicator — where aggression is elevated during feeding, check whether subordinate fish are being excluded from feeding areas and whether feed waste is elevated.
- When aggressive behavior is observed, note the specific behaviors observed to provide more context.

- To facilitate consistency between observers, it is recommended to conduct periodic calibration sessions (scoring the same cage simultaneously and then discussing any points of disagreement).
- **Validation:** The thresholds are based on expert opinion.

Key evidence

Source	Summary of source	Key result
Noble et al., 2007	Experimental study investigating the effects of different self-feeding regimes on growth, behavior, and fin damage in 100 juvenile rainbow trout (90 g) in freshwater laboratory conditions over 48 days.	Fish were provided with either a single 2-hour access period to self-feeders, three 2-hour access periods to self-feeders, or unlimited access to self-feeders. The authors found a significant increase in aggressive behavior in the "one access period" group.
Noble et al., 2020	Literature review on many rainbow trout welfare indicators, including aggressive behavior.	The review highlighted aggression during certain life stages or handling routines as a sign of social stress. Increased current speed can reduce aggression in salmonids, as fish expend more energy maintaining position.

Feeding behavior

Active feeding with minimal waste can indicate a healthy population with appropriate feed management. Feed motivation can be reduced by stressors, which can lead to reduced feed intake and growth.[30] Reduced feeding can also indicate disease or poor water quality.[31] Fish often have a decreased appetite after experiencing handling procedures such as crowding, pumping, or transport. Uneaten feed can also contribute to deteriorating water quality. The amount of time taken to recover to normal feeding behaviors after such an event is also a useful indicator of welfare.[32]

Producers should understand normal feeding behaviors in their production to better identify and score feeding behavior.

Feeding behavior

Level 0	Level 1	Level 2	Level 3
Active anticipatory behavior before feeding **OR** Active behavior during feeding	Some anticipatory behavior before feeding **OR** Mostly active behavior during feeding	Reduced anticipatory behavior before feeding **OR** Slow or reluctant feeding	Absent anticipatory behavior before feeding **OR** Minimal activity during feeding

Feed waste

Level 0	Level 1	Level 2	Level 3
Feed is consumed efficiently, with < 2% feed waste within the normal feeding period	Most feed is consumed, with 2–5% of feed uneaten	5–10% of the feed remains uneaten	> 10% of the feed remains uneaten

Overall: Overall score is determined by the most severe subindicator

Protocol application

Use a consistent method for observing and assessing behavior to ensure data are standardized, including the location and time of observation. An example protocol could be: observe behavior for five minutes before feeding, five minutes during feeding, and then five minutes at another time in the day. Aggressive behavior should also be scored during this time. For consistency, conduct observations at the same time each day.

Prevalence percentage estimates are based on the visible portion of the population, acknowledging that using camera systems or surface observation will be unable to capture the behavior of all individuals in a given enclosure.

Using non-intrusive methods is preferred (e.g., underwater camera) as this avoids altering fish behavior through human presence.

For consistency in measuring feed percentage, measure at the same time each day (e.g., 30 minutes after feeding). Calculate the waste percentage by dividing uneaten feed by total feed offered. Feed waste can be estimated using visual observation (e.g., with a camera or from the surface) or collected from waste feed collectors.

Two scoring systems are provided. It is recommended to score using both and then take the worst score. When in doubt, assess conservatively and use the more severe threshold level.

Scoring notes

- Anticipatory behavior before feeding includes behaviors such as:
    - Increased swimming activity in the minutes before feeding.
    - Movement towards the feeding area when staff approach.
    - Schooling behavior near the expected feeding location.
- Active anticipatory behavior means a clear increase in the above activities before feeding; some anticipatory behavior means some increase in activity; reduced anticipatory behavior means minimal behavioral change; and absent anticipatory behavior means no response.
- A lack of anticipatory behavior should not necessarily be interpreted as low appetite if fish otherwise feed well.
- Feed waste percentage is a more objective, evidence-based measure than anticipatory behavior. However, how applicable the feed waste score is depends on whether the amount of feed provided is appropriate.
    - Feed waste may not always be measurable, particularly in sea cages. In these cases, rely on the feeding behavior score.
- Feeding behavior may be impacted by one-off events such as handling and weather events, which the assessor should also note. Daily appetite can also vary naturally, for example based on how much was eaten the previous day. In these cases, daily feeding behavior scores should be interpreted cautiously.
- Persistent level 2 or level 3 feeding behavior may indicate a chronic welfare problem requiring investigation of disease, water quality, or chronic stressors.
- Reduced feeding behavior may result from aggressive exclusion — see also the aggressive behavior indicator.

- To facilitate consistency between observers, it is recommended to conduct periodic calibration sessions (scoring the same cage simultaneously and then discussing any points of disagreement).
- **Validation:** The behavioral thresholds are based on expert opinion, while the feed-waste percentage thresholds are based on Øverli et al. (2006) and informed by expert opinion.

Key evidence

Source	Summary of source	Key result
Øverli et al., 2006	Experimental study investigating how two selectively bred lines of 300 rainbow trout (bred to be either highly responsive or lowly responsive to stress) (456–493 g) relate to feed waste in laboratory conditions over 12 days. Fish were hand-fed once daily over 20 minutes with graduated feeding regimes: 0.5% body mass/day (days 1–3), 1.0% body mass/day (days 4–6), and 2.0% body mass/day (days 7–12). Uneaten pellets were collected from PVC grid pellet traps 15 minutes after feeding ended.	Fish from the highly stress-responsive line wasted approximately 11% of feed, while fish from the low-stress responsive line wasted approximately 4% of feed. The difference in feed waste increased progressively as rations increased. The highly-responsive fish also demonstrated slower growth rates and poorer feed conversion efficiency. There was also a positive correlation between initial size variation within tanks and total feed waste.
McKay and Gjerde, 1985	Experimental study investigating the effects of different salinities on growth in 90 rainbow trout (50–150 g) over 12 weeks. Fish had been reared in freshwater and then transferred to salinities of 0, 10, 20, 24, 28, and 32 ppt.	Appetite was significantly better at 0 ppt than at higher salinities. At 10 ppt, fish were leaving 39.5% of feed uneaten. At 20 ppt, fish were leaving 36.8% of feed uneaten, and at 32 ppt, fish were leaving nearly half of feed uneaten. Mortality increased with increasing salinity, from 0% at 0 and 10 ppt, but reaching 13% mortality at 32 ppt.
Noble et al., 2020	Literature review on many rainbow trout welfare indicators, including feeding behavior.	A poor or absent feed response can indicate disease or stress. While increased swimming speed before or at the start of feeding can indicate positive anticipation, persistence during meals or over several days may reflect competition or resource scarcity, signaling reduced welfare.

Swimming behavior

Normal swimming behavior reflects good physiological condition and the ability of fish to cope with their environment. Disease, injury, or deformity can severely affect swimming behavior due to physiological changes the fish may be struggling to adapt to.

Level 0	Level 1	Level 2	Level 3
Normal, active swimming structure with appropriate cage use	Mostly normal swimming structure with minor variations	Clear disruption of normal swimming structure	Severe disruption of normal swimming structure

Protocol application

Use a consistent method for observing and assessing behavior to ensure data are standardized, including the location and time of observation. An example protocol could be: observe behavior for five minutes before feeding, five minutes during feeding, and then five minutes at another time in the day. For consistency, conduct observations at the same time each day.

Swimming behavior is assessed based on the visible portion of the population, acknowledging that using camera systems or surface observation will be unable to capture the behavior of all individuals in a given enclosure.

Using non-intrusive methods is preferred (e.g., underwater camera) as this avoids altering fish behavior through human presence.

When variations from normal swimming are observed, they should be documented in notes.

When in doubt, assess conservatively and use the more severe threshold level.

Scoring notes

- When assessing swimming behavior, focus on organization and schooling rather than specific geometric patterns.
- Activity level changes are more welfare-relevant than specific swimming patterns.
    - Normal variation is expected with feeding and changing environmental conditions.
    - Abnormal hyperactivity may indicate a chronic stressor, poor water quality, or disease.
    - Lethargy may also indicate disease, severe stress, or poor nutrition.

- **Level 0:** Normal active swimming structure includes organized schooling and responsiveness to stimuli.
- **Level 1:** Minor variations from normal swimming structure include unusual avoidance of enclosure areas, some disruption to schooling, and occasional increased activity.
- **Level 2:** Clear disruption to normal swimming structure includes fish clustering at the bottom or in the corners of the enclosure, disorganized movement, and episodes of hyperactivity or lethargy.
- **Level 3:** Severe disruption to normal swimming structure includes fish avoiding open or feeding areas, constant bottom-dwelling, no schooling structure, extreme lethargy, and frantic swimming.
- Good use of the enclosure space indicates comfort and environmental adequacy.
    - Use of the whole water column indicates comfort throughout the cage.
    - Bottom-dwelling may indicate low oxygen, poor water quality, or disease.
    - Corner-clustering may indicate water flow problems, aggression, or fear response.
- To facilitate consistency between observers, it is recommended to conduct periodic calibration sessions (scoring the same cage simultaneously and then discussing any points of disagreement).
- **Validation:** The thresholds are largely based on expert opinion.

Key evidence

Source	Summary of source	Key result
Noble et al., 2020	Literature review on many rainbow trout welfare indicators, including swimming behavior.	Unstructured swimming at the bottom of the tank indicates acute stress, deviating from the normal circular schooling pattern typically seen in healthy fish.
Oppedal et al., 2011	Review article on the behaviors of Atlantic salmon.	Salmonids typically form a circular swimming pattern during the day and avoid both the innermost part of the cage, the corners, and the bottom (though this structure breaks down during feeding). Fish that remain at the bottom of the tank may be experiencing environmental stress, such as low dissolved oxygen. It is normal for swimming speed to be reduced at night. A lack of reduced swimming speed at night may indicate disrupted circadian rhythms due to artificial lighting.

Environmental indicators

Indicators relating to the water quality and environmental conditions of the farm.

Stocking density

Appropriate stocking density provides fish with sufficient space to swim, feed, and behave normally while maintaining good water quality. Stocking density, expressed as fish biomass per unit volume of water (kg/m³), is a complex indicator of welfare. The indicator involves multiple parameters that change over time as fish grow in a static volume. The search for a 'golden' stocking density has been a subject of much academic discussion, as it is not a good indicator of welfare per se. This is because the negative consequences of higher stocking densities can be mitigated through good management practices.[33]

However, across the literature, there is a clear trend: increasing stocking density results in poor welfare outcomes for rainbow trout (e.g., higher mortality and lower growth). Separating the direct impacts of high stocking density from associated issues, such as deteriorated water quality, can be very difficult. Further, some studies have found poor welfare outcomes at very low densities (e.g., at initial densities of 0.03 kg/m³ or 0.4 kg/m³).[34] Producers should proceed with caution when considering very low density levels.

Level 0	Level 1	Level 2	Level 3
≤ 10 kg/m³	> 10 to ≤ 15 kg/m³	> 15 to ≤ 25 kg/m³	> 25 kg/m³

Protocol application

Calculate stocking density by dividing total fish weight (kg) by total water volume available to fish (m³). Recalculate density regularly (ideally weekly) using predicted fish growth data.

Scoring notes

- At very low densities for commercial settings (e.g., < 5 kg/m³) and at level 2 and level 3, it is recommended to increase monitoring frequencies for other welfare indicators such as aggressive behavior, water quality impacts, and mortality.
- When other important welfare indicators (such as dissolved oxygen, mortality, and aggressive behavior) score poorly, consider adjusting stocking density.
- **Validation:** The prevalence thresholds are derived from considering the results in the studies listed below, as well as regulatory thresholds (the RSPCA Assured rainbow trout standard sets a limit of 15 kg/m³ over the site, and 17 kg/m³ in any one enclosure, and Norwegian regulation[35] sets the maximum allowable stocking density for ongrowers in sea cages at 25 kg/m³). The level 0 threshold has been set at 10 kg/m³ as a precaution, however no papers cleanly test this exact density against higher densities.

