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Guidelines on important operational welfare indicators for key European species used in aquaculture research

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This deliverable report is a summary of a review article published by the above authors in Reviews in Aquaculture entitled "Welfare indicators for aquaculture research: toolboxes for five farmed European fish species" DOI: 10.1111/raq.70109 as an output of the AQUAEXCEL3.0 project which supported the work.

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Deliverable Objective:

The goal of this deliverable is to concisely present guidelines for a harmonised documentation toolbox for input- and outcome-based welfare indicators for farmed fish in aquaculture research.

This summary constitutes a deliverable of the AquaExcel3.0 project that provides a condensed summary of the recent article that has been accepted for publication in Reviews in Aquaculture "Welfare indicators for aquaculture research: toolboxes for five farmed European fish species" DOI: 10.1111/raq.70109. It contains text and tables that are reproduced and adapted with permission from the above open access article under the CC BY license: Noble, C., Abbink, W., Alvestad, R., Ardó, L., Bégout, M.-L., Bloecher, N., Burgerhout, E., Calduch-Giner, J., Chivite-Alcalde, M., Císař, P., Durland, E., Espmark, Å. M., Falconer, L., Føre, M., Georgopoulou, D., Heia, K., Helberg, G. A. N., Izquierdo Gomez, D., Johansen, L.-H., Johansson, G. S., Jónsdóttir, K. E., Kolarevic, J., Krasnov, A., Kumaran, S. K., Kvæstad, B., Larsson, T., Lazado, C. C., Madaro, A., Moroni, F., Måge, I., Nilsson, J., Ortega, S., Papandroulakis, N., Pérez-Sánchez, J., Prentice, P. M., Planellas, S. R., Roth, B., Smith, A., Solberg, L. E., Stavrakidis-Zachou, O., Stien, L. H., Striberny, A., Svalheim, R. A., Sæther, B.-S., Timmerhaus, G., Toften, H., Tschirren, L., van de Vis, H., Ytteborg, E., Zena, L. A., Østbye, T.-K. K. (in press). Welfare indicators for aquaculture research: toolboxes for five farmed European fish species. Reviews in Aquaculture, DOI: 10.1111/raq.70109. © 2025 The Authors. Published by John Wiley & Sons Australia, Ltd. Please refer to the above article for the original, extended text, DOI: 10.1111/raq.70109.

Abstract:

Ensuring fish welfare in laboratory and operational research settings is both a legal and ethical obligation under the European Directive 2010/63/EU. Central to this directive are both the 3Rs (Replacement, Reduction, and Refinement), which guide decisions on husbandry, care, and trials, as well as indicator-based assessment of fish welfare to inform these decisions. However, assessing welfare in fish is complex, even in controlled experimental environments, due to prevalent gaps in knowledge about species- and life-stage-specific needs of fish and a missing standardisation of welfare assessment methods. This deliverable aims to introduce the reader to the content of the associated review article, which aims to develop harmonised, practical welfare indicator (WI) toolboxes for five key aquaculture species: Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), and the common carp (*Cyprinus carpio*) to address species- and life-stage-specific welfare needs and go beyond the current guidelines in Annex III of the Directive. The toolboxes include input-based (e.g., environmental conditions) and outcome-based (animal responses) indicators, which are both essential tools for monitoring welfare. Each toolbox includes robust, repeatable, and easily interpretable WIs that effectively reflect fish welfare, especially during critical periods in husbandry and research.

1. Introduction

Monitoring and auditing are fundamental for safeguarding and improving animal welfare in aquaculture research, not only to fulfil ethical obligations and legal requirements, but also to guarantee scientific quality (Hawkins et al., 2011), reliability, and reproducibility (Prescott et al., 2022). This is particularly important in applied aquaculture research, where an in-depth understanding of species-specific needs and welfare indicators is essential to support an industry that farms phylogenetically very diverse species. This deliverable addresses the five key species for the European sector with regard to production volume - the Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and the common carp (*Cyprinus carpio*).

1.1 Animal welfare indicators, assessment and monitoring

The first step in monitoring fish welfare is defining what welfare means for a fish and ways to assess or audit it (J. Turnbull & Kadri, 2007). A popular definition of animal welfare is "the quality of life as perceived by the animal itself" (Bracke et al., 1999). This definition is clear, concise and intuitive and has also been adopted for fish (Noble et al., 2018; Stien et al., 2013). By extension, an animal's welfare state is the sum of its positive and negative feelings, its conscious subjective experience (Kristiansen et al., 2020; Mellor et al., 2020, 2009; Stien et al., 2020). The feelings generated by the brain are at the core of guiding the animal toward fulfilling its needs, thereby maximising its chance of survival (and, in the long term, producing offspring). The needs, or requirements, that are monitored in this way by the emotional and cognitive systems in the brain are termed welfare needs, and welfare indicators are defined as all parameters that can be measured or observed that give information about the fulfilment, or change in fulfilment, of a single or numerous welfare needs (Kristiansen et al., 2020; Noble et al., 2018; Stien et al., 2020). The list of possible welfare needs for fish is long, but for simplicity they can be grouped into four domains (1) nutrition, (2) physical environment, (3) health, and (4) behavioural interactions, which then contribute to a fifth domain, (5) the mental state of the animal, termed the Five Domains Model, e.g., (Mellor et al., 2020), similar to the Five Freedoms (Farm Animal Welfare Council (FAWC), 1993). To ensure a more complete welfare audit, sufficient indicators must be included to conclude about the fulfilment or dissatisfaction of all main welfare needs.

One way to categorise WIs is to differentiate between input- and outcome-based indicators (Noble et al., 2018). Input-based welfare indicators include observations describing the resources, environment and procedures the fish are exposed to. In contrast, outcome-based indicators are animal-based and outline how welfare needs are being met. For example, environmental parameters such as water oxygen saturation and water temperature are input-based indicators influencing the need for an appropriate water environment, while reduced appetite, growth, gill health can be an outcome of this need not being fulfilled. Outcome-based welfare indicators can be further divided into individual- and group-based indicators. Individual-based indicators describe the individual behaviour, health status or physical appearance of each fish. Group-based welfare indicators are applicable at the population/group level, for instance, schooling behaviour, population mortality or how much feed the fish consume each day as a group.

Another approach to classifying welfare indicators is to divide them into operational welfare indicators (OWIs) and so-called laboratory-based welfare indicators (LABWIs). OWIs are easy and practical for experimental and farm use (e.g. appetite, growth), whilst LABWIS are more complex, requiring further analysis in the laboratory or other specialist facilities (e.g. cortisol, microbiome), see (Noble et al., 2018).

1.2 Fish welfare in relation to the EU Directive 2010/63/EU

Animal research in the EU is regulated by Directive 2010/63/EU, which aims to protect animals used for scientific purposes while promoting the development of alternatives and it has been amended by Commission Delegated Directive (EU) 2024/1262 of 13 March 2024. The Directive acknowledges that although replacing live animals is the ultimate goal, their use remains necessary to safeguard human and animal health and the environment (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes., 2010; European Commission., 2024). Central to the Directive are the principles of the 3Rs, which must guide decisions in animal care, husbandry, and scientific procedures. It includes detailed regulations on facility standards, procedural obligations, and animal welfare, covering aspects such as housing, nutrition, and transport.

The European 2010/63/EU Directive and its amendment Commission Delegated Directive (EU) 2024/1262 have a specific, if somewhat brief annex (ANNEX III Guidelines for fish) for fish and their use in scientific procedures. They offer a general overview of a limited number of welfare parameters, primarily environmental (input-based) indicators, to follow and adhere to (Table 1). While there is no species- or life stage-specific information that researchers can use in their welfare monitoring and auditing practices (aside from those for zebrafish) the directive states that some of the water quality parameters should be appropriate/optimal/adapted to each specific species and does acknowledge the need for information on this.

Several authors have therefore collated summaries of how the Directive can be applied to individual species, outlining applicable welfare indicators for a range of species, including Atlantic salmon, rainbow trout, European sea bass, gilthead seabream and the common carp, Atlantic lumpfish (*Cyclopterus lumpus*), ballan wrasse (*Labrus bergylta*), Nile tilapia (*Oreochromis niloticus*), three-spined stickleback (*Gasterosteus aculeatus*), goldfish (*Carassius auratus*), guppy (*Poecilia reticulata*) and zebrafish (*Danio rerio*) (Golledge & Richardson, 2024; Toni et al., 2019).

This current deliverable summarises the work by (Noble et al., In press) and builds on previous work by proposing a Welfare Indicator toolbox that includes both input- and outcome-based indicators at both group and individual levels, suggesting methods for scoring and auditing welfare, such as injury scoring schemes. A broader range of behavioural indicators is also proposed.

