French Reference Centre
for Animal Welfare

FRCAW literature review on the welfare of farmed fish in connection with their slaughter

Full title: Opinion of the FRCAW on slaughter conditions for farmed fish

Requested by: French Reference Centre for Animal Welfare (FRCAW)

Updated: 7 March 2024

Background: At the first meeting of the fish welfare platform set up by the French Inter-professional Committee on Aquaculture Products (Comité Interprofessionnel des Produits de l'Aquaculture, CIPA), a need was identified for scientific information on fish welfare during slaughter, particularly in connection to stunning. To meet this need, the FRCAW, in agreement with CIPA, proposed a literature review.

Purpose of the expertise: Much work is already available on stunning and slaughter conditions for the various species of fish farmed in Europe. However, no literature review exists that specifically addresses the situation in France. Moreover, the pre-stunning phase is generally not dealt with in detail, despite its impact in terms of animal welfare. This document therefore aims to identify:

- the factors likely to compromise the welfare of fish in the French fish farming sector from the time they leave the rearing pond or tank to their death;
- the causes of these factors;
- the stages involved in the process, i.e., pre-stunning, stunning, killing, etc.;
- the impacts on animal welfare of the factors identified;
- methods to measure these impacts;
- preventive/corrective actions to limit negative outcomes for fish welfare.

This document provides the industry with a state-of-the-art report that is tailored to the situation in France.

Note on references: the evidence base for this document is composed of academic and scientific works and materials published by the industry. A core working list of relevant titles was produced by the coordinator of the expertise and was expanded by the members of the expert panel. The FRCAW does not necessarily agree with the opinions expressed in some works contained in the corpus, but these have been included because they contain relevant information that the majority of the expert panel considers worth communicating.

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Glossary

Degree-day: notation used in fish farming to measure duration as a function of water temperature, as fish are poikilothermic. Thus ‘100 degree-days’ means that incubation will last 5 days in water at 20°C, 7 days in water at 15°C, 10 days in water at 10°C. (This is valid within certain temperature limits: do not extrapolate this calculation to extreme temperatures) (Source: https://doris.ffessm.fr/Glossaire/Degre-jour/).

Hyperoxia: excessive levels of oxygen in the blood.

Hypoxia: reduction in the amount of oxygen delivered by the blood to the tissues

Killing: the process of causing the death of animals. In the case of slaughter that respects animal protection, killing follows stunning.

Poikilotherm: an animal whose body temperature varies with that of its environment.

Polyploidy: polyploidy enables sterile organisms to be bred (not 100%), some of which have higher growth and survival rates and are even of improved quality. Polyploidy involves a change in the number of chromosomes and can occur naturally, without human intervention (for example, triploid oysters are not GMOs) (Rasmussen & Morrissey, 2007).

Portion trout: trout with a life cycle of between 10 and 14 months and a slaughter weight of around 250 grams.

Raceway: linear pool with concrete walls.

Stunning: the process of rendering an animal unconscious, with or without killing it, immediately before it is slaughtered for consumption. Stunning should be distinguished from immobilisation which, although it also stops an animal's behavioural responses, does not cause it to lose consciousness.

Sellers: for the purposes of this summary, sellers are only businesses that sell directly to supermarkets, wholesalers and restaurants.

Slaughter: the killing of animals for human consumption.

Teleosts: family of ray-finned fish, representing 99.8% of fish species. Almost all fish species farmed in France are teleosts.

List of abbreviations

AHAW: Animal Health And Welfare

ANSES : Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (French Agency for Food, Environmental and Occupational Health and Safety)

CCA: Conseil Consultatif de l'Aquaculture (Aquaculture Advisory Council)

CIPA: Comité Interprofessionnel des Produits de l'Aquaculture (Interprofessional Committee for Aquaculture Products)

FRCAW: French Reference Centre for Animal Welfare

DDPP: Direction Départementale de la Protection des Populations (Departmental Directorate for the Protection of Populations)

DGAL: Direction Générale de l'Alimentation (Directorate-General for Food)

DGAMPA: Direction Générale des Affaires Maritimes, de la Pêche et de l'Aquaculture (Directorate-General for Maritime Affairs, Fisheries and Aquaculture)

ECG: electrocardiogram

EEG: electroencephalogram

EFSA: European Food Safety Authority

EMFAF: European Fund for Maritime Affairs, Fisheries and Aquaculture

FEAP: Federation of European Aquaculture Producers

LT: Large trout (1-2 kg)

HPI: hypothalamic-pituitary-interrenal

IFREMER: Institut Français de Recherche pour l'Exploitation de la Mer (French Research Institute for Exploitation of the Sea)

INRAE: Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (French National Research Institute for Agriculture, Food and the Environment)

ITAVI: Institut Technique des Filières Avicole, Cunicole et Piscicole (Technical Institute for the poultry, rabbit and fish sectors)

WOAH: World Organisation for Animal Health

WTO: World Trade Organisation

ONIRIS: Ecole Nationale Vétérinaire, Agroalimentaire et de l'Alimentation (Nantes-Atlantique National College of Veterinary, Agrifood and Food Sciences)

RAS: Recirculating Aquaculture System

RSPCA: Royal Society for the Prevention of Cruelty to Animals

SNGTV: Société Nationale des Groupements Techniques Vétérinaires (National Society of Veterinary Technical Groups)

SYSAAF : French Poultry and Aquaculture Breeders Union

RT: Rainbow trout

VLT: Very large trout (3-4 kg)

EU: European Union

VOR: vestibulo-ocular reflex

VER: visual evoked response

Introduction

There is growing interest in the issue of animal welfare, particularly in Western society. In response, the number of scientific publications in Europe on animal welfare doubled between 2003 and 2014 (Gautret et al., 2017). Fish welfare has been far slower to arouse the public's interest than that of the main terrestrial farmed species, despite the fact that threats to fish welfare in farming and at slaughter have been clearly identified. Indeed, every one of the 7 opinions on slaughter methods for the main species of farmed fish issued by EFSA (the European Food Safety Authority) made the point that there were problems in bringing actual practices into line with the recommended methods to protect animal welfare at the time of killing (EFSA, 2009a, 2009b, 2009c, 2009d, 2009e, 2009f, 2009g). These issues have now become more pressing for the public, notwithstanding the measures introduced in recent years by the industry to improve fish welfare and product quality. Meanwhile, discussions are ongoing at European Community level on the welfare of fish in aquaculture. Two reports have been published by the European Commission: the first in September 2017, 'Welfare of farmed fish: current transport and slaughter practices', and the second ,'Report from the Commission to the European Parliament and the Council on the possibility of introducing certain requirements for the protection of fish at the time of killing', in March 2018 (European Commission, 2017, 2018). A working group on fish has been set up as part of the European discussion platform on animal welfare, established in 2017. However, the French aquaculture industry lacks the indicators and technical solutions that will be needed if it is to fulfil the commitment it made to consolidate its progress concerning animal welfare following the French National Food Conference.

Although the scientific study of the sensitivities and welfare of fish may initially have lagged behind such work on the main farmed terrestrial species, it has seen rapid growth in recent years. Indeed, several European research projects on fish welfare have received financial support, either directly through the last two framework programmes (FP7[1] and H2020[2]) or indirectly through ERA-NET actions[3]. A number of COST Actions[4] have also been funded to

[1] The Seventh Framework Programme was the European Union's research and technological development funding programme for the period 2007-2013. https://cordis.europa.eu/programme/id/FP7/fr

[2] Horizon 2020 was the EU's research and innovation funding programme for the period 2014-2020. https://research-and-innovation.ec.europa.eu/funding/funding-opportunities/funding-programmes-and-open-calls/horizon-2020_en

[3] As part of its Horizon 2020 framework programme, the European Commission introduced a new financial instrument for European research: the ERA-NET Cofund. It is designed to increase the effectiveness of project-based research funding on a European scale. It combines the actions of the earlier ERA-NET and ERA-NET+ schemes.

[4] 'COST (European Cooperation in Science and Technology) is a funding organisation for research and innovation networks. [Its] Actions help connect research initiatives across Europe and beyond and enable researchers and innovators to grow their ideas in any science and technology field by sharing them with their peers. COST Actions are bottom-up networks with a duration of four years that boost research, innovation and careers' (https://www.cost.eu).

strengthen the links between researchers and stakeholders with an interest in the welfare of farmed fish (e.g. COST Action 846 'Measuring and Monitoring Farm Animal Welfare' and COST Action 21124 'Lifting farm animal lives - laying the foundations for positive animal welfare').

Despite this recent expansion in the scientific investigation of fish sentience and cognition, the extensive range of fish species farmed and variety of farming methods make it hard to draw general conclusions. Indeed, the many differences in physiology and neuroanatomy between farmed species suggest that fish sensitivities vary greatly among the species of interest. A further complicating factor lies in the varying degrees of domestication, which began less than 3000 years ago (Teletchea & Fontaine, 2012). When combined, our lack of knowledge concerning the particular sensitivities of each species and the diversity of species farmed present a major obstacle to technological innovation, particularly in slaughter methods, and thereby compromise progress in animal welfare.

This document answers the need created by this situation. It offers a summary of technical and scientific knowledge that can improve understanding of the welfare needs of fish during slaughter, encompassing preliminary fasting and crowding, transfer, transport and actions at the slaughter site. Beyond the updates it provides on the current state of knowledge, it analyses the stress and pain factors likely to affect the welfare of fish during slaughter, identifies possible knowledge gaps and suggests ways to improve slaughtering practices in the light of fish sensitivities. It takes into account the diversity of fish species, while focusing on the main species farmed in France[5], namely trout, carp and sturgeon (freshwater fish), and sea bass, sea bream, meagre, turbot and sole (saltwater fish). These species have different needs in terms of oxygen and water temperature, different sensitivities and different adaptive capacities. The summary is structured as follows. Beginning with a description of the situation of aquaculture in France (practices and market situation), it then moves on to a review of the regulatory aspects of welfare in fish farming and societal expectations. The focus then turns to the sensory and emotional sensitivities of fish, described in the light of scientific knowledge, before moving on to an account of fish slaughtering methods and their impacts on fish stress and pain. For ease of reading, the slaughtering process is dealt with in two sequential phases, the first covering the departure of the fish from the rearing tank or pond (fasting, batching and crowding of fish, their removal and, if necessary, transport etc.) and second involving the process of stunning and killing itself. Stress and pain factors are discussed at each stage.

[5] For simplicity, the term 'France' is used throughout this document to refer to mainland France.

1. Background

1.1. Fish farming in France

Viewed globally, fish farming is a relative newcomer compared with other types of farming. Nevertheless, the degree of domestication of the various farmed species varies considerably. Thus, carp farming is an ancient practice, particularly in Asia; salmonid farming (salmon, trout) expanded strongly in the 1980s and 1990s in North America and Europe, to be followed by South America; while in France, the domestication of aquatic species is recent, meaning that their characteristics are closer to those of their wild relatives. Selection is applied to certain species and has already improved their performance and ability to adapt to a farmed environment. However, the aquatic products market is the only one where wild and farmed animals of the same species are both sold, supplied through fishing and aquaculture respectively.

French fish farming is highly diversified, and the figures for 2021 show a total production of around 43,621 tonnes[6] of fish (34,718 tonnes of salmonids raised in freshwater, 300 tonnes of other freshwater fish raised in facilities other than ponds, including sturgeon and perch, 2,868 tonnes of pond raised fish, of which 44% is carp, and 5,735 tonnes of marine fish, 77% of which is sea bass and sea bream), 44 tonnes of caviar and 102 tonnes of trout eggs for consumption (Agreste, 2023a). France also produces juveniles and eggs for reproduction: 163 million trout embryonic eggs and 19 million trout juveniles, 16 million fry for species of freshwater fish other than salmonids (Agreste, 2023a), and, for marine fish, 100 million sea bass and sea bream fry (Federation of European Aquaculture Producers (FEAP), 2023).

1.1.1. <u>Species raised in France</u>

Table 1 lists the main species of fish farmed in France. These include Salmonidae farmed in freshwater: rainbow trout, brown trout, brook trout, Arctic char; marine fish: sea bass, gilthead (sea) bream, meagre, turbot, sole, salmon and trout farmed at sea; pond-farmed fish: carp, etc.; and other freshwater fish farmed in facilities other than ponds: sturgeon, perch.

[6] Fish production, excluding juveniles, is always expressed in terms of tonnage, as it is impossible to convert to numbers of animals due to the wide range of sizes. In addition, animals are systematically managed in batches, not by number of individuals.

Table 1. Main fish species farmed in France in 2021. *Data taken from (Agreste, 2023)*

Salmonidae	Rainbow trout (98% of freshwater salmonid production)	*Oncorhynchus mykiss*
	Brown trout, brook trout, Arctic char, etc. (around 2% of freshwater salmonid production)	*Salmo trutta, Salvelinus fontinalis, Salvelinus alpinus*
Marine fish	Bar	*Dicentrarchus labrax*
	Sea bream	*Sparus aurata*
	Meagre	*Argyrosomus regius*
	Turbot	*Scophtalamus maximus*
	Sole	*Solea senegalensis*
Pond fish	Carp (several species) and other pond fish	Cyprinidae and others
	Sturgeon (several species)	Ascipenseridae

Production takes place throughout France as shown in Figure 1, but is unevenly distributed by species. Some regions are more active, such as Nouvelle-Aquitaine and Hauts-de-France (Figure 1).

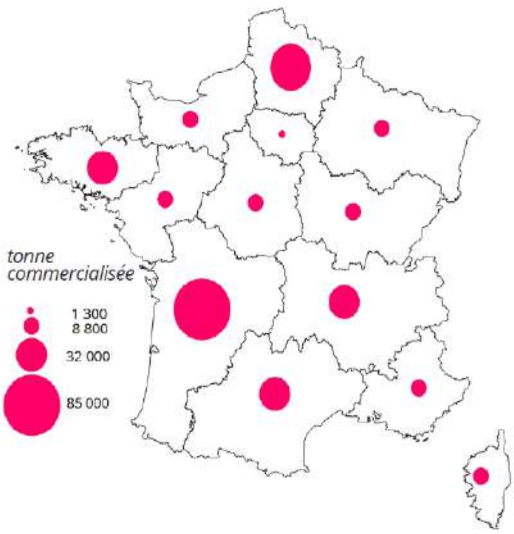


Figure 1. Breakdown of farmed fish production in tonnes by French region, excluding hatcheries/nurseries and pond fish production. *Source: (Agreste, 2020)*

N.B. It is not possible to provide a map of primary slaughtering sites. In the trout sector, some production sites also carry out their own slaughtering, either for direct retail sale or for sale to a distributor (wholesaler, supermarket, etc.). In such cases, no travel is involved between the farm and the slaughter site, only a transfer within the farm. Not all production sites have a processing plant (and therefore an abattoir), though. Moreover, not all processing plants slaughter the fish they process (e.g., smokeries may receive fish that have already been gutted or filleted). Where slaughter is not carried out on the production site, the fish are transported in tanks filled with water and oxygen (on open trucks, smaller road vehicles or boats); depending on the length of the journey, additional water treatment methods are used (cooling, etc.). It is common for fish reared in ponds to be transported to the abattoir in tanks filled with water and oxygen. For marine fish and sturgeon farms, slaughter is carried out on the production site.

Rainbow trout is the most farmed fish species in France. The length of the rearing period depends on the size of fish required. For example, it takes between 10 and 14 months for a fish to reach the "portion" stage, when the fish weighs around 250 grams. Increasingly, farming is moving towards the production of large trout (LT) or very large trout (VLT), obtaining animals weighing 1-2 or 2-3 kg respectively (Figure 2). These sizes are sold as freshly cut fish (steaks, supremes, fillets) or as smoked trout to meet growing consumer demand (Figure 3). The rearing period in these cases is around 2-3 years (depending on strain and rearing conditions, particularly water temperature).

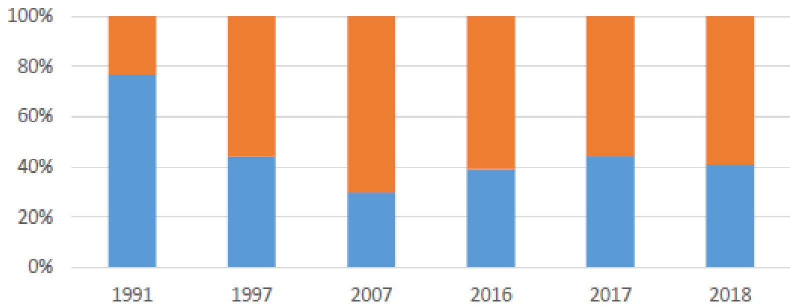


Figure 2. Trends in farmed rainbow trout sizes (TAEC) since 1991. *Portion trout are shown in blue and large and very large trout are in orange. Data from Agreste (2011, 2019)*

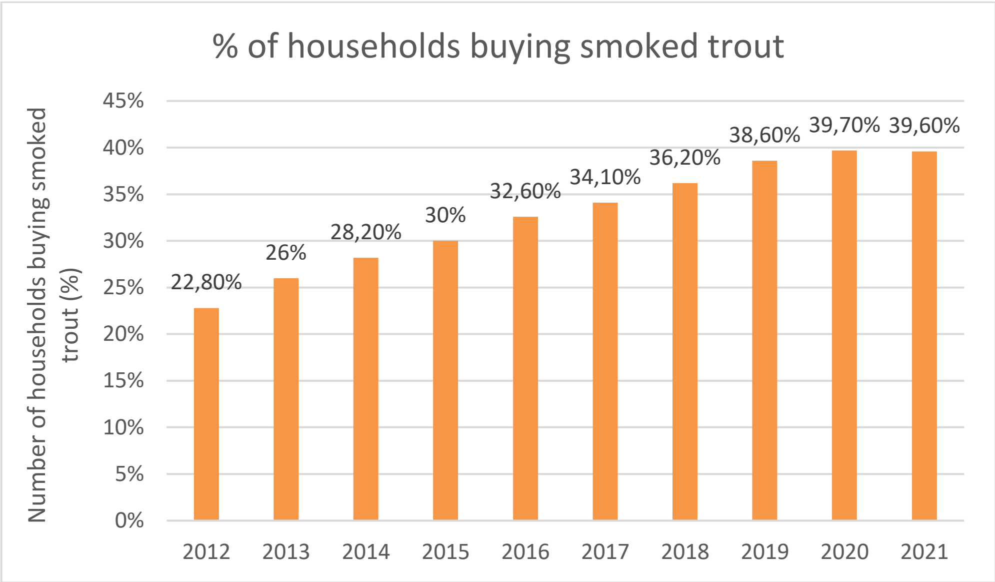


Figure 3. Trends in the proportion of households buying smoked trout for home consumption. *Data from FranceAgriMer (2013, 2014, 2015, 2016, 2017a, 2018, 2019a, 2020, 2021, 2022c).*

Marine fish farms have comparable rearing periods (Table 2).

Table 2. Indicative rearing periods in France

Species	Time in hatchery	growing period
Bass	90 to 150 days	18 to 36 months
Sea bream	90 to 150 days	18 to 36 months
Meagre	90 to 150 days	18 to 36 months
Turbot	90 to 150 days	18 to 36 months
Atlantic salmon	12 months to smolt stage N.B. reared in fresh water	18 months
Trout in the sea	12 months to freshwater juvenile stage	18 months
Sole	140 days	18 months

In the marine fish farming sector, specialist companies or sites operate hatcheries, since the control of reproduction and larval rearing require dedicated facilities, if only for the cultivation of the phytoplankton and zooplankton needed to feed the larvae (unlike salmonids, which can feed on inert compound food following the disappearance of their yolk bladder, marine fish larvae feed on living prey).

1.1.2. Breeding / production systems

Depending on the species being farmed, different production systems and water supply sources are used. For example, facilities for marine species (sea bass, sea bream, turbot, meagre) can be located on land, using pumped seawater from nearby, or can be established directly in the sea (using tank or cage systems).

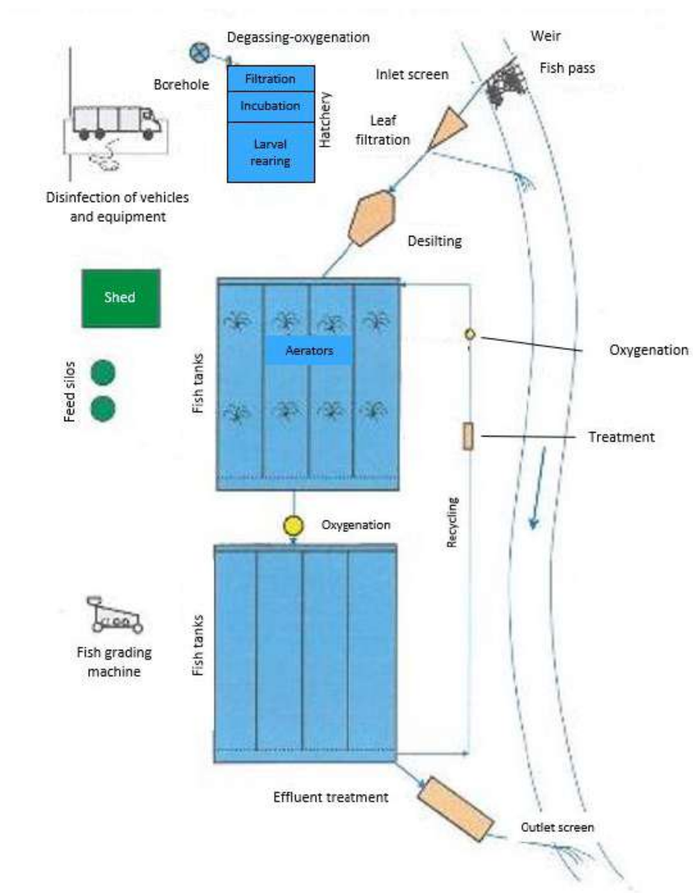
For freshwater species, different water supply systems are available, such as springs or boreholes, particularly for hatcheries (trout, sturgeon), diversion from a watercourse (trout, sturgeon) and pond farming (sturgeon, pond fish), while RAS (recirculating aquaculture systems) have recently been developed for use in part or all of a fish farm (trout and sturgeon).

In almost all cases, fish farms are heavily dependent on the quantity and quality of locally available water, since it is this water which, sometimes in untreated form (e.g. in ponds and offshore cage farms), supplies the environment in which the fish are reared. Apart from the specific case of farms using low water flow rates (farms with recirculating water systems, hatcheries in particular), it is not feasible to install treatment systems able to change the quality of the intake water to any great extent. Production systems therefore need to be designed for the type of production (hatchery, pre-growth, grow-out) and the environment in which they are installed. For example, trout hatcheries are mainly located on springs or boreholes to guarantee the quality of water entering the facility, since fry need to be reared in water of excellent quality and a stable temperature.

In land-based installations, the water circulates through tanks (such as cylindrical tanks or raceways[7]) at a flow rate set by the fish farmer to keep a steady current, before being returned to the environment. In recirculating systems, the water leaving the tanks passes through various treatment systems (mechanical, biological, degassing) before being returned to the tanks (Figure 4)

[7] Raceway: a linear pool with concrete walls

A.



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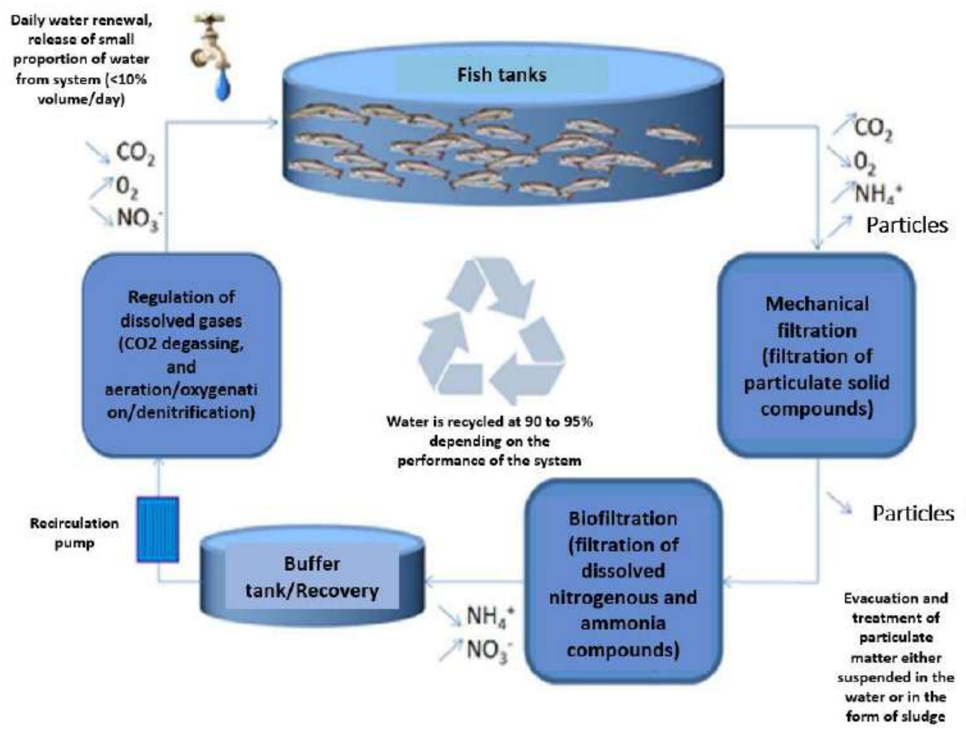

Figure 4. Layout of an open-circuit fish farm (with recirculation, where appropriate) Drawing: Jean DURET (Cemagref) (A) and diagram of a recirculating aquaculture system (RAS) (B). *Sources: (A) Guyennet (2000); (B) FranceAgriMer (2019b)*

1.1.3. Economic factors and the French market

Salmonids account for 55% of the value generated by French fish farming. Figure 5 shows the respective market shares of the various categories in the French industry, including marine fish and sturgeon, the sea bass, sea bream and meagre hatcheries in which France specialises, and caviar, for which France is the third largest producer worldwide.

