# WELFARE ASSESSMENT *study for* EUROPEAN SEA BASS


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ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ
UNIVERSITY OF CRETE

## UNIVERSITY OF CRETE

A scientific approach towards improvement of welfare status and welfare assessment of on-growing European sea bass *(Dicentrarchus labrax).*

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Curing EU aquaculture by co-creating health and welfare innovations

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# ABSTRACT

The aim of the study is to present a detailed set of specific indicators, methods of evaluation and units of measurement for the welfare assessment of farmed European sea bass, *Dicentrarchus labrax*. The study provides the user with prerequisites for welfare risk assessment, including risk tolerance, definition of uncertainty level, identification of relevant factors and elements of a risk management system. Finally, the study proposes a welfare assessment scheme, based on the welfare metrics of each specific welfare indicator, for on-growing fish that can be readily interpreted by all stakeholders and regulatory authorities involved in aquaculture.

CHAPTER 1

# INTRODUCTION

The latest production report[1] by the Federation of European Aquaculture Producers (FEAP) estimates that in 2022 the total farmed fish production in Europe was 2,865,072 tonnes, with marine cold-water species representing 67% of total production, while freshwater species and marine Mediterranean fish species accounted for 14 and 19%, respectively. The main farmed fish species are Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*) and common carp (*Cyprinus carpio*), which together represent 96% of the total European production[1].

The EU-27 aquaculture sector in 2021 sold 1.1 million tonnes in volume and €4.2 billion in value and employed around 57,000 people[2-3]. Shellfish makes up more than 50% of total aquaculture production, while fish production accounts for around 49% (with marine fish species and freshwater fish species representing 21% and 28% respectively)[2] of the total volume. The main farmed fish species are rainbow trout, European sea bass, gilthead sea bream, common carp and tuna. Greece ranks first in volume (137,000 tonnes in 2022) and in value (744 million euros) among the EU-27 in terms of fish farming[4].

Under the European Green Deal, aquaculture production is recognised as a source of "low carbon" protein for food and feed. EU Member States must comply with specific, strict requirements under EU and national legislation to ensure sustainable aquaculture production with respect to the environment, fish health and welfare. The welfare of farmed fish is of high priority in Europe and in January

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[1] https://feap.info/index.php/data/
[2] https://ec.europa.eu/eurostat/web/products-eurostat-news/w/ddn-20231002-2
[3] https://oceans-and-fisheries.ec.europa.eu/ocean/blue-economy/aquaculture/overview-eu-aquaculture-fish-farming_en
[4] https://fishfromgreece.com/en/annual-report-2023/

2024 the Commission designated an EU Reference Centre for animal welfare focusing on the welfare of aquatic animals[5].

Fish are sentient animals exhibiting remarkable cognitive skills, kin recognition and individual recognition, sophisticated social behaviours, numerical competency, pain perception and sensory perception[6]. Fish are moral subjects and their welfare is recognised under three kinds of regulatory and non-regulatory provisions[7], namely: (i) standards, codes of conduct or codes of good practice, (ii) recommendations by public institutions ("soft" rules) and (iii) laws ("hard" rules). However, the legislation regarding the welfare of farmed fish is still poorly developed compared to that of terrestrial farmed animals[7].

Nowadays, welfare of farmed fish is of prime importance for fish farmers, consumers, stakeholders and the competent authorities. The development of reliable welfare indicators and scoring systems to assess fish welfare on-site and the development of training courses is of high priority[7].

## **European sea bass** (*Dicentrarchus labrax,* Linnaeus 1758)

The European sea bass is typically a marine fish species inhabiting coastal lagoons and estuaries, shallow waters (2-10 m), coastal waters down to about 100 m in depth and occasionally river mouths. It is a eurythermic, eurihalyne, oceanodromous species with wide distribution from Norway to Morocco, the Canary Islands and Senegal, as well as from the Mediterranean to the Black Sea[8-9]. The European sea bass exhibits spatially structured genetic diversity, with Atlantic populations being more homogenous and with Mediterranean populations showing some differences between several sub-basins[9]. Wild and farmed populations show genetic differentiation, with farmed populations being fairly heterogeneous and having higher levels of differentiation[10].

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[5] https://food.ec.europa.eu/animals/animal-welfare/eu-reference-centres-animal-welfare_en

[6] Brown, C. (2015). Fish intelligence, sentience and ethics. Animal cognition, 18(1), pp. 1-17.

[7] https://www.europarl.europa.eu/thinktank/en/document/IPOL_STU(2023)747257

[8] https://fishbase.mnhn.fr/summary/63

[9] Pérez-Rufala, A. and C. Marcos, 2014. Ecology and distribution of *Dicentrarchus labrax* (Linnaeus 1758). In "Biology of European sea bass" (F. J. S. Vásquez & J. A. Muñoz-Cueto, Eds). CRC Press Taylor & Francis Group, pp. 3-33.

[10] https://www.sciencedirect.com/science/article/pii/S2352513422001417

Adult sea bass show demersal behaviour, are less gregarious than young fish and may swim considerable distances to offshore winter (Mediterranean) spawning areas. Eggs are pelagic and larvae recruit in coastal lagoons and estuaries in spring. Juveniles exhibit schooling behaviour and, as they grow, migrate offshore to deeper waters.

The main environmental factor affecting European sea bass distribution, migration and growth rate (assuming food is not restricted) is water temperature. The species is adapted to the greater and more frequent temperature changes that often occur in coastal lagoons and estuaries of the subtropical and tropical regions. That may be explained by the high Acclimation Response Ratio (ARR)[11] of 0.25-0.27, which is higher than that of several fish species in cold or temperate regions and like that of tropical species[12].

The European sea bass is considered to be a stress-sensitive species that displays both high basal (resting) and post-acute stress cortisol concentrations[13]. Heritability of cortisol is high (0.36) and quantitative trait loci (QTL) affecting cortisol levels have also been detected[14]. In addition, post-stress plasma cortisol concentration is a repeatable trait (r = 0.39) and individuals with constantly high response (HR) or low cortisol stress response (LR) have been identified[15]. HR individuals also show higher concentrations of total and free circulating cortisol levels than LR individuals, accompanied by higher sensitivity to adrenocorticotropic hormone (ACTH) signalling[16]. Accordingly, stress management under intensive rearing conditions is of prime importance for European sea bass health and robustness.

The European sea bass fish farming industry started in the late '80s to early '90s and sea bass is today one of the main farmed species. Türkiye and Greece lead the production of juveniles (230 and 153 million fish respectively in 2020) and commercially sized fish (95,000 and 52,000 tonnes respectively in 2020)[17].

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[11] ARR: the change in critical thermal limit with a given change in acclimation temperature; higher ARR absolute values indicate higher plasticity in thermal tolerance limits, i.e. greater acclimation capacity of the species.
[12] https://doi.org/10.1016/j.jtherbio.2011.11.003
[13] https://doi.org/10.1016/j.ygcen.2011.06.004
[14] https://www.mdpi.com/2076-2615/10/9/1668#B13-animals-10-01668
[15] https://www.nature.com/articles/srep34858
[16] https://doi.org/10.1371/journal.pone.0202195
[17] FEAP Production report 2021 V1.1

## Roadmap for Mediterranean fish welfare

In 2019, a guide to good practices and assessment indicators for the welfare of Mediterranean fish was published (in Greek, followed by an English version in 2020)[18]. That guide was developed by the University of Crete in collaboration with the Hellenic Aquaculture Producers Organization (HAPO) and has been adopted by the Hellenic Ministry of Rural Development and Food to serve as the National Welfare Guide for Mediterranean farmed species in Greek aquaculture (Figure 1). The guide described for the first time the current scientific data and ethical views on animal welfare, the welfare needs of the two main marine fish species reared in Mediterranean aquaculture (European sea bass and gilthead sea bream) and the available operational and laboratory welfare indicators. The need for the development of a welfare scoring system (welfare metrics) was noted.

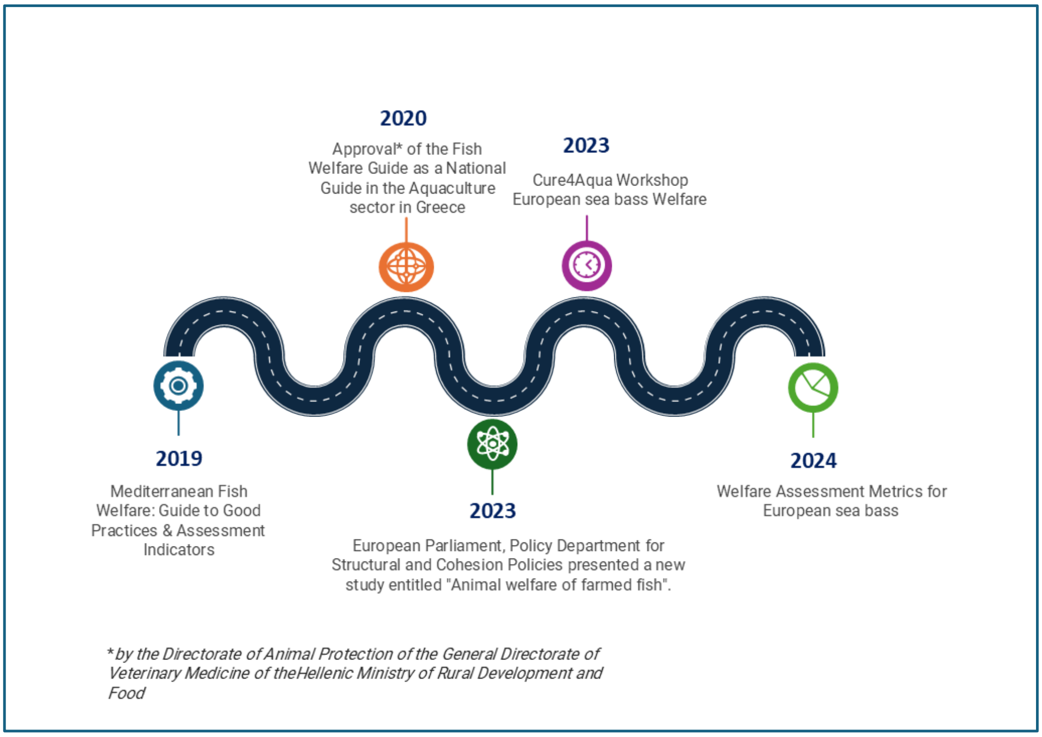


**Figure 1.** Roadmap for the development of welfare assessment scheme for European sea bass, *Dicentrarchus labrax.*

[18] M. Pavlidis and A. Samaras, 2020. Mediterranean Fish Welfare: Guide to good practices and assessment Indicators, pp. 90.

On 27 June 2023, the Policy Department for Structural and Cohesion Policies presented a new study entitled "Animal welfare of farmed fish"[7] (https://www.europarl.europa.eu/thinktank/en/document/IPOL_STU(2023)747257).

The study reviewed the current scientific knowledge, knowledge gaps, welfare needs and regulatory framework regarding the welfare of the main fish species reared in the EU-27 (rainbow trout, common carp, European sea bass, gilthead sea bream and Atlantic salmon) under various production systems and production phases. The study also provided species-specific welfare case studies, a SWOT analysis and policy recommendations relevant to EU decision-making.