Table 1. Summarising the welfare indicators (WIs) included in Annex III of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010, on the protection of animals used for scientific purposes (European Commission, 2010) and amended by the Commission Delegated Directive (EU) 2024/1262 of 13 March 2024 with regard to the requirements for establishments and for the care and accommodation of animals, and with regard to the methods of killing animals. Table reproduced with permission under the CC BY license from: Noble, C., Abbink, W., Alvestad, R., Ardó, L., Bégout, M.-L., Bloecher, N., Burgerhout, E., Calduch-Giner, J., Chivite-Alcalde, M., Císař, P., Durland, E., Espmark, Å. M., Falconer, L., Føre, M., Georgopoulou, D., Heia, K., Helberg, G. A. N., Izquierdo Gomez, D., Johansen, L.-H., Johansson, G. S., Jónsdóttir, K. E., Kolarevic, J., Krasnov, A., Kumaran, S. K., Kvæstad, B., Larsson, T., Lazado, C. C., Madaro, A., Moroni, F., Måge, I., Nilsson, J., Ortega, S., Papandroulakis, N., Pérez-Sánchez, J., Prentice, P. M., Planellas, S. R., Roth, B., Smith, A., Solberg, L. E., Stavrakidis-Zachou, O., Stien, L. H., Striberny, A., Svalheim, R. A., Sæther, B.-S., Timmerhaus, G., Toften, H., Tschirren, L., van de Vis, H., Ytteborg, E., Zena, L. A., Østbye, T.-K. K. (in press). Welfare indicators for aquaculture research: toolboxes for five farmed European fish species. Reviews in Aquaculture, DOI: 10.1111/raq.70109. © 2025 The Authors. Published by John Wiley & Sons Australia, Ltd, who selectively highlight the text below. The table is formulated using text directly reproduced from the Directive 2010/63/EU, and Commission Delegated Directive (EU) 2024/1262, acknowledging its copyright, and with permission.

Input-based Operational Welfare Indicator (OWI)	What Annex III of the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 amended by the Commission Delegated Directive (EU) 2024/1262 of 13 March 2024 states in relation to fish:
General text regarding water quality	*Adequate water supply of suitable quality shall be provided at all times. Water flow in re-circulatory systems or filtration within tanks shall be sufficient to ensure that* ***water quality parameters are maintained within acceptable levels, according to the characteristics of the husbandry system, to the species and life stage requirements****.* *Water supply shall be filtered or treated to* ***remove substances harmful to fish****, where necessary.* *Water-quality parameters* ***shall at all times be within the acceptable range that sustains normal activity and physiology for a given species and stage of development****.* *Appropriate measures* ***shall be taken to minimise sudden changes in the different parameters*** **affecting water quality**. *Appropriate water flow and water level shall be ensured and monitored.*
Oxygen	*Oxygen concentration shall be* ***appropriate to the species and to the context in which the fish are held. Where necessary, supplementary aeration of tank water shall be provided, depending on the husbandry system.***

Temperature	*Temperature shall be* ***maintained within the optimal range for the fish species and their stages of development and kept as stable as possible****.* ***Changes*** *in temperature* ***shall take place gradually****.*
Nitrogen compounds	*The concentrations … of nitrogen compounds, namely ammonia, nitrite and nitrate,* ***shall be kept below harmful levels****.*
Carbon dioxide	*The concentrations of carbon dioxide ...* ***shall be kept below harmful levels***
pH	*The pH level* ***shall be adapted to the species and monitored to be kept as stable as possible****.*
Salinity	*The salinity* ***shall be adapted to the requirements of the fish species and to the life stage of the fish. Changes*** *in salinity* ***shall take place gradually****.*
Lighting	*Fish* ***shall be maintained on an appropriate photoperiod****.*
Noise and vibration	*Noise levels* ***shall be kept to a minimum*** *and, where possible, equipment causing noise or vibration, such as power generators or filtration systems, shall be separate from the fish-holding tanks. For aquatic animals, equipment causing noise or vibration, such as power generators or filtration systems, shall not adversely affect animal welfare.*
Stocking density	*The stocking density of fish* ***shall be based on the total needs of the fish in respect of environmental conditions, health and welfare****.*
Water volume	*Fish shall have sufficient water volume* ***for normal swimming****, taking account of their size, age, health and feeding method.*
Water flow	*The water flow* ***shall be appropriate to enable fish to swim correctly and to maintain normal behaviour****.*

1.1. Practical considerations and implications for data sampling

Standardised methods for sampling should be operationally applicable and secure samples that are as representative as possible. Sample sizes and frequencies should also be operationally realistic in terms of i) the time required to conduct the sampling, and ii) the number of individuals affected by sampling (Nilsson et al., 2022).

1.1.1. Sampling input-based welfare data

When sampling input-based indicators, the aquaculture system type, location and timing of sampling must be considered, as they may influence the accuracy and relevance of the data.

In tanks, the water typically comes from a single inflow, so properties like temperature and salinity are uniform throughout, though they may change over time. In contrast, fish-influenced properties such as oxygen and CO_2 can vary both spatially (depending on fish distribution and water currents) and temporally due to fish activity, metabolism, feeding, or stress (Folkedal et al., 2010; Nilsson et al., 2012). Monitoring tank effluent is a standardised method for assessing water quality, ensuring measurements reflect water affected by all fish. The Norwegian Standard 9417 "Salmon and Rainbow Trout – Terminology and Methods for Documentation of Production" (Standard Norge, 2022) states that measurements in the effluent water should be done 5 cm outside the drain, while the point of measurements done inside the tank should be 1/3 into the tank at mid-depth. Therefore, measurements in scientific tank studies conducted in effluent water are a minimum standard. However, horizontal and vertical profiling may be needed for parameters that vary within the tank or are influenced by system placement and environmental conditions. Profiling variables like water velocity, oxygen, CO_2, pH, conductivity, ammonia, and nitrite across seasons or inlet settings can help reduce or account for data variability.

In net pens, water is primarily supplied from natural currents, and the physical properties of the water vary with depth and time, especially in stratified environments (Oppedal et al., 2011). In addition to the natural levels in the inflowing water, oxygen is affected by, amongst others, current velocity, tides, local stocking density, planktonic activity and the biomass the water has passed through, in addition to the fish's metabolic rate, and rapid local changes may occur within a net pen, also in the horizontal plane (Alver et al., 2023; Burke et al., 2021; Johansson et al., 2006, 2007; Oldham et al., 2018). It is important to consider where sensors are placed within the farm environment to capture the conditions to which the studied fish are exposed (Burke et al., 2021). As a minimum, measurements should be carried out daily and cover the main depth interval, for instance, as a vertical profile, and be audited at times where there is an expected minimum, i.e., at the highest fish density and when the current speed is at its lowest (Nilsson et al., 2022; Oppedal et al., 2011). For salmonids, the NS9417 (Standard Norge, 2022) farming standard states that measures should be taken at 3, 5, and 15 m, and at the maximum cage depth.

1.1.2. Sampling outcome-based welfare data

Outcome-based indicators can be sampled at the individual or group level. Manual sampling of fish for scoring outcome-based indicators at the individual level involves handling the fish, is laborious, and can be detrimental to the fish (Folkedal et al., 2016). Therefore, the EU Directive 2010/63/EU states that handling of fish in experiments should be kept to a minimum, which may potentially restrict sample size and the frequency of sampling events. Limiting both sample size and frequency may, however, lead to high uncertainty of the proportion of a given welfare score in the population. Using fewer, broader categories of indicators during routine evaluations may be beneficial for both the frequency of observations and the time spent per fish. Indicators with high or rising frequencies may then be focused on for more detailed investigations, while indicators of less concern are not, as suggested by (Nilsson et al., 2022; Stien et al., 2020). A common method to reduce biased sampling is to crowd parts of, or the whole fish group, to reduce their ability to flee (Thorburn, 1992). Crowding, however, is stressful, and the physical contact between fish and the rearing system may lead to injuries (Bagni et al., 2007; Erikson et al., 2016; Noble et al., 2018). Furthermore, sampling may still be biased even with the entire population crowded, in both tanks as well as in cages (Nilsson & Folkedal, 2019). The choice of sampling method and number of individuals sampled must therefore depend on the type of experiment and data collected and be based upon group size, the size of the rearing unit, acceptable level of stress on the fish, requirements for precision of the sample estimate, and so forth. Avoiding sampling bias is difficult, and its potential impacts should always be considered.

2. The Proposed WI toolbox

Input-based water quality parameters affect fish welfare (European Food Safety Authority (EFSA), 2008a) and can therefore serve as welfare indicators for all fish species in both experimental and applied settings. However, there are challenges related to applying thresholds to these and our Noble et al., (in press) article states:

i. Thresholds should be based on a broad knowledge base, audited, and applicable to each life stage, species, and experimental setting.
ii. There are still numerous knowledge gaps on how certain water quality parameters, either alone or in tandem with others, can impact fish welfare.
iii. There are various ways in which thresholds can be set and applied. Rather than specific limits, ranges can be introduced (MacIntyre et al., 2008) and applied to various water quality parameters (Toni et al., 2019; Tschirren et al., 2021).

In our review article (Noble et al., In press), "**We therefore propose and acknowledge that it is not always appropriate to attempt to impose thresholds (or threshold ranges) upon water quality parameters, especially if a range of connecting and interrelated factors need to be considered.** Where it is appropriate to do so, various examples will be provided for each species within each WI section".

One must also consider potential inter-relationships between differing input-based WIs. For example, temperature and oxygen interact (Remen et al., 2016), and pH influences levels of toxic compounds like ammonia, CO_2, and hydrogen sulphide, especially in intensive RAS. These interactions highlight the need for systematic monitoring to support early warning systems and safeguard welfare (see Noble et al., in press, for more information).

Acclimation is the response by an animal that enables it to tolerate a change in a single factor in its environment. Although not a welfare indicator *per se* in our review article (Noble et al., In press) we state "acclimation (the length of time that a fish has to acclimate to the conditions it is subjected to), in addition to the actual level of the parameter and speed of change, can have a striking influence on a fish's welfare state", and one should also consider this, see (Noble et al., In press) for more information.

2.1. Input-based WIs

This next section is also a summary and reproduction of the information contained in our recent review article, adapted and reproduced with permission from (Noble et al., In press) and we direct the reader to that article for more widespread information on each indicator and its application and interpretation.

