Using ethology to improve farmed fish welfare and production

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EXECUTIVE SUMMARY

The welfare of farmed fish is a topic that is gaining exponential attention in research, public awareness, production and policy, since the impacts on the animals are reflected the whole sector. To address it correctly it is important to define the terms: welfare is the state of the animal as it copes with its environment. This definition enables the measurement of welfare through different indicators, which in turn allow the testing, validation and implementation of measures to improve the welfare and production of farmed fishes. The focus of this report is on the behavioural indicators of welfare, which represent the state of the animal very accurately. To fully understand and interpret the behaviours of farmed fish, we propose an ethological approach by looking at behaviours under the 4 questions of ethology: causation, development, function and evolution. The first two questions (causation and development) tell *how* the behaviours are occurring, while the last two questions (function and evolution) explain *why* the behaviours are occurring. We also propose that the general reference point to assess the welfare of fish under farming conditions should be biology in the wild, always taking into account that there may be changes due to the domestication process of selected strains. Using the ethological approach allows the understanding of these processes. The indicators of welfare that are suitable to be used on farms are called Operational Welfare Indicators (OWIs). These can be variables measured on the animal (e.g. behaviours, skin condition, injuries), measure on the water (e.g. oxygen, ph, temperature) or measured through the management procedures (e.g. cleaning, culling, feeding). Behavioural indicators comply with all requirements to become OWIs: they are valid, reliable, repeatable, comparable, suitable and practical. To be used to their full potential, behavioural OWIs should interpreted using the ethological approach.

Here we present the summaries of welfare profiles of the five most cultivated species in the EU: seabass, seabream, salmon, trout and carp. The summaries are based on the information detailed on the FishEthoBase at http://FishEthoBase.net. We compare information from the wild with data from fish farming and identify the points where welfare can be improved in these species. We also identify the main challenges that fish face when in captivity: environmental (water quality, light, temperature, complexity), ethological (space, density, reproduction), physiological (pain, diseases, parasites, stress), and human-induced (stunning, slaughtering, handling, transport). Based on these challenges and the information on the species, we propose detailed actions to improve the welfare of these fish. These are divided into two main aspects: improvements *during the life in captivity of the fish*, namely through development and application of OWIs, improvements in handling procedures, transport protocols and environmental enrichment; and *at slaughter*, namely through the implementation of humane stunning protocols prior to slaughter. To integrate and expedite these actions, we propose a framework to gather and transfer knowledge, namely through the creation of training courses in several levels as well as the creation of European Reference Centre of Fish Welfare. Finally, we propose European priorities for research regarding welfare and production of farmed fish, and priorities for funding. The research priorities are: filling knowledge gaps about natural behaviour of farmed species that may be informative for their welfare in farms, developing species-specific OWIs and environmental enrichment strategies (and adapting them for each farming system), develop technological tools to monitor farmed fish (i.e. precision fish farming), advance on the study of consciousness indicators for humane stunning and slaughter, and detail the link between good welfare and good quality in fish. The funding priorities are: enable the research on the previous points, validate and assist the implementation of welfare improvements (including financial assistance for companies regarding equipment for humane stunning), fund training programs and create awards (or rewards) for good welfare practices in the industry.

The fishes are the centre of network of stakeholders in fish farming. Improving the welfare of the animals will positively affect the fish themselves and improve the state of the whole sector.

1- STATE OF THE ART IN FISH WELFARE

1.1- Operational definition of welfare and applications to fish farming.

Fish welfare is a hot topic in the aquatic food sector nowadays. However, the term 'welfare' is prone to different understandings, often depending on the person, the context and the type of contact with fish. In fact, it may represent very different things to a fish farmer, a biologist, a policy maker or a consumer. Only by clearly defining what welfare is can it be properly assessed, monitored and eventually incorporated into laws and regulations (Broom, 1991). Among the many definitions of welfare that have been debated over (see for example Carenzi and Verga, 2009; Hewson, 2003), the one proposed by Broom (1991, 1986) best fulfils the premises of clarity and objectiveness and can therefore be operationalised (i.e. put into practice). According to this definition, welfare is **the state of the animal as it copes with the environment**. This definition of welfare has important implications: (i) welfare is a characteristic of an animal, not something that is given to it; (ii) welfare will vary along a continuum, from negative to positive; (iii) welfare can be measured independently of ethical considerations; (iv) measures of difficulty in coping with the environment give information about the welfare of the animal concerned; (v) direct measurements of the state of the animal must also be used to assess its welfare, over and above knowledge of its biology; and (vi) coping mechanisms may vary among different species, and there are several consequences of failure to cope.

Three distinct approaches are used when addressing animal welfare (Fraser, 2009, 1999; Huntingford et al., 2006). A **feelings-based approach** requires that to be in a state of good welfare the animal should be free from negative experiences and have access to positive ones. This approach works under the assumption that fish are sentient animals, capable of feelings, emotions or equivalent affective or mental states. A **function-based approach** requires for good welfare that an animal can adapt effectively to its environment, such that all its biological functions are working effectively. Lastly, a **nature-based approach** assumes that each species has an inherent biological nature and that the ability to express it (particularly to express a natural repertoire of behaviour) is essential for good welfare. Applying each of these approaches separately has led to important improvements in animal welfare (Fraser, 2009). However, suffering, health problems and impairment of natural behaviour often accompany each other. An integrated, multi-disciplinary ethological approach could promote the objective measurements of welfare (Saraiva et al., 2018) – see section 1.2.

The original paradigm welfare in farmed animals relied largely on the concept of the Five Freedoms (freedom from thirst and hunger, freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behaviour and freedom from fear and distress), which was coined in 1965 in the Brambell Report in the UK (Brambell, 1965) on husbandry of livestock, and revised in 1979 into its present form. Importantly it paved the way for animals – including fish - to be considered by European law as sentient beings, in the Lisbon Treaty of 2007 (European Union, 2007). Even so, the approach is open to criticism: it implies that captive animals are passive within their environment (Ohl and van der Staay, 2012) when it is clear that they are not. In addition, understandably at the time, the emphasis was very much on protecting animals from negative experiences, epitomising the view that 'free from harm equals good'. In fact, the idea that animals can experience positive states that culture conditions should accommodate has historically been underrepresented in current frameworks and, to an extent, in current welfare research. A step in the direction of acknowledging positive welfare states was the rationale around the Five Domains (nutrition, environment, health, behaviour and mental state) which looks into the five freedoms in a gradient within each one, from negative to positive. This proposal then extends into the concept of Quality of Life, where captive animals experience a "life worth living" once their welfare needs are met, and may even have a "good life" if they experience welfare above the minimum requirements (Mellor, 2016; Mellor et al., 2020). These concepts, although appealing, still contain a rather passive view of the animals towards their environment and introduce subjective terms into their construction, rendering the evaluation of welfare even more complicated. A more dynamic perspective on welfare, which in some senses overrides such complications, is the allostasis concept (Korte et al., 2007), according to which challenges in the environment within certain limits (allostatic loads) may be beneficial to an animal, because they represent stimulation. Too much stimulation represents a load that the animal fails to cope with, but too little stimulation may also be harmful (Fig. 1). In addition, the allostasis concept also sets a theoretical framework for environmental enrichment. This view integrates well with the definition of welfare by Broom, which puts welfare along a continuum and predicts that what is now called positive welfare may arise as a result of proper environmental stimulation.

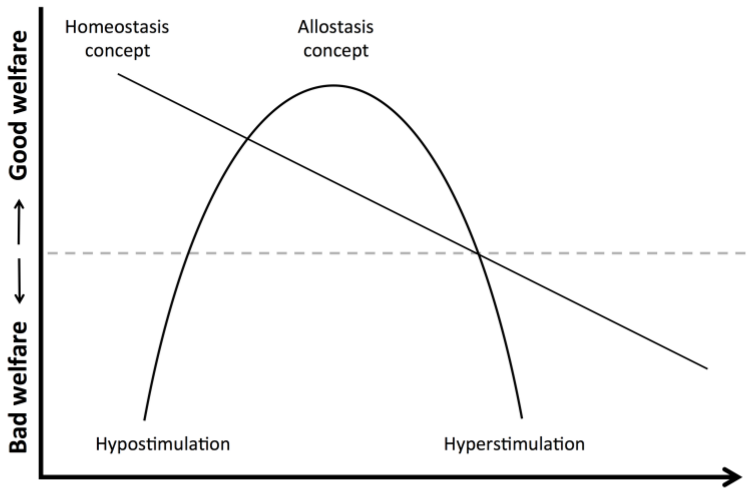

Figure 1 - Animal welfare in relation to environmental challenges as shown by the out-dated concept based on homeostasis and the new concept based on allostasis. Adapted from Saraiva et al. (2018) and Korte et al. (2007).

The view that animals may sometimes do things 'because it feels good' (rather than seeking specific goals such as food or safety) and that they experience mental and physical states that exceed what is strictly necessary for short-term survival (aka positive welfare) has been discussed by many authors (e.g. Lawrence et al., 2019; Yeates and Main, 2008). The application of positive welfare to fish was proposed by Franks and Fife-Cook (2019), based on the assumption that fish (as other vertebrates) are naturally equipped to seek positive affective states. According to this view, knowledge of their biology and behaviour should allow the identification of such positive affective states. Examples of behaviour that fish may perform because it feels good include preferential attachment, aka 'friendship' (Heathcote et al., 2017), social buffering of fear aka 'seeking comfort' (Faustino et al., 2017), social motivation (Galhardo et al., 2011; Maia et al., 2017), or free-choice exploration (Graham et al., 2018).

The concept of positive welfare, and the feeling-based approach (see text above for a definition) applied to fish welfare generally, are based on the assumption that fish are sentient animals, otherwise they would not have feelings or emotions, positive or negative. Clearly, in the case of positive emotional states, tight operational definitions are needed to develop this approach to fish welfare (Franks et al., 2018). In fact, there is accumulating evidence of some commonality in neuroendocrine and cognitive correlates of affective states in fish of several species and mammals. For example, Gilthead seabream exhibit different behavioural, physiological and neuromolecular states that are specific to the core emotional states recognised in humans (Cerqueira et al., 2017). Given that fish have general neural systems that are similar to those that modulate the feeling of well-being and seeking positive reinforcement in mammalian brains (e.g., the serotonergic and dopaminergic systems), it is plausible to expect some capacity for positive feelings in fish (Braithwaite et al., 2013; Fife-Cook and Franks, 2019). In fact, the use of the indicators we propose below is well based in a functional approach.

Special features regarding fish welfare

While sentience is a common trait shared with land farm animals, fishes have some special traits that have implications for welfare, for example: many species have complex life histories, with marked changes in habitat and form at critical points, meaning that optimal conditions for culture vary with life stages within a single species; although mammals, birds and fish are all intimately exposed to their environment through a large respiratory surface, in fish there is no buffering prior to contact with the gills, so that health and welfare are particularly compromised by poor water quality; and the fact that many fish naturally spend their lives in dense schools means that, up to a point, culture of such species at high density may not be necessarily detrimental, if combined with good water quality and good management practices (Saraiva et al., 2022b).

1.2- The ethological approach: addressing fish welfare through the 4 questions of ethology (evolution, adaptation, function, mechanisms).

Behaviour is the first and foremost indicator of the biological state of an animal, and behavioural observations are the best tool to understand not only the physiological state of the individual but also its mental state (Cerqueira et al., 2017; Dawkins, 2008; Martins et al., 2012). Therefore, the knowledge on the ethology of farmed fish species is fundamental for the correct evaluation of their welfare (Saraiva et al., 2019).

The 4 questions of ethology (function, causation, development and evolution) represent 4 non-exclusive perspectives to study behaviour (Bateson and Laland, 2013; Tinbergen, 1963). Using an ethological approach we can decipher the behaviour of fish in farms, understand what the animals are experiencing and what physiological mechanisms are in action. More importantly, we can understand why those behaviours are occurring, and act accordingly. For example, asking whether animals suffer if deprived of the opportunity to perform natural behaviour might require an understanding of how behaviour is triggered and controlled, the effects of early experience and genetics, the behavioural and hormonal effects of deprivation, a knowledge of how that species behaves in the wild, its brain activity and probably more. A simple 'applied' question about the welfare of a fish in a cage could therefore lead to pure research questions in several different disciplines and the ethological approach allows the integration of the possible links between them, whereas narrower perspectives would miss them (Dawkins, 2008). In summary, the ethological approach towards welfare provides an integrative perspective on the state of the animal as it displays its behaviours. The use of behaviours as indicators of welfare is described in detail in section 1.3 In fact, the analysis of behaviour allows the answering of two important questions when it comes to welfare: 1- are the animals healthy and 2 – do they have what they want (Broom, 2010; Dawkins, 2004, 2003). It is important to highlight that the evaluation of welfare using behavioural observations should be accompanied by other indicators (Broom, 2010; Saraiva et al., 2018) and must rely on solid knowledge on the biology of the animal and a clear reference point, which we propose to be behaviour in the wild (see section 1.4).

The assessment of the welfare state of farmed fish must rely on a clear operational definition of welfare such as the one provided in 1.1, and on robust indicators that are able to measure unambiguously the variables they are assumed to be measuring. Optimal welfare indicators should take into account not only the health of the fish (i.e., a function-based approach to welfare; Segner et al., 2012) but also reflect the animals' emotional state (i.e., a feelings-based approach; Duncan, 2004) as well as their biological needs (i.e. a nature-based approach; Ashley, 2007; Saraiva et al., 2019; Sneddon, 2007). These indicators, and the essential role they play in this story, will be discussed in the next section.

1.3- Operational welfare indicators, their use, application and meaning in the "real world" of EU aquaculture. The case for behaviour as a potential key indicator.

Although fish behaviour and communication abilities are highly developed, they cannot express themselves in words and therefore it is impossible to directly ask how they are coping with their environment. To assess their welfare, we must use **welfare indicators** to extract information about their welfare state. These indicators should be:

i) valid: their result should be directly related to the welfare need or aspect they are assumed to be measuring;

ii) reliable: their result should be the same independently of who measures it and how the measure is taken;

iii) repeatable: the measurement should be possible to be taken multiple times, while providing a consistent result;

iv) comparable: the result should allow comparisons between contexts;

v) suitable: the measurement method should be adequate for the specific rearing systems or husbandry routines.

Welfare indicators may rely on observations made:

- on the animals themselves (animal-based or group-based)
- on the aquatic environment they are reared in (resource-based)
- or on the routines and protocols performed on-site (management-based).

These three types of data source provide complementary information about the welfare state of the fish. Indicators observed on the animals are also called *Direct* or *Output* indicators, while the other two types are also called *Indirect* indicators.

Indicators that comply with all the previous assumptions and, in addition, are designed to be used on farm - and therefore prone to be easily taken up by the industry - are called Operational Welfare indicators, or OWIs) (Noble et al., 2018). These OWIs contrast with other kinds of indicators that, reliable and sharp as they may be, require time consuming, expensive, and/or complex analysis, usually away from the farm context. Behavioural variables may fulfil all the previous premises (Table I), provided that the collection, storage and accessibility of data is performed correctly, and its interpretation is made by trained staff. Although it may seem as a big ask, the benefits of an ethological approach towards welfare in fish farming are large.

Table I – Rationale for the use of behaviour as OWIs

Assumption	Reason for fulfilment	Requirements
Validity	Behaviours are hard-wired, often species-specific, biological responses to external and internal stimuli (i.e. animals cannot fake them).	Observers must be trained to interpret species-specific behaviours.
Reliability	Farming stimuli evoke consistent behavioural responses to animals.	Observers must be trained to interpret behavioural responses to stimuli.
Repeatability	Animals respond with the same behaviour to the same stimulus.	Observers must be trained to interpret behavioural responses to stimuli.
Comparability	Behavioural categories are species-specific across animals and populations. Major types of behaviours are similar across taxa.	Observers must be trained to interpret species-specific behaviours.
Suitability	Most fish farms have the conditions to use at least some behaviours as indicators	Observers must have visual access to the fish, either directly or indirectly, live or recorded (see text).
Practicality	Behaviours are cheap and easy to observe, do not require labs or expensive behaviour for basic data acquisition	Observers need visual access to fish. Quality of data increases with quality of images.

It should be noted that behavioural OWIs are not perfect and may fail to address more specific aspects of poor welfare (e.g. disease types and causes, or particular management stressors). Therefore, although the scope of this report is focused on behaviour, it is of utmost importance that other types of indicators are combined when assessing the welfare of farmed fish. In addition, there may be disadvantages of behavioural welfare indicators: they are often variable over time and difficult to quantify, the observers need the sufficient skills to observe the animals' 'body language', and there can be large individual differences in behaviour within animal groups. It is also worth noting that some behavioural responses can be considered normal coping activities and therefore contributing to welfare, whereas other responses may be considered abnormal or maladaptive, therefore hampering welfare. The differences between normal (i.e. part of an adaptive response) and abnormal behaviour are frequently unclear (Martins et al., 2012). It is therefore imperative that the establishment and interpretation of behavioural indicators should be made by trained personnel on the ethology of the target species.

Nonetheless, behavioural observations were probably the first indicators of health and welfare to be used in animal production, and they still are (Broom, 2010; Dawkins, 2004, 2003) including in fish (Martins et al., 2012; Noble et al., 2020, 2018). Behavioral observations are cheap, accessible and offer direct indications on the state of the animal that may be observed on site and in real time. The evidence that supports the adequacy of behavioural observations as welfare indicators when joined by deep ethological knowledge of the species has been in fact mounting in recent years.

There are general behavioural patterns associated with poor welfare states (including diseases, infections, fear, pain or negative cognitive states) that are transversal to several taxa (Kent et al., 1992; Sneddon, 2020; Sneddon et al., 2014). The neural networks underpinning these behaviours have even been recently identified (Ilanges et al., 2022). The use of behavioural variables as operational indicators of negative welfare is therefore increasingly rooted on solid neurophysiological evidence, which provides ever growing reliability for their use in industry context. Although far less is known for positive welfare states and while much attention has been given to recognising negative states (perhaps as a legacy of the Five Freedom concept), we believe that positive welfare states are a goal worth pursuing and therefore should be able to be identified and assessed.