In November 2023, the University of Crete, in collaboration with PRORATA (https://prorata.gr/) and Common/space (https://www.commonspace.gr/), held a workshop in Athens, Greece for the development of operational welfare indicators (OWI) for farmed European sea bass[19]. The workshop was run in the framework of the EU Horizon Europe programme Cure4Aqua[20]. The workshop involved a Delphi consultation to address the development of OWI for European sea bass in the on-growing phase in open sea cages through the presentation of experts' opinions and the identification of consensus among the scientific community, the aquaculture industry, the competent authorities, relevant NGOs and consumer associations.

## Aim of the study

The overall aim of this report is to develop a set of reliable operational welfare indicators, as well as a welfare scoring system, that will allow for welfare assessment of on-growing European sea bass by both producers and the competent authorities.

The study consists of the following chapters:

**Chapter 2** presents working definitions, i.e. a welfare glossary of the commonly used welfare-relevant terms (sentience, stress, pain, welfare indicators).

[19] https://cure4aqua-workshop.eu/2023/
[20] https://cure4aqua-project.eu/

**Chapter 3** addresses the prerequisites for welfare risk assessment, including risk tolerance, definition of uncertainty level, identification of relevant factors and elements of a risk management system.

**Chapter 4** presents a welfare assessment matrix (a modified version of a risk assessment matrix) for farming practices.

**Chapter 5** describes a list of factors affecting welfare state, with their definitions and consequences (welfare risk).

**Chapter 6** provides a welfare assessment scheme, based on the welfare metrics of each specific welfare indicator, for on-growing European sea bass that can be readily interpreted by all stakeholders involved in aquaculture.

CHAPTER 2

# WELFARE GLOSSARY

## 2.1 Coping, pain, suffering and distress

### Cope / Coping

A conscious use of cognitive, affective or behavioural efforts to deal effectively with externally imposed events and demands that the individual perceives as unpleasant or potentially harmful[21].

In a broader sense, this means dealing successfully with a demanding situation[22].

### Coping style (or behavioural syndrome or personality)

A correlated set of individual behavioural and physiological characteristics that is relatively consistent over time and across situations and that may affect an individual's emotional or functional reaction to a stressor (modified from Coppens et al., 2010)[23].

### Proactive coping style

Proactive, bold individuals display high aggression, a "fight-or-flight" strategy when stressed, as well as rigid and routine-forming behaviour, high risk taking, low post-stress corticosteroid production and high levels of circulating catecholamines (adrenaline and noradrenaline).

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[21] Anshel, M.H., 2012. Coping with Stress. In: Seel, N.M. (eds) Encyclopedia of the Sciences of Learning. Springer, Boston, MA. https://doi.org/10.1007/978-1-4419-1428-6_473
[22] https://dictionary.cambridge.org/dictionary/english/cope
[23] Coppens, C.M., de Boer, S.F. and Koolhaas, J.M., 2010. Coping styles and behavioural flexibility: towards underlying mechanisms. Philos Trans R Soc Lond B Biol Sci., 365(1560): 4021-4028

**Reactive coping style**

Reactive, shy individuals display low aggression, a "freeze-and-hide" strategy when in danger, behavioural flexibility, low risk taking, high(er) post-stress corticosteroid production and low levels of circulating catecholamines.

**Appraisal**

An automatic, often unreflective assessment of what is happening and what it may mean for the individual or those the individual cares about. It involves evaluation of stimuli as positive, negative or neutral and evaluation of the controllability of the stressor and an individual's coping resources[24].

**Robustness**

Animal robustness is a complex trait composed of multiple components. Robustness is of importance for livestock production systems and genetic selection[25] and corresponds to the ability to cope with perturbations and maintain productive and functional traits selected by breeding programmes in a broad variety of environments[26].

**Stress / Distress**

A state of either real (systemic/physiologic) or perceived (processive/psychogenic) threat to the well-being of an organism; a state resulting from an "imbalance between demands and resources" or occurring when "pressure exceeds one's perceived ability to cope"[27].

Stress is an inferred internal state[28] and should be considered a process that includes the stimulus, the perceptual processing of that input and the behavioural and physiological output[29].

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[24] Lazarus, R.S. and Folkman, S., 1984. Stress, Appraisal and Coping. Springer Publishing Company, pp. 460.
[25] De La Tore, A. et al., 2022. Exploration of robustness indicators using adaptive responses to short-term feed restriction in suckling primiparous beef cows. Animal, 16(7), 100556
[26] Strandberg, E., 2009. The role of environmental sensitivity and plasticity in breeding for robustness: lessons from evolutionary genetics. In Breeding for robustness in cattle (Klopčič, M., Reents, R., Philipsson, J., Kuipers, A., Eds.). Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 17–34.
[27] Folkman, S., 1984. Personal control and stress and coping processes: A theoretical analysis. Pers. Soc. Psychol., 46: 839-852.
[28] https://www.ncbi.nlm.nih.gov/books/NBK4027/
[29] Levine, S., 2005. Developmental determinants of sensitivity and resistance to stress. Psychoneuro-endocrinology, 30: 939-946

Distress can be defined as an aversive, negative state in which coping and adaptation processes fail to return an organism to physiological and/or psychological homeostasis[28].

**Stress response**

The physiological and psychological stress response is one of nature's fundamental survival mechanisms. The short-term stress response (or fight-or-flight response) enhances performance, adaptation and protection of a given individual when challenged and/or under threat. The stress response engages complex molecular, physiological, neuro-endocrine, metabolic, immunological and behavioural mechanisms and networks to cope with the challenge.

**Pain**

An unpleasant sensory and/or emotional experience associated with or resembling that associated with actual or potential tissue damage[30].

**Nociceptive pain**

Pain resulting from physical damage or potential damage to the body[31].

**Neuropathic pain**

Pain occurring when an injury or medical condition damages the nervous system or prevents it from working properly[31].

**Suffering**

A negative emotional state which derives from adverse physical, physiological and psychological circumstances, in accordance with the cognitive capacity of the species and of the individual being and its life experience[32].

**Anxiety-like behaviours**

An animal's behavioural responses resembling anxiety behaviours in humans.

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[30] Raja, SN, Carr, DB, Cohen, M, Finnerup, NB, Flor, H, Gibson, S, et al., 2020. The revised International Association for the Study of Pain definition of pain: concepts, challenges and compromises". Pain, 161 (9): 1976-1982
[31] https://www.medicalnewstoday.com/articles/319895
[32] https://www.humane-endpoints.info/en/glossary-detail-page/suffering

### Anxiety

Anxiety is a feeling of uneasiness and worry, usually generalised and unfocused as an overreaction to a situation that is only subjectively seen as menacing[33]. It is an emotion characterised by feelings of tension, worried thoughts and physical changes like increased blood pressure[34].

## 2.2 Cognition, sentience, consciousness

### Sentience

The quality of being capable of having sense perceptions, of experiencing sentiments or feelings[35]. Sentience draws a "*dividing line in nature that isn't just the ability to feel pain and pleasure, but also the ability to have subjective perceptual experiences of many types, emotional experiences and the cognitive awareness of good and bad*"[36].

### Emotion

There is no consensus in philosophical and sociological reviews or in psychology and biomedical published literature on a definition of emotion.

A complex reaction pattern, involving experiential, behavioural and physiological elements, by which an individual attempts to deal with a personally significant matter or event[37].

A tendency felt towards anything intuitively appraised as good (beneficial) or away from anything intuitively appraised as bad (harmful)[38].

### Feelings

Feelings are self-contained subjective experiences that are evaluative and independent of the sensations, thoughts or images evoking them[39]. Feelings

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[33] Bouras, N. and Holt, G., 2007. Psychiatric and Behavioral Disorders in Intellectual and Developmental Disabilities (2nd ed.). Cambridge University Press.
[34] https://www.apa.org/topics/anxiety
[35] https://dictionary.cambridge.org/dictionary/english/sentience
[36] Nussbaum, M., 2023. Justice for Animals: Our Collective Responsibility. Simon & Schuster, pp 400
[37] https://dictionary.apa.org/emotion
[38] Arnold, M.B., 1960. Emotion and personality. Columbia University Press. Arnold 1960 (From: https://plato.stanford.edu/archIves/sum2020/entries/emotion/)
[39] https://dictionary.apa.org/feeling

differ from emotions in that they are purely mental, whereas emotions are designed to engage with the world.

**Consciousness**

The term "consciousness" is notoriously ambiguous and difficult to define[40]. A being is conscious only if there is "*something that it is like*"[41] to be that creature, i.e. a subjective experience of life or being for that being, a proprietary perspective that individuals have of their own perceptual, cognitive and emotive processes[40].

**Cognition**

The possession of conscious mental processes[42], all forms of knowing and awareness, such as perceiving, conceiving, remembering, reasoning, judging, imagining and problem solving[43]. Animal cognition is often understood to be what permits flexible, goal-oriented behaviour through information processing[44].

## 2.3 Welfare

**Welfare**

Three main concepts (function-based, feeling-based and nature-based) and various definitions of animal welfare can be found in the respective literature. Moreover, different observers tend to emphasise different elements and concerns in relation to welfare. The welfare of an animal is a condition intrinsic to the animal; it is not a set of values transferred to the animal by humans[45].

*Working definition 1*: The welfare of an individual is its state as regards its attempts to cope with its environment[46].

*Working definition 2*: A balance or cumulation of pleasant and unpleasant experiences over time[47].

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[40] https://plato.stanford.edu/entries/consciousness/
[41] Nagel, T., 1974. What Is It Like to Be a Bat? The Philosophical Review. 83 (4): 435-450
[42] https://dictionary.cambridge.org/dictionary/english/cognition
[43] https://dictionary.apa.org/cognition
[44] https://plato.stanford.edu/entries/cognition-animal/
[45] COM (95)711 final, 1995. COMMUNICATION FROM THE COMMISSION TO THE COUNCIL AND THE EUROPEAN PARLIAMENT on the welfare of calves
[46] D. Broom, 1991. Animal welfare: concepts and measurement. Journal of Animal Science, 69(10) 4167-4175
[47] Reimert, L.E. et al., 2023. Review: Towards an integrated concept of animal welfare. Animal, Aug:17 Suppl 4:100838. doi: 10.1016/j.animal.2023.100838

**Welfare state**

An individual animal's experienced affective state at a given moment in time; aggregate quality ("quality of life") of an individual's subjective experiences over a given time. An animal is in a good state of welfare if (as indicated by scientific evidence) *"it is healthy, comfortable, well-nourished, safe and able to express innate behaviour and is not suffering from unpleasant states such as pain, fear or distress"*[48].

**Affective state**

Longer-lasting mood states (emotions and other feelings that are experienced as pleasant or unpleasant) which are not caused by a single stimulus but are the result of an accumulation of experiences over time.

**Welfare needs**

A requirement, as a consequence of the biology of the animal, to obtain a particular resource or respond to a particular environmental or bodily stimulus[49].

The term "needs" describes both requirements which are essential for life and requirements which are not essential for life, but which are significant to the animal[46].

**Welfare consequences**

These are the outcome of a factor or factors that affect an animal's welfare status.

Measurements or observations of animal's features or qualities which provide information about the welfare status of an individual. Welfare indicators assess the welfare consequences.

[48] World Organisation for Animal Health, 2008
[49] Broom, D.M and Johnson, K.G., 2000. Stress and Animal Welfare. Springer/Kluwer Scientific & Business Media, pp. 211

## 2.4 Welfare measurement

**Overall welfare assessment**

A systematic, independent and documented process for monitoring and evaluating the degree of fulfilment of all welfare needs, as well as the welfare status of animals associated with infrastructures, procedures and operations for a selected group, during a defined period.