2.1.1. Temperature

Water temperature is a key indicator, with both the absolute optimal value and temporal and spatial changes to consider. Noble et al., (Noble et al., In press) state "Most fish are classified as ectotherms, meaning their metabolic heat production and retention mechanisms are insufficient to increase their body temperature. Consequently, water temperature has a major impact on their metabolism and other body functions, and influences swimming capacity, growth, sexual maturation, immune response and more". It primarily affects welfare needs related to the physical environment and health.

Table 2. Summarising the range of temperatures that are preferred and tolerated by each species outlined in this report. Table adapted from text contained in the following article with permission under the CC BY license: Noble, C., Abbink, W., Alvestad, R., Ardó, L., Bégout, M.-L., Bloecher, N., Burgerhout, E., Calduch-Giner, J., Chivite-Alcalde, M., Císař, P., Durland, E., Espmark, Å. M., Falconer, L., Føre, M., Georgopoulou, D., Heia, K., Helberg, G. A. N., Izquierdo Gomez, D., Johansen, L.-H., Johansson, G. S., Jónsdóttir, K. E., Kolarevic, J., Krasnov, A., Kumaran, S. K., Kvæstad, B., Larsson, T., Lazado, C. C., Madaro, A., Moroni, F., Måge, I., Nilsson, J., Ortega, S., Papandroulakis, N., Pérez-Sánchez, J., Prentice, P. M., Planellas, S. R., Roth, B., Smith, A., Solberg, L. E., Stavrakidis-Zachou, O., Stien, L. H., Striberny, A., Svalheim, R. A., Sæther, B.-S., Timmerhaus, G., Toften, H., Tschirren, L., van de Vis, H., Ytteborg, E., Zena, L. A., Østbye, T.-K. K. (in press). Welfare indicators for aquaculture research: toolboxes for five farmed European fish species. Reviews in Aquaculture, DOI: 10.1111/raq.70109. © 2025 The Authors. Published by John Wiley & Sons Australia, Ltd.

Water temperature [°C]		Preference range	Tolerance range	Reference
Atlantic salmon	Fry	12–14	0–20	(European Food Safety Authority (EFSA), 2008a)
	Parr, smolts	12–14 / 13–16	3–18 / 2–22	(Arnesen et al., 1998; Elliott & Elliott, 2010; Handeland et al., 2003)
	Post-smolts	13–18 / 16–18 / 16–17.5	7–17 / 3–18 / 1–18	(European Food Safety Authority (EFSA), 2008a; Hines et al., 2019; Hvas et al., 2017; Johansson et al., 2009; Noble et al., 2018)
	Broodstock	5-8 / 6–8	8–12 / 1.5–12	(European Food Safety Authority (EFSA), 2008a; Heggberget, 1988)
Rainbow trout	Fry, fingerling	7–13 / 11–13 / 16–18 / 13 / 16 / 17	3–15 / 4–15 / 0–22 / 7–17 / 8–20 / 13–19 / 14–19	(Bear et al., 2007; European Food Safety Authority (EFSA), 2008d; Janhunen et al., 2016; Lewis et al., 2010; Schurmann et al., 1991; Sutterlin & Stevens, 1992; Woynarovich et al., 2011)
	Ongrowers	10–16 / 12–18 / 16–18	0–22 / 1–25 / 7–18	(European Food Safety Authority (EFSA), 2008d; MacIntyre et al., 2008; Raleigh, 1984; Wedemeyer, 1996)
	Broodstock	16–18 / 10–13	0–22	(European Food Safety Authority (EFSA), 2008d) and references therein
European sea bass	Juveniles	17–24	8–32	(Dülger et al., 2012; European Food Safety Authority (EFSA), 2008c; Person-Le Ruyet et al., 2004; Stavrakidis-Zachou et al., 2022)

	Ongrowers	18–24	8–28	(Dülger et al., 2012; European Food Safety Authority (EFSA), 2008c; Sánchez Vázquez & Muñoz-Cueto, 2014)
	Broodstock	13–16*	8–28 / 9–16*	(Dülger et al., 2012; European Food Safety Authority (EFSA), 2008c; Jennings & Pawson, 1991) (*when spawning)
Gilthead seabream	Juveniles	17–22	8–30	(European Food Safety Authority (EFSA), 2008c; Feidantsis et al., 2020)
	Ongrowers		8–30	(European Food Safety Authority (EFSA), 2008c)
	Broodstock	15–17*	13–20	(European Food Safety Authority (EFSA), 2008c) (*when spawning)
Common carp	Fingerlings	20–28	2–38	(Bauer & Schlott, 2004; European Food Safety Authority (EFSA), 2008b)
	Ongrowers		2–36	(European Food Safety Authority (EFSA), 2008b)
	Broodstock	20–28		(European Food Safety Authority (EFSA), 2008b)

2.1.2. Oxygen

Dissolved Oxygen (DO) availability "is essential for fish, and most fish absorb oxygen from the water rather than from the air. They do this by gulping large amounts of water through the gills, where the gill filaments absorb the dissolved oxygen and transport it into the bloodstream." (Noble et al., In press). This oxygen uptake via diffusion across the gills is mainly determined by oxygen saturation rather than concentration, and thus saturation is the more relevant criterion when using dissolved oxygen levels in the water as a welfare indicator (Stien et al., 2013). It primarily affects welfare needs related to the physical environment.

Table 3. Summarising the range of dissolved oxygen saturations that are preferred and tolerated by each species outlined in this report. Table adapted from text contained in the following article with permission under the CC BY license: Noble, C., Abbink, W., Alvestad, R., Ardó, L., Bégout, M.-L., Bloecher, N., Burgerhout, E., Calduch-Giner, J., Chivite-Alcalde, M., Císař, P., Durland, E., Espmark, Å. M., Falconer, L., Føre, M., Georgopoulou, D., Heia, K., Helberg, G. A. N., Izquierdo Gomez, D., Johansen, L.-H., Johansson, G. S., Jónsdóttir, K. E., Kolarevic, J., Krasnov, A., Kumaran, S. K., Kvæstad, B., Larsson, T., Lazado, C. C., Madaro, A., Moroni, F., Måge, I., Nilsson, J., Ortega, S., Papandroulakis, N., Pérez-Sánchez, J., Prentice, P. M., Planellas, S. R., Roth, B., Smith, A., Solberg, L. E., Stavrakidis-Zachou, O., Stien, L. H., Striberny, A., Svalheim, R. A., Sæther, B.-S., Timmerhaus, G., Toften, H., Tschirren, L., van de Vis, H., Ytteborg, E., Zena, L. A., Østbye, T.-K. K. (in press). Welfare indicators for aquaculture research: toolboxes for five farmed European fish species. Reviews in Aquaculture, DOI: 10.1111/raq.70109. © 2025 The Authors. Published by John Wiley & Sons Australia, Ltd.

Dissolved Oxygen Saturation [%]		Preference range	Tolerance range	Reference
Atlantic salmon	Fry, parr	>70% (12.5°C)	>39% (12.5°C)	(European Food Safety Authority (EFSA), 2008a; Stevens et al., 1998)
	Post- smolts	42% (7°C) / 53% (11°C) / 66% (15°C) / 76% (19°C)	24% (7°C) / 33% (11°C) / 34% (15°C) / 40% (19°C)	(Remen et al., 2016)
	Broodstock	>70% / >80%		(European Food Safety Authority (EFSA), 2008a; Noble et al., 2018)

Rainbow trout	Fry	81-100% (17-19°C)	> 30%	(Poulsen et al., 2011)
	Ongrowers	80-120%	60-80% and 120-160%	(Tschirren et al., 2021)
European sea bass	Juveniles	>80% (22°C)	40% (22°C)	(Pichavant et al., 2001; Thetmeyer et al., 1999)
Gilthead seabream	Ongrowers		17% (12°C) / 22% (16°C) / 36% (20°C) / 40%	(European Food Safety Authority (EFSA), 2008c; Remen et al., 2015)
Common carp	All life stages		> 20%	(European Food Safety Authority (EFSA), 2008b)

2.1.3. Ammonia, Nitrite, Nitrate

Ammonia is toxic to fish (Ip et al., 2001; Twitchen & Eddy, 1994) and has a negative impact upon, e.g., the central nervous system, gill function, behaviour, feeding, and can lead to mortality (Thorarensen & Farrell, 2011). Nitrite can also be toxic to the fish (F. B. Jensen, 2003; Kroupova et al., 2005) and can have a negative impact upon, e.g., oxygen transport, cardiovascular function and various excretory and endocrine tasks (F. B. Jensen, 2003; Svobodová et al., 2005). Nitrate "is the ultimate product of nitrification and can build up in RAS if the water exchange levels in the production system are low." (Noble et al., In press). It is less harmful than the other two nitrogenous compounds, but it may disrupt endocrine function (Edwards & Hamlin, 2018). These nitrogenous compounds primarily affect welfare needs related to the physical environment.

Table 4. Summarising the range of ammonia levels that affect each species outlined in this report. Table adapted from text contained in the following article with permission under the CC BY license: Noble, C., Abbink, W., Alvestad, R., Ardó, L., Bégout, M.-L., Bloecher, N., Burgerhout, E., Calduch-Giner, J., Chivite-Alcalde, M., Císař, P., Durland, E., Espmark, Å. M., Falconer, L., Føre, M., Georgopoulou, D., Heia, K., Helberg, G. A. N., Izquierdo Gomez, D., Johansen, L.-H., Johansson, G. S., Jónsdóttir, K. E., Kolarevic, J., Krasnov, A., Kumaran, S. K., Kvæstad, B., Larsson, T., Lazado, C. C., Madaro, A., Moroni, F., Måge, I., Nilsson, J., Ortega, S., Papandroulakis, N., Pérez-Sánchez, J., Prentice, P. M., Planellas, S. R., Roth, B., Smith, A., Solberg, L. E., Stavrakidis-Zachou, O., Stien, L. H., Striberny, A., Svalheim, R. A., Sæther, B.-S., Timmerhaus, G., Toften, H., Tschirren, L., van de Vis, H., Ytteborg, E., Zena, L. A., Østbye, T.-K. K. (in press). Welfare indicators for aquaculture research: toolboxes for five farmed European fish species. Reviews in Aquaculture, DOI: 10.1111/raq.70109. © 2025 The Authors. Published by John Wiley & Sons Australia, Ltd.