In fish, the major types of behavioural indicators of welfare are explored in section 3.1

One essential aspect that should always be present is that different species may have different manifestations of each behavioural OWI. In other words, behavioural OWIs should always be considered in a species-specific manner when performing detailed analyses. That is in fact the purpose of the ethological approach, as explained above in section 1.2.

1.4- Domestication, plasticity and selection of farmed fish species: why biology in the wild is still a benchmark for welfare needs of farmed fish.

Domestication is a human-induced process that gradually changes a cultured organism. It extends over generations and involves developmental effects within each generation, culminating generally in genetic changes across generations. Land farm animals have been under the domestication process for millennia (Zeder, 2012). To put things in perspective, fish domestication is a much more recent process: while exceptions such as carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*) and goldfish (*Carassius auratus*) may have possibly been artificially selected for hundreds of years, and a few species such as cod (*Gadus morhua*), salmon (*Salmo salar*) and trout (*Oncorhynchus mykiss*) since the 1800s (Duarte et al., 2007), the vast majority of farmed fish has been under domestication merely since the middle of the twentieth century (Balon, 2004; El-Sayed, 2006; Teletchea, 2015; Teletchea and Fontaine, 2012). Although the gap between the time domestication has been underway in terrestrial farm animals and in farmed fish is enormous, there has been a considerable effort towards fish domestication in recent times (Duarte et al., 2007). However, the main component in the domestication process is the generation interval (i.e. the average age of the parent animals at the birth of their offspring—note that this is *not* the age at maturity). In farmed fish, this can vary from 6 to 8 months in tilapia(Eknath et al., 1998), 3–4 years in salmon and trout (Gjedrem, 2000), 4–6 years in sea bass and sea bream (Haffray et al., 2007; Janssen et al., 2015) and eventually from 12 to 33 years in some species of sturgeon (Harkness and Dymond, 1961).

Fish are highly susceptible to artificial selection pressures. It is known that fish farmed under well-managed systems (i.e. providing conditions that enhance growth and survival while supplying the correct nutrition regimes for the species) can maximise growth to nearly their physiological maximum, suffer lower mortality rates than in the wild and are usually less prone to infectious diseases (Lorenzen et al., 2012). For example, fast growing Atlantic salmon (*Salmo salar*) is the result of 40 years of research and artificial selection for fast growing progeny, with an increase of 10–15% in each generation (Gjedrem, 2005). Yet even those 40 years account for solely 10-15 generations of selected salmon, according to its generation interval. This is over hundred-fold behind the domestication efforts of land animals. Considering this, determining whether and how this process affects fish welfare is not a straightforward task. In fact, most artificial selection goals are related to production traits that, by a series of factors, may influence welfare in unknown ways. Even the simple fact of being born under farming conditions (whatever they may be) will evoke differential survival of larvae and the emergence of certain typical phenotypes, usually bolder and more aggressive (Huntingford, 2004). The implications of the domestication process on the behavioural perspective of welfare are therefore far from simple. Behavioural changes due to generations in captivity do seem to occur but (1) they are accompanied by physiological and cognitive modifications that are challenging to accommodate in good welfare, and (2) while the behavioural phenotypes of wild fish are adaptive and selected throughout stable evolutionary pressures, captive phenotypes are responding to extremely different settings that are artificially rapid and that can often push welfare needs into collision with traits required for production (see Saraiva et al, 2018 for a review).

In summary, the selection efforts of farmed fish are mostly related with production traits, yet all farmed fish species are still in early stages of domestication. Therefore, regardless of the species, strain and the domestication stage, *all farmed fish may be very similar to their wild counterparts in their essential functions and needs*, and the possible differences are in range but not in type. That is why we propose that biology in the wild is still the best possible benchmark for major species-specific welfare indicators. The existence of such a benchmark is actually very good for fish welfare science because it gives us a point of reference, general as it might be. While we cannot turn to wolves to assess the welfare of a poodle, we still can turn to wild sea bream to compare the welfare of their captive counterparts.

2- FISH FARMING IN THE EU: CASE STUDIES

2.1-Review of scientific literature on the wild ethology and welfare needs of key fish species farmed in the EU: European seabass, Gilthead seabream, Rainbow trout, Common carp and Atlantic salmon.

The welfare of farmed fish may seem understudied and under-represented in academia and practice when compared to land animals (Sánchez-Suárez et al., 2020). There is however quite a comprehensive body of knowledge of the biology of fish species that are used for farming, yet this information requires integration into a framework focused on welfare. On the other hand, many aspects directly related to welfare in aquaculture remain poorly known to science, industry, policy makers and/or the general public. The FishEthoBase aims to tackle both these issues. This open-access database on fish ethology and welfare provides a platform where scientific knowledge is scrutinised and summarised in order to answer relevant criteria regarding welfare in aquatic animal farming. The aim is to cover all fishes farmed nowadays as well as other aquatic species, delivering concrete solutions for fish farmers, pointing to knowledge gaps for researchers and providing awareness for the general public and other stakeholders (Saraiva et al., 2019). To achieve this, a set of core criteria was chosen to portray the variables in fish farming most likely to affect the welfare of fish. Each criterion is divided into the general life stages of the animal, which may generally correspond to rearing stages in the farming environment: Eggs, larvae (hatchery), juveniles (nursery, grow-out), adults (grow-out) and spawners (broodstock). The entries for each life stage then refer to the knowledge in nature (i.e., in the "Wild") and under aquaculture conditions (i.e., in "Farm"), ideally discriminating the various farming methods when literature is available. For each criterion, the existing knowledge on the biology in the wild is, therefore, overlapped with the existing knowledge on farming conditions, which will implicitly allow drawing conclusions on the welfare conditions of the species regarding that criterion.

All entries in the database are referenced. When there is no reliable information on any of the sections described above, a standard sentence 'no data found yet' is entered. When findings are contradictory or insufficient, the entry becomes 'further research needed…' to highlight the existence of knowledge gaps.

The basic rationale for the short profiles is that the catalogue of questions, or criteria, designed to achieve a rapid evaluation of the welfare state of a farmed species should be as short and sharp as possible. We arbitrarily set the cut-off line at 10 critical questions, which should (i) depict the major limitations imposed to the lives of fish under farming conditions and, therefore, directly impact their welfare and (ii) be able to be applied to all farmed species. These criteria were designed to take into account not only the multidimensional nature of welfare (mental, physiological and natural) but also common conceptual guidelines towards animal welfare in practice—namely the five domains and the allostatic model described above. We classified the major types of constraints imposed on fish in any farming method in: restricted space, manipulation and handling, low complexity of the environment, unnatural aggregation of individuals, and slaughter. The selection of the core criteria, therefore, reflects these impositions.

A full paper explaining and exploring the FishEthoBase was published in the journal Fishes (Saraiva et al., 2019). The next sections summarise the welfare profiles detailed in the FishEthoBase https://FishEthoBase.net/

Summary of welfare profile of *Dicentrarchus labrax*

For a complete version of this profile, including further criteria, technical summaries, detailed information, welfare scores and full list of references, please visit: https://FishEthoBase.net/db/14/

European Seabass *(Dicentrarchus labrax)*, a moronid from the Eastern Atlantic and the Mediterranean, is a valuable species for aquaculture, dominating the Mediterranean marine finish culture together with *Sparus aurata*.

Home range: Seabass larvae and fry are planktonic and hence moved by the water current. Seabass juveniles and adults, though, traverse an area of up to several (hundreds of) kilometres. It is unclear whether the provision of safety from predators, feed, mates, and preferred water parameters overrides the need to cover the home range it moves across in the wild. As a precautionary principle, larger enclosures should provide better welfare. In this respect, earthen and seawater ponds with up to 10,000 m2 represent a larger overlap with the wild range than raceways. Cages with diameter of 50 m, though, are also promising alternatives to provide nearly natural home-ranges

Depth range: Similarly to the home range, the depth range in the wild covers needs of food as well as shelter from adverse water parameters or predators. Already larvae and fry of Seabass can be found in up to 15 m depth; juveniles and adults, although generally roaming in 1-3 m, may dive down to 60 m. Just like in home range, it is unclear whether providing the species in captivity with all needs overrides the need for depth. Therefore, sea cages covering a larger part of the wild range than tanks, raceways or ponds are potentially beneficial. Further research is needed to find out whether Seabass prefers deeper facilities over shallower ones. Submerged cages result in lower stress than surface cages.

Migration: As an amphidromous species, Seabass adults live and spawn in the open sea. The fry migrate inshore to benefit from estuaries, lagoons or boardering rivers as nursery grounds. After a couple of years, juveniles migrate back offshore to mature and spawn. Once again, it is not known if providing the different age classes with the conditions of the respective habitat is sufficient to satisfy their needs or whether the individuals have to experience the transition itself. Since migration distances by far exceed home range distances, the need to migrate might be one of the hardest ones to accommodate in captivity. Providing current to give the impression of moving through water requires delicate precision, as too high a current might lead to malformations.

Reproduction: Seabass spawns in winter-spring depending on latitude. Nothing is known about the mating system and courtship rituals in nature. Common reproductive dysfunction in captivity indicates, though, that Seabass is sensitive during the spawning season. Photoperiod, temperature or hormonal manipulation to induce spawning are common practices but natural spawning is possible and even egg quality increased if the spawners are accommodated to their spawning environment for several years and then left alone for spawning. Stripping is a stressful procedure that should be avoided as well.

Aggregation: Seabass in all age classes may congregate in unstructured casual shoals, yet juveniles more often tightly school whereas adults more often live solitarily. Even in the absence of specific densities, laboratory studies suggest that densities in raceways and tanks >15 kg/m^3 may potentially induce stress . Lower stress at stocking densities in cages and ponds needs to be verified in the farming context.

Aggression: Under farming conditions, cannibalism may occur from 30 to 60 days post-hatching, after weaning from live feed. Little or no aggression is reported for juveniles, adults or spawners.

Substrate: Seabass does not build nests but releases eggs in the water which then live pelagic – independent of bottom and shore. Juveniles and adults, on the other hand, may be found in proximity to aquatic vegetation, and in the lab, juveniles readily sought shelter. Earthen ponds inherently provide substrate. In tanks and cages, suspending plant-fibre ropes could result in a more stable social structure and consequently in higher welfare. This application should be validated in full scale commercial context.

Handling and slaughter: transfer between rearing containers, sampling, monitoring, transport to the slaughter system require confinement, crowding, air exposure. All of this is highly stressful for Seabass – even more so than for other Mediterranean farmed species – and should therefore be avoided or protocols refined as much as possible. Environmental enrichment can potentially mitigate the effects of such stressors, yet all strategies need verification for the farming context. For slaughter, electrical stunning followed by immersion in ice water is less stressful than asphyxia on ice or hypothermia in ice-water slurry and leads to death while the individual is unconscious. The specific protocol needs to be validated for farming conditions and should be verified at each farm.

Summary of welfare profile of *Sparus aurata*

For a complete version of this profile, including further criteria, technical summaries, detailed information, welfare scores and full list of references, please visit: https://FishEthoBase.net/db/49/

Gilthead seabream (*Sparus aurata*) is a sparid from the eastern Atlantic and the Mediterranean, representing one of the most frequently farmed species in Mediterranean marine finfish aquaculture besides Seabass (*Dicentrarchus labrax*).

Home range: Seabream juveniles and adults live in (one or more) coastal lagoons or estuaries where lagoons are usually <700 m in diameter. Nothing is known about home range in the time they are at sea and also not about the home range of larvae, fry and spawners. Whether providing the individuals in captivity with their needs (e.g., water parameters, food, mates, safety from predators) will override the urge to traverse a home range is not known. Therefore, larger facilities will potentially provide higher welfare. Earthen ponds of 2,000 m^2, but also sea cages with 50 m diameter, are more promising than tanks and raceways.

Depth range: For food, to shelter from predators, and to adjust to preferred water parameters, Seabream juveniles and adults use the upper 0-5 m, but may also be found up to 30 m and occasionally up to 130 m. Depth range for larvae, fry, and spawners is not known. Similarly to home range, it is not clear whether the need to dive is overwritten as soon as all needs are cared for in captivity. Again, deeper facilities potentially provide better welfare. Sea cages have the advantage that they can be lowered to different depths and therefore cover a larger part of the natural depth range than tanks, raceways, and earthen ponds. Since environmental conditions are not controlled with sea cages, though, it might happen that individuals crowd in layers of preferred water temperature, for example, and are stressed by temperature outside their preference range.

Migration: Seabream is amphidromous, meaning that juveniles and adults migrate between the coast and the open sea independent of spawning. After hatching at sea, fry move towards the coast to benefit from lagoons and estuaries as nursery grounds. Juveniles and adults stay there most of the year, only to return to the sea in winter. This need to migrate serves the purpose of providing the best possible environmental conditions for each life stage. It is not clear, though, that the urge disappears when the needs are taken care of in captivity. Just as in home range, larger facilities are potentially more in line with natural needs, although migration distances are even harder to cover. There is no research on welfare effects of providing current or changing salinity to mimic migration.

Reproduction: although it is known that Seabream in the wild spawns in winter-spring, there is no knowledge on the mating system and courtship rituals. In the farm, keeping female spawners in small groups with male spawners stabilised the spawning rate of eggs and increased the rate of fertilisation by males. Hormonal manipulation is uncommon, but photoperiod and temperature manipulation is standard and should be avoided due to their likely welfare reduction. It is unclear whether stripping takes place.

Aggregation: how closely Seabream larvae, fry, and spawners aggregate is not known. Juveniles and young adults probably organise in tight schools, older adults can also appear solitarily. Without specific densities in the wild, it is hard to make recommendations for farms. From laboratory studies, one may conclude that densities >15 kg/m^3 probably decrease growth and increase stress. Sea cages and ponds rather stay below this threshold than tanks.

Aggression: all of Seabream's age classes display aggression under farming conditions or in laboratory studies. In fry, size grading might decrease cannibalism. In juveniles and adults, a certain density might reduce adverse behaviour which has to be carefully balanced with the density threshold in aggregation (see above).

Substrate: since spawning takes place in the sea, nest building is unlikely. Seabream juveniles and adults, however, are found over sandy or muddy bottoms, over rocks and stones or in seagrass beds. Adults are suspected to bury themselves in sand at night. Earthen ponds, per their design, provide substrate. Laboratory studies confirm the positive influence of providing substrate: glass gravel, especially of blue colour, increased growth and decreased aggression and probably stress in tanks; plant-fibre ropes increased welfare in tanks and cages and decreased aggression and interaction with the net in cages. Further research is needed to confirm these findings for the farming context.

Handling and slaughter: Seabream is prone to stress by handling, confinement, crowding, and air exposure. Because these stressors are typical steps of husbandry, they should be reduced to a minimum. Environmental enrichment can potentially mitigate the effects of such stressors, yet all strategies need verification for the farming context. For a humane slaughter including fast loss of consciousness and death while unconscious, a) percussive stunning followed by bleeding, b) spiking followed by ice slurry or c) electrical stunning followed either by bleeding or by immersion in ice-water slurry are recommended over direct immersion in chilled water or in ice-water slurry without stunning.

Summary of welfare profile of *Oncorhynchus mykiss*

For a complete version of this profile, including further criteria, technical summaries, detailed information, welfare scores and full list of references, please visit: https://FishEthoBase.net/db/30/

Rainbow trout (*Oncorhynchus mykiss*) is one of the dominant salmonids farmed in Europe and North America, second only to Atlantic salmon (*Salmo salar*). There are two strains in *O. mykiss*: the anadromous and the potamodromous. Aquaculture populations probably combine genes of both strains.

Home range: Rainbow trout larvae and fry probably stay close to the redd; juveniles, adults, and spawners all move up to several kilometers to satisfy their needs of food, preferred water parameters, mates, and shelter form predators. Since it is unknown whether the impulse to move disappears once all needs are satisfied in captivity, larger farming systems are recommended over smaller ones. Raceways and ponds of 1,200-1,300 m^2, but also sea cages with 50 m diameter, overlap to a larger degree with the natural home range than tanks.

Depth range: Rainbow trout builds nests at around 0.1 m depth from which alevins emerge. Juveniles and adults, which remain in fresh water, search for food, take shelter and adjust to preferred water parameters in 0-5 m, up to 100 m. Smolts that migrate to the sea stay within 1 m of the sea surface and return to shallow fresh water to spawn. Again, deeper systems are to be preferred over shallower ones as long as it is unclear whether providing Rainbow trout with all needs makes the urge of diving deep redundant. Sea cages in up to 50 m depth overlap with a larger part of the natural depth range than ponds or raceways which are usually not deeper than 1.5 m max. Sea cages, though, come with their own challenges, as stocking density does not allow all individuals access to layers of preferred temperature, pH or oxygen. Those individuals which manage to get access might crowd to such a degree that welfare deteriorates.

Migration: The potamodromous Rainbow trout remains in fresh water and moves up- and downstream for several kilometres. The anadromous strains of trout spend their life in fresh water for up to 4 years, then smoltifies (i.e. the process of physiological changes that allow salmon to adapt from living in fresh water to living in seawater) to sustain seawater conditions, and migrates downstream to stay at sea for up to 3 years before migrating back to natal rivers to spawn. Needs of neither strain may be met in captivity due to the enormous distances covered, and it is uncertain that the migration need is suppressed when providing each age class with their respective natural conditions in captivity. Therefore, larger facilities are to be preferred over smaller ones. Whether the provision of changing salinity to simulate migration improves welfare has not been researched. Providing currents to simulate a riverine environment could decrease stress.

Reproduction: Rainbow trout spawns at 2-7 years in different seasons depending on latitude. The male stays close to the female, while she builds the nest, and either chases away competitors or stimulates her. Keeping sexes separated in farming conditions does not allow natural reproductive behaviour and should be avoided. Similarly, keeping sex-reversed all-female populations has a unknown effect on welfare. Although it avoids applying hormonal manipulation to those individuals that will be sold, it prevents the species of performing its natural reproductive behaviour. Instead of hormonal manipulation that large farms sometimes apply, small farms mimic environmental changes (decrease water level, increase water current) to stimulate natural reproduction in line with the wild. Stripping of eggs and semen – even if under anaesthesia – should be avoided in favour of natural spawning.

