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FARMED FISH SLAUGHTER METHODS REPORT

Recommendations for Rainbow trout, Atlantic salmon, European sea bass and Gilthead sea bream

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The following report about the slaughter practices of the main species of farmed fish reared and consumed in France was completed by the animal welfare studies department of Welfarm.

It is the first part of a larger work about the French fish farming sector, conducted thanks to the financial support of Eurogroup for Animals.

A second report focused on rearing practices of farmed rainbow trout and subsequent welfare guidelines for this species is under way. A similar work will be conducted for sea bass and sea bream later on.

This work constitutes the basis for the recommendations that Welfarm will put forward to improve fish welfare through dialogue with the fish farming industry, and by lobbying retailer purchasing offices about their supply policies.

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This report was written by Welfarm's animal welfare studies department. Pricillia Durbant, Gautier Riberolles, Françoise Burgaud and Léa Ritter all contributed to its completion.

Table of contents

Introduction

In France, 20 to 66 million farmed fish are slaughtered every year by the fish farming sector (Fishcount). Slaughter methods, performed with or without prior stunning, are very diverse. This diversity of slaughter methods is also observed within the European Union, and more widely, at a global scale.

In practice, the choice of a particular slaughter method is guided by several considerations: the morphology and anatomy of the considered species (bone resistance, shape of the fish etc.), the production output, the pace of the slaughter line, financial investments and expected margins, certification requirements, goals about flesh quality and end product characteristics etc. Numerous methods are being used, and they do not all require the same kind of equipment. In fact, certain methods do not actually require any (e.g asphyxia in air), whereas other methods rely on equipment of varying degrees of sophistication according to the level of mechanisation and automation.

The diversity of stunning and killing methods, as well as the specificities of each species makes designing humane slaughter recommendations complex. Even though some stunning and killing methods have no benefit at all in terms of animal welfare, some methods have both strengths and weaknesses.

Scientific research about stunning methods, adequate stunning parameters, and their impacts on fish is not very advanced, although publications on the topic have been increasing in recent years. However, it already appears that several methods, either traditional or more recent, are criticized due to the stress and pain they inflict upon animals.

The analysis described in this report seeks to describe the characteristics of the different stunning and killing methods in terms of animal welfare for the following four target species: rainbow trout (*Oncorhynchys mykiss*), Atlantic salmon (*Salmo salar*), European sea bass (*Dicentrachus labrax*) and gilthead sea bream (*Sparus aurata*).

These four species have been selected due to their importance in terms of production and/or consumption in the French fish farming sector (Fishcount estimates, FranceAgriMer 2020, FranceAgriMer 2021).

In order to identify the stunning and killing methods that ought to be phased out, improved or recommended, an analytical framework has been developed. It is based on the main welfare hazards, i.e factors likely to induce suffering, that have been identified in the scientific literature. Each stunning and killing method has been assessed in regard to those welfare hazards, according to a methodology based on a grading system. This allowed us to divide slaughter practices in three categories. The first two categories are ethically unacceptable methods and recommended methods. For the latter group, a hierarchy of methods has been designed based on their potential in terms of animal welfare. Finally, the third category includes methods that are characterised by a level of uncertainty which is too high for them to be recommended for the time being.

This report is divided into four sections. In the first part, the main basis of scientific knowledge about the ability of fish to suffer are summed up. Then, the state of the legal situation surrounding farmed fish slaughter, and the components that are necessary to call a slaughter method "humane" are presented. The second part presents the methodology that we used and the welfare hazards that were considered within our analytical framework. Stunning and killing methods are then described and reviewed in the light of our analytical framework in the third part. Finally, the recommendations resulting from our analysis of the different stunning and killing methods are presented.

I. Principles of animal welfare at the time of slaughter

The ability of fish to suffer has been the subject of controversy in the past but is nowadays well established. The scientific background and institutional position statements about this topic are briefly addressed here. The legal framework about the conditions in which farmed fish are slaughtered, and the components required for a slaughter method to be considered "humane" are then discussed.

1. Pain sensibility in fish

The existence of pain sensibility in fish has been the subject of controversy but is now close to a scientific consensus. It is in particular well established for teleost fish (Sneddon 2019, Sneddon 2015). This group includes the vast majority of fish species, among which those consumed in France and in Europe like salmonids, sea bass and sea bream, but it excludes rays, sharks and lampreys.

