Gilthead Sea Bream Operational Welfare Indicator (OWI) Protocol: Ongrowing, Seawater

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Centre for Aquaculture Progress

Gilthead Sea Bream Operational Welfare Indicator (OWI) Protocol: Ongrowing, Seawater

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

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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 a tool for assessing the welfare of farmed gilthead sea bream (*Sparus aurata*), 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 the Aquaculture Stewardship Council (ASC).

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, strengthened consumer trust, and a stronger and more sustainable aquaculture industry.

Scope

Developed specifically for ongrowing gilthead sea bream, this protocol may be applied to juveniles or to lower-salinity brackish water 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 gilthead sea bream welfare has been published by the Spanish and Greek aquaculture associations APROMAR[2] and HAPO,[3] existing resources lack comprehensive, standardized, and threshold-based assessment across a wide range of indicators for gilthead sea bream producers. This protocol addresses that gap.

This protocol includes:

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

For most indicators, the thresholds have not yet been tested against welfare outcomes in gilthead sea bream, so they should be treated as provisional. We will revise them as farm data and new studies become available.

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

Gilthead sea bream, 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 gilthead sea bream. Users may apply this protocol to juveniles, though application in this context 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 judgments.

This protocol is a living document that will be updated and refined over time as gilthead sea bream 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.

This 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]

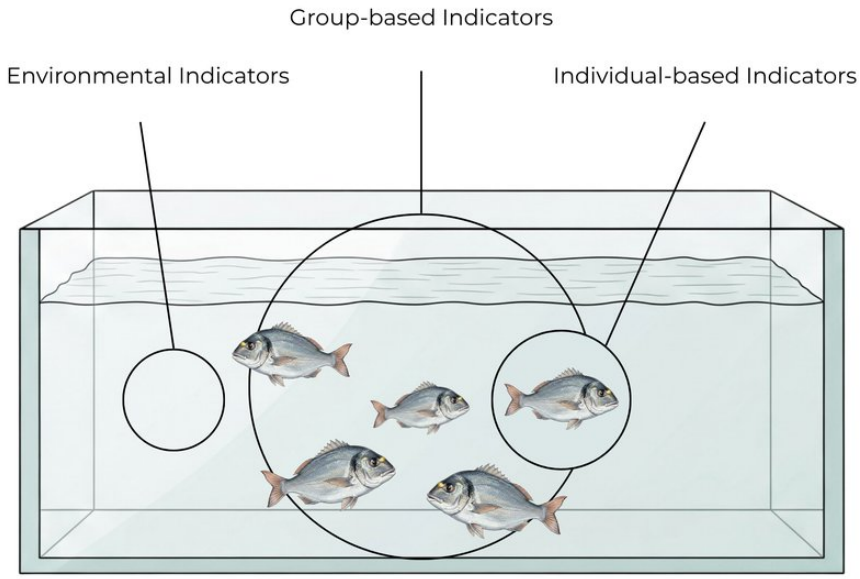
1. **Individual-based indicators** (animal-based outcome measures): These assess the physical condition of individual fish (e.g., fin and skin condition) through sampling or camera-based observations. Individual-based indicators directly reflect how fish have been affected by their environment and management, providing outcome measures of their welfare risk.
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. Each indicator should be interpreted as a 'welfare risk indicator'. No single indicator can describe the internal welfare state of an animal, but each one affects 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 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 gilthead sea bream, while others rely on evidence from different species, alternative life stages, or laboratory studies that does 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 risk 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 risk. 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 environmental conditions. Some environmental parameters, like oxygen saturation, may also vary significantly in both space and time (at least in sea pens), with the most critical levels easily going undetected.
7. **Routine assessment only:** This protocol focuses on routine welfare assessment. It does not provide guidance on assessing welfare during high-risk events such as crowding, handling, transport, stunning, or slaughter. These events require separate assessment methods. Further, no concrete recommendations are provided for addressing identified

welfare risks. Producers should therefore ensure they have strong management plans in place that enable them to act upon identified welfare issues.

8. **Positive welfare:** This protocol assesses welfare risk through indicators of injury and deformity, behavioral deviations, and environmental risks. It does not include positive welfare indicators.

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:

- **Gilthead sea bream-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 conditions. This column is about the strength of evidence that the metric is a valid indicator of welfare in gilthead sea bream.
  - Example scoring:
    - **High:** Multiple empirical studies (e.g., 4+) on gilthead sea bream, with some testing substantial welfare-relevant outcomes, usually with at least one testing ongrowing gilthead sea bream (depending on how relevant it is for the indicator)
    - **Moderate:** Some empirical studies (e.g., 1–3) on gilthead sea bream testing substantial welfare-relevant outcomes
    - **Low:** Some gilthead sea bream-specific evidence, but substantial welfare-relevant outcomes are not tested (e.g., production metrics only)
    - **Very low:** No empirical gilthead sea bream-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	Gilthead sea bream-specific evidence	Threshold validation
Individual-based indicators		
Vertebral deformity	Moderate	Low (extrapolated from salmon)
Body condition	High	Moderate
Scale loss	Low	Low (extrapolated from salmon)
Skin hemorrhaging	Moderate	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	Moderate	Low (extrapolated from salmon)
Eye injury	Moderate	Low (extrapolated from salmon)
Operculum damage	Moderate	Low (extrapolated from salmon)
Gill damage	Moderate	Low (extrapolated from salmon)
Fin damage	Moderate	Low (extrapolated from salmon)
Group-based indicators		
Mortality	Moderate	Low
Aberrant behavior	Low	Low
Aggressive behavior	Low	Very low
Feeding behavior	Low	Low
Swimming behavior	Low	Very low
Environmental indicators		
Stocking density	High	Moderate
Dissolved oxygen	Moderate	Moderate

Indicator	Gilthead sea bream-specific evidence	Threshold validation
Temperature	High	Moderate
Carbon dioxide	Moderate	Low
pH	Moderate	Low
Turbidity	Very low	Low (Secchi depth extrapolated from salmon)
Total suspended solids	Very low	Very low
Ammonia	Moderate	Low
Nitrite	Low	Very low
Nitrate	Very low	Very low
Metals	Low	Very low
Water velocity	Moderate	Moderate
Salinity	Moderate	Low
Hydrogen sulfide	Very low	Very low (extrapolated from salmon)

Many thresholds for individual-based indicators in particular have been informed by the Laksvel protocol for Atlantic salmon. As gilthead sea bream and salmon are distantly related and farmed in different conditions, these thresholds should be interpreted with caution. We started with Laksvel as a base because it is the most developed operational welfare indicator protocol available for farmed fish. The alternative was to set thresholds without any existing guidance. Over time, as more evidence about individual-based indicators in gilthead sea bream is generated, this protocol will be updated to reflect that new understanding.

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 risk within production systems. This table underscores the gaps in gilthead sea bream 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.

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:

- Structured review of indicators used in established protocols (e.g., Laksvel for Atlantic salmon and APROMAR’s ‘A guide on fish welfare in Spanish aquaculture - volume 3: Welfare of gilthead sea bream’).[13]
- Review of other materials considering operational welfare indicators, including those published by industry.
- Analysis of certification requirements from major schemes (e.g., ASC).
- Consideration of commercial need for the indicator to be included, scientific validity, practical feasibility, and welfare relevance for gilthead sea bream.

Threshold development process

Scoring thresholds were aligned with the system used in our previously published Rainbow Trout Operational Welfare Indicator (OWI) Protocol: Ongrowing, Seawater,[14] which in turn was based on Laksvel. Threshold recommendations for each OWI were developed through an indicator-specific structured review of the current scientific literature, prioritizing evidence from ongrowing gilthead sea bream in commercial conditions. Where such evidence was limited (which was often the case), the analysis incorporated evidence from laboratory settings, alternative life stages, or other species, 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 gilthead sea bream in commercial 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 gilthead sea bream) 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 scoring system in our previously published Rainbow Trout Operational Welfare Indicator (OWI) Protocol: Ongrowing, Seawater,[14] which in turn is aligned with the Laksvel protocol for Atlantic salmon.

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

Where a fish or group meets the description of more than one level, assign the highest level.

Each score reflects the welfare risk for one indicator, considered in isolation, at the time of assessment. A score describes the current extent of injury or deviation. 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 risk 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 how welfare risk is distributed within a group.

A fish with a level 3 score for one or more individual-based indicators should be assessed immediately for pain and likelihood of recovery. Where the fish is severely compromised or unlikely to recover, it should be humanely culled.

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. No single score represents a clear pass or fail. The purpose of scoring is to help producers identify times of higher risk and increase the ability to take preventative action.

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 assessment 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 assessment of welfare risk. Where producers do not assess every indicator, they should choose a set that covers each of the welfare domains (see "Defining fish welfare and Operational Welfare Indicators"). Any fish with a severe injury or deviation seen during assessment should be recorded, even if that indicator is not part of the routine set.

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 (at least for salmon).[15] Certification schemes also provide guidance: for morphological assessments, ASC requires monthly assessments; the Best Aquaculture Practices Farm Standard recommends weekly checks; and the RSPCA requires a minimum of four assessments in the freshwater stage and monthly in seawater for salmonids.

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). Ideally, morphological indicators should be assessed **monthly** (and for an individual site, no less than any applicable certification standard requires). Frequent assessment allows early detection of trends (e.g., rising deformity prevalence or declining condition factor) before they become serious welfare problems. This frequent sampling may not be achievable on every site, as morphological assessment requires handling, which is itself a stressor. Handling fish for no other purpose than morphological scoring should be avoided where possible. Where it is necessary, the welfare cost of that handling should be weighed against the value of the additional data. In the case that the interval between assessments is extended, the sample size should be increased proportionally. 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. When fish are assessed out of water, time out of water should be kept as short as possible, and applicable certification or legal limits on air exposure should always be respected. Where longer assessment is needed, sedation should be considered.

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 risk.

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 risk across 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 (e.g., sampling at different positions and depths in the pen).

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

Number of fish per pen	Suggested sample (where practical)
≤ 20,000	30
> 20,000 to ≤ 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 pen at the site. The number of pens assessed may be reduced over time if consistent results and strong correlations are observed across pens, and pens are assessed on a rotating basis. Where pens are sampled on a rotating basis, producers should record which pens were sampled and why, and give priority to pens with higher expected risk (e.g., after handling events, during disease outbreaks, or where environmental or behavioral indicators have worsened).

To keep scoring consistent, assessors should periodically score the same fish, or the same set of images, and discuss any differences.

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),[16] 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 pen, 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).

Observe at the same time of day and from the same position on each visit, and before feeding unless feeding behavior is being assessed. Where possible, repeat observations at different times of day, as a single short observation may miss behaviors that come and go. Recording video allows scores to be checked later and compared over time.

Producers should understand what normal behavior looks like at each site, across seasons and production stages. A clear change from this baseline should prompt further investigation.

Individual-based indicators

Indicators assessed on individual fish through visual examination or measurement.

1. Vertebral deformity 25
2. Body condition 29
3. Scale loss 32
4. Skin hemorrhaging 34
5. Wounds 37
6. Change of coloration 41
7. Snout damage 43
8. Jaw deformity 46
9. Eye opacity 49
10. Eye injury 52
11. Operculum damage 54
12. Gill damage 57
13. Fin damage 60

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 may cause ongoing stress.

Level 0	Level 1	Level 2	Level 3
No externally visible vertebral deformity	Minor or suspected vertebral deformity, noticeable 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.

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, deviations, or curves where the fish's swimming will likely be impacted.
    - A clear vertebral deformity in a fish that is noticeably smaller or larger than its cohort (e.g., a shortened or rounded body) is scored as level 3, as abnormal size indicates the deformity is affecting the fish.
- Vertebral deformities are often chronic conditions, though they can improve over time.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

Key evidence

Source	Summary of source	Key result
Fragkoulis et al., 2019	Longitudinal study investigating the prevalence and recovery of lordosis in 1,700 gilthead sea bream (initial weight 6 g) in commercial conditions over 434 days, reaching a standard length of 26.2 cm. Lordosis was examined using both external morphology and x-rays. For visual observation of external morphology, assessors categorized the fish as "normal", "uncertain" or "lordotic".	At the beginning of the experiment, 10.5%, 2.5%, and 87% of the 1,700 fish were classified externally as 'lordotic', 'uncertain', and 'normal'. In a separate validation sample of 100 juveniles, all 'lordotic' fish had severe lordosis when examined internally, all 'uncertain' fish had light lordosis, and 4 of the 78 'normal' fish had light lordosis. The prevalence of fish with external signs of lordosis decreased from 10.5% at the beginning of the experiment to 5.2% at the end, resulting in an external recovery rate of 43.6%. X-raying this group, it was determined that 26.8% of the fish showed recovery in the x-rays, 48.8% showed light internal lordosis, and 24.4% showed a counterbalancing kyphosis. There was no significant difference in the mortality rates between the fish, though fish without lordosis at the beginning of the experiment had a lower mortality rate (18.7%) than the fish with lordosis at the beginning of the experiment (21.8%).
Andrades et al., 1996	Experimental study investigating the prevalence of lordosis in larvae and 50 adult and juvenile gilthead sea bream	Lordosis was identified as a significant problem that originated early in the fish's life but had lasting effects. 27% of

Source	Summary of source	Key result
	in seawater laboratory conditions over 3 months. Fish were sampled periodically, and also weighed and examined using an x-ray system to visualize their skeletons and swim bladders.	larvae had an axial deformity. These larvae had a high mortality rate, with ~80% dying. However, the remaining percentage survived and developed, mostly maintaining the lordosis. All juveniles with lordosis had an uninflated swim bladder and displayed abnormal swimming behavior, as they had to constantly swim to avoid sinking. However, they later developed functional swim bladders, but this did not correct the lordosis. It was hypothesized by the authors that an uninflated swim bladder was the consequence of lordosis rather than the other way around which had been suggested in some of the existing literature. Deformed fish were shorter than others and were also identified as more susceptible to trauma. Lateral lines were noted as a useful visual indicator to identify otherwise not externally visible lordosis in fish, particularly juveniles, by the lateral line having an irregular trajectory.
Can, 2013	Experimental study comparing the effects of intensive and semi-intensive rearing systems on vertebral deformities in 120,000 juvenile gilthead sea bream over a 50-day period in commercial conditions in Turkey. At weaning (60 dph), 120,000 fish were divided into two groups of 60,000: an intensive group (reared in three replicate outdoor tanks with an initial stocking density of 5 fish/L) and a semi-intensive group (reared in three	At the end of the study, 32% of the intensive group had lordosis, while 0% in the semi-intensive group had lordosis. In the intensive group, the frequency of lordosis and the daily mortality of lordotic fish increased over the study period. At the end of the study, the semi-intensive group achieved a significantly higher survival rate (92%) compared to the intensive group (68%), with cannibalism being a primary factor of inducing mortality. Mean weight was also significantly higher in the semi-intensive group.

Source	Summary of source	Key result
	replicate outdoor tanks at an initial stocking density of 0.15 fish/L). 1000 specimens from each group were sampled at the beginning and the end of the experiment. Weekly, 30 individuals per tank were sampled and radiographed using x-rays to determine the severity of the vertebral deformity if applicable, which was categorized as “light lordosis” or “severe lordosis”.	The author hypothesized that higher stocking densities in the intensive system increase competition, elevating swimming activity and stress, increasing tail-beat frequency and amplitude, exerting force on the vertebral axis, leading to increased vertebral deformities.

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
> 1.9	> 1.7 to ≤ 1.9	> 1.5 to ≤ 1.7	≤ 1.5

Emaciation

Level 0	Level 1	Level 2	Level 3
No clear signs of emaciation	Slightly lean	Lean appearance	Signs of emaciation
			

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 of emaciation.