**Factors (or input factors)**

Any aspect of the physical environment, rearing system, management practices and resources available to the animal having potential positive or negative effects on its welfare status (modified EFSA Panel on Animal Health and Welfare (AHAW))[50]. Factors may impact the welfare status of the farmed fish either positively or negatively.

**Indicators**

Welfare indicators are empirical measurements of specific traits or states that, based on evidence, are deemed to be correlated to a greater or lesser extent with an individual's affective state. There are various types of indicators based either on input related to the production process and production system identified or expressed as physiological, health and behavioural outcomes. The indicators are mutually exclusive, meaning that they do not present overlaps and are collectively exhaustive, meaning that there is no single indicator that can show whether an overall welfare status (positive or negative) is achieved.

**Input-based welfare indicators**

Resource-based and management-based (indirect) indicators that are used to survey the environment and the management (operations, treatments etc.) the animals are subject to; all measurements or observations that describe factors that influence the degree of fulfilment of a welfare need (modified from Stien et al., 2020)[51].

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[50] https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2012.2767

[51] Stien, L.S., Bracke, M., Noble, C. and Kristiansen, T.S., 2020. Assessing fish welfare in aquaculture. In The Welfare of Fish (T.S. Kristiansen, A. Ferna, M.A. Pavlidis & H. van de Vis, Eds). Springer, p. 515

**Outcome-based welfare indicators**

Indicators that are collected directly from the animal; these indicate how the animal copes with the influencing factors (outcome-based) and also describe the result or consequence of the degree of fulfilment of the animal's welfare needs. They are typically, but not exclusively, based on behavioural, health-related, morphological or physiological (e.g. stress indicators) measurements (modified from Stien et al., 2020)[51].

**Operational welfare indicators**

Welfare indicators that are reliable, replicable, practical and feasible for use in farming. Examples of operational indicators are mortality related to cannibalism, traumas, wounds or anticipated swimming behaviour (e.g. aggressive, passive).

**Laboratory-based welfare indicators**

Welfare indicators that need to be evaluated by someone with access to a lab or other analytical facilities. The majority of laboratory-based welfare indicators are related to measurement of physiological parameters such as glucose, cortisol or lactate.

**Group-based welfare indicators**

Outcome-based indicators based on measurements or observations at the group level (e.g. a batch of fish within the same net-pen sea cage), such as blood, scales or excessive mucus in the water, swimming behaviour, feeding patterns, mortality.

**Individual-based welfare indicators**

Outcome-based indicators based on measurements or observations performed on individual animals, such as morphological features, condition factors, health status, physiology, behaviour of individual fish within a batch/group.

**Welfare metrics**

Quantitative constructs used to evaluate and compare animal welfare across different situations or over time. They are often combined to create composite scores that are easily interpreted by stakeholders, producers and the public.

**Welfare score**

A score that indicates how well a fish farm unit fulfills a welfare criterion or principle.

CHAPTER 3

# PREREQUISITES FOR WELFARE RISK ASSESSMENT

An overall fish welfare risk assessment should include the identification of relevant factors, i.e. hazards that may have an impact on the welfare status of the individual animal, the characterisation of risk tolerance and uncertainty level, the development of a risk management system and state-of-the-art training activities.

**Risk tolerance**

Aquaculture operations, by design, introduce artificial environments that diverge from the natural habitats where aquatic organisms thrive in the wild. To ensure the sustainability of such farming practices and their alignment with broader human welfare goals, it is imperative to establish a welfare risk tolerance level for farming of aquatic organisms. This level of tolerance must be contingent upon the depth of understanding of aquatic animals' welfare needs and expertise in farming practices specific to aquatic animals. Consequently, it should acknowledge the inherent variability across species and production systems, facilitating the exploration of novel farming techniques and technologies. This nuanced approach not only fosters innovation and ensures improved fish welfare, but also creates avenues for responsible investment in the burgeoning aquaculture sector. The description of tolerance of consequences of factors in European sea bass farming is as follows:

*Mortality*

Depending on the specific farming practices, a mortality rate of less than 10% during the production cycle is tolerated. Any rate above that raises concerns.

*Pain, injuries*

Socially accepted levels involve implementing measures to ensure that pain or injuries are managed effectively when observed. In this context, unnecessary animal suffering is considered a minimum moral standard for responsible management of European sea bass farming practices.

*Disease*

Disease prevalence should be kept below 10% through good management practices.

*Stress, discomfort*

Optimal environmental conditions to ensure low levels of chronic stress should be ensured. Efforts should be made to minimise discomfort and/or acute stress when observed.

*Fasting*

European sea bass in nature often experience food deprivation lasting several weeks, especially during low water temperatures. Short periods of feed withdrawal prior to ordinary farming operations are not therefore of significant welfare concern. On the other hand, voluntary fasting that may continue for a week or more poses serious concerns for the health and welfare of individual fish.

**Definition of uncertainty level**

The welfare assessment needs to be justified by research data and publications. The level of uncertainty about a factor that improves or impairs animal welfare or the impacts of a factor needs to be recognised, either to avoid inconclusive decisions or by filling the knowledge gaps. The levels of uncertainty defined by the World Organisation for Animal Health, formerly OIE (OIE 2004)[52] are presented in Table 1.

[52] OIE 2004. Handbook on Import Risk Analysis for Animals and Animal Products Volume 2. Quantitative Risk Assessment pp 126.

**Table 1.** Description of uncertainty levels[52].

| LEVEL | DESCRIPTION |
|---|---|
| LOW | Solid and complete data available; strong evidence provided in multiple references; authors report similar conclusions |
| MEDIUM | Some but not complete data available; evidence provided in a small number of references; authors' conclusions vary from one to the other; Solid and complete data available for other species can be extrapolated to the species concerned |
| HIGH | Scarce or no data available; evidence tends to be provided in unpublished reports, based on observations or personal communications; authors' conclusions vary considerably |

**Identification of factors**

A prerequisite for any risk assessment is description of the problem, including identification of factors. Identification of factors is the recognition of aspect(s) in the environment of the animals that can impair or improve their welfare (EFSA 2012)[53]. A group of various experts, literature review or even legislative framework can be used to identify factors that may affect the welfare of the animals concerned. Clear identification and description of the factor is necessary. A list of environmental, husbandry and health factors in the European sea bass farming process is presented in the following chapters.

*Risk monitoring and auditing*

*Accountability*

Any facility engaged in the farming of aquatic organisms shall designate at least one employee in charge ("welfare officer") who has the appropriate educational background and training as responsible for ensuring the welfare of the farmed animals. The owner of the establishment will delegate the responsibilities associated with implementing welfare recommendations and overseeing the proper functioning of the welfare system in place.

[53] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2012.2767

**Risk management tools**

An indicator-based welfare monitoring system shall be in place to provide records and reporting on fish welfare-related outputs, including mortalities, injured animals and disease outbreaks in each production phase. The system needs to be part of an ordinary internal auditing system and of any official auditing system in place.

**Training**

Support shall be provided to delegated personnel through training on welfare by authorised educational institutions and training centres, such as (a) short-term executive training for welfare officers and (b) generic training for staff who manage fish with regard to welfare aspects and operational working instructions.

**Risk assessment**

In the event of unforeseen circumstances, significant redesigns of production methodologies or the introduction of new equipment, raw materials and other substantial changes, the competent staff of the establishment is required to conduct a new welfare risk assessment. Additionally, regular reviews of the existing welfare system are expected to be conducted, thereby ensuring its effectiveness and alignment with up-to-date standards and good practices.

**Power analysis for sample size**

The assumptions and the parameterisation of power analysis aimed at calculating the sample size at cage level are presented below:

1. Fish hosted in a cage are assumed to be a homogeneous study group regarding the presence of animals with a low or high welfare status level.
2. In the power analysis, we define a 95% probability of asserting that there are no fish with adverse animal events (low welfare status) in the population sample considered if there really are no fish with low welfare status compared to the whole population examined (cage population).
3. For individuals in a given cage population with a low welfare status, a confidence interval with a 95% confidence level is set and a design prevalence level of up to 33% is assumed.

4. Using the G*Power 3.9.1.4.[54] analysis, the sampling size is calculated as 120 animals per cage. This is the suggested number of fish to be sampled to estimate the proportion of adverse animal events. Larger sample sizes are required for adverse events with low frequency (i.e. < 5%)[55].
5. It is possible to conclude low welfare status by demonstrating that the frequency of an adverse outcome recorded is less than the specified value for the adverse outcome (e.g. < 2% mortality during live fish transport task)[55].

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[54] Faul, F., Erdfelder, E., Lang, A.-G. and Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral and biomedical sciences. *Behavior Research Methods*, *39*, 175-191. Download PDF

[55] J.O.Hampton, D.I.MacKenzie and D.M.Forsyth (2019). https://doi.org/10.1371/journal.pone.0211417

CHAPTER 4

# WELFARE ASSESSMENT MATRIX

The welfare assessment matrix is a modified version of a risk assessment matrix, also known as a Probability and Severity or Likelihood and Impact risk matrix. It can be used as a tool to identify potential risks affecting a business.

The welfare assessment matrix is based on three intersecting factors: the probability of the welfare threat (risk) event occurring, the frequency of exposure and the severity (potential impact) of the event for the individual fish.

The overall welfare assessment score is calculated according to the formula:

$$WS = P \times S \times F$$

Where WS = Welfare Score, P = Probability, S = Severity, F = Frequency of exposure

**Probability:** The likelihood that the projected consequences will occur given the frequency of exposure. Scores range from 1 to 5, where:

- ① = Rare under normal conditions
- ② = Unlikely under normal conditions
- ③ = Moderate (50/50 chance)
- ④ = Likely (greater than 50% chance)
- ⑤ = Almost certain, unavoidable

**Severity:** The degree of the consequences in terms of cost and impact on performance, health, welfare and animal life. Scores 1 to 3 are presented in Table 2 below:

**Table 2.** Levels of welfare impacts – consequences.

| Impacts | LOW (Score 1) | MODERATE (Score 2) | HIGH (Score 3) |
|---|---|---|---|
| **Mortality** | < 1% mortality/incident | 1-5% mortality/incident | > 5% mortality / incident |
| **Pain** | Acute pain events | Occasional instances of acute pain | Prolonged instances of pain |
| **Injuries** | < 1% injuries / incident | 1-3% injuries / incident | >3% injuries / incident |
| **Disease** | Disease prevalence < 5% | Disease prevalence 5-15% | Disease prevalence > 15% |
| **Stress** | Low frequency or brief duration of behavioural alterations | Moderate frequency or duration of behavioural alterations | High frequency or prolonged duration of behavioural alterations |
| **Fasting** | Fasting < 60DD* prior to handlings | Short-term fasting (60-100DD*) | Prolonged fasting (>100DD*) |

** DD: Degree days (sum of mean daily water temperatures in °C for total number of days measured or duration in days x average temperature in measured period)*

**Frequency:** The duration (amount of time) of exposure to a welfare risk. Scores 1 to 4, where:

- ① = Once per on-growing period
- ② = 2-3 repeats
- ③ = Many repeats
- ④ = Constant

A list of welfare risks for on-growing European sea bass is presented in Table 3.