Key evidence

Source	Summary of source	Key result
Zahedi et al., 2019	Experimental study investigating the effects of stocking density on health status in female ongrowing rainbow trout (405 g) in freshwater laboratory conditions over 60 days. Sampling was conducted every 20 days. Three different stocking densities were tested: 12, 24, and 44 kg/m³. One additional group was considered the 'reduced density' group, which moved from a density of 44 to 12 kg/m³ on day 40 for the final 20 days. To maintain the desired stocking density, the required number of fish was removed weekly.	High density significantly reduced the final weight and condition factor. The reduced-density group did not have different growth parameters compared to the high-density group. The low-density group had no mortality, whereas the medium- and high-density groups had mortality rates of 1.5% and 2.5%, respectively. The gene expression of trout in the high-density group was indicative of chronic stress, with the low-density group exhibiting the lowest stress marker expression.
Liu et al., 2016	Experimental study investigating the effects of stocking density on health status in ongrowing rainbow trout (114 g) in laboratory conditions over 300 days. Fish were initially housed in triplicate groups at three stocking densities: 4.6, 6.6, and 8.6 kg/m³. At the end of the experiment, densities reached 31.1, 40.6, and 49.3 kg/m³. 12 fish per density group were sampled at regular intervals.	There were limited differences between the groups before 60 days, when all groups had densities below 16 kg/m³ (8.9, 12.7, and 15.9 kg/m³, respectively). At day 120, the groups had densities of 12.0, 16.4, and 20.0 kg/m³, respectively. At this point, the highest-density group (20 kg/m³) showed a significantly higher cortisol response compared to the lowest-density group, though this difference was downregulated by day 300. Low-density fish achieved significantly higher weights and lengths. High-density groups also had significantly higher mortality (3.35% vs. 2.21% in low-density groups). High-density fish also showed elevated plasma levels, indicating chronic stress.
Aksakal et al., 2011	Experimental study investigating the effects of stocking density on stress	The study demonstrated significantly elevated kidney G6PD activity and

Source	Summary of source	Key result
	and oxidative proteins on ongrowing rainbow trout (130 g) in laboratory conditions over 2 months. Four different stocking densities were tested: 15, 20, 25, and 30 kg/m³. Consistent water quality parameters were maintained.	significantly depressed muscle G6PD activity at 20 kg/m³ and above, with HSP70 mRNA expression significantly increased at 25 kg/m³ and 30 kg/m³. These changes indicate that increasing stocking densities beyond 15 kg/m³ induces additional stress in rainbow trout.

Dissolved oxygen

Adequate dissolved oxygen is fundamental for all metabolic processes in fish, and therefore dissolved oxygen is one of the most critical environmental parameters to measure. Dissolved oxygen levels can fall due to high stocking density, elevated water temperature, poor water exchange, or other environmental factors such as algal blooms. Both hypoxia (low oxygen) and hyperoxia (excessive oxygen) are major physiological stressors, compromising health, immunity, and overall welfare, and can lead to morbidity and mortality.[36]

Level 0	Level 1	Level 2	Level 3
> 90 to ≤ 110 %	> 80 to ≤ 90 % **OR** > 110 %	> 60 to ≤ 80 %	≤ 60 %

Protocol application

Regular (e.g., weekly) calibration of monitoring devices against known standards is required to ensure accurate measurements.

Measuring dissolved oxygen at various points is crucial for a comprehensive understanding of the system's environment. This involves taking readings at different depths (e.g., surface, mid-column, and bottom) as well as at different horizontal locations (e.g., center and cage edge). Readings should also be taken from areas with the poorest expected circulation, such as the depth with the highest fish density. This is important because oxygen levels can stratify in specific areas due to factors such as temperature and stocking density.

It is recommended to monitor oxygen levels continuously, with manual verification at least twice daily, at dawn and dusk due to the photosynthesis and respiration cycles of algae/plankton impacting oxygen levels. The duration of suboptimal conditions should also be noted, with increased monitoring of fish behavior during these periods. When taking multiple readings in a pen, use the worst-scoring reading within the assessment period for overall scoring.

Scoring notes

- If the oxygen monitoring device measures the absolute oxygen level and does not provide a saturation reading, temperature compensation must be applied. Specific calculators can be used to determine the saturation.
- Due to diurnal variation, expect daily fluctuations, with morning readings typically being the lowest.

- Depth stratification can occur, with oxygen depletion possible in deeper layers.
- Feeding may affect the availability of oxygen, with temporary oxygen depletion occurring post-feeding in intensive systems.
- At level 2 and level 3, monitor fish behavioral changes, such as an increase in surface swimming.
- Acute oxygen depletion or oversaturation (e.g., < 4 hours) is less concerning than sustained exposure.
- Oxygen interacts with temperature. Higher temperatures reduce the water's oxygen capacity while increasing fish oxygen demand, meaning monitoring oxygen levels is especially important during high-temperature periods.
- **Validation:** The above oxygen levels have been validated only for temperatures for 13–21°C, based on available rainbow trout studies. The effects of oxygen levels outside this range are unclear, though as temperature declines, lower saturation levels should be tolerable. If using these thresholds for temperatures outside of this range, proceed with caution. Further, very high saturations (e.g., > 110%) have only been studied to a limited extent. Very high saturation systems should be treated with caution. The health and welfare status of the fish is also important, as gill issues or high stress levels may impact the levels of oxygen tolerated, with the above thresholds needing to be treated conservatively in these cases.

Key evidence

Source	Summary of source	Key result
Waldrop et al., 2020	Experimental study investigating the impacts of dissolved oxygen concentration and swimming exercise on rainbow trout (from 18 g to 1 kg) in freshwater laboratory conditions over 341 days. Dissolved oxygen was measured at high (100% saturation) and low (70% saturation) levels. Swimming exercise was also tested at high levels (1.5–2.0 body lengths/second) and low levels (~0.5 body lengths/second).	Fish in the high dissolved oxygen groups showed significantly better growth, reaching 1019 g vs. 878 g. The higher oxygen group also had reduced caudal fin damage. Mortality in the 100% dissolved oxygen group was 1.95%, and in the 70% dissolved oxygen group was 1.30%, but this was not statistically significant.
Park et al., 2023	Experimental study investigating the impact of dissolved oxygen concentration on 1080 rainbow trout (123 g) in freshwater RAS over 8 weeks. 4 different dissolved oxygen levels were tested: 5–6 mg/L, 9–10 mg/L, 14–	Fish in the lowest oxygen group had significantly reduced growth rates, weight gain, length growth, and daily feed intake. They also had elevated growth hormone and hemoglobin/ hematocrit levels, indicating physiological stress.

Source	Summary of source	Key result
	15 mg/L, and 17–18 mg/L. The temperature was 17.6°C. Converting these to oxygen saturation levels (assuming 0 salinity and sea-level atmospheric pressure of 101.32 kPa), the four oxygen saturations tested were: ~52%–62%, ~93%–104%, ~145%–155%, and ~176%–186%.	The higher oxygen groups did not show significant differences in growth performance. The ~93%–104% group had the best growth performance and the most balanced hormone levels. The hormone Insulin-like Growth Factor-1 was also highest in this group, indicating better conditions.
Jiang et al., 2021	Experimental study investigating the impact of temperature and oxygen on 360 ongrowing rainbow trout (110 g) in freshwater RAS over 28 days. Three temperatures (13°C, 17°C, and 21°C) and two dissolved oxygen levels (4.2 mg/L (hypoxic) and 9.6 mg/L (hyperoxic)) were tested. There were 6 treatments for each combination of temperature and oxygen, with 3 replicates each, resulting in 18 tanks with 20 fish per tank. Converting to oxygen saturation levels (assuming 0 salinity and a sea-level atmospheric pressure of 101.32 kPa), the low-oxygen treatments (4.2 mg/L) resulted in 40% saturation at 13°C, 43% saturation at 17°C, and 47% saturation at 21°C. The high-oxygen treatments (9.6 mg/L) resulted in 91% saturation at 13°C, 99% saturation at 17°C, and 108% saturation at 21°C.	High dissolved oxygen levels improved survival at all temperatures and enhanced growth performance and feed consumption. Under low oxygen conditions, the 13°C treatment (40% saturation) achieved 86.7% survival with 0.89% growth rate, the 17°C treatment (43% saturation) achieved 86.7% survival with 1.15% growth rate, and the 21°C treatment (47% saturation) showed severely compromised welfare with only 70% survival and 0.36% growth rate. Under high oxygen conditions, the 13°C treatment (91% saturation) achieved 93.3% survival with 1.20% growth rate, the 17°C treatment (99% saturation) achieved the best performance with 96.7% survival and 1.46% growth rate, and the 21°C treatment (108% saturation) maintained 90.0% survival with 0.95% growth rate.

Temperature

Water temperature influences the physiological processes in rainbow trout. As ectothermic animals, rainbow trout rely on the external temperature of water to regulate their own body temperature. Deviations from preferred temperatures (particularly if rapid) can cause physiological stress, including changes in metabolic rates, respiration, blood pH, and osmoregulation. Temperature is a compounding welfare indicator because water's capacity to hold dissolved oxygen decreases with increasing temperature. Consequently, it can be challenging to separate the direct effects of temperature from those of reduced oxygen availability.

Level 0	Level 1	Level 2	Level 3
> 13 to ≤ 16 °C	> 10 to ≤ 13 °C **OR** > 16 to ≤ 18 °C	> 4 to ≤ 10 °C **OR** > 18 to ≤ 20 °C	≤ 4 °C **OR** > 20 °C

Protocol application

Measuring temperature at various points is crucial for a comprehensive understanding of the system's environment. This involves taking readings at different depths (e.g., surface, mid-column, and the bottom).

It is recommended to monitor temperature continuously, with manual verification at least twice daily, at dawn and dusk. This is to capture the minimum and maximum water temperatures for the day. Where temperature is measured at a single depth only, use the 24-hour average temperature from all readings to determine the score level. Where temperature is measured at multiple depths, take the 24-hour average separately for each depth, and then use the most favorable of these per-depth averages as the final score level.

Extreme readings (e.g., 2°C above or below the daily average) and rapid temperature changes (e.g., a change of 3°C in 24 hours) should be flagged for investigation. The duration of suboptimal conditions should also be noted, with increased monitoring of fish behavior during these periods.

Scoring notes

- Temperature tolerance in fish depends on factors such as species, past acclimation, physiological capabilities, and health status.
- Natural thermal tolerance varies seasonally. Anticipate temperature challenges during extreme weather.

- Gradual temperature changes are better tolerated than rapid fluctuations.
- Poor water movement can create temperature gradients.
- Low temperatures can reduce feeding and slow growth, while high temperatures can increase respiratory rates, reduce oxygen-carrying capacity, cause stress, and increase the risk of mortality.
- **Validation:** The thresholds are primarily based on evidence from rainbow trout in freshwater systems and have not been validated for rainbow trout in seawater conditions.