Ammonia [mg NH_3-N/L]		EC	LC50	Reference
Atlantic salmon	Parr	0.02-0.05		(Fivelstad et al., 1993; Kolarevic et al., 2012, 2013)
	Post-smolt	0.14	0.24–0.34 (48h)	(Alabaster et al., 1979; Knoph, 1996)
	All	0.04–0.08 / < 0.05		(European Food Safety Authority (EFSA), 2008a; Fivelstad et al., 1995; Knoph & Olsen, 1994; Knoph & Thorud, 1996)
Rainbow trout	Fry	0.05–0.19		(Burkhalter & Kaya, 1977)
	Juveniles	>0.01–0.03 / >0.001 to 0.005		(European Food Safety Authority (EFSA), 2008d; Haywood, 1983; Klontz, 1991; MacIntyre et al., 2008; Tarazona & Muñoz, 1995; Vosylienė & Kazlauskienė, 2004)

	Ongrowers	>0.05 / >0.01–0.05		(Becke et al., 2019; European Food Safety Authority (EFSA), 2008d; MacIntyre et al., 2008; Vosylienė & Kazlauskienė, 2004)
	All		0.13–0.90 (96h)	(Thurston & Russo, 1983)
	Fingerlings	0.05		(European Food Safety Authority (EFSA), 2008c)
European sea bass	Juveniles, ongrowers	0.13–0.5 / 0.06–0.26	0.97–2.30 (96h)	(Dosdat et al., 2003; European Food Safety Authority (EFSA), 2008c, p. 2019; Kır et al., 2019; Lemarié et al., 2004)
Gilthead seabream	Juveniles, ongrowers	0.5–0.7	0.80–2.73 (96h)	(Kir & Sunar, 2018; Person-Le Ruyet et al., 1995; Wajsbrot et al., 1991, 1993)
Common carp	Fingerlings	0.05–0.40	1.74–2.33 (96h)	(European Food Safety Authority (EFSA), 2008b; Guan et al., 2010; Hasan & Macintosh, 1986; Svobodová et al., 1993)
	Juveniles, ongrowers	1.00 / 0.05–0.50	1.74–2.33 (96h)	(European Food Safety Authority (EFSA), 2008b; Guan et al., 2010; Hasan & Macintosh, 1986; G. Jeney et al., 1992; Zs. Jeney et al., 1992; Svobodová et al., 1993)

Table 5. Summarising the range of nitrate and nitrite levels that affect each species outlined in this report. Table adapted from text contained in the following article with permission under the CC BY license: Noble, C., Abbink, W., Alvestad, R., Ardó, L., Bégout, M.-L., Bloecher, N., Burgerhout, E., Calduch-Giner, J., Chivite-Alcalde, M., Císař, P., Durland, E., Espmark, Å. M., Falconer, L., Føre, M., Georgopoulou, D., Heia, K., Helberg, G. A. N., Izquierdo Gomez, D., Johansen, L.-H., Johansson, G. S., Jónsdóttir, K. E., Kolarevic, J., Krasnov, A., Kumaran, S. K., Kvæstad, B., Larsson, T., Lazado, C. C., Madaro, A., Moroni, F., Måge, I., Nilsson, J., Ortega, S., Papandroulakis, N., Pérez-Sánchez, J., Prentice, P. M., Planellas, S. R., Roth, B., Smith, A., Solberg, L. E., Stavrakidis-Zachou, O., Stien, L. H., Striberny, A., Svalheim, R. A., Sæther, B.-S., Timmerhaus, G., Toften, H., Tschirren, L., van de Vis, H., Ytteborg, E., Zena, L. A., Østbye, T.-K. K. (in press). Welfare indicators for aquaculture research: toolboxes for five farmed European fish species. Reviews in Aquaculture, DOI: 10.1111/raq.70109. © 2025 The Authors. Published by John Wiley & Sons Australia, Ltd.

Nitrite, Nitrate [mg/L]		Recommended safe limits:		
		NO_2^--N	NO_3-N	Reference
Salmon			100	(Davidson et al., 2017; Freitag et al., 2015, 2016; Good et al., 2017)
Trout	Fingerlings Adults	0.001-0.009 0.003	5.6-16.9 11.3-33.8	(European Food Safety Authority (EFSA), 2008d; Tschirren et al., 2021; Wedemeyer, 1996; Westin, 1974)
Sea bass	Adults	50*	125*	*Effect concentrations (Saroglia et al., 1981; Scarano et al., 1984; Torno et al., 2018)
Seabream		<0.02–0.06	<50	(European Food Safety Authority (EFSA), 2008c; Parra & Yúfera, 1999)
Carp		0.05	80	(Staykov et al., 2015; Svobodová et al., 1993)

2.1.4. pH

"Extreme as well as fast-changing pH levels can occur in aquaculture and impair fish welfare, which makes pH a crucial parameter when auditing fish welfare", it also "affects the equilibria of multiple compounds with different toxicity (e.g., ammonium/ammonia, carbon dioxide/bicarbonate), particularly in systems with low water exchange" (Noble et al., In press). It primarily affects welfare needs related to the physical environment.

Table 6. Summarising the range of pH values that are optimal and tolerated by each species outlined in this report. Table adapted from text contained in the following article with permission under the CC BY license: Noble, C., Abbink, W., Alvestad, R., Ardó, L., Bégout, M.-L., Bloecher, N., Burgerhout, E., Calduch-Giner, J., Chivite-Alcalde, M., Císař, P., Durland, E., Espmark, Å. M., Falconer, L., Føre, M., Georgopoulou, D., Heia, K., Helberg, G. A. N., Izquierdo Gomez, D., Johansen, L.-H., Johansson, G. S., Jónsdóttir, K. E., Kolarevic, J., Krasnov, A., Kumaran, S. K., Kvæstad, B., Larsson, T., Lazado, C. C., Madaro, A., Moroni, F., Måge, I., Nilsson, J., Ortega, S., Papandroulakis, N., Pérez-Sánchez, J., Prentice, P. M., Planellas, S. R., Roth, B., Smith, A., Solberg, L. E., Stavrakidis-Zachou, O., Stien, L. H., Striberny, A., Svalheim, R. A., Sæther, B.-S., Timmerhaus, G., Toften, H., Tschirren, L., van de Vis, H., Ytteborg, E., Zena, L. A., Østbye, T.-K. K. (in press). Welfare indicators for aquaculture research: toolboxes for five farmed European fish species. Reviews in Aquaculture, DOI: 10.1111/raq.70109. © 2025 The Authors. Published by John Wiley & Sons Australia, Ltd.

pH		Optimal range	Tolerance range	Reference
Atlantic salmon	Fry	6.5–7 / 6–8.5	5 / 5.4	(European Food Safety Authority (EFSA), 2008a; Noble et al., 2018)
	Smolts	6–8.5	5.4	(European Food Safety Authority (EFSA), 2008a)
	Post-smolts	7–8.5	5.4	(European Food Safety Authority (EFSA), 2008a)
Rainbow trout	Fingerling	5.5–8.5	4–9	(European Food Safety Authority (EFSA), 2008d)
	Adults	7–7.5 / 5.5–8.5	6–8.5 / 4–9	(European Food Safety Authority (EFSA), 2008d; Tschirren et al., 2021)
European sea bass	Fingerling, adults	8–8.2	6.5–8.5	(European Food Safety Authority (EFSA), 2008c)
Gilthead seabream	Ongrowers	8	7.5–8.5	(European Food Safety Authority (EFSA), 2008c)
Common carp	Fingerlings	7.5–8	5.9–9.5	(European Food Safety Authority (EFSA), 2008b; Heydarnejad, 2012; Sapkale et al., 2011)
	Juveniles, adults	7–8	5.5–10	(European Food Safety Authority (EFSA), 2008b; Heydarnejad, 2012; Sapkale et al., 2011)

2.1.5. CO_2

Elevated environmental CO_2 concentrations can negatively impact feed intake, digestion and growth rates (Skov, 2019) as well as behaviour. It primarily affects welfare needs related to the physical environment.

Summarising the data outlined in (Noble et al., In press):
Atlantic salmon: Adverse effects have been observed above 15 mg/L (Fivelstad et al., 2015; Mota et al., 2019) and recommended safe limits are reported to range from below 10–15 mg/L (Fivelstad et al., 2003; Skov, 2019).
Rainbow trout: Adverse effects have been observed above 34.5 mg/L, but not at concentrations below this level (Danley et al., 2007; Good et al., 2010). Recommended safe limits are 9–30 mg/L for fry and fingerlings (Heinen et al., 1996; MacIntyre et al., 2008; Smart, 1981; Wedemeyer, 1996) and 5–30 mg/L for ongrowers (Tschirren et al., 2021).
European sea bass: Adverse effects have been observed at 75 mg/L, and the LC_{50} was indicated at 115.5 mg/L (48h) and 104.8 mg/L (120h) (Cecchini et al., 2001; Grøttum & Sigholt, 1996). A safe limit of 40 mg/L is recommended for all life stages (Blancheton, 2000).
Gilthead seabream: Growth depression has been observed at > 20 mg/L (Ben-Asher et al., 2013).
Common carp: Concentrations < 25 mg/L have been reported to be within the carp's tolerance ranges (Svobodová et al., 1993).