The ability of fish to feel pain has been officially recognised by various scientific institutions. In 2009, a report dedicated to fish sentience by the European Food Safety Authority (EFSA) concluded that fish can feel pain (EFSA 2009e). This same report also considers that they have the ability to feel fear. A collective expert opinion by the French National Agronomic and Environmental Research Institute (INRAE) in 2017 about consciousness in animals also concluded that fish possess cerebral structures "that likely allow them to consciously feel pain" and that they are likely to be able to feel emotions (Le Neindre & al. 2017). The British Farm Animal Welfare Council also favours the thesis that fish can feel pain, or at least claims that the level of evidence supporting it is serious enough to require the establishment of guidelines in order to minimize fish suffering. The existence of such guidelines coming from the World Animal Health Organisation (OIE), the British Veterinary Association (BVA), the National Veterinary Institute of Norway, and the scientific council for animal welfare from the Swedish University of Agricultural Sciences (SLU) expresses by implication the same position from those institutions.

In fact, fish possess nociceptors that have been shown to react to mechanical, thermal, chemical, and electrical stimuli. Furthermore, they behave differently when they are submitted to noxious stimuli compared to non-noxious stimuli, and there is evidence that they actively seek to avoid pain or to reduce its intensity. Finally, the behavioural modifications that occur after they receive noxious stimuli are reduced if they are provided with pain relieving drugs (Sneddon 2015).

Despite this, some authors still argue that fish cannot feel pain (Brian Key 2015, 2016, Rose 2002, Rose & al. 2014). However, such authors are a minority within the scientific community. Thus, Brian Key's article arguing that fish cannot feel pain generated no less than 30 academic articles published as a response, among which 27 argued that fish can indeed feel pain (Brown 2016). The rationale behind the claim that fish cannot feel pain usually relies on three main arguments: the absence of neocortex in fish, the fact that some behavioural indicators used to assess pain sensibility in fish are also present in decerebrated mammals that may be unconscious, and the difference between fish and mammals about the ratio of A-delta and C nervous fibers. However, each of these arguments elicited compelling academic refutations (Brown 2016ab, Sneddon & Leach 2016).

2. Legal framework surrounding fish welfare at the time of slaughter

Two recent published reviews offer a synthesis of the legal framework surrounding the conditions under which farmed fish are slaughtered (Gimenez-Candela & al. 2020, Riberolles RSDA 02/2020).

- ***International scale: OIE standards***

Though legally non-binding, the aquatic code of the OIE contains standards about "welfare aspects of stunning and killing of farmed fish for human consumption" (Section 7, Chapter 7.3). In particular, the following points are mentioned:

- Workers must be experienced, competent and trained
- A back-up stunning system must be available
- Effective stunning should be verified by the loss of consciousness, which can be assessed through the following signs 1) absence of body and opercular motion (loss of the opercular beat), 2) absence of "visually evoked potentials " (VEP), 3) loss of the vestibulo-ocular reflex
- The use of mechanical stunning methods including head percussion[1], brain destruction and gun shooting, is recommended
- The use of electrical stunning is recommended
- In the case of dry or semi-dry electrical stunning, fish must enter head first into the system
- The following methods: *"chilling with ice in holding water, carbon dioxide (CO_2) in holding water; chilling with ice and CO_2 in holding water; salt or ammonia baths; asphyxiation by removal from water; exsanguination without stunning"* should not be performed if mechanical or electrical stunning methods are available

Generally speaking, the legal requirements of the European Union are below OIE standards regarding the welfare of farmed fish at the time of slaughter.

- ***European scale: Council of Europe***

Fish are excluded from the European convention for the protection of animals for slaughter. However, the standing committee of the European convention for the protection of animals kept for farming purposes adopted a Recommendation concerning farmed fish on December the 5th of 2005. This recommendation, which is legally non-binding, sets the following standards regarding emergency slaughter on the farm:

- Requirement to stun fish and make them insensible before killing
- Immersion in CO_2 saturated water shall not be performed except when large numbers of fish have to be killed rapidly (depopulation)
- Exsanguination shall not be performed without prior stunning

- ***European scale: European Union regulation***

The slaughter of farmed animals must be performed in compliance with Council Regulation (EC) 1099/2009 on the protection of animals at the time of killing. Fish have not been properly