**Table 3.** List of welfare risks for on-growing European sea bass in net-pen sea cages.

| WELFARE RISKS | PROBABILITY | SEVERITY | FREQUENCY | OVERALL RISK | COMMENTS |
|---|---|---|---|---|---|
| **A. Environmental factors** | | | | | |
| High light intensity | | | | | |
| High turbidity (suspended solids in the water) | | | | | |
| Prolonged exposure to unfavourable water temperatures | | | | | |
| Rapid and intense change in water temperature | | | | | |
| Prolonged exposure to unfavourable water oxygen | | | | | |
| Strong waves | | | | | |
| Insufficient depth/Site selection | | | | | |
| High presence of predators | | | | | |
| **B. Husbandry factors** | | | | | |
| Rearing in inappropriate stocking densities | | | | | |
| Handling | | | | | |
| Air exposure | | | | | |
| Use of anaesthetics | | | | | |
| Net damage | | | | | |
| Feed quality | | | | | |
| Competition for access to food | | | | | |
| No access to food/fasting (i.e. a period of time when the fish does not have usual access to food) | | | | | |
| Harvesting procedure | | | | | |
| **C. Health factors** | | | | | |
| Presence of diseases | | | | | |
| Amplification/transmission of diseases | | | | | |
| Use of antibiotics | | | | | |
| Use of antiparasitic agents | | | | | |
| Presence of aggression | | | | | |

Depending on the probability, frequency and severity, the welfare values and risks can be categorised according to Table 4.

**Table 4.** Welfare risk values, risk level and impact based on the probability, frequency and severity of exposure.

| VALUES | RISK LEVEL | ACTION |
|---|---|---|
| **48-60** | Remarkably high | Discontinue/STOP |
| **36-47** | High | Immediate correction |
| **27-35** | Substantial | Correction required |
| **13-26** | Possible | Attention needed |
| **1-12** | Slight | Acceptable |

CHAPTER 5

# FACTORS AFFECTING WELFARE AND EXPECTED CONSEQUENCES

This chapter explores the key factors influencing fish welfare, focusing on resources utilized and management practices that may impair well-being. It provides a comprehensive overview through tables that define and describe these factors, highlighting their potential impact on welfare. In addition, the chapter presents indicators used to assess welfare state, both at the group level and for individual animals, ensuring a holistic understanding. Finally, it offers a detailed list of laboratory indicators, serving as essential tools for precise welfare evaluation.

**Table 5.** List and definitions of resource-based welfare factors.

| | FACTOR | WELFARE RISK(S) | DEFINITION |
|---|---|---|---|
| 1 | Water temperature: absolute level | Stress | The actual, observed level of the sea water temperature on the day of measurement |
| 2 | Water temperature: duration outside of limits | Stress | Overall duration of the period in which the temperature is outside of normal limits |
| 3 | Water temperature: rapidity and intensity of change | Stress, disease | Rate of change of temperature during the last 24 hours |
| 4 | Oxygen saturation | Stress, disease, mortality | The amount of free oxygen ($O_2$) that is dissolved in the water in relation to the carrying capacity of the sea water |
| 5 | pH | Stress, mortality | Refers to the alkalinity or acidity of the water |
| 6 | Turbidity | Stress | The disturbance or reduction in light transmittance in water resulting from suspended, colloidal or dissolved matter or the presence of planktonic organisms |

| | FACTOR | WELFARE RISK(S) | DEFINITION |
|---|---|---|---|
| 7 | Lighting | Stress | Refers to the light conditions in terms of intensity, quality (spectrum) and periodicity (photoperiod) of the light |
| 8 | Presence of predators | Stress, pain, injuries | Predator species and number of days on which present |

**Table 6.** List and definitions of management-based welfare factors.

| | FACTOR | WELFARE RISK(S) | DEFINITION |
|---|---|---|---|
| 9 | Stocking density | Stress, pain, injuries | Fish biomass stocked per unit (sea cage) of volume ($m^3$) of water |
| 10 | Food quality/type | Stress, mortality, susceptibility to disease | Refers to the food used to feed fish in terms of composition (macro- and micro- nutrients), digestibility, size etc. |
| 11 | Food delivery | Stress, pain, injuries | Refers to feed management in relation to quantity, feeding frequency and feeding delivery method |
| 12 | Feeding frequency | Stress | The number of times per day that feed is provided; dates and duration of fasting |
| 13 | Handling | Stress, pain, injuries, mortality, diseases | The duration, frequency and severity of the handling operations |
| 14 | Mitigation measures against predators | Stress | Presence or absence of mitigation measures |

**Table 7.** List and definitions of fish group-based impacts – indicators of consequences.

| | CONSEQUENCE INDICATOR | WELFARE RISK | DEFINITION |
|---|---|---|---|
| 15 | Overall mortality | Mortality | The number of dead fish during the whole on-growing phase (from transfer from the weaning installations to sea cages up to harvest) |
| 16 | Three-day mortality | Mortality | The number of dead fish during three consecutive days after handling or transport |
| 17 | Growth rate | Stress | The increase in the fish body weight or size over time |

| | CONSEQUENCE INDICATOR | WELFARE RISK | DEFINITION |
|---|---|---|---|
| 18 | Swimming behaviour | Stress, pain, disease | The way that fish swim in the cage (i.e. group swimming, aggressive swimming, passive swimming) |
| 19 | Feeding behaviour | Stress, disease | Appetite – response to food |
| 20 | Distribution in the vertical water column | Stress, disease | The position of the school of fish in the cage. Specifically, whether it is closer to the surface, centre or bottom of the cage |
| 21 | Presence of aggression | Stress, disease, pain, injuries | The observation of aggressive behaviours, such as chasing and biting, among conspecifics |
| 22 | Presence of scales/mucus/ blood in the cages' water | Stress, disease, pain, injuries | Refers to the observation of biological materials like fish scales, mucus and/or blood in the water of the cage |
| 23 | Presence of diseases | Disease, stress, injuries | |

**Table 8.** List and definitions of individual fish-based impacts, i.e. consequences.

| | CONSEQUENCE INDICATOR | WELFARE RISK(S) | DEFINITION |
|---|---|---|---|
| 24 | Condition factor | Stress, disease, injuries | The condition of the fish in terms of body weight and total length ratio, according to the formula $K = 100*W/L^3$, where W is the total body weight of the fish and L is its total length |
| 25 | Emaciation state | Stress, disease, injuries | Refers to the body condition of the fish in terms of extreme thinness due to the absence of body fat and muscle |
| 26 | Obesity | Stress, disease | An accumulation of excessive amounts of adipose tissue in the body |
| 27 | Skin condition | Stress, disease, injuries, pain | Skin condition: intact skin or presence of wounds, lesions, injuries, blood spots, infection and/or scale loss |
| 28 | Eye condition | Stress, disease, injuries, pain | Eye condition: intact eye, presence of cataract, injury, exophthalmia and/or loss of one or both eyes |
| 29 | Fin condition | Stress, disease, injuries, pain | Fin condition: intact, normal fins or presence of injury, infection, malformation and/or loss |
| 30 | Vertebral deformities | Stress | Deformities that present themselves in the vertebral column of the fish (e.g. lordosis, scoliosis) |

| | CONSEQUENCE INDICATOR | WELFARE RISK(S) | DEFINITION |
|---|---|---|---|
| 31 | Jaw deformities | Stress | Deformities that present themselves in the lower or upper jaw of the fish |
| 32 | Operculum status | Stress | Operculum condition in terms of the covered surface area of the gill chamber |
| 33 | Lateral line detection | Stress | External morphology of the body canal: normal lateral line: continuous; slightly curved |
| 34 | Gill colour | Stress, disease | The colour of the gills, gill filaments and lamellae |

**Table 9.** List and definitions of laboratory health/welfare impacts, i.e. consequences.

| | CONSEQUENCE INDICATOR | WELFARE RISK(S) | DEFINITION |
|---|---|---|---|
| 35 | Haematocrit | Stress, disease | The ratio of the volume of red blood cells to the total volume of blood |
| 36 | White Blood Cell and Differential Count | Stress, disease | Measurement of the number of white blood cells and the percentage of each type of white blood cell present in fish blood |
| 37 | Cortisol | Stress, disease | Measurement of cortisol in fish blood or scales |
| 38 | Glucose | Stress, disease | Measurement of glucose in fish blood, serum or plasma |
| 39 | Lactate | Stress, disease | Measurement of lactate in fish blood, serum or plasma |
| 40 | Cholesterol | Stress, disease | Measurement of cholesterol in fish serum or plasma |
| 41 | Osmolality | Stress, disease | A measure of the body's electrolyte–water balance; it can be measured in fish plasma by using an analytical instrument called an osmometer and expressed in mOsm $kg^{-1}$ |
| 42 | Visceral fat content | Stress, disease | The content of fat in the viscera of the fish. |
| 43 | Liver condition | Stress, disease | The condition of the liver in terms of size (hepatosomatic index) and fat content (steatosis) |

# CHAPTER 6

# WELFARE ASSESSMENT SYSTEM

An evaluation system for the welfare status of animals kept in specific infrastructure based on a) the existence of prerequisites, b) the existence of factors and c) on magnitudes of impacts (consequences) is proposed. In order to reach a conclusion about the welfare status of a population examined, the sample size to be assessed is based on the power analysis described in Chapter 3. The current chapter provides a numeric evaluation system for the range of well justified welfare risk factors and the magnitudes of possible poor welfare impacts, related to European sea bass farming activities.

The numeric rating score is a screening tool to assess the severity or magnitude of welfare impairment at that moment in time on a scale from 0 to 3, in which 0 represents the lowest and 3 the highest rating.

For individual-based welfare indicators, where the proportion of animals with a different score is accurately recorded, a weighted sum must be calculated, with weights increasing with severity. In the case of group-based indicators (e.g. swimming or feeding behaviour), the score attributed to the fish unit is equal to the worst score obtained at group level as long as at least 15% of the observed animals are in groups that obtain this score or a lower one.

For an overall assessment of fish welfare on a particular unit (fish farm), it should be noticed that some indicators may be interrelated. For example, emaciation (e.g. low condition factor) can be the result of limited access to food (hunger) or disease or both. Another example is the resource-based indicator "water temperature"; it is well known that as water temperature levels increase, the amount of dissolved oxygen in water decreases and therefore less oxygen is available to farmed fish. In an overall assessment scheme and in order to avoid double counting, measures should be allocated to only one criterion either the one with higher severity or the one with worst welfare evaluation.

Another important issue is that some indicators may be considered as more important than others. For instance, injuries and wounds are considered more important than incomplete or branching lateral line. In a similar way, "absence of disease" may be considered more important than presence of vertebral deformities. However, as absence of disease does not compensate for injuries, vertebral deformities and lateral line deficiency, synthesis does not allow compensation between scores.

The scores obtained by a fish unit should be mathematically processed to assign that farm to a welfare category. However, the evaluation of (i) the weight of different indicators and (ii) the range of welfare categories, from the worst (unacceptable welfare) to the best (highest welfare level), remains to be developed, and the weighting factors to be defined. This will be included in the next update of the proposed welfare assessment protocol, with consultation among aquaculture scientists, social scientists, stakeholders, and consumers.