2.1.6. Lighting

Light affects many biological factors in fish. It has three components: quantity (intensity), quality (spectrum and distribution), and periodicity (photoperiod), see (Noble et al., In press) and references therein. High light quantities (intensities) can be stressful or lead to mortality (Boeuf & Le Bail, 1999). Light quality can affect growth (Karakatsouli et al., 2007; Papoutsoglou et al., 2005; Ruchin, 2004), behaviour (Marchesan et al., 2005) and the physiology of the fish (Karakatsouli et al., 2007). Periodicity can affect, e.g., the immune response (Ceballos-Francisco et al., 2020) and spawning (Imsland et al., 2014). Sudden changes should also be avoided when fish are held under a light:dark cycle, as this can be a stressor for the fish (Mork & Gulbrandsen, 1994). Light primarily affects welfare needs related to behavioural interactions and nutrition.

Atlantic salmon: are categorised as primarily diurnal, and prolonged exposure to high quantities of light (intensities) can damage their retinas (Vera & Migaud, 2009), and periodicity (a change in day length) is often required to initiate smolt development and later seawater performance (Ebbesson et al., 2007; Handeland & Stefansson, 2001; Striberny et al., 2021).
Rainbow trout: are categorised as mostly diurnal, but juveniles can be nocturnal in winter at low temperatures (Riehle & Griffith, 1993). Continuous 24-h light can be immunosuppressive in juvenile rainbow trout (Leonardi & Klempau, 2003).
European seabass: are categorised as mostly diurnal, but low water temperature can lead them to become nocturnal (Sánchez-Vázquez et al., 1998). High light quantities can increase cortisol levels and cause retinal damage (Vera & Migaud, 2009). In larvae, continuous light can cause swim bladder problems and jaw deformities (Villamizar et al., 2009).
Gilthead seabream: are categorised as mostly diurnal, but low water temperature can lead them to become nocturnal (Paspatis et al., 2000). It remains unclear whether continuous light conditions favour the development of skeletal deformities (Mhalhel et al., 2023).

Common carp: A 12:12 light cycle for all life stages is recommended by numerous sources (Chakraborty et al., 1992; Ghomi et al., 2011; Ruchin AB, 2019; Toni et al., 2019).

2.1.7. Noise

In our article (Noble et al., In press) we state "There are several definitions of noise (Fink, 2020; Van Geel et al., 2022), and for the purpose of this review, we define noise as any unwanted sound that has a detrimental effect on the fish". It primarily affects welfare needs related to the physical environment and behavioural interactions.

Atlantic salmon; rainbow trout; European sea bass; gilthead seabream; common carp: as far as we are aware, there is no published knowledge on the effects of different noise levels on welfare.

2.1.8. Stocking density

Stocking density can be described as the density of fish within a rearing system (Ellis et al., 2002). Both high and low stocking densities can affect fish welfare (Adams et al., 2007; Ellis et al., 2002; Johansen et al., 2006; L. R. Sveen et al., 2016). Using stocking density as a singular WI is problematic, as water quality and behavioural considerations should be taken into account. Hence, the (European Food Safety Authority (EFSA), 2008d) states, "stocking density *per se* should not be used as an indicator for good welfare as it is difficult to set appropriate levels of stocking densities, the monitoring of the conditions of the fish should be regarded as a preferred option".

Atlantic salmon; rainbow trout; European sea bass; gilthead seabream; common carp: in our Noble et al., (Noble et al., In press) article, we state that we "do not wish to make recommendations on numerical thresholds for different stocking densities in relation to their impacts upon welfare. For aquaculture research, if densities are not a specific objective of the experiment, they should be defined in relation to water quality, fish health, and other welfare indicators, including behavioural considerations and injury levels, and a focus should be on monitoring and documentation". It primarily affects welfare needs related to the physical environment and behavioural interactions.

2.1.9. Water velocity

Water velocity in tanks can aid system cleaning and fish welfare, but is often unevenly distributed due to design factors like inlet and outlet placement (Gorle et al., 2020; Gorle et al., 2018). Large tanks often require methods to homogenize flow. In net pens, it can aid water exchange and can exercise the fish, but it must not be greater than the sustained swimming capacity of the fish in the rearing system. Velocity varies with system size, and both extremes can harm welfare (Espmark et al., 2017). While high velocity may boost heart health and growth (Castro et al., 2011; Nilsen et al., 2019), it may also impair skin health (Timmerhaus et al., 2021). It primarily affects welfare needs related to the physical environment and behavioural interactions.

Table 7. Summarising the range of water velocities that are optimal and tolerated by each species outlined in this report. Table adapted from text contained in the following article with permission under the CC BY license: Noble, C., Abbink, W., Alvestad, R., Ardó, L., Bégout, M.-L., Bloecher, N., Burgerhout, E., Calduch-Giner, J., Chivite-Alcalde, M., Císař, P., Durland, E., Espmark, Å. M., Falconer, L., Føre, M., Georgopoulou, D., Heia, K., Helberg, G. A. N., Izquierdo Gomez, D., Johansen, L.-H., Johansson, G. S., Jónsdóttir, K. E., Kolarevic, J., Krasnov, A., Kumaran, S. K., Kvæstad, B., Larsson, T., Lazado, C. C., Madaro, A., Moroni, F., Måge, I., Nilsson, J., Ortega, S., Papandroulakis, N., Pérez-Sánchez, J., Prentice, P. M., Planellas, S. R., Roth, B., Smith, A., Solberg, L. E., Stavrakidis-Zachou, O., Stien, L. H., Striberny, A., Svalheim, R. A., Sæther, B.-S., Timmerhaus, G., Toften, H., Tschirren, L., van de Vis, H., Ytteborg, E., Zena, L. A., Østbye, T.-K. K. (in press). Welfare indicators for aquaculture research: toolboxes for five farmed European fish species. Reviews in Aquaculture, DOI: 10.1111/raq.70109. © 2025 The Authors. Published by John Wiley & Sons Australia, Ltd.

Water velocity [BL/s]		Optimal range	Tolerance range	Reference
Atlantic salmon	Fry	0.10–0.25 m/s		(Heggenes & Traaen, 1988)
	Smolts	0.10–0.5 m/s		
	Post-smolts	0.8–1		(Solstorm et al., 2016; Timmerhaus et al., 2021)
Rainbow trout	Fingerling	0.9 / 0 – 1 / 0.75 – 1.5		(Farrell et al., 1991; Houlihan & Laurent, 1987; Larsen et al., 2012; McKenzie et al., 2012)
	Adults	0.5–1	0.2–3 / 0.5–3	(Hafs et al., 2012; Parker & Barnes, 2015; Tschirren et al., 2021)
European sea bass	Fingerling, adults	2		(Palstra et al., 2020)
Gilthead seabream	Juveniles	1.5		(Ibarz et al., 2011)
Common carp	Adults		>2.5	(Martin & Johnston, 2006)

2.1.10. Water exchange rate

In our Noble et al., (Noble et al., In press) article, we state "water exchange in closed production units can be expressed as the volume of water flowing into and exiting the unit per unit of time (e.g., L/min) or as the percentage of the water volume exchanged per day. Alternatively, it can be linked to the biological production and expressed as the volume of water flowing into and out of the unit per kg fish per time (e.g., L kg^{-1} min^{-1}), a quantity referred to as specific water flow". It replenishes oxygen saturation levels in closed systems as well as facilitating the removal of waste products, which can build up if the water exchange rate is too low. It primarily affects welfare needs related to the physical environment.

Atlantic salmon: Studies, conducted in flow-through tanks with oxygen supplementation, show reduced growth in Atlantic salmon fry at 0.7 L kg^{-1} min^{-1}, while smolts tolerate flows down to 0.15 L kg^{-1} min^{-1} (Fivelstad et al., 1999, 2004). Post-smolts exposed to flows ≤0.3 L kg^{-1} min^{-1} show elevated immune and stress responses, and 0.3 L kg^{-1} min^{-1} is recommended as a lower limit in closed systems (Calabrese et al., 2023; L. R. Sveen et al., 2016).

Rainbow trout: Sufficient flow related to biomass is an important factor in maintaining welfare and performance. Subadult rainbow trout welfare was improved at 1.5 to 2.5 exchanges per hour (Ross et al., 1995).

European sea bass: In sea bass (100–150 g), waste accumulation becomes problematic below ~0.33 L kg^{-1} min^{-1} in flow-through tanks with oxygen supplementation (Lemarie & Toften, 2002).

As far as we are aware, relevant information on gilthead seabream and carp is lacking or scarce, but in general lower specific water flows lead to the accumulation of deleterious waste products (Damsgård et al., 2011).

2.1.11. Salinity

The Directive states that "salinity shall be adapted to the requirements of the fish species and its life stage, and changes in salinity shall take place gradually" (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes., 2010). Euryhaline fish can tolerate a wide range of salinities, while stenohaline fish need a narrow and relatively steady range of salinities (Kültz, 2015). It primarily affects welfare needs related to the physical environment.

Atlantic salmon: Euryhaline. Have problems tolerating seawater before smoltification and after they start maturing (Persson et al., 1998; Stien et al., 2013). Fry–smolts and broodstock have a reported tolerance range of 0-10 ppt (Craik & Harvey, 1988; European Food Safety Authority (EFSA), 2008a). Post-smolts can cope with both freshwater and full-strength seawater, but moderate salinities promote better growth and stress regulation (Hvas et al., 2018; Ytrestøyl et al., 2020).