Aggregation: little is known about aggregation in Rainbow trout in the wild. Whereas trout alevins school, probably for safety reasons, the older parr are rather solitary. Smolt and adult behaviour requires further research. After spawning, kelts may seek lose company of conspecifics if not immediately returning to the sea. Based on this, stocking density in captivity probably exceeds natural conditions. Laboratory studies indicate that densities between 10 and 25 kg/m^3 (lower for spawners) potentially result in better welfare than higher densities. Although some studies demonstrate that higher densities may still be possible under acceptable welfare standards, these should be approached with caution, and OWIs (animal-, resource- and management-based) thoroughly monitored. Earthen ponds and some tanks with densities below these limits are to be preferred over raceways, sea cages, and other tanks. Also, enriching tanks with PVC pipes, plastic plants, and stones potentially increases shoal cohesion probably indicating positive emotions. This application needs to be verified for farming conditions.

Aggression: Rainbow trout is aggressive and territorial in all life stages, although nothing is known about life at sea. Food competition is one reason for aggression, so care should be taken to find a sufficient ration size and ideal distribution strategy and amount. Another method to potentially decrease aggression is through enrichment with a) bubbles to which individuals are highly attracted or b) PVC pipes, plastic plants, and stones.

Substrate: Rainbow trout uses substrate throughout its life: spawners cut redds in gravel; alevins stay hidden in gravel until yolk sac absorption; juveniles and adults are found over rubble to boulder substrate and take shelter in macrophyte beds, woody debris, and below undercut banks. In farms, for fry, enriching raceways with stones may reduce fin erosion and covering tanks may result in sheltering. For juveniles and adults, by design, earthen ponds provide substrate. Enriching pond bottom with cobble potentially increases growth and tolerance to seawater transfer compared to barren raceways or asphalt-bottom ponds. Raceways may decrease predator-avoidance behaviour and fear by adding netting to protect from avian predators. Tanks benefit from enrichment with a) randomly fired currents which potentially decrease cortisol, b) bubbles which may keep individuals occupied or c) PVC pipes, plastic plants, and stones which could reduce fin erosion, fear, and neophobia. These applications need to be tested in farming context. For spawners, substrate should be provided for nest building.

Handling and slaughter: Rainbow trout husbandry may require transfer between facilities, regular size grading, and harvest. These come with handling, air exposure, crowding, confinement, and transport. Rainbow trout is sensitive to all of these which should therefore be avoided as much as possible. Transport stress may be lowered through addition of salt. Environmental enrichment can potentially mitigate the effects of such stressors, yet all strategies need verification for the farming context. Handling and air exposure for weight monitoring can be avoided by infrared technology or other remote precision fish farming techniques. At harvest, to induce unconsciousness fast and kill while still unconscious, percussive or electrical stunning followed by a) evisceration, b) evisceration and exsanguination, c) ice slurry or d) percussive killing is preferred over asphyxia in air or on ice, carbon dioxide stunning or hypothermia in ice slurry.

Summary of welfare profile of *Salmo salar*

For a complete version of this profile, including further criteria, technical summaries, detailed information, welfare scores and full list of references, please visit: https://FishEthoBase.net/db/1/

Atlantic salmon (*Salmo salar*) is a salmonid from both coasts of the northern Atlantic, migrating into boardering rivers to spawn. It is the most frequently farmed fish in Europe which represents 50% of the worldwide *S. salar* production.

Home range: Atlantic salmon fry stay close to the redd, whereas parr may cover an area of several thousand square meters. Home range for smolts and adults at sea is unknown. Grilse or kelts move up to several kilometers up- or downstream after spawning. While in the wild traversing a home range is all about satisfying needs of specific water parameters, food, shelter or mates, in captivity, these needs are provided for. Still, it is unknown whether the need for space completely vanishes. Thus, larger farming systems are a safer bet than smaller ones. For parr and grilse, tanks with 20-25 m diameter and for parr, freshwater cages with 25 m diameter cover a lot of the natural home range. Without knowledge on home range for smolts and adults at sea, no recommendations for farming facilities are possible.

Depth range: while in fresh water, Atlantic salmon lives in shallow rivers in up to 0.6 m max, sometimes in deeper pools of 1 m or more. At sea, smolts and kelts stay mostly within 1-3 m, occasionally up to 5-6 m, sometimes much deeper. Although deep dives to avoid predators, adjust to water parameters or find food are not necessary in captivity, it is uncertain whether diving deep serves other purposes or is rewarding in itself. Nevertheless, tanks of 1.5 m for fry, tanks of up to 4.5 m and freshwater cages of up to 5 m for parr will cover most of these needs. Some sea cages for smolts may be submerged to up to 50 m, yet the lack of control of water parameters might result in stress if individuals cannot flee adverse conditions or if they crowd in layers with preferred conditions.

Migration: Atlantic salmon usually is an anadromous species hatching and spending up to 5 years in fresh water before migrating to the sea for up to 3 years and returning to natal rivers to spawn. There do exist landlocked populations, though, which remain in fresh water, at most migrating up- and downstream or into adjacent tributaries or lakes. Although reasons for these migrations (e.g., mates, water parameters, food, shelter) do not apply in captivity, it remains unclear whether all reasons are identified or whether the urge to migrate persists. Therefore, the larger the facilities the better. The biggest tanks and cages cannot cover the enormous distances observed in the wild, though.

Reproduction: Atlantic salmon is flexible in spawning age, ranging from 2 to almost 8 years. In nature, spawning takes place in autumn-winter, yet this may differ depending on latitude. The male courts the female. Keeping spawners in captivity in mixed-sex groups adheres to natural conditions, whereas hormonal manipulation does not and should be avoided. In Rainbow trout (*Oncorhynchus mykiss*), as an alternative to hormonal manipulation, small farms decrease water level and increase water current as a way to encourage natural reproduction. Further research is needed to determine whether this works in Atlantic salmon as well. Stripping prevents individuals from displaying and experiencing natural spawning behaviour and should be avoided.

Aggregation: in the freshwater phase, Atlantic salmon is rather solitary, although conspecifics live in the vicinity. For migration to the sea, smolts form tight schools, probably as protection from predators. Even if data on specific densities in the wild are missing, laboratory studies help identify 10-20 kg/m^3 as a threshold from which on welfare may start to deteriorate. Accordingly, sea cages and some tanks which stay below this level are promising.

Aggression: Atlantic salmon is aggressive in almost all life stages, but loses the tendency during downstream migration after smolting where aggregation in schools does not allow for agonistic behaviour. Level of aggression at sea is unclear. In farms, aggression seems to decrease with sufficient amount of feed and a density that has to be carefully adjusted to avoid crowding on the one hand and aggression on the other.

Substrate: as for aggregation and aggression, substrate use differs between the freshwater and the marine phase. Female spawners cut redds in gravel and cover eggs; alevins stay hidden until yolk sac absorption. Fry and parr use "home stones" of different sizes over which they hover in summer and under which they hide in autumn. For cover from predators, high current velocities as well as low temperature, they also use rooted aquatic macrophytes, woody debris or overhanging riparian vegetation. In contrast, smolts and adults at sea are probably pelagic. Providing alevins in captivity with hatching substrate like stones, gravel, astroturf, biomatting, etc. accommodates this dependence on substrate. Stripping of spawners should be avoided in favour of providing nest-building material. Covering outdoor tanks of parr not just deters predators, but may potentially decrease fear and stress. Shelters (even simple ones) may increase growth. This application needs verification for the farming context.

Handling and slaughter: husbandry procedures or their side effects like handling, size grading, crowding, confinement, noise, transport, and sudden parameter changes are potentially stressful for Atlantic salmon. These procedures should therefore be performed with the largest care and avoided if possible. In smolts, (temporarily) submerging cages can circumvent rough surface conditions (low oxygen levels, storms, ice, algal bloom, sea lice larvae). The resulting increased jumping behaviour once the cage is lifted back up can be used as a stress-free delousing method using the treatment floating at the surface. During slaughter, to avoid prolonged suffering and achieve fast unconsciousness, percussive or electrical stunning followed by bleeding should be preferred over hypothermia in chilled water.

Summary of welfare profile of *Cyprinus carpio*

For a complete version of this profile, including further criteria, technical summaries, detailed information, welfare scores and full list of references, please visit: https://FishEthoBase.net/db/12/

Common carp (*Cyprinus carpio*) is the oldest and one of the most frequently farmed aquatic species worldwide. The origin is debated, some placing it in fresh waters of China, others in Europe.

Home range: how much space Common carp larvae, fry, and spawning adults cover to satisfy their needs is unknown. Juveniles and adults traverse up to an average of 30,000-4,000,000 m^2 for shelter, food, beneficial water parameters, and mates. Providing this space in captivity might not be necessary, as all of these needs can be concentrated in a much smaller area. Since it is unclear whether the need to move will indeed be eliminated, precaution calls for rather larger than smaller rearing systems. Earthen ponds of up to 1,000 m^2, even better the ones for spawners of up to 25,000 m^2, are thus to be preferred over cages, tanks, and recirculating systems.

Depth range: Common carp is a bottom grazer but also feeds in the water column. Depth use ranges from an average 1.5 m in spring and summer to 2-3 m in winter. Spawning takes place at the surface up to 1.7 m. This spectrum may be rather covered in earthen ponds of 2.5 m or deeper than in tanks. Even if providing the whole depth range in farms is not called for because all needs are taken care of, it is still recommended and feasible. It is uncertain that all motives for diving deep are identified and satisfied in captivity.

Migration: Common carp migrates within fresh water, referred to as a potamodromous migration type. For the exact migration distance, further research is needed. Some strains migrate less than others, which is promising for aquaculture, as providing sufficient living space is then, in fact, feasible. Earthen ponds rather fulfill space requirements than other facilities (see home range). Clues for effects on welfare when presented with water currents simulating migration are still missing.

Reproduction: females and males of Common carp mature at 2-3 years or later and are able to spawn year round depending on latitude. The male courts the female. Therefore, the farming procedure of separating sexes goes against natural behaviour and is not recommended. Also, applying hormonal manipulation to induce spawning, followed by stripping, deviates from wild conditions and should be avoided. In fact, natural spawning is possible in captivity.

Aggregation: whereas the aggregation type for Common carp larvae and fry is unknown, juveniles and adults occasionally gather in lose groups and also disperse into a solitary lifestyle. Seeking the proximity of conspecifics might be for protection, as it was observed during the night, under ice cover, and during spawning, among other occasions. In absence of specific densities in the wild, hints from laboratory studies may help in recommending stocking densities in captivity. Better growth and less stress may be expected at <1 individual/m^2 than at higher density, which is in line with how many extensive ponds are stocked. Further research is needed.

Aggression: in general, Common carp is considered not aggressive, yet there are some hints on aggression, especially with stocking density >1 individual/m^2. Further research could shed light on the extent and the triggers of this tendency. Even if aggression should not be a dominant problem, solutions to decrease it with the help of environmental enrichment, for example, would be interesting.

Substrate: Common carp lives over sand and mud. To shelter, juveniles and adults use aquatic vegetation, submerged macrophytes, and dead trees. Spawners release eggs over submerged vegetation to which the eggs stick. Some farms accommodate the spawning type by fitting ponds with (artificial) substrate and then transfer it – complete with eggs – to hatching or nursery ponds. In the lab, spawners also accepted ribbons of plastic sheets as spawning substrate. Whether this could be applied in farms requires verification. For grow-out, ponds are more in line with the substrate-preferring Common carp than tanks, cages or recirculation systems. The ponds should have earthen bottoms, though, instead of concrete, plastic sheets, etc. Still, adding opportunities to shelter (whether natural or artificial) can add even more overlap with wild conditions.

Handling and slaughter: harvest of Common carp includes steps of confinement, crowding, and netting followed by transport. Common carp is stressed by all of these, so they should be kept to a minimum or avoided if possible. Selling carp alive to consumers is unacceptable, as it prolongs the suffering and leaves the slaughter procedure to a lay person – if the fish has not died of asphyxia in the meantime. Instead, only properly stunned and slaughtered individuals should be sold. Percussive or electrical stunning followed by evisceration, gill cut or destruction of the heart introduces unconsciousness instantly, performs killing during this insentient period, and is less stressful than asphyxia or live chilling. For electrical plus percussive stunning followed by either of the named slaughter methods, a protocol is needed.

2.2- Challenges and responses associated with positive and negative outcomes of established farming protocols.

The following section is largely based on the work of Saraiva et al (2022a), where the authors addressed welfare challenges in farming systems of the five main species in European Aquaculture.

On a global scale, aquaculture generally relies on a relatively small number of rearing systems. There are land-based systems, such as ponds (natural and artificial), various flow-through systems (tanks and raceways) and recirculating aquaculture systems (RAS). Alternatively, fish can be reared in water-based systems, such as freshwater/inshore/offshore floating net pens, or semi-closed containment systems (S-CCS). Within every farming system, fishes experience various husbandry routines and operations. Each of these systems can present different welfare challenges or risks to the fish, which in turn are dependent upon both the species and its life stage (van de Vis et al., 2020).

Aquaculture systems vary in their degree of production intensity, ranging from extensive to super-intensive systems. In general, extensive systems are characterized by minimal inputs and relatively low yields (close to natural yields), whereas with increasing intensification, additional feed is required to maintain higher stocking rates in semi-intensive conditions. Intensive and super-intensive systems rely to a large extent or even completely on supply of external inputs and technologies. Intensification also implies higher costs for investment and management, be it for the construction of advanced aquaculture technologies (e.g. industrial pond farms, raceways or offshore cage farms) or for the maintenance of such highly stocked systems (e.g. costs for feed inputs; fuel or electricity for aeration) (Ottinger et al., 2016).

Atlantic Salmon and Rainbow trout are generally farmed intensively and mainly destined for consumption. The most common farming systems used are flow-through systems, RASs and floating cages (e.g. Jones, 2004; Vandeputte and Labbé, 2012), and the interest in S-CCSs has been increasing during last years (van de Vis et al., 2020) (Table II). The intensive system is characterised by high production, at high fish densities, with many parameters under human control. In intensive breeding systems, selected broodstocks are held in large freshwater ponds or tanks (usually flow-through or RAS systems) where they release eggs and milt (i.e. seminal fluid containing semen), which will be mixed to produce fertilised eggs. The fertilised eggs are then placed in purpose-built incubators until hatching. After hatching, the fry absorb nutrients from a yolk sac attached to their bodies, and they remain in the hatching environment until they are able to feed independently. Then, larval fish are directly transferred to the first-feeding tanks. At the nursery stage, Atlantic salmon and Rainbow trout have different rearing requirements that will dictate the type of containment, but the source of water available will determine whether flow-through, semi-closed containment systems or recirculation systems are best. Normally, Atlantic salmon are kept on land in freshwater tanks after hatching, before smoltification starts naturally or is induced artificially. The smolts or post-smolts are then transferred mostly to sea cages, or RAS and S-CCS systems for the final grow-out phase until harvest. In Rainbow trout, fry are moved to outdoor grow-out facilities, which can comprise concrete raceways, ponds, RASs, cages in lakes or sea cages with different sizes and characteristics according to site availabilities, environmental conditions, and specific company targets. Atlantic salmon and Rainbow trout are grown on to a marketable size usually within nine months in sites dedicated to the production of portion-size trout of 450 g average weight. Some fish, though, are grown on to larger sizes over twenty months to be harvested at three kg plus. In addition, small-scale Rainbow trout farms can use semi-intensive systems for on-growing where young stock are brought in by road and grown-out for either food or re-stocking markets. Extensive salmonids production is quite rare on a commercial scale, and mostly consists of releasing juvenile fish for downstream migration at the smolt stage.

Gilthead seabream and European seabass are each mostly produced in three different aquaculture systems, whose techniques and procedures are very similar for the two species (e.g. Bagni, 2005; Basurco et al., 2011; Colloca and Cerasi, 2005) (Table X). Intensive systems are characterised by a high production at high fish densities, where many parameters are under human control. To secure a reliable and sufficient supply of good quality fish eggs, most hatcheries have established their own broodstock units, where breeders of different age groups are maintained under long-term stocking conditions. Parent animals may come from the wild, but nowadays most of them come from a selective programme at the farm. After hatching, the larvae will absorb their yolk sac and, once they start feeding, weaning usually takes place in a dedicated section of the hatchery (i.e. nursery area) equipped with larger round or rectangular tanks. Juveniles are pre-fattened intensively with a controlled diet and at high densities until they reach the size for the on-growing phase. In intensive production, on-growing units are supplied with juveniles, which may be purchased from separated hatcheries, but large production units normally rear their own. Intensive on-growing phases can be carried out in land-based installations (tanks or raceways) or in coastal floating cages. Semi-intensive farming systems are usually carried out in net enclosures within limited areas of the lagoons or in earthen ponds, where human control of the farming environment is much lower than in intensive systems but greater than in the extensive ones. This technique involves artificial enrichment with fry collected by specialised fishermen or seeding with pre-fattened juveniles in intensive systems to minimise mortality and shorten farming time. Extensive systems are based on the natural migration of euryhaline fish between the open sea and coastal lagoons, brackish ponds or salt marshes, and they have been widely developed in northern Italy ('vallicoltura') and in southern Spain ('esteros'). This traditional extensive method of lagoon management places special traps or barriers made of reeds, nets or cement in appropriate lagoon sites to capture fish during their autumn migration to the open sea.

Common carp is a freshwater species that is generally reared in ponds in intensive, semi-intensive, or extensive monoculture or polyculture systems, or in integrated carp culture with other agriculture systems (e.g. Peteri, 2004) (Table II). Spawning can either occur in large ponds, where fry can be harvested or left there until they reach fingerling size and are moved to prepared ponds, or can take place in hatcheries, where ovulation and spermiation are artificially induced (i.e. hormonal injections) and eggs are artificially fertilised, then fry are moved from tanks into ponds when they reach the feeding fry stage. The fry are nursed in ponds or alternatively, if predators are present in the ponds (i.e. larger conspecifics, other fish species in polyculture systems, or even potential avian predators), in tanks or in industrial raceways or water recirculating systems. Then fingerling production takes place in semi-intensive ponds, and from there they can be moved to ongrowing systems, where growing carp to reach market size can take place in 1) extensive monocultural production systems in stagnant water ponds; 2) intensive monocultural production systems in cages, irrigation reservoirs, running water ponds/tanks, or in recirculating systems; 3) polycultural systems with other species; or 4) systems integrated with animal husbandry and/or plant production. From here they can either be transported to be sold live to consumers or restaurants, or to be slaughtered in an abattoir.