Key evidence

Source	Summary of source	Key result
Sutterlin and Stevens, 1992	Experimental study investigating thermal preferences of 6 triploid rainbow trout (1.2–2.3 kg) in commercial sea cages in a stratified Newfoundland fjord (10–24 ppt) across two trials of 4–5 days each, during summer. Fish distribution was measured using acoustic telemetry tags.	The average selected temperature was 13.3°C, regulating body temperature between 13°C–14°C. Individual trout ranged from 10.8°C (somewhat of an outlier) to 14.5°C mean temperatures, with a total range of 7.2°C to 17.3°C. A diel cycle was observed, with 5 trout showing a 12-hour rhythm, spanning 3–4°C around the mean. Fish tended to stay in the upper 2m of the water (where it was warmer), and 94% of observations were in the top 4m. Salinity also increased with depth, and the authors noted that temperature preference could not be fully separated from salinity avoidance.
Jiang et al., 2021	Experimental study investigating the impact of temperature and oxygen on 360 ongrowing rainbow trout (110 g) in freshwater RAS over 28 days. Three temperatures (13°C, 17°C, and 21°C) and two dissolved oxygen levels (4.2 mg/L (hypoxic) and 9.6 mg/L (hyperoxic)) were tested. There were 6 treatments for each combination of temperature and oxygen, with 3 replicates each, resulting in 18 tanks with 20 fish per tank.	Although temperature did not have a statistically significant impact on mortality, mortality rates correlated with temperature, particularly at the low dissolved oxygen levels. At high dissolved oxygen levels, survival rates were 93.3% at 13°C, 96.7% at 17°C, and 90.0% at 21°C. At low dissolved oxygen levels, survival rates were 86.7% at 13°C, 86.7% at 17°C, and 70% at 21°C. 17°C also had the highest growth rates and feed consumption, with 21°C having the lowest final weights,

Source	Summary of source	Key result
		growth rates, and feed intake. 13°C and 17°C yielded generally good welfare indicators with minimal stress responses, whereas 21°C showed some welfare concerns.
Cocherell et al., 2014	Experimental study investigating temperature preferences of 40 ongrowing rainbow trout (568 g) using a freshwater chamber which had a temperature gradient from 12°C to 24°C. 3 groups of fish were acclimated to different temperatures (12°C, 15°C, and 18°C) for 15–22 days, then individually tested in the gradient chamber for 1 hour.	12°C- and 15°C-acclimated fish preferred temperatures of 15.8–16.2°C. 18°C acclimated trout preferred a significantly warmer temperature of 18.4°C. The 12°C and 15°C groups actively avoided temperatures higher than 19°C. The 18°C group avoided temperatures below 16°C and above 20°C.
Bear, 2005	Experimental study investigating the survival and growth at different temperatures in juvenile rainbow trout (mean weight ranging from 13.9 to 38.3 g) in freshwater laboratory conditions over 60 days. Survival and growth were assessed at 2°C increments between 8 and 30°C. 36 tanks were used, with 50 fish per tank and 3 replicates per temperature.	The study found that the best growth and condition occurred between 10–18°C, with 12–16°C being the optimal performing range. Feed conversion efficiency declined significantly above 20°C, and growth ceased entirely above 24°C. Survival remained high between 8–18°C, with these temperature groups showing survival rates of 96.3%–100%. However, at 20°C, survival dropped to 92.9%, but increased again to 98.6% at 22°C. Above 24°C, conditions became rapidly lethal for rainbow trout. At 24°C, the survival rate fell dramatically to 72.8%, but at 26°C, there was a massive die-off with only 1.3% survival, and at 28°C, there was complete mortality.
North et al., 2006	Experimental study investigating the impact of stocking density on 100 female rainbow trout (starting at 180 g) in freshwater laboratory conditions over 9 months.	There was a correlation between increased plasma cortisol and water temperatures below 4°C between January and April, particularly in the 10 kg/m^3 group. The authors noted

Source	Summary of source	Key result
	Three stocking densities were tested: 10, 40, and 80 kg/m^3. Water temperature ranged from 0.1°C to 13.2°C.	evidence that cortisol levels are elevated during the winter months in juvenile salmon.

Carbon dioxide

Carbon dioxide (CO_2) is a waste product of fish respiration and so will always be present to some degree in water where fish are produced. However, elevated levels can pose a threat to fish welfare, particularly when other water quality parameters, such as low dissolved oxygen, are present. High CO_2 levels can impede a fish's ability to excrete CO_2 from its blood, which is vital for maintaining blood pH and the blood's capacity to transport oxygen effectively. Rainbow trout in seawater systems are more sensitive to CO_2 than in freshwater systems.

Level 0	Level 1	Level 2	Level 3
≤ 5 mg/L	> 5 to ≤ 8 mg/L	> 8 to ≤ 15 mg/L	> 15 mg/L

Protocol application

Regular calibration of the sensor is especially important for detecting low CO_2 levels.

It is unlikely that CO_2 will need to be assessed in sea cages, and so only needs to be monitored on a risk-assessed basis. It is recommended to measure CO_2 in areas of highest fish density and poorest water circulation (near the bottom in stratified systems, such as in sea cages).

Use a reliable method to measure CO_2, rather than calculating it from pH levels, for example. A temperature correction may be needed, as CO_2 solubility and toxicity vary with temperature.

Scoring notes

- Account for carbonate buffering in seawater measurements.
- Reduced appetite is an early indicator of CO_2 stress.
- Monitor for reduced growth rates and changes in blood pH under moderate CO_2 exposure, indicating physiological impacts.
- Diurnal variation results in CO_2 being typically highest in the early morning.
- CO_2 and pH are closely linked, and both should be measured.
- Post-feeding spikes of CO_2 may result in temporary increases, but should resolve within 2–4 hours.
- Acute CO_2 increases (e.g., for less than 4 hours) are less concerning than sustained exposure.
- Monitor for behavioral changes, including reduced feeding and surface gasping during elevated CO_2 periods.

- **Validation:** Limited rainbow trout-specific studies exist, with evidence from Atlantic salmon also relied upon. Threshold validation is also limited.

Key evidence

Source	Summary of source	Key result
Pfalzgraff et al. (2025)	Experimental study investigating growth, appetite, and mineral deposition under different CO_2 regimes in juvenile rainbow trout in RAS over 5 weeks. Three trials were conducted, each involving 360 trout: two in freshwater and one in seawater. 4 CO_2 conditions were tested: control (≤ 3 mg/L CO_2), medium (10 mg/L CO_2), high (25 mg/L CO_2), and fluctuating (daily oscillation between 10–25 mg/L CO_2).	In freshwater, only 25 mg/L CO_2 significantly reduced growth (by 8–13%). However, the effects were more severe in seawater: 10 mg/L CO_2 reduced growth, and 25 mg/L reduced growth by 44%. Condition factor was also measured in the seawater trial. The high CO_2 group had a significantly lower condition factor than the control. Similarly, in the high CO_2 group, blood pH was significantly higher compared to all other groups. In seawater, fish had a 35% reduction in feed intake. In freshwater, fish ate their full ration. Blood pH increased in high CO_2 groups, and kidney mineral deposits also increased.
Khan et al., 2018	Experimental study investigating the effects of acute and long-term exposure to CO_2 on 32 juvenile Atlantic salmon (20 g) in laboratory conditions over 47 days. For the chronic exposure experiments, fish were raised in 4 different CO_2 concentrations, 2.9, 10, 15, and 25 mg/L.	There was a 17% decrease in growth performance and a 21% increase in feed conversion between 2.9 mg/L and 25 mg/L CO_2. There was a linear relationship between decreased growth performance and feed conversion across all doses, with both 10 mg/L and 15 mg/L being progressively worse than the 2.9 mg/L control. The condition factor was significantly higher at 2.9 mg/L than in the other groups.
Good et al., 2010	Experimental study investigating the effects of chronic exposure to CO_2 on	The control achieved a survival rate of 100%, while the low- and high-CO_2

Source	Summary of source	Key result
	4800 rainbow trout in freshwater RAS over 6 months. Fish were raised in replicated RAS systems at either 8 mg/L or 24 mg/L CO_2 exposure, with flow-through tanks serving as controls.	groups had 96.5% and 98.3% survival rates, respectively. The final weight was 938 g in the control group, approximately 13% lower in the low CO_2 group (817 g), and 10% lower in the high CO_2 group (849 g). Lymphocytic hepatitis was significantly more likely in the high CO_2 group, while epithelial hyperplasia was significantly more likely in the low CO_2 group. The prevalence of lesions associated with reduced water quality was similar across all groups, if not higher in the control.

pH

Stable pH within an appropriate range supports normal gill function, osmoregulation, and blood pH regulation in fish. pH is a measure of the acidity or alkalinity of water, reflecting the concentration of hydrogen ions. pH in seawater typically ranges from 7.5 to 8.5. Rapid changes to pH should be avoided. Extreme pH values can cause direct tissue damage, particularly to gill structures, compromising oxygen uptake and ion regulation. Maintaining stable, appropriate pH levels is essential for minimizing stress and ensuring proper physiological function in sea cage ongrowing systems.

Level 0	Level 1	Level 2	Level 3
> 7.8 to ≤ 8.2 pH	> 7.5 to ≤ 7.8 pH **OR** > 8.2 to ≤ 8.5 pH	> 7 to ≤ 7.5 pH **OR** > 8.5 to ≤ 9 pH	≤ 7 pH **OR** > 9 pH

Protocol application

Calibrate the pH meter or probe (glass electrode recommended for accuracy in seawater) daily, using at least two buffer solutions spanning the expected range (e.g., pH 4.0, 7.0, and 10.0).

It is recommended to measure pH once a day. Measuring pH at various points is crucial for a comprehensive understanding of the system's environment. This involves taking readings at different depths (e.g., surface, mid-column, and the bottom) and at different horizontal locations.

When taking multiple readings in a pen, use the worst score within the assessment period for overall scoring.

If there is a change in pH, note whether it is due to a brief event (e.g., a storm or algal bloom) or to a chronic condition.

Scoring notes

- Acute (e.g., < 24 hours) and infrequent (e.g., once per month) exposure to suboptimal pH levels will have less severe welfare impacts than chronic (e.g., > 7 days) or frequent (e.g., > 30 days per year) exposure.
- If level 3 pH occurs frequently at the site, the site is not suitable as welfare cannot be maintained at the location.
- pH levels should not be considered in isolation, but also in reference to total alkalinity, dissolved carbon dioxide, and other substances such as ammonia.

- **Validation:** The evidence on the impacts of different pH levels for rainbow trout in seawater is extremely limited, with the majority of data investigating rainbow trout in freshwater. These findings were adjusted upwards to account for naturally higher pH levels in seawater. Level 0 was based on typical seawater pH ranges.

Key evidence

Source	Summary of source	Key result
Laurent et al., 2000	Experimental study investigating the effect of highly alkaline freshwater on the morphology of the gills in ongrowing rainbow trout (175–250 g) in laboratory conditions over 72 hours. Fish were either exposed to pH 9.5 or to pH 8 (control).	Fish in the 8 pH group showed normal physiological functions. The 9.5 pH group had negative welfare impacts, even in the first 10 hours. This included a reduction in essential ion uptake, decreased ammonia excretion, and loss of functional gill cells. After 24 hours, blood ammonia rose to nearly seven times normal.
Winter et al., 2005	Experimental study investigating the effects of aluminium exposure on juvenile rainbow trout (~0.6 g) in laboratory conditions over ~2 days. Water pH ranged between 4 and 10. The trout were exposed to 3 μmol Al/L in ion-poor water. There was a control group with no aluminium, with a pH of 7.2.	Aluminium accumulation was pH-dependent. The highest was at pH 6–8, then pH 5 and 9, with no accumulation at pH 4 and 10. The pH 7–8 groups had no mortality over the course of the experiment, despite aluminium accumulation. There was a 20% mortality rate in the groups with pH 9, 6, 5, and the control. There was a 100% mortality rate in the pH 10 and 4 groups, caused by gill damage, decreased plasma ion concentrations, impaired ammonia elimination, and disrupted ion homeostasis. It is difficult to separate the effects of pH and aluminium.
Mota et al., 2016	Experimental study investigating the effects of water exchange rate and pH on hormonal levels in 250 juvenile rainbow trout (90 g) in laboratory conditions over 70 days. Three treatments were tested: High	Fish in low-pH systems had significantly higher plasma cortisol levels at the end of the experiment, indicating stress. Water cortisol concentrations and testosterone levels were also

Source	Summary of source	Key result
	water exchange and neutral pH (~pH 7.3), low water exchange and neutral pH (~pH 7.3), and low water exchange and low pH (~pH 5.8).	significantly higher in low pH systems halfway through the experiment. These concentrations were also higher at the end of the experiment, but these results were not significant. No significant differences were found between survival, growth, or feed conversion.
Calabrese et al., 2023	Experimental study investigating the effects of water flow rate on 2500 post-smolt Atlantic salmon in intensive seawater laboratory flow-through systems. Four treatments were tested: 0.2, 0.3, 0.4, and 0.6 L/kg of fish/min. In these treatments, pH was 6.94, 7.2, 7.37, and 7.46 respectively. With increased water flow, there was lower carbon dioxide and ammonia.	Blood pH was significantly elevated in the lowest flow treatment (and therefore lowest pH), indicating compensation. After 2 weeks, blood pH had been restored in the 0.3, 0.4, and 0.6 L/kg of fish/min (corresponding to pH of 7.2, 7.37, and 7.46), but not in the 0.2 L/kg of fish/min treatment (corresponding to a pH of 6.94).