Rainbow trout: Euryhaline. Their osmoregulatory capacity depends on their body weight; seawater tolerance increases from when the fish are 50 g to 150 g (Lee & Lee, 2020). Salinity acclimatisation is recommended (Lee et al., 2022) if they are exposed to higher salinity. It is not recommended to hold maturing broodstock in seawater, as it negatively impacts the survival of both broodfish and egg; salinities of 10-13 ppt had the best results (Albrektsen & Torrissen, 1988).

Table 8. Summarising the range of salinities that are preferred and tolerated by European seabass, gilthead seabream and common carp. Table adapted from text contained in the following article with permission under the CC BY license: Noble, C., Abbink, W., Alvestad, R., Ardó, L., Bégout, M.-L., Bloecher, N., Burgerhout, E., Calduch-Giner, J., Chivite-Alcalde, M., Císař, P., Durland, E., Espmark, Å. M., Falconer, L., Føre, M., Georgopoulou, D., Heia, K., Helberg, G. A. N., Izquierdo Gomez, D., Johansen, L.-H., Johansson, G. S., Jónsdóttir, K. E., Kolarevic, J., Krasnov, A., Kumaran, S. K., Kvæstad, B., Larsson, T., Lazado, C. C., Madaro, A., Moroni, F., Måge, I., Nilsson, J., Ortega, S., Papandroulakis, N., Pérez-Sánchez, J., Prentice, P. M., Planellas, S. R., Roth, B., Smith, A., Solberg, L. E., Stavrakidis-Zachou, O., Stien, L. H., Striberny, A., Svalheim, R. A., Sæther, B.-S., Timmerhaus, G., Toften, H., Tschirren, L., van de Vis, H., Ytteborg, E., Zena, L. A., Østbye, T.-K. K. (in press). Welfare indicators for aquaculture research: toolboxes for five farmed European fish species. Reviews in Aquaculture, DOI: 10.1111/raq.70109. © 2025 The Authors. Published by John Wiley & Sons Australia, Ltd.

Salinity [ppt]		Optimal range	Tolerance range	Reference
European sea bass	Juveniles		3–30	(Dalla Via et al., 1998)
	Adults	30	0–60	(Eroldoğan & Kumlu, 2002; M. K. Jensen et al., 1998; Sinha et al., 2015)
Gilthead seabream	Juveniles	12		(Laiz-Carrión et al., 2005)
	Ongrowers	5–30		(Claireaux & Lagardère, 1999)
Common carp	Fingerlings	0.5–2.5	2.5–7.0	(Salati et al., 2011; Wang et al., 1997; Whiterod & Walker, 2006)

2.2. Outcome-based WIs at the Group Level

This next section is also a summary and reproduction of the information contained in our recent review article, adapted and reproduced with permission from (Noble et al., In press) and we direct the reader to that article for more in-depth information on each indicator and its application and interpretation.

2.2.1. Behaviour

Behaviour is fundamental to assessing fish welfare and can reflect the fish's response to the rearing environment, the husbandry procedures, and its conspecifics (Martins et al., 2012). Behaviour provides key insights into the subjective experiences of fish, is a non-invasive measure in most situations, and can be indicative of the fish’s internal state in real-time. At the group level, these behaviours include, e.g., swimming speed, shoaling behaviour, orientation/polarisation, spatial distribution, feeding behaviour and activity, agonistic behaviours, freezing, fleeing and panic behaviours (Barreto et al., 2022; Martins et al., 2012; Noble et al., 2018). Where observation tools or practices allow, many group behaviours can also be classified at the individual level, including swimming speed, orientation, feeding behaviour and activity, agonistic behaviours, freezing, fleeing and panic behaviours, in addition to ventilation rate (Martins et al., 2012). Significant changes in these behaviours have been linked with acute and chronic stress in aquaculture and are established signs of disease and poor welfare states (Martins et al., 2012).

When monitoring behaviour, a clear understanding of the behaviours the fish can exhibit in their given rearing system is crucial. In this regard, we would like to draw the reader’s attention to existing ethograms. Saraiva et al., (2022) have assembled a comprehensive OWI guide for the aquaculture species that we consider in this report and they have kindly given their permission for us to reproduce and adapt their ethograms in Noble et al., (Noble et al., In press), which we also reproduce here under the CC By licence (see Tables 9a and 9b). Behaviours primarily impact on welfare needs related to nutrition, the physical environment, health and behavioural interactions.

Tables 9a (top) and 9b (below). A general ethogram for aquaculture research. Reproduced and adapted with kind permission from Saraiva JL, Volstorf J, Cabrera-Álvarez MJ, Arechavala-Lopez P. Using ethology to improve farmed fish welfare and production. Report produced for the AAC. (2022) 67 pp + annexes https://aac-europe.org/en/publication/using-ethology-to-improve-farmed-fish-welfare-and-production-2/. Italics indicate original text from Saraiva et al. (2022) and non-italic text is our adaptation of their ethogram. This table is reproduced with permission under the CC BY license from: Noble, C., Abbink, W., Alvestad, R., Ardó, L., Bégout, M.-L., Bloecher, N., Burgerhout, E., Calduch-Giner, J., Chivite-Alcalde, M., Císař, P., Durland, E., Espmark, Å. M., Falconer, L., Føre, M., Georgopoulou, D., Heia, K., Helberg, G. A. N., Izquierdo Gomez, D., Johansen, L.-H., Johansson, G. S., Jónsdóttir, K. E., Kolarevic, J., Krasnov, A., Kumaran, S. K., Kvæstad, B., Larsson, T., Lazado, C. C., Madaro, A., Moroni, F., Måge, I., Nilsson, J., Ortega, S., Papandroulakis, N., Pérez-Sánchez, J., Prentice, P. M., Planellas, S. R., Roth, B., Smith, A., Solberg, L. E., Stavrakidis-Zachou, O., Stien, L. H., Striberny, A., Svalheim, R. A., Sæther, B.-S., Timmerhaus, G., Toften, H., Tschirren, L., van de Vis, H., Ytteborg, E., Zena, L. A., Østbye, T.-K. K. (in press). Welfare indicators for aquaculture research: toolboxes for five farmed European fish species. Reviews in Aquaculture, DOI: 10.1111/raq.70109. © 2025 The Authors. Published by John Wiley & Sons Australia, Ltd.

Behaviour	Definition	Level	Effect upon welfare	Source data
Spawning	*Movements, actions and/or displays that lead to reproduction. May include courtship, nest building, egg releasing, fertilisation, parental care or other species-specific behaviours.*	*Group or Individual*	*Positive*	Saraiva et al. [34] and relevant for all five species
Foraging	".. a complex behavior that ranges from detecting and searching for food, capturing prey, and determining if it should be swallowed or rejected" [419].	*Group or Individual*	*Positive*	Martins et al. [406] and relevant for all five species
Group structure	*Shoaling (in a group but not directional or coordinated); schooling (in a polarised, directional and coordinated swimming) or disperse (no clear group formed).* Aspects of these behaviours are further outlined below, in addition to group cohesion.	*Group*	Coordinated swimming outside feeding is generally considered positive in aquaculture research settings	Martins et al. [406] and relevant for all five species
Vertical distribution	Vertically distributed *close to surface, midwater, bottom, etc* or throughout the rearing system.	Group or Individual	Negative and Positive	Oppedal et al. [83]; Saraiva et al. [34] and relevant for all five species
Horizontal distribution	Horizontally distributed e.g., in the centre or periphery of the rearing system or near system features that may have utility such as feeding delivery points or tank water inlets. See also 'thigmotaxis'.	Group or Individual	Negative and Positive	Martins et al. [406] and references therein. Relevant for all five species
Group cohesion	"... describes the distance between individuals within the group" [420].	Group	Negative and Positive	e.g., Ward et al. [420] and relevant for all five species
Surface activity	Refers to the "...number of rolls and jumps the fish make" [4] where jumps (leaps) can be described "leaping, with the whole body breaking clear of the water" and rolling which can be described as "only the dorsal part of the body breaking the surface" [421]. Rolling has also been defined as where the surface is "...broken by the fish...rolling through with a larger body proportion" [422].	*Group*	Low can be positive but also negative if fish need to refill the swim bladder (e.g., in A. salmon). High can be positive if it indicates e.g., the filling of the swim bladder or feeding motivation, but can also be indicative of stress or high parasite load	Furevik et al. [421]; Bui et al. [423]; Noble et al. [4]