[1] This recommendation is considered applicable to carps by the OIE which may be a problem considering that common carps have a particular skull anatomy that makes them very resistant to percussive stunning (CIWF 2018)

considered by the European legislator as they should have been: fish are explicitly excluded from most of the regulation. In fact, the definition of the word "slaughterhouse" excludes places where farmed fish are slaughtered. The only requirement from the regulation which is applicable to farmed fish is the following *"Animals shall be spared any avoidable pain, distress or suffering during their killing and related operations.*" (Chapter II, article 3.1)

The Regulation (EU) 2018/848 of 30th May 2018 on organic production and labelling of organic products mentions slaughter practices in aquaculture. It states that "*"Any suffering shall be kept to a minimum during the entire life of the animal, including at the time of slaughter"* and requires that "*Slaughter techniques shall render fish immediately unconscious and insensible to pain. Handling prior to slaughter shall be performed in a way that avoids injuries while keeping suffering and stress at a minimum. Differences in harvesting sizes, species, and production sites shall be taken into account when considering optimal slaughtering methods."* Specific methods to be used are not indicated. This regulation has come into force on January the 1st 2022, supplanting the former Commission Regulation (EC) 710/2009 laying down detailed rules on organic aquaculture animal and seaweed production (**<u>no longer in force</u>**) which stated that *"Slaughter techniques shall render fish immediately unconscious and insensible to pain. Differences in harvesting sizes, species, and production sites must be taken into account when considering optimal slaughtering methods".*

Thus, it must be observed that fish are not properly protected by specific requirements in the European legal norms related to animal welfare at slaughter.

- ***<u>National scale</u>***

France does not have any legal requirement regarding farmed fish welfare at the time of slaughter.

Moreover, similarly to the European regulation, in Section 4 "Slaughter", from Chapter IV of the first section of book II of the regulatory part of the "Code rural et de la pêche maritime", the definition of the terms "slaughterhouse" and " slaughter facility " exclude places where farmed fish are slaughtered. Therefore, all the legal texts using the terms "slaughterhouse" and "slaughter facility" are not applicable to farmed fish.

However, **there is an ambiguity surrounding the inclusion of fish in the following requirements:** the requirement to stun animals, the requirement that slaughter plant workers must be trained or supervised by someone with animal welfare training, the requirement to perform bleeding as quickly as possible before the recovery of consciousness, the ban on suspending animals before stunning, and the requirement to immobilise animals. This ambiguity is related to the definitions of the words and the name of the various sections and sub-sections. In practice, this ambiguity is interpreted as an exclusion of farmed fish from those requirements.

Before the uptake of the European regulation on organic aquaculture, standards of organic aquaculture in France used to be defined by a national text which is **<u>no longer in force</u>**. This text was the Production requirements concerning the organic production and processing method of aquaculture species and their derivatives, approved by the ministerial ruling of February the 2nd of 2007 and included the following sentences: *"Fish must be stunned before any processing (bleeding and/or evisceration). Fish may be stunned by performing an electrical shock, a blow to the head, by inducing numbness through chilling, or by asphyxia in CO_2 saturated water".*

Additional Observations

Slaughter practices are also regulated for animals used for scientific purposes by article 6 of Directive 2010/63/EU of the European parliament and of the council of 22 September 2010, incorporated into French law by article R214-89 of the "Code rural et de la pêche maritime". Methods allowed for fish in this context are anaesthetic overdosing with prior sedation, concussion/percussive blow to the head and electrical stunning.

On November the 1st of 2020, a European resolution relative to the safeguarding of animal welfare within the European Union has been adopted by the French National Assembly. Point 19 of this resolution addressed to the European Commission: "Demands to extend the applicability of regulation (EC) 1099/2009 of 24 September 2009 to facilities where farmed fish are slaughtered and encourages reflection about the conditions under which fish are slaughtered in wild capture fisheries".

3. Defining "humane slaughter"

Various publications from animal welfare NGOS like the Royal Society for the Prevention of Cruelty to Animals (RSPCA) in the UK or the Humane Slaughter Association (HSA), as well as scientific articles and legal texts were used to establish a set of criteria necessary for a slaughter method to be considered humane. Thus, a slaughter method, irrespective of species, can be considered humane if it meets the following requirements:

- Stunning prior to killing must result in a loss of consciousness and sensibility without any pain
- Stunning must result in an immediate loss of consciousness
- Stress before stunning must be limited both in duration and in intensity
- Animal welfare must be safeguarded during the complete process of stunning and killing
- Killing must be quick and effective in order to prevent any recovery of consciousness
- The efficacy of each step of the process must be guaranteed

Although those principles apply to all farmed animals, animal welfare considerations are rarely mentioned for farmed fish to this day in regulations and other official texts.