Condition factor (often denoted as 'K') is calculated as follows: $\mathbf{K = 100 \times W / L^3}$, 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 1.9 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 2.0 to 1.8 is a warning sign.
- Signs of emaciation include visible spine or ribs and reduced muscle mass. Inspect the fish's belly fat.
- Very high condition factors may indicate deformity (e.g., when a vertebral 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 condition factors.
- 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.
- Total length is approximately 1.072 × fork length (calculated by the authors of this protocol based on the mean total and fork lengths of 3,312 farmed sea bream in Karakatsouli et al., 2024). K on fork length is therefore approximately 1.23 × K on total length ($1.072^3 = 1.23$). This was used to inform the thresholds, where papers reported K in terms of total length.
- **Validation:** The condition factor thresholds are based on the studies set out below. The visual scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

Key evidence

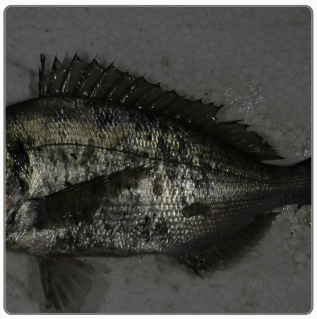
Where a study calculated condition factor using total length, fork-length equivalents are given instead. These were calculated by the authors of this protocol by multiplying condition factor by 1.23 (Karakatsouli et al., 2024).

Source	Summary of source	Key result
Fazio et al., 2018	Cross-sectional study investigating growth and welfare indicators associated with different temperatures on 60 gilthead sea bream (145 g) in commercial flow-through seawater tanks. There were two sample periods from a farm in Sicily, one in October and one in February.	Condition factor was 1.91 in October and 2.01 in February. The difference was not statistically significant. The authors stated that a condition factor below 1.0 indicates poor condition and above 1.4 indicates good to excellent condition. They provided no source or reasoning for these cut-offs.
Fetouh and El-Far, 2023	Field study investigating the condition factor in 13 species of wild-caught fish, including 222 gilthead sea bream (107	Gilthead sea bream had the highest average condition factor of the 13 species assessed. The minimum

Source	Summary of source	Key result
	g). Condition factor was calculated using the total length.	condition factor was 1.38 and the maximum was 2.23, with the average being 1.78. The condition factor also varied slightly with the season, with a condition factor of 1.76 in autumn, 1.72 in spring, and 1.82 in summer.
Bavčević et al., 2010	Experimental study investigating recovery after food deprivation in 460 gilthead sea bream (155 g) in commercial seawater conditions over 60 days. There were 4 groups: One group was fed to satiation with a target of 1.8% of body weight per day for the entire 60 days, the R1.8 group. The other groups were starved at different levels for the first 30 days and then fed to satiation for the remaining 30 days. R1.4 (target of 1.4% of body weight per day), R0.5 (target of 0.5% of body weight per day), and R0 (completely starved). Condition factor was calculated on days 1, 30, and 60 using total length.	All groups started with a condition factor between 1.96–1.98. After 30 days of starvation, the R0 group's condition factor decreased to 1.71, the R0.5 group's condition factor decreased to 1.85, and the R1.4 group's condition factor increased to 2.03. The control increased to 2.08. At the end of the 60 day period (after starved fish were fed to satiation again for 30 days), all groups recovered to reach a condition factor of 2.09–2.1, except for the R0 group, which achieved a condition factor of 2.02. The groups that were starved gained weight faster than the control group once being fed to satiation, although their growth rate in length was not significantly different.

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, 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	Scale loss dominates the overall impression of the fish
			

Protocol application

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

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.
- Where scales are missing as part of a wound, score the area under wounds and exclude it from the scale loss estimate.
- Scales floating in the water indicate that scale loss is a problem.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

Key evidence

Source	Summary of source	Key result
Mateus et al., 2017	Experimental study investigating the impact of chronic crowding stress over 4 weeks on skin healing after scale removal in 64 gilthead sea bream (87 g) reared in laboratory seawater conditions over 7 days. There were two treatment groups: the control, maintained at a normal stocking density of 16 kg/m^3, and the chronically stressed group, maintained at a high stocking density of 45 kg/m^3 for 4 weeks. Fish were distributed into 4 fish tanks with 2 replicates per treatment group. After 4 weeks in these conditions, fish in both groups had approximately 50% of scales on the left side of the body above the lateral line removed using the blunt side of a knife. The right side was left undamaged.	After 4 weeks, crowded fish had lower plasma cortisol than controls (4.0 vs 9.1 ng/mL) and lower expression of the glucocorticoid receptor in skin. 3 days after scale removal, chronically stressed fish had significantly lower plasma osmolality than the control. 7 days after scale removal, stressed fish showed elevated cortisol (7 times higher) than the control group. In both groups the epidermis was re-established within 3 days. Crowding reduced the expression of skin genes for wound repair and inflammation.
Vieira et al., 2011	Experimental study investigating the effects of scale removal on gene expression in 64 gilthead sea bream (93 g) in laboratory conditions over 7 days. Scales were removed on 60–70% of the body by using a wet paper towel to wipe the scales off. There were 4 treatment groups: fed, fed with scales removed, fasted, and fasted with scales removed.	The fish demonstrated rapid tissue repair. On day 3 after scale removal, the epidermis was re-established, and the scale pocket was reformed (without the scale). By day 7, a thin regenerated scale was visible. Fasted fish with scales removed showed an inflammatory response, osmotic barrier repair, and energy metabolism reallocation. 429 gene probes were upregulated on day 3 compared to fasted fish with scales intact. By day 7, only 10 were upregulated in the same comparison. The authors noted there were no evident signs of stress and no mortality occurred.

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	Only 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.

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
Holhorea et al., 2023	Experimental study investigating the effects of stocking density on welfare in 462 gilthead sea bream (initial body weight 480 g) over 53 days in flow-through systems. Prior to the experiment, the fish were reared at a density of 12–16 kg/m^3. Fish were grown at three different final stocking densities: low density / LD: 8.5 kg/m^3, medium density / MD: 17 kg/m^3, and high density / HD: 25 kg/m^3. Dissolved oxygen fell with density.	35 fish per treatment were scored for external condition scoring. HD fish showed significantly worse skin status than both the LD and MD groups, with LD fish having the lowest scores. The authors did not observe biting-chasing activities before or after feeding, and concluded skin damage was primarily due to active feeding behavior and involuntary collisions during feeding. Within the HD group, faster-growing fish had more skin damage. The authors suggested that active feeding with involuntary collisions during feeding caused the damage, though collisions were not measured. Within the HD group, there was also a positive correlation between plasma glucose and skin damage.
Aly et al., 2025	Study investigating the impacts of vibriosis on 250 gilthead sea bream from commercial fish farms in Egypt.	Vibriosis prevalence was highest in summer (35%) and spring (26%). Clinical signs observed included hemorrhagic protruded anus, hemorrhages in the pectoral, dorsal,

Source	Summary of source	Key result
		and tail fins, skin discoloration, and skin ulcers.
Aly et al., 2019	Study investigating the impacts of vibriosis on 200 moribund gilthead sea bream from commercial fish farms in Egypt. Fish were collected during disease outbreaks.	Erosion in the skin of dorsal muscles and hemorrhages in the pectoral fins and tail were observed.

Wounds

Wounds are 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** One 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.

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 gray fungal growth, excessive debris or mucus, visible discharge, redness extending further than 0.5 cm from the wound edge, or necrotic tissue.
    - Any suspected infection should be checked by a fish health professional.
- 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 for Atlantic salmon:[17]
  - Small wounds are visible only on close inspection.
  - Moderate wounds are ≤ 1 cm in diameter.
  - Large wounds are > 1 cm in diameter.
    - For the Centre for Aquaculture Progress' previously published protocol on ongrowing rainbow trout in seawater,[18] wound size classification used a 2 cm diameter as the threshold, but this has been adjusted down for the smaller size of gilthead sea bream.
  - For smaller fish, it is appropriate to use stricter thresholds to differentiate between a moderate wound and a large wound. While imprecise, as a rule of thumb, a diameter of 5% of the fish's total length can be considered as the difference between moderate and large wounds.
- When scoring, note the wound location to further identify trends (e.g., dorsal surface, lateral sides, ventral surface, head). In two laboratory studies, wounds below the lateral line triggered a stronger immune and tissue-repair response, and the authors of one study suggested they may be more serious because they lie closer to internal organs.
- **Validation:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

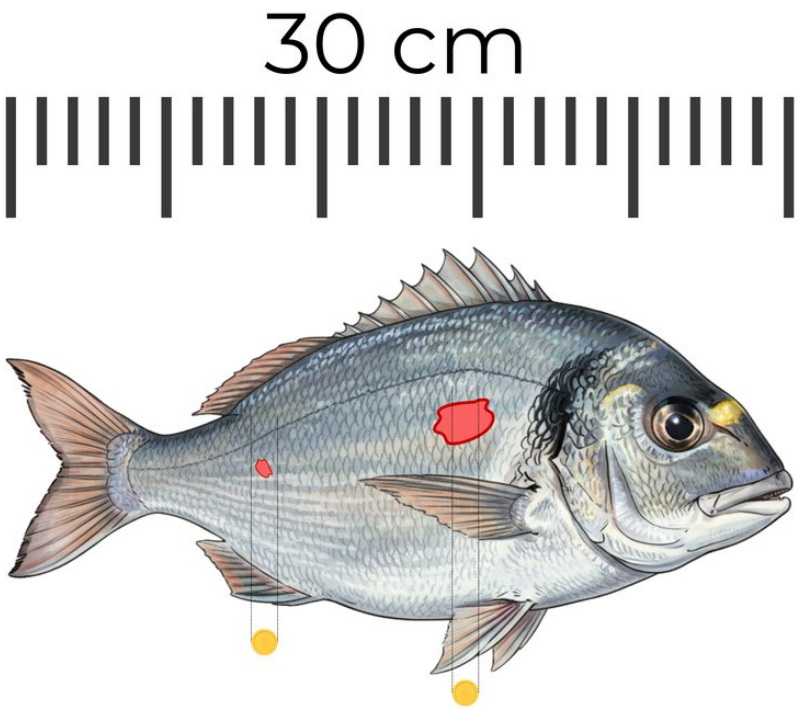


Figure: *Wound size reference on gilthead sea bream. The coin represents a 1 cm diameter, approximating the threshold between a moderate wound (≤ 1 cm) and a large wound (> 1 cm).*

Key evidence

Source	Summary of source	Key result
Chen et al., 2020b	Experimental study investigating the impact of skin wounds in 24 juvenile gilthead sea bream (22 g) in seawater laboratory conditions over 1 week. Half of the fish were on a probiotic diet. Fish received a 2 mm deep, 8 mm diameter circular wound just below the lateral line.	Wounded fish on the control diet had intestinal inflammation, showing signs of mild intestinal disease. The gut of these fish also showed a significant upregulation of three pro-inflammatory genes. Wounded fish on the probiotic diet did not show these symptoms. This demonstrates that localized skin wounds can impact fish homeostasis.
Piñera-Moreno et al., 2024	Experimental study investigating gene expression in response to superficial	The location of the wound triggered a different biological response, with

Source	Summary of source	Key result
	wounds in 45 ongrowing gilthead sea bream (100 g) in seawater laboratory conditions over 20 days. The fish received a 4 mm diameter superficial wound either above or below the lateral line, behind the dorsal fin.	below the lateral line triggering a stronger response. Key markers were more pronounced in the group with the wound below the lateral line, and tissue repair markers were also stronger in this group, though these were mostly not significant due to high variability. The authors suggested that wounds below the lateral line may be more critical to welfare due to its proximity to vital organs and susceptibility to infection.
Ceballos-Francisco et al., 2017	Experimental study investigating healing and mucosal immunity of gilthead sea bream (138 g) in seawater RAS laboratory conditions over 7 days. Wounds (8 mm diameter, 2 mm depth) were made either above or below the lateral line of the sea bream. Sampling occurred daily over the 7 days.	The healing process was faster for wounds below the lateral line, but the differences were not statistically significant due to high variability. The wound location affected the mucosal immune response. Fish wounded below the lateral line showed higher peroxidase and esterase activity peaks during the inflammatory phase. The authors suggested that skin cells are more sensitive to physical aggression in the area below the lateral line.

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.[19] 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, gilthead sea bream 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., silvery body with normal patterns and color intensity)	Very mild color variations that are stable over time 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.

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 gilthead sea bream for that specific site. It may be useful to compare to photos taken from past healthy cohorts.
- Consider environmental factors (such as turbidity, lighting, and substrates) and nutritional factors (such as specific diet) that can affect natural coloration.
- Change in coloration is a nonspecific indicator and must be interpreted alongside other indicators. A coloration change on its own does not establish a welfare problem, but should prompt closer inspection.

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

Key evidence

Source	Summary of source	Key result
Kalogianni et al., 2011	Experimental study investigating the skin's cellular responses to high concentrations of ammonia in 300 adult gilthead sea bream (50 g) in seawater laboratory conditions over 96 hours, including a 24 hour exposure period and a 72 hour recovery period. In addition to the control group, there were two different concentrations of total ammonia nitrogen: a medium exposure group (3.34 mg/L) and a high exposure group (13.1 mg/L).	Gilthead sea bream showed a significant increase in the dispersion of melanosomes, causing the fish to become darker. This was strongest in the medium ammonia group and started almost as soon as exposure began. The medium ammonia group showed a stronger effect than the high ammonia group. This was most pronounced at 24 hours of exposure. The authors suggested that an increase in melanosome dispersion is a response to stress with a warning function to other fishes.
Pavlidis et al., 2011	Book chapter on pigmentation physiology and discoloration problems, with a focus on Sparidae.	Skin pigmentation is an indicator of animal welfare. The two main pigments in fish skin are melanin (black/brown) and carotenoids (red/yellow). Carotenoids are not produced by fish on their own, but are stored from diet. Several farmed Sparidae become darker under intensive rearing, and in gilthead sea bream, discoloration has been reported as a lack of pink or red pigmentation.
Leclercq et al., 2010	Review article on morphological skin color changes in teleost fish, including gilthead sea bream.	Dark/light backgrounds and stressors could lead to color changes in gilthead sea bream.

Snout damage

The snout and mouth of fish are essential for feeding, respiration through the buccal-opercular pump, 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 cartilage or bone **OR** Any infected damage
			

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.

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 gray 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 active bleeding and tissue loss creating a depression in the snout profile.
- Severe snout damage includes any exposed cartilage or bone, open wounds covering a large area, tissue loss that changes the shape of the snout or jaw, damage that prevents the mouth from closing, or signs of infection. Signs of infection include any white or gray 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
Aly et al., 2025	Study investigating the impacts of vibriosis on 250 gilthead sea bream from commercial fish farms in Egypt.	Vibriosis prevalence was highest in summer (35%) and spring (26%). Clinical signs observed included hemorrhagic protruded anus, hemorrhages in the pectoral, dorsal, and tail fins, skin discoloration and skin ulcers, including ulcers in the mouth. The authors noted ulcerations on the mouth could be more lethal than other skin ulcerations, as they could

Source	Summary of source	Key result
		cause the fish to lose appetite and starve.
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 took about 170 minutes to resume feeding, against about 80 minutes in the other groups, and their gill ventilation rate nearly doubled. 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 was interpreted by the authors as an attempt to relieve pain. These behaviors were absent in controls and were significantly reduced by morphine.
Noble et al., 2012	Literature review on injuries and deformities in fish, including snout injuries.	Mouth damage can inhibit a fish's ability to ingest food, impacting growth and survival. Mouth damage may also result in a reduced ability to use the buccal-opercular pumping system to enable proper ventilation of the gills.

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.

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.