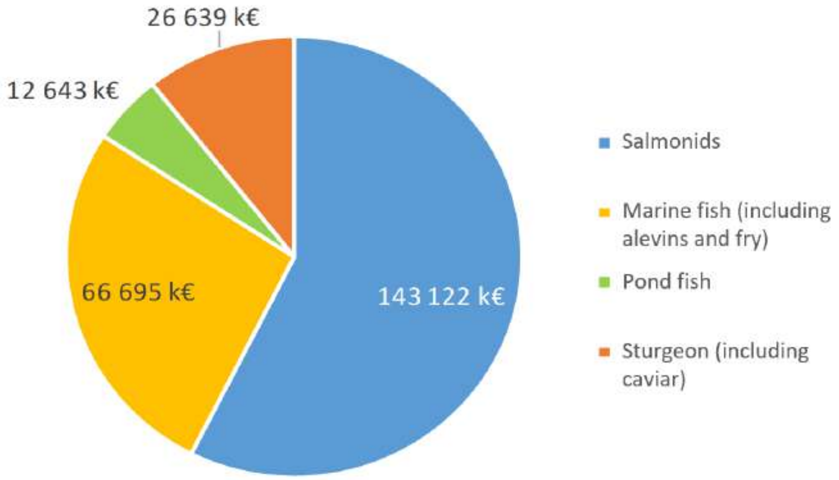

Figure 5. Turnover for the French fish farming industry (including eggs for consumption and juveniles) in 2020. *Data from Agreste (2023)*

Following a period of sharp decline, the volume of French freshwater fish production has generally stabilised since the mid-2000s. In recent years, there has been a slight increase in production volume, without returning to the levels seen in the mid-90s (Figure 6).

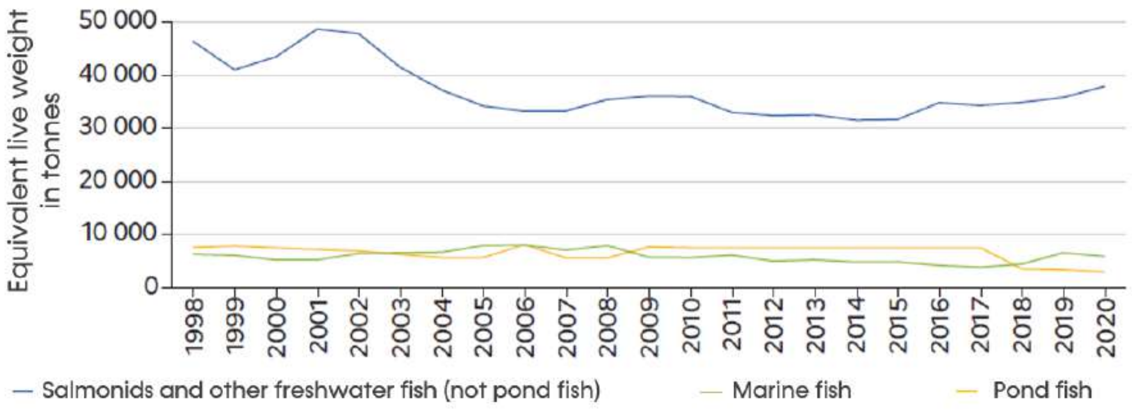

Figure 6. Fish production in mainland France between 1998 and 2020. NB: Volumes are in live weight equivalent. Figures for pond production were not updated between 2008 and 2017. *Source: Agreste (2022).*

A significant proportion of production, almost 6%, is organic (Table 3).

Table 3. Share of organic production (number of companies and production figures) in the French fish farming industry. *Source: Agreste (2023)*

Species	Number of organic producers	Organic production (tonnes)	Non-organic production (tonnes)
Salmonids (for consumption of meat)	23	1 198.90	33 518.71
Salmonids (for consumption of eggs)	7	5.38	92.58
Other freshwater fish not raised in ponds, including sturgeon and perch (meat)	0	0.00	300.42
Sturgeon (caviar)	0	0.00	43.96
Pond Fish	5	45.94	2 822.41
Bass	1	s	s
Other saltwater fish, including meagre, gilthead bream, etc.	0	0.00	3 094.16

The main principles of organic production, as described in Regulation (EU) 2018/848, apply to fish farming:

- Use of genetically modified organisms (GMOs), polyploid animals[8] obtained artificially, and hormones or hormone derivatives, is banned
- Allopathic and antiparasitic treatments are restricted (prevention is preferred)[9] .

In fish farming, 100% of the juveniles introduced onto a farm operating under organic regulations must be organic; organic and non-organic units must be separated (France has opted for the principle of non-mixed farms, except for hatcheries); grow-out facilities may not use recirculation systems or artificial forms of water heating or cooling; restrictions are placed on on the use of artificial light; the use of oxygen is authorised only in exceptional cases; maximum densities are set for each species; for feed, priority is given to raw materials derived from co-products, such as fish meal and oils; killing techniques must immediately render fish unconscious and unable to feel to pain. Additionally, particular attention is paid to the knowledge and skills of the farmer, handling (kept to a minimum and carried out with care[10]), equipment and procedures to avoid stress or physical damage, and measures to reduce transport times.

Total production volumes (organic and non-organic) in France are, nevertheless well below those of some other European countries, and French production trends are not keeping pace with the growth seen worldwide, due to the difficulty of increasing production at existing sites

[8] Polyploids are sterile (although not 100%) and some exhibit higher growth and survival rates, or even improvements in quality. While they may be produced artificially, the change in the number of chromosomes that constitutes polyploidy can also occur naturally, without human intervention (for example, triploid oysters are not GMOs). (Rasmussen & Morrissey, 2007)

[9] The use of allopathic treatments is limited to two per year, excluding compulsory vaccinations and mandatory treatment programmes. However, in the case of animals with a production cycle of less than one year, only one allopathic treatment per year is authorised.

[10] For example, the regulations on organic production state that “Handling of aquaculture animals shall be kept to a minimum and shall be carried out with the utmost care. Appropriate equipment and procedures shall be used to avoid stress and physical damage to the animals. [...] Grading operations shall be kept to a minimum and shall be carried out in a manner compatible with the welfare of the animals."

and of setting up new processing concerns. Among the obstacles are the complexity and length of administrative procedures (it takes at least three years to obtain a renewal of a prefectorial production decree, and the average is more like five years) and a regulatory framework that is not tailored to fish farming in terms of the environment: official requirements are based on the full gamut of texts arising from the 2006 Law on Water and Aquatic Environments and call for studies that exceed the limits of what can be dealt with by fish farmers. This explains why, other than a single pre-fattening site in 2010, no new fish farming sites have been created in the past 30 years.

Looking at the market shares of the various fish farming countries who are members of the FEAP (Federation of European Aquaculture Producers, including the European Union, Norway, the United Kingdom and Turkey), with the exception of trout, for which France supplies 20% of EU produce, French fish-farming production is insignificant at European level. Within the European Union, the other two trout-producing countries are Italy and Denmark (where trout are farmed at sea following a pre-growth phase in freshwater). The main producers of bass and sea bream are Turkey, Greece and Spain. Although French production accounts for around 8% of Mediterranean sea bass and sea bream fry, it represents between 1 and 2% of the volume of sea bass and sea bream fry produced by FEAP members (FEAP, 2023). By way of comparison, Turkey produces approximately 37% of Mediterranean sea bass and sea bream fry. Thus, most of the sea bass and sea bream consumed in France come from EU or third countries (see below).

France is a major consumer of aquatic products. It is estimated that the per capita annual consumption of aquatic products in France (including 11% of fish from aquaculture) in 2017 was 33.5 kg (FranceAgriMer, 2022a). To meet these consumption requirements, France relies overwhelmingly on imports which, in 2018, represented 93% by volume of the supply of aquatic products to French customers (FranceAgriMer, 2021).

Fish from French aquaculture accounted for just 1.9% of aquatic products consumed in France in 2018 (CIPA et al., 2023) (Table 4).

Table 4. External trade[11] figures for the main species of fish farmed in France in 2021. Figures are shown by volume (T) and value (k€). *Data from FranceAgriMer (2022b)*

	Imports	Exports	Balance of trade
Trout	11,760 T (60% from Spain)/€72,001 k	7,642 T (55% to Germany)/€31656 k	- 4,118 T/-€40,348 k
Sea bass and sea bream	21,524 T (44% from Greece)/€107,090 k	2,966 tonnes (29% destined for Italy)/€28,804 k	- 18,558 T/-€78,286 k
Salmon	245,752 T (47% from Norway)/€1,684,243 k	40,715 T (39% destined for Poland)/€376,322 k	- 205,037 T/-€1,307,920 k
Carp	402 T/988 k€	63 T/€288 k	- 339 T/-€700 k

1.1.4. Slaughter methods by species

The slaughter methods used in France vary by species (Table 5).

Table 5. Slaughtering practices used in France (number of sites)[12]. *Source Agreste (2021)*

Means of death or slaughter technique	Salmonid farms	Sturgeon and other freshwater fish farms excluding ponds	Pisciculture in ponds	Marine pisciculture
Slaughter practice				
Electrical stunning/killing	88	3	6	1
Bleeding	32	3	2	6
Stunning or percussion	76	5	4	4
Live chilling	3	0	3	15
CO2	2	1	0	0
No specific killing technique	19	2	189	5
Total	**365**	**21**	**211**	**28**

N.B.: The source data from Agreste includes all producers (i.e. fish farms) in the sector. Processing/packaging plants with no fish farming facilities are therefore not included. That said,

[11] Figures provided in this table include live fish, whole or cuts of fresh fish and smoked fish. The difference in the average prices of fish imports and exports can be explained by the fact that more fish or "products" with a higher price per kg are exported (or imported, depending on the species) than imported (or exported, depending on the species). For example, for trout, France mainly exports live fish in bulk (cheaper) and mainly imports fresh cuts of fish (more expensive), hence the difference in price per kilo.

[12] The last row of Table 5 indicates the total number of businesses surveyed for each category. Many of these businesses do not slaughter the fish themselves. Also, some companies use several techniques, meaning that the final row figures do not correspond to the sum of the other rows in the table.

the processing/packaging businesses included do frequently carry out the slaughter of fish themselves, accounting for 70 to 80% of market suppliers[13]. Meanwhile, fish farms without slaughtering facilities also responded to this survey, a fact that probably explains the number of responses selecting the option "no specific slaughter technique".

Electrical and percussive stunning and killing are the main methods used in salmon farming, while live chilling (commonly called 'thermal shock' in French) is the most widespread technique for marine fish (the main species in France, sea bass and sea bream, are "warm" water species that are stunned and killed through immersion in ice slurry). Different publications and practitioners use a number of terms to describe this method: "live chilling", "thermal shock", "ice asphyxiation", "ice water bath", "ice slurry", etc.

The source does not specify whether "electrocution" as a slaughter practice includes electronarcosis or electrocution, or both (cf. 3.2.2.3).

With regard to "bleeding" techniques, the information provided in the source does not make it possible to determine whether or not this is preceded by stunning. In practice, all stunning methods can be followed by bleeding for fishes above a certain size (small fish sold whole are not bled).

A full description of stunning and slaughter techniques is provided in section '3.2. Stages 2 and 3: stunning and killing'.

1.1.5. <u>Changes in expectations regarding the welfare of farmed fish</u>

For its aquatic products image barometer, FranceAgriMer regularly surveys a representative panel of the French population in mainland France. In December 2017, the focus was on questions relating to the welfare of aquatic animals (FranceAgriMer, 2017a).

The results of this survey suggest that the three main challenges for a more sustainable and equitable production of fish, shellfish and crustaceans are:

- *"Good environmental status of the marine environment (water quality, protection of the seabed, etc.)*" (92% of respondents)

- *"Product health quality*" (91% of respondents)

- *"Preservation of threatened species (cetaceans, turtles, etc.)*" (91% of respondents)

These issues are considered even more important by consumers over 65 and retired people.

It should be noted that animal welfare comes in fourth place, judged to be one of the main issues for a more sustainable and fairer production of fish, shellfish and crustaceans by 88% of respondents.

Panel members were then asked specifically about their perception of animal welfare. The results show that 2/3 of French people say they are sensitive to the welfare of fish, crustaceans

[13] Here, market suppliers are processing plants that sell directly to supermarkets, wholesalers and restaurants.

and shellfish (the three categories of animals being grouped together in the same question). This sensitivity is more marked among women, people aged between 50 and 64, and people not in work.

The survey also showed that consumers take animal welfare into account mainly by "*consuming aquatic products with quality-related labels (logos, organic, etc.)*" (36% of respondents) and by "*consuming fish products rather than aquaculture products*" (34% of respondents). For most French people, fish farming can be compatible with animal welfare, but only if practices are improved (58% of respondents).

The same survey revealed that the main information proving attention to animal welfare that consumers expect to see is:

- Information on the living conditions of fish (88% of respondents, first choice for 34%)
- Information on fish "stocking" levels (88% of respondents, first choice for 13%)

We also note that information on slaughter conditions for "live aquatic products" is very important to consumers as proof that animal welfare has been taken into account (84% of respondents, first choice for 14%).

Last, there is little knowledge on initiatives to promote the welfare of fish and shellfish, with almost half of respondents having never heard of any such initiative.

We would point out the benefits of clarifying the terms used in this type of survey, so that a better understanding of consumer expectations with regard to animal welfare can be gained.

At European level, fish welfare is receiving increasing attention, as is demonstrated by the work of the Aquaculture Advisory Council (AAC).

Advisory Councils are stakeholder-led organisations that contribute to European objectives, created under Common Fisheries Policy Regulation (EU) No 1380/2013. The AAC guides the European Commission and the Member States on all matters relating to the management, socio-economics, and conservation aspects of aquaculture.

The AAC's recommendations represent the views of European aquaculture stakeholders in the following proportions:

- ✓ 60% - Sector organisations (aquaculture, food, veterinary, trade unions, etc.)
- ✓ 40% - Other interest groups (environmental, consumer, animal welfare, etc.)

(list of members available at: https://aac-europe.org/fr/a-propos/membres)

In 2019, the AAC issued a recommendation on fish welfare at slaughter. It recommends, at various levels (European and national), "Supporting the development of fish slaughter technology", "Supporting the development of best practices"[14] , "Maximising value from best

[14] No good practice is described or highlighted in these recommendations, but the AAC recommends consolidating the knowledge base and identifying good practice, and establishing "a platform [through] the Commission for the sharing of best practices, on an ongoing basis, as they are developed by industry, experts and relevant authorities".

management practices", "Ensuring the efficacy and benefits of fish slaughter practices", entrusting a number of tasks to an "EU Animal Welfare Reference Centre" and promoting "the need for species specific standards at international fora" (AAC, 2019). Such recommendations, which are the product of consensus among all participating stakeholders, are sent to the European Commission as a matter of course.

In 2022, the AAC issued recommendations on the welfare of fish during transport (AAC, 2022b) and on the creation of a European reference centre for fish welfare (AAC, 2022a). Since then, the European Commission has launched a call for applications for the creation of a European Reference Centre for Aquatic Animal Welfare on 12/04/2023.

This AAC activity demonstrates the active involvement in this issue of both the professional sector and animal welfare NGOs in Europe.

Conclusions:

The French fish farming industry is small compared with those of other animal species and other countries, with a variety of methods employed in production, farming, transport and slaughtering. This variety is linked in particular to the wide variety of species farmed, and the differences in farming environments (in particular the difference between freshwater and seawater).

The industry needs to take account of growing societal expectations regarding developments in the protection and welfare of fish. In particular, it would be useful to clarify the detail of the diverse slaughter methods and practices, and to provide a better technical and regulatory framework for them.

1.2. Regulatory matters and fish welfare initiatives

1.2.1. Global: the recommendations of the World Organisation for Animal Health (OIE)

WHOA (formerly OIE) is responsible for setting intergovernmental animal health standards and, since the creation of the World Trade Organisation (WTO) in 1995, has been recognised as a WTO reference in the category of health measures.

In 2002, WHOA broadened its mandate to include animal welfare. It should be noted that, although WHOA's codes and standards serve as international references, they are not binding.

According to the WHOA Terrestrial Code, "animal welfare means the physical and mental state of an animal in relation to the conditions in which it lives and dies". The WHOA (Terrestrial Animal Health Code) guidelines on animal welfare also refer to the "Five Freedoms", published in 1965 (Brambell, 1965) to describe the right to welfare of animals under human control. According to this concept, an animal's primary welfare needs can be met by ensuring:

- Freedom from hunger, malnutrition and thirst,
- Freedom from fear and distress,
- Freedom from heat stress or physical discomfort,
- Freedom from pain, injury and disease, and
- Freedom to express normal patterns of behaviour.

With regard to aquatic animals, the Aquatic Code is not as developed on welfare as it is for terrestrial animals, but it does include a chapter (7.2) on transport and a chapter (7.3) on welfare aspects of stunning and killing (WHOA, 2022).

Chapter 7.2 on the welfare of farmed fish during transport provides recommendations to minimise the effect of transport on the welfare of farmed fish and applies to their transport by air, sea or on land within a country and between countries. It includes sections on responsibilities, competence, transport planning, documentation, loading, transport and unloading of fish and post-transport activities. It clearly states that there should be species-specific recommendations.

Chapter 7.3 on welfare aspects of stunning and killing of farmed fish for human consumption provides recommendations that also cover transport and holding immediately prior to stunning. The principles also apply to the stunning and killing of farmed fish for disease control purposes. The chapter includes sections on personnel, transport, design of holding facilities, unloading, transferring and loading, and stunning and killing methods with a summary table of methods used for fish and their respective animal welfare issues (Table 6).

Table 6. Summary table of some stunning/killing methods for fish (not including ice slurry and gas methods) and their respective welfare issues[15] . *Source: WHOA (2022)*

Stunning/ killing method	Specific method	Key fish welfare concerns/requirements	Advantages	Disadvantages
Mechanical	**Percussive stunning**	The blow should be of sufficient force and delivered above or adjacent to the brain in order to render immediate unconsciousness. Fish should be quickly removed from the water, restrained and given a quick blow to the head, delivered either manually by a club or by automated percussive stunning. The effectiveness of stunning should be checked, and fish be re-stunned if necessary. It can be a stun / kill method.	Immediate loss of consciousness. Suitable for medium to large sized fish.	Hand operated equipment may be hampered by uncontrolled movement of the fish. Mis-stunning may result from a too weak blow. Injuries may occur. Manual percussive stunning is only practicable for the killing of a limited number of fish of a similar size.
	Spiking or coring	The spike should be aimed on the skull in a position to penetrate the brain of the fish and the impact of the spike should produce immediate unconsciousness. Fish should be quickly removed from the water, restrained and the spike immediately inserted into the brain. It is a stun / kill method.	Immediate loss of consciousness. Suitable for medium to large sized fish. For small tuna, spiking under the water avoids exposure of fish to air. The pineal window of tuna facilitates spiking for this species.	Inaccurate application may cause injuries. Difficult to apply if fish agitated. It is only practicable for the killing of a limited number of fish.
	Free bullet	The shot should be carefully aimed at the brain. The fish should be positioned correctly and the shooting range should be as short as practicable. It is a stun / kill method.	Immediate loss of consciousness. Suitable for large sized fish (e.g. large tuna).	Shooting distance; calibre need to be adapted. Excessive crowding and noise of guns may cause stress reaction. Contamination of the working area due to release of body fluids may present a biosecurity risk. May be hazardous to operators.
Electrical	**Electrical stunning**	Involves the application of an electrical current of sufficient strength, frequency and duration to cause immediately unconsciousness. It can be a stun / kill method. Equipment should be designed and maintained correctly.	Immediate loss of consciousness. Suitable for small to medium sized fish. Suitable for large numbers of fish, and the fish do not have to be removed from the water.	Difficult to standardise for all species. Optimal control parameters are unknown for some species. May be hazardous to operators.
	Semi-dry electrical stunning	The head of the fish should enter the system first so electricity is applied to the brain first. Involves the application of an electrical current of sufficient strength, frequency and duration to cause immediately unconsciousness. Equipment should be designed and maintained correctly.	Good visual control of stunning and the ability for re-stunning of individual fish.	Misplacement of the fish may result in improper stunning. Optimal control parameters are unknown for some species. Not suitable for mixed sizes of fish.

[15] WHOA specifies that "*the terms small, medium and large fish should be interpreted relative to the species in question"*.

WHOA also specifies that the methods "*chilling with ice in holding water"*, "*carbon dioxide (CO2) in holding water*", "*chilling with ice and CO2 in holding water*", "*salt or ammonia baths*", "*asphyxiation by removal from water"* and "*exsanguination without prior stunning*" are also used but are not mentioned in this table because they result in poor fish welfare and it is preferable not to use them if it is feasible to use one of the methods listed in the table, as appropriate to the fish species.

1.2.2. European regulations on animal welfare

At European Union level, animals are recognised as sentient beings in Article 13 of the Treaty on the Functioning of the European Union (Council of the European Union, 2007). As such, in implementing the Union's policies in the fields of agriculture, fisheries, transport, internal market, research and technological development and space, the Union and the Member States must pay full regard to the welfare requirements of animals.

Fish are therefore covered by this provision, but European law does not specifically include them in its legal framework. One Directive (98/58/EC) and two Regulations (EC 1/2005 and EC 1099/2009) comprise the totality of European legislation relating to fish welfare.

Article 2 of Council Directive 98/58/EC of 20 July 1998 states that fish are covered by the Directive. Article 3 is the only article that applies to fish, stating that "*Member States shall make provision to ensure that owners or keepers take all reasonable steps to ensure the welfare of animals under their care and to ensure that those animals are not caused any unnecessary pain, suffering or injury".* (Council of the European Union, 1998). Member States had until 31 December 1999 to bring this Directive into force.

In France, the texts making the relevant provisions for fish are as follows:

- Decree of 30 March 2000 amending the Order of 25 October 1982 on the breeding, rearing and keeping of animals
- Law No. 2001-6 of 4 January 2001 containing various provisions to comply with Community law on animal health and the safety of products of animal origin for human consumption, and amending the Rural Code.

Council Regulation EC/1/2005 on the protection of animals during transport and related operations applies to all vertebrate animals and therefore applies to fish. Article 3 sets out the general conditions for the transport of animals, stating that no transport shall cause injury or undue suffering to the animals. Article 4 lists the documents required for the transport of live animals and Article 5 stipulates that welfare must not be compromised by insufficient coordination of the different parts of the journey or by failure to take account of weather conditions. Part 2.3 of Annex I Chapter V "Watering and feeding interval, journey times and resting periods" specifies that "*Species other than those referred to in point 2.1. or 2.2. shall be transported in accordance with instructions about feeding and watering and taking into account any special care required".* (Council of the European Union, 2004). For fish, in the absence of a definition of such "special care", the general rules apply, i.e., those relating to feeding and watering.

Given that these requirements in Council Regulation EC/1/2005 do not apply to fish (indeed, fish should not be fed during transport – the resulting excretion (faeces, urine) would be toxic to the fish, given that the water is not renewed – and watering is a meaningless exercise for fish), a draft guide to good transport practice was discussed between the industry and the French Ministry of Agriculture and Food. Work on this was carried out between 2013 and 2015, but ultimately came to nothing, a particular problem being the wide variety of transport methods employed, which made it hard to cover all modes of transport. Nevertheless, on 14 December

2016, the DGAL (Direction Générale de l'Alimentation) issued service note "DGAL/SDSPA/2016-955" on the rules applicable to the transport of live fish and their products on French soil. This service note applies to all movements, including the restocking of the natural environment, but does not apply to movements involving health inspections, exchanges or exports. This leaves room for further work on fish transport.

Council Regulation (EC) No 1099/2009 on the protection of animals at the time of killing covers the critical factors in the lives of animals farmed on land during their transport and slaughter. In its first Article (1.1), it stipulates that only the requirements laid down in Article 3.1 apply to fish: "Animals shall be spared any avoidable pain, distress or suffering during killing and related operations". This general obligation confirms the difficulty of laying down specific welfare and protection rules for fish, due to the lack of knowledge on the subject and the diversity of the species involved. Nevertheless, Article 27(1) of the Regulation states that "*the Commission shall submit to the European Parliament and to the Council a report on the possibility of introducing certain requirements regarding the protection of fish at the time of killing, taking into account animal welfare aspects as well as the socioeconomic and environmental impacts".* (Council of the European Union, 2009b).

In 2018, the European Commission published a report to the European Parliament and the Council on the possibility of introducing certain requirements relating to the protection of fish at the time of killing due to the very general nature of Regulation (EC) 1099/2009. It concludes that, "*Overall the general requirements contained in Regulation (EC) No 1099/2009 applicable to the welfare of fish at slaughter have contributed to the development of a framework in terms of national legislation and guidance for the welfare of farmed fish in the EU particularly for Atlantic salmon.*" It also notes that *"The level of achievement of OIE standards at slaughter varies with the species considered. For Atlantic salmon, best practices are mostly achieved, with a few exceptions, in the case-study countries. For common carp and rainbow trout, the level of achievement varies between methods used. For European sea bass and gilthead sea bream, OIE standards are not achieved in the case-study countries. […] The commissioned study report findings have also shown that the industry as a whole is gradually but continuously improving fish welfare as evidenced by the increasing use of more humane methods such as electrical stunning, the phasing out of others such as CO2stunning[2], and the adoption of private standards.*" These findings allow the Commission to conclude that: "*At this stage, the Commission considers that the evidence suggests that it is not appropriate to propose specific requirements on the protection of fish at the time of killing, taking into account that the objectives of the Regulation may equally be achieved by voluntary measures, as evidenced by the improvements introduced by industry in recent years. It is also important to note that this is a comparatively new and very diverse sector compared to other traditional farmed animal production systems, and technology for improved welfare is currently progressing. In view of these ongoing developments the Commission concludes that if further guidance is required this would be best achieved at Member State level. In any event the Commission will continue to monitor progress in this area."* (European Commission, 2018).

In the specific case of organic production, mention should also be made of Commission Regulation EC/710/2009, which lays down detailed rules for aquaculture animals and algae and includes species-specific parameters in Annex XIIIa (Council of the European Union. Most of these provisions are included in the consolidated version of Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on organic production and labelling of organic products, which came into force on 1 January 2022 (European Union 2018).