II. Methodology applied to assess the humaneness of slaughter methods

All slaughter methods currently used imply both benefits and drawbacks in terms of animal welfare. To acknowledge this complexity in our assessment process, a standardised methodology based on welfare hazards analysis has been developed.

First, the different welfare hazards considered are listed and the grading system for those hazards is described. Then, each considered welfare hazard is more thoroughly presented, along with its grading specificities. Finally, the full analytical grid used to assess slaughter methods is presented as a table.

1. Welfare hazards

Using various publications from official expert bodies (EFSA, OIE, FAWC, SLU, NVI) and animal welfare organisations (RSPCA, HSA, CIWF), as well as a thorough review of the scientific literature, the main slaughter methods used in France and the E.U for our target species have been listed. On this basis, the main **welfare hazards**, i.e risk factors likely to impair animal welfare, were identified. Eleven welfare hazards were selected to be used for a systematic analysis of slaughter methods.

The considered welfare hazards are the following: exposure to noxious chemicals, immediateness of the loss of consciousness, failure rate, risk of recovery of consciousness (reversibility), air exposure, handling, poor water quality, high or extreme densities, tissue damage, thermal shock, reliance on workers' skill.

An analytical framework based on these welfare hazards has been constructed to allow a rigorous comparison of the different stunning and killing methods according to their potential to minimize fish suffering. In other words, welfare hazard analysis was used to identify the methods that give rise to suffering, and those which are more humane.

The slaughter methods that are being used are not always the same across species. For instance, full-automatic and semi-automatic percussive stunning machines are used to stun salmonids (EFSA 2009ab, European commission 2017 – 2018) but not sea bass and sea bream (EFSA 2009c). Furthermore, for the same method, the welfare outcome on fish can vary across species. Therefore, the mark attributed to the different methods for each welfare hazard can be different depending on the considered species.

For most welfare hazards, a grading system relying on three possible marks from "A" to "C" ("A" being the best mark) was used to express to what degree the method is compatible with welfare requirements. Only the welfare hazard called "air exposure" has a grading system based on four possible marks from "A" to "D".

For some welfare hazards, the grading system is binary: the mark can either be "A" or "B". In such cases, the mark "A" means that the welfare hazard is absent, whereas "B" means that it is present.

Therefore, depending on welfare hazards, the mark "A" can mean that the considered welfare hazard is irrelevant for the considered method (e.g no handling or no air exposure at all), or that the method implies the presence of this welfare hazard but at a level which is deemed low or acceptable in view of other available methods (e.g : failure rate below or equal to 1%, low reliance on workers' skills).

In addition, due to their nature and in light of the scientific literature, it has been considered that some welfare hazards were particularly important, and that controlling them is absolutely essential to achieve a humane slaughter. Therefore, those welfare hazards have been considered to be disqualifying factors. Consequently, the attribution of the lowest mark (either B, C or D depending on hazards) for those hazards means that the considered method is rejected irrespective of its marks on other welfare hazards. The disqualifying welfare hazards are the following: exposure to noxious chemicals, immediateness of the loss of consciousness, failure rate, air exposure, high or extreme densities, tissue damage, thermal shock.

This does not mean that the remaining welfare hazards are of no importance to fish welfare, but for those, the attribution of the lowest mark does not disqualify the considered method. However, it has an impact on its position in the final recommendation ranking.

Sometimes, bringing nuances to the marks that were attributed was deemed necessary, among other things, when marked differences are observed across species. Side notes were used to explain those nuances.

2. Definition of welfare hazards and their related grading

2.1 Exposure to noxious chemicals

Exposure to noxious chemicals can induce vigorous aversive behavioural reactions such as escape attempts or panicking which can be expressed by numerous jumps, a dramatic increase in swimming speed, oxygen seeking behaviour or violent head shaking movements. Such reactions are the result of high levels of stress (EFSA 2009abc). In fact, depending on their nature and dose, noxious chemicals can cause discomfort to fish when they are introduced in their environment. In the specific case of the saturation of water with CO_2, the resulting major drop in pH can be considered to be an exposure to a noxious chemical. Chemicals can also cause irritation to the gills and skin, with different effects depending on species tolerance.

Meanwhile, stress biomarkers can rise (increase in plasma cortisol, glucose, and lactate, decrease in blood and muscle pH, increased speed of the post-mortem drop in muscle pH, depletion of ATP energetic reserves etc…) as a result of exposure to a noxious chemical.