Behaviour	Definition	Level	Effect upon welfare	Source data
Exploration	"..individual willingness to investigate novel environments, food items or objects" [424]. *Movements or actions that apparently serve to collect information* on new environments and objects.	*Individual*	*Positive*	Martins et al. [406] and relevant for all five species
Anticipation	*Movements or actions that precede an occurrence and indicate that the fish are aware of routine procedures taking place imminently. The most common is food anticipatory behaviour, where the fish* increase activity *before feeding.* Can be indicative of feeding motivation.	*Individual*	*Positive*	Martins et al. [406] and relevant for all five species
Swimming speed	*slow, regular, fast, erratic bursts*	*Individual*	Negative and Positive	Martins et al. [406] and relevant for all five species
Ventilation frequency	*Rate at which the opercula open and close, as a measure of the respiratory needs of the fish.*	Individual	High can be both *negative* if fish are gasping (poor water quality or respiratory problems), or positive if indicative of increased respiratory needs (exercise)	Millidine et al. [425]; Martins et al. [406] and relevant for all five species
Aggression	"..behaviour that actually or potentially causes harm to another animal" Huntingford and Damsgård, in [418]. *Agonistic interaction between two or more individuals. Can occur without physical engagement (i.e. Low Intensity Aggression: fin erection, colour changing, displays etc.) or including physical interaction (High Intensity Aggression: chasing, biting, fighting)*	*Individual*	*Negative* for the recipient	Axling et al. [426]; Carbonara et al. [427]; Duan et al. [428]; Flood [429]; Neofytou et al. [430]; Newcombe and Hartman [431]; Oikonomidou et al. [432]; Solstorm et al. [355]; Wagner et al. [299]; Øverli et al. [433] and relevant for all five species
Stereotypical behaviours	"Stereotypies are repetitive, invariant behaviour patterns with no obvious goal or function" [434]	*Individual*	*Negative*	Martins et al. [406] and relevant for all five species
Freezing	Fish cease swimming and become immobile e.g., [435].	Individual	Negative	Maximino et al. [435] and relevant for all five species
Thigmotaxis	*Strong avoidance of open areas and preference for moving in very close proximity of the walls of the rearing environment.*	*Individual*	Mostly *Negative*, unless e.g., a water inlet or other potentially beneficial flow dynamic parameters are close to a wall	Saraiva et al. [34] and references therein. Relevant for all five species
Scototaxis	Preference for dark instead of light substrates e.g., [435].	Individual	Negative	Maximino et al. [435] and relevant for all five species
Apathy	"the animal ceases to respond to stimuli that would normally elicit a response" [436].	Individual	Negative	Browning [437] and relevant for all five species

2.2.2. Hunger and Appetite

Hunger can be termed as "the drive to consume" (Beaulieu & Blundell, 2021) and appetite can be termed as "food intake, selection, motivation and preference " (Blundell et al., 2010). If appetite drops or is lost, it can be associated with, e.g., poor health (Damsgård et al., 2004), poor water quality (Thetmeyer et al., 1999) or stress (Höglund et al., 2022). However, fish may choose not to eat because they are already full or have recently eaten (Noble et al., 2020). Low appetite may also simply be due to low water temperature or maturation (Huntingford et al., 2006; Jobling et al., 2012). So, whilst it has excellent utility as a WI, these factors should be paid close attention to. Appetite primarily affects welfare needs related to nutrition, the physical environment, health and behavioural interactions.

2.2.3. Scales or blood in the water

Fish scales can be associated with aiding, e.g., bodily defence, biofouling prevention and flow management as the fish moves through the water, see e.g., (Wainwright & Lauder, 2017). Scales can be lost if the fish are handled (Conte, 2004; Ellis et al., 2002) and can be observed in or around the rearing system or operation (Noble et al., 2018). Observations of free-floating scales should be considered a group-level WI if the individual fish or fishes that are the source of scales cannot be identified. Fish can bleed from the gills if they have, e.g., been subjected to mechanical trauma (Gismervik et al., 2019; Poppe, 1999) or have health problems (Currie et al., 2022). Observations of blood in the water can therefore be an indicator of these problems, but as with scales in the water, it is considered a group-level WI. Scales or blood in the water are primarily linked to welfare needs related to the physical environment, health and behavioural interactions.

2.2.4. Health

Health is a key welfare domain in the five domains model (Mellor et al., 2020). It can be defined as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (World Health Organization, 1946). Its utility as a WI has been thoroughly addressed by Segner et al., (Segner et al., 2012) and they highlight its impact on resilience, immunocompetence, and homeostasis, among others. Health status is primarily linked to welfare needs related to nutrition, physical environment, health and behavioural interactions.

2.2.5. Mortality

Mortality has some utility as a WI when comparing differing, e.g., production systems (Noble et al., 2018) or operations (Bui et al., 2022). However, there are some caveats and in the Noble et al., (Noble et al., In press) article, we state "Although it is relatively straightforward to use mortality as a welfare indicator comparing the outcome for two groups, the fact that experiments often are conducted with relatively few fish can create artefacts." Mortality and its cause (if possible) should be monitored and recorded throughout the whole experiment (Bui et al., 2022). It is very challenging to set thresholds in relation to what mortality levels are high, normal, or low mortality; to do so, one would potentially require extensive datasets from previous species- and life-stage specific generations, such as sources utilising industry data (Soares et al., 2011). Mortality data can also be reported as percentage survival (J. F. Taylor et al., 2011). Mortality is primarily linked to welfare needs related to nutrition, the physical environment, health and behavioural interactions.

2.3. Outcome-based WIs at the Individual Level

This next section is a summary and reproduction of the information contained in our recent review article, adapted and reproduced with permission from (Noble et al., In press) and we direct the reader to that article for more in-depth information on each indicator and its application and interpretation.

Scoring schemes for manually measuring morphological outcome-based WIs at the individual level allow rapid evaluations of the indicator in question. A widely used injury scoring system in aquaculture is the FISHWELL scoring scheme (Noble et al., 2018), which categorises different morphological welfare indicators into a 0-3 scoring scheme. This scheme was updated and replaced by the LAKSVEL scoring scheme, which has a more extensive picture and text-based guide on how to score each injury or morphological trait at each of the four 0-3 levels (Nilsson et al., 2022). However, this granularity may not be sufficient for scientific studies, and a stepwise approach with adapted granularity may be needed. We therefore propose a secondary-level scoring for the LAKSVEL scoring scheme as a case study on how scoring schemes can be refined and applied in aquaculture research settings, see (Noble et al., In press) for more details:

- Scale loss, haemorrhaging and wounds: audited on each side of the fish in addition to dorsal or ventral of the lateral line and/or posterior/anterior to the dorsal fin.
- Jaw deformities and injuries: scored on the upper and lower jaw separately.
- Eye damage (exophthalmos, cataract, keratitis and haemorrhaging): scored on each eye separately.
- Opercular erosion or haemorrhaging: scored on each operculum separately.
- Gill injuries or paleness: scored on each gill separately.
- Fin damage: scored on each of the dorsal, adipose, caudal, anal, pelvic and pectoral fins separately. Paired fins scored separately.
- Fin damage: further categorised as healed or active in the form of splitting, erosion, haemorrhaging (see Noble et al., 2018 and references therein).

2.3.1. Gill status

The gills are vital for i) gas exchange, ii) osmoregulation, iii) acid–base balance, iv) ammonia excretion, and v) immunity, amongst other factors (Evans et al., 2005; Olson, 1991). They are also covered by mucus, which has roles related to defence and behaviour (Reverter et al., 2018).

The gills are affected by numerous gill diseases and disorders, and there are currently seven distinguishable types of gill disease, including but not limited to amoebic gill disease (AGD), parasitic gill disease, viral gill disease, bacterial gill disease, zooplankton (cnidarian nematocyst)-associated gill disease, and others, listed in (Boerlage et al., 2020). Poor water quality can also negatively affect gill form, morphology and function (Lazado et al., 2021; Stiller et al., 2020).

Gill status can be evaluated macroscopically as an OWI or microscopically as a LABWI. OWI gill scoring is generally straightforward, but as with other manual scoring, experience and/or diligence are needed. It is therefore preferable that a single or small group of trained observers score it throughout an experiment to limit inter-observer variability in scoring. Gill status primarily affects welfare needs related to the physical environment, health and behavioural interactions.

2.3.2. Opercular deformities

The opercular plate covers the gills and is used to seal the opercular and buccal cavities (Noble et al., In press). Deformities to this plate have been noted for all species covered in this report (Abdel et al., 2004;

Andrades et al., 1996; Beraldo et al., 2003; Blaker & Ellis, 2022; Kužir et al., 2015; Noble et al., 2018; Prestinicola et al., 2013), and include partial or complete opercular shortening or erosion, inward or outward folding of the plate, and uni- or bilateral damage. Drivers for these deformities can be genetic (Negrín-Báez et al., 2015), nutritional (Mazurais et al., 2009), poor husbandry or poor water quality (Lindesjöö et al., 1994), high water velocity at early life stages (Beraldo et al., 2003; Koumoundouros et al., 1997) or even behavioural (Blaker & Ellis, 2022).

A deformed operculum does not close properly (Blaker & Ellis, 2022), and if the deformity involves curling inwards, it can interfere with gill function and damage the gills (Beraldo et al., 2003; Koumoundouros et al., 1997). Exposing gills may also increase pathogenic infection risks (Beraldo et al., 2003). Opercular deformities primarily affect welfare needs related to nutrition, the physical environment and health.

2.3.3. Skin damage

The skin protects the fish from its external environment, as reviewed in (Sveen et al., 2020) and the skin and its mucus play a role in sensing the surrounding environment, movement and homeostasis (Groff, 2001), 2001). It can heal and regenerate (Richardson et al., 2016; Sveen et al., 2016), but severe skin damage can be lethal. Wound healing ability is affected by temperature, nutrition stress and other factors such as wound severity (Jensen et al., 2015; Sveen et al., 2018).

Wounds to the skin can be due to mechanical trauma or caused by ulcer-induced diseases (Groff, 2001), and they can be deep or superficial, as reviewed in (Sveen et al., 2020). Wounds and scale loss can be easily detected and monitored as an OWI, but micro-damage, such as e.g., missing epidermis, must be diagnosed using LABWIs (Karlsen et al., 2018). Skin damage primarily affects welfare needs related to the physical environment, health and behavioural interactions.