Turbidity

Clear water allows fish to see their surroundings, detect feed, and respond to social and environmental cues. Turbidity is a water quality parameter that relates to water clarity, indicating how materials in the water scatter light. Turbidity is most accurately measured by nephelometry in Nephelometric Turbidity Units (NTU). High turbidity can reduce fish's ability to see, affecting behaviors such as feeding.

While turbidity and Total Suspended Solids are often discussed together, they do not necessarily always correlate with each other.[37]

It is difficult to give recommendations that accurately reflect welfare in all cases, as turbidity can increase from suspending different materials, each of which may have varying impacts on welfare. The given thresholds should be applied with caution.

Turbidity (NTU)

Level 0	Level 1	Level 2	Level 3
≤ 4 NTU	> 4 to ≤ 20 NTU	> 20 to ≤ 40 NTU	> 40 NTU

Secchi disc depth

Level 0	Level 1	Level 2	Level 3
> 6 m	4–6 m	≥ 2 to < 4 m	< 2 m

Overall: Overall score is determined by the most severe subindicator

Protocol application

Turbidity can be measured with a nephelometer. Handheld nephelometers can provide spot checks, while submersible sensors can provide continuous monitoring. Secchi discs are often used in sea cage systems, being lowered into water until no longer visible, measuring visibility depth in meters. Scoring under both measures has been provided.

It is recommended to measure turbidity once a day, with increased monitoring during high-risk periods (such as storms, nearby construction, algal blooms). Turbidity should be measured at consistent times, avoiding post-feeding periods, as turbidity may temporarily increase due to leftover feed. Measuring turbidity at various points is crucial for a comprehensive understanding of the system's environment. This involves taking readings at different depths (e.g., surface, mid-column, and the bottom), upstream and downstream of cages, and at the place of highest fish density.

Note the turbidity source when identifiable (e.g., sediment, algae, organic matter, or feed waste), as well as the spatial distribution (e.g., whether it is localized or system-wide).

Scoring notes

- Nephelometers should be calibrated using a consistent methodology, with the same measurement angle and depth.
- Inorganic sediments are generally more harmful than organic particles, causing gill irritation and reducing feeding efficiency. Algal turbidity may indicate water quality issues, but it is less physically damaging. Localized, temporary waste particles are less concerning than other sources of environmental turbidity. It is critical to identify the source of turbidity for an appropriate management response.
- Storm- or construction-related turbidity is usually temporary, but can be severe and require immediate attention.
- Acute exposure to elevated turbidity levels (e.g., < 24 hours) has less severe welfare impacts than chronic exposure (e.g., > 7 days).
    - Short-term elevations (e.g., < 24 hours) are generally minimally harmful to welfare if not recurring, especially at levels of 10–20 NTU.
    - Sustained exposure (e.g., 20–40 NTU for 24–48 hours at the higher end, or multiple days to weeks at the lower end) increases the risk of impaired feeding, behavior changes, and gill stress. Sustained exposure at this level requires management action.
    - Chronic exposure over multiple weeks to months can result in severe impairment, especially at levels > 25–30 NTU.
    - Where there is chronic exposure, consider temporary fish relocation if possible.
- **Validation:** There is limited rainbow trout-specific data and the majority of data comes from freshwater sediment studies, which may not translate to turbidity in sea cages. The Secchi disc depth thresholds have been developed based only on one paper (Montes et al. (2018)), which was conducted on Atlantic salmon in Chilean fjords, where the focus of the paper was the impact of algal blooms. The thresholds represent expert judgment and precautionary principles rather than validated, evidence-based welfare boundaries. Thresholds should be applied with caution. The thresholds also do not account for particle composition, which is more important than absolute NTU values.

Key evidence

Source	Summary of source	Key result
Birtwell et al., 2008	Report and literature review investigating the appropriateness of using turbidity as an indicator to protect aquatic organisms (including	Canadian and British Columbia Guidelines consider clear water to be water with < 8 NTU, allowing an increase of 8 NTU over the long term

Source	Summary of source	Key result
	salmonids) during land development in the Pacific and Yukon Region (freshwater).	and 2 NTU over the short term (< 24 hours). < 3 NTU is assessed to have no risk, and < 8 NTU is assessed to have a low risk of significant impairment for clear water fish if maintained for a duration of at least 3 weeks. Continuous exposure to 9–20 NTU was considered to carry a moderate risk of significant impairment if maintained for a duration of at least 5 days. Another reference considered slight impairment on behavioral effects to manifest after exposure to 10 NTU for 1 day, 15 NTU for 8 hours, or 23 NTU for 3 hours. An exposure to 21 NTU or higher for over 3 hours was considered to pose a significant threat to significant impairment. Exposure to 25 NTU (for dry conditions) and 100 NTU (for wet conditions) was considered a harmful level. Cited studies included results demonstrating that turbidity of 25 NTU resulted in reduced growth rate for juvenile coho salmon exposed for about 2 weeks. Additionally, experimental results showing a 62% reduction in brook trout growth at 40 NTU were extrapolated to estimate a 50% reduction in growth at 25 NTU. Rainbow trout exposed to 23 NTU turbidity exhibited altered behavior, including more frequent movement between cover and feeding areas, longer exposure to predators, and reduced foraging efficiency. Growth was also negatively impacted, likely due to impaired prey capture and increased metabolic demands during feeding.

Source	Summary of source	Key result
Bash et al., 2001	Literature review on the impacts of turbidity and suspended solids on salmonids, with a focus on salmonids in freshwater environments.	Turbidity levels can have a range of effects on salmonids. This includes gill trauma, osmoregulation, behavioral avoidance, and territoriality. However, salmonids are able to cope with some level of turbidity. One reviewed paper suggested that salmonid populations not normally exposed to high levels of natural turbidity could be negatively affected by relatively low levels of turbidity (18–70 NTU). Several papers reviewed suggested that even very low levels of turbidity (e.g., 6–7 NTU) had some negative impacts on welfare, e.g., reduced feeding and avoidance behaviors. In one study, coho salmon juveniles were exposed to ~60 NTU, at which point disruption of social interactions, including aggressive behaviors, was observed. Once turbidity dropped to ~20 NTU, these interactions were re-established, marking a behavioral threshold for visibility and social stability. A number of studies indicated that aggressive behaviors decreased at higher turbidities, although fish preferred to avoid high-turbidity environments.
Sigler et al., 1984	Experimental study investigating the effects of turbidity on the preference and growth of juvenile rainbow trout and coho salmon in laboratory conditions over 14 to 21 days. There was a treatment group that had a turbidity level of nearly 0 NTU. The exposure group was exposed to	Generally, fish in clear waters had significantly higher growth rates than fish in turbid waters. The authors noted that large amounts of suspended particles could reduce fish's ability to see food. Consistently fewer fish chose to stay in the turbid channels compared to the clear water channels.

Source	Summary of source	Key result
	turbidities between 25 NTU and 86 NTU.	
Montes et al., 2018	Statistical analysis of an extensive database spanning from 1989–2016 in southern Chile, investigating harmful algal bloom thresholds for farmed Atlantic salmon. The primary focus was to quantify critical thresholds of *A. catenella* and *P. cf. verruculosa*. Covariates included Secchi depth readings and fish behavior (where the data was recorded as ‘normal’ or ‘abnormal’).	Secchi depth was a fixed factor in the analysis with four levels: 1–2m, 2–4m, 4–6m, and > 6m. The deeper the Secchi reading, the higher probability of observing ‘normal’ behavior. At Secchi readings with a depth of 1–2m, there was at least a 50% chance of observing abnormal behavior, regardless of the abundance of algal cells. The authors noted that this Secchi depth should be treated as a critical threshold. Fish had lower rates of abnormal behavior at greater Secchi depths, even where the algal cell abundance was the same. For example, at around 568 algal cells/mL, where the Secchi reading was 1–2m there was approximately an 80% probability of abnormal behavior, while where the Secchi reading was > 6m, there was only approximately a 30% probability of abnormal behavior.

Total suspended solids

Total Suspended Solids (TSS) refers to the amount of solid material above 1 μm in diameter that is suspended in the water.[38] These solids can consist of various substances, including uneaten feed, fish fecal matter, algae, and other waste. The impact of TSS on fish welfare is complex and not only depends on the absolute amount of TSS, but also on the characteristic makeup of the suspended particles (such as size, composition, and abrasiveness).[39] In sea cages, TSS levels are largely determined by environmental conditions, stocking density, feeding practices, and water flow, though farmers have limited ability to control TSS once elevated. Increased TSS and turbidity levels often co-occur, but measure different aspects. Turbidity primarily affects feeding behavior through visual impairment, while TSS primarily affects gill function through particle irritation and bacterial load.

Level 0	Level 1	Level 2	Level 3
≤ 10 mg/L	> 10 to ≤ 25 mg/L	> 25 to ≤ 40 mg/L	> 40 mg/L

Protocol application

Measuring TSS typically requires expensive equipment or sending samples to a laboratory. Turbidity can be used as a proxy indicator where it is not possible to measure TSS, though turbidity and TSS do not necessarily correlate.

It is recommended to measure TSS in high-risk periods, (such as storms, nearby construction, algal blooms, and where turbidity readings are high). Measuring TSS at various points is crucial for a comprehensive understanding of the system's environment. This involves taking readings at different depths (e.g., surface, mid-column, and bottom) and at multiple horizontal locations (e.g., at the center and near the net edges).

Note the TSS source when identifiable (e.g., clay, sand, organic matter, or feed waste), as well as the spatial distribution (e.g., whether it is localized or system-wide).

Scoring notes

- Acute exposure to elevated TSS levels (e.g., < 24 hours) has less severe welfare impacts than chronic exposure (e.g., > 7 days).
- **Validation:** The thresholds were decided based on expert opinion and evidence, mostly considering typical organic biosolids (such as feed waste and fecal matter). Abrasive inorganic sediments (such as clay, silt, or sand) or algal blooms may cause welfare impacts at lower concentrations. Further, there is limited rainbow trout-specific evidence

(particularly in seawater) assessing the full range of TSS levels, e.g., the 10–30 mg/L range has not been tested.

Key evidence

Source	Summary of source	Key result
Becke et al., 2019	Experimental study investigating the effects of suspended solids (system-generated organic particles) and unionized ammonia on 785 juvenile rainbow trout (87 g) in RAS over 13 weeks. The control group had ~5 mg/L TSS, while the treatment had > 35 mg/L. Unionized ammonia increased at week 6 (by ~0.012 mg/L) and week 11 (by ~0.025 mg/L) in both groups.	The higher TSS levels did not show any detrimental histological alterations in the gills. Even at levels up to nearly 70 mg/L, no direct issues were observed in the fish. Increased TSS levels caused indirect effects, increasing turbidity and suppressing feeding behavior. A higher bacterial load in the gills and spleen was found in the higher TSS group.
Ross et al., 1985	Experimental study investigating the impact of estuarine silt on respiratory behavior in 15 juvenile rainbow trout (66–115 g) in laboratory conditions with 60-minute exposures. For suspended solids treatment, 5 fish were exposed to estuarine silt at three concentrations (10, 100, 1000 mg/L) for 1 hour.	All three groups showed increases in ventilatory activity (40–65%) and coughing activity (150–270%) with exposure. The 100mg/L group had a 690% increase in oxygen, higher than the 1000 mg/L group, which had a 480% increase. Oxygen consumption data were not collected for the 10 mg/L group. In terms of recovery, cough rates in the 1000 mg/L treatment group did not fully return to baseline levels after 3 hours, whereas they did in the lower-concentration groups. The 1000 mg/L group also noted a marked increase in swimming activity.
Noble et al., 2020	Literature review on many rainbow trout welfare indicators, including TSS.	A definitive threshold value for an acceptable level has not been agreed upon. Still, an upper limit of 15 mg/L has been suggested for Atlantic salmon, and the RSPCA recommends a maximum concentration of non-

Source	Summary of source	Key result
		suspended solids of < 25 mg/L for all life stages of rainbow trout.