It is possible to observe changes in stress biomarkers without it being associated with marked behavioural reactions. However, in such case, it is not possible to exclude the possibility that fish may feel pain, discomfort or at least some level of stress. To avoid thinking of these two possible sets of reactions as a hierarchical escalation of the aversive response, we decided to attribute the same mark "B" to both situations (B-Phy & B-Phy-Bh).

However, only the mark "B-Phy-Bh" was considered to be disqualifying as the severe distress of fish is more certain in this case.

Possible marks for this hazard:

A : The considered method does not involve exposure to noxious chemicals, or this exposure does not induce physiological stress nor behavioural reactions
B-Phy: The considered method induces a rise in stress biomarkers in reaction to exposure to noxious aversive chemicals present in the environment of fish. This physiological stress is not necessarily accompanied by marked behavioural reactions.
B-Phy-Bh: The considered method triggers vigorous behavioural reactions like escape attempts or panicking (jumps, very rapid swimming, oxygen seeking, head shaking movements etc…) indicating high levels of stress. Those behavioural reactions are accompanied by a rise in stress biomarkers.

This welfare hazard has been considered to be disqualifying only when fish display vigorous behavioural reactions in addition to physiological stress (B-Phy-Bh). Therefore, any method obtaining the mark "B-Phy-Bh" for this hazard is rejected.

2.2 Immediateness of the loss of consciousness

So as to prevent suffering, stunning methods should result in an immediate and painless loss of consciousness. As part of the slaughter process, sometimes fish become immobilised after displaying behavioural aversive reactions, but it does not necessarily mean that they become unconscious. In some cases, fish become immobilised but remain conscious for several minutes, as shown by the

persistence of “visually evoked potentials” (VER) i.e electromagnetic patterns measured by electro-encephalography (EEG) indicating brain activity in response to visual stimulation (EFSA 2009ab).
Possible marks for this hazard:
A: The loss of consciousness is immediate i.e occurring in less than one second
B: The loss of consciousness is not immediate i.e it lasts more than one second, and occurs after significant prior stress and/or pain

This welfare hazard has been considered to be disqualifying, due to the potential suffering of fish. Therefore, any method obtaining the mark “B ” for this hazard is rejected. Nonetheless, the non-immediateness of a method is less problematic if the time period before the loss of consciousness is not associated with significant suffering.

2.3 Risk of recovery of consciousness (reversibility)

A stunning method can be reversible or irreversible. A method is said to be reversible if its application induces a temporary loss of consciousness of a given duration. If an effective killing method is performed after a reversible stun within the time period when animals are still unconscious, then, they will die without suffering. However, it must be guaranteed that the killing method used is able to effectively induce death within a duration that is inferior to the duration of unconsciousness resulting from the reversible stun. Reversible stunning methods always come with a risk (most often not quantified) that animals may recover consciousness before or during killing, which may result in suffering.

A method is said to be irreversible if performing it induces a permanent loss of consciousness without any possibility for animals to recover consciousness later on. Therefore, irreversible stunning methods have the benefit of avoiding risks of suffering related to the recovery of consciousness before or during bleeding after stunning. Irreversible stunning methods can also be called killing methods.

Many stunning methods can be reversible under certain conditions and irreversible under other conditions. In particular, gas exposure methods, hypothermic methods, percussive methods, chemical methods as well as electrical stunning methods can be reversible if they are applied for a short duration and/or with specific parameters or dosage and can be irreversible if applied for a longer duration and/or with other parameters. Depending on the way they are used, these methods can be more or less effective, resulting in an irreversible stun on some individuals and a reversible stun on others. Sometime, methods which are normally irreversible can become reversible if they are not correctly executed, in particular in cases where the duration of application is insufficient due to an overly high slaughter line pace or if the correct procedure is not known by operators.

Possible marks for this hazard:
A: The considered method results in an irreversible stun. The risk of recovery of consciousness is absent or very low
B: The considered method is reversible even if correctly performed by workers. There is a risk of recovery of consciousness before or during killing.

This hazard has not been considered to be disqualifying because reversible stunning can still be humane if it is quickly followed by an effective killing method before fish recover consciousness.

2.4 Failure rate

Stunning methods most often do not have a 100% efficacy. Therefore, the assessment of the welfare outcome of a stunning method must take into account the failure rate i.e the percentage of individuals that are not correctly stunned.