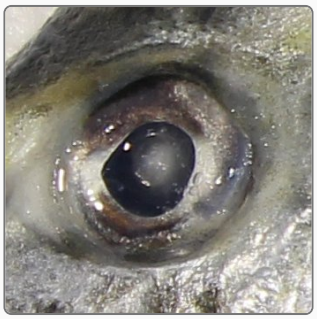
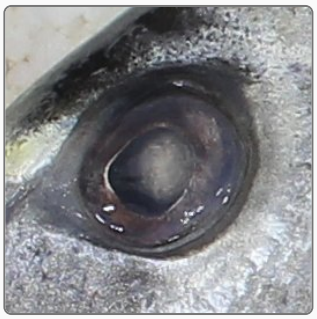
Key evidence

Source	Summary of source	Key result
Geladakis et al., 2025	Longitudinal study investigating the recovery of jaw abnormalities in 527 gilthead sea bream (13.8–15.2 g at time of seawater transfer) over one year of ongrowing in a commercial sea pen.	No gilthead sea bream with a shortened lower jaw deformity at initial observation recovered one year later. Pugheadedness was classified into two different categories, either with or without gross defects on the maxillary and premaxillary bones. In fish without gross defects, 30.4% recovered, but fish with gross defects had a 0% recovery rate. At the end of the ongrowing period, the normal group had a 31.5% rate of tag loss/mortality, which could be treated as a baseline. Fish with pugheadedness but without gross defects had a tag loss/mortality rate of 37%, fish with pugheadedness with gross defects had a tag loss/mortality rate of 43%, and fish with lower jaw deformity had a tag loss/mortality rate of 47.5%. The difference from the normal group was statistically significant only for fish with lower jaw deformity. Of the lower jaw deformity fish, those that did not survive the grow-out stage were significantly smaller than those that did (averaging 12.8 g in weight vs 15.7 g in weight at the juvenile stage). Pugheadedness did not significantly impact growth, while lower jaw deformity did (the authors hypothesized this was due to increased difficulty with feed ingestion).
Fragkoulis et al., 2018	Cross-sectional study investigating the heritability of upper jaw abnormalities in 1400 gilthead sea bream larvae at 39 days post-hatching.	For externally visible abnormalities, pugheadedness had a 6.7% prevalence (with a range of 3.0–13.5% across tanks), lateral displacement of upper jaw had a 2.3% prevalence (with a

Source	Summary of source	Key result
	Larvae were raised in 6 tanks. 200–300 larvae were randomly sampled per tank at 39 days post hatching.	range of 0–10.0% across tanks), and crossbite had a 0.9% prevalence. There was no significant heritability for these abnormalities, however internal abnormalities associated with lateral displacement of the upper jaw had a significant heritability of 0.22. Abnormalities were also observed microscopically, with the most common abnormality being the narrowing of maxillaries, with a prevalence of 89%. Genetic correlations between growth and deformities were low to moderate and negative.
Mhalhel et al., 2023	Review article on deformities in gilthead sea bream.	Jaw deformities can restrict the fish's ability to move their mouth and ingest food, which leads to reduced growth and reduced respiratory efficiency. Although the causes of deformities in gilthead sea bream are uncertain, the authors suggest that environmental factors, nutritional imbalances, genetic disorders, unsuitable rearing conditions, traumatic injury, and parasite infections are most likely.

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 of 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.

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 affected, the welfare risk is significantly higher than for unilateral cases. Where both eyes are scored as level 2 or 3, 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
Carrillo et al., 1999	Observational study investigating unilateral eye abnormalities in 8000 ongrowing gilthead sea bream (199 g) in seawater laboratory conditions (simulating commercial conditions) over 18 months. A random sample of 20 live fish was taken from each tank every 15 days and examined. Additionally, any fish showing abnormalities at other times were also examined.	15% of the randomly sampled fish had unilateral eye abnormalities. An additional 764 targeted fish were examined after being identified for abnormalities. 91.2% of affected fish had cataracts, most of which were in the first stage (63.3%). Gas bubbles were observed in 1.1%, and severe exophthalmos in 7.7%. All affected fish displayed behavioral issues including disorientation, rolling, and positioned near the wall or the central filter of the tank. The authors suggested the eye abnormalities were caused by trauma from aggressive social behavior. 67.3% of the affected fish were male, and the observed abnormalities occurred during the spawning preparation season, a time of increased aggressive behavior.
Jehan et al., 2019	Experimental study investigating the effects of bacteria causing mortalities by experimentally infecting 160 juvenile gilthead sea bream (60 g) in commercial seawater conditions. The researchers infected the fish with bacteria potentially causing mass mortalities in an Egyptian fish farm (*V. alginolyticus* and *P. fluorescens*). 80 fish were infected with each, with 20 left as controls.	Clinical signs of infected fish included uni- or bilateral eye opacity, exophthalmia, dropped eyes, or even complete eye loss. Cataract severity ranged from minor to completely opaque. Further signs included abnormal swimming behavior and organ damage.

Source	Summary of source	Key result
Bjerkås et al., 2000	Cross-sectional study investigating cataracts in 152 sea bream (35–300 g) raised in commercial conditions.	11.9% of examined gilthead sea bream were diagnosed with small cataract changes. These changes were found in the anterior and posterior cortex of the lens. Sea bream were more frequently affected than sea bass.

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.

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

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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 to ≤ 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).

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.

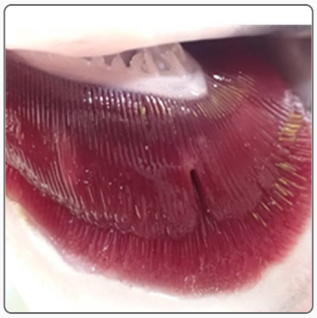
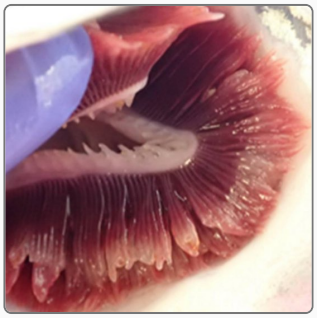
Key evidence

Source	Summary of source	Key result
Vermeylen et al., 2023	Experimental study investigating whether an altered mechanical use of the gill cover could be associated with opercular deformities in 540 juvenile gilthead sea bream. Three dissolved oxygen levels (80%, 100%, and 200%) were tested.	At 48 and 55 days post hatching, 27.2% of 191 fish assessed had a deformed operculum across all treatment groups. Those in the 80% dissolved oxygen conditions had the most severe opercular shape, but the difference was not statistically significant. However, in the hyperoxic conditions of 200%, there was the highest prevalence of deformities (50% at 48 days and 30.3% at 55 days). There was a drop in opercular deformities from 48 days to 55 days.
Beraldo and Canavese, 2011	Longitudinal experimental study investigating the severity of opercular deformities in 4100 gilthead sea bream (starting weight 0.8 g) held in seawater tanks, raceways, and then floating sea pens over 16 months. Fish with opercular malformations were specifically selected for the study. The anomalies were grouped into LA (low level, with a gap of less than 1 mm), MA (medium level, with a gap of more than 1 mm), HA (high level, where the operculum complex was completely folded into the gill chamber), and bilateral anomaly	There was no statistical difference between the mortality rates of the groups, ranging from 3.2%–3.8% over the period. A nearly linear recovery process was observed over time. 7 months into the study, 50% of the fish had regained anatomical integrity of their operculum. At the end of the study, 61% of the population with unilateral anomalies achieved this, with the split being 91%, 54%, and 37.5% for LA, MA, and HA groups respectively. Only 13.6% of fish with

Source	Summary of source	Key result
	groups.	bilateral anomalies regained an intact operculum, and a further 43.6% became unilateral.
Beraldo et al., 2003	Observational study investigating abnormalities of the opercular complex in 582 gilthead sea bream (aged 25–600 days) from 3 commercial fish farms. The fish were categorized into different groups based on age and assessment methodology: Group A (200–600 days old, observed with a stereomicroscope, with bony plates isolated from the opercular and branchiostegal complex), Group B (50–70 days old, subject to staining of the bony-cartilaginous skeleton in toto), Group C (25–60 days old, light-microscope observation), and Group D (70–140 days old, scanning electron microscope).	All fishes older than 60 days in the sample clearly showed opercular abnormalities. In Group A, opercular abnormalities occurred bilaterally 22.7% and unilaterally 77.3% of the time. Abnormalities included inward opercular folding, outer concave depression, partial lack of operculum, and a combination of a partial lack or folding of the operculum in its lower third. In 120 fish aged 25–40 days old in Group C, chosen as they were deemed likely to show the defect, 98.3% had a folding of the loose edge of the operculum into the gill chamber.

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 to ≤ 50% of gill area) **OR** Any small lesion	Severe alterations (affecting > 50% of gill area) **OR** Any large lesion
			

Protocol application

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

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

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

Scoring notes

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

- For assessors familiar with a 0–5 level clinical gill scoring system (e.g., Tassal's gill score guide for amoebic gill disease),[20] 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.
- Reference photos show Atlantic salmon gills, as suitable gilthead sea bream images were not available. This will be updated over time when better images become available.
- **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
Kapetanović et al., 2006	Case study investigating gill disease in 10 gilthead sea bream (10–313 g) in commercial seawater conditions.	The only external signs of the disease observed on individual fish were brown to yellowish brown, swollen, and clumped areas of the gills. The hematological analysis reflected this, with the serum protein being higher in fish without visible gill damage. Reduced hematocrit was also reported in affected fish.
Araújo-Luna et al., 2018	Experimental study investigating the effects of different rearing conditions on welfare in ongrowing gilthead sea bream (300 g) in seawater laboratory conditions. Three different stocking densities were tested: 5, 10, and 20 kg/m^3, with each group maintaining a dissolved oxygen level of at least 70%. This trial ran for 9 weeks.	Gill histopathology scores were 7.8, 3.5 and 2.8 from the lowest to the highest oxygen group, which the authors classed as moderate, mild and no significant pathology. Only the 40–60% group differed significantly from the others. Increasing stocking densities did not have a significant impact on the health of the gills. Using the same scoring system, scores of 1.3, 1.4, and

Source	Summary of source	Key result
	In a separate trial, three different dissolved oxygen levels were tested: 40–60%, 60–80%, and 80–100%. The stocking density was kept constant at 10 kg/m^3. This trial ran for 6 weeks.	2.5 were obtained for stocking densities of 5, 10, and 20 kg/m^3.
Abou-Okada et al., 2023	Observational study investigating gill histopathologies from co-infection of *Ergasilus sieboldi* and *Vibrio alginolyticus* in 45 gilthead sea bream (250 g) from a semi-intensive earthen pond seawater farm in Egypt. 15 additional fish that did not exhibit any behavioral or clinical signs were also sampled as a control.	The fish exhibited signs of abnormal behavior, respiratory distress, and skin ulceration. The cumulative mortalities ranged from 15–20%, with an irregular daily mortality pattern. Clinical examinations of infected fish showed skin darkening, detached scales, skin hemorrhaging, skin abrasion, and pale gills. The infections caused gill architectural deformation, gill lamellae with edematous fluid, lesions in the gill filament, mucus, epithelial fusion, and hyperplasia. The control fish had normal gills.
Riera-Ferrer et al., 2023	Experimental study investigating the effects of *Sparicotyle chrysophrii* in gilthead sea bream exposed to the parasite in RAS compared with unexposed controls in flow-through systems. Two trials were run with 28 exposed fish each: trial 1, 118 g over 81 days, and trial 2, 20 g over 58 days.	The parasite caused gill pathology (e.g., lamellar synechiae, clubbing), significant anemia and increased inflammatory cells in gill tissue.

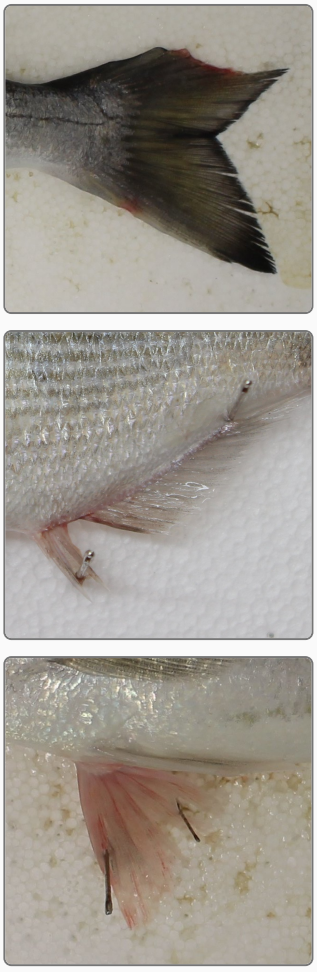
Fin damage

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.

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:** This scoring system has been adapted from the Laksvel scoring system for Atlantic salmon.

Key evidence

Source	Summary of source	Key result
Holhorea et al., 2023	Experimental study investigating the effects of stocking density and limited oxygen on 462 ongrowing gilthead sea bream (480 g) over 53 days. Fish were grown at three different final stocking densities: low density (8.5 kg/m^3), medium density (17 kg/m^3), and high density (25 kg/m^3).	The highest density group had worse fin damage, with the low and medium density groups not differing. In the high density group, the caudal fin scored the worst, followed by the pectoral fin and pelvic fin, and then dorsal fin. The authors hypothesized that increased contact in a high-density environment could lead to physical injuries and aggressive behavior. In this study, aggressive indicators were not monitored, but there were no observations of biting or chasing activities before or after feeding in any group. The authors therefore hypothesized that the physical signs of welfare impairment were primarily due to active feeding behavior with involuntary collisions.
Arechavala-Lopez et al., 2013	This study compared the fin condition of 300 gilthead sea bream (100 wild, 100 farmed, 100 escaped). The 100 farmed gilthead sea bream were obtained from open sea pens with a rearing density of	For fin erosion, farmed gilthead sea bream scored the highest with an average of 2.1. Escaped gilthead sea bream were next with an average score of 1.6, and wild gilthead sea bream had an average score of 0.8.

Source	Summary of source	Key result
	approximately 15 kg/m³. Fin erosion was scored on a numerical scale of 0 to 4 (with 4 being the worst score) and fin splitting was recorded as the mean number of splits per fish. The anterior dorsal fin was excluded as it is commonly damaged by handling.	For fin splitting, the escaped gilthead sea bream had the highest splitting with 2.3 splits per fish, with farmed gilthead sea bream the next highest at 1.9 and wild gilthead sea bream the lowest at 1.2. The authors suggested that the reason why escaped gilthead sea bream scored the highest in fin splitting was due to their fins being more fragile and weaker after farmed conditions than the fins of wild fish.
Bordignon et al., 2025	Cross-sectional study investigating animal-based indicators in 540 gilthead sea bream (367 g) from 3 commercial suppliers in Croatia, Greece, and Turkey, harvested in autumn or spring.	Caudal fin erosion occurred in 77% of fish, with an average erosion index of 0.72. Fish originating from Greece had the highest occurrence (88%) and severity (0.92). Sea bream harvested in autumn showed a higher fin erosion index than those harvested in spring (0.75 v 0.65).

Group-based indicators

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

1. Mortality 65
2. Aberrant behavior 67
3. Aggressive behavior 71
4. Feeding behavior 75
5. Swimming behavior 79

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.[21] In 2024, annual mortality in the marine phase was around 15% for both Atlantic salmon and rainbow trout.[22] In 2021, approximately 5% of all Norwegian locations for Atlantic salmon and rainbow trout achieved mortality rates below 5% in the sea phase.[23] Individual farmers, scientific institutions, and others have argued that this is a feasible mortality rate to achieve.

Mortality rates for commercial gilthead sea bream production are less clear, as public reporting is not required in key production countries. Surveys and production data suggest average mortality of about 6–14% per year, with reported values from about 1% to 30%.[24,25,26,27]

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 ≤ 0.045 %/day	> 0.045 %/day

Mortality per annum

Level 0	Level 1	Level 2	Level 3
≤ 5 %/year	> 5 to ≤ 10 %/year	> 10 to ≤ 15 %/year	> 15 %/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%, initiate immediate investigation.
- 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. While culls are not included in these scoring thresholds, a rising cull rate should be investigated in the same way.
- Being aware of seasonal trends is also essential for making improved management decisions.
- At the end of each production cycle, compare the number of fish stocked with the numbers harvested, dead, culled, escaped, and otherwise removed. A large unexplained difference suggests that deaths are going unrecorded or that counts are inaccurate, and should be investigated.
- **Validation:** These thresholds are based on Norwegian industry aspirations and expert opinion, and checked against Mediterranean production data where it exists. Annual figures for gilthead sea bream assume an 18-month ongrowing period where studies reported mortality per cycle.