Ammonia

NH_3 is the unionized form of ammonia, which can cross from water into fish. Where there are high levels of ammonia in the environment that the rainbow trout are in, this can mean that they reduce the amount of ammonia they excrete, or even uptake ammonia from the water. The pH and temperature of the water significantly affect the amount of NH_3, which increases with higher pH and temperature. Higher salinities result in lower NH_3 levels. Ammonia can cause physiological welfare issues, such as gill damage, osmoregulatory stress, and even mortality. In sea cages, ammonia levels are typically low due to high water exchange rates, but can accumulate in areas of poor flow, high stocking density, or during harmful algal blooms that elevate pH.

Level 0	Level 1	Level 2	Level 3
≤ 0.01 mg/L	> 0.01 to ≤ 0.02 mg/L	> 0.02 to ≤ 0.04 mg/L	> 0.04 mg/L

Protocol application

Most test kits measure TAN (Total Ammonia Nitrogen), not NH_3 directly. If using such a kit, calculate NH_3 from TAN by also recording pH and temperature. The simplified formula for this is: **NH_3 (mg/L) = TAN × f**, where f is the fraction of NH_3 that depends on salinity, pH, and temperature.

It is recommended to measure ammonia monthly in the winter (or other low-risk periods) and weekly in the summer, with increased monitoring during high-risk periods (such as periods of elevated pH or temperature).

It is recommended to take readings at different depths (e.g., surface, mid-column, and bottom), at multiple horizontal locations, and at any location where ammonia accumulation is suspected. Take the worst measurement from the assessment period.

Note the ammonia source when identifiable, as well as the spatial distribution (e.g., whether it is localized or system-wide).

Scoring notes

- Some examples of the value of f at different salinities, pH, and temperatures are set out on the FAO website.
- Online calculators can also be used by entering the TAN and relevant water quality parameters, such as those published by the Florida Department of Environmental Protection.

- Even at low TAN concentrations, high pH can produce high levels of NH_3. For example, at 15°C and pH 7.5, the percentage of TAN that is NH_3 is 0.665%. At pH 8.5, the percentage is about 10 times higher, at 6.28%.
    - During periods of elevated pH (e.g., during algal blooms), especially at pH > 8.5, increased monitoring frequency is essential, as even low TAN readings can result in high NH_3 levels.
- Acute exposure to elevated ammonia levels (e.g., < 24 hours) has less severe welfare impacts than chronic exposure (e.g., > 7 days).
- Persistent level 2 or level 3 scores for ammonia suggest the site's water exchange is insufficient for the current stocking density or feeding practices. Consider reducing stocking density, improving water flow, or reassessing site suitability.
- **Validation:** While a limited number of studies were relied on, a range of ammonia concentrations was tested on adult and juvenile rainbow trout.

Key evidence

Source	Summary of source	Key result
Vosylienė and Kazlauskienė, 2004	Experimental study investigating the sublethal effects of ammonia on adult rainbow trout (1 year old) in laboratory conditions over 14 days. Ammonia concentrations between 0.012–0.09 mg/L NH_3 were tested. The control group had a concentration of 0 mg/L NH_3.	At 0.012 mg/L, there was minimal impact, with most parameters remaining at or near control levels. At 0.024 mg/L, there was a significant increase in old and damaged red blood cell forms, and fewer juvenile red blood cells were being produced. Concentrations of 0.044–0.09 mg/L caused anemia (reduced red blood cells and hemoglobin), immunosuppression, and increased respiratory effort. There was also a tendency for gill ventilation frequency to increase after exposure to levels of 0.024 mg/L or higher.
Thurston et al., 1984	Experimental study investigating the effects of low levels of ammonia on rainbow trout over 5 years. Over these 5 years, the rainbow trout had two generations of offspring. Five ammonia levels ranging from	Histopathological lesions were common at ≥ 0.04 mg/L NH_3, including significant tissue damage in the gills and kidneys. Blood ammonia levels were positively correlated with water ammonia

Source	Summary of source	Key result
	0.01–0.07 mg/L NH_3 were tested under water conditions with a mean pH of 7.7 and a temperature of 9.3°C.	concentrations. The second generation of offspring had significant welfare impacts at a concentration of 0.02 mg/L NH_3, with histopathological lesions common. However, these fish had a severe protozoan infection, which could have made them more vulnerable. However, the 2 preceding generations did not have significant lesions at this level.
Grobler and Wood, 2018	Experimental study investigating the effects of ammonia on dominance hierarchy formation of 84 juvenile rainbow trout (9.56 g) over 11 days in freshwater laboratory conditions. Fish were size-matched to create 21 groups of 4 fish each. There were 5 treatment groups: the control, 0.0515 mg/L, 0.0884 mg/L, and 0.1105 mg/L NH_3, as well as a sodium bicarbonate control.	Ammonia effects were concentration-dependent: at 0.0515 mg/L NH_3, stable hierarchies formed despite a 50% reduction in aggression, with lower growth, oxygen consumption, and cortisol (subordinate fish lost weight). At 0.0884 mg/L NH_3, aggression was 75% lower, and hierarchies were severely disrupted. At 0.1105 mg/L NH_3, aggression was abolished and hierarchies could not form. Studies at higher concentrations terminated at 5 days without physiological measurements. The authors attributed decreased swimming ability to an important cause of the lack of aggressive acts and the absence of a social hierarchy in the NH_3-exposed groups. The lower plasma cortisol at 0.0515 mg/L NH_3 was unexplained, with authors suggesting possible reasons being that the effects of reduced aggression in lowering plasma cortisol were higher than the effects of elevated NH_3 in increasing it, NH_3 having a 'calming' effect due to overall reduced activity, NH_3 impairing the ability to mobilize cortisol, and acclimation to ammonia.

Nitrite

Rainbow trout can absorb nitrite (NO_2^-) from the water through the gills, where it enters the bloodstream. This results in nitrite reacting in the blood, reducing its ability to carry oxygen, which can cause respiratory and cardiovascular stress and eventually anemia. This can happen even if there is otherwise sufficient dissolved oxygen in the water. While nitrite can be a problem in freshwater systems, in sea cages, nitrite is not usually a problem due to the flowing, changing water, and because chloride ions compete with nitrite for transport.[40]

Level 0	Level 1	Level 2	Level 3
≤ 0.5 mg/L	> 0.5 to ≤ 2.5 mg/L	> 2.5 to ≤ 5 mg/L	> 5 mg/L

Protocol application

Nitrite can be measured using colorimetric test kits (e.g., diazotization method) or photometric analysis.

It is generally unnecessary to monitor nitrite levels in seawater conditions. Nitrite only needs to be observed in high-risk scenarios, for example, where poor water exchange is suspected, or a harmful algal bloom is present.

It is recommended to take readings at different depths (e.g., surface, mid-column, and the bottom). Take the worst measurement from the assessment period.

Scoring notes

- Nitrite is generally unlikely to impact rainbow trout welfare in sea cages.
- In lower-salinity systems such as brackish water systems (e.g., 10–20 ppt), chloride protection is reduced, so more conservative thresholds may need to be applied.
- **Validation:** These thresholds have not been validated for rainbow trout and should be applied with extreme caution in lower-salinity systems.
    - The thresholds have been developed by first considering freshwater rainbow trout data (Kroupova et al., 2008 and Wedemeyer and Yasutake, 1978). From that data, thresholds were determined to be < 0.01, 0.01–0.05, 0.05–0.1, and > 0.1 mg/L, corresponding to level 0, level 1, level 2, and level 3.
    - Nitrite was determined to be 50–100 times less toxic in seawater than in freshwater, based on a study conducted on Chinook salmon (Crawford and Allen, 1977), with a seawater salinity of 32.5 ppt.
    - The freshwater rainbow trout thresholds were multiplied by 50 (taking the most conservative approach).

- However, this does not account for any species-specific differences between rainbow trout and Chinook salmon, although chloride-competitive inhibition mechanisms are well established across teleost species.

Key evidence

Source	Summary of source	Key result
Kroupova et al., 2008	Experimental study investigating the effects of subchronic nitrite exposure on 240 juvenile rainbow trout (19 g) in freshwater laboratory conditions over 28 days. 5 nitrite concentrations were tested: 0.01, 0.1, 0.6, 1.0, and 3.0 mg/L. A control group was kept in nitrite-free tap water.	There was no mortality up to 1.0 mg/L nitrite; however, 65% mortality occurred at 3.0 mg/L nitrite. However, even at the lowest tested concentration (0.01 mg/L), trout showed significant welfare impacts, including respiratory damage (abnormal cell growth in gill tissue) and elevated blood glucose levels. As concentrations increased, gill damage progressively worsened, hemoglobin and hematocrit decreased, and white blood cell counts increased.
Wedemeyer and Yasutake, 1978	Experimental study investigating the effects of acute and chronic nitrite exposure on juvenile rainbow trout in freshwater systems. For the acute exposure tests, static 96-hour bioassays were conducted, using duplicate groups of 20 fish per nitrite level. In the chronic exposure tests, trout were exposed to 0.015, 0.03, and 0.06 mg/L of nitrite over 6 months.	The 96-hour LC50 ranged from 0.5 mg/L to 12.1 mg/L depending on water hardness and pH. Acute exposure caused methemoglobinemia (brown blood disease). For the chronic exposure experiments, the study concluded there were no significant reductions in growth or the ability of smolts to adapt to seawater following the chronic exposure. While exposure to 0.06 mg/L caused minimal gill epithelial changes, these issues began to resolve after 7 weeks as the fish adapted to the new water conditions.
Crawford and Allen, 1977	Experimental study investigating the effects of acute nitrite exposure on 140 Chinook salmon (10.2–13.4 g) in both	In freshwater, 19 mg/L NO_2^- caused 50% mortality, and 27 mg/L NO_2^- caused 70% mortality over 48 hours.

Source	Summary of source	Key result
	seawater and freshwater laboratory environments over 48 hours. 31 fish were treated in seawater environments (32.5 ppt), and 109 fish were treated in freshwater environments. In freshwater, 4, 19, and 27 mg/L NO_2^- were tested. In seawater, 100, 815, and 1070 mg/L NO_2^- were tested.	In seawater, 815 mg/L and 1070 mg/L caused 10% mortality in 48 hours. Nitrite was determined to be 50–100 times less toxic in seawater than in freshwater.

Nitrate

Nitrate (NO_3^-) is less toxic than nitrite, but elevated levels can still be a cause for concern for welfare by reducing blood oxygen-carrying capacity. The toxicity of nitrate is influenced by environmental factors, with higher temperatures, lower dissolved oxygen, and higher TSS levels potentially exacerbating nitrate stress. In sea cages, nitrate accumulation is generally not a concern due to continuous water exchange and dilution. However, in areas with agricultural runoff, upwelling events, or localized pollution, nitrate levels may impair fish welfare.

Level 0	Level 1	Level 2	Level 3
≤ 25 mg/L	> 25 to ≤ 50 mg/L	> 50 to ≤ 100 mg/L	> 100 mg/L

Protocol application

Most test kits measure nitrate-nitrogen, not NO_3^- directly. If using such a kit, calculate NO_3^- from nitrate-nitrogen. The simplified formula for this is: **NO_3^- (mg/L) = NO_3-N (mg/L) × 4.43**, ensuring measurements use the same units.

It is recommended to measure NO_3^- monthly in the winter (or other low-risk periods), with increased monitoring during high-risk periods (such as periods of agricultural runoff).