1.2.3. EFSA recommendations on the slaughter of aquaculture fish

Most of EFSA's work is undertaken in response to referrals from the European Commission, the European Parliament and EU Member States.

The Panel on Animal Health and Animal Welfare (AHAW), nominated by EFSA, published five expert opinions in 2008 on the risks for welfare of different farming systems and life stages of farmed salmon, rainbow trout, carp, eel, sea bream and sea bass (EFSA, 2008a, 2008b, 2008c, 2008d, 2008e). These will be revised over the period 2027-2029 (European Commission, 2021b).

In 2009, the AHAW panel also published expert opinions on the welfare aspects of fish stunning and slaughter for farmed salmon, rainbow trout, turbot, tuna, carp, eel, sea bream and sea bass (EFSA, 2009a, 2009b, 2009c, 2009d, 2009e, 2009f, 2009g). Their conclusions are presented in the following paragraphs.

Salmon

Methods assessed: carbon dioxide (CO_2), ice slurry, percussive stunning, electrical stunning, bleeding.

EFSA concludes, among other things, that carbon dioxide and ice slurry are the most risky methods with regard to the welfare of salmon, in particular because :

- high concentrations of CO_2 produce strong adverse reactions in salmon and it is therefore difficult to prescribe conditions that would reduce suffering,
- ice slurry does not cause immediate unconsciousness.

The CO_2 method was considered to present the greatest risk to salmon.

Conversely, the most favourable methods for the welfare of salmon appear to be percussive stunning and killing methods and certain electrical stunning methods. EFSA nevertheless points out that electrical stunning and killing methods can lack efficacy, particularly when the electrical current applied is too low.

Rainbow trout

Methods assessed: percussion, electrocution, carbon dioxide (CO_2), ice slurry (described in the opinion as “asphyxia in ice, ice slurry”), asphyxia, bleeding.

EFSA concludes, among other things, that carbon dioxide, water-ice mixtures and asphyxia in air are the most harmful methods for trout welfare.

Conversely, mechanical and electrical stunning cause trout to lose consciousness immediately if they are properly performed. These methods are therefore more conducive to trout welfare.

Sea bass and sea bream

Methods (commercial) assessed: asphyxia in air, ice, ice slurry.

EFSA concludes, among other things, that the three commercial methods investigated do not result in immediate loss of consciousness and include a prolonged period of consciousness for a period of several minutes, during which indications of poor welfare are apparent (physiological and behavioural responses). Alternative methods such as carbon dioxide, exposure to nitrogen and electrical stunning have been used only experimentally (at the time of publication). Of these alternative methods, only electrical stunning can induce immediate loss of consciousness and recovery is prevented by subsequent chilling of stunned fish.

Tuna

Methods (commercial) assessed: underwater shot to the head (lupara), shooting at the head from the surface, and spiking or coring the brain. The size of the fish and intended market are key factors in the choice of slaughter methods.

EFSA concludes, among other things, that underwater shooting (lupara) causes less suffering to large tuna than shooting from the surface, due to the large number of fish and the percentage of fish that require a second shot. For small tuna, coring and spiking in shallow water cause the lowest level of suffering, but they could be improved.

Turbot

Methods (commercial) assessed: bleeding, asphyxia on ice.

EFSA concludes, among other things, that the existing methods of killing turbot (bleeding and asphyxiation on ice) involve prolonged periods of consciousness during which stress responses have been observed. For this reason, these methods constitute a considerable welfare risk for turbot. Trials involving electrical stunning, which induces immediate loss of consciousness followed by chilling in ice water slurry, have shown promising results for turbot welfare and meat quality.

Eel

Methods (commercial) assessed: salt, ammonia, ice-salt mixture, electrical stunning in water. All these methods are followed by evisceration.

EFSA concludes, among other things, that there are currently no commercially available stunning methods that induce immediate loss of consciousness in all eels until death. Electrical stunning immediately followed by a killing method is the preferred commercially available method with regard to the welfare of eels.

Carp

Methods (commercial) assessed: asphyxia followed by percussion, electrical stunning in water, percussion stunning. All these methods are followed by evisceration.

According to EFSA, it is commonly accepted that the majority of carp are sold alive or as whole fish by retailers (supermarkets, market sales) or at the farm and that less than 15% of carp produced for human consumption is processed commercially.

EFSA nevertheless concludes that, of the methods evaluated:

- The electrical stunning method used commercially does not appear compatible with immediate unconsciousness in carp, due to the short duration of application of the electrical current and insufficient current/voltage.
- Aversive responses in carp to air asphyxia are a major welfare hazard.
- The risk assessment shows that percussive stunning with minimal exposure to air produces the least negative impact on carp, if properly performed.

Table 7 summarises the advantages and disadvantages of the methods used for stunning and/or killing fish by species, according to EFSA. Only methods considered non-experimental by EFSA and studied in this summary are described further in this table.

Table 7. Description of the methods used for stunning and/or killing, and their advantages and disadvantages, according to EFSA[16] . *Only methods considered non-experimental by EFSA and studied in this summary are developed in this table. (Original illustration by CNR BEA)*

Method of stunning or killing	Fish species studied by EFSA	Advantages of the method for fish welfare	Disadvantages of the method for fish welfare	Source
Electric stunning or stunning and killing: Passing of an electric current through the fish's brain to disrupt neural activity and cause loss of consciousness.	Atlantic salmon	- Unconsciousness induced in less than one second when electrical parameters are sufficient (for portioned trout, a current of between 3 and 6 V/cm should be applied between 30 and 60 sec).	- Potential exposure to pre-stun shocks. - Effectiveness of stunning depends on electrical parameters and water conductivity. - Potential paralysis and ineffective stunning if electric currents produce insufficient voltage. - Fish can recover from stunning, requiring a killing method to be applied immediately afterwards to prevent the fish regaining consciousness. - For carp: it would appear that the current/voltage and duration of application used are not compatible with instantaneous unconsciousness.	(EFSA, 2009a) p.21-22 et p. 37-38
	Rainbow trout			(EFSA, 2009d) p.30-31
	Eel			(EFSA, 2009c) p.25-26
	Carp			(EFSA, 2009b) p10-12 et p.22-23
Percussion: Striking the fish's head with an object with sufficient force to cause haemorrhaging in the brain	Salmon	- Instantaneous loss of consciousness and death when correctly applied. - Overall robust methods in terms of fish welfare.	- Potential paralysis before loss of consciousness. - Difficulty in applying the correct stun (due to varying fish sizes for automatic systems and need to immobilise the fish in manual systems), potentially leading to ineffective or reversible stunning. - Leaves fish exposed to air.	(EFSA, 2009a) p.21 et p. 37-38
	Rainbow trout			(EFSA, 2009d) p.14-15 et p.30-31
	Carp			(EFSA, 2009b) p.22-23
CO2 : Immersion of the fish in a tank of water saturated with CO_2	Atlantic salmon		EFSA states that **this method should not be used to stun and kill** any species of fish. - Slow loss of consciousness (around 6 minutes for salmon and over 4 minutes for trout). - Aversive for fish (aversive reactions sometimes last up to 4 minutes for salmon and more than 3 minutes for trout). - Fish hypoxia linked to the fact that the water is not changed between 2 batches. - Haemorrhage of the gills, loss of mucus, high metabolic activity and stress. - Potential paralysis before loss of consciousness.	(EFSA, 2009a) p. 18-19 et p. 37-38
	Rainbow trout			(EFSA, 2009d) p.16-17 et p.30-31

[16] The practical advantages and disadvantages of the methods studied by EFSA are not included in this table, which focuses on animal welfare.

Method of stunning or killing	Fish species studied by EFSA	Advantages of the method for fish welfare	Disadvantages of the method for fish welfare	Source
Live chilling: Immersion of the fish in a tank filled with ice/ice slurry.	Atlantic salmon		EFSA states that **this method should not be used to stun and kill** Atlantic salmon. - Slow loss of consciousness (5 minutes on average for sea bream). - Slow death (20 to 35 minutes for sea bream and 18 to 35 minutes for sea bass). - Aversive to fish (aversive reactions can last up to 4 minutes for salmon, sea bass and sea bream).	(EFSA, 2009a) p. 19-20 et p. 37-38
	Rainbow trout			(EFSA, 2009d) p.17 et p.30-31
	Sea bass			(EFSA, 2009e) p.17-18
	Sea bream			(EFSA, 2009e) p.17-18
Asphyxia in air: Fish are removed from the water and left to die in the open air.	Rainbow trout		EFSA states that **this method should not be used to stun and kill** any species of fish. - Slow death (up to 128 minutes for sea bass and 4 hours for turbot). - Extremely aversive to fish.	(EFSA, 2009d) p.22 et p.30
	Sea bass			(EFSA, 2009e) p.15-16
	Sea bream			(EFSA, 2009e) p.15-16
	Turbot			(EFSA, 2009g) p.13 et p.20
Bleeding: Severing of fish gill arteries.	Atlantic salmon	- When preceded by a stunning method, bleeding prevents the fish from regaining consciousness.	EFSA indicates that **this method should not be used without prior stunning** for all fish species. - Slow death if not preceded by a stunning method. - Aversive to fish if not preceded by a stunning method.	(EFSA, 2009a) p. 23-24 et p. 37-38
	Rainbow trout			(EFSA, 2009d) p.17
	Turbot			(EFSA, 2009g) p.13 et p.20
Spiking or coring: The brain of the fish is pierced manually with a sharp tool.	Tuna	- Unconsciousness induced in less than a second when the procedure is carried out correctly	- Ineffective stunning if performed incorrectly. - Aversive to fish if bled before the brain is destroyed.	(EFSA, 2009f) p.13-15 et p.22

1.2.4. <u>Fish welfare in aquaculture: a new priority for the European Commission</u>

As part of its "Farm to Fork" strategy, the European Commission is committed to reviewing animal welfare regulations and considering different possibilities for labelling.

To inform its thinking, it launched a number of projects, including a "fitness check" of current European rules on animal welfare at farm level, during transport and at slaughter. The results were published in October 2022, their main objective being to "assess whether the existing rules are still fit for purpose, in particular the extent to which they are relevant, efficient, effective, coherent, and have added value". To do this, the European Commission based its work on:

- "in-depth documentary research" (including EU legislation, European Commission staff working documents, peer-reviewed scientific articles and university theses, statistical studies and raw data supplied by Member States),
- field surveys, in particular a targeted stakeholder consultation and a public consultation
- an "independent study to support the cost-benefit analysis of the current EU legislation on animal welfare".

The fitness check concludes that, although European legislation on animal welfare has "improved the welfare of many of Europe's animals", it "is not fully adequate to meet current and future needs" because "current rules do not fully reflect society's growing expectations and ethical concerns, scientific and technological evidence, developments and future sustainability challenges". Indeed, farmed fish are cited as one of the species whose welfare in the EU is still at a "sub-optimal level", in particular because they are not the subject of targeted legislation (European Commission, 2022).

Given that one of the aims of the review of EU animal welfare legislation is to broaden the scope of current rules with the latest scientific evidence, the European Commission has published a roadmap listing the future mandates for EFSA to be issued between 2022 and 2030. According to this roadmap, between 2027 and 2029 EFSA will publish six reports on the welfare of different species of farmed fish: salmon and trout (June 2026), carp (June 2027), sea bass (June 2028), sea bream (June 2028), eel (June 2028), and tuna (December 2029). These reports will not, however, consider slaughter practices (European Commission, 2021b).

In 2017, the European Commission also set up a European Animal Welfare Platform comprising an interactive network of experts to assist it on issues directly related to animal welfare and to promote dialogue between stakeholders.

Last, on 12 May 2021, the European Commission published its new 2021-2030 strategy for aquaculture. While the concept of animal welfare was not mentioned in the previous strategy, an entire section (Section 2.2.2) has now been devoted to the issue. The strategic guidelines for more sustainable and competitive EU aquaculture for the period 2021-2030 will be used to direct the allocation of subsidies from the European Maritime Affairs, Fisheries and Aquaculture Fund (EMAFAF). The section on fish welfare includes the following focus areas:

- "developing good practices on fish welfare during farming, transport and killing;

- setting common, validated, species-specific and auditable fish-welfare indicators throughout the production chain (including in transport and slaughtering);
- further research and innovation, in particular on species-specific welfare parameters, including nutritional needs in different rearing systems; and
- providing knowledge and skills on fish welfare to aquaculture producers and other operators that handle live farmed fish". (European Commission, 2021a)

1.2.5. Specific French regulations and initiatives for the welfare of fish

Under French legislation, Article 515-14 of the Civil Code states that "animals are sentient living beings". However, the protection of fish is poorly regulated in France and relies more on the initiatives of the industry.

In 2018, CIPA expanded and reactivated the "welfare referents" working group dedicated to discussions and reflections on fish welfare. In order to gain a better understanding of welfare criteria, CIPA has joined forces with scientific and technical partners to report on knowledge of the welfare of fish species farmed in France and on work in progress, to establish priorities for work and to encourage the emergence of projects based on these fish welfare priorities. Since 2019, the "Fish welfare" platform has brought together, at the initiative of CIPA and CNR BEA, AgroParisTech, ANSES, DGAL, DGAMPA, IFREMER, INRAE, ITAVI, ONIRIS, SNGTV and SYSAAF. The priorities of the French fish farming industry in terms of fish welfare are as follows:

- Identifying welfare indicators for farmed fish by species and by farming system,
- Characterisation of the links between stocking density and fish welfare[17] ,
- Improving slaughtering conditions and methods to ensure the fish welfare.

The aim of creating the fish welfare platform is to build bridges between farmers' approach to this issue and recent knowledge acquired by the scientific community.

In addition, the French fish farming industry has introduced a single set of production specifications ("Quality Charter - Aquaculture de nos Régions®") , the requirements of which are verified by a third-party organisation at farms and processing and packaging plants. It covers 70% of production volumes intended for consumption. The specifications include obligations of means based on the 4 main principles of animal welfare (good health, good food, good environment and respect for the specific behaviour of the species) which characterise the needs of the fish to be satisfied in order to maximise their welfare:

- Hygiene control, application of the guide to good sanitary practice in fish farming
- Adapting breeding to the amount of water available, maximum densities

[17] Density has been used by default (for lack of operational indicators) as a welfare criterion, particularly in production specifications (quality and origin identification signs, retail chains). French fish farming professionals would like to see scientific publications on the subject provide information on the links between stocking density and fish welfare.

- Monitoring water quality, fish numbers and weights, growth and mortality (daily removal of dead fish)
- Facilities and equipment designed to promote the well-being and limit the stress of fish
- Creation of batches of uniform size
- Handling limited to that which is strictly necessary
- Preventing predation by wild animals
- Adapting and monitoring your diet
- Fasting before transport and/or slaughter

In the absence of available operational welfare indicators (currently being developed as part of the work being done by the fish welfare platform), these obligations of means should ensure that fish are farmed under good conditions.

Lastly, health approval for packaging and processing plants covers slaughter. Checks carried out by the DDPP (Direction Départementale de la Protection des Populations) are designed to assess slaughter methods from both a health and an animal welfare point of view.

En conclusion :

La réglementation en matière de bien-être des animaux est souvent peu adaptée aux poissons et non spécifique. Cela s'explique entre autres par la diversité des espèces élevées, par le manque de connaissances sur leurs sensibilités spécifiques, et par l'intérêt relativement récent du consommateur pour la prise en compte de leur bien-être.

Dans la perspective de la modification prochaine de la réglementation européenne, il est indispensable de disposer de connaissances scientifiques précises sur la sensibilité et les besoins physiologiques et comportementaux des poissons afin de proposer des pratiques adaptées pour une protection optimale des espèces aquatiques.

2. Sensory and emotional sentience in fish

European regulations and, consequently, French law are based on the recognition of the sentience of fish, following the wider principle of "animal sentience". As for other animal species defined as sentient living beings, the science divides fish sentience into two dimensions, one purely sensory and the other psychological, both of which need to be taken into account when designing slaughter procedures to respect fish sentience. The teleost family of ray-finned fish accounts for 99.8% of all fish species and therefore includes almost all fish species farmed in France (Figure 7). In this report, the sensory and emotional world of fish is described mainly in terms of teleosts.

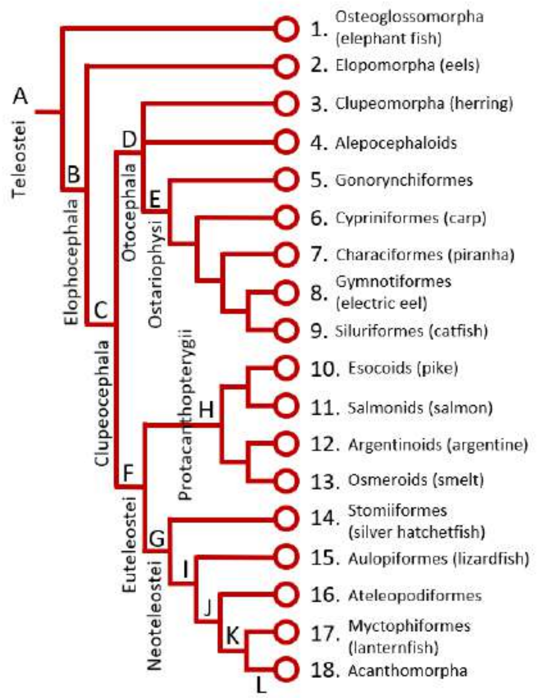

Figure 7. Classification phylogénétique de la famille des Téléostéens *Based on Lapert (2010)*

2.1. The sensory dimension of sentience

Compared with terrestrial vertebrates, fish have a particularly sophisticated sensory system. They have six senses: sight, smell, taste, hearing and touch, along with a mechanosensory organ, the lateral line, that enables them to perceive vibrations in the water and changes in pressure. Some species can, moreover, emit and perceive electrical fields. Fish are also poikilothermic (their body temperature is identical to that of the surrounding water), which makes them highly sensitive to temperature change. They use their sensory abilities for behavioural decision-making and to communicate with other fish. These abilities can vary greatly from one fish species to another, thus affecting fish reactions to slaughtering practices in different ways. As a consequence, it is essential to take into account the sensory capabilities specific to each individual species when attempting to understand how a fish perceives and interprets the various events associated with a particular slaughtering practice.

2.1.1. Vision

The eye of a fish works in much the same way as that of most vertebrates: light passes through the cornea and is then directed towards the retina by the crystalline lens. Unlike the lenses of mammals, which are oval, fish lenses are spherical, preventing them from seeing properly at a distance but allowing them to perceive shapes nearby. In most species, the eye adjusts to distance vision by using ligaments and retracting muscles that pull the lens towards the retina (Figure 8a). The retina is equipped with photoreceptor cells comprised of rods and cones, respectively allowing fish to perceive low-intensity lights and distinguish colours (Figure 8b). The density of the cones enables many species of fish, including salmonids, to detect ultraviolet light. These photoreceptors are in contact with intra-retinal neurons and ganglion cells whose axons extend from the retina via the optic nerve, carrying information to the optic tectum, the primary visual centre in a fish's brain (Montgomery & Carton, 2008). However, eye morphology and the neural circuits involved in light perception can vary between species as the result of differing light levels in the environments in which the various species live. In general, the eyes of deep-water species are larger in diameter than those of fish that live nearer the surface (Montgomery & Macdonald, 1998). Unlike mammals, most fish species do not adjust the diameter of their pupils in response to variations in light intensity. Instead, a retinomotor response occurs, involving morphological changes and pigment migration in the photoreceptors and epithelium. Nor do fish have eyelids (except certain species of shark), making them particularly sensitive to sudden changes in luminosity.

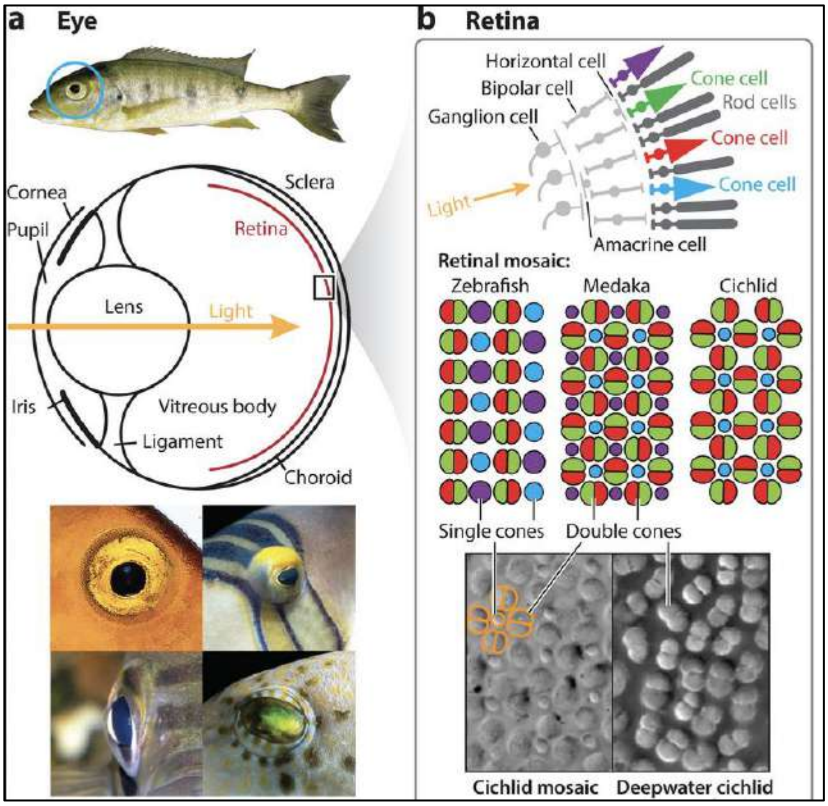

Figure 8. The visual sensory system of teleost fish. *Source: Musilova et al, 2021. (a) The lens in teleost eyes is spherical. (b) The retina is equipped with photoreceptor cells made up of rods (Rod cells) and cones (Cone cells), which may be single or double, depending on species.*

2.1.2. Smell

In teleosts, the olfactory organ is located on the dorsal part of the head. It consists of a cavity connected to the external environment by two openings (anterior and posterior nostrils, or nares), which direct the flow of water into the organ when the fish is swimming. The olfactory cavity is made up of lamellae, themselves composed of an epithelium. The epithelium contains olfactory receptor neurons (ORNs) whose axons are linked directly to synapses in the olfactory bulb that send information to the olfactory regions of the forebrain. Five main classes of non-volatile chemical compounds can be detected by the olfactory organs of the teleost family: amino acids, gonadal steroids, bile acids, prostaglandins etc. (Laberge & Hara, 2001), and polyamines (Rolen et al., 2003). A fish's perception of these odorous compounds influences its feeding behaviours, reproduction, migration, social behaviours and avoidance of predators.

2.1.3. Taste

In most fish species, the sense of taste is divided between two sets of receptors: taste buds and solitary chemosensory cells. Taste buds form part of the epidermal sensory organs, which respond to a variety of chemical compounds as well as tactile stimuli (Barlow & Northcutt, 1997). In general, taste buds are found in the oropharyngeal cavity but in some groups, for example catfish, they are also found throughout the body. Oropharyngeal taste buds are innervated mainly by the vagus nerve, which projects from the vagal lobe into the hindbrain, while external taste buds are innervated by the facial nerve. The main chemical compounds detected by fish are amino acids, carboxylic acids, nucleotides and bile salts. Taste sensitivity to CO_2 , H^+ and marine toxins has also been reported (Hara, 1994). Unlike the taste buds, solitary chemosensory cells are not sensitive to tactile stimuli. They are found across the entire surface of the fish's body and are innervated by the facial or spinal nerves. Clusters of solitary chemosensory cells have been identified on the rays of the dorsal and pectoral fins, mainly responding to chemical compounds such as bile acids.

2.1.4. Hearing

Sound travels in water three times faster than in air. The main auditory sense used by fish is otolith hearing (Montgomery & Carton, 2008). The otoliths found in the inner ear of the fish are very dense and together act as an accelerometer, detecting the animal's movements in the sound field. In most species, the sacculus is specialised in sound detection, using sets of hair cells to detect the relative movements of the fish and the two saccular otoliths. The different orientations of these populations of hair cells enable fish to determine a source of sound vibration in a three-dimensional environment. The swim bladder also has a role to play in the auditory system by mechanically detecting variations in pressure and communicating with the inner ear. It acts as a resonance chamber for sound vibrations, amplifying the sound. Most fish species can detect very low-frequency sounds (infra-sound) down to frequencies of 500 to 800 Hz. Species with the most highly developed auditory systems can also hear ultrasound (> 2kHz) (Montgomery & Carton, 2008). Some species (cod, haddock, red mullet) are also able to emit sounds for intra-specific communication by vibrating the muscles that touch the swim bladder or the swim bladder itself, or by using various methods to produce stridulatory sounds.

2.1.5. Touch

Cutaneous sensory receptors linked to a profusion of nerve endings located across the entire skin, particularly around the mouth, provide fish not only with a sense of touch but also with the means to detect water temperature and salinity.

Particular sensitivity to temperature

Fish are poikilothermic animals in that their body temperature is identical (± 0.1-1°C) to the temperature in their immediate environment, making them extremely sensitive to any temperature changes (Quigley & Hinch, 2006). They can detect variations in ambient temperature of less than 0.05°C. As a group, fish species are able to adapt to a wide range of temperatures, from 0 to 40°C. A curve is often used to represent the thermal sensitivity of fish, summarising tolerance (survival rate, maintenance of balance, sensitivity to touch) and performance (fertility, growth, metabolic rate, swimming performance). For each species, (i) the optimal temperature for performance, (ii) the thermal window that designates the limits of the temperature range beyond which performance decreases and (iii) the zone of tolerance between the maximum and minimum temperatures that an individual fish can tolerate and survive during short-term exposure have been defined (Schulte, 2011). In fish accustomed to a warm maritime environment, a cold thermal shock (7 to 14°C below the usual environmental temperature) causes physiological stress and reduces metabolic performance and the ability to swim (a state known as 'cold coma'), sometimes even ending in death. The more rapid and greater the drop in temperature, the greater the negative effect (Szekeres et al., 2014). In most teleost fish, temperature receptors are located in the lateral line, but cartilaginous fish have a specialised organ, the ampullae of Lorenzini, which enables them to detect temperature gradients and electromagnetic fields.