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 to ≤ 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 to ≤ 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 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

- This indicator scores individual fish showing abnormal behavior. Changes in the swimming structure of the group as a whole are scored under swimming behavior. A behavior seen in most of the group can raise both scores.
- 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, while accounting for natural variation due to the time of day or year).
    - Reduced schooling participation.
    - Vertical swimming in upper layers.
    - Escape attempts.
  - **Major aberrant behaviors** can include:
    - Surface gasping (indicating respiratory distress).
    - Body rocking or stereotypies (repeated movements with no clear purpose).
    - Side swimming.
    - Severe lethargy (minimal movement, poor responsiveness).
    - Frantic swimming (fast, uncoordinated swimming).
    - Resting on the bottom of the enclosure.
    - Loss of balance or equilibrium.
    - Erratic movements (sudden, unpredictable changes in speed or direction).
    - Increased thigmotaxis (staying close to the net or tank wall instead of in open water).
    - Visibly rapid or exaggerated mouth and opercular movements (increased ventilation).
- 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 pen simultaneously and then discussing any points of disagreement).
- The percentages set out in the thresholds are based on the observed fish. For example, if 100 fish are observed, then 1 or 2 fish displaying minor aberrant behaviors would score level 1. A single fish displaying a major aberrant behavior scores level 1 only if at least 1,000 fish are observed; otherwise it scores level 2.
- **Validation:** The prevalence thresholds are derived from Pettersen et al. (2014) 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. Due to limited gilthead sea bream-specific evidence, the types of behaviors described above are largely informed by salmonid studies.

Key evidence

Source	Summary of source	Key result
Glaropoulos et al., 2012	Experimental study investigating escape behaviors and coping ability due to food supply in 405 gilthead sea bream (about 31 g) over 21 days. Three feeding level groups were used: 2%, 1%, and 0% of body weight per day, under 3 net conditions: intact mesh, mesh with a cut twine, and mesh with three adjacent cut twines, forming a tear. With the tear, the fish could escape the net. Fish were observed for 15 minutes each day right before the feeding time.	Fish inspection and biting of the net was observed in all experiments. Biting the twine consisted of violent pulling and tearing movements. Limited-fed sea bream being fed 1% daily showed net inspection, biting, and escape attempts, which the authors attributed to an intensified search for food. Starved fish became lethargic, with slow swimming near the net. Well-fed fish being fed 2% daily displayed high inspection with limited escape attempts. The presence of a tear decreased biting (as fish would choose to cross rather than to bite). Most escapes occurred between days 17–19.
Goldan et al., 2003	Experimental study investigating food competition in gilthead sea bream (250–1,100 mg) in seawater laboratory	Subordinate fish mostly avoided the lower feeding zone and displayed vertical swimming in the upper layers,

Source	Summary of source	Key result
	conditions over 16 days. Fish were observed in eight different groups, including four single-size groups and four mixed-size groups. Food was dispersed in one corner and for a limited amount of time, where all feed was consumed within 15 minutes. Behavior was observed on days 1, 4, 10, and 16, for 8 minutes before feeding and 8 minutes during feeding.	interpreted as avoidance of social interactions.
Pettersen et al., 2014	Review paper presenting a welfare index to assess welfare of Atlantic salmon in sea pens. Aberrant fish were included as an indicator.	The authors set four levels for the proportion of aberrant fish: 0%, < 0.1%, 0.1–2%, and > 2%. > 2% was considered very poor welfare and a knockout level in the index. Citing other studies, the authors described aberrant fish as showing changed swimming, rapid breathing or darker skin, and sometimes abnormal behavior such as bumping into the nets, circling or spiraling.

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.[28] 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	Moderate aggressive behavior	Elevated aggressive behavior	Severe aggressive behavior **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	≤ 5% of fish involved in aggressive interactions **OR** Normal aggressive behavior	> 5 to ≤ 20% of fish involved in aggressive interactions **OR** Moderate aggressive behavior	> 20% of fish involved in aggressive interactions **OR** Elevated or severe aggressive behavior **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 level.

Scoring notes

- **Normal aggressive behavior** can involve brief chasing, threat postures, brief confrontations, or positioning disputes, such as when a feeding hierarchy is established. These behaviors typically do not result in physical harm.
- **Moderate aggressive behavior** can involve minor fin nipping or extensive chasing.
- **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 of individual fish, 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, so behaviors score one level higher outside feeding than during feeding. The prevalence thresholds in the non-feeding subindicator also reflect this: ≤ 5% is likely due to individual personalities with some potential for emerging issues, > 5 to ≤ 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 pen simultaneously and then discussing any points of disagreement).
- **Validation:** Due to limited gilthead sea bream-specific evidence, the types of behaviors described above are informed by salmonid studies and expert opinion.

Key evidence

Source	Summary of source	Key result
Oikonomidou et al., 2019	Experimental study investigating the effects of food quantity and distribution method on 180 gilthead sea bream (39 g) in laboratory conditions over 13 days. There were 4 treatment groups, a combination of low (1% of body mass) and high (3% of body mass) food quantity, and localized distribution and dispersed distribution. Behavior was video recorded for 7 minutes surrounding the 1 minute feeding time, which occurred twice a day.	Gilthead sea bream showed heightened aggression in the low ration groups, regardless of distribution. Aggression was observed to be significantly higher during feeding than before or after feeding. Biting was the main aggressive behavior during feeding, when chasing was almost absent. Before feeding, biting was usually more common than chasing. After feeding, chasing was as common as biting at the low ration and more common at the high ration. Chasing was rare during feeding, more frequent before feeding, and most frequent after feeding. More fish had dark vertical bands after feeding compared to before feeding, which quickly appeared / disappeared, though the authors noted there was a possibility that some fish retained the bands they developed during feeding.
Batzina et al., 2014	Experimental study investigating the effects of environmental enrichment on aggressive behaviors in 306 gilthead sea bream (initial weight 25 g) over 3 months. There were 6 treatment groups, covering combinations of environmental enrichment (blue substrate, red-brown substrate, or no	The lower density groups had higher aggression and mass variability. The blue substrate groups reduced aggression and had better growth. Blue substrate also reduced brain serotonergic activity at both densities, and dopaminergic activity at the lower density. The authors concluded that substrate and density effects were

Source	Summary of source	Key result
	substrate) and stocking density (lower stocking density of 4.9 kg/m³ and higher stocking density of 9.7 kg/m³).	socially induced. The red-brown substrate reduced aggression, but did not produce a significant growth improvement.
Folkedal et al., 2018	Experimental study investigating the impact of habituation and conditioning to light flashes on behavior in 168 gilthead sea bream (247 g) in laboratory conditions over four days. There were two treatment groups: fish exposed to a light stimulus 30 seconds before feeding and fish exposed to the same light stimulus 30 minutes before feeding. Each group received 10 equally sized meals every hour from 09:00 until 18:00.	Some fish developed dark vertical bands and performed mock charges, signaling territoriality rather than overt aggression. The paper did not report this causing any injury or feeding exclusion. The number of territorial fish (identified by their dark bands) did not change over the experiment but varied from trial to trial within the same tank. Tanks with more territorial fish had a higher baseline swimming speed, suggesting the social hierarchy raised the activity level of the whole group.

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.[29] Reduced feeding can also indicate disease or poor water quality.[30] 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.[31]

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 to ≤ 5% of feed uneaten	> 5 to ≤ 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.

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

For consistency in measuring feed waste, 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. Where fish are fed to satiation, little feed will be left uneaten and the leftover feed estimates will not be useful. In these cases, calculate the shortfall instead: (planned ration − feed eaten) ÷ planned ration. For example, if 25 kg was planned and fish stopped feeding at 21 kg, the shortfall is 16%.

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 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, or following staff.
  - Schooling behavior near the expected feeding location.
- Active anticipatory behavior means a clear increase in the above activities before feeding with most visible fish responding (e.g., gathering at the feeding area or following staff); some anticipatory behavior means some increase in activity (a clear response from a significant part of the visible group); reduced anticipatory behavior means minimal behavioral change; and absent anticipatory behavior means no response.
- A lack of anticipatory behavior can be an early warning sign and should be checked against behavior during feeding. It should not 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 pens. In these cases, rely on the feeding behavior score.
- Feeding behavior may be impacted by one-off events such as handling, weather events, or predators, 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 pen simultaneously and then discussing any points of disagreement).
- **Validation:** Due to limited gilthead sea bream-specific evidence, the thresholds are informed by salmonid studies and expert opinion.

Key evidence

Source	Summary of source	Key result
Sánchez et al., 2009	Experimental study investigating the effects of feeding schedule on locomotor activity rhythms and stress response in 48 gilthead sea bream (108 g) in laboratory conditions over 60 days. There were 2 treatment groups: scheduled daytime feeding, where fish would receive a single meal at 2PM, or random daytime feeding, where fish would receive a single meal at a random time during the light period.	Fish in the scheduled group showed a clear increase in locomotor activity several hours before feeding time, while the random feeding group showed sustained activity throughout the light phase with no anticipatory peaks. At day 30, the scheduled feeding group had a significantly higher weight, but at day 60 there was no significant difference. Plasma cortisol was significantly higher, with levels being approximately 8 times higher in the randomly fed group. Glucose levels were also significantly elevated in the random group. The authors suggest that a scheduled feeding cycle is beneficial for fish welfare as they can prepare themselves for the feed, enabling fish to prepare and optimize food intake and nutrient use. This could also save energy, as the fish with a feed schedule are not required to be on constant alert to avoid missing out on food.
Andrew et al., 2004	Experimental study investigating the impacts of increased competitive behavior on productivity in gilthead	Lower rations resulted in increased competition including increased feeding intensity, increased density

Source	Summary of source	Key result
	sea bream (32 g) over 1 month. There were five different treatments. Ration sizes of 3.5%, 3%, 2.5% and 2% body weight per day were fed at a low feed delivery rate of 10 g per minute. In the other group, a ration size of 3% was fed at a high feed delivery rate of 20 g per minute.	under the feeder, and a higher frequency of sharp-angled turns. Increasing the feed delivery rate increased competition (though to a lesser degree compared to reducing ration size), requiring fish to conduct more sharp turns to stay in the feed area. Aggressive interactions such as chasing/nipping and fin damage were low and did not differ between treatments.
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 9% of feed (ranging from 6–11% across rearing units), while fish from the low-stress responsive line wasted approximately 4% of feed (ranging from 2–6% across rearing units). 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 trend towards a positive correlation between initial size variation within tanks and total feed waste, though it was not statistically significant.

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.

How swimming behavior indicates welfare in gilthead sea bream is not well understood. One experiment on gilthead sea bream broodstock in enriched environments found that there was more individual swimming behavior and reduced schooling behavior in enriched environments and that this could be indicative of improved welfare, as in nature gilthead sea bream live in small groups or independently.[32] The same paper mentioned that in some species, higher density shoaling is associated with acute stress, and that physical and cognitive stimulation is lost compared to independent swimming. Another paper, citing work on salmon, suggests that schooling might indicate an adaptive behavior to high stocking densities (rather than schooling actually being the preferred method of swimming) as it minimizes collisions.[33]

Level 0	Level 1	Level 2	Level 3
Normal, active swimming structure with appropriate pen 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 level.

Scoring notes

- When assessing swimming behavior, focus on organization and schooling rather than specific geometric patterns.
- This indicator scores the swimming structure of the group as a whole. Individual fish behaving abnormally are scored under aberrant behavior. A behavior seen in most of the group can raise both scores.
- 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 pen.
  - 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 pen 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
Muñoz et al., 2020	Experimental study investigating the effects of environmental enrichment on swimming behavior of 360 gilthead sea bream (218 g) over one month. 10 fish were surgically implanted with acoustic tags. The experiment was conducted in an experimental sea	There was a clear difference in swimming patterns in the day and night. In the day, fish swam closer to the surface, likely due to the continuous daytime feeding. At night, fish descended deeper into the sea pen and exhibited similar swimming

Source	Summary of source	Key result
	pen. For the first 2 weeks, there was no environmental enrichment. For the next 2 weeks, structural enrichment was added to the sea pen (four vertical ropes hanging in the center). There was automatic continuous feeding during the day.	activity as in the day. Environmental enrichment increased the vertical range of swimming depths during the night, but had no significant effect during the day. The fish's space distribution was denser in the central part of the sea pen near the enrichment structure and often away from the net. The authors noted that fin abrasion may be minimized by enough space and by avoiding stress.
Remen et al., 2015	Experimental study investigating the effect of temperature on oxygen requirements in 99 gilthead sea bream (397 g) in laboratory conditions over 45 days. Fish were distributed into 3 replicate tanks, with each tank going through sequential temperature changes, with 8–10 days acclimation at each temperature. In each tank, oxygen saturation was dropped over a period that ranged from 2 hours at 20°C to 5 hours at 12°C.	There was no change in swimming speed with oxygen dropping towards the limiting oxygen saturation. However, as it continued to drop below this level there was a steady decline in swimming speed. The fish did not demonstrate typical signs of severe acute stress such as resting on the tank bottom, escape attempts, gulping at the surface, losing equilibrium, or having a startle response when the tank lid was lifted.
Mignucci et al., 2021	Experimental study investigating the cardiac and behavioral responses to hypoxia and warming in 10 gilthead sea bream (534 g). Each fish experienced 4 treatments in a combination of being held in cylindrical tanks in groups of 3 or being held in a respirometer, a confined individual chamber, and being subjected to gradual warming (from 21°C to 31°C) or hypoxia (from 100% to 15% saturation).	In the hypoxia experiments, the tank group showed normal heart rate from 100–40% saturation and the respirometer group elevated heart rate (with the authors theorizing this was due to confinement stress), but both groups experienced severe slowing at 30%, and further slowing at 20–15% to similar heart rates. In the tank, fish moved slowly around the perimeter and then tended to begin resting on the bottom at hypoxic levels. There was no sign of agitation at any hypoxic levels in the

Source	Summary of source	Key result
		respirometer. There was no significant difference between the tank and respirometer at any level of hypoxia, with activity being low. In the warming experiments, as with the hypoxia experiments, the tank group showed normal heart rate and the respirometer group elevated heart rate at 21°C. At 23°C, there was a significant increase in heart rate in both groups and a significant increase in activity in the tank. Heart rates increased significantly from 21°C up to 27°C in the tank group, but only up to 23°C in the respirometer group. In the tank group, activity also increased with increasing temperature, including occasional bursts / escape attempts. The fish in the respirometer were confined so restricted in increasing behavior.

Environmental indicators

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

1. Stocking density 85
2. Dissolved oxygen 89
3. Temperature 93
4. Carbon dioxide 98
5. pH 102
6. Turbidity 106
7. Total suspended solids 110
8. Ammonia 113
9. Nitrite 117
10. Nitrate 120
11. Metals 122
12. Water velocity 126
13. Salinity 130
14. Hydrogen sulfide 134

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/m^3), 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.[34]

However, across the literature there is a clear trend: increasing stocking density results in poor welfare outcomes for gilthead sea bream (e.g., higher mortality and lower growth). Some isolated studies have found better or neutral welfare outcomes at higher densities (e.g., higher survival rates at 36–44 kg/m^3 compared to 12–15 kg/m^3).[35] Separating the direct impacts of high stocking density from associated issues, such as deteriorated water quality, can be very difficult.

Level 0	Level 1	Level 2	Level 3
≤ 10 kg/m^3	> 10 to ≤ 15 kg/m^3	> 15 to ≤ 20 kg/m^3	> 20 kg/m^3

Protocol application

Calculate stocking density by dividing total fish weight (kg) by total water volume available to fish (m^3). Recalculate density regularly (ideally weekly) using predicted fish growth data.

Scoring notes

- At very low densities for commercial settings (e.g., < 2 kg/m^3) 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.
- Stocking density should always be interpreted alongside other important welfare indicators (such as dissolved oxygen, mortality, and aggressive behavior). In RAS or raceways, higher stocking densities may pose smaller welfare risks due to higher control of important parameters such as dissolved oxygen.
- Stocking density will increase over time until harvest. The time spent at different densities should also be noted, as long periods at higher density are likely to carry more risk than a short peak before harvest.
- **Validation:** The thresholds are precautionary management thresholds, derived from considering the results in the studies listed below. Studies often test stocking densities in

smaller laboratory tanks, rather than in larger systems replicating commercial conditions, so results should be interpreted with caution. Typical commercial sea pens range in densities of 10–20 kg/m³, with densities from 8–70 kg/m³ having been reported (Muniesa et al., 2020).