It is recommended to take readings at different depths (e.g., surface, mid-column, and bottom) and at multiple horizontal locations. Take the worst measurement from the assessment period.

Scoring notes

- The standard measurement methods for nitrate are either cadmium reduction (colorimetric) or ion-selective electrode methods.
- High nitrate levels are unlikely in sea cages.
- Where ammonia and nitrate levels are elevated, combined nitrogen stress occurs. In these cases, monitor closely and consider taking action.
- Acute exposure to elevated nitrate levels (e.g., < 24 hours) has less severe welfare impacts than chronic exposure (e.g., > 7 days).
    - When chronic exposure lasts more than 30 days per year, the site may be unsuitable or require seasonal production planning.
- **Validation:** There are few studies on adult rainbow trout, and the thresholds have been derived from studies on juveniles.

Key evidence

Source	Summary of source	Key result
Papadopoulos et al., 2024	Experimental study investigating the influence of water nitrate concentration and elevated temperature on 160 juvenile (8 g) rainbow trout in laboratory conditions over 20 days. Nitrate concentrations of 40 mg/L and 110 mg/L were compared at both 17°C and 21°C.	The condition factor at 110 mg/L was lower than at 40 mg/L. At 110 mg/L, rainbow trout showed multiple stress responses compared to 40 mg/L: inflammatory activation (elevated TNF-α), cellular stress (increased heat shock protein production - HSP70), oxidative stress (higher catalase and Cu/Zn SOD in early phases), and metabolic disruption (reduced glucokinase expression for the majority of the trial period).
Freitag et al., 2015	Experimental study investigating the effects of elevated nitrate on endocrine function in 54 juvenile Atlantic salmon (102 g) in freshwater laboratory conditions over 27 days. Three concentrations were tested: 23.0, 45.6, and 451.0 mg/L nitrate.	Plasma nitrate levels increased significantly with increasing concentration. There was minimal impact on welfare in the lowest nitrate group. Testosterone was significantly elevated in fish exposed to 45.6 mg/L nitrate. In the highest nitrate group, testosterone levels returned to normal. Plasma nitrate was 23 times higher than the control.

Metals

In a healthy aquatic environment, metal concentrations remain low enough to avoid toxic effects on fish. Many metals are present in open seawater and at toxic levels can significantly affect fish welfare, for example, by damaging gills. Metals are generally more damaging and toxic to fish in freshwater conditions than in seawater conditions, as the higher salinity can act as a protective mechanism.

Thresholds are provided for cadmium and copper only, as these have the strongest evidence. Cadmium accumulates in tissues (particularly liver, kidney, and gills) and causes chronic toxicity, including immunosuppression, growth impairment, and organ damage, even at low concentrations. Copper is acutely toxic to fish, causing gill damage, osmoregulatory failure, and mortality at relatively low concentrations, and its toxicity is strongly influenced by water hardness and dissolved organic matter. Although thresholds are not provided due to limited available scientific evidence, it is also recommended that producers measure other metals (including iron, aluminum, and zinc) to alert them to potential welfare problems.

Cadmium

Level 0	Level 1	Level 2	Level 3
≤ 1 µg/L	> 1 to ≤ 5 µg/L	> 5 to ≤ 10 µg/L	> 10 µg/L

Copper

Level 0	Level 1	Level 2	Level 3
≤ 1 µg/L	> 1 to ≤ 5 µg/L	> 5 to ≤ 20 µg/L	> 20 µg/L

Overall: Overall score is determined by the most severe subindicator

Protocol application

Field test kits are available, but are unlikely to be accurate at low concentrations for thresholds in the µg/L range. For more accurate measurements at low concentrations, send samples to labs and use acid-washed bottles. Samples should be stored refrigerated until analysis.

It is recommended to measure metals in high-risk periods (e.g., spring runoff or storm season). Outside of these periods, monitor metals annually, as they rarely vary. If the site is located near potential metal sources, monitor quarterly or monthly, depending on proximity to industrial areas.

Measuring metals at various points is crucial for a comprehensive understanding of the system's environment. This involves taking readings at different depths (e.g., surface, mid-column, and bottom) and at multiple horizontal locations (e.g., at the center and near the net edges). It is recommended to monitor near pollution sources and any painted structures.

Scoring notes

- Cadmium accumulates in fish tissues. Repeated exposure, even if brief, contributes to a chronic toxic burden.
- Antifouling paint can act as a hidden source of copper.
- For copper, the thresholds should be interpreted conservatively when pH < 7.0, as acidic conditions increase copper toxicity. If pH is high (e.g., > 8.5), then copper toxicity reduces.
- When copper approaches level 2 thresholds, farm managers should investigate the causes of copper, as pH shifts can rapidly escalate toxicity.
- When fish are exposed to multiple problematic metals (e.g., cadmium and copper, or other metals), welfare risk is elevated beyond the sum of individual metal effects. Increase monitoring frequency and urgently investigate sources. Multiple metals at concerning levels indicate a systemic water-quality problem requiring immediate intervention.
- **Validation:** The suggested thresholds do not account for other parameters, such as water quality (particularly pH), which can significantly influence toxicity of metals. Apply these thresholds with caution. The thresholds have been largely informed by results in freshwater (and adjusted down). Nowak and Duda (1996) investigated rainbow trout in brackish waters, however reported measurements in total copper rather than dissolved copper. These results have been treated as referring to the maximum possible dissolved copper. The thresholds are therefore conservative.

Key evidence

Source	Summary of source	Key result
Haux and Larsson, 1984	Experimental study investigating the effects of and recovery from long-term sublethal cadmium exposure in 135 rainbow trout (100g) in brackish water conditions (around 7 ppt) over 87 weeks. Fish were exposed to either 0, 10, or 100 µg/L of cadmium for 30 weeks, and then recovered in clean water for 57 weeks. Fish were sampled from each	The liver and kidneys in the exposure groups continuously accumulated cadmium. Exposure groups experienced anemia (with the higher exposure group being slightly worse), hypocalcemia (only the higher exposure group), hypermagnesemia (only the higher exposure group), blood glucose elevation (higher in the higher exposure group) and muscle glycogen depletion (with the higher exposure

Source	Summary of source	Key result
	treatment group at weeks 18, 30, 55, and 87, with 9–12 fish being sampled per occasion.	group being more severely depleted). Anemia and ion balances normalized within 25 weeks of recovery, however blood glucose elevation and muscle glycogen depletion were persistently present more than 1 year after exposure ceased.
Majewski and Giles 1981	Experiment investigating the effects of cadmium exposure on 96 adult (375 g) rainbow trout in freshwater laboratory conditions over 129 days. Two different treatments were used: 3.6 μg/L and 6.4 μg/L.	At the higher concentration (6.4 μg/L), fish exhibited significant increases in ventilation rate, hematocrit, and hemoglobin levels over time, suggesting elevated physiological stress and possible compensation for impaired oxygen transport or uptake. Additionally, erythrocyte ATP levels decreased significantly at the higher concentration, indicating potential disruption to cellular energy metabolism. Many of the measured parameters worsened progressively over the course of the trial, suggesting a compounding effect on welfare with chronic cadmium exposure. Overall, the lower dose (3.6 μg/L) appeared to cause mild-to-moderate acute physiological stress, while the higher dose (6.4 μg/L) led to more severe and chronic welfare impairments.
Heydarnejad et al., 2013	Experimental study investigating the effect of cadmium exposure over 30 days on 60 juvenile rainbow trout (19.5 g) in laboratory conditions. Three different treatments were conducted: control (0.07μg/L), low 1 μg/L, and high 3μg/L.	No recorded welfare issues in the control group. AST, ALT, and ALP tended to increase over time with higher exposure, indicating higher stress. Weight gain, specific growth rate, and condition factor reduced with increasing exposure to cadmium. Feed conversion ratio increased with increasing cadmium, indicating poorer efficiency.

Source	Summary of source	Key result
Dethloff et al., 2001	Experimental study investigating the effects of dissolved copper in wild conditions on ~45 ongrowing rainbow trout in freshwater creeks. The dissolved copper concentrations were 0.56, 1.71, 3.82, and 10.06 µg/L.	The two lowest copper exposures had limited impacts on welfare. Increased copper exposure negatively affected key blood and immune parameters, including reductions in muscle protein and leucocrit, as well as hepatic copper accumulation.
Sommers et al., 2016	Experimental study investigating the effects of salinity on olfactory toxicity and behavioral responses of 39 coho salmon (30.8g in freshwater, 56.7g in seawater) and 54 Chinook salmon (23.5g) in laboratory settings. Olfactory function was measured using electro-olfactograms after an acute 30-minute exposure to 50µg/L of dissolved copper. This was conducted on coho salmon in freshwater, at 10 ppt, and in seawater (28–30 ppt), and Chinook salmon in seawater (28–30 ppt). Avoidance behavior was assessed in Chinook salmon in both freshwater and seawater over 60 minutes using a multi-chambered tank, where dissolved copper was added to the segment over time. A physical structure was also added in the exposed segment vs. a control, to test whether the fish's attraction to the structure would be overridden by the presence of copper. In the freshwater experiment, the background copper levels were 2.6–3.0µg/L, with the exposure segment reaching a concentration of 7.9µg/L after 30 minutes. In seawater, the background level of copper was 0.4µg/L initially, and reached 16.9µg/L in the treated segment after 30 minutes.	The acute 30-minute exposure to 50µg/L of copper significantly reduced electro-olfactogram responses in freshwater, but not at 10 ppt or seawater, indicating salinity protects against olfactory toxicity. Fish avoided the copper exposed segment in freshwater and seawater. In the non-exposed groups, Chinook salmon preferred the submerged structure in freshwater but not in seawater. However, in the exposed freshwater group, the presence of copper negated the preference for the structure.

Source	Summary of source	Key result
Nowak and Duda, 1996	Experimental study investigating the effects of chronic exposure to copper in 200 rainbow trout (65–375g) in laboratory conditions. Two experiments were conducted. The first experiment was conducted at 19.6 ppt with 15 fish per tank with 8 tanks (including two controls) over 6 weeks, exposing fish to concentrations of 30, 70, and 200µg/L of nominal copper. This corresponded to 10.75–22.00µg/L, 12.6–17.12µg/L, and 41.55–46.77µg/L of total copper respectively. The second experiment was conducted at 14.6 ppt (though this was variable, ranging from 0–23 ppt) with 10 fish per tank with 8 tanks over 7 weeks, also exposing fish to 30, 70, and 200µg/L of nominal copper. This corresponded to 16.00–17.50µg/L, 33.25–43.50µg/L, and 44.50–70.75µg/L of total copper, respectively.	In the first experiment, except for fish exposed to the highest concentrations of copper, mortality was 0%. However, high mortality was associated with total copper of 56–60µg/L (reflecting a spike in copper levels), with mortality rates of 20 and 27% respectively. In experiment two, at a total copper concentration of 17.5µg/L, survival was 100%, but then at 16µg/L, survival was 80% (however, this may have been caused by salinity stress). There were very high mortality rates above 33.25µg/L, ranging from 30–80%. Growth was consistently reduced at total copper concentrations of 22.00µg/L or higher. Sodium and potassium levels were elevated in fish exposed to the highest copper concentrations, and gill histology showed increased mucous cells.

Water velocity

The water velocity that fish are exposed to impacts how they swim and also plays a critical role in providing sufficient dissolved oxygen and carrying away waste. Water velocity is often measured in body lengths per second (BL/s), as current speed has a relative impact depending on the fish's body size. While some flow is beneficial as exercise, excessively high water velocity can quickly exhaust rainbow trout, making them unable to maintain position. This can result in the current forcing the trout into uncomfortable areas of the cage for prolonged periods (such as against net walls or other equipment), which can cause mechanical damage.