Not a lot of information is available regarding failure acceptability thresholds of the different methods. In her audit framework for captive-bolt stunning of cattle, Dr. Temple Grandin has set acceptability thresholds about failure rates (Recommended animal handling guidelines & audit guide 2013). The stunning performance is deemed “excellent” if the failure rate at first shooting is below or equal to 1%, “acceptable” if it is between 1% and 5%, and “unacceptable ” if it is above 5%. Dr. Temple Grandin considers that there is a “serious problem” for animal welfare if failure rates are superior to 10%.

Due to a lack of information about similar audit thresholds for farmed fish, the values from Dr. Temple Grandin’s cattle audit guide were taken as a reference for the grading of this welfare hazard, irrespective of methods.

Stunning failure rates are not always known for all methods. For this reason, this hazard has been considered only for the methods for which information about failure rates was available. The values reported by the EFSA regarding failure rates are in great part educated guesses based on expert opinion, and therefore **<u>not always data coming from empirical measures</u>** (EFSA 2009ab). Furthermore, the values provided by the EFSA date back to 2009, which means that it is possible that some progress may have been achieved to reduce failure rates in the meantime without it being formally reported in the scientific literature.

It must be said that for certain methods (gas exposure methods, electrical methods, hypothermic methods), assessments of consciousness through EEG revealed that it sometimes happens that fish are quickly paralysed yet remain temporarily or indefinitely conscious (EFSA 2009ab, Berg & al. 2021). In those situations, it is difficult for workers to detect that fish are still conscious. Electro-immobilisation is the word used to describe this phenomenon when occurring as a result of an electrical shock (EFSA 2009ab). This phenomenon can be a limitation to the accuracy of failure rate assessments.

Possible marks for this hazard:
A: Excellent, the stunning failure rate is inferior or equal to 1%
B: Acceptable, the stunning failure rate is between 1% and 5%
C: Unacceptable, the stunning failure rate is above 5%
?: no information is available about the stunning failure rate for the considered method

This welfare hazard has been considered to be disqualifying, due to the potential suffering of fish. Therefore, any method obtaining the mark “C” for this hazard is rejected.

2.5 Air exposure

Except for a few species that developed special abilities, fish gills are not made in a way that allows them to function in air. During air exposure, gas exchanges are compromised. The gills collapse because they are no longer supported by the lift of an aqueous medium and oxygen intake soon becomes insufficient. Panic movements by the fish may worsen this phenomenon. In fact, our target species suffocate and display aversive reactions expressed through vigorous movements and agitation when submitted to air exposure (Robb & Kestin 2002). Fish end up exhausted until they lose consciousness due to hypoxia. Death can be caused by a prolonged lack of oxygen in the brain if the air exposure lasts long enough. In some cases, some species are used to air exposure when they jump out of water to escape, to go through obstacles or waterfalls, or when they swim through overly shallow waters, which can all happen to salmonids during migration. However, such instances of air exposure are usually brief, and numerous species never leave water throughout their whole life cycle.

Minimising air exposure is part of the guidelines on water quality and handling for the welfare of farmed vertebrate fish (E.U platform on animal welfare 2020). Being kept out of water results in a significant stress for fish, deemed to be of **high intensit**y by the EFSA (EFSA 2009ab). Even air exposure of relatively short duration can have an impact. For instance, rainbow trout previously submitted to exhaustion show somewhat increased mortality rates if they are also submitted to a 30-seconds-long air exposure (Ferguson & Tufts 1992). This effect is even stronger in the case of a 60-seconds-long air exposure. Air exposure lasting only 10 seconds can even have a small impact on fecundity in Atlantic salmon (Cook & al. 2015). Some authors recommend keeping the duration of air exposure under 10 seconds when handling fish for all species (Cook & al. 2015). The EFSA also recommends keeping air exposure below 10 seconds for rainbow trout (EFSA 2009a) and considers that air exposure longer than 15 seconds is a significant welfare hazard (EFSA 2009a table 6). RSPCA farmed fish standards as well as Friends of the Sea audit guidance for fish welfare standards require to keep air exposure below 15 seconds.

Possible marks for this hazard:
A: No air exposure at all
B: Fish are submitted to air exposure for less than 15 seconds
C: Fish are submitted to air exposure for more than 15 seconds but less than 4 minutes
D: Fish are submitted to air exposure for at least 4 minutes or more

The mark “D” was added to distinguish between methods leading to fish being exposed to air during a few seconds to a few minutes, and those which result in an air exposure that can last ten minutes, or even tens of minutes for certain species.