Key evidence

Source	Summary of source	Key result
Holhorea et al., 2023	Experimental study investigating growth and genetic markers response to different stocking densities and dissolved oxygen levels in 462 gilthead sea bream (480 g) in laboratory conditions over 53 days. Three stocking densities were tested: LD (6 kg/m³, ending at 8.5 kg/m³), MD (12 kg/m³, ending at 17 kg/m³) and HD (22 kg/m³, ending at 25 kg/m³). Dissolved oxygen was also maintained at different concentrations for each group. The low density group had a saturation of 70–95%, the medium density group 55–75% and the high density group 45–60%.	Growth and feed intake significantly decreased from LD to HD. HD fish had a final body weight of 598 g compared to 662 and 669 g in the MD and LD groups respectively. Epidermal status and fin damage worsened with increased stocking density. HD fish had substantially worse fin damage than MD fish, which in turn had slightly more damage than LD fish. Plasma cortisol levels in HD fish were slightly higher than MD fish, with both nearly double that of LD fish. Swimming behavior was more homogeneous in HD fish, reflecting improved social cohesion.
Roncarati et al., 2006	Experimental study investigating the impacts of stocking density on 344,520 gilthead sea bream fingerlings over 17 months in intensive seawater commercial raceway conditions and semi-intensive seawater earthen ponds. Gilthead sea bream were reared with the goal of obtaining three different final densities: 40 kg/m³, 20 kg/m³, and 0.2 kg/m³, with initial densities of 0.4 kg/m³, 0.2 kg/m³ and 0.003 kg/m³ (calculated by the authors of this protocol based on data presented in the study). The two higher density	The final densities for the groups were 48 kg/m³, 21 kg/m³, and 0.27 kg/m³. The survival rates were 89%, 91%, and 87% respectively, which was not statistically significant. Final weight did not differ significantly between groups (326.5, 340.3 and 314.9 g from highest to lowest density). Towards the end of the experiment as the final density was reached, the highest density group exhibited a marked spike in plasma glucose, LDH, and transaminase levels compared to the other groups, suggesting crowding stress. Plasma triglycerides

Source	Summary of source	Key result
	groups were reared in commercial raceways and fed 0.4–1.2% daily, while the lowest density group was reared in an earthen pond and fed 0.4% five days a week. Every three months and at harvest, blood was sampled from 10 fish per group. At harvest, biometrics were recorded from 150 individuals per group.	and total cholesterol were consistently and significantly higher in the two higher density groups throughout the trial. The authors concluded that a density of 20 kg/m³ can be considered acceptable for raising healthy ongrowing gilthead sea bream.
Martos-Sitcha et al., 2019	Experimental study investigating the effect of stocking density and hypoxia on gilthead sea bream (34 g) in RAS laboratory conditions over 22 days. Two stocking densities were tested, a low density of 9.5 kg/m³ and a high density of 19 kg/m³. Two different oxygen levels were also tested at each density level, 75–85% saturation and 42–43% saturation.	Cortisol levels were significantly higher in the high density groups than in the low density groups. Growth was significantly lower in the high density group.
Araújo-Luna et al., 2018	Experimental study investigating the effects of stocking density on 388 gilthead sea bream (initial weight 269–272 g) in laboratory conditions in flow-through tanks over nine weeks. Three stocking densities were tested: 5 kg/m³, 10 kg/m³ and 20 kg/m³, reaching final densities of 8 kg/m³, 16 kg/m³ and 31 kg/m³. In each group, dissolved oxygen saturation was maintained above 70% for the majority of the experimental period, though oxygen did decline significantly with increasing density (78%, 76%, and 74%).	Specific growth rate declined borderline significantly as stocking density increased. Feed conversion ratio improved significantly with increasing density. Gill histopathology scores also worsened with increasing density (scores of 1.3, 1.4, and 2.5 with increasing density), though this result was not statistically significant. Scores between 0–3 were considered to be normal healthy gills. There were no significant differences based on density in hematocrit, hemoglobin, plasma glucose, or plasma cortisol results. A second trial in the same study tested dissolved oxygen. The authors concluded that dissolved oxygen had a stronger influence on welfare than stocking

Source	Summary of source	Key result
		density, and that density up to 20 kg/m^3 did not affect growth or physiology while dissolved oxygen stayed above 70%.

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
> 85 to ≤ 110 %	> 70 to ≤ 85 % **OR** > 110 to ≤ 150 %	> 60 to ≤ 70 % **OR** > 150 to ≤ 200 %	≤ 60 % **OR** > 200 %

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 pen 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. During periods of higher water temperature, monitoring frequency should be increased, as the combination of reduced oxygen solubility and increased metabolic demand creates a high welfare risk. 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

- Example mg/L values required for each saturation at different temperatures in seawater at 38 ppt:

Level	Dissolved oxygen (% saturation)	mg/L at 14°C	mg/L at 18°C	mg/L at 24°C	mg/L at 28°C
0	> 85 to ≤ 110	> 6.9 to ≤ 9.0	> 6.4 to ≤ 8.3	> 5.8 to ≤ 7.4	> 5.4 to ≤ 7.0
1	> 70 to ≤ 85	> 5.7 to ≤ 6.9	> 5.3 to ≤ 6.4	> 4.7 to ≤ 5.8	> 4.4 to ≤ 5.4
1	> 110 to ≤ 150	> 9.0 to ≤ 12.2	> 8.3 to ≤ 11.3	> 7.4 to ≤ 10.2	> 7.0 to ≤ 9.5
2	> 60 to ≤ 70	> 4.9 to ≤ 5.7	> 4.5 to ≤ 5.3	> 4.1 to ≤ 4.7	> 3.8 to ≤ 4.4
2	> 150 to ≤ 200	> 12.2 to ≤ 16.3	> 11.3 to ≤ 15.1	> 10.2 to ≤ 13.5	> 9.5 to ≤ 12.7
3	≤ 60	≤ 4.9	≤ 4.5	≤ 4.1	≤ 3.8
3	> 200	> 16.3	> 15.1	> 13.5	> 12.7

- 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.
- At higher temperatures, fish have a higher oxygen demand.
- 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., for less than 4 hours) is less concerning than sustained exposure (e.g., more than 24 hours or recurring daily over multiple days). Brief dawn dips that recover within a few hours of daylight are expected and less concerning than conditions that persist throughout the day.
- The upper thresholds refer to oxygen saturation only. Total gas supersaturation (including gases other than oxygen) can cause gas bubble disease.
- Sustained level 2 or 3 scores should trigger an immediate management intervention.
- **Validation:** In many of the papers dissolved oxygen co-varied with stocking density, making it difficult to isolate the welfare effects of dissolved oxygen alone. The 70% dissolved oxygen saturation separating level 1 and level 2 follows APROMAR (2024), which recommends dissolved oxygen saturation of at least 70%. However, no study tested 70% specifically, with the available studies testing dissolved oxygen levels in broad bands. The 60% boundary reflects gill pathology and reduced growth at 40–60% saturation. Although

mortality was not observed at these 40–60% levels of saturation in experimental studies, these studies tended to be short-term.

Key evidence

Source	Summary of source	Key result
Araújo-Luna et al., 2018	Experimental study investigating the effects of dissolved oxygen levels on gilthead sea bream (316 g) over 6 weeks. Three oxygen levels were tested: 40–60%, 60–80%, and 80–100%. The stocking density was fixed for all treatments. Water temperature was between 24.4–27.8°C.	There was no mortality in any of the groups. Growth was 36% in the lowest oxygen treatment and 40% in the two higher dissolved oxygen groups. Only fish in the 80–100% group exhibited healthy gills with a score of 2.8 (indicating no significant pathology). The gill histology score was significantly worse in the lowest oxygen group, with a score of 7.8, indicating moderate pathology of clinical significance. The 60–80% group was non-significantly worse than the 80–100% group, scoring 3.5 (suggesting mild pathology only). There was no difference in plasma cortisol between the groups, although there was high variability within treatments. Hematocrit was significantly higher in both the 40–60% and 60–80% groups compared to the 80–100% group.
Samaras et al., 2023	Experimental study investigating the effects of dissolved oxygen on growth rates and other welfare indicators in 315 gilthead sea bream (79 g) in RAS over 3 months. Three oxygen levels were tested: 40–60%, 60–80%, and 80–100%, all at a temperature of 26.5°C.	Growth performance was stronger in the higher oxygen levels than the lower oxygen levels, with an overall similar weight between the 60–80% and 80–100% groups. Cortisol levels in fish plasma and scales were similar between the different groups.
Vermeylen et al., 2023	Experimental study investigating the association between dissolved oxygen and opercular deformities in juvenile gilthead sea bream. Three dissolved oxygen levels were tested: 80%, 100%,	The hyperoxic 200% treatment had the highest prevalence of opercular deformities (50% at 48 days and 30.3% at 55 days). Fish held at 80% dissolved oxygen had the most severe opercular

Source	Summary of source	Key result
	and 200%. 540 fish were sampled between 20 and 55 days post hatching. Deformity prevalence was assessed in the 191 fish sampled at 48 and 55 days, which had fully developed opercula.	shape, but the difference was not statistically significant. Across all treatment groups, 27.2% of 191 sampled fish had a deformed operculum.
Pavlidis and Samaras, 2020	Literature review on many gilthead sea bream and European sea bass welfare indicators, including dissolved oxygen.	It is noted that moderate hyperoxia of around 150% has not caused mortalities or significant changes in growth or the immune system of European sea bass, though hyperoxia of over 200% has caused these changes. No literature investigating hyperoxia in gilthead sea bream was cited.

Temperature

Water temperature influences the physiological processes in gilthead sea bream. As ectothermic animals, gilthead sea bream 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
> 18 to ≤ 24 °C	> 16 to ≤ 18 °C **OR** > 24 to ≤ 26 °C	> 14 to ≤ 16 °C **OR** > 26 to ≤ 28 °C	≤ 14 °C **OR** > 28 °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 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 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.
- Temperatures below 14°C are associated with a shift to anaerobic metabolism, and below 12°C with increased susceptibility to winter syndrome. Prolonged exposure to low temperatures poses a severe welfare and survival risk.
- In regions where summer water temperatures routinely exceed 28°C, extended level 3 scoring may occur. This reflects genuine welfare risk during these periods and should prompt heightened monitoring of other welfare indicators, particularly dissolved oxygen and mortality, rather than being dismissed as unavoidable. Sustained level 3 scores should trigger an immediate management intervention.
- **Validation:** The thresholds have been based on many papers testing gilthead sea bream exposed to different temperatures. Fish can acclimate to different temperatures, and depending on what they are used to, may have different thermal preferences (within reasonable thermal ranges).
    - Much of the welfare evidence at high temperatures comes from laboratory studies where fish were warmed within one to two days. Farmed fish tend to warm gradually over the season and are likely to tolerate a given temperature better given longer acclimation periods. Level 3 has therefore been set above 28°C after discussion with industry experts.
    - Some growth studies and farm growth models place the growth optimum around 25°C, although results vary with life stage and salinity. Signs of cellular stress and a shift towards anaerobic metabolism have been reported from 22–24°C, so level 1 (> 24 to ≤ 26°C) flags the start of that risk.

Key evidence

Source	Summary of source	Key result
Vargas-Chacoff et al., 2009	Experimental study investigating the metabolic response to various salinities and temperatures in 90 gilthead sea bream (150 g) in laboratory conditions over 35 days. Overall, there were nine different treatment groups, each with a combination of salinity (low salinity at 5 ppt, seawater at 38 ppt, and high salinity water at 55 ppt) and temperature (12°C, 19°C, and 26°C). The	Plasma glucose and plasma lactate levels tended to be substantially higher at 26°C than at other temperatures, indicating high energy demand. Low temperature overall reduced metabolic demand, and high temperature increased metabolic demand. The authors noted that 12°C could be

Source	Summary of source	Key result
	fish were acclimated for 14 days, and then the experiment was carried out for a further 35 days.	the breakpoint of when gilthead sea bream are susceptible to winter syndrome.
Feidantsis et al., 2015	Experimental study investigating the impacts of carbon dioxide, temperature, and pH on cellular stress responses and mortality in 720 gilthead sea bream (324 g) in laboratory conditions over 10 days. Fish were initially kept at a temperature of 18°C, and then were exposed to either 18, 24, or 28°C at either pH 8.05 or pH 7.3, with the lower pH groups exposed to elevated carbon dioxide. For the higher temperature groups, the temperature was gradually raised over 24 to 48 hours.	Both the control fish and fish exposed to elevated carbon dioxide with low pH levels showed no mortality at 18°C. Fish exposed to 24°C (with normal pH/ carbon dioxide levels) showed a 7% mortality from day 3, but no further mortality was observed by the last day of the experiment. For fish exposed to carbon dioxide at this temperature, mortality reached 15% by the end of the experiment. At 28°C, a gradual increase in fish mortality started on day 4 and reached 70% at the end of the experiment. In the 28°C group with elevated carbon dioxide, mortality reached 100% on the first day. Note: in treatments combining elevated temperature with elevated CO_2/low pH, mortality was substantially higher than with temperature alone, indicating synergistic effects between these stressors.
Ibarz et al., 2003	Experimental study investigating the effects of reduced temperature on oxygen consumption and feeding rates in 100 gilthead sea bream in seawater laboratory conditions. Two experiments were run. The first investigated how the rate of a temperature drop from 18°C to 8°C impacted gilthead sea bream, with one group having a gradual drop of 1°C per day over 10 days, and the other	Decrease in oxygen consumption was directly related to the fall in water temperature. The gradual or sharp rate of cooling had no significant effect on the overall drop in metabolic activity, although the metabolic depression was more pronounced when temperature dropped below 12°C compared to when it dropped above 12°C. Fish appetite gradually decreased as

Source	Summary of source	Key result
	group having sharper drops, with the first drop from 18°C to 12°C, being held at that temperature for one week, and then another sharp drop from 12°C to 8°C. The second experiment investigated prolonged cold exposure, where the temperature was reduced from 16°C to 8°C or 12°C, at which the fish were held for 20 days.	the temperature dropped below 18°C. At 18°C, feed ration was 1% of body weight, but at 13°C, feed ration was 0.3% of body weight. Below 13°C, fish stopped eating and did not resume feeding even after 20 days of maintenance at 12 or 8°C. The authors noted that general metabolic depression, together with fasting, could favor opportunistic pathogens, potentially initiating the signs of winter disease.
Feidantsis et al., 2021	Review of the potential impacts of global warming on marine fishes using gilthead sea bream as a case study, integrating multiple laboratory and field studies.	14°C corresponds to a lower critical temperature, being the temperature at which anaerobic metabolism is triggered in gilthead sea bream. At temperatures below 12°C, gilthead sea bream develop winter syndrome. 18°C was noted to be within the optimal temperature range. Temperatures between 22–24°C indicate an upper limit for gilthead sea bream, with some cellular stress indicators observed. At 24°C, indications of transition from aerobic to anaerobic metabolism are observed. Survival is compromised at temperatures above 26°C, with oxidative defense compromised at prolonged exposure to higher temperatures, and increased mortality and sluggishness observed in temperatures above 28°C.
Tort et al., 2004	Experimental study investigating the effects of temperature on feeding rates, immune indicators, and histopathological changes in gilthead	Feed intake dropped substantially at low temperatures, although the authors noted that the results suggest that once the fish have reached the

Source	Summary of source	Key result
	sea bream (85 g, with 64 fish sampled) over 4 weeks in controlled experimental conditions. Fish were fed two types of feed, control and a specifically prepared winter feed. Fish were acclimated at 18°C for 2 weeks, then were subjected to a temperature ramp of 1°C per day down until 11°C. The fish were held at 11°C for 13 days, and then temperature was ramped back up to 18°C.	new stable temperature, feeding behavior is re-established and feed intake is recovered after a certain period of time. However, no complete recovery of food intake was achieved during the recovery period (where the temperature was increased from 11°C back up to 18°C). Lysozyme activity decreased significantly at low temperatures and only partially recovered when the temperature reached 18°C again. Histological examination showed mild mucous cell hyperplasia of the intestinal mucosa after the temperature drop, which the authors interpret as a possible preliminary stage of winter syndrome.