Table II. Farming systems and production phases for the top-five most farmed finfish species in Europe: Atlantic salmon (*Salmo salar*), Rainbow trout (*Oncorrhynchus mykiss*), Gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*) and Common carp (*Cyprinus carpio*). Adapted from Saraiva et al. (2022a).

	System	R. trout	A. salmon	G. seabream	E. seabass	C. carp
Land-based	Polyculture or natural/rice fields	-	-	-	-	on-growing
	Artificial and natural ponds	on-growing	on-growing	on-growing	on-growing	hatchery nursery on-growing
	Flow-through tanks and raceways	hatchery nursery on-growing	hatchery nursery on-growing	hatchery nursery on-growing	hatchery nursery on-growing	hatchery nursery on-growing
	Recirculating tanks and raceways	hatchery nursery on-growing	hatchery nursery on-growing	hatchery nursery on-growing	hatchery nursery on-growing	nursery on-growing
Water-based	Semi-closed containments	-	on-growing	-	-	-
	Floating cages (marine-freshwater)	on-growing	on-growing	on-growing	on-growing	on-growing
	Off-shore cages	-	on-growing	on-growing	on-growing	-
Others	Integrated multitrophc farming systems (IMTA)	-	-	on-growing	on-growing	on-growing

Welfare constraints exist throughout the production cycle of any farmed fish species. When identifying the welfare challenges the fish are exposed to throughout the production cycle, we use the framework proposed by the FishEthoBase (Saraiva et al., 2019) and Huntingford (2020): various criteria are used to evaluate the challenges imposed on any farmed fish species, by taking the ethology of those species as a standard to compare how well those species may cope with those challenges. We propose a grouping of these major challenges into four main categories (with examples):

1. Ethological
    - Spatial limitations
    - Reproduction
    - Density / aggregation / social issues

2. Physiological
    - Pain
    - Infectious disease / immunocompetence*
    - Parasites*
    - Stress

3. Environmental
    - Water parameters
    - Light parameters
    - Temperature parameters
    - Environmental complexity

4. Human-induced /procedural
    - Standard Operational Protocols
    - Slaughter methods

The categories marked with an asterisk (*) are mainly health-related issues that are more related to a veterinary approach, and in that sense will generally not be addressed here as they fall outside the scope of this report. These four main categories are often interlinked (Fig. 2) and are applicable to all fish farming systems- They may yet differ in intensity and severity depending on the combination of species and method. In the following sections we review those challenges in a species-specific approach for the five most farmed fish in Europe, while highlighting possible suggestions for improvement. Species-specific information can be traced back to the welfare profiles from section 2.1.

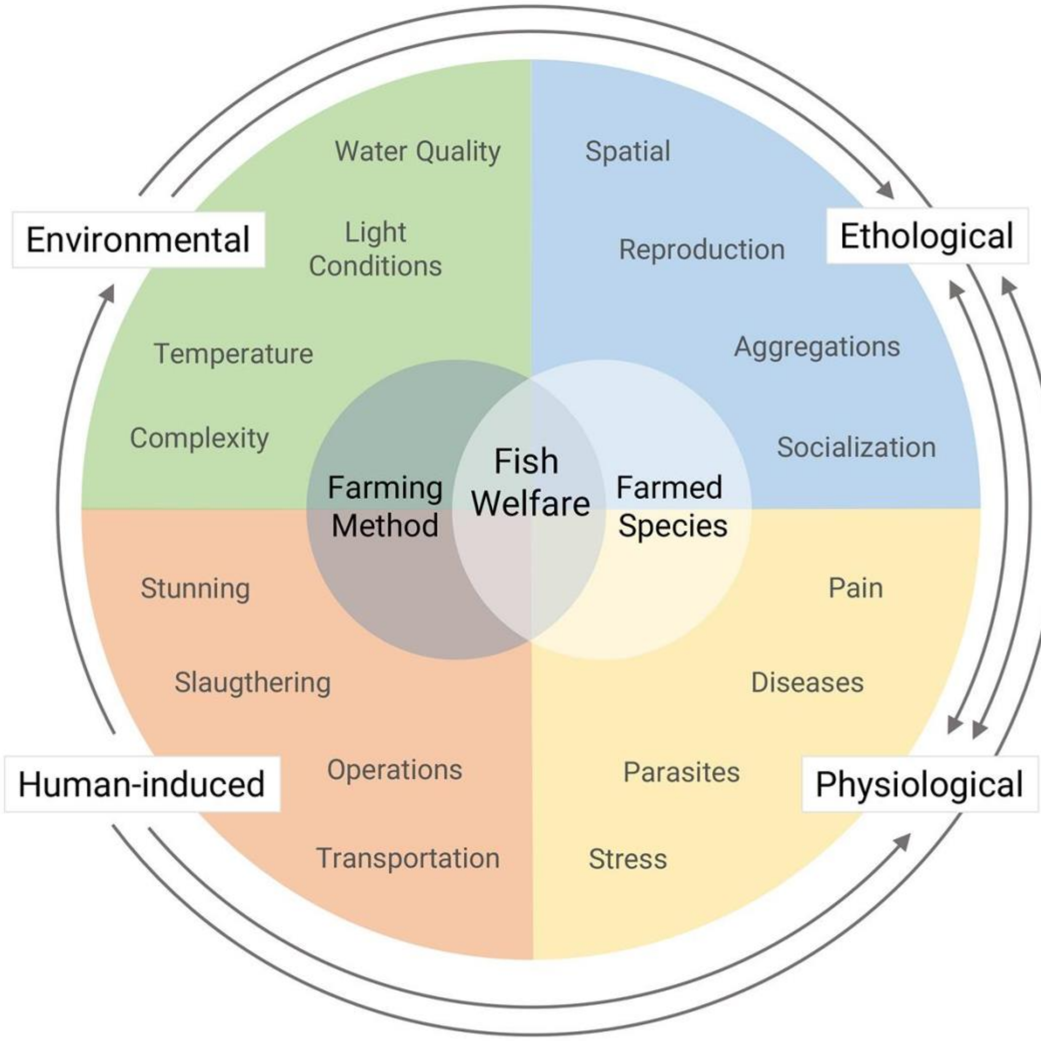

Figure 2. Representation of the links between different ethological, physiological, environmental and human-induced challenges that fish are exposed to throughout their lives under farming conditions. The arrows highlight the inter-links among categories. The type of challenges within each category of challenge are detailed in the text. Adapted from Saraiva et al. (2022a).

1- *Ethological challenges*

We define ethological challenges as those that impair behavioural functions directly or indirectly, considering the four classical ethological questions posed by Tinbergen (1963): function, causation, development and evolution. Captive environments impose constraints that challenge fishes' ability to cope with their environments, for example, restricting free movement of animals. Some species may cope better with such **spatial restrictions** because they have evolved in restricted areas and are adapted to confined areas. This may be the case with Common carp for example, yet this applies only for certain life stages (Flajšhans and Hulata, 2017). Many others, however, are not found in spatially restricted environments and may not be equipped to cope with the spatial challenges of fish farming (Saraiva et al., 2018). This may be especially true for migratory species.

Another immediate aspect of fish farming is the **aggregation** of animals. Fish under culture conditions are stocked using various methods described in previous sections, sometimes in very high and mostly artificial densities (Saraiva et al., 2018, 2019). This results not only in a technical challenge to maintain water quality (see point three) and monitor disease outbreaks, but also in ethological challenges to cope with proximity to a very large number of conspecifics. Some species encounter near-natural density conditions in specific life stages under some farming methods, such as juveniles of Seabream and Seabass (Abecasis and Erzini, 2008; Bégout Anras et al., 1997) and therefore may be naturally equipped to deal with such a social context. However, in other cases such as alevins and fry of Rainbow trout this never happens in nature, and such artificial crowding may lead to maladaptive behavioural responses such as aggression (Berejikian et al., 2000), abnormal behaviours, immune impairments, poor feeding and/or stress (Andersson and Höglund, 2012). The most appropriated density should rely on indicators of welfare and take into account the specific farming conditions on a case-by-case basis (Saraiva et al., 2022b).

Reproduction is often highly artificial, either because some species are not known to be able to spawn naturally in captivity, for example salmon (Stead and Laird, 2002), or because standard industry procedures dictate artificial spawning inductions as the best means to achieve a regular supply of gametes (Zohar and Mylonas, 2001). These procedures often involve stressful handling, such as prolonged emersion, manipulation and mechanical damage from stripping (see point four). However, there are species who spawn spontaneously in captivity and there is evidence that egg quality is higher in these cases as in the case of Seabass (Forniés et al., 2001).

Cognition in captive environments is challenged mainly due to the absence of stimulation (Korte et al., 2007). Rearing facilities are usually barren for sanitary and practical reasons, but this may impair cognitive aspects in several species, particularly those that evolved and are adapted to complex environments. For example, sea bream have impaired cognition, brain function and spatial orientation in barren tanks, which are improved by environmental enrichment (Arechavala-Lopez et al., 2022a, 2020, 2019). Similarly, positive effects of rearing fish in complex environments have been reported for salmonids, carps, and other species of aquaculture interest (Jones et al., 2021; Näslund and Johnsson, 2016). Finally, life in captivity may induce negative **emotional states** in fish. It is known that fish may experience emotion-like affective states such as fear (Cerqueira et al., 2017; Tatemoto et al., 2021) or pain (Sneddon, 2015) that may hinder their welfare in ways we are only now starting to unravel. Specifically, the lack of cognitive stimulation in fish farms may be especially relevant for broodstocks, since these animals may spend several years in captivity. The rearing of these fish for extended periods (their whole natural lifetime in some cases) in highly predictive and stable environments, usually barren for hygiene reasons, may lead to the appearance of symptoms usually associated with boredom (Meagher, 2018), such as stereotypical behaviours (Mason and Rushen, 2006) or apathy (Wemelsfelder and Birke, 1997). It is therefore highly likely that extended understimulation may impair mental and physical mechanisms due to chronic lack of opportunities to interact with the environment. On the other hand, it should also be noted that excessive environmental stimulation can sensitise coping mechanisms, enhancing permanent arousal and leading to a generalised state of exhaustion, which is the cause of many anxiety-related pathologies (Meehan and Mench, 2007). Therefore, inappropriate stimulation induces an allostatic overload of different types which is incompatible with good welfare (Galhardo and Oliveira, 2009; McEwen and Wingfield, 2003). Appropriate stimulation methods, such as environmental enrichment, should take species-specific requirements into account when they are planned (Arechavala-Lopez et al., 2022a).

2- *Physiological challenges*

The function-based approach to welfare has been the basis for much of the existing industry standards regarding health (Huntingford and Kadri, 2014). Farmed fish face serious physiological challenges that have significant effects on their health (infections, parasites, etc). The veterinary and health plans for fish farms are efficient at dealing with these kinds of situations and this is why we consider diseases to be mostly beyond the scope of this section. However, there are other physiological aspects of fish farming that are well within our approach to welfare, for example stress responses and pain. The physiological stress response is adaptive when animals face acutely stressful events, which in the wild are natural, sporadic, short-term and unforeseen but in farming environments are artificial, prolonged and repetitive. This may lead to **distress** and often to chronic stress, with important negative effects on fish welfare. It is important to highlight that farmed fish populations are not a homogeneous mass of animals. Yet this individual variation may be classified in the proactive-reactive continuum, where more proactive animals respond similarly among them to stressors and differently from reactive ones (Castanheira et al., 2017). It is therefore expectable that artificially selected lineages may cope better with stress if selection pressures work towards that objective. However the selection for these less reactive animals, who also have higher feed efficiency (and therefore grow better) and seem to have higher disease resistance, often induces a co-selection for aggressiveness and may trade off against welfare (Macaulay et al., 2022). In highly competitive environments this may lead to stunted growth of part of the population, although this effect may vary in range depending on the species, life-stage and method. For example, the appearance of 'loser fish' in salmon farms, that present slow growth and abnormal behaviours, is linked to poor welfare practices and severely impairs production (Madaro et al., 2022). What seems to be particularly effective on buffering stressors and providing good performance opportunities, especially for reactive individuals, sems to be environmental enrichment (Arechavala-Lopez et al., 2022a; Castanheira, 2017). Some of these human-induced stressors (see below) may in fact induce **pain**, which not only has an obvious strong immediate impact on welfare but may lead to long lasting negative effects such as avoidance, withdrawal, fasting, immune depression etc, especially when combined with chronic distress responses to traumatic events (Ashley and Sneddon, 2008). The physiology of farmed fish is subjected to a constant test to maintain **homeostasis** when we consider that the quality of water, the critical environmental component of fish farming, is influenced by the factors addressed next.

3- *Environmental challenges*

The regulation and monitoring of water as a holding medium for captive fish is paramount: water provides the basic life support for farmed fish. Indeed, there are many aspects to address in **water quality,** but we focus on (arguably) the most important ones: salinity, oxygen (O_2), carbon dioxide (CO_2), nitrogen compounds and pH. Regarding salinity, some of the species addressed in this chapter are euryhaline (i.e. able to adapt to a wide range of salinities) throughout most of their life cycle, such as Seabream and Seabass, while others have sensitivity windows when they perform migrations from freshwater to saltwater and vice versa (McCormick, 2001). Others are stenohaline (i.e. cannot tolerate a wide fluctuation in the water salinity), such as carp. Providing farmed fish with appropriate salinities in the appropriate life stage will prevent osmotic stress - that can pose a major physiological challenge. Regarding oxygen, while some species can tolerate low oxygen saturations, such as carp (Stecyk and Farrell, 2002), others (e.g. salmon) are very sensitive and experience poor welfare under 50% saturation (Oldham et al., 2019). CO_2 is a by-product of aerobic respiration and accumulates in waters with poor renovation, aeration or flow. It is highly toxic by itself but also because it lowers the pH of the water, it impairs the senses and overall physiology of fish (Ishimatsu et al., 2004). Finally, the accumulation of toxic nitrogen compounds from excretion in poorly filtered water can be deadly for fish (Ip and Chew, 2010).

All these aspects require technical (and, depending on the method and intensification level, often technological) solutions for monitoring and correction. Additionally, they are highly synergistic, so even small changes in any of these variables may have dramatic effects on welfare or even survival of fish. And this becomes even more critical when **temperature** is entered into the equation. As fish are ectotherms, their physiology is strongly affected by the environmental temperature. In some procedures within usual farming protocols, however, the fish are subjected to temperatures well outside their comfort range, both at the lower end in the common slaughter method of asphyxia on ice, and at the higher end in crowding, harvesting or other handling events where usually fish are crowded in low volumes of water, prone to severe temperature increases. Unfortunately, increasing the water temperature lowers the O_2 saturation, which triggers a cascade of both physical and biological reactions that ultimately lead to severe and rapid degradation of water quality: higher respiratory rates and metabolism cause rapid O_2 depletion and increase in CO_2, as well as increase in release and accumulation of faecal matter and urine, with build-up of ammonia compounds. The accumulation of these bioactive stress signals may also function in a positive feedback mechanism (i.e. the presence of such compounds increases fish metabolism and consequentially their release) and this cocktail can be extremely harmful, often deadly for fish in extreme events (Huntingford et al., 2006). Production units must therefore constantly monitor the water parameters and be able to correct them when deviations are found.

Light is also a fundamental aspect to consider when farming fish. Light intensity for example can have dramatic effects on the physiology and behaviour of farmed species. Photoperiod is also a critical environmental cue that fish use to read their environment. It is therefore not a surprise that manipulation of photoperiod (often combined with temperature) is one the most used techniques to induce spawning, delay maturation and control the life cycle of farmed species. Some manipulations of photoperiod and light intensity seem to be innocuous or positive, while other highly artificial settings (for example, 24h light for extended periods, sometimes in species which never experience such conditions in the wild, or bright lights in species adapted to deeper, darker waters) may hinder the welfare of some of the species in ways yet to be properly evaluated ((Huntingford et al., 2006).

It has been mentioned above that barren environments impair ethological aspects of fish welfare. One of the ways to counteract this effect is by environmental enrichment (EE), i.e., the deliberate addition of **complexity** to the captive environment. For several species, EE improves the overall welfare of farmed fish by providing stimuli and opportunities for choice, exploration and interaction with their surroundings (Arechavala-Lopez et al., 2022a, 2020, 2019). However, there are also reports of negative effects that may be due to incorrect interpretations of the ethology of the target species and/or inappropriate deployment of EE measures (Saraiva et al., 2021a) EE measures should not interfere with farming protocols, or the latter should be changed to accommodate EE measures. To summarise, while EE remains a favourable tool to improve the welfare of farmed fish, its implementation must take into account 1) understanding of species-specific requirements and 2) the farming protocols at each facility (Saraiva et al., 2021a).

4- *Human induced challenges*

Standard fish farming protocols have been developed and optimised largely from a production perspective. These protocols include human-induced challenges to the welfare of fish, stressors which never occur in nature and therefore fish are not naturally equipped to cope with them. Here we can divide these into two main components: the **handling**, where we consider all the operations that the fish are subjected to during their lifetimes (transport, grading, vaccinations, moving, crowding, harvesting, etc) and the **slaughter**, including stunning (if any) and the procedure that ultimately leads to the killing of the fish.