## 6.1 Water temperature

### *Why?*

European sea bass is an ectotherm, i.e. an animal that depends mainly on external heat sources and whose body temperature changes with the temperature of the environment. The sea bass is a eurythermal species that is able to cope with a wide range of temperatures from 5 to 28°C[56], with minimum and maximum survival water temperatures reported as 5 to 34 °C[57]. From spring to autumn, juveniles inhabit estuaries and lagoons, while during the winter they migrate from the coastline to deeper waters, as they prefer temperatures above 9-10°C[58-59]. The optimum water temperature for on-growing fish is between 22 and 24°C[60] or 26-27[61] while sexually mature fish prefer spawning temperatures of

[56] http://doi.org/10.4194/1303-2712-v20_5_01
[57] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.844
[58] Pickett, G.D. and Pawson, M.G. (1994). Seabass – Biology, Exploitation and Conservation. Chapman & Hall, London.
[59] https://doi.org/10.1111/age.12779
[60] https://doi.org/10.1017/S0025315400071289
[61] https://doi.org/10.1016/j.aquaculture.2004.04.021

8.5-9°C (at the start of the spawning period) and 15°C (triggering the end of the spawning period)[62].

Farmed sea bass is reared within the standard temperature range of 10-13 to 24-27°C for Mediterranean aquaculture, so the absolute level of the water temperature is of no major welfare concern. However, the overall duration that the temperature is outside of preferable limits in the specific productive/life-cycle phase is of welfare concern as it may demand extra metabolic resources, alter oxygen consumption and may subsequently affect the physiological performance of the fish. More importantly, abrupt changes in water temperature may cause an internal shock reaction, which may be influenced by the genotype, recent thermal history, physiological condition and health status of the fish. Acclimation to colder or warmer conditions has been reported to take several weeks in fish; that is the case for European sea bass too, which require a substantial time, exceeding one month[63]. As a rule of thumb, temperature changes over ± 1.5°C per day should be avoided[64], even though European sea bass are able to cope with acute thermal stress exhibiting $CT_{max}$[65] as high as 40°C for fish previously acclimated to elevated temperatures[66].

## *Welfare risks*

Stress, immunocompetence

## *Indicators*

### *6.1.1 Water temperature – absolute level*

The actual, observed level of the sea water temperature on the day of measurement.

Measuring method: Thermometer.

Measuring unit: °C.

[62] https://doi.org/10.1016/j.fishres.2023.106884
[63] https://doi.org/10.1371/journal.pone.0272510
[64] https://doi.org/10.1016/B978-0-12-409527-4.00020-1
[65] $CT_{max}$: Critical temperature that is lethal to fish when there is a rapid increase or decrease in temperature
[66] https://doi.org/10.1371/journal.pone.0272510

#### 6.1.2 Water temperature – duration outside of limits

Overall duration of the period that the temperature is outside of normal limits.

Measuring method: Thermometer.

Measuring unit: days.

#### 6.1.3 Water temperature – rapidity and intensity of change

Rate of change of temperature during the last 24 hours.

Measuring method: Thermometer.

Measuring unit: °C/day.

**Scoring system**

| INDICATOR/SCORE | 0 | 1 | 2 |
|---|---|---|---|
| ABSOLUTE LEVEL | 13-25°C | <13°C or >25°C | |
| DAYS OUTSIDE OF LIMITS | Between 13-25 for more than 15 days during the last 30 days | <13°C or >25°C for more than 15 days during the last 30 days | |
| DAILY CHANGE | < 1°C/24h | 1-3°C/24h | >3°C/24h |

## 6.2 Water oxygen content

**Why?**

Oxygen consumption depends on the life-cycle phase, size and physiological condition of the fish, as well as on environmental (ambient temperature and salinity) and managerial (stocking density, water renewal, feeding rate, stressful operations) conditions of the farm. European sea bass copes well with a broad range of dissolved oxygen concentrations (3 to 7.4 mg $L^{-1}$)[57], but long term exposure to oxygen saturation below 80% (at 22 °C and 26°C) has been found to impair feed intake and growth[67-68]. It is reported that oxygen consumption in European sea bass reared under routine conditions increases linearly with increasing temperatures, from 140 to 300 mg $kg^{-1}$ $h^{-1}$ in the range of 13-29°C[57] Acute hypoxia has been reported at 1.9 mg $L^{-1}$ following 4 h of exposure and

[67] https://doi.org/10.1111/j.1095-8649.2001.tb00158.x
[68] https://doi.org/10.1016/j.aquaculture.2022.738830

chronic hypoxia at 4.3 mg $L^{-1}$ after 15 days of exposure[69]. During on-growing in net-pen sea cages, dissolved oxygen may be a factor threatening animal welfare when the renewal of water is low and the stocking density and the water temperature are high.

### Welfare risks

Stress, disease, mortality

### Indicator

The amount of free oxygen ($O_2$) that is dissolved in the water in relation to the carrying capacity of the sea water.

Measuring method: Oxygen meters, measurements taken at 5 m depth.

Measuring unit: %.

### Type of indicator

Input-based, resource-based indicator.

### Scoring system

| INDICATOR/SCORE | 0 | 1 | 2 |
|---|---|---|---|
| OXYGEN SATURATION | 60-110% | 40%-60% for more than 10 days | < 40% for more than 10 days |

## 6.3 Presence of predators

### Why?

The main predators that are found near the net-pen sea cages are fish-eating birds (gulls, cormorants and herons), marine mammals (seals, dolphins) and occasionally piscivorous fish (such as tuna and greater amberjack). Predators' attacks, due to the lack or inappropriateness of mitigation measures, lead to chronic stress, poor feed conversion efficiency and reduced growth[70].

### Welfare risks

Pain-Injuries (Bird/predators hits) and/or Stress (predators' presence).

[69] https://doi.org/10.1016/j.aquaculture.2008.03.041
[70] https://doi.org/10.1016/j.aquaculture.2011.02.001

## Indicators

### 6.3.1 Presence of predators

Measuring method: Visual observation

Measuring unit: Records of the species and frequency (days) of presence.

### 6.3.2 Mitigation measures against predators (Yes/No)

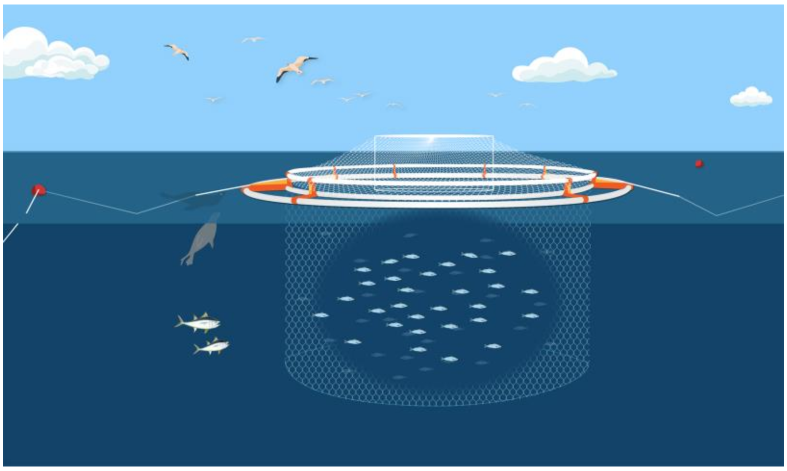

***Figure 2.*** *Graphical representation of indicative anti-predatory measures in E. sea bass cages.*

## Type of indicator

Input-based, resource-based, management-based indicator.

## Scoring system

| INDICATOR/SCORE | 0 | 1 |
|---|---|---|
| PRESENCE OF PREDATOR | Low frequency (once per week) | High frequency (≥ once per week) |
| MITIGATION MEASURES | Present | Absent |

## 6.4 Stocking density

### *Why?*

Stocking density expressed as kg of fish biomass per unit of volume or surface ($m^3$ or $m^2$) is not per se a helpful management-based welfare indicator[71]. The complex interrelation between the biology of the species, the available water volume for expressing its physiological and behavioural needs, water quality and managerial practices make it challenging to propose *a priori* a precise stocking density for on-growing fish. However, published data show that rearing of European sea bass in small numbers and water (cage) volumes results in high cortisol values compared to fish held in large numbers under the highest rearing volume[67]. Other reports show that high densities (45 to 60 kg $m^{-3}$) induce stress, as well as fin and skin damage[72-73-74], a situation that can be reversed under proper water quality and good maintenance conditions[69].

As stated above, the number of individuals or fish biomass per unit volume is not necessarily a good indication of what the fish experience. Therefore, for raceways or other type of tanks with water flowing through them, a more precise expression of stocking density is available, such as the carrying capacity (kg of fish per L of water per minute) or the flow index (kg of fish per L per min per cm)[75]. However, such a concept is not yet available for on-growing fish in open sea cages, so we will use the ordinary measuring unit for stocking density.

### *Welfare risks*

Pain, injuries (Fin/skin damage), Stress (High cortisol levels)

### *Indicator*

Fish biomass stocked per unit (sea cage) of volume ($m^3$) of water.
Measuring method: Measurement of body weight in a fish sample at regular intervals.
Measuring unit: kg $m^{-3}$.

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[71] https://doi.org/10.1111/are.12942
[72] https://doi.org/10.1016/j.aquaculture.2007.12.012
[73] https://doi.org/10.1016/j.aquaculture.2009.11.007
[74] https://doi.org/10.1051/alr/2009047
[75] https://doi.org/10.1111/j.1095-8649.2002.tb00893.x

### *Type of indicator*

Input-based, management-based indicator.

### *Scoring system*

| INDICATOR/SCORE | 0 | 1 | 2 |
|---|---|---|---|
| STOCKING DENSITY (KG $M^{-3}$) | 5-15 | < 5 or 15-20 | > 20 |

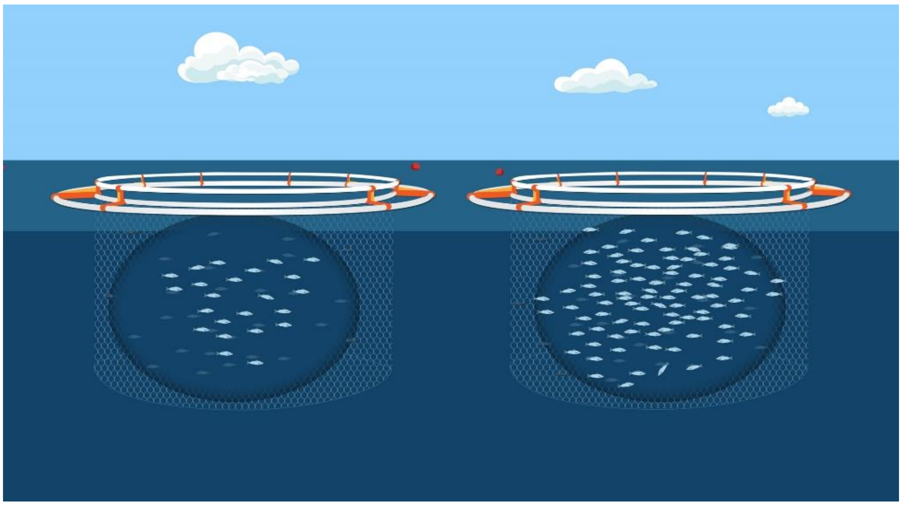


***Figure 3.*** *Graphical representation of two different stocking densities in sea cages.*

## 6.5 Food – Feeding

### *Why?*

The fulfilment of nutritional needs is of prime importance for European sea bass welfare. Feeds should be of optimal quality, based on the available scientific knowledge and empirical data / good husbandry practices. Daily feeding is a prerequisite to minimise competition, aggression and ensure fish welfare, involving the provision of prime-quality commercial feeds and of adequate food administration and distribution among the reared fish.