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 alongside other poor water quality conditions, such as low dissolved oxygen. 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.

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

Protocol application

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

CO_2 is unlikely to need assessment in sea pens, so it 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 pens).

Use a reliable method to measure CO_2 rather than calculating it from pH levels. A temperature correction may be needed, as CO_2 solubility and toxicity vary with temperature. Thresholds refer to dissolved free CO_2 in mg/L.

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 exposures (e.g., more than 24 hours or recurring daily over multiple days).
- Monitor for behavioral changes, including reduced feeding and surface gasping during elevated CO_2 periods.
- **Validation:** Limited gilthead sea bream-specific studies exist, so thresholds have been developed conservatively. No available study has isolated the effects of CO_2 from co-

occurring pH changes in gilthead sea bream. All cited studies used CO_2 injection to manipulate levels, which simultaneously lowers pH (except for Ben-Asher et al. (2013)). The thresholds therefore reflect the combined welfare risk of elevated CO_2 and reduced pH rather than CO_2 effects in isolation.

- This is acceptable for operational purposes, as CO_2 and pH typically co-vary in production conditions, but it means that CO_2 thresholds should always be interpreted alongside pH measurements.
- The most comprehensive study (Ben-Asher et al., 2013) was conducted in brackish water and the authors noted that seawater pH scale constants would yield CO_2 values approximately 45% lower. This complicates direct application of their concentration thresholds to seawater sea pen conditions and supports a conservative threshold approach. The full-scale RAS result, where fish in tanks with CO_2 maintained at ~16 mg/L grew approximately 10% more than fish exposed to fluctuating 19–37 mg/L, provides the strongest commercial-scale evidence supporting the level 3 threshold of > 15 mg/L, although this was also in brackish water conditions.
- Background CO_2 levels in commercial sea pen conditions have not been well characterized. If monitoring reveals that levels routinely exceed 1 mg/L in well-managed sea pen systems with good water exchange, the level 0 threshold may need to be reconsidered.

Key evidence

Source	Summary of source	Key result
Ben-Asher et al., 2013	Experiment investigating the effects of sublethal CO_2 concentrations on performance markers in 255 adult gilthead sea bream (310 g) over 71 days and 1500 juvenile gilthead sea bream (4 g) over 64 days in brackish water flow-through tanks. In adults, the CO_2 levels tested were: 7.2, 22.2, and 39.3 mg/L. In juveniles, the CO_2 levels tested were: 5.2, 20.5, 32.3, and 56.3 mg/L. A further full-scale RAS study was also conducted, with 180,000 fish split between pH-controlled tanks (with constant ~16 mg/L CO_2) and pH-uncontrolled tanks (with fluctuating 19–37 mg/L CO_2) over 197 days.	In both life stages, growth rate decreased with increasing CO_2, but growth was only minimally inhibited up to ~20 mg/L. At 32.3 mg/L, juvenile growth was reduced by 39% relative to the lowest treatment; at 56.3 mg/L, by 56%. Survival was unaffected except at the highest concentration for juveniles with 87.9% survival, with other groups having survival rates of 97.6–100%. In the full-scale experiment, the fish in the controlled pH tanks were ~10% heavier at the end of the experiment compared to the uncontrolled tank. The authors noted that using seawater pH scale constants would give CO_2

Source	Summary of source	Key result
		values ~45% lower than recorded in this experiment (which used brackish water).
Feidantsis et al., 2015	Experimental study investigating the effects of CO_2, temperature, and pH on cellular stress responses and mortality in gilthead sea bream (324 g) in RAS over 10 days. There were 6 treatment conditions (Henry's Law and the solubility coefficient from Weiss (1974) were applied by the authors of this protocol to calculate dissolved CO_2 concentrations from pCO_2 values, using the salinity reported for each treatment and a seawater density of 1.024 kg/L): - 18°C + pH 8.05 + CO_2 0.69 mg/L - 18°C + pH 7.3 + CO_2 4.72 mg/L - 24°C + pH 7.95 + CO_2 0.58 mg/L - 24°C + pH 7.3 + CO_2 4.16 mg/L - 28°C + pH 7.94 + CO_2 0.56 mg/L - 28°C + pH 7.3 + CO_2 4.14 mg/L	Exposure to elevated CO_2 levels above 24°C increased fish mortality. In the lower CO_2 group at 24°C, there was a mortality of 7%, while there was a mortality of 15% in the higher CO_2 group. In the 28°C groups, in the lower CO_2 group there was a 70% mortality over 10 days, while there was a 100% mortality in the first day of exposure in the higher CO_2 group. There was no mortality in either of the 18°C groups. Heat Shock Proteins were significantly higher at higher CO_2.
Ruiz-Jarabo et al., 2021	Experimental study investigating the impact of ocean acidification on energy management in 54 gilthead sea bream (262 g) in laboratory seawater conditions over 5 weeks. There were three experimental groups with the following water quality parameters (Henry's Law and the solubility coefficient from Weiss (1974) were applied by the authors of this protocol to calculate dissolved CO_2	There was no mortality in any treatment. There were no changes in body condition or plasma osmoregulatory markers, though the authors noted that longer periods of exposure may reveal stronger differences. The authors also noted that homeostasis was achieved by costly regulating processes at higher CO_2 levels. At the highest CO_2 level, liver and

Source	Summary of source	Key result
	concentrations from pCO_2 values, using the salinity reported for each treatment and a seawater density of 1.024 kg/L): pH 8.17 and CO_2 0.55 mg/L, pH 7.87 and CO_2 1.23 mg/L, and pH 7.62 and CO_2 2.32 mg/L.	muscle glycogen decreased, an important energy source to adapt to the hypercapnia conditions, showing metabolic cost. Plasma CO_2 increased 63% in the highest CO_2 group, with significant linear correlation to water CO_2. Plasma lactate increased 176% at the highest CO_2 level.

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.

Level 0	Level 1	Level 2	Level 3
> 7.8 to ≤ 8.2 pH	> 7.7 to ≤ 7.8 pH **OR** > 8.2 to ≤ 8.3 pH	> 7.3 to ≤ 7.7 pH **OR** > 8.3 to ≤ 8.5 pH	≤ 7.3 pH **OR** > 8.5 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. In RAS, nitrification

consumes alkalinity, which makes pH more sensitive to CO_2, so alkalinity should also be monitored.

- **Validation:** Most studies used CO_2 to decrease pH, which confounded results. It is difficult to separate these results and to understand whether welfare impacts were due to the effect of increased CO_2 or reduced pH. This is acceptable for operational purposes, as CO_2 and pH typically co-vary in production conditions, but it means that pH thresholds should always be interpreted alongside CO_2 measurements.
    - The thresholds for pH above 8.2 are based on expert judgment and the general principle that extreme alkalinity can impair gill function and increase ammonia toxicity. No study has tested the welfare effects of elevated pH (above 8.2) on gilthead sea bream, though APROMAR (2024) recommends a pH of 8.5 as the upper limit. These thresholds should be interpreted as precautionary.

Key evidence

Source	Summary of source	Key result
Araújo et al., 2018	Experimental study investigating the effects of temperature and pH on survival and proteome in 48 gilthead sea bream (38 g) in RAS laboratory conditions over 42 days. There were 4 treatment groups: 18°C at pH 8 (control), 22°C at pH 8 (warming), 18°C at pH 7.5 (acidification), and 22°C at pH 7.5 (warming and acidification). Each treatment used a single tank of 12 fish.	Mortality was 0% in the control and warming group. However, in the acidification group and the warming and acidification group, the mortality was 17% and 50% respectively. Condition factor did not differ between the treatments. The proteome remained stable in the control, but had some moderate changes in the warming group, with more intense changes in the acidification group.
Eshchar et al., 2006	Experimental study investigating the growth of gilthead sea bream at high ammonia and low pH in two trials in land-based seawater conditions. In the first trial, 300 fingerlings (0.9 g) were held for 16 days under three conditions: high TAN at pH 6.8, low pH (6.8) with negligible TAN, and ambient seawater (pH not reported). In the second trial, gilthead sea bream (84 g) were grown for 250 days in two commercial-scale flow-through tanks,	In the first trial, growth and survival did not differ between treatments. In the second trial, final weight (332 g vs 371 g) and feed conversion ratio (2.50 vs 2.27) did not differ significantly between the experimental and control tanks, with no apparent mortalities.

Source	Summary of source	Key result
	one per treatment. The experimental tank had low water exchange, averaging pH 7.22, 5.55 mg N/L TAN and 7.4 mg/L CO_2. The control tank had normal water exchange, averaging pH 7.52, 1.34 mg N/L TAN and 2–5 mg/L CO_2, at about 24°C (22–25°C). Each treatment used a single tank.	
Feidantsis et al., 2015	Experimental study investigating the effects of CO_2, temperature, and pH on cellular stress responses and mortality in gilthead sea bream (324 g) in RAS over 10 days. There were 6 treatment conditions (Henry's Law and the solubility coefficient from Weiss (1974) were applied by the authors of this protocol to calculate dissolved CO_2 concentrations from pCO_2 values, using the salinity reported for each treatment and a seawater density of 1.024 kg/L): - 18°C + pH 8.05 + CO_2 0.69 mg/L - 18°C + pH 7.3 + CO_2 4.72 mg/L - 24°C + pH 7.95 + CO_2 0.58 mg/L - 24°C + pH 7.3 + CO_2 4.16 mg/L - 28°C + pH 7.94 + CO_2 0.56 mg/L - 28°C + pH 7.3 + CO_2 4.14 mg/L	Both 18°C groups had no mortality, but mortality was higher at higher temperatures. In the 24°C group, pH 7.95 had 7% mortality over 10 days, and pH 7.3 had 15% mortality over 10 days. In the 28°C group with pH 7.94, mortality started on day 4 and reached 70% on the last day, and the pH 7.3 group had mortality of 100% after the first day.
Rodríguez et al., 2026	Experimental study investigating bone density and growth in response to low pH levels in 200 juvenile gilthead sea bream (24 g) in laboratory conditions over 68 days.	Specific growth rate was significantly higher in the control group compared to the 7.3 pH group, with the largest reduction in the first half of the trial. The authors interpreted this as the initial energetic cost of adjusting to

Source	Summary of source	Key result
	Two pH levels were tested: 7.9 (control) and 7.3.	the lower pH. Vertebra opacity and slenderness ratio were significantly different in the pH 7.3 group, suggesting altered skeletal structure. Plasma potassium was significantly elevated in the pH 7.3 group, which the authors noted has been observed under stress conditions. Mortality was not reported.

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
≤ 10 NTU	> 10 to ≤ 20 NTU	> 20 to ≤ 40 NTU	> 40 NTU

Secchi disc depth

Level 0	Level 1	Level 2	Level 3
> 6 m	> 4 to ≤ 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 pen 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 pens, 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.
- A clear change in turbidity should prompt investigation of the cause, even within level 0 or level 1.
- 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 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 > 40 NTU.
    - Where there is chronic exposure, consider temporary fish relocation if possible.
- **Validation:** Gilthead sea bream are naturally found in coastal and estuarine environments where moderate turbidity is common. There is no gilthead sea bream-specific data available. Thresholds have been developed based on evidence from other Sparidae species. The thresholds also do not account for particle composition, which is more important than absolute NTU values. The thresholds represent expert judgment and precautionary principles rather than validated, evidence-based welfare boundaries. Thresholds should be applied with caution.
    - As NTU levels cannot be converted into Secchi disc depth, the Secchi disc depth thresholds have been developed separately 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. These thresholds have no validation for gilthead sea bream in different conditions and should be treated as a provisional reference only. Montes et al. (2018) studied harmful algal blooms, so shallow Secchi readings in that study partly reflected bloom density, not only particle load.

Key evidence

Source	Summary of source	Key result
Lowe et al., 2015	Experimental study investigating the effects of sediment-induced turbidity on 45 silver sea bream in laboratory conditions over 30 days. An additional field survey of 73 fish across 7 estuaries was also conducted to validate the results. In the experimental trial, 5 conditions were tested: ≤ 10 (control), 20, 40, 80, or 160 NTU. The field conditions ranged from 1–2 NTU (4 mg/L TSS) to 17 NTU (37 mg/L TSS).	Foraging success declined exponentially with increasing turbidity, from ~77% in the control, ~73% in the 20 NTU group, to ~8% in the 160 NTU group. Chronic weight loss increased up to 40 NTU (at 14%) and plateaued at that turbidity. Gill alterations, such as epithelial hyperplasia and lamellar fusion increased with increasing turbidity. Ventilation rate also tended to increase with increasing turbidity. Behavioral distress (such as gulping and lethargy) appeared at 80–160 NTU, with a 17% mortality. In the field observations, body condition declined linearly with increasing turbidity, and diet shifted from pelagic to benthic prey at 12–17 NTU. The authors noted that negative health impacts in the field began at 12–17 NTU and at ≥ 40 NTU in the laboratory, noting laboratory results may be conservative due to contaminated sediments and cumulative storm events in the field.
Cumming and Herbert, 2016	Experimental study investigating the effects of chronic turbidity exposure on 75 silver sea bream (15–60 g) in laboratory conditions over 30 days. Fish were exposed to ≤ 10 (control), 20, 40, 60, or 80 NTU.	Gill damage increased with increasing turbidity: lamellar density decreased significantly at 40 NTU and above, epithelial lifting elevated at 80 NTU, and oxygen diffusion distance increased at 60 and 80 NTU, though oxygen uptake did not change. All turbidity-exposed fish lost weight, which was significant at 40 NTU and above. Specific growth rate was 0% in

Source	Summary of source	Key result
		the control group and around -0.35% per day in the 40 NTU group.
Cyrus and Blaber, 1987	Experiment investigating turbidity preferences in various species, including goldlined sea bream, Cape stumpnose, and goldsilk sea bream. 4 turbidity ranges were tested: < 10, 10–50, 50–80, and > 80 NTU.	The goldlined sea bream spent 48% of their time at < 10 NTU, 17% at 10–50 NTU, 25% at 50–80 NTU, and 10% at > 80 NTU. Cape stumpnose and goldsilk sea bream were classified as 'indifferent' to turbidity, but still spent minimal time at > 80 NTU. Some individuals became less active, moved to the bottom, and appeared unable to navigate at > 50 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: 1–2 m, 2–4 m, 4–6 m, and > 6 m. The deeper the Secchi reading, the higher probability of observing 'normal' behavior. At Secchi readings with a depth of 1–2 m, 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 1–2 m there was approximately an 80% probability of abnormal behavior, while where the Secchi reading was > 6 m, 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. 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).[38] In sea pens, 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. TSS and turbidity levels often increase together, 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 ≤ 20 mg/L	> 20 to ≤ 35 mg/L	> 35 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).
- Turbidity and TSS often rise together from the same event; they may be interpreted together.

- **Validation:** There is no gilthead sea bream-specific data available. Thresholds have been developed based on evidence from other Sparidae species.
  - To develop thresholds, reported turbidity in the literature was converted to TSS levels by the protocol authors.
    - The Cyrus and Blaber (1987) paper cites previous papers which converted between the two: 20 NTU ≈ 15 mg/L of suspended solids; 124 NTU ≈ 130 mg/L of suspended solids; and 266 NTU ≈ 250 mg/L of suspended solids. This results in ratios of 0.75, 1.05, and 0.94 (converting from NTU to mg/L), the average of which is 0.91. These involved estuaries, and the approximations were derived by converting silt concentrations. This ratio was used to calculate the amount of total suspended solids in other papers.
    - This NTU to TSS conversion ratio of 0.91 derived from estuarine silt samples may not apply accurately to all suspended particle types. In ponds with phytoplankton blooms, the ratio may differ substantially. Where possible, measure TSS directly rather than converting from turbidity readings.
  - The thresholds represent expert judgment and precautionary principles rather than validated, evidence-based welfare boundaries. Thresholds should be applied with caution. The particle makeup of TSS also has a critical impact on the effects.