Sensitivity to water salinity

Marine fish are sensitive to the concentrations of salts in their environment and can detect salinity levels as low as 0.5 ppm. They have calcium receptors that detect variations in the density of cations in the water. These receptors enable them to detect changes in concentrations of Ca^{2+}, Mg^{2+} and Na^{+}.

The lateral line: touch at a distance

The lateral line is a mechanosensory organ formed of canals equipped with receptors that detect variations in pressure, currents, vibrations, movement and obstacles in the vicinity of a fish. It is visible in most species as a line that extends along each side of the body between the operculum and the caudal fin. Water enters the canals through multiple pores located along the line that open onto sensory receptors known as canal neuromasts (Figure 9a, c), formed by hair cells that communicate with the brain via the lateral nerve. In addition to the canal neuromasts located within the lateral line, superficial neuromasts are distributed across a fish's body (Figure 9a, b). Neuromasts are covered by a cupula, a gelatinous structure that acts as a biomechanical interface between hair cells contained in them and the surrounding water. The approach of an object creates movements in the water that bend the cupula and the ciliary bundles within the hair cells, triggering a nerve impulse to the brain via the associated neurons that form the nerves of the lateral line, giving the fish an almost instantaneous motor response. It is the lateral line that enables some species to form coordinated shoals of fish that can move rapidly without colliding. The hair cells of the neuromasts resemble the hair cells of the inner ear, indicating that the lateral line and the inner ear share a common origin and function, although the lateral line tends to detect obstacles and movements closer to the fish (at a distance of less than one fish length).

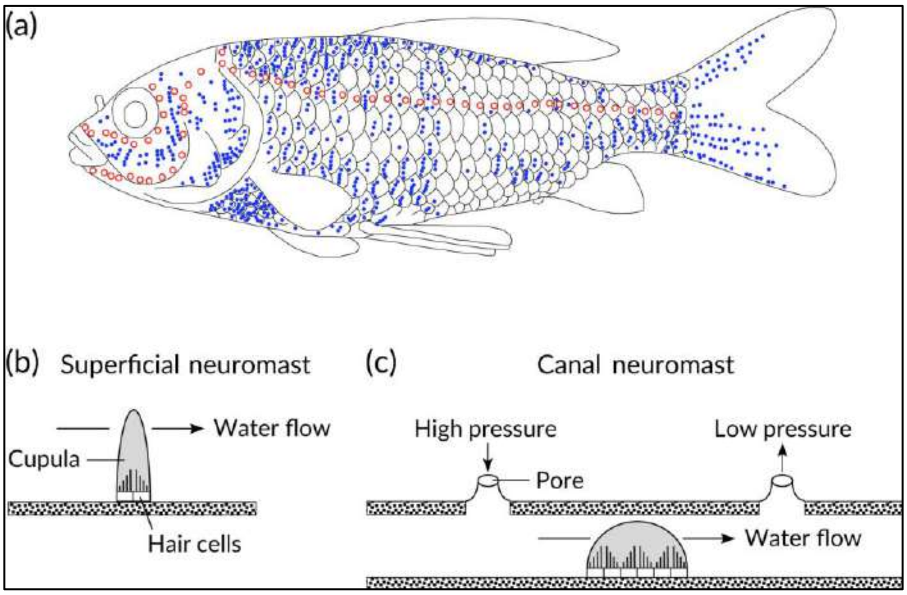

Figure 9. (a) Distribution of neuromasts in the teleost fish, *Carassius auratus*, (b) a superficial neuromast and (c) a canal neuromast. *Source: Mogdans, 2019 ; ● superficial neuromasts; ○ pores connecting to the canal neuromasts that extend (internally) along the lateral line. Superficial neuromasts are directly stimulated directly by the flow of water at the surface of the fish's skin, whereas canal neuromasts respond to differences in pressure generated by the flow of water between pores.*

2.1.1. Generation and perception of electric fields

All fish generate weak electric fields due to the exchange of ions between their bodies and their environments, but not all can perceive them. In weakly electric fish (producing a few hundred millivolts/cm), an electrical organ generates discharges controlled by the electromotor system located in the brain stem (Caputi et al., 2005). They thus emit a weak electric field that allows them to perceive the presence of obstacles or animals crossing their path. Known as active electroreception, this characteristic, found in certain groups of fish (Siluriformes, some Osteoglossiformes and Gymnotiformes), Figure 7), provides information that helps fish to orient themselves in their environment and detect prey. Some fish are capable of emitting far more powerful electric fields (a few hundred volts/cm) that are used for predation, defence or even communication, and can, in some cases, stun a prey animal (Caputi et al., 2005). Other families of fish (sturgeons and cartilaginous fish, not members of the teleost family) use passive electro-reception, which enables a fish to detect the electric fields emitted by its prey using receptors such as its ampullae of Lorenzini. The perception of these electric fields seems to be limited to nearby prey, i.e., no further away than the length of the fish's body (Montgomery & Carton, 2008).

2.2. The psychological dimension of sentience

The psychological dimension of sentience reflects an individual's ability to feel emotions. An emotion is an intense, transient affective response to an inciting situation. As for terrestrial vertebrates, the emotion felt by a fish will depend on its assessment of the inciting situation; this emotion is not directly measurable, but can only be inferred from its behavioural and physiological reactions in conjunction with the situation to which the animal is exposed (Boissy et al., 2007). Of the various emotions identified in mammals (fear, anxiety, frustration, pleasure, etc.), fear, anxiety and pain are those that have been described, as they appear in many species of fish. Under European regulations, all vertebrates, including farmed fish, are considered to be sentient living beings (Lisbon Treaty, 2007). This status has appeared in the French Civil Code since the addition of Article 515-14[18] in 2015.

The study of fish welfare at the time of slaughter is a relatively recent phenomenon compared with the more longstanding body of work on this area for terrestrial animals (Terlouw et al., 2008). Studies are now available, though, that draw on both neuro-anatomical evidence and the expression of behaviours to suggest that fish, like mammals and birds, experience pain and stress (cf. 0. and 2.2.4.). Neurobiological studies have also identified brain structures in the telencephalon of fish that are homologous with the amygdala and hippocampus, the parts of the mammalian brain linked to the emotions, while neurotransmitters associated with the emotions in humans, such as dopamine, serotonin and isotocin (the mammalian equivalent of oxytocin), have also been identified in fish (Thompson & Walton, 2004; Winberg & Nilsson, 1993). Despite these advances, some researchers still remain sceptical concerning the capacity of fish to feel pain or suffering, citing the absence in fish of the cerebral cortical structures involved in the perception and awareness of pain in mammals (but see 2.2.3. and 2.2.4.) and asserting that the results of studies designed to characterise pain in fish cannot be replicated (Diggles et al, 2023; Rose, 2007).

2.2.1. __Fear__

Fear is a fundamental emotion for the survival of the individual, producing adaptive behaviours that protect animals from possible threats. Certain behavioural responses to threatening stimuli (the sudden dropping of an object, the appearance of a new object, predation) have been described for fish (Table 8). These responses are also elicited by a conditioned stimulus previously associated with the occurrence of a threat, indicating that

[18] "Animals are sentient living beings. Subject to the laws that protect them, animals fall under the rules governing property.

cognitive processes such as anticipation and prediction are involved rather than mere reflexes. For example, individual rainbow trout and Atlantic salmon placed in a chamber swim away to a different chamber when a light cue associated with a plunging net is initiated, thus expressing conditioned fear (Yue et al., 2004). Moreover, following ablation of the medial and lateral pallia of the telencephalon, changes were observed in the conditioned fear responses of goldfish (Portavella et al., 2004). This points to the involvement of the central nervous system in the fear responses of the fish in that study.

Table 8. Physiological and behavioural indicators of fear in fish *(table created by FRCAW)*

	Species	Indicators	References
Physiological	Trout (*Onchorhynchus mykiss*)	↑ ventilation (gill movements/beat rate)	(Sneddon et al., 2003)
	Zebrafish (*Danio rerio*)	↑ ventilation (gill movements/beat rate)	Summarized in (Kalueff et al., 2013)
	Zebrafish (*Danio rerio*)	Change of colour	Summarized in (Kalueff et al., 2013)
Behavioural			
Reflexive/automatic responses	[European] Sea bass (*Dicentrarchus labrax L.*)	↑ maximum speed (flight), followed by ↓ swimming activity	Millot et al., 2009
	Zebrafish (*Danio rerio*)	Immobilisation (freezing)	Summarized in (Kalueff et al., 2013)
	Zebrafish (*Danio rerio*)	↑ erratic swimming (zig-zag swimming)	Summarized in (Kalueff et al., 2013)
	Zebrafish (*Danio rerio*) adult, goldfish (*Carassius auratus*), guppy (Poecilia reticulata) and Nile	Scototaxis (preference for areas of darkness)	(Maximino et al., 2010)
	Zebrafish (*Danio rerio*)	Thigmotaxis (preference for contact with tank/pond sides)	Summarized in (Kalueff et al., 2013)
	Zebrafish (*Danio rerio*)	Geotaxis (position at the base of the water column/ bottom of the tank)	Summarized in (Kalueff et al., 2013)
	Salmon (*Oncorhynchus tshawytscha*)	↓ swimming activity, ↓ dietary intake, geotaxis	(Berejikian et al., 2003)
	Trout (*Onchorynchus mykiss*)	↑ maximum speed (flight), followed by immobilisation, erratic swimming (zig-zag swimming), avoidance of new objects	(Poisson et al., 2017)
	Trout (*Onchorynchus mykiss*)	↓ swimming activity, ↓ dietary intake	(G. E. Brown & Smith, 1997)
	Tilapia (*Oreochromis niloticus*)	Inhibited feeding anticipation	(Silva et al., 2015)
	Tilapia (*Oreochromis niloticus*)	Neophobia (avoidance of new objects)	(Martins et al., 2011)
	Tilapia (*Oreochromis niloticus*)	Anorexia	(Martins et al., 2011)
Conditioned fear responses	Trout (*Onchorynchus mykiss*) and salmon (*Salmo salar*)	Conditioned flight response to a light cue associated with a plunging net	(Yue et al., 2004)
	Trout (*Onchorynchus mykiss*) and goldfish (*Carassius auratus*)	Conditioned avoidance response to a zone/area associated with an electric shock	(Dunlop et al., 2006)
	Tilapia (*Oreochromis niloticus*)	Conditioned flight response to cessation of water flow associated with confinement	(Martins et al., 2011)
	Tilapia (*Oreochromis niloticus*)	↑ plasmatic cortisol in response to a light cue associated with confinement	(Moreira & Volpato, 2004)

2.2.2. Anxiety

Anxiety, too, is attributed to fish or, rather, a state of anxiety, since this emotion can be relatively long-lasting. Anxiety is a negative emotion caused by the fear of future danger (whether real or not). In zebrafish placed in social isolation or exposed to an alarm pheromone (signifying a risk of predation), a reduction in exploratory behaviour, the appearance of erratic movements ('zig-zag' swimming), an increase in the time spent motionless (freezing), and a position at the bottom of the water column were observed. These behaviours are accompanied by an increase in plasma cortisol. Such behavioural and physiological measures resemble the responses associated with fear, with the difference that they can occur when no danger is present. What is more, they have been shown to disappear when anxiolytics (diazepam) or antidepressants (fluoxetine) are administered to zebrafish exposed to anxiety-provoking situations (Egan et al., 2009). Anxiolytic effects of such substances have also been reported in other model fish species (*Pimephales promelas*: Margiotta-Casaluci et al., 2014; *Oryzias latipes*: Ansai et al., 2016; *Carrassius Auratus*: Simmons et al., 2017). Similar effects have also been observed in sea bass and zebrafish following exposure to nicotine at certain concentrations (Alfonso et al., 2020; Levin et al., 2007).

2.2.3. Pain

Pain has recently been re-defined by the International Association for the Study of Pain as ‘an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage’ (see Raja et al., 2020). Pain therefore goes beyond nociception – which is the non-conscious processing of noxious stimuli by the peripheral and central nervous systems (Tracey, 2017) – in that its definition presupposes that an individual can feel emotions. The ability to feel emotions (fear, anxiety) has been demonstrated in certain species of fish using both physiological and behavioural approaches (cf. 2.2.1., 2.2.2.), suggesting that the definition of pain is also applicable to this class of vertebrates. Several studies have shown that teleost fish possess the neuroanatomical features required to feel pain. Nociceptors that respond to extreme heat, mechanical pressure and chemical lesions have been identified in rainbow trout, distributed chiefly around the mouth (Ashley et al., 2007; Sneddon et al., 2003). The main circuits that carry nociceptive information from the peripheral system to the brain are the spinothalamic tract (body) and the trigeminal tract (head), which have been identified not only in teleosts but also in elasmobranchs and agnathans (Sneddon, 2004). In trout, for example, two types of nociceptor (A delta and C fibres) are present in the trigeminal nerve. An opioid circuit (receptors and endogenous ligands) can be found in the nervous system of fish, strongly suggesting their ability to modulate pain (Gonzalez-Nunez & Rodríguez, 2009). In the course of a noxious stimulus, the differential expression of genes has been observed, mainly in the forebrain (the site of pain in mammals), with the global changes continuing for up to 6 hours (Reilly et al., 2008b). With regard to physiology, the injection of acidic substances has been shown to lead to an increase in ventilation rate (gill beats) in trout and zebrafish (Sneddon, 2009) and an increase in plasma cortisol levels in trout (Ashley et al., 2009) and tilapia (Roques et al., 2012).

Behavioural changes also occur in fish following potentially painful stimuli, and such changes differ according to the type of nociceptive stimulus and fish species studied (Table 9). For example, tilapia reduce their swimming activity following an electric shock compared with initial activity levels (Roques et al., 2012), but their activity increases after a fin biopsy (Roques et al., 2010). Atlantic salmon (Bjørge et al., 2011) and rainbow trout (Sneddon, 2003a, 2003b) respond to nociceptive stimulation by decreasing their activity levels. Other behavioural changes have also been observed following the injection of acid into the mouth, namely, rubbing of the injured area (trout and goldfish) and rocking of the body on the substrate at the bottom of the tank (trout, carp and carassin) (Newby et al., 2009; Reilly et al., 2008a; Sneddon, 2003a). However, in other species exposed to the same nociceptive stimulus, these behavioural responses were not observed (cod (Gadus morhua) in Eckroth et al., 2014; and pike (Esox lucius in Pullen et al., 2017). In trout, food intake was also suspended for more than 3 hours, and the neophobia usually observed in this species when faced with a new object was inhibited (Sneddon et al., 2003). These behavioural changes can last for more than 6 hours, indicating that they are not merely reflexes, and they do not appear when an analgesic (morphine) is administered (Mettam et al., 2011; Sneddon et al., 2003).

Experiments have shown that teleost fish are also capable of avoiding a zone in their environment where they have been subjected to a painful experience (prior exposure to an electric shock), and this avoidance can last for up to three days (Dunlop et al., 2006). After three days of food deprivation, they will risk entering the area to feed (Millsopp & Laming, 2008), thereby choosing a compromise. These studies demonstrate that fish are capable of memorising pain experiences and anticipating them by actively avoiding the associated area, reflecting complex and continued changes in their information-processing capacity following nociceptive stimulation.

Table 9. Physiological and behavioural indicators of pain in fish *(Table created by FRCAW)*

	Species	**Indicators**	**References**
Physiological	Trout (*Onchorynchus mykiss*)	↑ ventilation (gill movements)	(Ashley et al., 2009; Sneddon, 2003a, 2003b)
	Zebrafish (*Danio rerio*)	↑ ventilation (gill movements)	(Reilly et al., 2008a)
	Goldfish (*Carassius auratus*)	↑ ventilation (gill movements)	(Newby et al., 2009)
	Trout (*Onchorynchus mykiss*)	↑ plasma cortisol	(Ashley et al., 2007)
	Tilapia (*Oreochromis mossambicus*)	↑ plasma cortisol and glucose	(Roques et al., 2012)
Behavioural	Trout (*Onchorynchus mykiss*)	↓ swimming activity	(Reilly et al., 2008a; Sneddon, 2003a, 2003b)
	Zebrafish (*Danio rerio*)	↓ swimming activity	(Correia et al., 2011; Maximino, 2011; Reilly et al., 2008a)
	Tilapia (*Oreochromis mossambicus*)	↓ swimming activity	(Roques et al., 2012)
	Salmon (*Salmo salar*)	↓ swimming activity	(Bjørge et al., 2011)
	Piaussu (*Leporinus macrocephalus*)	↑ swimming activity	(Alves et al., 2013)
	Tilapia (*Oreochromis niloticus*)	↑ swimming activity	(Roques et al., 2010)
	Salmon (*Salmo salar*)	Anorexia for 6.5h and swimming at the base of the water column	(Bjørge et al., 2011)
	Trout (*Onchorynchus mykiss*)	Anorexia	(Mettam et al., 2011; Sneddon, 2003a)
	Trout (*Onchorynchus mykiss*)	Unusual behaviours (rocking, rubbing)	(Sneddon, 2003a, 2003b)
	Carp (*Cyprinus carpio*)	Unusual behaviours (rocking, rubbing)	(Reilly et al., 2008a)
	Tilapia (*Oreochromis mossambicus*)	Unusual behaviours (rubbing)	(Roques et al., 2012)
	Goldfish (*Carassius auratus*)	Unusual behaviours (rubbing)	(Newby et al., 2009)
	Zebrafish (*Danio rerio*)	Unusual behaviours (tail movements)	(Maximino, 2011)
	Trout (*Onchorynchus mykiss*)	↓ response against predators	(Ashley et al., 2009)
	Trout (*Onchorynchus mykiss*)	↓ aggressive behaviours	(Ashley et al., 2009)
	Trout (*Onchorynchus mykiss*)	↓ neophobia (new objects)	(Sneddon, 2003a)
	Goldfish (*Carassius auratus*) and trout (*Onchorynchus mykiss*)	Avoidance of an electric shock zone for 3 days	(Dunlop et al., 2006)
	Carp (*Cyprinus carpio*)	Avoidance of fishing hooks for up to a year	(Beukema, 1970)
	Goldfish (*Carassius auratus*)	Compromise decision (electric shock vs food)	(Millsopp & Laming, 2008)
	Carp (*Cyprinus carpio*)	Reduced responses when treated with analgesics (opioides)	(Chervova & Lapshin, 2011)
	Goldfish (*Carassius auratus*)	Reduced responses when treated with an analgesic (morphine)	(Newby et al., 2009)
	Trout (*Onchorynchus mykiss*)	Reduced responses when treated with non-steroidal anti-inflammatory drug (carprofen) and anesthetics (lidocaine)	(Mettam et al., 2011)
	Trout (*Onchorynchus mykiss*)	Reduced responses when treated with an analgesic (morphine)	(Sneddon, 2003a, 2003b)
	Zebrafish (*Danio rerio*)	Reduced responses when treated with an analgesic (morphine)	(Correia et al., 2011)

2.2.4. Stress

Like other vertebrates, fish respond to events that may cause them harm with a suite of adaptive physiological reactions known as the 'stress response'. These neuroendocrine adjustments trigger reversible metabolic and behavioural changes that enhance a fish's ability to overcome or avoid threats and are beneficial, at least in the short term. When the stress response is prolonged, though, it becomes detrimental, leading in the long term to reduced growth, reproductive dysfunction and immunosuppression (Braithwaite & Ebbesson, 2014). Stress is often associated with a negative emotional state.

Fish generally manifest the same neuroendocrine and behavioural strategies as terrestrial vertebrates when faced with a stressor (Wendelaar Bonga, 1997). Initially, we see a primary response in which the catecholaminergic system and the hypothalamic-pituitary-interrenal (HPI) axis are activated (Figure 10). Catecholamines are released rapidly from the chromaffin tissue in the anterior kidney (via the catecholaminergic system), and CRH (corticotrophin-releasing hormone) is released from the central nervous system via the hypothalamus (HPI axis). CRH is a neuropeptide that stimulates the release of pituitary ACTH (adrenocorticotropic hormone). ACTH stimulates the synthesis and secretion of cortisol by the interrenal gland (homologous to the mammalian adrenal cortex) in association with other pituitary hormones such as MSH (melanocyte-stimulating hormone, which stimulates melanophores and the secretion of β-endorphins). Of these two neuroendocrine systems, the activation of catecholamines results in immediate behavioural responses (immobilisation, escape, increased gill beats and heart rate), while responses are slightly delayed when cortisol is activated (reduced appetite) (Wendelaar Bonga, 1997; Ellis et al., 2012).

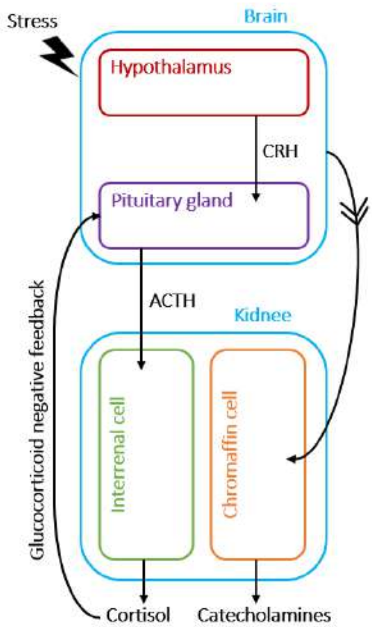

Figure 10. Activation of the hypothalamic-pituitary-interrenal axis in fish. *Based on Delfosse (2017)*

An increase in the concentration of cortisol is a commonly used indicator of acute stress in fish, with peaks from 30 minutes to 4 hours after an acute stress-inducing event. Differences in peak times can be due to the type of stressor (fishing, confinement, transport), the duration of the stressful event, and the species of fish. It is possible to measure cortisol not only in fish blood plasma, but also in mucus, faeces, scales or water discharged through the gills (Sadoul & Geffroy, 2019). Following these primary responses, further, secondary responses arise that may or may not be directly caused by the primary endocrine response. They include changes in the concentrations of glucose, lactate, lactic acid and the major ions (e.g., chloride, sodium, potassium, etc.) present in the blood, as well as changes in glycogen and chaperone protein (HSP) levels in tissues. These responses are all related to physiological adjustments, for example, changes in metabolism, respiration, acid-base status, hydromineral balance, immune function and cellular responses (Barton, 2002). If the stressor is prolonged and the fish is unable to adapt (e.g., chronic stress due to poor water quality or high stocking density), tertiary responses linked to the animal's overall performance then appear, such as reduced growth, reduced activity, inhibition of reproduction, and reduced resistance to disease (Barton, 2002).

A state of stress can alter the cognitive evaluative capacities that lead to affective states (Baciadonna & McElligott, 2015; Harding et al., 2004). Such judgement biases have been demonstrated for several animal species (rodents: Neville et al., 2021; chickens: Hernandez et al., 2015; sheep: Doyle et al., 2011; pigs: Murphy et al., 2013). The underlying principle involves the individual's response to an ambiguous situation, which may be perceived as negative or positive depending on the individual's level of stress. Recently, a species of cichlid (*Amatitlania siquia*) used in the laboratory has been shown to display decision-making judgement biases induced by stress linked to social separation (Laubu et al., 2019). Emotional reactions and states in fish are therefore not reflexive or automatic responses. On the contrary, how the animal perceives and feels about events occurring in its environment depends on cognitive processes.

2.3. Indicators of stress

Under stressful conditions, fish display physiological and behavioural responses that may resemble those that occur in circumstances where negative emotions (fear, anxiety) are triggered, or pain is felt. To determine the cause of such responses, it is therefore important to know in what context they appear. In the case of the slaughter process, behavioural measurements or physiological and metabolic parameters can be used to assess the level of stress in fish.

2.3.1. Physiological indicators

As stated above, it is possible to use the effectors of neuroendocrine responses to stress as stress indicators. For example, during the primary response phase, activation of the catecholaminergic system and the HPI axis can be detected by measuring catecholamines (adrenaline and noradrenaline) or corticosteroids, the latter being mainly found in the form of cortisol in teleost fish (Galhardo & Oliveira, 2009). Accordingly, plasma cortisol is the most frequently measured indicator, but gill cortisol or the cortisol in mucus or water can also be measured (Sadoul & Geffroy, 2019). The resultant activation of cardiovascular and respiratory functions can be measured by heart rate or gill beat rate respectively. Since the HPI axis response is linked to energy metabolism and hydromineral balance, plasma metabolites (glucose, lactate, etc.) or metabolites from peripheral organs such as the liver (liver glycogen) or muscle (muscle glycogen) can also be quantified. Haematocrit, the leucocyte count and the partial pressure of oxygen or carbon dioxide (pO_2 or pCO_2) are also indicators frequently measured by scientists.

2.3.2. Behavioural indicators

Fish behavioural responses to stress are similar to the responses described for several fish species in situations that trigger negative emotions (Table 8, Table 9). Chief among them are 'freezing' (total immobilisation often accompanied by an increased gill beat rate) and erratic swimming. A colour change linked to the contraction or dilation of chromatophores and attributable to the release of plasma catecholamines can also be observed in certain species (Egan et al., 2009; Kalueff et al., 2013). Last, a study has appeared very recently on the sensitivity of QBA (Qualitative Behaviour Assessment) as a welfare indicator for stress in Atlantic salmon (Wiese et al., 2023). QBA uses qualitative criteria to characterise the emotional state of individuals within a group of animals, employing a predetermined list of descriptive adjectives that are scored based on observations of the expressive behaviours of individual animals by several observers. According to the study, QBA shows promise as an evaluation method for well-being and stress in farmed fish.