This welfare hazard has been considered to be disqualifying, due to the potential suffering of fish. Therefore, any method obtaining the mark “C” or “D ” for this hazard is rejected.

2.6 High or extreme densities

When held at high densities, animals cannot avoid physical contact with each other, and the risk of water quality deterioration (toxicity, nitrogenous waste, pH shift, hypercapnia and hypoxia) is increased. Furthermore, studies about stocking densities during the rearing phase show that fish held at high stocking densities display high levels of fin erosion, which leads researchers to think that higher densities increase aggression levels between conspecifics (Ellis & al. 2002).

Compressions are the result of extreme densities where fish are crammed on top of each other. The weight pressure applied on the fish on the bottom can be significant (tens or hundreds of Kg), which can result in fractures, organ rupture, crushing of tissues etc. In addition, as fish get agitated, they can injure each other: contusions, ripped off scales, fin ray damage, skin damage (in particular with sea bass due to their dorsal spikes), eye damage etc.

Possible marks for this hazard:
A: This hazard is not involved in the considered method or only at a low level
B: Fish are submitted to stress due to an overly high density, available swimming space is severely limited
C: Fish are submitted to severe stress as well as pain due to compressions by other fish as a result of extreme density. Some fish may also face air exposure if they happen to be on top of a batch crammed with fish without enough water

This welfare hazard has been considered to be disqualifying, due to the potential suffering of fish. Therefore, any method obtaining the mark “C” for this hazard is rejected.

2.7 Handling

Stress is elicited every time fish are handled. Depending on the slaughter method, fish may be manually oriented on a table, held firmly in order to immobilise them or grasped by the gills or tail. In the wild, fish are very rarely in physical contact with conspecifics, and they possess efficient mechanoreceptors on their lateral line that allow them to feel water pressure waves caused by nearby movements. Those sensors trigger escape reflex when facing predators and can also be used to coordinate movements so that fish can swim close to each other in a school but without touching. Physical contacts mostly occur only as part of reproductive and grooming behaviour. Any handling event includes the risk of contusions, ripping off scales or compressions.

In rainbow trout, mechanonociceptors react to very light pressure thresholds (sometimes as small as 0,1 g), inferior to what is seen with mechanonociceptors located in mammalian skin (Sneddon 2003). This means that even light pressures may be perceived as painful by fish (Sneddon 2003). Therefore, any handling event may cause some level of pain, at least for rainbow trout. In addition, there is also a risk that fish may slip off the hands of workers and fall onto the ground which entails a painful shock.

Reducing occurrences of handling, and the duration of handling events is part of the European platform on animal welfare guidelines about farmed fish (E.U platform on animal welfare 2020). Even handling events of a very short duration can trigger a physiological stress response lasting up to several hours (Pickering & al. 1982, Barton 2000, Brydes & al. 2009). Stress caused by handling is considered to be of a **low intensity** by the EFSA when referring to carefully maintaining or orienting fish prior to stunning (EFSA 2009ab).

Possible marks for this hazard:
A: Fish are not handled by workers
B: At least one step of the considered method involves fish being handled while they are conscious for a given duration

This welfare hazard has not been considered to be disqualifying.

2.8 Poor water quality

The water environment of fish can deteriorate due to an elevation of the rates of carbon dioxide, ammonia, pH variations, or a decrease in dissolved oxygen for various reasons. The presence of suspended matters, pieces of scales or faeces, can also contribute to water quality deterioration. Issues linked to the massive drop in pH in relation to methods involving immersion in CO_2 saturated water are addressed within the hazard “exposure to noxious chemicals”.

Some fish are still able to efficiently maintain homeostasis under small variations of physicochemical water parameters, or short-term water quality deterioration. Over long periods of time, poor water quality can result in chronic stress which entails dysfunction regarding growth and reproduction, or even death.

In the context of slaughter practices, fish are usually not maintained long enough in holding facilities to allow a severe elevation of ammonia levels. For instance, nitrogenous wastes are limited in this context because fish are usually fasted for several days before transportation and slaughter, and therefore come at the slaughter plant with empty guts. However, some suspended matter may still be present.

Stress due to poor water quality in the context of slaughter has been considered to be of **low or moderate intensity** by the EFSA (EFSA 2009ab). The impact on animals depends on the duration of exposure to poor water quality, and to the degree of water quality deterioration.