Key evidence

Source	Summary of source	Key result
Li and Shen, 2012	Experimental study investigating the impact of suspended solids on blackhead sea bream (0.46 g) in laboratory settings. Two experiments were run: a 10-day mortality experiment (300 individuals) and a 20-day experiment for sublethal end points (10 days of exposure, then 10 days in clean seawater). In the mortality experiment, 0, 50, 100, 500, 1000, and 2000 mg/L of suspended solids were tested. In the sublethal experiment, 0, 100, and 1000 mg/L were tested. Sediment particles were < 63 µm, and made of fine silt / clay.	Mortality was dose-dependent over the 10 days. In the control group, setting the baseline mortality rate, mortality was 7%. Mortality increased with increased suspended solids, at 18% in the 50 mg/L group, 34% in the 100 mg/L group, and increasing to 66% in the 2000 mg/L group. At 1000 mg/L, gill filaments were covered with mucus and fine sediment, causing lamellae to stick together and reducing respiratory surface area. The authors noted that even at a lower level of 100 mg/L, fish may experience minor respiratory distress.
Cumming and Herbert, 2016	Experimental study investigating the effects of chronic turbidity exposure	Gill damage increased with increasing turbidity: lamellar density decreased

Source	Summary of source	Key result
	on 75 silver sea bream (15–60 g) in laboratory conditions over 30 days. Fish were exposed to ≤ 10 (control), 20, 40, 60, or 80 NTU, corresponding to 9, 18, 36, 54, and 72 mg/L of suspended solids (converted by the authors of this protocol using a ratio of 0.91).	significantly at 36 mg/L and above, epithelial lifting elevated at 72 mg/L, and oxygen diffusion distance increased at 54 and 72 mg/L. All turbidity-exposed fish lost weight, which was significant at 36 mg/L and above. The control group had a specific growth rate of 0%, while the 36 mg/L group had a specific growth rate of around -0.35% per day.
Cyrus and Blaber, 1987	Experiment investigating turbidity preferences in various species, including goldlined sea bream, Cape stumpnose, and goldsilk sea bream. 4 turbidity ranges were tested: < 9, 9–45, 45–72, and > 72 mg/L.	The goldlined sea bream spent 48% of their time at < 9 mg/L, 17% at 9–45 mg/L, 25% at 45–72 mg/L, and 10% at > 72 mg/L. Cape stumpnose and goldsilk sea bream were classified as 'indifferent' to turbidity, but still spent minimal time at > 72 mg/L. Some individuals became less active, moved to the bottom, and appeared unable to navigate at > 45 mg/L.

Ammonia

NH_3 is the unionized form of ammonia. Where there are high levels of ammonia in the environment that the gilthead sea bream are in, this can mean that they reduce the amount of ammonia they excrete, or even take up 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 pens, 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
≤ 0.05 mg/L	> 0.05 to ≤ 0.1 mg/L	> 0.1 to ≤ 0.3 mg/L	> 0.3 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 × 1.214**, where f is the fraction of NH_3 that depends on salinity, pH, and temperature, and 1.214 converts ammonia nitrogen to ammonia.

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). In RAS, measure ammonia daily.

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, in seawater 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:** The thresholds are largely informed by expert opinion. While a limited number of studies investigate the impacts of ammonia on gilthead sea bream welfare, they do not test threshold-relevant concentrations over a long period of time. The thresholds are therefore precautionary.
    - The longest study on the species (Wajsbrot et al., 1993) used small juveniles over 20 days and did not test concentrations below 0.12 mg/L NH_3, where growth was already reduced by 18% (not significant). At 0.33 mg/L NH_3, 15% of fish died within 20 days (not significant).
    - Level 0 is set at the 'permissible (harmless)' level of 0.05 mg/L unionized ammonia for marine fish given by Haywood (1983) cited by Wajsbrot et al. (1993), and supported by Seo et al. (2020), where red sea bream at 0.03 mg/L NH_3 had the best outcomes, although this was confounded by other parameters.
    - The upper limit for level 1 (0.1 mg/L NH_3) is informed by safe levels estimated from lethal concentrations (about 0.09 mg/L NH_3 at 30 ppt in Kir and Sunar, 2018, and about 0.08 mg/L NH_3 in Wajsbrot et al., 1991), and by the observations in Seo et al. (2020) that welfare indicators (cortisol, white blood cells, liver enzymes) were compromised at 0.07 mg/L in red sea bream.
    - Level 3 aligns with the long-term safe limit of 0.26 mg/L NH_3-N (about 0.32 mg/L NH_3) used by APROMAR (2024).
    - No study has tested the chronic effects of low-level ammonia exposure (≤ 0.1 mg/L NH_3) over production-relevant timescales (months) in gilthead sea bream. The level 0 and level 1 thresholds are conservative extrapolations and should be validated through field monitoring data.

Key evidence

Source	Summary of source	Key result
Wajsbrot et al., 1993	Experimental study investigating the toxicity of ammonia to gilthead sea	Survival was 100% in the control and at 0.12 mg/L NH_3, 85% at 0.33 mg/L, 90%

Source	Summary of source	Key result
	bream (0.26 g) over 20 days. 5 different unionized ammonia nitrogen (NH_3-N) concentrations were tested: 0.1, 0.27, 0.47, 0.74, and 0.89 mg/L as well as a control group with undetectable levels of ammonia nitrogen. This is converted into NH_3 by the authors of this protocol (multiplying by a factor of 1.214), providing ammonia levels of 0.12, 0.33, 0.57, 0.9, and 1.08 mg/L respectively. There were two replicate tanks per concentration. Seawater with a salinity of 40.5 ppt was used, and all groups had high oxygen levels at a minimum of 90% saturation throughout the entire experimental period. The temperature was 26°C and pH 8.1.	at 0.57 mg/L, 60% at 0.90 mg/L, and 50% at 1.08 mg/L. At 0.12 mg/L NH_3, growth was 82% of the control, and at 0.33 mg/L 94%, though neither was significant. At 0.57, 0.9, and 1.08 mg/L, growth was significantly suppressed, at 68%, 61%, and 50% of the control respectively. The gills and kidneys showed no histopathological effects at any concentration. At 0.90 mg/L NH_3, fish showed clear histopathological effects in the liver, with responses starting at 0.57 mg/L. The authors noted gilthead sea bream appeared to be less sensitive to ammonia than other marine fish, and cited 0.05 mg/L unionized ammonia as a permissible level for marine fish.
Kir and Sunar, 2018	Experimental study investigating the effects of salinity and acute ammonia and nitrite exposure in 720 juvenile gilthead sea bream (1.1 g) in laboratory conditions over 96 hours. Three different salinities were tested: 10 ppt, 20 ppt, and 30 ppt. The TAN levels tested were 6, 8, 10, 12, 14, 16, 20, and 24 mg/L. The temperature was 20°C and pH 8.2.	The experiment calculated the concentration lethal to 50% of the test population in 96 hours (LC50). At a salinity of 10 ppt, the LC50 NH_3-N level was determined to be 0.25 mg/L. For a salinity of 20 ppt, it was 0.50 mg/L. For a salinity of 30 ppt, it was 0.76 mg/L. This corresponds to NH_3 levels of 0.3, 0.6, and 0.9, achieved by multiplying the NH_3-N levels by 1.214. Tolerance to ammonia significantly increased with increasing salinity. The authors estimated safe levels (LC50 ÷ 10) of 0.5, 1.1 and 1.9 mg/L TAN at 10, 20 and 30 ppt, equal to about 0.03, 0.06 and 0.09 mg/L NH_3 (converted by the authors of this protocol).

Source	Summary of source	Key result
Seo et al., 2020	Experimental study investigating the effect of ammonia and nitrite accumulation on 495 red sea bream (220 g) in seawater laboratory conditions over 20 days. Fish were held in different stocking densities: 2.5 kg/m^3, 5 kg/m^3, 10 kg/m^3, and 10 kg/m^3 with a protein skimmer to remove inorganic nitrogen compounds. Otherwise, total ammonia nitrogen was let to increase naturally, without constant levels. NH_3 levels have been calculated by the authors of this protocol from the TAN figures provided in the study (maximum 1.66 mg/L TAN), assuming a salinity of 38 ppt, pH of 8.1 and temperature of 18°C.	Higher stocking densities led to higher ammonia levels. Cortisol increased with higher stocking densities and ammonia. At the end of the experiment, the low and medium stocking density groups had about 0.03 mg/L of NH_3, and the highest stocking group without the skimmer had 0.07 mg/L. At the higher levels of ammonia and stocking density, cortisol levels were increased, white blood cells were reduced, and liver damage was suggested by changes in enzymes. Note that ammonia, nitrite, and nitrate levels co-varied with stocking density in this study. Welfare effects cannot be attributed to ammonia alone.

Nitrite

Gilthead sea bream 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 pens, nitrite is not usually a problem due to the flowing, changing water, and because chloride ions compete with nitrite for transport.[39]

Level 0	Level 1	Level 2	Level 3
≤ 1 mg/L	> 1 to ≤ 15 mg/L	> 15 to ≤ 30 mg/L	> 30 mg/L

Protocol application

Nitrite can be measured using colorimetric test kits (e.g., diazotization method) or photometric analysis. Thresholds are in mg/L nitrite (NO_2^-). If a test kit reports nitrite-nitrogen (NO_2-N), multiply by 3.29.

It is generally unnecessary to monitor nitrite levels in seawater conditions. Nitrite only needs to be measured 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 gilthead sea bream welfare in sea pens.
- 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.
- Gilthead sea bream are highly tolerant of nitrite in seawater: the 96-hour LC50 for juveniles at 30 ppt was about 2,650 mg/L NO_2^- (Kir and Sunar, 2018). However, welfare problems will occur before reaching this concentration.
- **Validation:** Evidence for the impacts of nitrite on gilthead sea bream is very limited, and the thresholds rely heavily on studies from other species and expert opinion. These thresholds have not been specifically validated for gilthead sea bream and should be

treated as provisional. They should be revised if substantial gilthead sea bream-specific data become available.

- Level 0 (≤ 1 mg/L NO_2^-) reflects background levels. In red sea bream, stress markers rose in the group where nitrite reached about 2.3 mg/L NO_2^-, though nitrite co-varied with ammonia and stocking density (Seo et al., 2020).
- Level 3 (> 30 mg/L NO_2^-) is set at the level below which APROMAR (2024) expects no toxicity problems at normal marine salinity.

Key evidence

Source	Summary of source	Key result
Kir and Sunar, 2018	Experimental study investigating the effects of salinity on acute ammonia and nitrite toxicity in 720 juvenile gilthead sea bream (1.1 g) in laboratory conditions over 96 hours. Three salinities were tested: 10 ppt, 20 ppt, and 30 ppt. Nominal nitrite concentrations were 600–1,200 mg/L NO_2-N (about 1,970–3,950 mg/L NO_2^-, calculated by the authors of this protocol by multiplying by 3.29). The temperature was 20°C and pH 8.2.	The experiment focused on calculating the lethal concentration required to kill 50% of the test population in 96 hours (LC50). The 96-hour LC50 was 370.8 mg/L NO_2-N at 10 ppt, 619.5 mg/L at 20 ppt, and 806.3 mg/L at 30 ppt. This corresponds to about 1,220, 2,040, and 2,650 mg/L NO_2^- (converted by the authors of this protocol by multiplying by 3.29). The authors estimated safe levels (LC50 ÷ 10) of 37, 61, and 80 mg/L NO_2-N (about 120, 200, and 265 mg/L NO_2^-) at 10, 20, and 30 ppt. Nitrite was about 2.2 times more toxic at 10 ppt than at 30 ppt.
Shen et al., 2024	Experimental study investigating the effects of chronic nitrite exposure to 450 spotted sea bass (29 g) in laboratory conditions over 28 days. The fish were reared at either 0, 8, or 16 mg/L of nitrite concentration. Fish were held at 33°C. Salinity levels were not mentioned. Given the spotted sea bass is a euryhaline fish that is cultivated off coasts in China, seawater conditions were assumed by the authors of this protocol.	Hemoglobin decreased as nitrite concentration increased. Damaged hemoglobin also increased with increasing concentration. Exposed fish also showed signs of damage to the gills, increasing in severity with increased concentration of nitrite. There were also signs of ion imbalance and oxidative stress markers in the two exposure groups.

Source	Summary of source	Key result
Deane and Woo, 2007	Experimental study investigating the effects of nitrite on 24 silver sea bream (250–400 g) in seawater laboratory conditions over 7 days. The fish were divided into 3 groups, and exposed to seawater containing either 0, 82.3, or 164.5 mg/L of nitrite (calculated by multiplying the nitrite-nitrogen levels by 3.29).	The nitrite-exposed groups suffered endocrine disruption, kidney dysfunction and cellular stress (measured through increase of HSP70 in gills, kidney, and liver, and of HSP90 in kidney and liver). The higher nitrite group had more severe welfare impacts.

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 pens, 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 ≤ 70 mg/L	> 70 to ≤ 150 mg/L	> 150 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^- 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 pens.
- 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 very few studies on the impacts of nitrate on gilthead sea bream, and the thresholds have been derived from studies on other species. These thresholds should be applied with caution and treated as provisional.
  - The 25 mg/L and 70 mg/L thresholds are interpolations between expected background levels and the APROMAR guideline of 150 mg/L. No study has isolated the welfare effects of nitrate at these specific concentrations in any Sparidae species.

Key evidence

Source	Summary of source	Key result
Seo et al., 2020	Experimental study investigating the effect of ammonia and nitrite accumulation on 495 red sea bream (220 g) in laboratory conditions over 20 days. Fish were held in different stocking densities: 2.5 kg/m³, 5 kg/m³, 10 kg/m³, and 10 kg/m³ with a protein skimmer to remove inorganic nitrogen compounds. Otherwise, total ammonia nitrogen was let to increase naturally, without constant levels.	In the 10 kg/m³ group, nitrate-nitrogen rose to 28.2 mg/L (or ~125 mg/L nitrate), while in the other groups it did not go above about 18 mg/L (or ~81 mg/L nitrate). In the 10 kg/m³ group with the higher nitrate levels, TAN and nitrite were highest, and cortisol, AST, ALT, and ALP were significantly increased. There were also signs of oxidative stress in the liver. Nitrate co-varied with ammonia, nitrite, and stocking density, so welfare effects cannot be attributed to nitrate alone.
Vectesi et al., 2012	Experimental study investigating the physiological response to nitrate in 45 European sea bass (155 g) over 48 hours. Nitrate levels of about 1 (control), 100, and 700 mg/L were compared.	There were no mortalities and no statistically significant differences. There were minor signs of immunological suppression in the two exposure groups, including lower white blood cell count, higher hemoglobin concentration, and more thrombocytes. The authors noted the true welfare risk of nitrate is in combination with other stressors such as high stocking density, handling, and poor water quality.
APROMAR, 2024	Review of gilthead sea bream welfare indicators, including nitrite and nitrate.	Nitrate below 150 mg/L is not a toxicity problem at normal salinities.

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 have been provided for copper and mercury only, as these have the strongest evidence. Mercury is unlikely to be an issue, but high levels can impact the liver, gills, and kidneys of gilthead sea bream. 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 cadmium, iron, aluminum, and zinc) to alert them to potential welfare problems.

Copper

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

Mercury

Level 0	Level 1	Level 2	Level 3
≤ 0 µg/L	> 0 to ≤ 0.5 µg/L	> 0.5 to ≤ 1 µg/L	> 1 µg/L

Overall: Overall score is determined by the most severe subindicator

Protocol application

Copper thresholds refer to dissolved copper (sample filtered before preservation). If only total copper is available, it can be used as a conservative estimate. Mercury thresholds refer to total mercury.