2.4. The consequences of negative emotional experiences for product quality

2.4.1. Impact of a negative emotional experience

Stress at the time of slaughter accelerates the drop in muscle pH and the onset of *rigor mortis*. The initial muscle pH, measured just after slaughter, is lower in stressed fish. However, the final pH, measured after 2 or 3 days of storage and acting as an indicator of muscle glycogen reserves, is generally unaffected (Lefevre et al., 2008, 2016). The stress-induced acceleration of post-mortem muscle degradation can affect the colour of the fillets, which are often less glowing and colourful, also affecting their mechanical strength (firmness), which may be reduced (Erikson et al., 2018; Kiessling, 2004; Lefevre et al., 2008, 2016; Roth, 2002). A moderate effect on odour and flavour (the combination of odour and taste) has also been reported (Sigholt et al., 1997; Concollato, 2016; Concollato et al., 2019). Preference tests comparing products from different slaughtering methods reveal that higher quality is associated with the least stressful method (Marx et al., 1997; Terlouw et al., 2021). An impact on the occurrence of 'gaping', where holes appear in the fillet due to the separation of the muscle layers, has been reported for stress (Roth, 2006) but has not been systematically observed (Kiessling, 2004; Erikson et al., 2018). On the other hand, the slaughter method has no effect on either lipid content or fatty acid composition, meaning that it does not affect the nutritional quality of the products (Duran, 2008; Simitzis et al., 2014). In terrestrial species, the effects of stress on quality also depend on genetic origin and the conditions under which the animals have previously been reared (Terlouw et al., 2021). Such a link has yet to be demonstrated for fish.

2.4.2. <u>Quality as an indicator of stress?</u>

The factors that contribute to the quality criteria affected by slaughter conditions are complex. Variations in these quality parameters can have several causes and it is difficult to attribute changes in them to a single factor such as stress at the time of slaughter (Terlouw et al., 2021). For example, stress at the time of slaughter is often associated with increased muscle activity but we know that muscle activity leads to the formation of lactates and a drop in muscle pH, whether stress is present or not. The impact of slaughter conditions on initial muscle pH, an often-measured parameter that is considered to be a stress indicator, may be the consequence of muscle activity alone, not of stress. The absence of a direct relationship between physiological stress levels (measured by plasma cortisol) and muscle pH has been observed in salmon, which exhibited very high and variable plasma cortisol levels but little difference in muscle pH (Erikson, 2016). Similarly, trout that were genetically selected to respond weakly (low rise in plasma cortisol) or strongly (high rise in plasma cortisol) to acute confinement stress showed similar responses to acute confinement stress immediately prior to slaughter when quality parameters were measured (Lefevre et al., 2016). Moreover, how an animal assesses a new situation, for example the stages that precede slaughter, will depend on its state of well-being, and it is its reaction to this assessment that may subsequently affect whether it meets flesh quality criteria (Terlouw et al., 2021). Certain quality parameters can therefore indicate that stress, and physiological stress in particular, may have been experienced by fish at the time of slaughter, but they cannot prove that a fish has suffered stress, nor can they be used to measure the level of stress experienced.

Conclusion:

Fish have a particularly sophisticated sensory system. In addition to the five senses (sight, smell, taste, hearing and touch) that they share with other vertebrates, they also have a lateral line, that enables them to perceive vibrations and changes in pressure. Some species can also emit and perceive electric fields. Fish are poikilothermic, making them highly sensitive to changes in temperature. Beyond these sensory capacities, they are able to process information via cognitive processes and the central nervous system, enabling them to feel emotions such as fear or anxiety and to perceive pain. Fish are also capable of developing states of stress, which can have an impact on product quality at the time of slaughter.

Both the sensory and psychological dimensions of fish sensitivity need to be considered if we are to develop slaughter procedures that respect the welfare of fish as recognised sentient beings.

3. Discussion of slaughter methods employed in France with regard to the sensitivities of farmed fish: factors causing stress and pain, and their effects

The slaughter process for farmed fish involves several stages. The first of these, referred to in this report as the 'pre-stunning stage', includes all activities commonly carried out prior to stunning, i.e., fasting of the animals, batching and crowding, loading, transport and unloading. This is followed by the stunning and killing stages, which may in some cases be carried out as a single procedure. The potential sources of pain and stress at each of these stages, as well as the consequences for fish welfare, are discussed in the following sections.

3.1. Pre-stunning stage (fasting, crowding, transfer and loading, transport)

The following sections dealing with pre-stunning practices draw particularly on the review article by Lines and Spence (2014).

3.1.1. <u>Fasting</u>

A fasting period, the length of which is dictated by species and water temperature (ranging from a single day to sometimes over a week), is necessary before slaughter to empty the contents of the digestive tract. This maintains good water quality during transport (keeping the water free of faeces) and ensures the subsequent sanitary quality of the product. Recommended fasting periods are available for certain species (54 °C days[19] for trout, for example, e.g. 5.4 days at 10°C), notably those established by the Royal Society for the Prevention of Cruelty to Animals (RSPCA, 2020). In practice, fish can end up being fasted for longer than necessary, primarily for operational reasons.

While many studies have examined the consequences of fasting fish prior to slaughter in relation to product quality, for example, (Einen et al., 1998 and Morkore et al., 2008) for Atlantic salmon and (Alvarez et al., 2008 and Lippe et al., 2021) for sea bream, few have investigated the effects of this practice in terms of the stress suffered by the fish.

Given the need for fish to have an empty gut before slaughter, several studies have attempted to determine the optimal fasting times for rainbow trout. These studies have made use of physiological stress indicators to evaluate the impacts of different fasting regimes on the trout.

López-Luna et al. (2013), for example, observed the effects of different fasting durations (24, 48 and 72 hours at 19°C, i.e.,19.5, 38.8 and 58°C days) and different slaughter times (morning

[19] This notation involving degree days (°C d), is used in fish farming to express duration as a function of water temperature, since fish are poikilothermic. For example '100 degree days' expresses a duration of either 5 days in water at 20°C, 7 days at 15°C, or 10 days at 10°C. This formula is valid only within a certain temperature range and must not be extrapolated to extreme temperatures *(Source: https://doris.ffessm.fr/Glossaire/Degre-jour/)*.

(08:00), afternoon (14:00) or evening (20:00)) on the physiology of rainbow trout. Within 24 hours (minimum fasting period), all fasting groups displayed a similar relative body weight loss compared to the control, with no significant further loss thereafter, leading the authors to conclude that a trout’s gut is emptied in less than 24 hours. For the majority of biochemical stress indicators measured in the study (plasma cortisol, glucose and blood lactate concentrations, along with haematocrit and leucocyte counts), no significant difference was observed between a morning, afternoon or evening cull either within a regime (control or fasting) or between regimes. Only leucocyte numbers fell slightly in fish fasted for three days, suggesting that the immune system becomes depressed beyond 3 days of fasting (58°C days). The number of degree days required to empty the intestine was similar in a study by Bermejo-Bermejo-Poza et al. (2017) based on a different water temperature. Here, the trout were reared in water at 6.15°C and the authors concluded that the trout's digestive system was emptied after 4 days of fasting (i.e., 22.3 °C days). In this latter study, liver colour (i.e., paleness) was observed as a potential indicator of stress: the livers of trout fasted for more than 5 days (28.6°C days) were significantly paler than those of trout fasted for 3 or 4 days (17.2 and 22.3°C days respectively).

Most studies on fasting observed no major physiological impacts on the fish under investigation for fasting times under 58°C days (Bermejo-Poza et al., 2016, 2017, 2019; López-Luna et al., 2013). However, no use was made in these studies of behavioural indicators of emotional reactivity, due to the difficulties involved in assessing how fish experience fasting. It has nevertheless been shown elsewhere that fasting exacerbates the emotional reactivity of cows in response to sudden events (Bourguet et al., 2011). It would be feasible to carry out similar tests for fish, taking investigation beyond the use of physiological measurements in the studies cited above. In general, the physiological state of animals can provide information on their physiological capacity to adapt. However, even in situations where their adaptive capacities are not exceeded, animals may still undergo a negative emotional experience. It should also be noted that for farmed fish accustomed to regular food distribution, a period of fasting does not meet their expectations and can therefore cause negative emotions (ANSES, 2018).

3.1.2. Crowding and transport to the slaughter site

3.1.2.1. Crowding

The purpose of crowding the fish within the rearing unit is to facilitate their capture and transfer to the slaughter unit. Techniques differ depending on whether fish have been reared in cages, or in ponds, tanks or raceways. Caged fish are crowded either by gradually and slowly lifting part of the net from the cage, or by inserting a second net into the cage (Lines & Spence, 2014). In other cases, the fish are encircled using a seine net, or are pushed back into a given area using panels or grids For fish in tanks, raceways or ponds, the water level is lowered beforehand (EFSA, 2009e).

The main threats to the welfare of fish during crowding result from their confinement at high densities. As an example, the density of commercially farmed sea bass and sea bream during crowding was estimated in 2009 to be 250 kg/m^3 (EFSA, 2009e). Confinement leads to:

- reduced water quality (EFSA, 2009e). In particular, oxygen availability decreases rapidly in reduced volumes of water as fish become agitated and stressed and their oxygen consumption rises. Concentrations of ammonia and other biological waste increase with the density and duration of the crowding operation;

- intense short-term stress in fish. A study of gilthead sea bream showed that, when densities were increased to the values encountered during commercial crowding operations, even for a short period (2 hours), this resulted in intense stress and affected the immune system of the fish, which took three days to recover their usual immunity levels (Ortuño et al., 2001). In cod, confinement leads to an increase in haematocrit and plasma lactate concentrations (J. A. Brown et al., 2010). In sea-farmed rainbow trout, confinement causes intense physiological stress, affecting blood cortisol concentrations and pCO_2 (Merkin et al., 2010). Stress is also expressed through behaviours such as accelerated swimming, escape behaviours, agitation manifested by splashing, and even jumping out of the water. In cod, confinement and the agitation it causes in the fish lead to inflation of the swim bladder, which can affect how a fish swims and upset its balance, sometimes irreversibly. To reduce this risk, it is recommended that the depth of the cage be gradually reduced in stages (J. A. Brown et al., 2010).

Crowding should therefore be carried out slowly and calmly to avoid a panic reaction, injury or death, and the length of time fish are crowded at high densities should be kept to a minimum (EFSA, 2009e). To maintain water quality, oxygenation and/or a water renewal system can be used (EFSA, 2009e). Last, as different species react differently to confinement, it is recommended that the specific characteristics of each should be taken into account (Bagni et al., 2007; Lines & Spence, 2014).

3.1.2.2. Transfer and loading

After crowding, fish are transferred either directly to a stunning and/or slaughtering unit or onto a vehicle for onward transport. Different methods are used, depending on the species. For example, salmon are transferred mainly by pumping (EFSA, 2009a), sea bass and sea bream are transferred mainly by using either small hand-operated nets or larger crane-operated brail nets and baskets, and are only rarely transferred by pumping (EFSA 2009e), while carp are mainly transferred using hand nets (EFSA 2009b).

‘Pumping is achieved by placing a large bore tube amongst the crowded fish. The pump sucks up water and fish through this tube, using a centrifugal or vacuum-pumping mechanism’ (Lines and Spence, 2014, Figure 11). Pumping is viewed as a welfare-friendly technique because the fish are not exposed to air during transfer. However, the design of the pumping equipment and its operation must avoid any risk of injury. For example, the WOAH Aquatic Code states that injury and ‘unnecessary’ stress caused by ‘equipment (such as nets, pumps, pipes and fittings) that are improperly constructed (e.g., sharp bends or protrusions) or improperly operated (e.g., overloading with fish of incorrect size or number of fish)' should be avoided (Article 7.2.6. of the WOAH Aquatic Code, 2023). It should be noted that salmon tend to swim against a current, which can be an advantage when encouraging this species to move voluntarily around a site. However, when salmon are being pumped along a pipe, this can lead to welfare problems, as their desire to swim against the current commonly causes them to remain in the pipe for too long, leaving them exhausted (EFSA, 2009a). Pumping distances from the crowding location to the stunning or slaughter unit vary considerably, ranging from a few metres to over a kilometre (Lines & Spence, 2014 and Figure 11). The risk to welfare increases with distance, due to the reduction in water quality (reduced oxygen, increased concentrations of excreted metabolic waste) and the risk of high densities if the pump fails to provide a continuous flow of water, if pipes become blocked, or if there is an uneven or turbulent flow of water through the pipes.

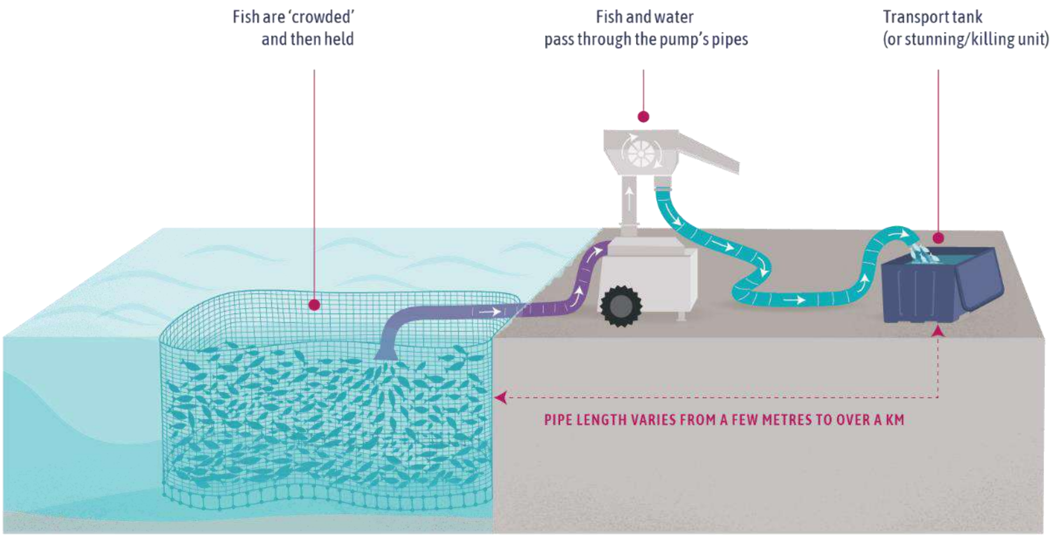

Figure 11. Transfer of fish by pumping *(original illustration created by FRCAW)*

Other methods of transferring fish from the holding site to the transport or slaughter tanks include the use of a dipping net or a larger scoop net, where a few kilograms of fish are transferred at a time by hand (Figure 12A), or a crane-operated net or basket that lifts up to several hundred kilograms of fish from the water (Figure 12B) (Lines and Spence, 2014). These transfer methods generally call for lower densities of fish at the crowding stage than transfer by pumping. Methods involving exposure to air are the most commonly used for practical reasons, but these represent severe threats to animal welfare, not least asphyxiation. They involve very high densities that are both a potential source of injury and are intensely stressful (EFSA, 2009e). Injuries (bruising, crushing, punctures, abrasions) can result from the strong pressure and friction as the fish are forced against each other or against or through the net. Transfer methods using 'wet brails' or baskets containing a certain amount of water avoid asphyxiation and reduce the risk of crushing, but are only feasible for transfers over short distances (Lines and Spence, 2014). Generally speaking, transfers using hand-held dipping and scoop nets are carried out in extensive or semi-intensive farming systems (EFSA, 2009e). If performed by skilled operators, these are less stressful for the fish, even though they take longer than the transfers using crane-operated brails that are common on commercial farms (EFSA, 2009e).

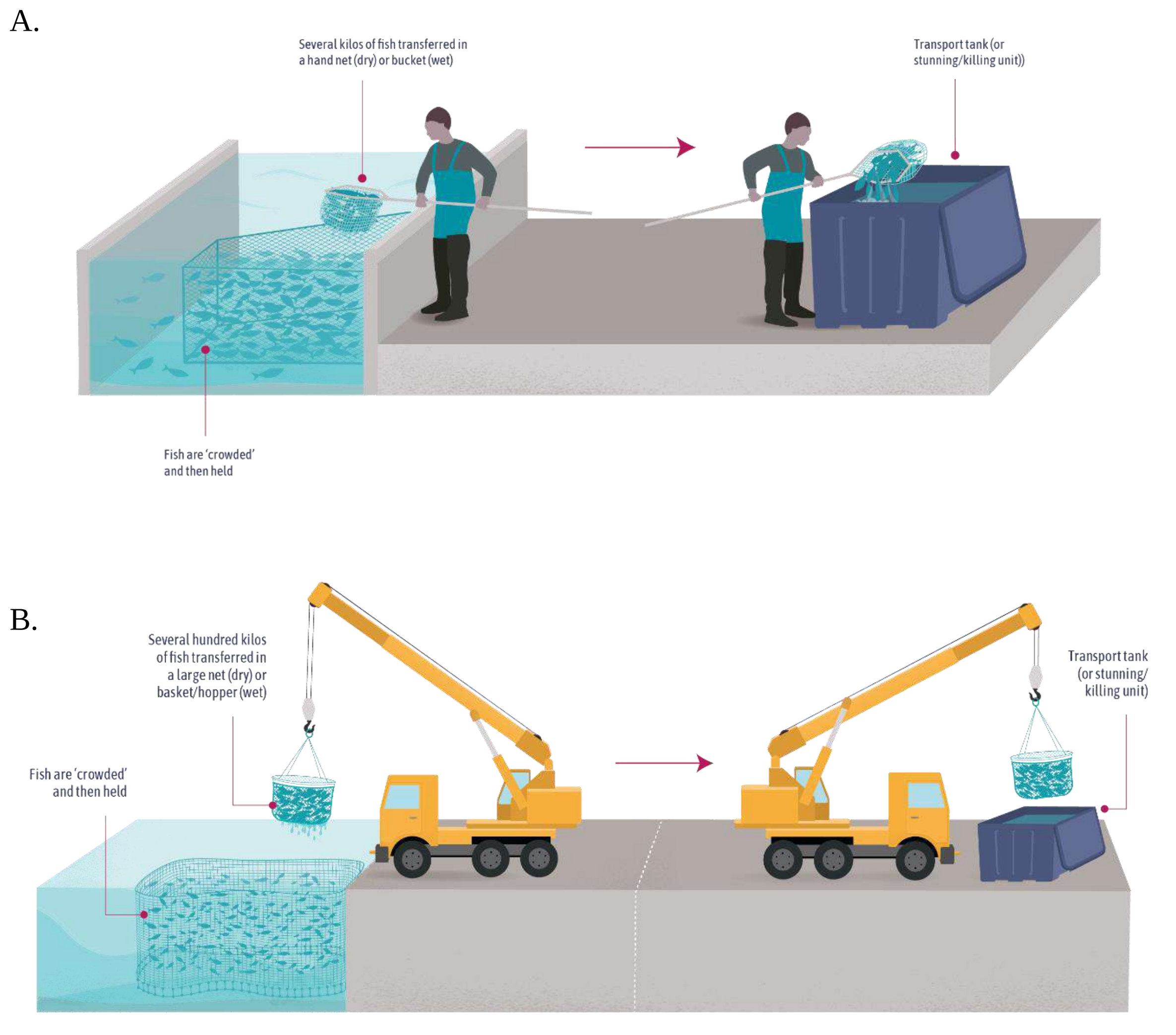

Figure 12. Transfer of fish using a hand net (A) and a crane-operated brail or basket (B) *(original illustration created by FRCAW)*

It should be noted that in some cases, conveyor belts (open to the air but covered with a thin film of water) may be used to transfer the fish from the end of the line into the transport tank or the stunning or killing unit. Depending on the species, the various loading techniques (pump, landing net, basket, belt) induce different stress levels.

3.1.2.3. Transport

While it is possible for some farmed fish in France to be slaughtered on site because the processing plant is located close by (marine fish reared on land-based sites; trout and sturgeon reared on sites with slaughtering and processing facilities), other fish must be transported to an external slaughtering site, or to a shared processing/packaging facility (marine fish reared at sea, trout and sturgeon reared on sites without slaughtering and packaging facilities) whose distance from the farm will vary. Transport times thus range from a few minutes to several hours. Live fish are transported by sea in well boats (salmon), in boats equipped with tanks, or by road in tanks. For all modes of transport, multiple studies have shown that transport prior to slaughter is a major stressor for fish (M. C. Gatica et al., 2008), as it is for all terrestrial species (Gregory, 2008). For example, high plasma concentrations of cortisol and lactate and a sharp increase in haematocrit and blood sugar levels have been observed following the transport by boat of rainbow trout reared at sea, indicating activation of the HPI axis (Merkin et al., 2010). While most studies on the negative impacts on fish welfare occurring during transport have concentrated on Atlantic salmon (M. C. Gatica et al., 2008), the pre-slaughter procedures followed for sea-farmed rainbow trout are generally the same (EFSA, 2009e).

In addition to the loading and unloading procedures described in the previous section, several other factors contribute to the exhaustion and stress associated with transport, such as the sensitivities of the species under consideration, the length of the journey, water quality, and fish density (Terlouw et al., 2008).

Good water quality management is crucial to ensure fish during transport. Dissolved oxygen concentrations and temperatures need to be maintained at levels close to those on the fish farm, carbon dioxide levels must be low, and nitrogenous waste must be controlled (Terlouw et al., 2008). The critical dissolved oxygen concentration is defined as the threshold value above which oxygen consumption is independent of concentration. Below this threshold, oxygen consumption is increasingly limited by concentration, until it reaches the lethal level. Critical and lethal oxygen levels vary according to the species of fish, and are higher in cold water fish due to the shape of their oxyhaemoglobin dissociation curve (Boyd, 1982). Conversely, if oxygen levels in the water of the transport tank reach saturation, this results in excessively high oxygen levels (hyperoxia) in the fish, accompanied by behavioural changes (Alfonso et al., 2020; Varga, 2014). Fish also excrete nitrogen in the form of ammonia, which reacts with water to form ammonium ions in an equilibrium reaction. Un-ionised ammonia is toxic to fish and high levels of un-ionised ammonia can cause physiological stress and escape responses (Danley et al., 2005).

In order to control these biochemical constants, water treatment and/or renewal systems need to be place during transport. Lines and Spence describe the results of investment by the salmon industry in the design and development of well boats: ‘Modern vessels are designed to enable water quality to be monitored and controlled, and so that the tanks can be emptied without leaving fish without water at the bottom of the tank. Under some circumstances the fish are cooled during the journey. This may reduce the fishes’ need for oxygen and their rate of ammonia production’ (Lines and Spence 2014). To provide a reference framework for the protection of farmed salmon during transport, standards have been produced in the UK (RSPCA, 2021). These standards recommend that cooling should be no faster than 1.5°C per hour and that water temperature should not be reduced below 50% of ambient temperature. As pointed out by Foss et al (2012), it is hard to distinguish between the effects of manipulation and those of a reduction in temperature on fish (reported in Lines and Spence (2014)).

Density levels are generally high for fish during transport and are stressful for the fish, as has been shown for sea-farmed salmon (Skjervold et al., 2001). At present, there are no density standards for transporting fish to the slaughter site, especially as the maximum acceptable density depends on water quality (and therefore on control systems to regulate parameters), journey time, temperature and species.

Along with the hazards of high densities and poor water quality, road transport also involves vehicle vibrations, possible physical shocks and rapid temperature changes. It has been shown that an increase in water temperature, for example, causes increased cortisol and plasma glucose concentrations in sea bass and gilthead sea bream (Papaharisis et al., 2019).

Like terrestrial animals, fish benefit from being held for a certain amount of time after transport and before slaughter under conditions suited to their needs, as this provideds a rest period in which they can recover from the stress of transport (Terlouw et al., 2008). Following transport by sea, it is the usual practice to hold fish for one or even several days for reasons of product quality. This holding period also improves fish welfare: in salmon, plasma cortisol and lactate concentrations, which are high after transport, were shown to fall after fish were held in tanks for 24 hours prior to slaughter (Gatica et al., 2010). The same recuperative effects have been observed in rainbow trout reared at sea (Barton, 2000).

Conclusions:

La The pre-stunning phase consists of fasting the animals to empty their gut, crowding them, and moving them from the farm to the slaughter facility. Animal welfare is affected by the length of fasting, the process of crowding (density stress, risk of lesions), transfer (risk of lesions during pumping or manual removal using a dip net, stress) and often also during journeys of all lengths (risk of deterioration in water quality, confinement, stress, asphyxiation, vibration, etc.).

3.2. Stages 2 and 3: stunning and killing

3.2.1. Definitions

3.2.1.1. Stunning

Stunning renders animals unconscious immediately before they are slaughtered for consumption and may also kill the animal. It must be distinguished from immobilisation (caused by an electric shock of inappropriate voltage/intensity, cold, or particular molecules), since the latter does not guarantee loss of consciousness. Stunning that results in unconsciousness is essential to prevent animal suffering before slaughter. Unconsciousness is a state in which brain functions are temporarily or permanently damaged and the individual does not react to stimuli, including pain (Terlouw, 2020).

The choice of most appropriate stunning method (gas, electronarcosis, percussion, etc.) is essentially dictated by species, since the biology of some fish species makes them highly resistant to cold or lack of oxygen, for example.

3.2.1.2. Awareness indicators

In order to identify an effective stun, certain indicators are used, most of which are based on the animal.

Following the use of a stunning or killing method, if a fish is still able to quickly regain its balance when inverted (turned on its back in the water), displays species-specific coordinated swimming activity, exhibits escape behaviour or responds to painful stimulation (from a needle or fin pinch), then the method is inappropriate or has been incorrectly executed. The absence of the above behaviours or responses is considered to be an indicator of unconsciousness. Caution should be exercised, however, as some stunning methods (live chilling in water-ice slurry, electronarcosis) may immobilise fish or induce paralysis, i.e., loss of muscular coordination and/or spontaneous physical activity, without rendering them unconscious (Lambooij et al., 2002; Croft, 1952). Fish that have only been immobilised or paralysed still experience pain but are unable to express it through their behaviour (van de Vis et al., 2003).

The vestibulo-ocular reflex (VOR) (commonly known as eye rolling) and respiratory reflexes have been used as indicators of brain function (Kestin et al., 2002). In the case of VOR, eye movement is observed when the fish is tilted from side to side. In a dead or unconscious fish, the eye remains motionless, parallel to the skull. In a fish that retains some brain function, the eye rotates dorso-ventrally when turned over (Figure 13). Lambooij et al (2010) have suggested that caution should be exercised when interpreting VOR, as electroencephalogram (EEG) recordings have shown that some Atlantic salmon regained consciousness during the absence of VOR. Neurophysiological measurements such as EEGs, visual evoked response (VER) recordings and electrocardiograms (ECGs) should be used to complement VOR in preliminary experimental studies. Similarly, with regard to the respiratory system, movements of the operculum and the lower jaw are to be observed when a conscious fish is placed in water or held in the air. In a dead or unconscious fish, the operculum and lower jaw exhibit no rhythmic movements, although vibrations may still be observed. If eye-roll reflexes/VOR and respiration are absent, the fish is probably dead or unconscious (Kestin et al., 2002).