In the handling component, one important aspect to take into account is the farming method. While culturing fish in ponds may be theoretically less invasive (in the sense that fish experience a more 'natural' environment with less handling, as they go through most of their life in the same enclosure at relatively low densities). At the other end of the spectrum are intensive RAS systems where the fish are usually crowded, transported, graded, vaccinated and treated several times before they reach the end of their production cycle (see, for example Saraiva et al., 2021b). These procedures may cause internal and external damage to the fish due to contact with other animals' skin and spines, promote excretion and accumulation of urine and faeces due to stress, impair immune functions due to erosion of mucus layers and wounds, which in turn can promote infections and disease outbreaks, and overall severely increase stress due to handling and emersion. There are ways to mitigate the harm inflicted during these procedures: for example, the use of passive methods to grade and move fish, fish pumps instead of brailing, the use of anaesthetics or sedation in transport, manipulation and emersion (when allowed by competent authorities), and all of these techniques demonstrate good results (van de Vis et al., 2020).

In the slaughter phase, the traditional method of asphyxia on ice without prior stunning has been demonstrated to be the worst, not only in terms of welfare but also in terms of flesh quality. Different species require different technical approaches towards the stunning procedure, however existing evidence shows that, if the fish are effectively stunned prior to slaughter, then the killing occurs painlessly, the flesh quality is better, rigor mortis is delayed, and the shelf life is longer (Poli, 2009). Percussive or electrical stunning solutions exist for all the major species (Saraiva et al., 2019). Regardless of the farming method, both handling and slaughter components depend on staff training and technical capabilities and may sometimes require changes in operational protocols. However, the benefits in terms of welfare and product quality are evident.

2.3- International survey: What is the industry standpoint on welfare?

One of the objectives of the present work is to obtain insights on how the industry is addressing the issue of welfare of their farmed fishes, as well as knowing what good practices are being implemented to investigate their feasibility at a larger scale. For that purpose, an online survey is taking place in collaboration with FEAP and the results will be known by end of November. Once sufficient replies are gathered, the results will be analysed and they will be made available to the present report as an Annex.

The formulation of the survey is as follows:

Dear fish farmer,

The Aquaculture Advisory Council, together with the Federation of European Aquaculture Producers and the FishEthoGroup, are performing a survey to fish farmers around Europe to understand how companies are addressing the welfare of their animals. Specifically, we seek to understand the industry perspective on the use of fish behaviour and other non-invasive indicators of welfare in aquaculture, how (and if) the companies see welfare as a part of their operation and which good practices are already taking place. The target species are sea bream, sea bass, trout, salmon and carp.

This short survey is completely anonymous and voluntary, and your honest contribution is extremely valuable, as this study will be used to inform the European Commission for upcoming improvements for the sector.

Thank you.

1- Species farmed in your company (please choose all that apply):

a) Sea bream

b) Sea bass

c) Trout

d) Salmon

e) Carp

2- Farming method used in your company (please choose all that apply):

a) Earth pond

b) Sea pens

c) Concrete raceways or tanks

d) RAS

3- Production size of your company (tonnes/year):

a) 0-100 t

b) 100-500 t

c) 500-1,000 t

d) 1,000-5,000 t

e) Above 5,000 t

4- In a scale of 0 to 5, how would you rank the importance of fish welfare for your company? (0- not important at all; 5 - extremely important)

5- What do you think are the major points of concern regarding welfare in the activity of your company? (please choose all that apply)

a) Diseases

b) Growth issues

c) Feeding issues

d) Signs of fish stress

e) Insufficient staff training

f) Handling procedures

g) Transport

h) Stunning and Slaughter

6- In a scale of 0 to 5, how would you rank the importance of welfare for the overall profitability of your operation? (0- not important at all; 5 - extremely important)

7- Of the following parameters, which ones do you use as signs of the welfare of your fish? (please choose all that apply)

a) Swimming behaviour

b) Feeding behaviour

c) Abnormal behaviours

d) Skin condition and appearance

e) Fin condition

f) External injuries, infections, parasites

g) Water quality or other environmental parameters

h) Others, which:

8- Please rank the following actions to improve welfare in farmed fish according to their priority for your company.

a) Development of welfare indicators based on the behaviour of farmed fish

b) Development of new treatments and vaccines

c) Development of environmental enrichment solutions

d) Development of technological tools to monitor fish welfare (cameras, sensors)

e) Establishment of humane slaughter methods

f) Training of staff to interpret behavioural indicators

g) Training of staff to understand effects of procedures

h) Training of staff for new technological monitoring tools

9- What do you already do to assess and improve the welfare of your fish in your farm? (please choose all that apply):

a) Less handling / more refined handling

b) Observe what the fish are doing and how they are behaving to monitor their welfare

c) Environmental enrichment (which: structures/colours/currents/sounds)

d) Check recorded data to take decisions

e) Regularly update routines and protocols

f) Continuous/Regular staff training

g) Regular check in with veterinarian

h) Selective breeding of strains resistant to stress

i) Other, which:

3- IMPROVING WELFARE IN EU FISH FARMING: TRANSLATING KNOWLEDGE INTO ACTION

To improve the welfare of farmed fish at EU level, we propose a framework that relies on a series of assumptions:

i. Fish welfare is important and a common objective for all stakeholders

ii. Fish welfare can be improved

iii. Knowledge gaps still exist

iv. Communication between parties is essential

While the first three points have been discussed in the previous sections, the communication issue is, from our perspective, where there is further room for improvement. In that sense, we believe that exchanging information and knowledge between parties (academia, producers, NGOs, retailers, certifiers, consumers, policy-makers) is fundamental to improve the welfare of farmed fish (note: it may be important to highlight that there is an important difference between knowledge and information. The latter refers to refined, filtered and useful data, whereas knowledge is the understating of the problem arising from integrating various sources of information. This is important because we will use these terms in *stricto sensu* in the following lines). We are aware that information exchange across and especially among stakeholders at the same level (e.g. among companies who might be direct competitors) may be less than straightforward. However, it is not only useful to acknowledge the issue upfront, but also desirable to encourage that the best indicators, methods or protocols to be shared as widely as widely as possible. The advancement of the sector would greatly benefit from such a collaborative approach.

We recommend that the translation of knowledge into practice regarding welfare in fish farming should be made in processes functioning in a logical, integrated framework (Fig Y).

- Gathering **knowledge** about welfare science and the biology of the target species;
- **Monitoring** of the welfare state of the fish, selecting and using the appropriate indicators;
- **Acting** on identified points where welfare can be improved, through planning, refining and/or creating standard operational protocols and methods;
- Exchanging information on **welfare** achievements between stakeholders to generate knowledge.

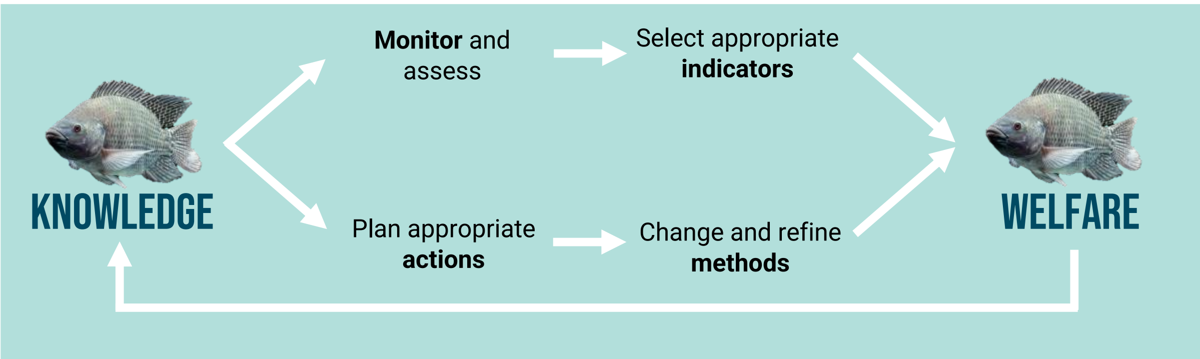

Figure 3 –Proposed framework to translate knowledge into practice in fish welfare. See text for details.

It should be highlighted that not only all these points must be integrated with each other but also they are part of a continuous process. Only through continuous gathering of reliable and representative data can we have an appropriate evaluation of fish welfare, that in turn should generate knowledge to inform the decisions and planning for improvement. In addition, this framework is applicable both at policy level and individual farm scale. Each point is further discussed in the following sections.

3.1 Gathering knowledge

Throughout the present text it has become clear that, other than large technological investment or disruptive new methods, the fundamental requirement to use behaviour as a tool to improve welfare and production in fish farming is in fact knowledge. Therefore, to this this purpose we propose the creation of **training programs**, designed and directed to all levels of staff that are directly or indirectly involved with the animals in a fish farm. Such program could be comprised of 3 levels:

Level I – Awareness (2h): general aspects of fish welfare (sentience, pain, behaviour, general OWIs) directed to technical staff (workers who work at feeding, cleaning, driving, etc)

Level II – Workshop (6h): biological basis of welfare (sensory systems, stress, ethology), OWIs, welfare challenges, technical solutions. Directed to veterinarians, biologists, production managers.

Level III – Full course (18h): sensory systems, sensory worlds, neuroscience of welfare, ethology, positive welfare; environmental enrichment, Precision fish farming, feeding and nutrition, disease, OWIs, technological solutions for handling and slaughter; legal aspects, regulation, ethics. Directed to R&D departments.

Many of these courses already exist to some extent, provided by certification schemes, universities, research centres, NGOs or producer associations. Identifying such courses is beyond the scope of this report, yet we do have a proposal on how to compile, organise and disseminate knowledge transfer, see 3.4. Knowledge and training should ideally be a continuous process. Apart from the general welfare training proposed above, companies should stimulate the technical preparation of their staff to face the challenges and changes the sector will be facing. Note that these challenges my not be only due to policy and regulations and may arise from economic cycles, societal developments or even climate change (Huntingford et al., 2023).

Another positive measure is the drafting of welfare guides of good practice at national level. As an example, the Spanish Association of Aquaculture Producers (APROMAR) recently published the first volume of a series that will not only provide the basic knowledge on fish welfare but also deliver species-specific information, tailored for the Spanish reality (APROMAR, 2022)

3.2 Monitoring and assessment

The theoretical foundations of OWIs and the reasons for behaviours to be good candidates for OWIs were discussed in section 1.3. It is never too much to highlight the importance of training on behaviour of the species and experience with the on-farm reactions of the animals at each facility.

The main assumption for the use behavioural OWIs is that farmers need to be able to see the fish directly (either from the surface or underwater, on site or through cameras, live or through recordings) or somehow infer their behaviour indirectly (for example through water movement upon feeding, harvesting, manipulation, etc). The degree of certainty and the robustness of the information will largely depend on the detail of these observations.

We have compiled a series of behavioural indicators that comply with the criteria to become OWIs for virtually any fish farm. They are presented in Table III.

Table III- Proposed behavioural OWIs. See text for details.

OWIs	rearing stage	base	level	Measurement type	Attributes	reference
Aggression	broodstock, hatchery, ongrowing	animal	individual	High/low	negative (high rate=poor welfare)*	Martins et al (2012)
Exploratory activity	broodstock, hatchery, ongrowing	animal	individual	high/low	Positive (high rate= good welfare)	Martins et al (2012), Roque et al (2020)
Anticipatory activity	broodstock, hatchery, ongrowing	animal	individual	high/low	positive	Martins et al (2012)
Foraging behaviour	broodstock, hatchery, ongrowing	animal	group	high/low	positive	Martins et al (2012), Marino et al (2020)
General appetite	broodstock, hatchery, ongrowing	animal, resource	Group	high/low	positive	Noble et al (2021), Marino et al (2020), Roque et al (2020)
Group swimming behaviour	broodstock, hatchery, ongrowing	animal	Group	shoal/school/disperse	positive	Martins et al (2012), Marino et al (2020), Roque et al (2020)
Individual swimming behaviour	broodstock, hatchery, ongrowing	animal	individual	sustained/prolonged/burst/erratic	positive	Martins et al 2012, Marino et al (2020)
Stereotypical behaviours	broodstock, hatchery, ongrowing	animal	individual	yes/no or high/low	negative	Martins et al (2012), Roque et al (2020)
Surface activity	broodstock, hatchery, ongrowing	animal	Group	calm/frenetic/frenzy/fins appearing	no surface breach=good; bodies emerge=terrible	Noble et al (2020)
Thigmotaxis	hatchery	animal	individual	yes/no or high/low	negative	FishEthoBase.net, Roque et al (2020)
Use of space	broodstock, hatchery, ongrowing	resource	Group	all space used/some parts avoided; species appropriate	positive	FishEthoBase.net, Roque et al (2020)
Vacuum behaviours	broodstock, hatchery, ongrowing	animal	individual	yes/no or high/low	negative	Martins et al (2012)

Ventilation rate	broodstock, hatchery, ongrowing	animal	individual	High/low	negative	Martins et al (2012), Noble et al (2018), Roque et al (2020)
Spawning behaviours	broodstock	animal	individual, group	yes/no or high/low	positive	unpublished

Although there are species-specific considerations to take into account, Table III provides a series of indicators that may be applied for the 5 species addressed in the present report.

In Table III, column 'OWI' refers to the name of the behaviour that is used as indicator. The ethogram (short, objective description of the behaviours) is presented in Table IV below. The rearing stage column identifies to which stages the OWI is suited. The base and level columns refer to which type of OWI they are (animal- or resource-based, measured at individual or group level, see section 1.3). The measurement type indicates if the measurements run from high to low (i.e., are continuous), if they are discrete (with examples of categories) or if they are binary (e.g., yes/no). The attribute designates how the indicator relates with welfare: if high values or presence of indicator have a negative attribute, it means welfare gets worse as values increase; if high values or presence of indicator have a positive attribute, it means welfare gets better as values increase. Finally, the reference column identifies which are the sources of information.

Table IV – General ethogram for selected OWIs

Behaviour (OWI)	Description
Aggression	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)
Exploratory activity	Movements or actions or along the tank that apparently serve to the collection of information about new objects and unfamiliar parts of the environment.
Anticipatory activity	Movements or actions that precede the delivery of feed and indicate that the animals are aware of routine procedures taking place imminently. The most common is food anticipatory behaviour, where the fish are agitated before feeding.
Foraging behaviour	Movements or actions or along the tank that apparently indicate that animal is searching for food. Whenever the animal finds food items it eats them.
General appetite	Food anticipatory behaviour + foraging behaviour + actual eating and/or feeding.
Group swimming behaviour	Type of swimming behaviour that the group of fish is displaying: shoaling (in a group but not directional or coordinated); schooling (in a polarised, directional and coordinated swimming) or disperse (no clear group formed).
Individual swimming behaviour	General type of movement each animal is performing when swimming: regular, fast, slow, erratic bursts, balanced/unbalanced, close to surface, midwater, bottom, next to walls, etc.
Stereotypical behaviours	behavioural pattern that is abnormally repetitive, invariant and with no obvious goal or function.
Surface activity	Movement of the group of fish at the surface upon handling, cleaning or feeding procedures. Varies from calm, with only fins surfacing, to a

	frenzy with whole bodies surfacing or even jumping as a sign of severe stress. (Note that salmon uses surface activity to voluntarily inflate their gas bladder. This species-specific behaviour should be taken in account when using this OWI).
Thigmotaxis	Strong avoidance of open areas and preference for moving in very close proximity of the walls of the rearing environment.
Use of space	Measure of how and how much space of the rearing environment is used by the animal. Related with exploratory behaviour.
Vacuum behaviours	Actions which apparently occur in the absence of any external stimulus or disengaged from their normal elements (e.g. nest building with no substrate)
Ventilation rate	Rate at which the opercula open and close, as a measure or respiration needs of the animal.
Spawning behaviours	Movements, actions and/or displays that lead to reproduction. May include courtship, nest building, egg releasing, fertilisation, parental care or other species-specific behaviours.

The ethogram presents the general behavioural patterns for each indicator but combining this information with species specific knowledge is essential. For example, mild surface activity in smolts and post-smolts of Atlantic salmon is normal and expectable, whereas in seabream or seabass it should interpreted as a warning sign. In addition, the type and intensity of each indicator should be validated as much as possible for every farm or company, since the combination of farming system, production size, technology available and methods used together with species, life stage and purpose renders a unique arrangement (Saraiva et al., 2022b). The internal transmission of information about OWIs is invaluable when interpreted at the light of appropriate training.

To conclude, it is never too late to highlight the need to use as many indicators as possible to monitor and assess the welfare of farmed fish. While the focus of this report is behavioural OWIs, other kinds of indicators should always be available for an integrated view on the welfare of the animals.

3.3 Actions to improve welfare

There are two critical points where the welfare of fish under farming conditions may be improved using ethological knowledge:

i. during their life in captivity
ii. at slaughter.

They require very different approaches and solutions, and the following section will address them.

3.3.1 Improving fish welfare during life in captivity

For the improvement of life in captivity, actions should be directed at improving the rearing environment (i.e. to match as much as possible the general physical, chemical and social conditions found in the environment where the species has developed adaptations for, see section 1.4) and reducing the human-induced stress factors from farming protocols to the minimum possible (see section 2.2), by changing or refining operational methods.

To improve the conditions of the environment, a good tool is **environmental enrichment** (EE): the deliberate addition of environmental stimuli to help captive fish meet with their physiological, behavioural and psychological needs, so they can better cope with the challenges of the farming environment. This definition and scope align seamlessly with all the theoretical background presented in the current report. It is clear that EE can improve the well-being of fish in captivity, providing adequate stimulation to help meet their biological needs, increasing resilience and consequently reducing factors that impair not only welfare but also production. While interest has been focused mainly on structural enrichment, there are many other enrichment strategies that merit attention (e.g. sensorial, occupational, social and dietary enrichment) and which may be of interest for fish farming. A review published recently by Arechavala-Lopez et al. (2022) has gone through the different types of EE strategies, their pros and cons and devised a decision-making scheme to be followed in farms. The following part is based on this work. For streamlining purposes many references are not presented here, yet all the entries are based on published data and they should be checked in the original paper.