Water velocity

Level 0	Level 1	Level 2	Level 3
> 1 to ≤ 1.4 BL/s	> 0.8 to ≤ 1 BL/s **OR** > 1.4 to ≤ 1.8 BL/s	> 0.5 to ≤ 0.8 BL/s **OR** > 1.8 to ≤ 2 BL/s	≤ 0.5 BL/s **OR** > 2 BL/s

Behavioral response to flow

Level 0	Level 1	Level 2	Level 3
Fish are swimming actively through the cage, with no forcing against structures	Fish are swimming actively throughout the cage, with occasional use of structures for rest	Fish aggregated in a small area of the enclosure **OR** Fish are occasionally forced against structures **OR** Fish displaying lethargy due to inadequate flow	Fish are frequently forced against structures **OR** Severe spatial restriction

Overall: Overall score is determined by the most severe subindicator

Protocol application

Velocity can be measured using a propeller flow meter or a Doppler velocimeter. Then **body lengths per second (BL/s)** can be calculated by dividing velocity (in cm/s) by fish body length (in cm). The estimated average body length in the population can be used to calculate this.

It is recommended to measure water velocity during high-risk periods (e.g., during storms or when water velocity is visibly very high). The water velocity should be assessed based on the measurements in the densest area.

Behavioral response assessment is mandatory for all evaluations, as water velocity is never uniform throughout the cage. Fish clustering in specific areas, forced positioning against structures, or lethargy indicates inadequate flow in those zones, even if measured velocity elsewhere is level 0 or level 1.

If calculating velocity in BL/s is not practical, behavioral assessment alone is sufficient for scoring using the qualitative methodology provided.

Scoring notes

- Water velocity will vary in different areas of the cage.
- Specialized current/velocity sensors are required to measure velocity, and many producers may not have access to this equipment.
- Examples of level 0 velocities depending on fish size:
  - 20 cm: 0.2–0.28 m/s.
  - 30 cm: 0.3–0.42 m/s.
  - 50 cm: 0.5–0.7 m/s.
- Given that there will undoubtedly be size variation in an enclosure, different proportions of the population will be affected differently by the water flow.
- Smaller individuals may tolerate a higher BL/s speed, but a lower m/s speed, meaning that the welfare impacts of swimming speed can be quite dependent on the size of the individual fish.
- **Validation:** Multiple studies investigating how ongrowing rainbow trout respond to different water velocities were relied upon. The qualitative thresholds were developed based on expert opinion.

Key evidence

Source	Summary of source	Key result
Fløtum Mittún et al., 2025	Experimental study investigating the preferred and optimal swimming speeds at three temperatures of 31 ongrowing (175 g) rainbow trout in seawater laboratory conditions (circular raceway) over 6 hours. The average body length was 25cm.	The preferred speed was between 1.18 and 1.24 BL/s for all temperatures. There was no significant effect of temperature on preferred swimming speeds. The critical swimming speed (maximum speed a fish can sustain before reaching complete exhaustion)

Source	Summary of source	Key result
	The three temperatures tested were 10°C, 15°C, and 20°C.	was between 2.1 and 2.6 BL/s for all temperatures.
Waldrop et al., 2020	Experimental study investigating the effects of swimming exercise and dissolved oxygen on growth, fin condition, and survival in juvenile rainbow trout (18 g) to harvest-size rainbow trout (~1 kg) in laboratory conditions over 341 days. Two water velocities were tested: high (1.5–2 BL/s) and low (~0.5 BL/s) at high (100%) and low (70%) oxygen saturations.	Swimming exercise at a higher velocity significantly increased the cardio-somatic index. The higher velocity group also had lower levels of aggression. Low-swimming exercise groups showed more damage to the right pectoral fin. There was no statistically significant impact of water flow on growth.
Hafs et al., 2012	Experimental study investigating the impact of carbon dioxide, water velocity, and other parameters on growth and fillet attributes in 600 ongrowing rainbow trout (300 g) in laboratory conditions over 90 days. The low-velocity group had a water velocity of 0.5 BL/s, and the high-velocity group had a velocity of 2 BL/s.	Overall, there were limited differences between the groups tested. For example, there was no significant difference in hematocrit or wet weight between the groups. Specific growth rate and feed conversion ratio were not adversely affected by the high velocity, and no fin damage was reported.

Salinity

Rainbow trout must constantly regulate their internal salt and water balance through osmoregulation, but this process works differently depending on their environment and smolt status. In freshwater, rainbow trout are hyperosmotic, meaning their body fluids have higher salt content than the surrounding water. In seawater, rainbow trout are hypoosmotic, meaning their body fluids have a lower salt content than the surrounding water. Pre-smolt trout lack osmoregulatory systems for seawater tolerance, meaning they can become severely dehydrated if exposed to high salinity. Rapid salinity transitions can cause osmotic shock.

Post-smolt trout have broader tolerance ranges, but high salinity levels can still be challenging to adjust to, particularly on transfer to seawater or for poorly smolted fish. There is minimal information on which salinity levels are best suited for post-smolt rainbow trout, but based on the evidence, they are likely best suited to brackish waters. Concrete salinity percentage thresholds could not be developed from the evidence, so instead are based on signs of osmoregulatory stress.

Level 0	Level 1	Level 2	Level 3
Fish have access to a salinity gradient, allowing self-regulation	No access to a salinity gradient, but fish are healthy acclimated adults with no osmoregulatory stress	Some signs of osmoregulatory distress	Active signs of osmoregulatory distress

Protocol application

Salinity can be measured using a digital salinity monitor.

It is recommended to measure salinity for rainbow trout in seawater at different cage depths (e.g., 3 m, 5 m, 15 m, and the maximum cage depth) and to monitor for large fluctuations.

It is recommended to measure salinity in high-risk periods (e.g., during the first 1–2 months after transfer to seawater or where there are signs of osmoregulatory distress). Outside of these, the fish are likely to have acclimated to the new salinity levels.

Scoring notes

- If there are many stratified layers of salinity, fish are unlikely to experience osmoregulatory stress because they can swim to their preferred salinity.

- Signs of osmoregulatory stress include:
    - Lethargy or abnormal behavior;
    - Reduced appetite;
    - Skin darkening;
    - Small post-smolts; or
    - Maturing fish.
- **Validation:** The threshold is based on the salinity-scoring methodology in the SWIM 1.0 protocol for salmon. It has not been validated for rainbow trout.

Key evidence

Source	Summary of source	Key result
Bahabadi and Morshedi, 2015	Experimental study investigating osmolarity, cortisol, and thyroid hormones in response to different salinity levels in 450 rainbow trout (93 g) over 60 days in laboratory conditions. The fish were initially held in freshwater. Salinities of freshwater (control), 10 ppt, 20 ppt, 30 ppt, and 40 ppt were tested. Upon transfer, the fish were gradually acclimated to the new salinity over 10 days.	Fish in the 30 ppt and 40 ppt treatment groups all died after the 10-day acclimation period. The freshwater, 10 ppt, and 20 ppt groups had very similar mortality rates of approximately 4% over the entire 70-day period. Significant reductions in final weight, daily growth rate, specific growth rate, and feed conversion ratio were observed up to a salinity of 20 ppt. Osmolality, cortisol, glucose, and thyroid hormones all increased with salinity, indicating stress.
Stien et al., 2013	Semantic model for overall welfare assessment of Atlantic salmon reared in sea cages, based on existing literature. 17 welfare indicators were selected, including salinity.	Salinity is scored as 1 (best) if fish have access to brackish water, scored as 2 if it is only adult fish with no access to brackish water, and scored as 3 (worst) if there are small post-smolts, maturing, or clearly impaired fish with no access to brackish water. On reviewing the literature, the authors concluded there was little evidence that salinity levels have significant effects on the welfare of adult Atlantic salmon in sea cages, while mature or small salmon may struggle to adapt to high salinities, and so may benefit from access to brackish water, citing

Source	Summary of source	Key result
		evidence that small salmon display a preference for haloclines.
Gonçalves et al., 2006	Experimental study investigating the possible ameliorating effects of reduced salinity on 400 adult Atlantic salmon (802 g) over 15 days, initially reared in seawater (35 ppt). A total of 4 tanks were used, with 2 maintaining seawater at 35 ppt and the other 2 at 28 ppt. Before the experiment, fish displayed signs of osmotic stress (skin darkening, lethargy, reduced appetite), and mortality was approximately 3% per day.	During the first 3 days of the experiment, there was no significant difference in mortality between the groups. By the end of the experiment, the 28 ppt salinity group had a survival rate of 79.8%, while the control had a survival rate of 31.2%. Plasma cortisol was approximately 4 times higher at the end of the experiment in the 35 ppt group compared to the 28 ppt group. The condition factor was also improved in the 28 ppt group, whereas it remained low in the 35 ppt group.

Hydrogen sulfide

Hydrogen sulfide (H_2S) is a dissolved gas that is particularly toxic in its unionized form. Higher levels of hydrogen sulfide are more likely in seawater systems compared to freshwater systems due to higher sulfate concentrations. Hydrogen sulfide tends to be more of an issue in closed systems than in open systems, though accumulation has been noted in lower levels of fjords.[41] Hydrogen sulfide impedes respiration, and mass mortalities related to hydrogen sulfide have been reported in RAS farms.[42]

Level 0	Level 1	Level 2	Level 3
≤ 0 μg/L	> 0 to ≤ 0.3 μg/L	> 0.3 to ≤ 1 μg/L	> 1 μg/L

Protocol application

Measure H_2S directly using a portable colorimetric kit, methylene blue method, or electrochemical sensor. If measuring total sulfide, calculate the H_2S fraction using pH, temperature, and salinity (see scoring notes). Sample and analyze promptly, as H_2S is volatile and concentrations can change rapidly after collection.

Hydrogen sulfide is generally not required to be measured in sea cages or other open systems. If measuring hydrogen sulfide in sea cages, it is recommended to measure at different cage depths (e.g., 3 m, 5 m, 15 m, and especially at maximum depth, as hydrogen sulfide tends to accumulate near the bottom). Scoring classification should be based on the maximum detected concentration at any depth where fish are active.

Scoring notes

- If total sulfide is being measured, the hydrogen sulfide levels must be calculated from pH, temperature, and salinity, or derived from reference tables such as those in the Australian & New Zealand Guidelines for Fresh & Marine Water Quality.
- Water quality parameters such as dissolved oxygen, temperature, and pH can affect hydrogen sulfide levels. For example, low pH is a risk factor for increased hydrogen sulfide.
- While short-term exposure (e.g., for a few hours) to elevated hydrogen sulfide may be tolerated, exposure over days or months poses a greater risk to rainbow trout.
- **Validation:** Extremely limited rainbow trout-specific evidence exists. One study on juvenile rainbow trout in freshwater was published in 1976, but more conservative thresholds have been suggested based on a more recent salmon study in brackish water, and the possibility that hydrogen sulfide tolerance levels may have been overestimated in older literature.

Key evidence

Source	Summary of source	Key result
Lazado et al., 2024	Experimental study investigating the effects of hydrogen sulfide exposure on 450 post-smolt Atlantic salmon (162 g) in brackish water laboratory conditions over 4 weeks. The fish were split into 3 treatment groups, with exposure to 0 (background level < 0.3 µg/L recorded), 1, and 5 µg of hydrogen sulfide, respectively.	The low-exposure group had a mortality rate of 4.7% over the trial period, and the high-exposure group had a mortality rate of 16%. Mortalities were only observed in the third week, with the authors suggesting that the fish were actively deploying countermeasures until their defenses were depleted. The control group had less than 1% mortality. Both exposure groups had significantly lower weights than the control group, with the low-exposure group weighing 26% less than controls and the high-exposure group 22% less. Exposed fish also showed signs of lethargy and exhaustion, with significant tissue damage present in both exposure groups. The authors explicitly propose that hydrogen sulfide levels should not exceed 1 µg/L in brackish water RAS systems for salmon.
Smith et al., 1976	Experimental study investigating the effects of hydrogen sulfide exposure on juvenile rainbow trout in freshwater conditions over 4–17 days. Acute toxicity tests were conducted on two groups of fish with average lengths of 45 and 54mm, respectively, with hydrogen sulfide concentrations ranging from 1.4–29.2 µg/L over 4–17 days. Chronic exposure tests on 50-day-old juveniles were also conducted at exposure levels of 0, 1.1, 3.7, 5.9, and 10.2	In the acute exposure tests, it was determined that, for 50% of fish to suffer mortality within 96 hours, the hydrogen sulfide level was 12.5–13 µg/L. Over a more extended period, a lower hydrogen sulfide level was required to cause 50% mortality. For 17 days, only an 8.6 µg/L exposure was required. There were also some impacts on growth. Fish exposed to 6.2 µg/L or below showed equal or slightly higher growth compared to the control, while those exposed to 9.5 µg/L had about half the average weight compared to the control.