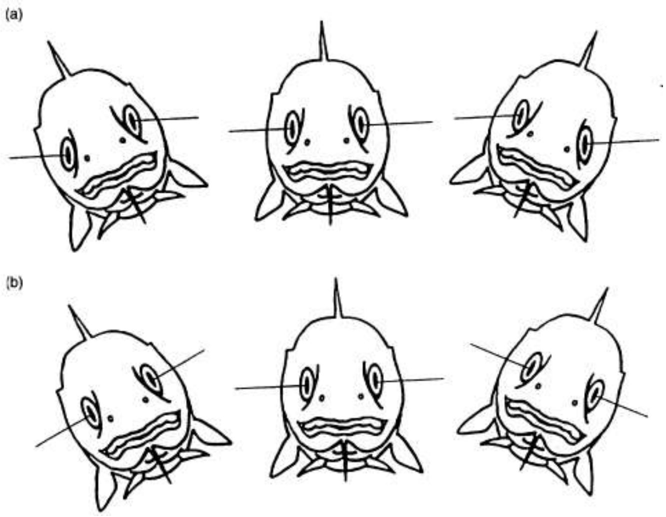

Figure 13. Diagram illustrating the vestibulo-ocular reflex ('eye roll') (a) in live cod and (b) its absence in dead cod, as indicated by the plane of the ocular axis in each image. *Source: Kestin et al (2002)*

To avoid pain and suffering, a fish displaying any one of the above consciousness indicators must be assumed to be conscious. To help the industry use these indicators, a number of publications provide tables of indicators to be used in monitoring loss of consciousness (Appendix 1).

Stunning must lead to a loss of consciousness and sensitivity in the shortest time possible (Robb & Kestin, 2002). Failing this, stunning methods, especially where they are not rapid, must cause no stress to the fish before it loses consciousness. Currently, farmed fish are slaughtered using a variety of methods that induce varying levels of stress depending on the species and the manner in which they are applied. The pre-stunning method chosen will depend on the species, for practical or physiological reasons. The regulations for fish stipulate that all unnecessary suffering must be avoided without going into further detail as the welfare and protection of fish depend at least as much on farming conditions (water quality, oxygen, temperature) as they do on slaughter procedures (Terlouw et al., 2008).

3.2.1.3. Killing

Killing follows the stunning phase. It should be noted that some of the stunning methods described below may also result in the death of the fish. A distinction can be made between individual stunning and/or killing methods where fish are processed one by one, and batch methods. This distinction often overlaps with that between 'dry' methods, which are often individual, and 'wet' methods, which are generally batch methods, but this is not always the case. Indeed, air asphyxiation is a dry batch method, while bleeding is more effective in water and predominantly involves an individual operation.

The key goal in the killing phase should be that it is as quick and efficient as possible for each individual in a batch while ensuring that animals do not regain consciousness before death. For slaughter to respect animal welfare and preserve the quality of the product, mastery of the slaughter process is essential.

For each method of slaughter, the failure rate (proportion of fish regaining consciousness) needs to be assessed to determine the method's effectiveness. The consciousness indicators referred to above (cf. 0) are used to measure this effectiveness.

3.2.2. The different methods of stunning and/or killing

3.2.2.1. Gas stunning

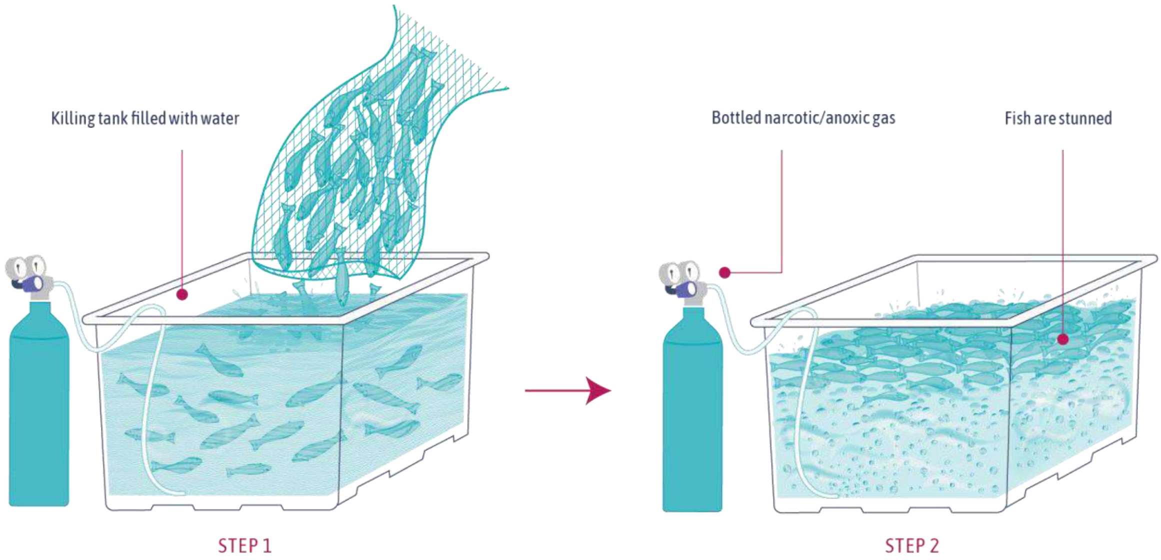

Figure 14. Gas stunning procedure *(original illustration created by FRCAW)*

According to Bjorlykke and his colleagues (2013) the sedation or stunning of fish with gas has several advantages, including an improved success rate for electrical or percussive stunning when it is used in advance of these techniques. However, fish tolerance of hypoxia differs markedly from that of mammals. Compared with terrestrial animals, it is particularly difficult to induce death by hypoxia in fish due to their general capacity for metabolic adaptation (Bjorlykke et al., 2013).

Use of carbon dioxide

Carbon dioxide (CO_2) has long been recognised as a powerful anaesthetic agent and is still widely used in the aquaculture industry in many countries to immobilise fish prior to slaughter (EFSA, 2009a, 2009d; Robb et al, 2000; van de Vis et al., 2003). CO_2 is an economically attractive alternative to other fish anaesthetics, with the major practical advantage that it leaves no harmful residues in fish produced for human consumption (Sandblom et al., 2013). However, the use of CO_2 has been questioned from an animal welfare perspective (EFSA, 2009a, 2009d, 2009e; van de Vis et al., 2003). A number of studies indicate that exposure to CO_2 triggers aversive behavioural responses, such as fight or flight, that continue for a significant time, lasting several minutes (EFSA, 2009a, 2009d, 2009e; Erikson, 2011; Robb et al, 2000; Roth et al., 2002; van de Vis et al., 2003). In addition to the above, there is evidence that exposure to anaesthetic levels of CO_2 induces a primary stress response in fish in the form of the release cortisol and catecholamines, indicating stress and therefore significant discomfort (Sandblom et al., 2013).

Robb and his team (2000) have evaluated the effectiveness of CO_2 stunning in salmon. When the salmon were immersed in water saturated with CO_2, they vigorously shook their heads and tails for around two minutes. The movements then decreased until the fish were completely immobile. However, at the point when the fish were bled (9 minutes after being immersed in water saturated with CO_2), some were still moving. The relatively slow loss of VER during CO_2 narcosis indicates that certain areas of the brain maintain functionality for more than 6 minutes, during which time the fish can feel pain and fear as they die. None of the fish displayed an immediate loss of VER, so it is probable that none lost consciousness immediately (Robb et al, 2000b). CO_2 narcosis is aversive to fish, as is clearly indicated by rapid and violent responses, for example frantic swimming, attempts to escape the tank and abnormal activity prior to stunning. Immobility is achieved in 2 to 4 minutes, but it has been demonstrated that the time taken for fish to lose consciousness varies according to species (2 minutes for salmon, 3 minutes for trout, 9 minutes for carp, 109 minutes for eel, 7 to 10 minutes for sea bass) (Poli et al., 2005). The stress caused by this method has also been confirmed by increased haematocrit levels and increased glucose and plasma cortisol levels in several species (Marx et al., 1999). In general, as for other animals, the exposure of fish to gas mixtures and anaesthetics in water does not immediately induce unconsciousness, unlike other available methods of euthanasia.

Use of nitrogen

The use of nitrous oxide (N_2) has been proposed as an alternative stunning method (Wills et al, 2006) and has been tested experimentally. No signs of frenzied activity were observed in rainbow trout, which became stunned after 6-8 minutes (Wills et al, 2006). However, this stunning method is not recommended for Atlantic salmon (Erikson, 2011).

Use of carbon monoxide

Carbon monoxide (CO) has been used for over 30 years to euthanise animals (Blackmore, 1993). No physiological or behavioural indicators of pain have been demonstrated for the use of CO as an euthanasia agent for animals (Bjorlykke et al., 2013). Although the use of CO appears to meet animal welfare requirements, it is nevertheless dangerous for the handler (in the event of exposure to a very high concentration of CO, respiratory arrest may occur immediately in humans), and is not currently used in France. According to Bjorlykke and his colleagues (2013), no aversive reaction would appear to have been observed with CO.

Use of gas mixtures

When mammals are anaesthetised or euthanised with CO_2 , additional oxygen is often added to the gas mixture (this technique is known as hyperoxic carbon dioxide anaesthesia) to prevent hypoxaemia (an abnormal reduction in the amount of oxygen in the blood) and asphyxia (the sensation of lack of oxygen) and thereby reduce stress and suffering (Coenen et al., 1995; Kohler et al., 1999). A mixture comprised solely of CO_2 + O_2 has not been tested in fish, however in one experiment, Roque and colleagues (2021) tested a gas mixture composed of 40% CO_2 + 30% N_2 + 30% O_2 on sea bream. When exposed to the gas mixture, the fish lost equilibrium at 1 min 12 s ± 32 s. Electroencephalogram recordings indicated that the fish began to lose consciousness when they lost equilibrium, sinking to the bottom of the tank.

3.2.2.2. Killing by 'live chilling'

This method is intended to induce a thermal shock that leads to the death of the fish. As pointed out by Hovda & Linley (2000), low water temperature can reduce fish activity, metabolic rate and oxygen consumption, immobilising fish until they die. Direct immersion in tanks filled with ice slurry (an ice-water solution composed of ice flakes and water in a ratio of between 1:2 and 3:1, temperature <2°C) (Figure 15) is the method most commonly used by fish farmers to kill Mediterranean species (mainly sea bass and sea bream) on a small scale (i.e., in batches of a few hundred kilos). The 'live chilling', or 'ice water bath', method involves refrigeration of the fish, improving product quality.

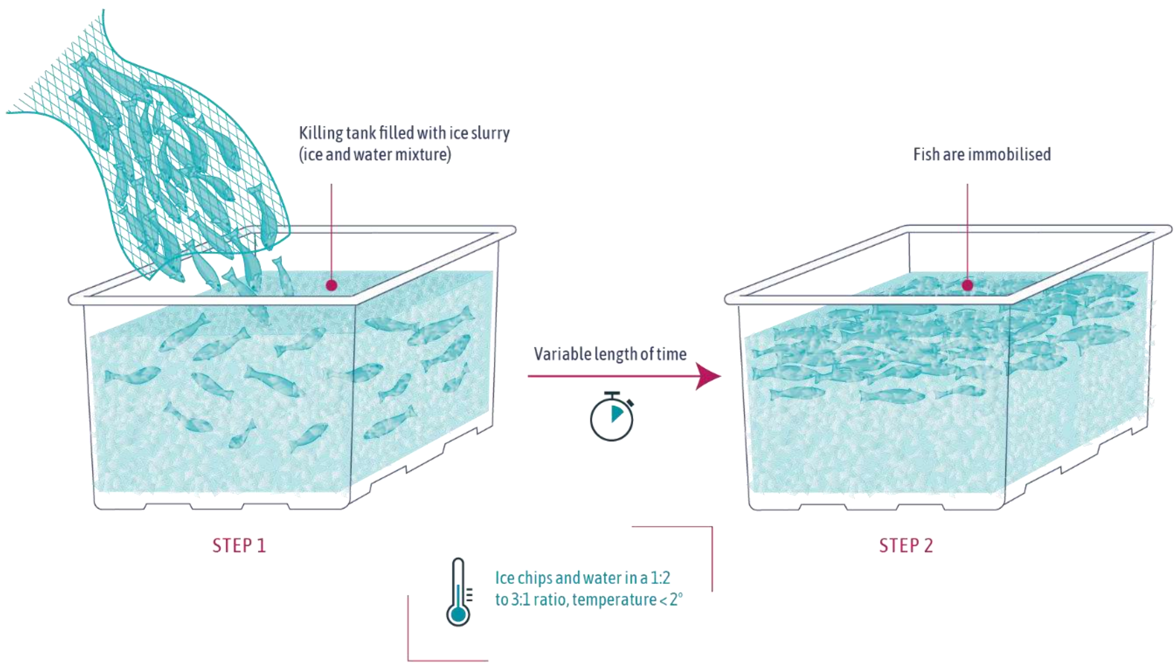

Figure 15. Killing procedure for 'live chilling' in ice slurry *(original illustration created by FRCAW)*

In practice, the volume of fish placed in the tanks and their initial temperature relative to that of the ice slurry can affect the efficiency of chilling. The rapid drop in temperature experienced (cold shock associated with direct immersion) can also lead to primary and secondary stress responses in fish, including elevated plasma levels of cortisol and catecholamines (Seth et al., 2013). It can take several minutes for death to occur. Neurophysiological measurement techniques such as EEG and ECG have been used for some species to measure the time taken to induce unconsciousness in fish following the use of an ice-water mixture as a killing method. A rapid heartbeat has been observed in eels and catfish immersed in ice-cold water (E. Lambooij et al., 2002, 2006). In these two species, low levels of brain activity (measured by EEG) and an absence of response to painful stimuli were observed in immobilised fish after 5 to 20 minutes' exposure to icy water (9°C drop in fish body temperature). A recent study on trout showed that the scores on loss-of-consciousness tests (breathing, balance, tail pinch response, VOR) remained higher (i.e., more fish were conscious) after immersion in ice slurry than when an electric current was used, even after over 20 minutes of immersion in the slurry (Bermejo-Poza et al., 2021). It is also hard to assess unconsciousness when using the live chilling method, due to the non-availability of some consciousness indicators, such as escape attempts and active swimming, as a direct result of the effects of cooling on fish mobility.

Immersion in ice slurry has practical advantages to offer: it is relatively simple, low-tech and inexpensive to carry out. The associated refrigeration of the fish also improves its sanitary quality and extends product shelf life. However, the critical features of live chilling by immersion in ice slurry are the lengthy period before loss of consciousness occurs and the lack of reliable indicators to assess an effective stun. Because of its slow action on fish activity and the lack of certainty that immobility indicates unconsciousness, the Norwegian Scientific Committee for Food Safety (VKM) describes this technique as inappropriate in terms of animal welfare (Hjeltnes et al., 2010). For this reason, the method is considered unacceptable for cold-water species (EFSA, 2009b ; Grigorakis, 2010); because the difference in temperature between the water in the rearing tanks and the ice slurry is smaller, it takes longer for death to occur. In the case of warm-water fish, a study focusing on tropical and subtropical species has concluded that this method is stressful and aversive for the species studied (Bowman & Gräns, 2019). For Mediterranean species (mainly sea bass and sea bream), EFSA ((2009e) did not propose a ban on this method, underlining the need for further research on effective and reliable alternative techniques for these species.

3.2.2.3. Electrical stunning and killing

Electronarcosis is a method of stunning fish before they are killed that involves the application of an electric current, either in water or out of water (wet or dry stunning). For this method to be effective, stun parameters (duration, intensity, frequency) must be adjusted to take account of the species and size of the fish, the method employed (wet or dry), the temperature and conductivity of the water, and the number of fish in the tank. Depending on the duration of the current, its intensity and frequency, electrical stunning can also be used as a method of killing; this is known as electrocution.

Electronarcosis is performed by passing an electric current through the brain, sometimes in conjunction with a current through the animal's heart. The electrical charge passing through the brain disrupts neuronal activity, producing a state that is similar to an epileptic seizure and detectable by ECG, causing cerebral function to cease, inducing unconsciousness and the cessation of respiratory reflexes (Lambooij et al., 2010). The passage of the current through the heart causes arrhythmia which also leads to loss of consciousness followed by the death of the animal (i.e. electrocution). Several electronarcosis systems are commercially available. Villarroel & Lambooij (2022) cite the following methods in particular:

- Head-only dry stunning: the fish are stunned by passing an electric current through the head, generated by two out-of-water electrodes. The operator positions the fish one by one, placing their heads between the two electrodes.
- 'Water bath' electronarcosis: the fish are placed with water in a tank equipped with electrodes. A current is generated between the electrodes, using the water and the fish as conductors. This 'in-tank' method can also be carried out without water, using only the bodies of the fish as a conductor (Figure 16).

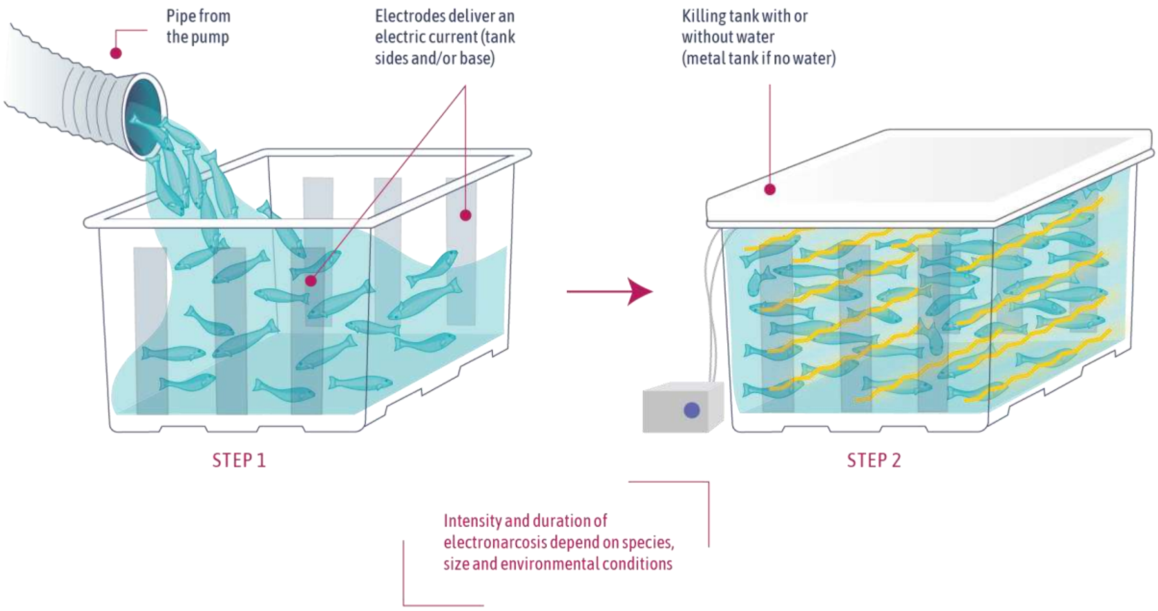

Figure 16. Electonarcotic stunning procedure for batched fish ('water bath' if the tank is full of water) *(original illustration created by FRCAW)*

- In-line in-water stunning: the fish are stunned by being passed through a pumped pipe in which an electric current is generated (Figure 17).

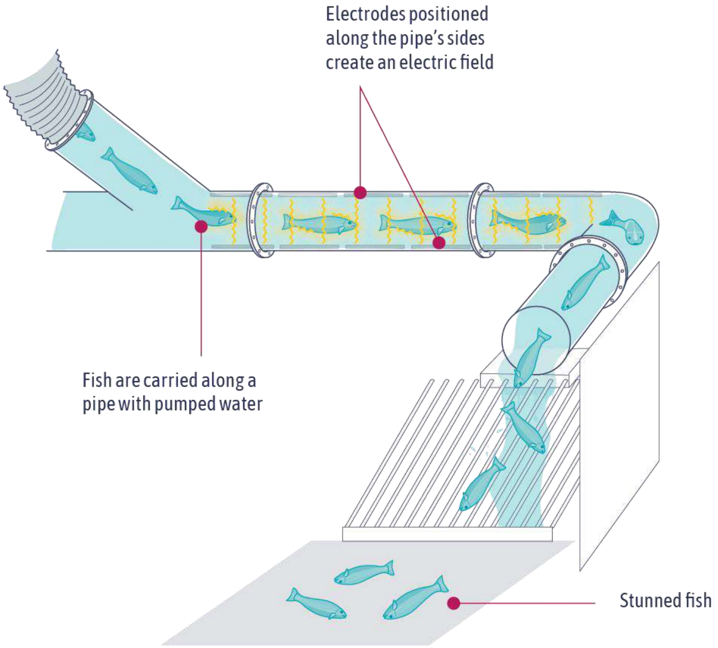

Figure 17. Procedure for in-line in-water stunning *(original illustration created by FRCAW)*

- Dry stunning: the fish are placed on a conveyor belt which carries them to a series of rows of electrodes that deliver an electric current (Figure 18).

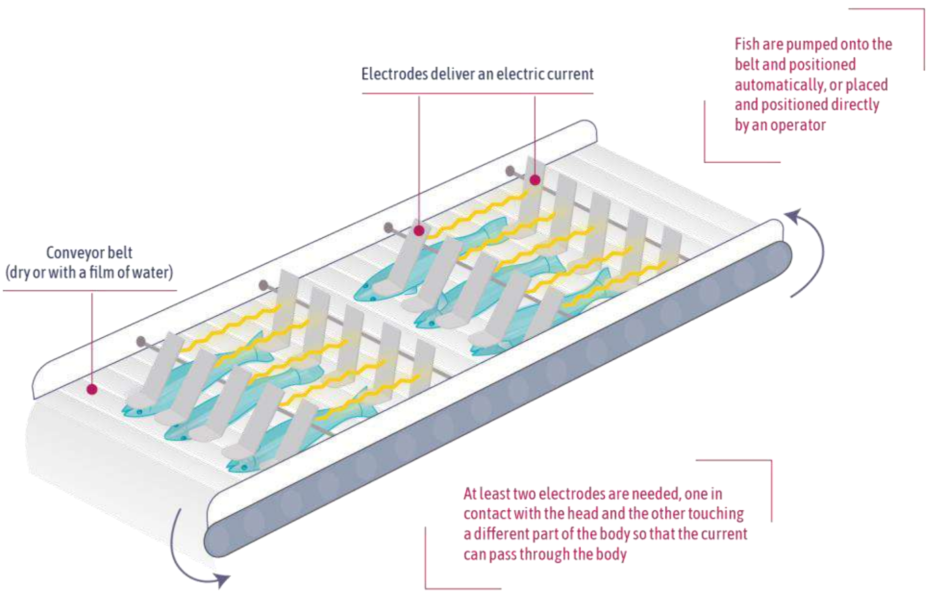

Figure 18. Procedure for dry stunning *(original illustration created by FRCAW)*

This list is not exhaustive (Welfarm, 2023). Each method has its advantages and disadvantages, and not all can be used under the same conditions for practical reasons. For example, the most common difficulties with dry stunning are to ensure that the fish are not exposed to pre-stun shocks as they enter the unit and that spasms do not cause them to lose contact with the electrodes. In the case of in-water stunning, it is important to ensure that the electric field in the water is homogeneous. The advantage of in-water methods is that they reduce the stress of exposure to air and light and reduce the likelihood of mechanical shocks (compared with dry methods). The choice of method and of the electrical parameters to be used must be adapted to the species of fish to be stunned, and to the size of individual fish to be stunned and their number.

In all cases, though, electronarctic systems are designed so that fish lose consciousness quickly. Under the right electrical conditions, some species of fish can lose consciousness in under a second (Llonch et al., 2012), but a prolonged electrical exposure ranging from ten seconds to more than a minute is necessary to ensure that the fish remain unconscious long enough to die of electrocution (Robb et al., 2002; Roth et al., 2003; Llonch et al., 2012), or for another slaughter method to be applied before any possible return to consciousness (Retter et al., 2018). As a rule, electrical stunning is reversible, so it is important for the fish to be killed immediately after the stun, either by electrocution or using another killing method.

Both wet and dry electrical stunning, depending on the study, have been tested on a considerable number of species, including carp (Retter et al., 2018), salmon (van de Vis et al., 2003; Roth et al., 2009; Grimsbo et al., 2014; Robb & Roth, 2003; Erikson et al., 2012), sea bass (Lambooij et al., 2008; Papaharisis et al., 2019), trout (Lines and Kestin, 2004; Lines & Kestin, 2005), sole (Llonch et al., 2012), perch (Llonch et al., 2012), sea bream (Papaharisis et al., 2019; van de Vis et al., 2003) and turbot (Morzel et al., 2003). Several of these studies used EEG to monitor or confirm the unconsciousness of the fish (B. Lambooij et al., 2008; Llonch et al., 2012; Retter et al., 2018; Robb & Roth, 2003). Other studies used physiological and biochemical indicators of acute stress, such as plasma glucose, blood cortisol and haematocrit levels, reduced glutathione and malondialdehyde (MDA) and energy status (ADP/ATP ratio) (Daskalova et al., 2016; Digre et al., 2010; Erikson et al., 2012; Grans et al., 2016; Mahmoud et al., 2019; Oliveira Filho et al., 2015; Papaharisis et al., 2019), as well as behavioural indicators such as escape responses, VOR, and the recovery of breathing and balance (Grans et al., 2016; Grimsbo et al., 2014; B. Lambooij et al., 2008; Llonch et al., 2012; Retter et al., 2018; Robb & Roth, 2003) to assess the effectiveness of stunning.