Field test kits are available, but are unlikely to be accurate at low concentrations for thresholds in the µg 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. In pen systems, 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

- Methylmercury is more toxic than inorganic mercury.
- The absorption of mercury through feed is more problematic than absorption of waterborne mercury.
- 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 sources 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.
- Very low levels of metals may be below the laboratory's detection limit. These results can be scored as 0 µg/L.
- **Validation:** The suggested thresholds do not account for other parameters, such as water quality (particularly pH), which can influence toxicity of metals. Apply these thresholds with caution. Most available literature investigated high acute concentrations of metals rather than aquaculture-relevant levels over time. With mercury, no papers examined doses for gilthead sea bream, except Guardiola et al., 2015, which examined sea bream exposed to 8 µg/L, measuring skin mucus immune activity only. Further, mercury is a difficult variable to control over time in experiments, as it gets absorbed.

Key evidence

Source	Summary of source	Key result
Gabbianelli et al., 2003	Experimental study investigating copper-induced DNA damage in erythrocytes of 17 gilthead sea bream in laboratory seawater conditions over 20 days. Fish were exposed to 0.1 ppm	After the 20 days, the treatment group had significantly more DNA strand breaks in their red blood cells: 19.5% of cells were above the damage cut-off, compared to 5% in the control.

Source	Summary of source	Key result
	$CuSO_4$, equivalent to ~25 µg/L of dissolved copper, assuming it is copper sulfate pentahydrate.[40]	However, antioxidant enzymes were not decreased significantly.
Kalantzi et al., 2016	Field study investigating the environmental and biological effects of copper alloy vs conventional nets at a commercial gilthead sea bream farm (478 g) in the Mediterranean over 1 year. Copper alloy nets were installed. Fish biomarkers and tissue copper were measured at 2 time points.	The background dissolved copper was 0.42 µg/L before cage installation. After installation, mean dissolved copper at the copper alloy cage peaked at approximately 1.8 µg/L declining over time. One episodic event caused the levels to increase to above 4.5 µg/L in the conventional net cage. No significant differences in fish biomarkers were observed between the cage types or over time.
Kim et al., 2018	Experimental study investigating the effects of waterborne copper on 150 red sea bream (27.2 g) in laboratory seawater conditions over 5 days. Waterborne copper levels of 10, 20, 30, and 40 µg/L were tested.	No significant changes in the health biomarkers were measured at 10 or 20 µg/L. At 30 µg/L, all biomarkers showed significant changes: cortisol was elevated, metallothionein nearly doubled, sodium-potassium ATPase (NKA) decreased indicating gill damage, and gill cell apoptosis occurred. At 40 µg/L, glucose increased by a factor of 1.6, indicating severe metabolic stress. The authors proposed 30 µg/L as the threshold concentration at which copper becomes acutely toxic to red sea bream.
EPA, 1984	Review of the impacts of mercury on many freshwater and saltwater species.	No Sparidae or Mediterranean aquaculture species were referenced in the document. The saltwater criterion was driven primarily by invertebrates and North American fish species. The Final Chronic Value for mercury for saltwater species was determined to be 1.106 µg/L.

Source	Summary of source	Key result
Pereira et al., 2016	Experimental study investigating the neurotoxicity of inorganic mercury in 168 white sea bream (146 g) in seawater laboratory conditions over 7 and 14 days. Fish were exposed to a concentration of 2 µg/L of mercury, with the concentration being refilled daily. When measured 24 hours after a refill, the concentration ranged from 0.05–0.36 µg/L, which would correspond to the minimum exposure concentration for the fish throughout the experiment duration. Fish were exposed to this concentration for either 7 or 14 days, and then were maintained in clean water. In total, the experiment lasted for 42 days.	No mortality was observed, and no alterations in fish feeding behavior were observed. Condition factor was not statistically significantly different between the treatment and control group. After 7 days of exposure, there was a significant reduction in total number of neurons and glial cells, as well as a significant decrease in cerebellum volume. However, a recovery of cellular loss was observed in the hypothalamus and cerebellum after 14 days of exposure, suggesting adaptive mechanisms. Fish exposed to mercury swam a smaller distance than controls after 7 days of exposure, with a lower velocity. At 28 days post exposure, previously exposed fish still swam less and for smaller distances than the controls. Cell loss in the optic tectum persisted after 14 days of exposure and 28 days after exposure ended.

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 gilthead sea bream, making them unable to maintain position. This can result in the current forcing the gilthead sea bream into uncomfortable areas of the pen 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
> 0.5 to ≤ 1 BL/s	0–0.5 BL/s **OR** > 1 to ≤ 1.5 BL/s	> 1.5 to ≤ 2 BL/s	> 2 BL/s

Behavioral response to flow

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

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 pen. 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 pen.
- 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: 0.1–0.2 m/s.
  - 30 cm: 0.15–0.3 m/s.
  - 50 cm: 0.25–0.5 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.
- Where flow can be controlled, consider lower flow at night to allow rest.
- **Validation:** Multiple studies investigating how gilthead sea bream respond to different water velocities were relied upon. Where evidence was lacking (such as the 1.5 BL/s threshold between level 1 and level 2) thresholds were developed based on expert opinion.

Key evidence

Source	Summary of source	Key result
Palstra et al., 2020	Experimental study investigating the impact of different velocities on the welfare of about 3,300 gilthead sea bream (initial weight 2 g, final weight 92–125 g) in RAS conditions over 8 months.	Growth increased with speed. The 2 BL/s group had a 36% weight increase compared to the control, and the 1 BL/s had a 15% increase. The percentage of fish with vertebral

Source	Summary of source	Key result
	Three flow regimes were tested: minimal flow, 1 BL/s and 2 BL/s.	lordosis increased substantially with an increase in flow. The minimal flow group had a rate of 13%, the 1 BL/s group had a rate of 23%, and the 2 BL/s group had a rate of 48%. Swimming at 1 BL/s had significantly lower baseline plasma cortisol levels than at 2 BL/s, but not significantly different from minimal flow. Fish did not show signs of fatigue.
Alfonso et al., 2021	Experimental study investigating the energetic costs associated with different swimming speeds in 79 gilthead sea bream (219–971 g) in laboratory conditions. The fish were divided into three groups and subjected to a critical swimming speed test (to determine at what speed the fish could no longer maintain position): untagged group, accelerometer tagged group, and EMG tagged group.	The accelerometer tag did not significantly decrease performance, though the EMG tag did decrease performance. Critical velocity was in the range of 2–4.79 BL/s across the different sized fish, with an average of 3–3.19 BL/s. 65% of critical velocity was determined as a crucial threshold by the EMG tag where the fish's metabolism shifted from aerobic to anaerobic. This point was determined at the point where the white fast-twitch muscles began to work harder than the red slow-twitch muscles. 65% of critical velocity therefore ranged from 1.3–3.1 BL/s, with an average of 1.95–2.1 BL/s (calculated by the authors of this protocol).
Svendsen et al., 2015	Experimental study investigating swimming speeds in 13 gilthead sea bream (79.77 g, 14.79 cm standard length). An 8.24 L swimming respirometer with a 32 cm long swimming section was used. Speed increased by 0.5 BL/s every 30 minutes until 2 BL/s, and then	There was a positive correlation between the sustained swimming speed (highest recorded speed fish could maintain for 0.5 hours) and optimum swimming speed (speed that minimized energy expenditure per unit of distance), suggesting individuals capable of higher sustained speeds also swim most

Source	Summary of source	Key result
	by 0.25 BL/s every 30 minutes until fatigue. After reaching 2 BL/s and every subsequent speed, velocity temporarily reduced to 0.5 BL/s to check for excess post-exercise oxygen consumption.	efficiently at higher speeds. Recovery times for sea bream lasted an average of 7.8 hours, with some individuals needing up to 20.9 hours. The sustained velocity ranged between 27 and 53.2 cm/s (equivalent to approximately 1.8 to 3.6 BL/s), with the optimum speed varying between approximately 13.5 to 39 cm/s (equivalent to approximately 0.9 to 2.6 BL/s, calculated by the authors of this protocol using the mean length).

Salinity

Fish must constantly regulate their internal salt and water balance through osmoregulation, but this process works differently depending on their environment and species adaptation. As a euryhaline species, gilthead sea bream are capable of tolerating a wide range of environmental salinities. However, rapid salinity transitions can cause osmotic shock and are not recommended.

Level 0	Level 1	Level 2	Level 3
> 12 to ≤ 40 ppt	> 8 to ≤ 12 ppt **OR** > 40 to ≤ 45 ppt	> 3 to ≤ 8 ppt **OR** > 45 to ≤ 55 ppt	≤ 3 ppt **OR** > 55 ppt

Protocol application

Salinity can be measured using a digital salinity monitor.

It is recommended to measure salinity for gilthead sea bream in seawater at different pen depths (e.g., 3 m, 5 m, 15 m, and the maximum pen depth) and to monitor for large fluctuations. When salinity stratification is present, the scoring should reflect the range of salinities available to the fish rather than the most extreme reading alone. If fish have access to a layer within the level 0 or level 1 range, the overall score should reflect this, even if surface or bottom readings fall into level 2 or level 3.

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

Increase monitoring frequency during and after periods of heavy rainfall or freshwater runoff, particularly at surface depths where salinity drops are most pronounced. In estuarine-influenced sites, seasonal freshwater input patterns should inform the monitoring schedule.

Scoring notes

- If there are many layers of salinity and salinity is stratified, fish are unlikely to experience osmoregulatory stress because they can swim to their preferred salinity (provided that preferred layer is not otherwise unsuitable, e.g., because it is low in oxygen).
- Rapid changes in salinity (e.g., after heavy rainfall) are more stressful than stable values to which fish acclimate over time.

- Extreme temperatures (low or high) can impact osmoregulation and exacerbate the welfare impacts of low or high salinities. When both temperature and salinity score level 2 or level 3 simultaneously, the combined welfare risk is greater than either indicator alone.
- **Validation:** While there are a number of scientific studies investigating the impacts of salinity on gilthead sea bream, applying these findings to commercial aquaculture can be complicated by a number of factors, including prior acclimation. The evidence base for salinity thresholds is substantially limited by the confounding effect of transfer stress. In most cited studies, fish were acclimated to seawater and then transferred to test salinities. Results therefore reflect the combined impact of salinity and transfer stress, and may overestimate the welfare impact of a given salinity on fish already acclimated to that environment. The thresholds should be applied with particular caution at sites where fish are gradually acclimated to ambient salinity conditions.
    - Level 0 extends to 40 ppt to include normal Mediterranean seawater (38–40 ppt; APROMAR, 2024). No study in the evidence reviewed tested salinities between 40 and 55 ppt.

Key evidence

Source	Summary of source	Key result
Vargas-Chacoff et al., 2009	Experimental study investigating the metabolic response to various salinities and temperatures in 90 gilthead sea bream (150 g) in laboratory conditions over 35 days, after 7 days at the new salinity (direct transfer) and 7 days of gradual temperature change. Overall, there were nine different treatment groups, each with a combination of salinity (low salinity at 5 ppt, seawater at 38 ppt, and high salinity water at 55 ppt) and temperature (12°C, 19°C, and 26°C).	In the 19°C groups, across the different indicators (plasma glucose, liver glycogen, gill glycogen, gills lactate, and amino acid use), low salinity and high salinity water cost more than seawater. For example, glucose was lower in both low and high salinities, indicating the high cost of osmoregulation.
Tort et al., 1994	Experimental study investigating the effects of acclimation to low salinity on gilthead sea bream in laboratory conditions over one week. The fish were previously held in seawater with a salinity of 37 ppt. There were three treatments: the control group (37 ppt), brackish (13	Ventilatory frequency had no significant change in the 13 ppt from the control, but significantly increased in the low salinity group, with a 40% increase. Oxygen consumption was similar in the control and 13 ppt group, with a significant increase in the low salinity group of 60%. Plasma osmolality and red blood cell count

Source	Summary of source	Key result
	ppt) and low salinity (3 ppt).	also did not differ significantly between the control and 13 ppt, but decreased significantly in the 3 ppt group. The authors concluded the 3 ppt group experienced increased metabolic cost.
Laiz-Carrión et al., 2005	Experimental study investigating the effects of different osmotic conditions on 480 male gilthead sea bream (20 g) over 100 days. The fish were divided into three groups based on environmental salinity: seawater (38 ppt), brackish water (12 ppt), and low salinity water (6 ppt). Each salinity treatment was performed in duplicate. Every three weeks, 10 fish from each tank were weighed and measured.	Fish acclimated to 12 ppt were found to have the highest body weight, weight gain, and specific growth rate. Fish in 38 ppt performed better than fish in 6 ppt. 12 ppt was considered to be the salinity in which gilthead sea bream is almost isotonic (i.e., the fish's body fluids have the same concentration as the surrounding water), and so the energetic cost of osmoregulation would be lower than in other salinities. Plasma osmolality and sodium levels were stable and similar between the 38 ppt and 12 ppt groups, but were lower in the 6 ppt group, indicating they had difficulty maintaining homeostasis. There were no significant changes in plasma metabolic markers after 100 days. No mortality was observed in any group.
Klaoudatos and Conides, 1996	Experimental study investigating the effects of abrupt transfer to different osmotic conditions in 1360 juvenile gilthead sea bream (1.92 g) over 21 and 90 days. Prior to the start of the experiment, all fish were initially maintained in natural seawater with a salinity of 38 ppt. Fish were then moved directly to tanks with salinities of 28, 18, or 8 ppt (or remained in the 38 ppt tank as a control) without acclimation.	For fish fed to satiation, best growth was found at 28 ppt. If feed was restricted to less than 2% of body weight per day, the 38 ppt group showed higher growth. Growth at 8 ppt was the poorest. The energy required to maintain body functions increased linearly as salinity decreased. The authors noted that the growth results were in line with the knowledge about gilthead sea bream fry, which are generally in coastal lagoon areas at that stage in their life.

Source	Summary of source	Key result
	A 21-day growth trial and a 90-day survival trial was conducted.	In the 8 ppt group, mortality rose sharply immediately, and reached around 65% by day 20. This timing suggests the issue was the abrupt transfer rather than the salinity levels themselves. In the 38 ppt group, mortality began at around day 5 and plateaued by day 30, reaching 20% at the end of the trial. Despite being the control group, the authors noted that 38 ppt gave higher mortality than 18–28 ppt (where mortality was minimal). The authors noted that the juvenile fish experimented on prefer lower-salinity environments early in their life.

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 deeper 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 pens or other open systems. If measuring hydrogen sulfide in sea pens, it is recommended to measure at different pen 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.
- Results below the detection limit can be scored as 0 µg/L.
- 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., a few hours) to elevated hydrogen sulfide may be tolerated, exposure over days or months poses a greater risk to gilthead sea bream.
- In sea pen operations, the most likely source of hydrogen sulfide is disturbance of anoxic seabed sediments (e.g., from storms, anchor repositioning, or seabed cleaning operations). Sites with heavy organic loading beneath pens may develop anoxic sediment layers that produce hydrogen sulfide.

- **Validation:** No gilthead sea bream-specific (or even Sparidae-specific) evidence for hydrogen sulfide tolerance exists. Evidence from other species has been relied on. The conservative thresholds have been suggested based on a more recent salmon study in brackish water (Lazado et al., 2024). While gilthead sea bream, as a marine species naturally exposed to higher sulfate concentrations than freshwater or brackish water salmonids, may have evolved higher hydrogen sulfide tolerance, in the absence of evidence precautionary thresholds are maintained.

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/L 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 in the high exposure group, and from the fourth week for the low and control groups, 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 higher olfactory organ damage scores present in both exposure groups. The authors explicitly propose that hydrogen sulfide levels should not exceed 1 µg/L in brackish water RAS 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.	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

Source	Summary of source	Key result
	Acute toxicity tests were conducted on two groups of fish with average lengths of 45 and 54 mm, 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 µg/L. 30 fish per treatment group were tested.	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. 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 Atlantic salmon smolt (114 g) in brackish water RAS conditions over 10 days. Fish were stocked at densities of 10 kg/m^3 in the control and one exposure tank, and 30 kg/m^3 in a second exposure tank. Fish were exposed to increasing hydrogen sulfide concentrations once daily for 10 consecutive days. The target concentration was doubled each day, and measured peaks rose from 4 µg/L to 68 µg/L on days 9–10. 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

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Vertebral deformity

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Body condition

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Skin hemorrhaging

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Snout damage

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Jaw deformity

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Eye opacity

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Eye injury

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Operculum damage

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Gill damage

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