Physical enrichment consists of adding physical complexity with structures, objects or any structural modification to increase heterogeneity of the rearing environment (Näslund and Johnsson, 2016). Some species use substrate or shelters in their natural environment, and, therefore, may also make frequent use of physical enrichment when in captivity. Such enrichment strategy can be created with a wide variety of features in many shapes and sizes, and they can be classified into two main types:

1) *Structures* – These objects can provide shelter or simply add heterogeneity and complexity to the rearing environment. Studies on different salmonids showed that **plastic tubes and shredding** not only can improve growth and survival, but also swimming agility and physiological stress response (e.g. reduced plasma cortisol levels) when presented with stressors (e.g. air exposure, handling, crowding), as well as decreased fin damage and related fin infections. Indeed, studies on the Atlantic salmon (*Salmo salar*) demonstrated that adding complexity in the rearing environment not only promotes cognitive abilities and improves brain plasticity but also decreases parasite occurrence and improves infection resistance and survival. In addition, the use of **nets** in hatchery and nursery tanks is known to be widely used in Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) farms (Arechavala-Lopez P. *pers*. *obs*.), with the aim of disrupting their circular swimming pattern when shoaling, thereby reducing vertebral malformations, although this has not yet been empirically tested. In these same two species, **suspended ropes** have been shown to have positive effects in a series of welfare indicators without any negative issues (Arechavala-Lopez et al., 2022b, 2020, 2019; Oliveira et al., 2022). It is also known that some aquaculture companies use submerged rings that release curtains of **air bubbles** inside the net-pen (Sea Pen Aeration systems; KAESER ®, https://www.kaeser.com/int-en/solutions/aquaculture/), which increase the oxygen saturation of the water, thus lowering the feed conversion ratio, as well as preventing algae and plankton (including planktonic stages of sea lice *Lepeophtheirus salmonis*) from entering the sea cage, and improving the overall health of the fish (Kadri, S. *pers*, *com*.). The air bubbles themselves (which may fall under the structural or occupational enrichment classification) have been demonstrated to have positive effects in the welfare of Rainbow trout (Kleiber et al., 2022).

2) Substrates - Providing floor substrate (**sand, pebbles, gravel, stones**, etc.) can be seen as another type of physical EE to improve or guarantee the welfare of fish, mostly for those species that regularly interact with the bottom or live closely associated to it during its whole life (e.g. benthic fish). Some studies on Gilthead seabream juveniles revealed that adding a uniform layer of single colour **glass gravel** as enrichment in rearing tanks can induce positive effects on fish condition and growth performance (i.e. final mass, specific growth rate, mass gain, food conversion ratio), and also reduces aggressiveness, increases fish-bottom interactions, improves the stress response (reduce brain serotonergic activity) and promotes better fillet quality. Although different **bottom colours** and densities can lead to different effects, some authors authors pointed out that improvement of the fish rearing environment with substrate may have multiple beneficial aspects for both fish welfare and producers. Substrate enrichment can also be applied to incubation pro- cesses. Salmonid alevins (yolk-sac fry) hatch from eggs buried in gravel and spend the first stage of their life within this substrate. Adding **hatching mats** to the bottom of the tanks provides a wide range of positive effects, as it has been demonstrated on different salmonid species. These hatching mats improve growth and survival of alevins, reduce yolk-sac constrictions and improve yolk conversion efficiency, reduce alevins swimming activity and malformations and permit resting on the bottom in normal body-position. Hatching mats also promote positive physiological changes, increase brain growth and decrease high activity and oxygen consumption due to stress. Indeed, several commercial salmon hatching mats are already available.

3) Combinations - Physical EE can provide shelter, substrate and complexity in a rearing environment at the same time, and can also allow the cohabitation of different species. This is the case of enrichment structures for cleaner fish in salmon aquaculture. Some authors demonstrated that juvenile lumpfish are able to adhere and rest on **smooth flat vertical or floating plastic surfaces**, which may mimic their natural requirements for surface adhesion. Structures made of pipes or artificial kelp are also provided to wrasse stocked in commercial salmon cages, providing shelter and resting places for their overnight inactivity. Consequently, and given the quick expansion of the use of cleaner fish in commercial salmon cages, several companies manufacture a varied range of vertical substrates or 'kelp curtains', resembling **artificial kelp made of PVC**. In this line, Leclercq et al (2015) developed sinking hides of plastic fake-kelp for ballan wrasse (*L. bergylta*) stocked in commercial salmon cages. These structures had hanging feeders or 'feed blocks' (**water-stable agar-based diet on PVC pipes or trays**), forming altogether a complex vertically suspended shelter and supplementary feeding for cleaner fish. These feed blocks were also specially designed for lumpfish—which quickly accepted and grazed on them—and successfully reduced the prevalence of cataracts compared to supplementary pelleted-commercial feed. Kelp-curtains, shelters and feed-blocks can be used for any cleaner-fishes in farming conditions. However, it is important to highlight that, given the behavioural and biological differences amongst species, they must be specifically designed for each cleaner-fish species. Moreover, the farming strategies and rearing conditions should also be taken into account to avoid undesirable effects. A further point worth considering when providing kelp-curtains, shelters, and feed-blocks for cleaner fish that coexist with Atlantic salmon in commercial cages, is that the complexity of the rearing environment is increased for both species and, of course, such provided structures must not cause any detrimental effect to either co-habitant's welfare.

In summary, structural enrichment is probably the best- known EE strategy and, therefore, the most used from laboratories to farms nowadays. Physical EE can provide shelter, substrate and complexity in a rearing environment at the same time if both types are combined. It is important to highlight that all these measures must be validated for each case before implementation, as their incorrect deployment can bring negative results.

Sensory enrichment is the addition of biologically relevant sensory stimuli to arouse the diverse senses of fish. To successfully provide sensory stimuli and implement sensorial EE in captive environments, it is essential to have a good knowledge of the biological needs and the sensory worlds of the targeted species. This is especially relevant for fish, given that there are substantial differences in their sensory systems compared to terrestrial animals, due to differing ecological and evolutionary pressures. In order to improve the welfare of captive fish, different sensory stimulations may be explored as potential methods of EE for these animals, including visual, auditory, chemical (olfactory, taste), hydromechanical, and electrical stimuli.

1) *Visual stimuli* - The diversity of fish visual systems, which might change during their life-history, or even within life stages, together with the enormous variety in eye anatomy and brain structures that process visual information make visual enrichment very challenging. In addition, light behaves differently underwater than at the surface, and can not only be influenced by many physical and biological factors, but also fluctuates within daytime, season or natural weather conditions. In indoor facilities, classic light bulbs (incandescent filaments) produce a reddish inefficient light underwater, whilst fluorescent tubes produce sharp peaks at specific wavelengths far from natural daylight. However, **modern light-emitting diode (LED)** technology provides versatile and better cost-effective lighting systems which can be used for different purposes in aquatic research and captive environments. Wright et al (2015) showed how Atlantic salmon instantaneously follow vertical light movements in sea cages. The authors suggested that positioning of lights may help move salmon away from fluctuating unsuitable depths (e.g. lice-rich depths) into temporary favourable depths (e.g. surface brackish waters to treat against stenohaline parasites), and throughout cages to avoid crowd- ing in narrow depth ranges. However, responses to different light conditions may vary depending on the species, especially due to specific physiological needs or behaviours (e.g. phototaxis), and also on the adaptation to different environments within the same species (e.g. living at different latitudes). Tank colour and depth, together with light source and water clarity, impact the degree at which light is absorbed, reflected, scattered and attenuated in the rearing environment. McLean (2021) reviewed the effects that **tank colour (floor and walls)** may have on various physiological and behavioural processes in larval and post-larval fishes. The au thor compiled a vast number of studies on a wide range of species demonstrating that different background colours can influence fish performance and survival, health, level of stress and even level of aggressiveness; effects that can be negative or positive depending on species and life-stage. Diverse patterns of wall and bottom tanks can be also applied as sensory enrichment, stimulating the visual system. Once again, all of these must be tested and validated *in situ* prior to implementation.

2) Auditory stimuli - human activities in aquatic environments generate a wide range of waterborne noises and, consequently, fish are subjected to ex- treme levels of acute (transient) and chronic (continuous) noise, both in natural and cultured conditions, which may negatively affect their stress level and welfare. Therefore, it is essential to take the appropriated measures (e.g. **isolation, insulation and adequate materials, spatial planning, etc.**) to reduce background noise-related impacts, ensuring good welfare conditions of farmed fish. This approach might be considered a strategy closely related to a kind of sensory enrichment, since it masks or reduces negative auditory stimuli. This **reduction of background noise** might also allow better communication through naturally-generated sounds of the captive species, which are produced in various behavioural contexts (agonistic interactions, court- ship, spawning, distress). On the other hand, adding **natural soundscapes** (background sounds specific to a species' natural habitat) is already considered an EE strategy through sensory stimulation in captive animals, and even though some studies suggested the potential use of sea soundscapes for marine species, further studies are needed in this matter. Some studies have assessed the potential effects of adding **background music** (i.e. rhythmic or systematic sound not typically found in the wild) on cultured fish. For example, it has been demonstrated that musical stimuli (**usually classical or downtempo music**) positively influence growth performance, feeding efficiency and stress reduction on common carp, Gilthead seabream and Rainbow trout, mostly reared in recirculating water systems (see Arechavala-Lopez et al, 2022 for a full list of references).

3) Chemical stimuli : olfaction, taste and chemosenses - Chemical senses play an essential ecological role (fish-environment interactions) and are extremely relevant in communication contexts in all fish taxa (cyclostomes, elasmobranchs and teleosts). In this context, **manipulation of odours or other chemical stimulations**, whether in the form of olfactory stimuli that are specific or non-specific to an animal's natural habitat, or pheromonal in nature, have been proposed as potential EE for captive fish. This form of EE must be taken with great caution, performed by trained specialists and throughly tested, since chemosensing is not familiar in humans and its effects on water are diffucult to predict. Regarding feed-related stimulation, food chemical signals may function in two ways as enrichments: **feed attraction and feeding stimulation**. In the first case, enrichment may rely on the use of attractants for faster detection, possibly reducing energy expenditure for the fish whilst mainly reducing waste (with the consequential positive effects on water quality and feed cost). In the second case, feeding stimulants have an effect on satiation and modulate food ingestion, with relevant effects on growth. These stimulants are different for carnivorous and herbivorous fish and there is at least theoretical potential to use chemicals to stimulate and enrich the environments of farmed fish whilst reducing the ecological and social impacts of forage fisheries. However, other ecological problems may arise in certain farming systems (cages, ponds) where feeding stimulants could be detrimental for local fauna.

4) Tactile stimuli - Fish are widely covered by tactile receptors and may also possess various tactile organs, mostly cutaneous outgrowths (e.g. barbels, free rays of fins, rostrum, breeding tubercles, or dermal teeth). Tactile organs are highly significant in orientation, reproduction, defence, social interactions, exploration and food searching behaviour. While in the wild there are multiple examples of beneficial effects of tactile stimulation between cleaner fish and their clients, in aquaculture, however, whether the **tactile stimulation of cleaner fish** (e.g. lumpfish and some wrasse species) reduces the stress and social conflicts on the clients (i.e. salmonids) or not, remains to be fully validated. A number of anecdotal reports suggest so, but require proper evaluation.

5) Hydromechanical and electro-sensing stimulation - the stimulation of these sensory systems in captivity is not yet assessed, and may be indirectly addressed through other enrichment strategies, such as social, occupational, or even physical enrichment, as well as by good welfare practices and management at fish farms.

Occupational enrichment is the introduction of diverse challenges into the rearing environment that are important to prevent monotony and, consequently, boredom. Occupational enrichment can encompass devices that provide animals with challenges or control over their environment, as well as enrichment items encouraging physical exercise. This can range from hydrodynamics (flows, currents, etc) that induce exercise, fine tuning variability and predictability in rearing protocols, or possibly introducing play features to allow play behaviour.

1) Hydrodynamism - The exercise levels and swimming capacity of fish cultured in ponds, recirculating systems, raceways and cages are generally lower than those in the wild, but depend heavily on species, life-stage of development, and rearing systems. Inducing the fish to swim in a certain **water-flow** can promote swimming exercise and could represent a natural, non-invasive, and economical approach to improve growth, resilience, robustness and welfare. Optimal exercise may have beneficial effects of major importance for aquaculture and, therefore, is a potential occupational enrichment strategy to be considered by the industry. Exercise-induced growth is optimal at specific speeds, most likely near optimal swimming speeds where the cost of transport is the lowest and the energetic efficiency the highest. At swimming speeds below optimum, energy is lost due to higher spontaneous activities (e.g. flight responses), whilst at higher speeds, swimming becomes unsustainable, stressful, and can finally cause fatigue. It must be noted that critical swimming speed varies with group shoaling behaviour, and also that fish densities and other structures can alter the water flow. In salmonid fish, the stimulatory effects of sustained moderate swimming on growth performance have been widely demonstrated. When juvenile salmonids are reared in flowing water (0.75–1.5 body length per second), they tend to grow faster, making more efficient use of the food and showing uniformity of growth rates and a reduced size range at harvest. It also known that these effects may be variable and sometimes even negative – for example there are reports of malformations being induced by continuous swimming in circular enclosures in Seabream. Currents, intensities and intervals must therefore be validated at each farm before deployment.

2) Predictability and variability – In fish farming, environmental predictability reduces the uncertainty that animals are exposed to, improves their cognitive skills, such as learning and spatial memory, and favours engrained behaviours. However, human-induced environmental predictability can create evolutionary traps that are detrimental to an animal's fitness; for example, poorly planned predictable feeding may induce high competition and thereby increase dangerous or lethal injuries as well as stunted growth. The right **balance between predictability and variability** is necessary, adjusting the variability within predictable events to ensure that animals do not get too accustomed to the same exact routines, and thus do not reach allostatic overload when they are exposed to unpredictable events. Several studies have shown that fish can be trained to predict events via classical conditioning. Thus, fish can be trained to predict negative events and then habituate to stressors, inducing a low physiological stress response, as shown in Atlantic salmon parr, and recently in trout (Kleiber et al., 2022). On the other hand, predictability of a positive event, such as feeding, can be detrimental for the welfare of fish in some species, such as Atlantic salmon parr, to which it induces higher levels of aggression. Moreover, spatially and temporally predictable feeding regimes in brown trout induced aggression and territoriality, which increased growth in individuals with high resting metabolic rate. However, implementing unpredictable feeding regimes as an alternative strategy can also be detrimental to welfare, as been shown for Salmon and Seabream. All these studies bring forward the importance of considering the stimulus valence (i.e. whether a stimulus is positive or negative) when studying stimulus salience (i.e. whether it is predictable or unpredictable). Cerqueira et al. (2020) showed in European seabass that an unpredictable negative stimulus (confinement) increased shoal cohesion and freezing and escape behaviours, reduced exploratory behaviour and increased cortisol levels and neural activation of brain areas related to fear, compared to a predictable negative stimulus, meaning that an unpredictable stressor triggers a stress response in this species. In light of such results, farmers should therefore test how predictable or variable their routines should be according to their species, life-stages, method, etc, in order to improve the welfare of their animals, and consequently their production.

3) Play and joy – Fish display behaviours likely to involve positively valenced experiences, or even likely to have the ability to play. Various studies identified three different play behaviour subtypes in fishes: locomotor (e.g. bubble jets/air stone), object manipulation (novel/stimulatory) and social (including human interaction). Regarding species of aquaculture interest, it is known that salmonids and other fishes can jump into the air from the water, which is highly relevant in net-pen culture since this be haviour can be related to buoyancy regulation, parasitic infections or stress. Some authors, however, suggested that some salmonid jumping behaviour may be also a form of play. Encouraging play behaviour, therefore, might be considered as occupational EE, though whether it involves positive emotions in fish is still under debate, and further research is still needed in this field.

Social enrichment comprises not only the presence of other individuals and their social interactions, but also the availability of space to interact or avoid other fish, either conspecifics or different species. In this sense, it is important to know whether the reared species is solitary, or likely to shoal in small or big groups at different life-stages, as well as if they usually co-habit with other fish species in the wild. For example, many fish species form shoals in the wild and thus, in captivity, these species may suffer in isolation or in inappropriate spaces to properly shoal. On the other hand, many farmed species that do not shoal in the wild, or associate with other species, are territorial and can engage in aggressive behaviours with conspecifics, which in both cases may be a big problem in the high stocking densities of captive environments. **Stocking density** is one of the major aspects to consider in improving fish welfare in monospecific intensive aquaculture, which is the most widespread practice. Diverse studies have demonstrated that different stocking densities can have direct effects on the stress response, growth rate, health and condition of intensively farmed fish. Appropriate density depends heavily on the behavioural and physiological requirements of each farmed species, as well as on life-stage, rearing system, food availability, social interactions and other environmental parameters (i.e. variations and alterations of water quality). The assessment of welfare using robust OWIs, combined with a realistic feasibility analysis and use of good practices is the best tool to determine the most suitable density for each case (Saraiva et al., 2022b). Social interactions are also influenced by the physiological and behavioural differences in stress responses of each individual within a population or rearing unit, namely stress copying styles or personalities, which can have relevant consequences for aquaculture. In addition, several studies have demonstrated the ability of diverse farmed fish to recognise familiar conspecifics and consequent positive effects on social interactions. Familiarity (i.e. **maintaining the reared population unchanged**) stabilises the hierarchical structure of a group, and governs behavioural modifications (e.g. agonistic behaviours) that promote feeding and growth, leading to higher fitness and survival. Therefore, besides being affected by densities in relation to space, food distribution and food quantity, social interactions are also affected by familiarity and personality, and all these factors might be modulated through social enrichment, but also through feeding strategies (see dietary enrichment).