The purpose of these studies was to determine the intensity, magnitude, frequency and duration of the current to be delivered for each fish species. Selection of the appropriate electrical parameters is essential to ensure effective stunning. For example, Robb et al (2002) demonstrated in rainbow trout that the duration of unconsciousness following the application of an electric field depended on current intensity, duration and frequency. They also showed that an increase in the intensity of the current applied resulted in an increase in the duration of unconsciousness. Use of strong currents (> 150 mA) led to death (irreversible unconsciousness). Similarly, when the duration of the application of the current was increased, this first increased the duration of unconsciousness, and then caused death (> 20 sec). By contrast, increasing the frequency of the current reduced the duration of unconsciousness and it is probable that, above a threshold frequency, the fish would not be stunned. Robb et al (2002) specified that to render a trout unconscious, a minimum current of 100 mA at 50 Hz must pass directly through the head for 1 second. If fish are to be stunned in a water bath, then a current density of at least 8.3 A m^{-2} at 50 Hz must be applied for at least 5 s to render all the fish unconscious, and for at least 30 s to kill all the fish. Last, when applying current densities of between 10.2 and 10.8 A m^{-2} for 5 s, a waveform frequency of 2000 Hz (or less) was required to stun the fish. By combining the three parameters, it is possible to stun or stun/kill trout for portioning.

Villarroel & Lambooij (2022) have recently compiled a list of the electrical parameters required to stun different species of fish (including carp, sea bass, salmon, sole and turbot) in a tank and using a dry method. However, before electronarctic stunning is carried out, it is important to test the parameters on a sample group of animals to validate their effectiveness. To do this, Noble et al (2020) propose control indicators relating either to the environment (E), the group of fish (G), or the individuals (I) of which key checkpoints are:

- The electrical parameters must comply with the manufacturer's instructions and be updated on the basis of practical experience (E),
- After stunning: no VOR, no regular gill movement, few muscle spasms, no ‘tail-grab’ reflex, no swimming movement or balance recovery (I),
- Absence of animal recovery in a test on 20 fish for 10 minutes post-stun (G).

The authors also specify that adequate back-up equipment must be provided in the event of a control failure.

It should be noted that the electric stunning of fish can affect the quality of the products, particularly in the form of residual blood in the muscle (Marx et al., 1999; Roth et al., 2003; Digre et al., 2010; Erikson et al., 2012).

3.2.2.4. Percussive stunning and killing

‘Percussion’ in this context describes the striking of the skull with a solid instrument. The purpose of percussive stunning is to induce immediate unconsciousness. In the guide ‘Humane Harvesting of Fish’ (HSA, 2005) percussive stunning is defined as follows: ‘When a fast, heavy blow is correctly applied to the skull it produces a rapid acceleration of the head, causing the brain to collide against the inside of the skull. This causes disruption of normal electrical activity resulting from a sudden, massive increase in intra-cranial pressure followed by an equally sudden drop in pressure. The consequent damage to the nerves and blood vessels causes brain dysfunction and/or destruction and impaired blood circulation. The duration of insensibility depends on the severity of damage to the nervous tissue and the degree to which the blood supply is reduced.’

Manual percussion involves the striking of the back of the skull with a club or ‘priest’ (Figure 19).

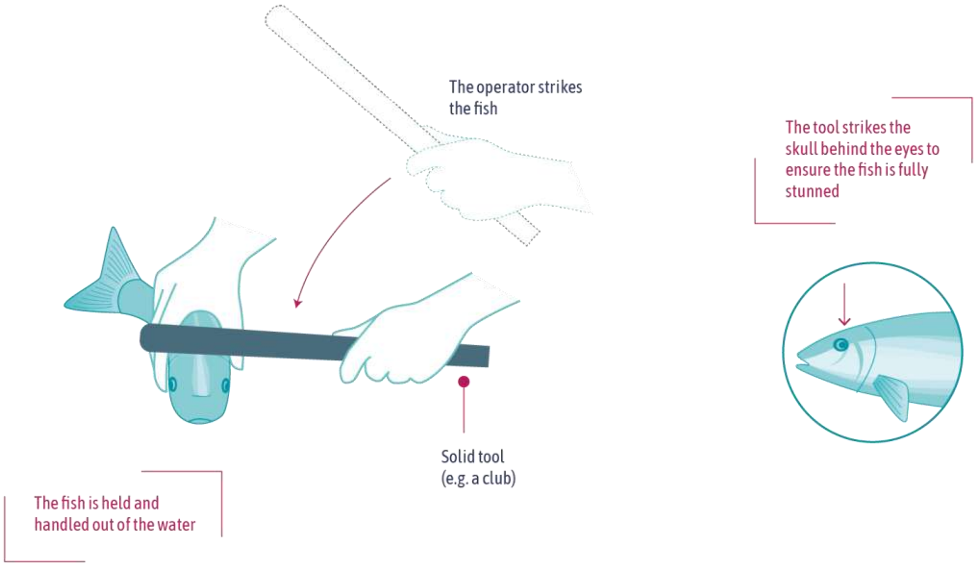

Figure 19. Procedure for manual percussive stunning *(original illustration created by FRCAW)*

Percussive stunning can also be carried out automatically or semi-automatically (Figure 20). Modern stunners take the form of flow-through machines that deliver a non-penetrative blow. The most commonly-used automated stunners are powered by compressed air at pressures between 90 and 120 psi (6-8 bar). Newer models automatically guide the fish to swim into the entry channels of the machine opening, ensuring that they are in an upright position. The fish activates the trigger system, causing the piston to strike the fish on the head, rendering it immediately unconscious (Figure 21). The automatic systems currently available were developed for large salmonids such as salmon and trout (over 1 kg). (HSA 2005)

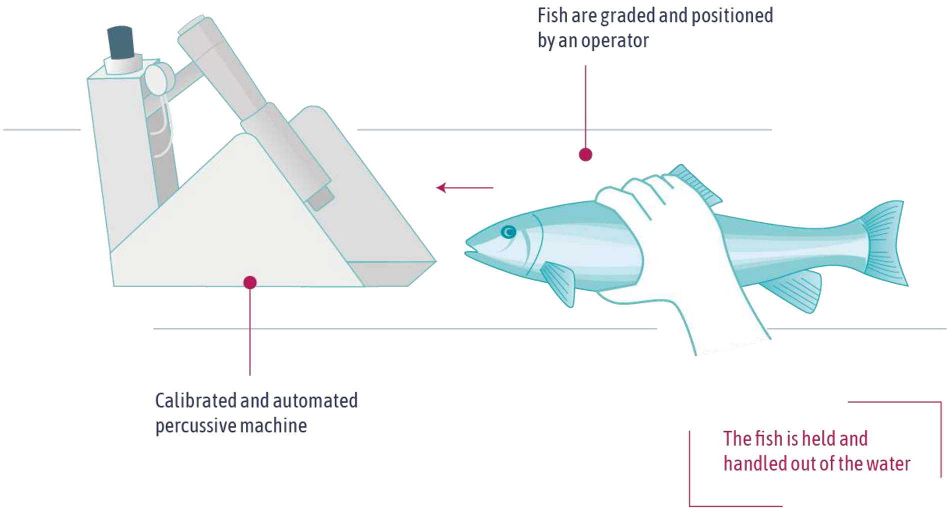

Figure 20. Procedure for percussive stunning using a semi-automatic stunner *(original illustration created by FRCAW)*

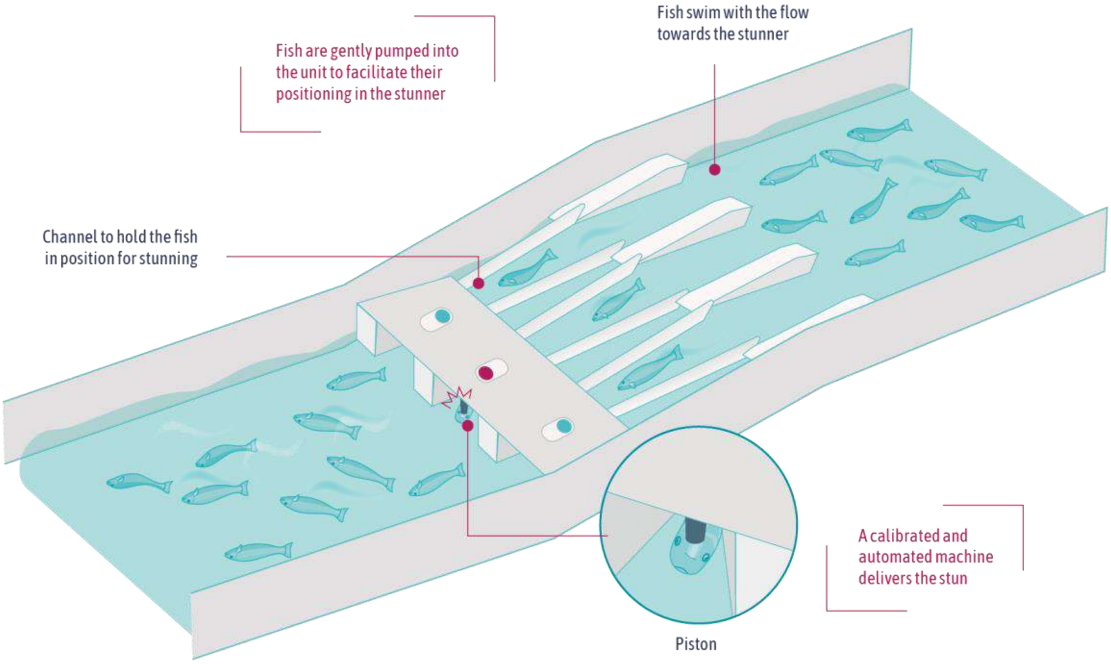

Figure 21. Procedure for percussive stunning using an automatic stunner *(original illustration created by CNR BEA)*

Percussive stunning is frequently followed by bleeding.

When a fish is struck with sufficient force and the blow is correctly positioned, stunning is irreversible. For stunning to be effective and avoid suffering, the fish must be held in place and delivery must be precise and apply measured force (Roth et al., 2007). A spasm running through the entire body of the fish is commonly interpreted as a sign of the animal's death. However, for certain species such as sea bream, eel and African mackerel, the morphology of the skull inhibits sufficient concussive force to induce loss of consciousness (van de Vis et al., 2003). The signs of effective percussive stunning are the absence of opercular movement, the absence of eye movement and a short-lived bulging of the muscle ring near the pectoral fin (HSA, 2005).

Manual percussion is simple to carry out for small batches of animals of sufficient size, but is not very fast. The fish must therefore be held in confinement. This method is used for large fish (WHOA, 2023). The main risk is the effects of repeated movements on the operator (which may lead to musculoskeletal disorders or MSDs), resulting in a loss of precision over time. If a movement is poorly executed, this can have significant repercussions for the fish, such as head injuries (WHOA, 2023), and may require it to be repeated several times (Wall, 2001).

Automation has proved useful for several species, but longer stun-to-bleed times can make drainage difficult during bleeding (due to the need to maintain blood circulation) (van de Vis et al., 2003). Care must also be taken when calibrating the machine to the fish. A miscalibration can cause the trigger to be activated at the wrong time, resulting in the incorrect positioning of the blow.

3.2.2.5. Stunning and killing by spiking

This method involves destroying the brain at the back of the head with a rod or blade (Figure 22).

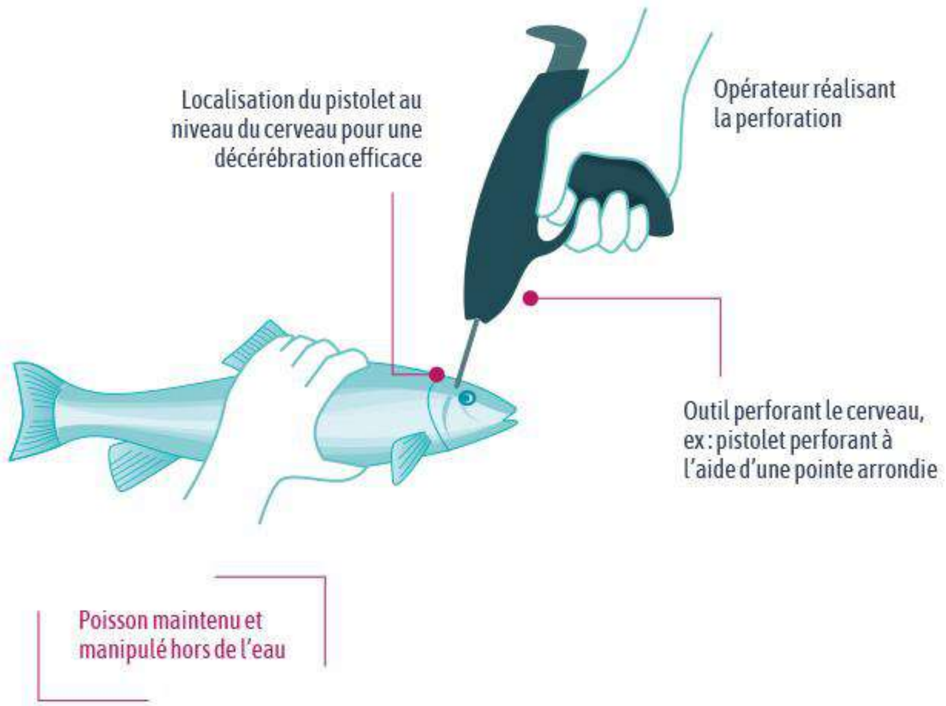

Figure 22. Procedure for stunning and killing of fish by spiking *(original illustration created by FRCAW)*

Delivery must be precise to be effective. If the technique is mastered, it causes rapid death in salmon (Robb et al, 2000) and sea bream (Nakayama et al., 1996). On the other hand, following poorly controlled delivery, signs of pain and intense muscular activity can be observed (Robb et al, 2000). This method has the same advantages and disadvantages as those described above for percussive stunning. Because of the precision required, the spiking method is better suited to large species that can be held individually (salmon, tuna, sturgeon, etc.). This technique requires training and experience to be mastered by the operator and thereby ensure animal protection (rapid death of the animal) and operator safety (avoiding injury from tools). In sea bass, this technique preserves product quality better than live chilling (Tulli et al., 2015).

3.2.2.6. Ikejime

This method, a particular application of the more general practice of spiking described above, is a traditional Japanese technique. As with spiking, it involves the destruction of the brain with a spike to induce unconsciousness and death in the fish. But this method also includes destruction of the spinal cord by passing a metal wire along the vertebral column's neural canal

(Figure 23). Ikejime therefore comprises two stages: spiking and pithing. These stages are often followed by bleeding.

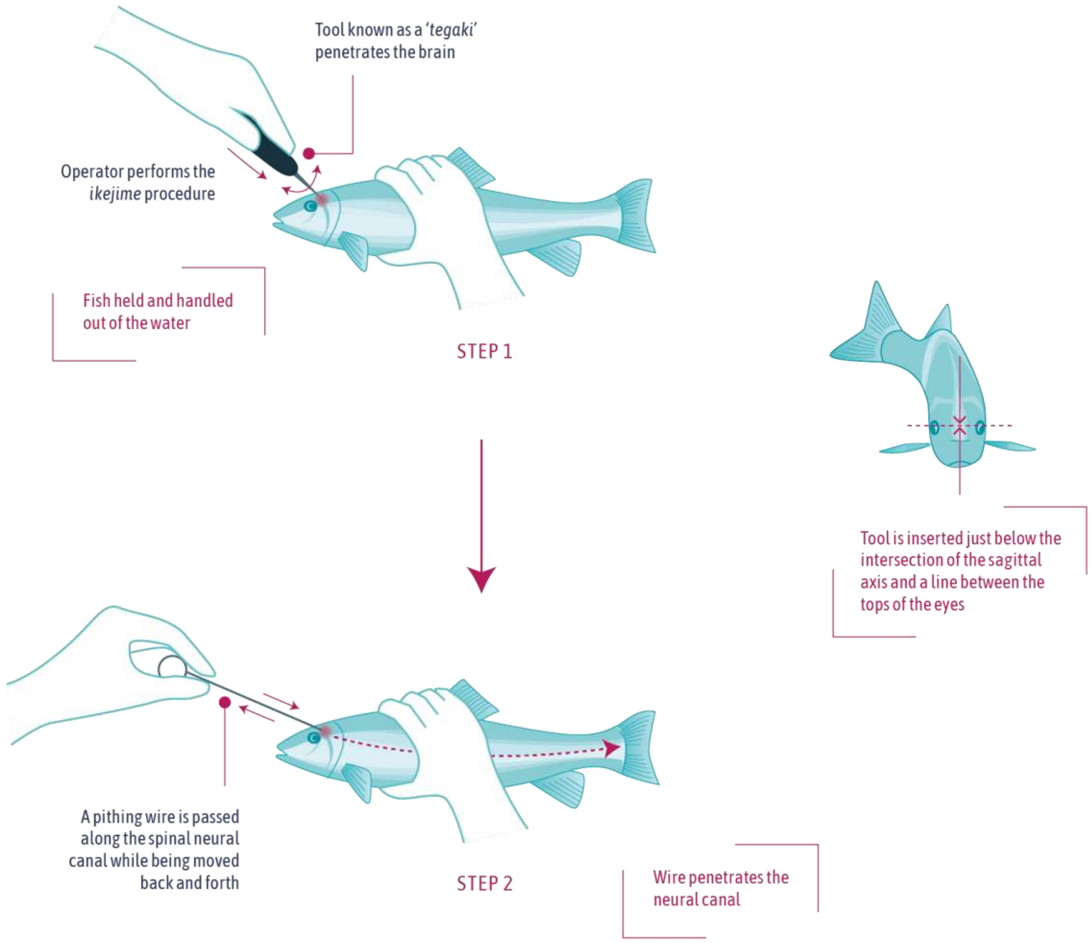

Figure 23. Procedure for ikejime stunning and killing *(original illustration created by FRCAW)*

Ikejime is an emerging slaughter method (outside Japan), particularly in the fishing industry. It requires precision and know-how on the part of the operator, and a knowledge of the anatomy of the nervous system of the species concerned to ensure that the method is used correctly (Robb & Kestin, 2002).

Pithing avoids reflex muscular contractions and slows down the food spoilage process (Terlouw et al., 2021). However, there are currently not enough scientific studies to support the value of this method compared with spiking alone, either in terms of the effectiveness of stunning and killing or in terms of product quality.

3.2.2.7. Killing by asphyxiation in air

Killing by asphyxiation in air is considered cruel and should not be used (Villarroel & Lambooij, 2022). Fish breathe aquatically via their gills, and most cannot survive outside the water. Keeping them out of the water therefore leads to their death after a long period of agony. Loss of brain function can take from 3 to 10 minutes in trout, depending on temperature, and 5 to 6 minutes in sea bream (Robb & Kestin, 2002). This period can even be measured in hours for species that adapt to hypoxia (carp, eel).

3.2.2.8. Killing by bleeding

Bleeding, which is essential for the quality and sensory characteristics of fish products, should only take place after the fish has been stunned. This process, which consists of severing a large artery, often at the gill arches (Figure 24), leads to death only after several minutes (Robb & Kestin, 2002; Robb et al, 2000). If there is no prior stunning, bleeding induces vigorous responses in a fish for about 30 seconds. The movements then subside, but a total time of 7 min for complete cessation of movement to be achieved has been measured in salmon (Robb et al, 2000). Additionally, EEG measurements show that VERs persist for a long time, around 280 sec in salmon (compared with 16 sec for percussion or 27 for spiking) (Robb et al, 2000).

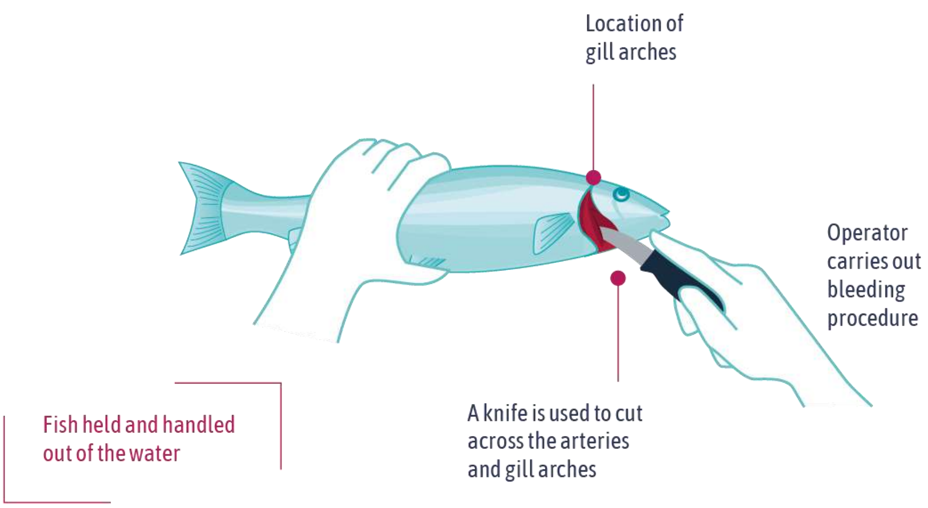

Figure 24. Procedure for killing by bleeding *(original illustration created by CNR BEA)*

3.2.2.9. Anaesthetic overdose

In Europe, the administration of an overdose of anaesthetic to bring about the death of animals cannot be used as a method of slaughter for human consumption but may be used under certain conditions (culling of deformed or dying animals). Before fish can be slaughtered and marketed, the farmer must ensure that withdrawal periods are respected if veterinary medicine has been prescribed during the rearing period. The withdrawal period that must be allowed is fixed by the marketing authorisation (MA) for each pharmacologically active substance. In the absence of a marketing authorisation, withdrawal periods are set at a flat rate by the authorities or the veterinary surgeon. These ensure compliance with the maximum residue limits (MRLs) that may or may not be set for different active substances. In Europe, for example, it is not possible to use anaesthetics or anaesthetic substances to euthanise animals for consumption, because the mandated withdrawal periods would not be respected. However, a number of countries, such as Australia and New Zealand, accept the use of the iso-eugenol-based anaesthetic AQUI-S in fish for human consumption, with no withdrawal period.

In Europe, to reduce the stress and pain of animals culled during batching due to deformations for example (e.g., deformed spine, shortened operculum, poor metamorphosis in flatfish, etc.), this is the preferred option to limit negative outcomes for the fish. Anaesthetic and euthanasia baths can be prepared and positioned close to the operators, allowing this measure to be included as a stage in the animal batching process. Two medicinal substances, tricaine and benzocaine, are permitted in France. The substance is placed in a vat of water in which the animals are then immersed. An initial dose is used to anaesthetise the fish. When the fish are deeply anaesthetised, a further dose of anaesthetic is added to obtain the lethal dose, and the fish are then euthanised (for fish used for scientific purposes: Directive-2010-63-EU). The doses/concentrations to be used to anaesthetise and then euthanise the animals depend on the species and rearing stage of the fish to be euthanised (Neiffer & Stamper, 2009). It is also important to take into account the temperature and quality of the water to ensure good uptake of the anaesthetic. Induction should take less than 5 minutes. It should be noted that certain substances may be perceived as aversive by some species of fish and may generate stress before inducing unconsciousness (Readman et al, 2013 ; Priborsky & Velisek, 2018 ; Welfarm, 2023).

3.2.3. Combining different methods

In practice, the stunning and/or killing methods described above are often combined. The aim is often to reduce the time taken to induce unconsciousness and to optimise killing conditions by combining the advantages of different methods and limiting the possible negative consequences for fish protection.

3.2.3.1. Combination of methods to increase stunning efficiency

Combinations of stunning methods include the addition of nitrogen gas to the ice water bath to reduce the dissolved oxygen concentration more rapidly and/or the addition of a moderate amount of carbon dioxide for its neurotoxic action which may be useful in reducing the duration of unconsciousness induction (Zampacavallo et al., 2015). Similarly, it has been shown in several species (notably Atlantic salmon and sea bass) that hypothermia in an ice water bath combined with a moderate dose of carbon dioxide reduces the level of physiological stress compared with the use of one of these two methods alone (Erikson et al., 2006; Merkin, 2014; Poli et al., 2005).

3.2.3.2. Combination of methods to kill effectively after stunning

Certain stunning methods, such as CO_2 or electronarcosis, may also be used for killing. Bleeding, described as a stressful method of killing, is very often applied as a method of killing after a stunning method inducing deep sedation.

Equipment designed to make the slaughter process less stressful for the fish has been developed and is used in some sectors. This is the case in France, where certain producers of large trout use a slaughtering machine that simultaneously knocks the fish unconscious (percussion) and cuts the gill arches (bleeding).

3.2.4. Comparison of different methods

3.2.4.1. Comparisons of methods in the scientific literature

To conclude this description of the different methods, we would ideally provide a comprehensive ranking of the welfare impacts of the methods discussed. With this in mind, we identified the many studies that provide comparisons of the consequences of different methods for the main fish species farmed in France, in terms of their biological impacts and/or product quality. The results of most of these comparative studies are compiled in Tableau 10. From each study, we have extracted data on species, growth stage (expressed most often as the average weight of the fish), the stunning and/or killing methods they compare, and the ranking of these methods, from least stressful to most stressful, for each criterion measured. It should be noted that anaesthetic substances feature in this table, since anaesthesia can be used as a control in experimental studies. The criteria measured in the selected studies include physiological stress indicators such as plasma cortisol and lactate, frequently-used indicators of quality such as the *rigor* index or initial muscle pH (immediately after slaughter), and far less frequently-used brain activity criteria such as VERs or behaviours.

Conclusions: We conclude from this work that it would appear very difficult to establish a hierarchy for the different methods tested. Each study is unique in terms of the species it examines, the physiological stage reached by the fish, and the technical parameters used for certain methods. Some studies use no control and compare and rank different methods, all of which have some negative impact on animal welfare. However, the table below serves to highlight **the detrimental effects of methods such as asphyxiation or bleeding without prior stunning, as discussed above**. It should also be noted that **percussive stunning often emerges as a less stressful method** in these comparative studies. The table also reveals the **lack of studies on certain species, such as sea bream and turbot**, for which the few existing studies report only very indirect quality indicators.