Source	Summary of source	Key result
	µg/L. 30 fish per treatment group were tested.	For the chronic exposure tests, the group exposed to 10.2 µg/L had a mortality rate of 57% over 50 days. In contrast, the other groups exposed to lower levels of hydrogen sulfide had mortality rates at or below 10%. The authors determined that to ensure no demonstrable adverse effect, the hydrogen sulfide levels should remain below 3–5 µg/L.
Ciani et al., 2024	Experimental study investigating the impact of short hydrogen sulfide exposure on 340 post-smolt Atlantic salmon (114 g) over 1 hour in brackish water RAS conditions. Fish were stocked at densities of 10 kg/m³ in the control and one exposure tank, and 30 kg/m³ in a second exposure tank. Fish were exposed to increasing hydrogen sulfide concentrations once daily for 10 consecutive days, with concentrations doubling each day, starting at 1 µg/L and reaching 68 µg/L. Swimming behavior was recorded using overhead and underwater cameras.	Fish rapidly responded to increases in hydrogen sulfide, exhibiting faster swimming speeds, erratic zigzagging patterns, and a loss of schooling. These behavioral changes increased linearly from no exposure to concentrations of 30–40 µg/L. Between 40–50 µg/L, behavior significantly shifted, with speed increasing by 65%, dispersion increasing by 33%, and pattern quality decreasing by 18% compared to the previous group. Further increases in hydrogen sulfide beyond this level did not result in additional changes in swimming parameters.

Appendix: OWI selection

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Vertebral deformity

Level 0 — Nilsson, Jonatan. 2026. Vertebral deformity in rainbow trout, score 0. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Vertebral deformity in rainbow trout, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan. 2026. Vertebral deformity in rainbow trout, score 2. Photograph. Personal communication, Institute of Marine Research.

Level 3 — Nilsson, Jonatan. 2026. Vertebral deformity in rainbow trout, score 3. Photograph. Personal communication, Institute of Marine Research.

Body condition

Emaciation, Level 0 — Nilsson, Jonatan. 2026. Healthy rainbow trout, body condition score 0. Photograph. Personal communication, Institute of Marine Research.

Emaciation, Level 1 — Nilsson, Jonatan, Kristine Gismervik, Kristoffer Vale Nielsen, Martin Haugsmo Iversen, Chris Noble, Jelena Kolarevic, Hilde Frotjold, et al. 2025. "Laksvel — a Standardised, Operational Welfare Monitoring Protocol for Atlantic Salmon Held in Sea Cages." Institute of Marine Research. https://www.hi.no/en/hi/nettrapporter/rapport-fra-havforskningen-en-2025-40.

Emaciation, Level 2 — Nilsson, Jonatan. 2026. Clearly emaciated rainbow trout, body condition score 2. Photograph. Personal communication, Institute of Marine Research.

Emaciation, Level 3 — Nilsson, Jonatan, Kristine Gismervik, Kristoffer Vale Nielsen, Martin Haugsmo Iversen, Chris Noble, Jelena Kolarevic, Hilde Frotjold, et al. 2025. "Laksvel — a Standardised, Operational Welfare Monitoring Protocol for Atlantic Salmon Held in Sea Cages." Institute of Marine Research. https://www.hi.no/en/hi/nettrapporter/rapport-fra-havforskningen-en-2025-40.

Scale loss

Level 0 — Nilsson, Jonatan. 2026. Rainbow trout with no scale loss, score 0. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Rainbow trout with minor scale loss, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan. 2026. Rainbow trout with moderate scale loss, score 2. Photograph. Personal communication, Institute of Marine Research.

Level 3 — Nilsson, Jonatan. 2026. Rainbow trout with severe scale loss, score 3. Photograph. Personal communication, Institute of Marine Research.

Skin hemorrhaging

Level 0 — Nilsson, Jonatan. 2026. Rainbow trout with no skin hemorrhaging, score 0. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Rainbow trout with minor skin hemorrhaging, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan. 2026. Rainbow trout with moderate skin hemorrhaging, score 2. Photograph. Personal communication, Institute of Marine Research.

Level 3 — Nilsson, Jonatan, Kristine Gismervik, Kristoffer Vale Nielsen, Martin Haugsmo Iversen, Chris Noble, Jelena Kolarevic, Hilde Frotjold, et al. 2025. "Laksvel — a Standardised, Operational Welfare Monitoring Protocol for Atlantic Salmon Held in Sea Cages." Institute of Marine Research. https://www.hi.no/en/hi/nettrapporter/rapport-fra-havforskningen-en-2025-40.

Wounds

Level 0 — Nilsson, Jonatan. 2026. Rainbow trout, score 0. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Rainbow trout with a small superficial wound, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan, Kristine Gismervik, Kristoffer Vale Nielsen, Martin Haugsmo Iversen, Chris Noble, Jelena Kolarevic, Hilde Frotjold, et al. 2025. "Laksvel — a Standardised, Operational Welfare Monitoring Protocol for Atlantic Salmon Held in Sea Cages." Institute of Marine Research. https://www.hi.no/en/hi/nettrapporter/rapport-fra-havforskningen-en-2025-40.

Level 3 — Bui, Samantha. 2026. Rainbow trout with a large wound, score 3. Photograph. Personal communication, Institute of Marine Research.

Snout damage

Level 0 — Nilsson, Jonatan. 2026. Snout damage in rainbow trout, score 0. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Bui, Samantha. 2026. Snout damage in rainbow trout, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Bui, Samantha. 2026. Snout damage in rainbow trout, score 2. Photograph. Personal communication, Institute of Marine Research.

Level 3 — Nilsson, Jonatan, Kristine Gismervik, Kristoffer Vale Nielsen, Martin Haugsmo Iversen, Chris Noble, Jelena Kolarevic, Hilde Frotjold, et al. 2025. "Laksvel — a Standardised, Operational Welfare Monitoring Protocol for Atlantic Salmon Held in Sea Cages." Institute of Marine Research. https://www.hi.no/en/hi/nettrapporter/rapport-fra-havforskningen-en-2025-40.

Jaw deformity

Level 0 — Nilsson, Jonatan. 2026. Healthy rainbow trout, jaw deformity score 0. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Rainbow trout with minor lower jaw deformity, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan. 2026. Rainbow trout with clear lower jaw deformity, score 2. Photograph. Personal communication, Institute of Marine Research.

Level 3 — Nilsson, Jonatan. 2026. Rainbow trout with severe lower jaw deformity, score 3. Photograph. Personal communication, Institute of Marine Research.

Eye opacity

Level 0 — Nilsson, Jonatan. 2026. Rainbow trout eye without any opacity, score 0. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Rainbow trout eye with minor opacity, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan. 2026. Rainbow trout eye with clear opacity, score 2. Photograph. Personal communication, Institute of Marine Research.

Level 3 — Nilsson, Jonatan. 2026. Rainbow trout eye with severe opacity, score 3. Photograph. Personal communication, Institute of Marine Research.

Eye injury

Level 0 — Nilsson, Jonatan. 2026. Healthy rainbow trout eye, score 0. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Rainbow trout with minor eye injury, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan. 2026. Rainbow trout with clear eye injury, score 2. Photograph. Personal communication, Institute of Marine Research.

Level 3 — Nilsson, Jonatan. 2026. Rainbow trout with severe eye injury, score 3. Photograph. Personal communication, Institute of Marine Research.

Operculum damage

Level 0 — Nilsson, Jonatan. 2026. Rainbow trout without operculum damage, score 0. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Rainbow trout with minor operculum shortening, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan. 2026. Rainbow trout with clear operculum shortening, score 2. Photograph. Personal communication, Institute of Marine Research.

Level 3 — Nilsson, Jonatan. 2026. Rainbow trout with severe operculum shortening, score 3. Photograph. Personal communication, Institute of Marine Research.

Gill damage

Level 0 — Fridman, Sophie, Smaragda Tsairidou, Nilantha Jayasuriya, Halina Sobolewska, Alastair Hamilton, Carlos Lobos, Ross D. Houston, Hamish Rodger, James Bron, and Tharangani Herath. "Assessment of Marine Gill Disease in Farmed Atlantic Salmon (Salmo salar) in Chile Using a Novel Total Gross Gill Scoring System: A Case Study." Microorganisms 9, no. 12 (December 16, 2021): 2605. https://doi.org/10.3390/microorganisms9122605.

Level 1 — Nilsson, Jonatan, Kristine Gismervik, Kristoffer Vale Nielsen, Martin Haugsmo Iversen, Chris Noble, Jelena Kolarevic, Hilde Frotjold, et al. 2025. "Laksvel — a Standardised, Operational Welfare Monitoring Protocol for Atlantic Salmon Held in Sea Cages." Institute of Marine Research. https://www.hi.no/en/hi/nettrapporter/rapport-fra-havforskningen-en-2025-40.

Level 2 — Nilsson, Jonatan, Kristine Gismervik, Kristoffer Vale Nielsen, Martin Haugsmo Iversen, Chris Noble, Jelena Kolarevic, Hilde Frotjold, et al. 2025. "Laksvel — a Standardised, Operational Welfare Monitoring Protocol for Atlantic Salmon Held in Sea Cages." Institute of Marine Research. https://www.hi.no/en/hi/nettrapporter/rapport-fra-havforskningen-en-2025-40.

Level 3 — Nilsson, Jonatan, Kristine Gismervik, Kristoffer Vale Nielsen, Martin Haugsmo Iversen, Chris Noble, Jelena Kolarevic, Hilde Frotjold, et al. 2025. "Laksvel — a Standardised, Operational Welfare Monitoring Protocol for Atlantic Salmon Held in Sea Cages." Institute of Marine Research. https://www.hi.no/en/hi/nettrapporter/rapport-fra-havforskningen-en-2025-40.

Fin damage

Level 0 — Nilsson, Jonatan. 2026. Rainbow trout pectoral fin, score 0. Photograph. Personal communication, Institute of Marine Research.

Level 0 — Nilsson, Jonatan. 2026. Rainbow trout dorsal fin, score 0. Photograph. Personal communication, Institute of Marine Research.

Level 0 — Cbunt biotactic. Rainbow Trout Georgian Bay. 2010. Photograph. https://commons.wikimedia.org/wiki/File:Rainbow_Trout_Georgian_Bay.jpg.

Level 0 — Nilsson, Jonatan. 2026. Rainbow trout anal fin, score 0. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Rainbow trout pectoral fin, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Rainbow trout dorsal fin, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Rainbow trout caudal fin, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 1 — Nilsson, Jonatan. 2026. Rainbow trout anal fin, score 1. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan. 2026. Rainbow trout pectoral fin, score 2. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan. 2026. Rainbow trout dorsal fin, score 2. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan. 2026. Rainbow trout caudal fin, score 2. Photograph. Personal communication, Institute of Marine Research.

Level 2 — Nilsson, Jonatan. 2026. Rainbow trout anal fin, score 2. Photograph. Personal communication, Institute of Marine Research.

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Level 3 — Nilsson, Jonatan. 2026. Rainbow trout dorsal fin, score 3. Photograph. Personal communication, Institute of Marine Research.

Level 3 — Nilsson, Jonatan. 2026. Rainbow trout caudal fin, score 3. Photograph. Personal communication, Institute of Marine Research.

Level 3 — Nilsson, Jonatan. 2026. Rainbow trout anal fin, score 3. Photograph. Personal communication, Institute of Marine Research.

Endnotes

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