Dietary enrichment refers to the food type or feeding strategy (distribution, quantity, periodicity, etc.) which mostly affects foraging behaviour or food intake, but it does not include the composition of the diet (which would be considered "internal or nutritional" enrichment). Feeding strategies play an important role here as dietary enrichment, given that feeding regimens, schedules and procedures can highly affect, positively or negatively, fish welfare status. An appropriate feeding strategy adjusted to the biological needs of each species and life-stage can help control foraging behaviour and reduce undesired behavioural responses and social interactions. However, foraging behaviour is one of the widest and most complex areas of investigation, and it is difficult to develop a universal feeding strategy. Many species-specific factors are involved in the feeding strategies and tactics of fish, such as feeding rhythms, food ratio and feeding time. In general, **self-feeding systems** improve fish welfare, allowing fish to choose their optimal feeding time and food ratio. However, automatic feeders can be used to deliver small quantities of feed at short intervals, whereas hand feeding allows better observation of fish reaction to the feed and reduces feed wastage, although increasing the labour demand. Farmers can also use **feed spreaders** that facilitate a more uniform and automatic distribution of feed throughout the rearing unit. It is noteworthy that a **combination of feeding strategies** appears most appropriate, but the observation of fish feeding response is essential and allows a quick adjustment of feeding strategies and diets, as well as a reduction of feed waste. Therefore, fish hunger and food availability are the main factors affecting fish welfare and effective production. Regarding formulated diets, they can be of different sizes and shapes, flavours, enhancers, texture, palatability and colour. Feeds can be also **formulated to sink or float** depending on where the fish usually feed within the water column. The actual act of eating may be rewarding in itself, in addition to the nutritional aspects, so the formulation of feeds according to how they behave in the other may be important to mimic natural prey behaviour for carnivorous fishes. Taste preference or evaluation of sensory quality of grasped food items is a well-developed sense in fish, consisting mainly of the gustatory system. Such preferences in fishes are widely known for the many carnivorous species of farming interest, but also for some herbivorous species, and such knowledge is highly relevant for the feeding behaviour and preferences of each farmed species, also considering its life-stage and rearing systems, in order to improve the welfare status of captive fish.

In summary, it should be highlighted that the effects of different EE often vary in direction and magnitude, and highly depend on each species and life-stage needs, preferences and natural history, combined with the characteristics of the fish farming system. To successfully implement an EE strategy there are several steps to follow, which are presented in Figure 4. **We highly recommend the reading of Arechavala-Lopez et al (2022a) for further information and a full list of references, that may not be present in this section for streamlining purposes.**

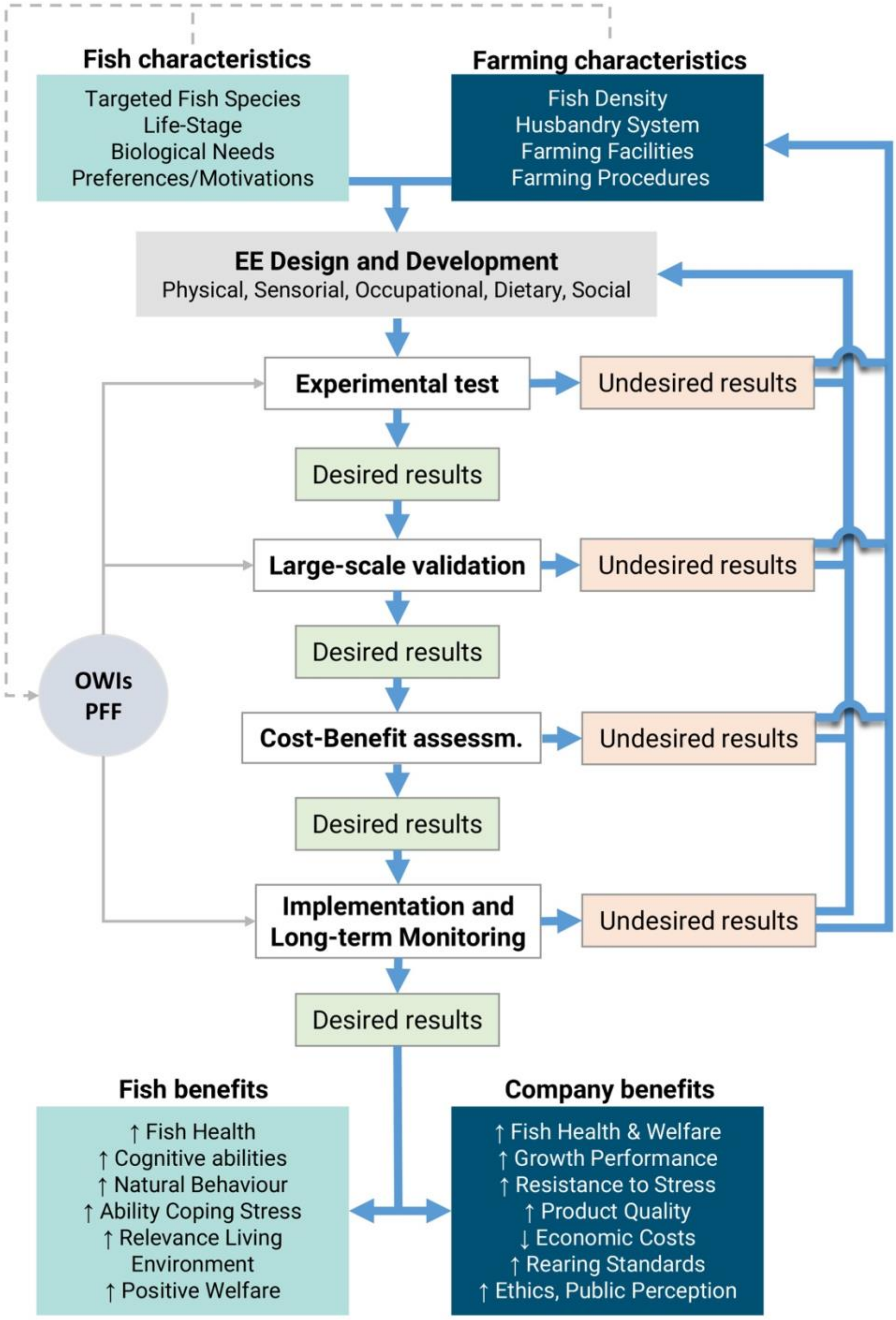

Figure 4. Decision-making scheme about the procedures from designing and developing environmental enrichment (EE) strategies to validation, final implementation, and monitoring at commercial scale, to obtain both fish and company benefits in fish aquaculture. OWIs: Operational welfare indicators. PFF: precision fish farming. Adapted from Arechavala-Lopez et al. (2022).

Another identified sensitive point is the handling, in which we include events described as 'Human induced challenges' from section 2.2, Fig 2: transport, vaccination, grading, sorting, etc. Keeping the ethological focus of this report in mind, the main stress inducing issues arise from the fact that farmed fish species are not equipped to deal with highly artificial factors such as those referred above. While the challenges have already been discussed in section 2.2, the actions proposed are generally to implement and develop **least invasive handling techniques** in all protocols, such as:

- passive grading methods whenever grading is necessary
- passive methods to move fish when tanks that are connected
- use of adequate pumps instead of brailing
- administration of anaesthetics whenever manipulation is required*
- managing and limiting crowding to the minimum time and density possible

* It should be noted that anaesthetics may reduce stress and thereby improve welfare but can also have unwanted side effects that reduce the welfare of the fish and should therefore always be used with caution. Based on the literature and our own experience, we recommend that anaesthetic protocols should always be tested on a few fish under prevailing conditions to ensure an adequate depth of anaesthesia, and that the secondary effects of anaesthesia do not override those of the procedure itself. For a review see Zahl et al (2012).

In any case where the fish should be handled, movements should be gentle and performed by trained staff, and animal welfare state must be monitored at all times, namely using the OWIs in Table III. Interestingly, the use of EE prior to and after transport has demonstrated to be an effective method to mitigate the effects of handling stress in many cases (Arechavala-Lopez et al., 2022a). The implementation of the recommendations above should nevertheless take into account the farming system, farm size, staff number, species, life-stage, etc.

For the specific case of live transport, there are several phases where welfare infringements may be an issue. Figure 5 summarises these, as well as the actions to be considered to safeguard the welfare of fish. The next lines are based on Saraiva et al. (2021b) and van de Vis et al. (2020).

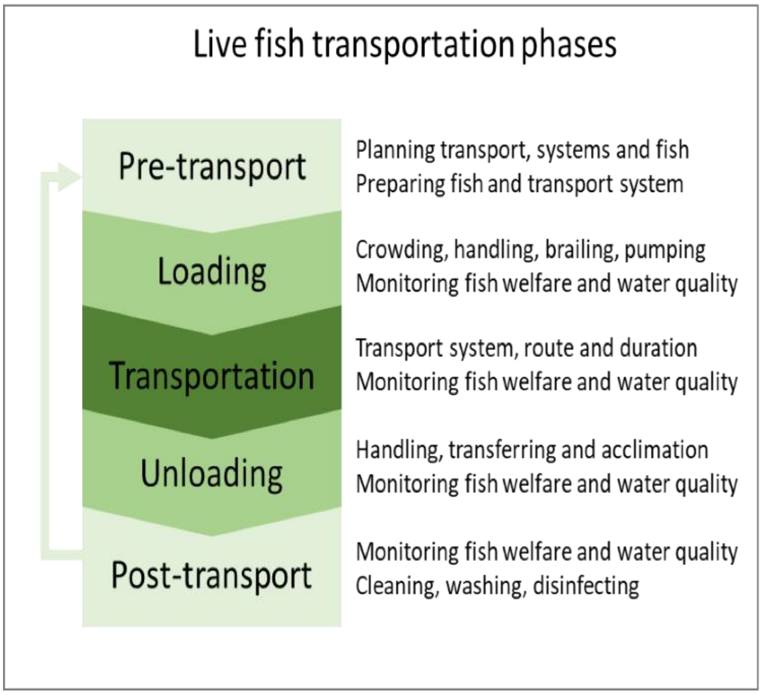

Figure 5- Transport phases, welfare issues and mitigation measures. Adapted from Saraiva et al. (2021a).

During the pre-transport phase, specific attention must be paid to planning the transport, especially to the quantity and condition of the fish, preparation of the animals prior to transport, its system and route, the loading and unloading procedures, the post-transport procedures, and welfare-monitoring program at each step. Planning every aspect related to live fish transportation in advance gives full control of the procedure, ensuring the best possible welfare conditions to farmed fish during transport and also ensuring mitigation and contingency plans. Time spent in each phase of live transport (pre-, loading, unloading, etc.) must be minimized whenever possible. Limits should be established and monitored by an aquatic animal veterinarian or trained staff to assess and correct any negative welfare implications throughout the process.

The loading of fish is a crucial phase with high possibilities of having an impact on the welfare of the fish, and evidence suggests that this part of the transport process is the most stressful phase for most farmed fish species. In many aquaculture systems, the loading process begins by crowding the fish using nets and then transferring them by hand-nets, brail nets or pumping into the transport container or vehicle. The welfare impacts of loading can be reduced by various methods that allow the fish to be maintained in water, or at least reduce the time the fish are out of the water to an absolute minimum. Physical contact between fish and other surfaces, dropping fish from pumps or elevators, handling before loading, and the loading itself cause poor welfare. Crowding can be particularly stressful and it should be performed in such a way that fish do not show signs of distress.

The transport phase consists of moving fish from the sender to the receiver and it can be challenging and unpredictable. During this phase the principal concern is for maintenance of satisfactory water quality (e.g. oxygen, carbon dioxide and ammonia levels, pH, temperature, and salinity) appropriate to the species being transported. Deterioration of water quality during transport is the most significant animal welfare issue for transporting live fish, especially the depletion of oxygen or accumulation of carbon dioxide and ammonia. Constant monitoring water parameters and the welfare status of the fish using OWIs is the key for less stressful transport events.

Unloading can be a critical phase and fish should be unloaded as soon as possible, given that similar to the loading phase, fish stress increases significantly during this phase. Unloading manoeuvres are diverse, but pumping fish and water through a pipe/hose into the new tank or net pen is the most common and extended practice, and fish are never exposed to air. Similar to loading, physical contact between fish and other surfaces from pumps or nets, and handling and dropping fish from pumps or elevators, cause poor welfare. Pumping and poor handling may result in physical injuries, particularly to the fins. Damages to the fish might also occur during unloading with pumps if water speed and g-force is too high, causing fish to hit walls, or misfitting joints, or sharp edges in the transport hose/pipe.

The post-transport phase is highly relevant to ensure good welfare conditions of transported fish and to identify possible problems and solutions. It can also potentially affect the welfare of the next fish group in the transport. The post-transport phase includes monitoring fish welfare, identifying any damage or poor condition, as well as losses due to any aspect of transport, including loading and unloading, and any increased incidence of disease in the days after unloading. After delivery, the haul and circulation systems are cleaned and washed after unloading, removing all organic material, followed by disinfection. Removal or disinfection of transport water is also considered in this phase.

Besides these general measures, we propose a continuous effort to refine protocols. This can be achieved through regular training of staff, good record keeping (including recording videos of operations) and general good management practices.

3.3.2 Improving welfare at slaughter

Regarding slaughter, it is imperative to **implement stunning methods before killing** the animals. The implementation of humane stunning methods, according to EFSA (2009a, 2009b, 2009c, 2009d) should take into account the welfare of the animals in the whole process: fasting, selection, collection, transport, handling and stunning protocol. The most important hazards in the pre-slaughter phase are associated with crowding and transfer by pumping or brailing. A period of fasting is needed to reduce the metabolic rate (and thus the physical activity of the fish which may reduce distress associated with transport) as well as to minimise the amount of faecal matter an ammonia compounds that is inevitably released during the pre-slaughter phase, namely at crowding and eventual transport. The accumulation of faeces, undigested feed and ammonia may quickly and severely compromise the water quality. It must be noted, however that food deprivation can result in the utilisation of body fat reserves and even functional tissue which is associated with poor welfare. In closed systems, there are a number of issues that need to be addressed to ensure good fish welfare at slaughter such as to ensure good water quality, e.g. adequate levels of dissolved oxygen. The effect of elevated levels of carbon dioxide, ammonium and total organic carbon, as well as low pH on the welfare of the fish needs to be addressed. Regarding crowding, this procedure is always a welfare hazard but can be minimised when handled properly. There is a high risk that fish are subjected to metabolic stress, handling stress and poor welfare (exhaustion) prior to slaughter. Crowding of fish should not be performed to a level that they show signs of distress, and indicators for distress include colour change, escape behaviour and air gulping. Exposing fish to air and mechanical forces during harvest (e.g. using brailing) should be avoided as it causes a major negative impact on their welfare. Instead, the use of pumps should be considered whenever possible. Fish should be monitored when exiting the pumping system where the presence of fresh injuries and exhaustion are indicators of poor welfare. After pumping, there should be visual checks for wounds and injuries and any causes of these rectified.

Independently of stunning methods, the consciousness state of stunned animals must be assessed. This is a crucial step, that will determine the success of the implementation of the stunning method. To be effective and humane, unconsciousness must be achieved instantly, regardless of method. Currently, we can only measure brain activity in laboratory settings, so in less controlled settings (i.e. the slaughter facilities and context) we must rely on pre-established animal-based indicators of consciousness (Retter et al., 2018). These are behaviours and clinical reflexes that are known to be associated with certain brain activity. Different indicators provide information on different aspects of brain function, and using multiple indicators in combination provides a more robust assessment than any single measure alone (Boyland and Brooke, 2017; Terlouw et al., 2016). The operational indicators of consciousness that can be used in farm environment are summarized in Table V.

Table V – Operational indicators of consciousness in fish that are applicable in farming context. Adapted and updated from Boyland and Brooke (2017).

indicator	observations	conclusions	reliability
Test for the **vestibulo-ocular reflex (VOR),** known as "**eye roll**" by rotating the fish and observing any eye movements.	Presence of eye roll	The fish is likely to be conscious	Good indicator of state of consciousness for many species as this is one of the last things to be lost during anaesthesia and one of the first to appear upon recovery (Kestin et al., 2002).
In an unconscious fish the eye is fixed in the skull when the fish is rocked from side to side. In a fish retaining some brain function, the eye rotates dorso-ventrally when the fish is rocked (EFSA, 2004)	Absence of eye roll	The fish is likely to be unconscious	Caution: unreliable for fish that have been live chilled (European Food Safety Authority (EFSA), 2004). Some species may retain brain activity after losing eye roll (Bowman et al., 2020).
Assess **opercular movement** (breathing)	Presence of opercular movement	The fish is likely to be conscious	Caution: This is an Insufficient indicator of consciousness. Fish may maintain brain activity without opercular movement (Bowman et al., 2020; Kallstenius and Grans, 2022). May however indicate sedation, or progress towards unconsciousness.
	Absence of opercular movement	The fish is likely to be unconscious, but **ONLY** if paired with absence of eye roll	

It must be noted there is a discrepancy between activity measured in the brain and operational indicators of consciousness that may be observed. However, the cessation of opercular movement coupled with the loss of eye roll are, to the best of our knowledge, the top operational indicators of unconsciousness that are measurable in farming context, similar to the palpebral (blinking) reflex in cattle (Verhoeven et al., 2015) that becomes absent only the deeper regions of the brain cease lose their activity. On the other hand, it is plausible that some brain activity may persist when animals are unconscious. We remain confident that the advances of research in the near future (see part 4 or this report) may bring new operational indicators that may confirm unconsciousness in a more reliable way.

There are many methods to stun fish before slaughter, although not all are effective nor are they applicable at commercial scale. In this sense we will exclude form our description asphyxia on air or on ice, death by hypothermia on ice slurry and gas stunning. These have been proven to be inhumane methods that cause intense suffering for a long time (in the case of ice) or in a shorter period, yet deemed unacceptable by EFSA standards (European Food Safety Authority (EFSA), 2009a, 2009b, 2009c, 2009d, 2004). However, several methods for humane stunning in fish farming are already available, that have the potential to induce rapid unconsciousness if correctly applied. Note: the following section is based on Boyland and Brooke (2017).

Automated percussive stunning

The principle of percussive stunning is that the head is struck with a non-penetrating device, at a force sufficient to stun or kill instantaneously. An effective blow causes the brain to strike the inside of the skull leading to disruption of normal electrical activity in the brain due to the sudden, massive increase in intra-cranial pressure followed by an equally sudden drop in pressure (Humane Slaughter Association, n.d.). The consequent damage to the nerves and blood vessels causes brain dysfunction and/or destruction and impaired blood circulation (Humane Slaughter Association, n.d.). This can be done manually with a 'priest' (a wooden or plastic club), or with an automated percussive stunning machine. The effect and duration of the stun depends on the severity of damage to the nervous tissue and the degree to which the blood supply is reduced (Humane Slaughter Association, n.d.). This is determined by the force and velocity of the blow, as well as the weight and shape of the hammer or club (EFSA, 2009b). Percussive stunning is often followed by a killing method – usually a gill cut. This may also be performed automatically by the percussive machine, within a few seconds following the percussive blow to stun.