2.3.4. Fin damage

Fish have rayed median fins, e.g., the dorsal or caudal fins and rayed paired fins, e.g., the pectoral and pelvic fins (Lauder & Madden, 2007). Each fin may have specific functions related to the control of, e.g., propulsion and manoeuvring (Lauder & Madden, 2007) and fins can possess nociceptors and mechanoreceptors (Koll et al., 2019; Roques et al., 2010).

Fin damage can affect fin function (Noble et al., 2012) and can be a bridgehead for pathogenic infiltration and infection (Loch & Faisal, 2015). Damage can be classified as erosion, thickening, splitting or haemorrhaging (Noble et al., 2012, 2018; J. F. Turnbull et al., 1996), both in isolation or in tandem. It primarily affects welfare needs related to the physical environment, health and behavioural interactions.

2.3.5. Snout/jaw damage

In the five fish species addressed in this report, the snout includes the mouth, jaws, nasal pit, and lateral line, which detects water movement and aids in behaviour (Coombs & Van Netten, 2005; European Food Safety Authority (EFSA), 2008a). Below the nostrils, olfactory rosettes connect to the central nervous system and guide behaviours like mating, feeding, and predator avoidance, while also playing a role in nasal immunity (Das & Salinas, 2020; Lazado et al., 2023; Whitlock & Palominos, 2022). The mouth, with jaws, tongue, and taste buds, is used for feeding and respiration, and its morphology varies by species and life stage (Abbate et al., 2020; Elgendy et al., 2016; Levanti et al., 2017; Noble et al., 2012).

Snout damage can occur in both aquaculture (Weirup et al., 2022) and aquaculture research (Moltumyr et al., 2022), and injuries can affect jaws, the region around the nasal pits, or spread outside these areas

(Nilsson et al., 2022). Mechanical trauma from handling, abrasion with rearing materials or equipment, and collisions with conspecifics can cause snout damage (Noble et al., 2020; Weirup et al., 2022), as can opportunistic bacterial pathogens such as *Tenacibaculum* spp. (Spilsberg et al., 2022).

Snout, nasal, mouth and jaw damage is a welfare issue, since (i) the area may have abundant nociceptors (Sneddon et al., 2003), (ii) it penetrates the skin (Spilsberg et al., 2022) causing osmoregulatory problems, pathogenic infiltrations or damage (Noble et al., 2012; Southgate, 2008), and (iii) can negatively affect how the fish captures and consumes feed (Branson & Turnbull, 2008). Snout/jaw damage primarily affects welfare needs related to nutrition, health and behavioural interactions.

2.3.6. Eye damage

Fish eyes are highly diverse in both form and function, reflecting their ability to adapt to a wide range of ecological niches. Comparative studies have revealed variations in eye size and structure among different fish species, including both diurnal and nocturnal forms, demonstrating how habitat and activity patterns can influence eye morphology (Moran et al., 2015; Pankhurst, 1989).

Eye damage can take many forms including bleeding in and around the cornea due to, e.g., parasites, mechanical/thermal damage or impact trauma (Karlsbakk et al., 2002; Overton et al., 2019). Opaque lenses, or cataracts, are seen in many aquacultural species (Bjerkås et al., 2000) and can be both short- or long-term challenges for the fish, depending on the cause (Noble et al., 2020). Eye damage can also lead to eye bulging, commonly referred to as pop-eye, or it can manifest as a sunken eye (Adamek et al., 2017; Hargis, 1991). There can be many causes of pop-eye, including viruses, parasites and gas bubble disease (Jones et al., 2023; Olsen et al., 2015) and sunken eye can be caused by, e.g., viruses (Adamek et al., 2017). Damage to the eye can cause blindness, secondary infections, and may be painful (Ashley et al., 2006; Neves & Brown, 2015; Pettersen et al., 2014). Eye damage primarily affects welfare needs related to nutrition, health and behavioural interactions.

2.3.7. Condition factor

In our recent review article (Noble et al., In press), we state "condition factor (K) is a morphometric index for evaluating length-weight relationships in fish and is calculated using the formula $K = 100 \cdot \text{weight} \cdot (\text{length}^3)^{-1}$. It is a well-established instrument for documenting changes in the nutritional status of animals (Nash et al., 2006), as it is generally assumed that if fish are identical in length, a heavier fish has more energy reserves than a lighter one and is in better condition (Bolger & Connolly, 1989). However, there are exceptions to this assumption as some studies have found no clear relationship between condition factor and lipid reserves in certain species or life stages".

Hence, a low condition factor may be indicative of malnutrition or lack of feed access, dietary deficiencies, poor water quality or poor health status (Dimitroglou et al., 2010; Hvas et al., 2022; Noble et al., 2008; Shin et al., 2018; Thetmeyer et al., 1999). However, an elevated condition factor may also be indicative of certain health conditions, such as the presence of vertebral deformities in species like Atlantic salmon (Hansen et al., 2010). Therefore, the body condition factor can be a reflection of the fulfilment of numerous welfare needs related to nutrition, the physical environment and health.

However, it is challenging to provide a threshold for what exactly is good or poor welfare in relation to the condition factor (Noble et al., 2018). Some sources state that condition factors of < 0.9 in Atlantic salmon, < 1.0 in rainbow trout, < 0.9 in European sea bass, and < 1.4 in gilthead sea bream (Bavčević et al., 2010; Folkedal et al., 2016; Noble et al., 2020; Stien et al., 2013; Yavuzcan Yildiz et al., 2021) can be indicative that the fish is emaciated. Regarding common carp, we could not find sources that specifically state condition factors indicative of good or poor welfare, possibly due to its several variants.

2.4. Other OWIs

2.4.1. Internal WIs for euthanised fish

In our recent review article, we state that "The health status of fish's internal organs is central to their health and welfare status (Tschirren et al., 2021 and references therein). Some authors have stated that all fish organs should be visually inspected for severe inflammation as a primary health and welfare auditing tool in experimental settings, before progressing to histological examination, where this is feasible, appropriate or where a more in-depth audit is needed (Johansen et al., 2006). Organs that can be potentially of interest, if the fish are being euthanised, include, but are not limited to, the heart (Johansen et al., 2006), liver (Mørkøre et al., 2020), spleen, kidney, stomach and intestines (e.g. Tschirren et al., 2021) or visceral fat levels around the pyloric caeca (Mørkøre et al., 2020). An audit of the buccal cavity can also provide an overview of any potential internal bleeding (e.g. Tschirren et al., 2021)."

Heart morphology: certain heart shapes have been associated with swift growth rates due to rearing temperatures in Atlantic salmon smolts, where differences in heart size and bulbus misalignment have also been observed (Frisk et al., 2020). A wide-ranging catalogue of different salmon heart shapes has recently been developed (Engdal et al., 2024).

The fish liver is key for metabolism, detoxification and immunity (Bruslé & Gonzàlez I Anadon, 2017; Taylor et al., 2022), and can be scored in relation to its, e.g., colour and shape. Liver colour can be explained by several factors, such as nutrition, genetics, or disease (Dessen et al., 2017; Thorud & Djupvik, 1988; Woo et al., 2002). Pale livers can have a higher fat content than dark livers in Atlantic salmon (Dessen et al., 2021) and fat accumulation may impair liver function and health.

Visceral fat serves as a fat repository for the fish, and their occurrence and severity can be scored in relation to how visible the pyloric caeca is (Dessen et al., 2017; Mørkøre et al., 2020). Fat deposition is affected by, e.g., diet and season (Bou et al., 2017; Rørvik et al., 2018).

2.4.2. Faecal consistency

Fish faecal collection and examination have been proposed as a WI by earlier authors (Johansen et al., 2006) and samples are often collected by stripping individual fish (Reid et al., 2024). This procedure may be stressful for the individual involved (Johansen et al., 2006; Stone et al., 2008) so should be conducted on euthanised/anaesthetised fish. Faecal samples can also be collected in certain rearing systems (Schumann et al., 2017). Poor faecal texture and stability can mean the fish have either voluntarily or involuntarily fasted for extended periods, which can be indicative of health and welfare problems (Reid et al., 2024; Zarkasi et al., 2016). Loose faecal consistency may also indicate health and welfare problems, often linked to inappropriate feed, causing gastrointestinal or osmotic issues (Olsen et al., 2006; Seibel et al., 2022). There are faecal scoring schemes available (Zarkasi et al., 2016) and this increases its potential utility as a health and welfare auditing tool.

3. Summary and conclusions

The welfare of fish used for scientific purposes in Europe is protected under Directive 2010/63/EU and its amendment Commission Delegated Directive (EU) 2024/1262 (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes., 2010; European Commission., 2024), with the needs of fish specifically addressed under Annex III of the Directive. Because these fish may undergo stressful procedures, and welfare indicators help assess and improve their condition, an operational welfare indicator toolbox that goes beyond the Directive is necessary. In this report, which is a brief summary of our recently published review article (see Noble et al., press), we therefore extend the range and scope of OWIs that should be considered in a welfare audit of fish used in scientific procedures, focusing especially on outcome-based welfare indicators at both the group and individual level. The WI toolbox also contains information that a user can use in relation to species- and life-stage-specific needs. It outlines a wider range of input-based OWIs than those covered in the directive, and also different behaviours to pay attention to when measuring and monitoring behaviour in different research settings. It also outlines a range of morphological OWIs to consider in a welfare audit, and ways to measure these in a simple and rapid manner.

As stated throughout this report, we strongly direct the reader to our review article, which was the outcome of this deliverable and formed the basis of this brief summary report (Noble et al., In press). The review article includes a wider range of LABWIs than we cover in this OWI report, outlines various technologies for streamlining WI monitoring both in relation to the fish and their rearing environment. It also contains case studies on how WIs can be used in applying humane endpoints.

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