European sea bass have high dietary protein requirements, ranging from 52 to 60 percent of their diet[76]. When adequate dietary digestible energy (DE) is provided, it is possible to reduce dietary crude protein levels to 42-48 percent of

[76] https://www.fao.org/fishery/affris/species-profiles/european-seabass/nutritional-requirements /en/

the diet for sea bass weight from 10 to 200 g, with best feed utilisation observed for the 45/16 crude protein/lipid diet[77]. The optimal protein to energy ratio of the diet for European sea bass should be 19 mg $kJ^{-1}$, in diets with at least 21 MJ $kg^{-1}$ digestible energy[72]. Water temperature has no significant effect on European sea bass protein requirements[72].

It should be noted that European sea bass is susceptible to high fat deposition in the liver and shows prolonged postprandial hyperglycemia[76]. It has been shown in several farmed fish that *ad libitum* feeding with excessive dietary fat levels has negative impacts on growth, feed efficiency, lipid accumulation in various tissues, antioxidant capacity and immune function and stimulates endoplasmic reticulum stress and suppressed autophagy in fish liver[78].

The impact of food quality and type (floating pellets, moist pellets, wet-extruded, dry-extruded pellets) on fish welfare can be assessed only in retrospect (*ex post facto*), using physiological, health and zootechnological indicators.

Regarding feeding practices, the feed should be spread over a large surface area to avoid overcrowding and competition from fish. The feed amount should be given gradually. The largest amount should be provided at the beginning and relatively quickly if the fish are aggregated on the surface. In this phase of intense activity, each fish should have a high probability of capturing feed.

However, if satiation occurs and the fish calm down, the amount of fish feed released can be reduced to satisfy any fish that may not have sufficient opportunity to feed and to limit the feed lost in the water column. Finally, it is recommended that the feeding time be kept relatively constant as fish adapt their biological rhythm accordingly.

European sea bass in nature often experience food deprivation lasting several weeks, especially during low water temperatures. Therefore, short periods of feed withdrawal prior to ordinary farming operations are not of significant welfare concern. On the other hand, voluntary fasting that may continue for days or weeks poses serious concerns for the health and welfare of individual fish.

[77] https://cordis.europa.eu/project/id/262155/reporting
[78] https://doi.org/10.1111/jpn.13759

Fasting periods should be applied when required by a given procedure (e.g. size sorting, prior to vaccination, prior to disease treatment) and should not exceed 24 to 48 h for each fish or 72 h for pre-slaughter[79], especially taking into consideration the digestive tract evacuation rates[80-81].

## *Welfare risk*

Stress

## *Indicator*

Feeding management in relation to feeding frequency.

Measuring method: Record the feeding frequency.

Measuring unit: The duration of fasting expressed in degree days (DD).

## *Type of indicator*

Input-based, management-based indicator.

## *Scoring system*

| INDICATOR/SCORE | 0 | 1 | 2 |
|---|---|---|---|
| **FEEDING FREQUENCY** | Fasting < 60DD[82] prior to handlings | Short-term fasting (60-100DD[82]) | Prolonged fasting (>100DD[82]) / Overfeeding |
| **LIVER CONDITION** | Pink pale liver coloration | Light pink liver coloration with spotted evidence of fat deposition | Whitish liver coloration with expanded evidence of fat deposition |

[79] https://www.compassioninfoodbusiness.com/improving-animal-welfare/fish/
[80] https://doi.org/10.1016/j.aquaculture.2009.01.015
[81] https://doi.org/10.1016/j.aquaculture.2018.05.017
[82] DD: Degree days (sum of mean daily water temperatures in °C for total number of days measured or duration in days x average temperature in measured period)

## 6.6 Mortality

### *Why?*

The mortality rate represents the percentage of fish that die during a specific production phase (e.g. larval rearing, weaning, on-growing) for reasons such as inadequate living conditions, inappropriate farming practices and weight at transfer to sea cages, extensive stress and anxiety, cannibalism and aggression by conspecifics, diseases, accidents, wounds, lesions and injuries or unexplained reasons.

It should be noticed that it is difficult to differentiate between underlying and immediate causes of mortality, and to reveal links between undetected poor fish welfare and fish mortality. More importantly, there are no available age-production phase-specific mortality patterns, which can be a useful indicator of the proportion of suffering in a given fish cohort. In a report by Waitrose & Partners, mortality of around 11.5% was reported for European sea bass at the end of on-growing phase in sea cages[83], which is in line with the 11.7% reported in Spain for 2018[84] and 15.8% from surveys across Mediterranean aquaculture[85]. If that is considered "baseline" mortality, it still represents a significant economic loss for producers and indicates a need to improve welfare for farmed European sea bass.

For all our farmers, mortality is recorded at regular intervals and investigated by a designated veterinarian.

### *Indicators*

#### *6.6.1 Overall mortality*

Measuring method: Record number of dead fish during the entire on-growing phase (from transfer from the weaning installations to sea cages up to harvest).

Measuring unit: Percentage, based on the initial number of fish at stocking.

#### *6.6.2 Three-day mortality*

Measuring method: Record number of dead fish in three-day periods after major handling (i.e. transport, transfer, vaccination, partial harvest).
Measuring unit: Percentage based on the number of fish currently in the cage.

---

[83] Animal Welfare at Waitrose & Partners – September 2020. Welfare Outcomes and Key Performance Indicators (KPI'S)
[84] https://doi.org/10.1016/j.aqrep.2022.101257
[85] https://doi.org/10.1111/tbed.13482

### *Type of indicator*

Output-based, animal group-based indicator.

### *Scoring system*

| INDICATOR/SCORE | 0 | 1 | 2 |
|---|---|---|---|
| OVERALL MORTALITY | < 15% | 15-25% | > 25% |
| THREE-DAY MORTALITY | < 2% | 2-3% | > 3% |

## 6.7 Growth performance

### *Why?*

In healthy, non-stressed fish, feed consumption is expected to lead to increased body weight. Growth rate is affected by a variety of abiotic and biotic factors and managerial conditions, as well as the genotype, life-cycle phase and physiological condition of the individual. Being one of the most important production traits, farmers use various methods to monitor growth rate throughout the growth period. Moreover, it is a customary practice to grade fish into groups of similar individual sizes.

If growth is lower than anticipated, that may indicate some problems with the living conditions of the fish, the causes of which should be thoroughly examined and identified in connection with additional indicators (such as feed conversion efficiency, health issues and social hierarchies).

Ordinary indicators used by scientists and farmers to evaluate growth performance are survival rate, body weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FE) and Fulton's condition factor (K).

### *Indicator*

The condition of the fish in terms of their body weight and total length ratio.

Measuring method: $K = 100*W/L^3$, where W is the total body weight of the fish and L is its total length (Fulton's condition factor).

Measuring unit: %.

## Type of indicator

Output-based, management-based indicator.

## Scoring system

| INDICATOR/SCORE | 0 | 1 | 2 |
|---|---|---|---|
| CONDITION FACTOR | > 1.0 | 0.9 - 1.0 | < 0.9* |

*Emaciation state*

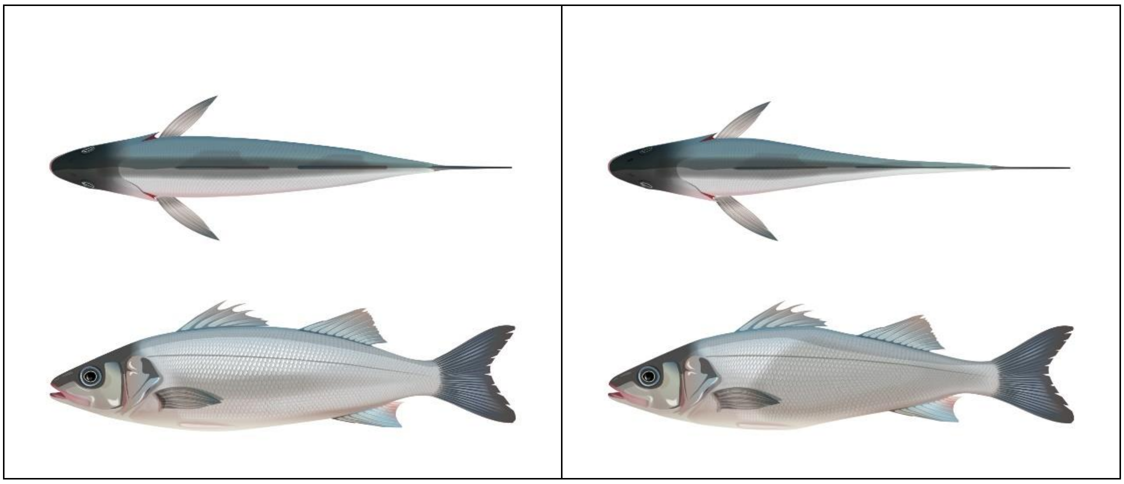


***Figure 4.*** *Graphical representation of a normal fish (left) and an emaciated fish (right).*

## 6.8 Swimming behaviour

## Why?

Changes in the behaviour of fish are an adaptive mechanism to cope with challenges and noxious stimuli. At the same time, they can be an early sign of stress, anxiety or health problems. Normal patterns of swimming, feeding and social behaviour associated with activity levels are all useful indicators of welfare. Group swimming, aggressive, passive or erratic swimming, freezing behaviour, time spent on the surface or at the bottom of the tank, exploration, bumping into one another or the sides of the tank and flashing[86] could serve as early signals of high stress or disease.

[86] When fish are resting in a shoal near the bottom and then suddenly move forward, turn on one side and appear to rub one flank on the substrate (Source: https://animaldiversity.org/ accounts/Dicentrarchus_labrax/)

Juvenile European sea bass form shoals that may range from a few dozen individuals to many thousands, depending on the strength of the year class and local conditions[87]. Such shoaling behaviour may continue for most of the sea bass's life.

## *Indicator*

The way that fish swim in the cage (i.e. group swimming, aggressive swimming, passive swimming).

Measuring method: Visual observance (a method highly dependent on the observer's experience).

Measuring unit: % of fish showing abnormal behaviour.

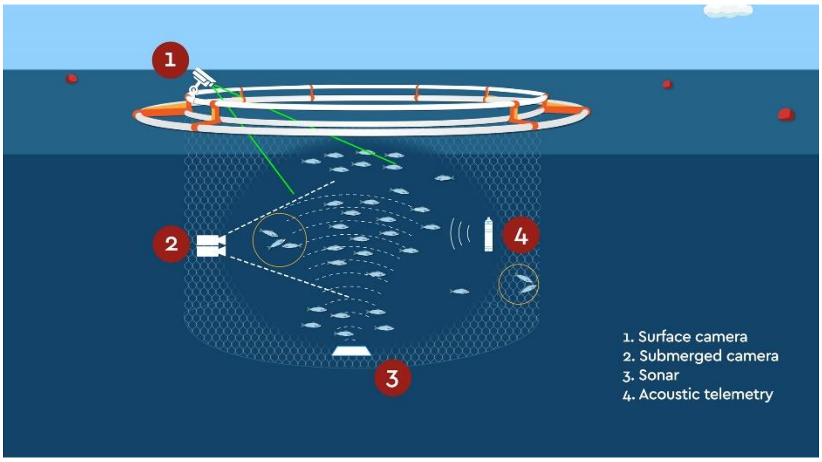



***Figure 5.*** *Graphical representation of swimming behaviour monitoring.*

## *Type of indicator*

Output-based, animal-based group indicator.