Tableau 10. Works comparing different slaughter methods for species reared in France / Europe. AnIE = IsoEugenol anaesthesia*, AnPE = Phenoxy Ethanol anaesthesia*, AnCO = clove oil anaesthesia*, As = asphyxia, BL = bleeding, CO = carbon monoxide, CO_2 = carbon dioxide, EN = electronarcosis, IS = ice slurry, N_2 = nitrogen, NS = not significant, pH3d = muscle pH measured 3 days after slaughter, pHi = muscle pH measured immediately after death (<2h), PS = percussion, SP = spiking, RT = Rainbow trout (*Oncorhynchus mykiss*), Salmon = *Salmo salar*, Sea bass = *Dicentrarchus labrax*, Sea bream = *Sparus aurata*, Turbot = *Scophthalmus maximus*, Carp = *Cyprinus carpio*, Tench = *Tinca tinca (original table created by FRCAW)*

Species	Growth stage	Comparative methods / techniques	Criteria measured	Ranking: - stress << + stress**	Reference
Salmonids					
Rainbow trout					
RT	≈ 300 g	IS, EN 200mA, EN 400mA	Loss of consciousness Plasma cortisol Onset of *rigor* pHi Muscle glycogen	EN<IS EN400<EN200, IS EN<IS EN<IS EN400<IS	(Bermejo-Poza et al., 2021)
RT	340 g	PS, N_2, As	ATP, pHi	PS<N_2< As	(Wills et al., 2006)
RT	≈ 350 g	PS, Ace	Plasma glucose and lactate, *rigor* index, pHi	PS<As	(Erikson et al., 2018)
RT	90-650 g	PS, CO_2, EN	pHi	PS<EN<CO_2	(Marx et al., 1997)
RT	≈ 700 g	CO, EN, As	pH3d	EN<As (med CO)	(Concollato et al., 2019)
RT	≈ 700 g	CO, EN, As	Plasma lactate *rigor* index, pHi	EN<CO, As EN, CO<As	(Concollato, 2016)
RT	730 g	CO, EN	ATP, fillet contraction	CO<EN	(Concollato et al., 2020)

Species	Growth stage	Comparative methods / techniques	Criteria measured	Ranking: - stress << + stress**	Reference
RT	≈ 900 g	PS, Ace	pHi	PS<As	(Duran, 2008)
RT	0.8-1 kg	As-Ice, EN, BL	pHi	EN, BL<As-Ice	(Giuffrida et al., 2007)
Atlantic salmon					
Salmon	≈ 800 g	PS, CO-1h, CO-2h	*rigor* index, pHi	PS<CO	(Bjorlykke et al., 2013)
Salmon	≈ 1 kg	PS, CO-8min, CO-20min	*rigor* index, pHi	PS<CO-20min	(Concollato et al., 2014)
Salmon	≈ 2 kg	PS, IS, IS+CO_2	*rigor* index, pHi	PS, IS<IS+CO_2	(Erikson et al., 2006)
Salmon	2.4 kg	PS, AnIE*, N_2, CO_2 low, CO_2 med, CO_2 high	plasma lactate pHi	PS<CO_2 low<CO_2 high<AnIE<CO_2 med<N_2 AnIE<PS, CO_2 low<CO_2 med<CO_2 high<N_2	(Erikson, 2011b)
Salmon	2.5-6 kg	PS, CO_2 -IS-PS, CO -IS-BL2	*rigor* index, pHi	PS< CO -IS-PS<CO -IS-BL2	(Roth, 2006)
Salmon	3.4 kg	PS, CO	*rigor* index pHi	PS<CO	(Bjørge et al., 2011)
Salmon	3-4 kg	7 groups: +/-IS, +/-CO_2, +/-PS, +/-BL	behaviour, pHi	Without CO_2 < with CO_2 (pH values ≥ 6.7)	(Olsen, 2006)
Salmon	4.4 kg	PS, pump-PS, pump-EN	*rigor* index, pHi	PS<pump-PS, pump-EN	(Roth et al., 2012)
Salmon	80 cm [!]	PS, EN, CO_2	*rigor* index	PS, EN<CO_2	(Roth, 2002)
Salmon	?	BL, CO_2 -BL, PS, SP	VERs	PS, SP<BL, CO -BL2	(Robb et al, 2000)

Species	Growth stage	Comparative methods / techniques	Criteria measured	Ranking: - stress << + stress**	Reference
Marine species					
Bass					
Bass	≈ 350 g	SP, IS	*rigor* index	SP=IS	(Tulli et al., 2015)
Bass	350 g	As, As-Ice, CO_2	plasma cortisol *rigor* index pHi	Ctrl<CO_2 <As-Ice<As CO_2, As-Ice<As CO_2 <As-Ice<As (pH values < 6.35)	(Acerete et al., 2009)
Bass	400 g	IS, IS+gas (70%N_2, 30%CO_2), IS+N2(100%)	time to death *rigor* index pHi plasma lactate (5h pm)	IS+gas, IS+N_2 <IS IS+gas<IS, IS+N_2 NS IS<IS+gas<IS+N_2	(Zampacavallo et al., 2015)
Bass	≈ 250-450 g	IS, EN	pHi	IS = EN	(Lambooij et al., 2008)
Bass	≈ 500 g	AnCO*, AnPE*, PS, IS, Ice, AnCO+IS	pHi (note: pH>7 for all)	Ice, IS, AnCO+IS<PS, AnCO, AnPE	(Simitzis et al., 2014)
Bass	550 g	IS, IS+gas(70%N_2 , 30%CO_2), EN 1 or 2 stages	time to death *rigor* index	IS+gas<IS IS<IS+gas<EN1, EN2	(Zampacavallo et al., 2015)

Species	Growth stage	Comparative methods / techniques	Criteria measured	Ranking: - stress << + stress**	Reference
			pHi plasma lactate (5h pm)	IS≤EN2≤IS+gas, EN1 IS<IS+gas, EN2, EN1	
Sea bream					
Sea bream	≈ 300 g	IS, As, PS-IS	*rigor* index pHi	IS, PS-IS<As PS-IS<IS<As	(Tejada & Huidobro, 2002)
Sea bream	420 g	IS, CO_2, saturated CO_2	pHi	saturated CO_2 <CO_2 <IS	(Giuffrida et al., 2007)
Sea bream	≈ 500 g	An*, IS	pHi	An<IS	(Matos et al, 2010)
Sea bream	?	IS, IS-CO_2	pHi	IS=IS-CO_2 (values<6.5)	(Panebianco, 2006)
Turbot					
Turbot	350 g	BL-IS, IS, PS-BL-IS	*rigor* index, pHi	PS<IS, BL-IS	(Ruff et al., 2002)
Turbot	≈ 500 g	PS, BL-Ice, EN	*rigor* index, pHi	PS<BL-Ice<EN	(Morzel et al., 2003)
Turbot	1.2-1.3 kg	PS, EN-5Hz, EN-80Hz, BL-Ice	*rigor* index pHi	PS<ES-80Hz<BL-Ice, EN-5Hz PS<<BL-Ice<EN-5Hz, EN-80Hz	(Roth et al., 2007)
Turbot	2.8 kg	IS, EN	pHi	IS = EN	(Knowles, 2008)
Other species (freshwater)					
Carp	150-330 g	PS, CO_2, EN	pHi, *rigor* index	PS<EN<CO_2	(Marx et al., 1997)
Carp	≈ 350 g	PS, Ace	pHi	PS<As	(Duran, 2008)

Species	Growth stage	Comparative methods / techniques	Criteria measured	Ranking: - stress << + stress**	Reference
Carp	≈ 1 kg	AnCO*, IS, CO_2, As	*rigor* index pHi	AnCO<IS<CO_2 <As AnCO, IS<CO_2 <As	(Rahmanifarah et al., 2011)
Carp	'market size'?	PS, IS, CO_2	plasma cortisol, pHi	PS<CO_2 <IS	(Varga, 2014)
Tench	≈ 80 g	PS, IS, CO_2, EN	*rigor* index	PS<CO_2, IS<EN	(Gasco et al., 2014)
Tench	≈ 160 g	CO, EN, PS	*rigor* gill cortisol pHi	PS<CO<EN CO<PS, EN PS<EN, CO	(Secci et al., 2018)

* In experimental studies, anaesthesia using other substances is used as a control.
** Method X < method Y indicates that method X has significantly less negative effect than method Y on the criteria measured.

4. Conclusion: assessment and recommendations

4.1. Summary of methods studied

Only the main stunning and slaughtering practices studied in the literature are referred to in this summary. Table 11 provides a summary of the practices studied in this report, listing their advantages and disadvantages in terms of fish welfare. Although fish welfare is the focus of the analysis of stunning and killing methods set out in this document, certain advantages and disadvantages from a technical perspective have also been included in Table 11 to provide the context for decisions made on the ground on whether to use or avoid the practices under review.

To appear in the table, fish welfare advantages and disadvantages must have been demonstrated for at least one species with no proven contrary effects for another species.

For each method, the table indicates whether it is used solely for stunning, solely for killing, or whether it can be used to both stun and kill. It should be noted that methods acceptable for stunning only must be followed by a killing method, and that methods suited only to killing must be preceded by a stunning method.

Last, it must be borne in mind that, for all the practices listed in the table below, the major contributory factors in effective stunning and the welfare of fish during slaughter are the correct training of the operating team, the use of suitable equipment that is properly calibrated and maintained, the ability to recognise when a fish has been effectively or poorly stunned, and the availability of emergency equipment (Appendix 2).

Table 11. Summary of methods studied for the stunning and killing of fish in aquaculture *(original illustration by the FRCAW)*

Methods used for slaughtering								
Method	**Brief description**	**Biological principle**	**Stunning / Killing**	**Relevant species in France**[20]	**Benefits**		**Disadvantages**	
					Fish welfare	**Other**	**Fish welfare**	**Other**
Gas (CO_2 and N_2)	Immersion of fish in a tank of water saturated with anoxic gas	Hypercapnia (too much CO_2) and/or hypoxia (too little O_2)	Stunning	Rainbow trout (all sizes) Brown trout Brook trout Arctic char	- Fish can remain in water from rearing to stunning	- Relatively inexpensive - Quick and easy to set up (batch stunning) **- CO_2** : Improves the success rate of other stunning methods	- Fish may take several minutes to lose consciousness - Painful/aversive method - Rapid return to consciousness	- Difficulty controlling effectiveness of the stun (linked to batch management) - Bleeding needs to be carried out quickly after stunning
Ice / ice slurry bath	Immersion of fish in a tank filled with ice or an ice-water mixture	Live chilling (thermal shock)	Killing	Sea bass Sea bream Meagre Sole	/	- Inexpensive - Quick and easy to set up (batch stunning) - 'Products' are clean	- Painful/aversive method - Some fish asphyxiate if there is insufficient water to submerge all individuals - Fish may take several minutes to lose consciousness	- Difficulty monitoring the effectiveness of the stun (linked to induced paralysis and batch management)

[20] What experts in the French fish farming industry have to say

Electric current	Passing of an electric current through the fish's brain (in or out of water)	Disruption of neural activity (electronarcosis) + cardiac arrhythmia (electrocution)	Stunning (electronarcosis) + Killing (electrocution)	Trout (all sizes) Sturgeon (male)	- Unconsciousness induced immediately or almost immediately in all individuals when parameters are properly calibrated **- In-water methods**: fish can remain in water from rearing to stunning.	**- Batch methods**: quick and easy to set up (batch methods)	- Effectiveness of stunning depends on use of electrical parameters appropriate to the species and size of the fish. **Dry methods:** - Asphyxiation (dry methods) - Pain, injury or psychological stress linked to possible pre-stun shocks **Tank method**: Pain, injury or psychological stress linked to overcrowding and possible pre-stun shocks	- Electrical parameters need to be adjusted to the species and size of fish stunned/killed - Relatively costly (varies depending on the system) - Bleeding needs to be carried out quickly after stunning (if fish not to be electrocuted) - Product quality impaired by use of certain electrical parameters
Percussion	Striking the skull of the fish with a solid instrument	Disruption of electrical activity and intracranial pressure leading to dysfunction and/or destruction of the brain	Stunning + Killing	Large salmonids (automatic and manual methods) Female sturgeon (manual method)	- Unconsciousness induced immediately or almost immediately in all individuals - Irreversible stunning when properly performed **- In-water methods**: fish can remain in water from rearing to stunning.	- Ease in controlling effectiveness of the stun **- Automatic percussion**: fast and automatic **- Manual percussion**: inexpensive	**- Dry methods**: pain, injury or psychological stress associated with removal from water **- Manual percussion**: pain, injury or psychological stress in the event of poor execution **- Manual and semi-automatic percussion**: potentially stressful confinement/crowding of fish prior to stunning	- Bleeding needs to be carried out quickly after stunning **- Automatic percussion**: expensive **Manual percussion**: - Risk of poor execution due to repetition of an action - Technical and precise movements required - Slow

Spiking/ decerebration	Destruction of the brain by inserting a sharp point or knife from just behind the skull	Destruction of the brain	Stunning + Killing	More suitable for large species (salmon, tuna)	- Unconsciousness induced immediately or almost immediately in all individuals when properly executed	- Ease in controlling effectiveness of the stun - Inexpensive	- Psychological stress linked to removal from water - Pain, injury or psychological stress in the event of poor execution - Potentially stressful confinement/crowding of fish prior to stunning	- Risk of poor execution due to repetition of the action - Technical and precise movements required - Slow
Ikejime	Destruction of the brain with a spike followed by destruction of the spinal cord with a wire passed through the spinal canal	Destruction of the nervous system	Stunning + Killing	Sea bass Large trout	- Unconsciousness induced immediately or almost immediately when properly executed	- Ease in controlling effectiveness of the stun - Inexpensive	- Psychological stress linked to removal from water - Pain, injury or psychological stress in the event of poor execution - Potentially stressful confinement/crowding of fish prior to stunning	- Risk of poor performance due to repetition of the action - Technical and precise movements required - Slow
Air asphyxiation	Fish are left in open-air trays or ice trays without water	Asphyxiation	Killing	All species	/	/	- Physical and psychological stress linked to asphyxiation in the air - Extensive period of agony (lasting up to several tens of minutes)	/
Bleeding	Sectioning of one or more arteries at the level of the branchial arches	Exsanguination which stops the heart	Killing	Large species	Painless when the fish is properly stunned	Helps maintain cleanliness and quality of products	If stunning is not carried out correctly : - Physical and psychological stress - Extensive period of agony (lasting up to several tens of minutes)	/

Methods used solely for experimental purposes or for purposes other than slaughtering								
Method	Brief description	Biological principle	Stunning / Killing	Relevant species in France[21]	Benefits		Disadvantages	
					Fish welfare	Other	Fish welfare	Other
Gas (CO)	Immersion of fish in a tank of water saturated with anoxic gas	Hypoxia (lack of O_2)	Stunning	/	Fish can remain in the water from rearing to stunning No aversive reaction observed	Quick and easy to set up (stunning by batch)	/	Difficulty controlling effectiveness of the stun (linked to batch management) Dangerous for the operator (CO)
Anaesthetic overdose	Immersion of the fish in a water bath containing an anaesthetic dose of tricaine or benzocaine, followed by the addition of a lethal dose.	Asphyxia due to lack of gill movement	Stunning + Euthanasia	/	Fish can remain in the water from rearing to stunning	/	/	Use banned in Europe for slaughter purposes Concentrations must be adjusted to take account of species, life stage, temperature and water quality for euthanasia to be effeictve.

[21] Information provided by experts in the French fish farming industry

4.2. General recommendations

This synthesis of available written evidence has been produced by FRCAW on its own initiative and responds to a need, among professionals in particular, for access to scientific information on fish welfare in light of the growing ethical and societal concerns on the subject. The work it contains is based on a corpus of 248 documents selected by a committee of 9 experts in aquaculture, slaughtering, physiology and fish behaviour. Its purpose is to:

- Report on the specific characteristics of the French fish-farming industry in relation to slaughtering processes and the associated regulations;
- Provide scientific information on the sensory and emotional sensitivities of fish and the indicators that can be used to assess these sensitivities;
- Report on the various processes undergone by fish during slaughter and provide information on their consequences for fish;
- Report on the various stunning and killing methods studied in the scientific literature and provide information on their consequences for fish.

Taking into account the findings of this review and the expert opinions expressed on the subject, this final section sets out the main conclusions and recommendations to be considered in the matter of the slaughter of farmed fish, as formulated by the expert committee.

4.2.1. **Lack of scientific and technical research, given the complexity of the subject**

This critical overview sets out the main methods used to slaughter fish as described in the literature. Based on the evidence provided by studies of various fish species, the chief critical concerns for fish in respect of the stunning and slaughtering methods described include, but are not limited to:

- Killing without prior stunning;
- Methods involving the crowding of live fish;
- Electric stun settings (intensity, duration) that produce little or no effective stunning;
- Too long a period between stunning and killing in the case of reversible stunning.

It is, however, impossible to focus more precisely on the critical aspects of each individual slaughtering practice because no studies exist on the reality of practices as they are carried out on the ground.

Recommendation 1: The stunning and killing practices, protocols and equipment employed in France should be more thoroughly inventoried and their respective impacts on the different species of fish concerned should be assessed. This could be achieved through surveys and interviews of equipment manufacturers or other professionals in the sector, coupled with observations on the ground.

Such an inventory of the practices and protocols currently in use would make possible the precise identification of those factors that cause pain and stress for the fish, thus making it possible to produce good practice guidance and establish reliable and rigorous protocols for fish welfare.

Recommendation 2: Technical guides and/or protocols should be drawn up by the industry, in partnership with technical institutes and research bodies. These guides should be based on scientific and technical studies using non-stressed controls to assess the effects of different practices on fish and to identify optimal stunning parameters. These guides should be reviewed and validated by scientific experts in fish physiology and behaviour.

Recommendation 3: Among the many research projects needed to improve fish welfare in the context of slaughter, more research effort should be invested in particular in:

- the study of alternative methods to the use of a water/ice mixture for killing without any other form of prior stunning;
- the study of the impact of fasting on fish behaviour to complement current studies on the welfare of fish during this stage of production;
- the verification of the effects of stress on product quality.

Not only the production of knowledge, but also the dissemination and valorisation of this knowledge are major tasks for fish protection. For example, knowledge concerning the effects of stress on product quality could also benefit the sector, where appropriate, as an additional positive consequence of animal welfare measures for fish farmers.

4.2.2. Pressing need to disseminate knowledge

It is important that current and future scientific knowledge on the issues identified in this summary should be widely disseminated to professionals in the fish farming and slaughtering sector, and that staff should be trained on the following aspects in particular (the list is not exhaustive):

- Sensitivities of different fish species;
- Distinction between immobility, unconsciousness and death;
- Fish stress indicators;
- Fish unconsciousness indicators;
- Fish death indicators.

Recommendation 4: Reliable indicators of stress and unconsciousness should be sought and studied for each species in the context of slaughter to determine their feasibility, repeatability and reproducibility in practice. In particular, specific behavioural indicators should be studied in greater detail to analyse the impact of different practices on fish.

Indicators that are feasible, repeatable and reproducible in practice could then serve as the basis for reliable self-monitoring or auditing tools.

4.2.3. Lack of regulations applicable to the transport and slaughter of fish

The European regulations on the welfare and protection of fish and their transposition into French law are too imprecise and lacking in specificity to guarantee real protection for fish during slaughter. These regulations reflected the state of knowledge at the time they were drawn up. Changes to the regulations, taking into account new knowledge and the feasibility of implementation, should be encouraged.

Furthermore, the conditions under which fish are transported do not fall within the European framework of animal welfare regulations, since Council Regulation EC/1/2005 on the protection of animals during transport does not apply to fish.

In view of these observations, the revision of European animal welfare regulations scheduled for 2023 constitutes a major opportunity to provide a better framework for the consideration of the welfare of farmed fish and to guarantee their welfare during slaughter.

Recommendation 5: The legal framework for the protection of fish, particularly around the time of slaughter, should be developed and clarified through the new European regulations on animal welfare in the light of recent scientific knowledge.

Recommendation 6: To inform its regulatory review of fish welfare, the European Commission should formally request an assessment from EFSA of the risks to fish welfare in the context of slaughter for all species farmed in Europe.

4.2.4. Constraints specific to the fish farming industry

The above findings can, in part, be attributed to the fact that the fish farming industry is subject to a number of specific constraints that make it difficult to conduct scientific investigations, to take ownership of the knowledge produced and to apply it. The aim of this section is to explain these constraints and suggest possible courses of action.

The major constraint on the monitoring and control of the slaughter of farmed fish lies in the wide variety of species and sizes of fish that are reared. For example, more than ten different species of fish are farmed in France. Yet fasting, loading, transport and slaughtering practices are rarely species-specific, despite the potentially very different sensory and emotional sensitivities among species (associated with the different ecological niche occupied by each). This failure to take account of the sensitivities specific to each species can cause stress and pain from the time the animals leave the home tank until they are killed. In the scientific literature, a limited number of practices and/or parameters tend to be studied for a single species. Conclusions translate poorly to other species, meaning not only that there is a general lack of knowledge but also that, where detailed knowledge of a species is available, it cannot be applied to many other farmed species. Moreover, in addition to species, developmental stage and size are important factors to be considered in addressing the sensitivities of individual animals.

Recommendation 7: The differences in sensitivities between fish species and sizes should be studied further, so that practices can be adapted to suit the needs of the individual fish sold commercially.

Regardless of their different sensory and emotional sensitivities, the aquatic environment common to all fish species itself imposes particular management constraints. This living environment produces numerous stressors for fish at the time of slaughter (fasting, exposure to air, etc.). In practice, during the pre-stunning phase, it is common to subject fish to fasting, primarily to maintain water quality but also to extend product shelf life by limiting faecal contamination, despite the fact that this fasting period is a potential source of stress for the animals. Additionally, the out-of-water handling and 'dry' practices associated with the pre-stunning, stunning and killing phases also critically effect these aquatic animals, leading to asphyxiation.

In terms of operational practicality for the sector, slaughtering in an aquatic environment also introduces constraints. One issue of note is the complexity of the task of observing the animals in the water to assess the effectiveness of stunning or the stress level of individual fish. This makes it hard to identify poor practice.

Recommendation 8: The handling of fish out of water should be limited to what is strictly necessary and ‘dry’ practices should not be used on non-stunned fish. The duration of the fast imposed on fish before they are killed should be adapted to the species and the water temperature to which a fish has been accustomed.

A further constraint common to all fish species is the practice of batching fish. Among other features, this form of management involves the animals being crowded together (crowding), a step that is particularly likely to cause stress and injuries to the fish, and that also encourages batch-based stunning and killing practices where fish can be crushed against each other in the event of high densities or where they are piled on top of each other, especially when removed from the water. Additionally, the management of fish in batches produces variable outcomes in individual fish, making it harder to judge whether a fish is unconscious or dead.

Recommendation 9: It is essential to take individual variability into account when managing batches of fish. Control testing should be carried out on sufficiently large samples of individuals representative of the batch to ensure the effectiveness of stunning methods.

4.2.5. Economic, practical and socio-cultural considerations

It should be noted that, in this summary, FRCAW has not addressed the economic, practical or socio-cultural aspects of the various slaughtering practices and their contexts, as this does not fall within its remit. Nevertheless, these aspects should also be studied, so that professionals may be provided with the best possible support in adopting more fish-friendly batching, transport and slaughtering practices. Public institutions (both European and French) that allocate funds to support actions in the field of aquaculture (e.g., EMFAF) could offer a useful route for the acquisition of greater knowledge and technico-economic information, as well as finance for fish farmers wishing to install welfare-friendly slaughter stations for large numbers of fish, which are currently still a costly investment.

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Appendix 1. Indicators for different degrees of consciousness adapted from an observation protocol for practitioners *Each indicator in the observation sequence (L to R) is scored for level of brain function, where (0) = full stun/unconsciousness, (1) partial stun/consciousness, (2) no stun/fully conscious. Based on (Kestin et al., 2002b)*

	Self-initiated behaviour		Response to stimuli			Clinical reflexes	
	Swimming	Equilibrium	Handling (restraint)	Piqure d'épingle	Swimming	Equilibrium	Handling (restraint)
Behaviour / reflex	Swimming	Righting ability	Response to handling	Behaviour / reflex	Swimming	Righting ability	Response to handling
Observation sequence indicators scoring (0)	No swimming	Unable to right	No response			Observation sequence indicators scoring (0)	No swimming
Observation sequence indicators scoring (1)	Slow or abnormal swimming (e.g. upside down)	Slow to right	Slow or feeble response after tail pinch	Observation sequence indicators scoring (1)	Slow or abnormal swimming (e.g. upside down)	Slow to right	Slow or feeble response after tail pinch
Observation sequence indicators scoring (2)	Normal swimming	Quickly rights	Immediate, vigorous escape attempt on first touch/ pinch	Observation sequence indicators scoring (2)	Normal swimming	Quickly rights	Immediate, vigorous escape attempt on first touch/ pinch

Appendix 2. **Overview of suitable welfare indicators for different fish killing methods, based on Noble et al., (2020)**

Environment-based indicators	**Group-based indicators**	**Individual-based indicators**
Correctly adjusted parameters for automated systems (voltage/amperage, percussive force, etc.)	Health status	Observation of eyeroll reflex, tail grab reflex, opercular movements, tail flapping → control of unconsciousness.
Water level, dose and holding time if using anaesthetics, fish density	Behaviour (fish are calm and not exhausted)	Observation of fin and snout injuries, skin or muscle haemorrhages, scale loss, crush injuries.
Suitable hydrological conditions (TºC, oxygen)	Fish enter machine correctly (head first)	Pre-rigor times, muscle and blood pH, other physiological indicators (levels of lactic acid, lactate, glucose, cortisol, etc.)
Length of time spent out of water	Blood or scales in water (indicating animal injuries)	

'Environment-based indicators adress the stunning machines or the bath with overdose anaesthetics; group-based indicators are what can be observed and checked during the euthanizing process, while individual-based indicators are based on sampling individual fish for close ups on missing reflexes and the correct blow/bleed where relevant' (Noble et al., 2020, p. 227).