According to the World Organisation for Animal Health (previously known as OIE), percussive stunning enables humane slaughter for several fish groups when applied correctly and when death ensues before consciousness can return (OIE - World Organisation for Animal Health, 2010). However, several risks to welfare are associated with this method. For fish killed by hand-held, manually-fed percussive systems there is a risk of asphyxia (suffocation) (European Food Safety Authority (EFSA), 2009b). Misstuns can occur, for example when the blow is delivered to the snout rather than the correct part of the head, and size variation between fish is one reason this may happen (EFSA, 2009b). Ineffective stuns can lead to paralysis without loss of consciousness and pain and distress from injuries. Possible injuries from percussive stunning include eye dislocation, eye bursting or rupture, and haemorrhaging (Roth et al., 2007). When ineffective blows are not followed by a corrective stun, fish may be exsanguinated (bled out, usually via gill cut) and/or eviscerated (gutted) while conscious (European Food Safety Authority (EFSA), 2009d). Therefore, percussive machines should not be used if fish are likely to be injured, not stunned effectively or not rapidly killed (often because of their size or orientation in the machine). Adjustment of percussive machines according to fish size should be done by skilled personnel (EFSA, 2009d).

Electrical stunning

According to the OIE (2010), electrical stunning can enable humane killing for some fish groups, providing that death occurs without fish regaining consciousness. Generally, electrical stunning works by stimulation of the higher nerve centres in order to "cause their dysfunction, either by induction of epileptiform activity or by complete cessation of function" (Robb et al., 2002). Electrical stunning should be followed by a separate killing method such as gill cutting, percussive blow or decapitation. The combination and timing of these two procedures will determine whether the overall slaughter method is effectively humane.

An electric current is delivered to fish via two electrodes in these systems, of which there are several variations:

- *Head-only electrical stunning*: fish are removed from their holding water and placed head-first into a stunner which delivers an electric current to the head.
- *Dry electrical stunning*: fish are removed from water and passed over a conveyor belt which acts as one of the electrodes, with a chain of plate electrodes (steel flaps) hanging above to complete the electrical circuit. This method is only considered fully humane if the fish enter the stunner head first. A variation on this system is what is often referred to as a 'semi-dry' system, which is as above but fish are sprayed with water before passing over the conveyor belt.
- *In-water electrical stunning*: fish are exposed to an electric current in water, e.g. while pumped through a pipe containing two plate electrodes (continuous flow system) or in a tank (batch system).

As handling and removal from water is a stressor to fish, systems that stun in-water may have the highest potential for humane electrical stunning (Lambooij, 2014). In dry and semi-dry stunning systems pre-stun shocks can be caused, for example, by fish entering the machine tail first or because muscle spasms cause them to lose contact with the electrodes.

Effectiveness of electrical stunning parameters is dependent on the species, number of fish, weight, size, and other variables. Water conductivity varies greatly and influences the strength of the stun; when water conductivity is high a lower field strength is required for stunning (Farm Animal Welfare Committee, 2014).

Insufficient electrical current, voltage or duration can lead to unsuccessful stunning which can be very painful and cause injuries to conscious fish (van De Vis et al., 2003). Alternatively it can mean fish regain consciousness after, for example, having their gills cut, and will experience significant pain and suffering. Ineffective electrical stunning can also lead to immobilisation, where the body is motionless and unresponsive in reflex tests but brain activity shows that the fish remains conscious and likely to be sensible to pain (Kestin et al., 2002; Robb and Kestin, 2002). Therefore, behavioural measures alone are not reliable for assessing electrical stun efficacy unless validated with brain activity data.

For commercial applicability, the effect of electrical stunning parameters on product quality will also be considered. Applying an electric current to a fish stimulates the muscles and causes them to contract. When incorrectly applied, this can lead to damage to the spine, dorsal aorta or veins, causing haematomas in the fillet (Hauck, 1949). The current direction (i.e. alternating or direct), field strength and frequency will determine the risk of injury to the fish and subsequent damage to the fillet (Lines and Kestin, 2005; Roth et al., 2004). For industry adoption, electrical parameters must be strong enough to stun effectively, while minimising any negative effects on quality. Modern methods and equipment, when properly applied and validated, do not present signs of carcass damage.

Combined electrical and percussive systems may be a good option for some species to reduce the risk of mis-stuns, as fish that are electrically stunned beforehand may be easier to align correctly in the percussive machines.

Anther potentially humane methods is **spiking** (or iki-jime, form the original Japanese expression). It essentially a manual method, where the brain is irreversibly damaged by pushing a solid, pointed metal rod (spiking) into the head which is then moved around to destroy the central nervous system (Farm Animal Welfare Committee, 2014). This method requires immobilisation of the fish to be performed, especially in smaller sizes, to accurately deliver the lethal puncture. It does not appear suitable to be implemented at a commercial scale due to the precision requirement. However, there is an emerging technology that claims to be able to perform spiking using artificial intelligence to accurately determine the position of the brain in each individual fish (Shinkei systems, n.d.). Although it is still in experimental phase, it shows a promising avenue adding to the suite of options available for commercial fish farming.

Each farm will have to determine the method that best fits their operation. It is recommendable and to consider realistically upfront that, apart from biological factors related to the species, there are financial and operational issues to take into account when deciding to choose the humane stunning and stunning method that best fits each case. Regardless, it is worth noting that welfare concerns are on the rise at EU level and should sooner or later become regulated and enforced (Giménez-Candela et al., 2020). More importantly, in terms of production, the application of good welfare practices at slaughter (as well as during the life of the fishes) has highly positive effects on product quality (Bermejo-Poza et al., 2021; Matos et al., 2010; Poli, 2009; Poli et al., 2005; Zampacavallo et al., 2015), and responds to requirements already in place by third-party certification labels (e.g. Friend Of the Sea, n.d.; Studer et al., 2020). It essential that the implementation of any change or new stunning protocol follows a strict training period. Failure to do so may compromise the whole procedure, and has the potential to backfire, causing poor(er) welfare for the fish and frustration for the farmer. The training suggestions for general welfare awareness in EU aquaculture are explained in section 3.1 of this document but specific tuition for technical aspects of equipment and protocols should always be performed.

3.4 Knowledge transfer

We propose the establishment of an EU-wide network of fish welfare R&D institutions, following up on an idea that has been advanced in several occasions but never formalised. The knowledge transfer within the network would enable the creation of an *EU-wide knowledge base* on indicators and protocols, integrating research and industry. At a later stage, this curated and centralised knowledge base could form the inception of an *EU fish welfare reference network*: a gathering of experts and institutions with the purpose to collect, organise, advance and transfer knowledge from and to the sector. The industry should play an essential role in such a network, as the practical expertise and knowledge that may be lost within fish farms is far too big to be disregarded (Medaas et al., 2021; Turnbull, 2022).

4- PREPARING THE FUTURE OF EU AQUACULTURE

4.1- Priorities for research to improve the welfare and production of farmed fish in the EU

In general terms, we believe that research should be directed to fill in identified research gaps in traits related to welfare that are directly impacted by farming activities. In that sense, and according to our ethological approach, many research gaps regarding behaviours in the wild can directly inform strategies to improve welfare. On the other hand, many aspects of welfare concerning specific challenges in farming remain unknown or warrant further research. It is always worth noting that better welfare is directly related to better production. Although putting this link into effect may present challenges, we strongly believe that when the welfare of animals is improved, both the quality of the product and its value increase. This is a case when the interest of the industry and the ethical standards underlying its activity walk hand in hand (Saraiva and Arechavala-Lopez, 2019).

These are the priorities for research that we propose, based on the contents and scope of the current report:

Behavioural traits in the wild

Many behavioural traits in the natural habitat of the five species addressed in this report remain unknown, despite being highly informative for the improvement of welfare in captivity. These include (but are not limited to):

- *Factors determining spatial ranges*: knowledge gaps found for all species
- *Biological and ecological drivers for migration*: gaps found for Seabass, Seabream, Trout and Salmon
- *Spawning behaviour*: gaps found for Seabass and Seabream
- *Natural aggregation and social preferences*: gaps found for all species

Species- and system-specific Operational Welfare Indicators

While the OWIs presented in Tables III and IV are general and may be adapted for all species, a collection of species-specific information such as those built by Noble et al (2020, 2018) for Salmon and Trout would be recommendable. Although some information is already present in the PerformFish and MedAid project reports regarding seabream (Marino et al., 2020; Roque et al., 2020), its validation, integration and peer-reviewed publication is still missing. For seabass, Yildiz et al (2021) tested an adaptation of the Salmon Welfare Index Model (SWIM 1.0) but species-specific details would benefit from further assessment. For Carp, to the best of our knowledge, there is no such approach yet. Furthermore, some indicators may not be present, relevant or (equally) functional in all farming systems. This evaluation would also be highly pertinent.

Species- and system-specific Environmental Enrichment strategies

Many EE strategies remain poorly understood, not only because some types of EE may be more suitable for certain farming systems and not others, but also because it is paramount to define the appropriate OWIs that will actually answer if the welfare of the animals is improved or not. Even within the strategies that appear to work, there are details about intensity and distribution that may determine their success or failure. For example, the use of suspended structures (Arechavala-Lopez et al., 2022b, 2019; Crank et al., 2019) is suggested to have positive effects in various species, but how many, what distribution and what type of materials are details that remain poorly known. The same could be applied for all other EE types detailed on section 3.3.1. Although this may seem a matter of detail, these knowledge gaps are often a barrier for the commercial application of EE in fish farms (see section 4.2).

Precision Fish Farming

The development of refined technological techniques to assess, monitor and improve the welfare of farmed fish is also worth pursuing. Although much has been done in Salmon, the remaining species would benefit from such approach. Besides allowing a deeper understanding of the causes and consequences of welfare under farming protocols, the implementation of cameras, sensors, Artificial Intelligence and other high-tech methods would enable the optimisation of resources to improve welfare and could streamline the identification of OWIs and development of EE strategies.

Consciousness indicators

The successful implementation of humane stunning and slaughter protocols in fish farming must rely on a solid identification of effective stunning. Although there are good reasons to believe that the absence of rhythmic respiration (opercular movements) and loss of the vestibular-ocular reflex (eyeroll) are reliable indicators of consciousness, there is data that suggests that brain activity is still present when these indicators disappear. Deeper research into the brain foundations of consciousness and sentience in fish will help to clear this issue, and allow the development, confirmation, or disproof of consciousness indicators in farming context(s). The practical and ethical implications are immense.

Relationship between Good Management Practices in welfare and production

Finally, the detailed understanding of how Good Management Practices (such as the use of appropriate environmental enrichment, good handling, good monitoring, humane stunning and slaughter, training, etc) impacts not only quality but also the profitability of commercial fish farming should be thoroughly researched. Detailed knowledge on technical and biological aspects of welfare and flesh quality, as well as the economical evaluation of financial cost versus benefit are worth researching. The inclusion of societal aspects such as reputational values and public awareness should also be considered.

4.2- Priorities for funding to improve the welfare and production of farmed fish in the EU

Apart from providing funding to accommodate the research priorities suggested in 4.1 (either by competitive calls or other types), we consider that the following topics warrant financial support. The results may be optimised if these topics are integrated in a possible funding program.

Validation of welfare solutions at commercial scale

As described in 3.3, there are several solutions that have the potential to improve the life of farmed fishes (EE, refined handling equipment and protocols, assessment tools, etc). However, only a very few of them reach the industry as available and tested solutions, ready to be deployed in farms. Part of this bottleneck may reside on the lack of validation at commercial scale for these instruments, and the fear that the implementation of these solutions may hinder established protocols, ending up either not working or becoming not practical, disrupting production. We propose the creation of financial incentives for companies to test, and eventually uptake, such methods while reducing the associated risks of testing them during commercial cycles. The form of such incentives is a matter of political decision, but we strongly believe that companies that adhere to them will have competitive advantages once the positive effects of welfare in production become clear.

Validation of humane stunning and slaughter solutions at commercial scale

The same rationale of the previous point applies here. The introduction of additional steps in established farming protocols may disrupt the production cycle, and this disruption may mask the potential benefits of the new technique. This is why we believe it would be positive to create financial incentives to study and validate humane stunning protocols at full commercial scale, for all farming systems and company dimensions. This measure would accelerate the assimilation of a fundamental measure to improve fish welfare as well as production.

Adoption of humane stunning and slaughter solutions in companies

While other methods to improve welfare of farmed fish may be achieved in a "cheaper" way, the purchase and application of stunning equipment is financially demanding, especially for small scale farms. Providing access to these equipments to as many companies as possible, as well as the training to operate them correctly (see next point), should become a fundamental priority to improve fish welfare and production.

Training

As stated throughout this report and detailed in 3.1, knowledge and training are the cornerstone of welfare. To maximise awareness for welfare issues and education at all levels, we suggest:

- The creation of grants for companies to train their staff, whether in the courses proposed in 3.1 or other training initiatives:
- The inclusion of welfare topics in professional and technical aquaculture learning programs

Good welfare awards

Finally, it could be positive to reward companies for their adoption of good welfare practices. The creation of an EU award to acknowledge Good Management Practices for industry stakeholders (producers, certifiers, retailers or similar) could not only spur the adoption of good welfare welfares but also serve as a dissemination marketing tool, both for the sector and the EU as a worldwide leader in fish welfare.

5- FINAL RECOMMENDATIONS.

Throughout this report we reviewed the welfare of the five most cultivated species in the EU under an ethological framework. The main takeaways to improve welfare and production of these fish under this ethological focus are:

- *Gather knowledge about the natural behavioural biology of the species*. Behaviour and biology in the wild are highly informative about the species needs and preferences under farming conditions. In addition, there may be traits that allow production protocols to be streamlined or even enhanced.

- *Gather knowledge about behavioural indicators under farming conditions*. Behaviour is a cheap indicator of the welfare state of the animals, yet requires training. Knowing the details of the behavioural responses of each farmed species is an effective way not only to assess their welfare at all stages but also to predict the impacts of changes in the rearing protocols

- *Acknowledge the challenges and limitations of farming methods*. The realistic acceptance that farming methods impose challenges to animals is the fist step to improve their welfare. This should be done at farm level, considering each farm as a particular case with its own characteristics.

- *Integrate knowledge from all stakeholders (including technical staff in fish farms) and translate it into practice*. A broad integration of experiences and concerns (from academia, policy-makers, NGOs, retailers, certifiers, consumers and producers, including the staff at fish farms) will enable informed decisions to be taken.

- *Implement measures to improve the life and the death of farmed fishes*. These measures include the definition of indicators to evaluate their welfare, adoption of EE, refinement of handling, transport and routine protocols, and implementation of humane stunning and slaughter.

- *Communicate and share information with all partners in the sector.* Communication is key to improve the welfare and production in farmed fish. The sharing of advances in protocols and discoveries of new methods should be encouraged, so that innovation in farmed fish welfare is accelerated.

The fishes are the centre of a strongly interconnected network of stakeholders in the fish farming sector (fig. 6). Improving the welfare of the animals will directly and positively affect the fish themselves, and indirectly improve the state of the whole sector.

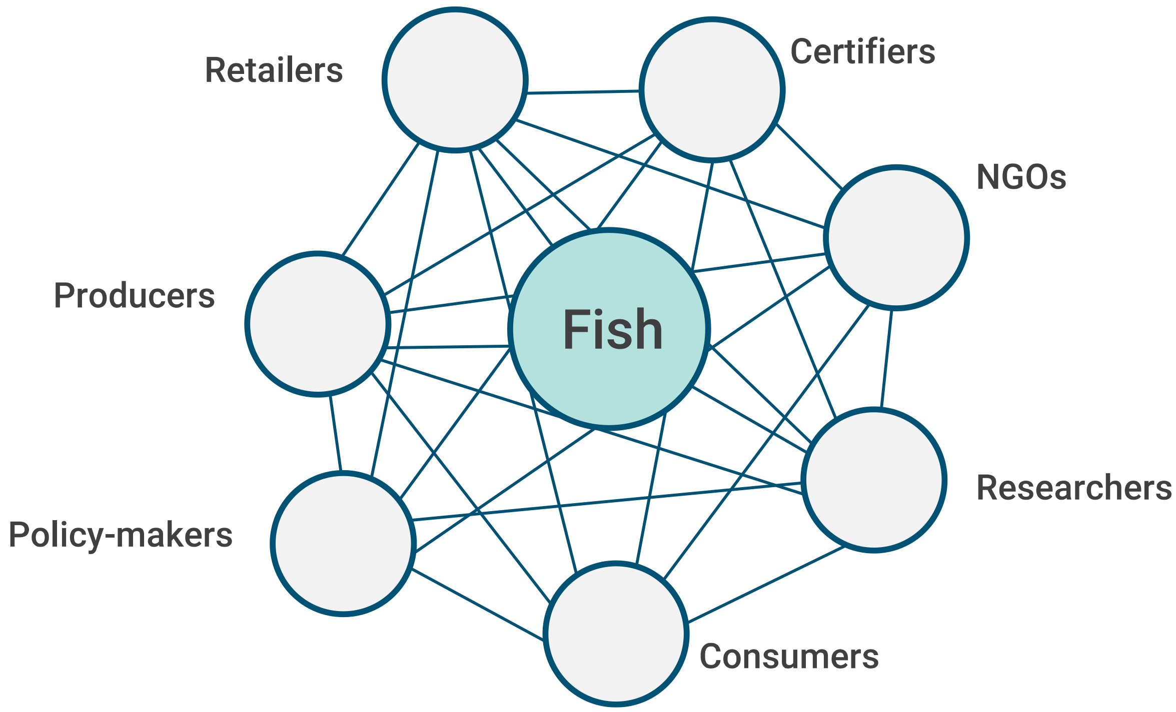

Figure 6- An interconnected stakeholder network in fish farming.

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