## *Scoring system*

| INDICATOR/SCORE | 0 | 1 | 2 |
|---|---|---|---|
| **SWIMMING BEHAVIOUR** | Normal | 0-10% of fish: (1) not schooling or (2) showing high swimming activity | > 10% of fish: (1) in the bottom of the cage and not responding to feeding or (2) not schooling or (3) showing lethargy |

87 https://animaldiversity.org/accounts/Dicentrarchus_labrax/

## 6.9 Feeding behaviour

### *Why?*

Feeding patterns in nature display marked seasonality, with European sea bass exhibiting diurnal feeding behaviour in summer-autumn and nocturnal feeding behaviour during the winter[88]. However, under intensive rearing conditions, some individuals show diurnal behaviour while others exhibit nocturnal behaviour and some may also invert the phasing of their daily feeding pattern[89]. Monitoring of feeding behaviour and fish appetite is of high importance to fish farmers, serving as a good indicator of fish health, welfare and performance.

Ordinary activities that take place in sea cages may stress the on-growing fish and have a negative impact on feed intake. These are of minor welfare concern as fish exhibit a compensatory response once the stressor has ceased[90]. However, chronic stress may modify daily feeding rhythms, induce reductions in daily and accumulated food intake[91] and affect feed conversion efficiency. Chronic stressful conditions may also lead to a reduced growth rate even when food intake levels are maintained[26].

Fish feeding intensity assessment (FFIA) aims to assess the intensity and magnitude of changes in behaviour during the feeding process, as an indicator of fish appetite[92]. While deep learning algorithms have been developed to assess appetite status (FFIA), at present human observation is the preferred method in fish farms. For example, fish appetite can be rated according to a four-step scale (Tables 10 & 11).

**Table 10.** Grading of behaviour of individual fish held in isolation and corresponding point scores in the feeding test[93].

| POINTS | BEHAVIOUR |
|---|---|
| 0 | Fish does not respond to food |
| 1 | Fish eats only pellets that fall directly in front of it and does not move to take food |
| 2 | Fish moves more than one body length to take food, but returns to original position in aquarium between each food item |
| 3 | Fish moves continuously between food items and consumes all food presented |

88 https://doi.org/10.3109/07420529808993197
89 https://doi.org/10.1007/s00360-011-0585-z
90 https://doi.org/10.1016/j.aquaculture.2009.10.031
91 https://doi.org/10.3389/fendo.2018.00451
92 https://ieeexplore.ieee.org/document/9943405
93 https://doi.org/10.1016/j.physbeh.2005.11.012

**Table 11.** The grading standards for fish feeding intensity[94].

| GRADING (INTENSITY) | BEHAVIOUR |
|---|---|
| None | Fish do not respond to food |
| Weak | Fish eat only pellets that fall directly in front of them but do not move to take food |
| Medium | Fish move to take food, but return to their original positions |
| Strong | Fish move freely between food items and consume all the available food |

### *Indicator*

Appetite – response to food.

Measuring method: Visual observance (a method highly dependent on the observer's experience).

Measuring unit: Compare appetite to normal appetite depending on conditions, season and physiological state.

### *Type of indicator*

Output-based, animal-based group indicator.

### *Scoring system*

| INDICATOR/SCORE | 0 | 1 | 2 |
|---|---|---|---|
| **APPETITE** | Normal (fish move freely between food items and consume food) | 1-2% of fish do not respond to food | >2% of fish do not respond to food |

## 6.10 Distribution in the vertical water column

### *Why?*

In various fish species, the distribution of the shoaling or individual fish in the vertical water column of the sea cage has been associated with their living conditions, i.e. environmental parameters, feeding motivation, potential predator

[94] https://doi.org/10.1016/j.aquaculture.2019.04.056

avoidance, husbandry practices, physiological state and health status of the fish[95]. For example, Atlantic salmon farmed in sea cages school during the day, avoiding the cage walls, the centre part (except when feeding takes place) and the bottom and prefer surface waters[96].

In European sea bass, the exact vertical behaviour in sea cages and the impact of environmental or managerial factors has been scarcely studied. However, it has been shown that European sea bass react to environmental factors (e.g. light, oxygen levels, waves and currents, presence of predators) and operational cues and distribute themselves inside sea cages based on environmental parameters, feeding motivation and stress state. It has been shown that European sea bass prefer to inhabit surface water before and during the morning feeding time, as a food anticipatory behaviour, as well as for a couple of hours afterwards and then again at dusk. Moreover, fish avoid the surface between 12:00 and 17:00, while the whole water column is used at night[97].

Regarding husbandry practices and stress, fish seem to respond to such stressors by moving to deeper water[95], therefore indicating the potential use of such movements as a welfare indicator. Finally, the spawning period, usually between December and March in Mediterranean aquaculture, may be accompanied by increased surface dwelling[95].

## *Indicator*

The position of the school of fish in the cage. Specifically, whether it is closer to the surface, centre or bottom of the cage.

Measuring method: Visual observance.

Measuring unit: Vertical position of the school in the water column (top/middle/bottom).

## *Type of indicator*

Output-based, animal-based group indicator.

[95] https://doi.org/10.1111/are.12103
[96] https://www.sciencedirect.com/science/article/pii/S0044848623011304
[97] https://doi.org/10.3389/fmars.2023.1168953

### Scoring system

| INDICATOR/SCORE | 0 | 1 | 2 |
|---|---|---|---|
| **VERTICAL POSITION OF THE SCHOOL** | Top/middle part depending on the time, husbandry operations etc. (e.g. top during feeding) | Bottom of the cage in specific periods only (e.g. during husbandry procedures) | Bottom of the cage for at least three days |

## 6.11 Presence of aggression

### Why?

European sea bass of the same size do not show a high degree of aggressive behaviour in nature. However, they are territorial when occupying summer feeding areas[9] and in sea cage farming there may be an intense expression of agonistic behaviour during feeding[98].

### Indicator

The observation of aggressive behaviours, such as chasing and biting, among conspecifics.

Measuring method: Visual observance (a method highly dependent on the observer's experience).

Measuring unit: Record the type of aggressive behaviour (links with injuries of fins, skin and eye condition).

### Type of indicator

Output-based, animal-based group indicator.

### Scoring system

| INDICATOR/SCORE | 0 | 1 | 2 |
|---|---|---|---|
| **AGGRESSION** | No aggression | Aggression during feeding (scramble competition) | Aggression during rearing (not only during feeding) |

[98] https://doi.org/10.1016/j.applanim.2019.02.010

## 6.12 Presence of diseases

### *Why?*

The legislation sets out a list of notifiable diseases and the species susceptible to them. WOAH (World Organisation for Animal Health) defines "listed diseases' as a disease, infection or infestation listed in Chapter 1.3 of the *Terrestrial* and *Aquatic Codes* after adoption by the World Assembly of Delegates. The list of aquatic animal diseases is selected based on the criteria detailed in the *Aquatic Code*[99].

Disease is an impairment of the normal state of an animal that interrupts or modifies its vital functions. In most cases, the manifestation of diseases is due to or influenced by the rearing conditions.

The development of a disease in each fish farming system is influenced by interactions among the farmed animals, the pathogen and the rearing environment. For a disease to occur there must be a potentially pathogenic agent, a susceptible host and environmental conditions that either increase the virulence of the pathogen or decrease the host's resistance.

Disease prevention measures must be in place to ensure the fish’s welfare. They should be recorded in the Veterinary Health Plan of each farm. According to Directive 2006/88/EC: “*More attention should be paid to preventive disease occurrence than to controlling the disease once it has occurred. It is therefore appropriate to lay down minimum measures of disease prevention and risk mitigation which should be applied to the whole production chain in aquaculture, from fertilisation and hatching of eggs to the processing of aquaculture animals for human consumption, including transportation*”.

The prevention measures are either specific to each disease or more general. They include screening methods, laboratory tests, vaccination protocols, biosecurity measures, stress reduction measures and educational programmes for the personnel taking care of the animals.

Concerning the disease diagnosis, the Veterinary Health Plan of the farm should include an emergency plan with written instructions. It shall describe the control measures that should be taken, starting by informing the responsible personnel,

[99] https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/

conducting laboratory tests and placing the farm in quarantine. If a disease is suspected, the emergency plan should be activated. This will minimise the reaction time, optimise the treatment outcome and reduce fish losses.

### *Indicator*

Indirect appetite and swimming behaviour indicators (see paragraphs 6.8-6.10), mortality (see paragraph 6.6), laboratory tests for disease diagnosis.

Measuring method: Visual observance (a method highly dependent on the observer's experience). Mortality records. Laboratory diagnostic tests.

Measuring unit: Compare swimming/appetite to normal swimming/appetite depending on conditions, season and physiological state (see paragraphs 6.8-6.10), mortality percentage (see paragraph 6.6). Laboratory results according to standard laboratory references.

### *Type of indicator*

Output-based, laboratory-based, animal-based group/individual indicator.

### *Scoring system*

| INDICATOR/SCORE | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| **HEALTH** | Normal (no signs of disease, fish swim, eat and behave normally; preventive measures in place) | Abnormal behaviour; no preventive measures in place | Disease diagnosis with adequate treatment | Disease diagnosis with non-adequate treatment |

## 6.13 Skin condition

### *Why?*

The skin is the largest organ of the integumentary system. It provides a protective barrier against microorganisms, hazardous substances, ultraviolet radiation, physical injury and mechanical damage. It also acts as a sensory organ (mechanoreceptors, nociceptors) and engages in several biochemical processes. Fish scales form part of the fish's integumentary system and serve a variety of functions from protection to locomotion (water dynamics).

Compromised skin integrity, defined as the combination of an intact cutaneous structure and a functional capacity that is high enough to preserve it[100], is associated with complications such as scale loss, presence of ulcers, lesions, skin erosion ("white patches") and petechia (small haemorrhagic spots in the skin), injuries, haemorrhages and infections. Maintaining skin integrity therefore equals maintaining skin health and it is an important indicator of farmed fish welfare. It should be noted that skin damage can also be caused by crowding/confinement/netting at harvest, as well as at slaughter in ice slurry.

## *Indicator*

Skin condition.

Measuring method: Macroscopic skin inspection.

Measuring unit: Record the extent of skin damage.

## *Type of indicator*

Output-based, animal-based indicator.

## *Scoring system*

Examination and individual evaluation of a population of fish (see reference in Chapter 3) following the scoring system below. The average value of the examined population provides the overall score of the indicator.

| **INDICATOR/SCORE** | 0 | 1 | 2 |
|---|---|---|---|
| **SKIN CONDITION** | Intact skin or less than 5% scale loss | 5-15% skin damage* | > 15% skin damage* |
| |  |  |  |

** Presence of wounds, lesions, injuries, blood spots, infection and/or scale loss*

[100] https://doi.org/10.1016/B978-0-12-815028-3.00011-0

## 6.14 Eye condition

### *Why?*

The eyes of farmed fish are sensitive to various injuries and damage due to slight protrusion from the head and the lack of eyelids. Nutritional deficiencies, imbalances in osmotic regulation, high light intensity and UV radiation, diseases, aggression and mechanical trauma are ordinary causes of eye damage. Accordingly, evaluation of eye condition is an important indicator of farmed fish welfare.

### *Indicator*

Eye condition (intact eye, presence of cataract, injury, exophthalmia and/or loss of one or both eyes).

<u>Measuring method</u>: Macroscopic eye inspection.

<u>Measuring unit</u>: Record of eye condition.

### *Type of indicator*

Output-based, animal-based indicator.

### *Scoring system*

Examination and individual evaluation of a population of fish (see reference in Chapter 3) following the scoring system below. The average value of the examined population provides the overall score of the indicator.

| **INDICATOR/SCORE** | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| **EYE CONDITION** | Intact eye | Unilateral slight injury or exophthalmia | Unilateral severe eye injury or lens with > 50% clouding (cataract) or blindness | Bilateral injuries, cataract or blindness |
| |  |  |  |  |

## 6.15 Fin condition

### *Why?*

Fins (external moving appendages) generate thrust and provide fish with balance, steering, defence and protection. Fin injury, damage, malformation and/or loss may be the outcome of genetic, environmental and husbandry interactions and can raise welfare issues related to imbalanced social hierarchies (aggregation, feeding competition, emaciation), increased water velocities, crowding, confinement, scratching on nets, improper handling or diseases. Fin damage is common in intensive fish farming and, apart from its significant impact on swimming ability, increases energy demand, reduces growth and can increase susceptibility to infection and mortality. Fin condition is therefore considered an indicator of the welfare status of fish.

Fin condition can be assessed by macroscopic examination. The length and/or fin profile damage (the percentage of missing rays, percentage or fraction of intact extremities present in each fin or in all fins or the percentage of severe or mild active erosion)[101] are parameters of interest.

### *Indicator*

Fin condition (intact, normal fin or presence of injury, infection, malformation and/or loss)

Measuring method: Macroscopic fin inspection.

Measuring unit: Record of skin condition.

### *Type of indicator*

Output-based, animal-based indicator.

### *Scoring system*

Examination and individual evaluation of a population of fish (see reference in chapter 3) following the scoring system below. The average value of the examined population provides the overall score of the indicator.

[101] https://www.researchgate.net/publication/45351194

| INDICATOR/SCORE | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| **FIN CONDITION** | No damage or most of fin remaining | Partially damaged – only half of fin remaining | Absent or little of fin remaining | More than one fin damaged, destroyed or absent |
| |  |  |  |  |

## 6.16 Vertebral deformities

Vertebral deformities are among the main skeletal malformations in many farmed species. Vertebral deformations such as kyphosis, scoliosis and lordosis affect the swimming behaviour of fish and have a negative impact on fish welfare and product quality.

Sorting fry with skeletal deformities in the weaning production phase, prior to transfer to sea cages, reduces the number of deformed on-growing European sea bass. However, empirical data, as well as limited published data[83], show that a low percentage (0,23 – 2,5%) of fish with fin and body damage can be found at harvest.

### *Indicator*

Deformities that appear in the vertebral column of the fish (e.g. lordosis, scoliosis).

Measuring method: Macroscopic inspection.

Measuring unit: Record of deformities.

### *Type of indicator*

Output-based, animal-based indicator.

### *Scoring system*

Examination and individual evaluation of a population of fish (see reference in Chapter 3) following the scoring system below. The average value of the examined population provides the overall score of the indicator.

| INDICATOR/SCORE | 0 | 1 |
|---|---|---|
| |  |  |

## 6.17 Jaw deformities

### *Why?*

Mouth deformities, lesions and haemorrhages, tissue erosion and snout deformation have been reported in both wild and farmed fish species. Mouth and/or jaw wounds can occur in relation to suboptimum handling procedures and managerial practices, contact with the walls of the rearing tank or catches to a sea cage net. Mouth damage, including jaw deformities, may result in an impaired ability to feed and to properly ventilate the gills, i.e. breathe, thereby raising welfare issues.

### *Indicator*

Deformities that appear in the lower or upper jaw of the fish.

Measuring method: Macroscopic jaw inspection.

Measuring unit: Record and extent of deformities.

### *Type of indicator*

Output-based, animal-based indicator.

## Scoring system

Examination and individual evaluation of a population of fish (see reference in Chapter 3) following the scoring system below. The average value of the examined population provides the overall score of the indicator.

| INDICATOR/SCORE | 0 | 1 | 2 |
|---|---|---|---|
| JAW CONDITION | No deformities | Mild deformities | Extreme deformities |

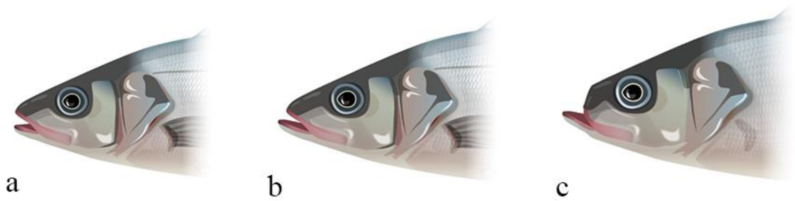



***Figure 6.*** *Graphical representation of normal jaws (a), mild jaw deformity through slightly protruding lower jaw (b) and extreme jaw deformity through absence of part of the upper jaw (c).*

## 6.18 Operculum status

## Why?

The operculum is a bony plate located on the posterior side of the head that covers the fish gills. The operculum protects the gills (fish breathing apparatus) from exterior injuries and damage, is an essential part of the buccal pump ensuring the efficiency of the respiratory mechanism and serves as a facial support structure. Operculum deformities such as shortened, missing and warped gill operculum have been reported in the case of farmed fish[102-103] and are associated with suboptimal rearing conditions and management practices, dietary deficiencies, water quality and pollution. Operculum damage makes gills more vulnerable to external threats and noxious stimuli, especially at high stocking densities, thus increasing the risk of health and welfare impairment.

[102] https://doi.org/10.1016/S0044-8486(97)89294-0
[103] https://doi.org/10.1016/S0044-8486(02)00416-7

## Indicator

Operculum condition in terms of covered surface area of the gill chamber.

Measuring method: Macroscopic operculum inspection.

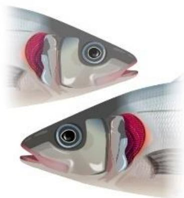
Measuring unit: Record and extent of operculum deformity.

## Type of indicator

Output-based, animal-based indicator.

## Scoring system

Examination and individual evaluation of a population of fish (see reference in Chapter 3) following the scoring system below. The average value of the examined population provides the overall score of the indicator.

| INDICATOR/SCORE | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| **OPERCULUM CONDITION** | Gill chamber full covered bilaterally | 1-15% of total unilateral gill area exposed | > 15% of total unilateral area exposed or 1-15% bilaterally | > 15% of total area exposed bilaterally |
| |  |  |  |  |

### 6.19 Lateral line detection

## Why?

The lateral line is a sensory system that allows fish to detect water motions and pressure gradients[104]. The lateral line enables fish to detect vibrations made by prey, orient fish in a water current (rheotaxis) and gain information about their spatial environment. In certain species it replaces vision at night or very deep in the water column where there is no daylight, allowing the fish to locate living prey. Moreover, it plays a vital role in normal swimming behaviour and schooling.

[104] https://doi.org/10.1111/j.1749-4877.2008.00131.x

Lateral line deformities have been reported in the case of both wild and farmed European sea bass[105-106]. Fish display two major deformities: incomplete lateral line (one or more missing sectors) or multiple (parallel or branching) lateral lines[105]. In another report, lateral line malformation was classified as: zigzag with missing sector, wavy with missing sector, several scattered missing sectors and several consecutive missing sectors[106]. The multiple lateral lines were reported only in the case of farmed specimens[105].

Histological observations have shown that the missing sectors of the lateral line are either empty "scale pockets", i.e. where the specialised scales (i.e. lateral line scales) are missing but the canal underneath is present and the scale print is obvious, or "somatic scales", where the missing lateral line is covered by normal somatic scales, like the rest of the body and there is no external sign of the lateral line[105]. "Scale pocket" malformation may be the result of an accident during the life-span[105] of the individual, while "somatic scale" deformities are the outcome of developmental irregularity[37].

## *Indicator*

External morphology of the body canal (normal lateral line: continuous; slightly curved).

Measuring method: Macroscopic inspection.

Measuring unit: Record of the number of fish with any discontinuity or anomaly of the lateral line.

## *Type of indicator*

Output-based, animal-based indicator.

## *Scoring system*

Examination and individual evaluation of a population of fish (see reference in Chapter 3) following the scoring system below. The average value of the examined population provides the overall score of the indicator.

---

[105] https://doi.org/10.1111/jai.12248
[106] https://doi.org/10.1016/S0044-8486(00)00454-3

| INDICATOR/SCORE | 0 | 1 |
|---|---|---|
| **LATERAL LINE (BODY CANAL)** | Normal* | Incomplete, multiple parallel or branching lateral lines |
| |  |  |

** Lateral line continuous and slightly curved from posterior end of the operculum to the base of the peduncle.*

## 6.20 Gill colour

### *Why?*

The fish gill is the main respiratory organ of most fish species. In addition to providing for aquatic gas exchange, the gills also play a significant role in essential physiological functions such as osmotic and ionic regulation, acid-base regulation and excretion of nitrogenous compounds[107].

Changes to gill structure in response to various infectious and/or non-infectious challenges (such as changes in salinity, pH, hypoxia, acidification) are often reported in the case of fish. Moreover, it is well known that the colour of a fish's gills is a commonly used quality indicator to assess freshness of the fish, i.e. the brighter the colour, the fresher the fish. Finally, it is also known that several physiological dysfunctions and/or pathologies (such as viruses, bacteria, parasites and harmful phytoplankton species) are associated with changes in gill colour (from bright red to pinkish, grey, green or pale) and gill morphology and structure (e.g. oedema, excessive production of mucus, anaemia, haemorrhages or necrosis)[108].

### *Indicator*

The colour of the gills, gill filaments and lamellae.

Measuring method: Macroscopic gill inspection.

Measuring unit: Record of gill colour.

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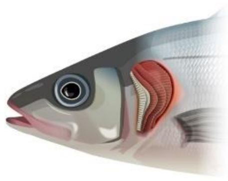
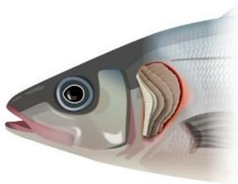
[107] https://doi.org/10.1152/physrev.00050.2003
[108] https://doi.org/10.17221/8763-VETMED

## *Type of indicator*

Output-based, animal-based indicator.

## *Scoring system*

Examination and individual evaluation of a population of fish (see reference in Chapter 3) following the scoring system below. The average value of the examined population provides the overall score of the indicator.

| **INDICATOR/SCORE** | 0 | 1 | 2 |
|---|---|---|---|
| **GILL COLOUR** | Bright red | Pale/Pinkish | Pale/Whitish with mucus |
| **GILL SCORE**[109] | No visible pathology, healthy, red-coloured gills or discrete focal white streaks or patches on individual filaments and slight erosion/damage to distal ends of filaments in less than 5% of the gill area. | More extensive coalescing white streaks or white focal patches on filaments, more extended erosion/damage to distal ends of filaments, grossly swollen or thickened filaments with localised areas of necrotic epithelium covering between 5-50% of the gill area. | Extensive grossly swollen or thickened filaments, shortened filaments (> 50% of filament length affected), pallor and areas of melanisation covering more than 50% of gill area. Widespread necrotic patches, near destruction of gill architecture due to severe loss of epithelium. |
| |  |  |  |

[109] https://doi.org/10.3390/microorganisms9122605

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## DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest.

## RESEARCH ETHICS

No animals were used for this research.

Cure4Aqua
Curing EU aquaculture by co-creating health and welfare innovations

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