Possible marks for this hazard:
A: The water used for the considered method has decent quality, or no water is used for this method
B: Water parameters are moderately deteriorated, suspended matter can be present, but the water is still relatively well oxygenated
C: Water parameters are severely deteriorated, high quantities of suspended matters may be present, and levels of dissolved oxygen may be insufficient.

This welfare hazard has not been considered to be disqualifying.

2.9 Tissue damage

Fish submitted to tissue damage endure pain and stress. Studies have shown that fish possess nociceptors in their skin that allow them to detect external stimuli damaging their tissues (see part I.1).

In rainbow trout, nociceptors have been particularly observed in the head region (Sneddon 2003). Rainbow trout react to noxious substances by modifying their behaviour (e.g : increased latency before eating, guarding behaviour, abnormal rocking motion, rubbing the sore area against tank walls). When fish endure skin, muscle, or bone injury, the EFSA considers that it results in severe pain if fish are conscious (EFSA 2009ab). This hazard, related to the pain it entails, is considered to be a **high intensity** stressor (EFSA 2009ab).

Possible marks for this hazard:
A: The considered method does not result in tissue damage or only at a very low level
B: Fish consciously endure tissue damage

This welfare hazard has been considered to be disqualifying, due to the potential suffering of fish. Therefore, any method obtaining the mark “B” for this hazard is rejected.

2.10 Pre-stun electrical shock

Electrical stunning works by exposing fish to an electrical shock which can induce immediate unconsciousness if it is strong enough and actually goes through the fish brain (EFSA 2009ab). However, if the electrical shock is not strong enough, or if it is not delivered at the right location in the body, fish may consciously endure the shock, which in such cases, is likely to be associated with suffering (EFSA 2009ab, personal communication with researchers Albin Gräns, Per Hjelmstedt & Jeff Lines). This type of event when fish consciously endure an electrical shock will be referred to as “pre-stun electrical shock” in this report.

Depending on cases, pre-stun electrical shocks can either last during the entire duration of the exposure to the electrical shock, or can be limited to only a few seconds and followed by a loss of consciousness.

There are several causes of pre-stun electrical shocks. They may occur if the electrical parameters used are inadequate, making the electrical shock too weak to induce immediate unconsciousness (EFSA 2009ab, personal communication with researchers Jeff Lines and Albin Gräns). When electrical stunning is carried out in-water, even if the electrical parameters are adequate, fish may still endure pre-stun electrical shocks if the electrodes are positioned in such way that the subsequent electrical field is heterogenous i.e the strength of the field varies in space, with areas where the field is strong enough and areas where it is too weak (EFSA 2009ab, personal communication with researchers Jeff Lines and Albin Gräns). In that case, fish located in areas where the electrical field is too weak to induce immediate unconsciousness are at risk of enduring pre-stun electrical shocks. If the electrodes are too far apart from one another, the electrical field may be too weak to induce immediate unconsciousness, putting the fish at risk to endure pre-stun electrical shocks (personal communication with researchers Albin Gräns and Per Hjelmstedt). In-water electrical stunning can also put fish at risk of enduring pre-stun electrical shocks if the parameters being used are not suited to the water conductivity (Lines & Kestin 2004b, Jung-Schroers & al. 2020, EFSA 2009ab, personal communication with researchers Jeff Lines, Albin Gräns and Per Hjelmstedt).

For dry electrical stunning, if the point of contact of the electrodes with the fish is far from the fish head (e.g if the point of contact is located on the tail), it is likely that most of the current will not go through the fish brain, putting the fish at risk of enduring an electrical shock without losing consciousness (EFSA 2009ab, Mejdell & Gismervik 2009b, personal communication with researcher Cecilie Mejdell). This phenomenon is all the more likely in cases where only a few fish are directly in contact with the electrodes and where the current indirectly reaches other fish which are not in contact with the electrodes by first travelling across the bodies of the fish directly in contact with the electrodes. In this case, not only is the point of entry of the electrical current not necessarily close to the brain, but the strength of the electrical shock will also be reduced and may be insufficient because the current will be losing some of its intensity due to the resistance of the bodies of the first fish it will travel through to finally reach the remaining fish. On another note, fish may also be at risk of enduring pre-stun electrical shocks if their muscle spasms result in the point of contact with the electrodes moving away from the fish head (EFSA 2009ab).

For some electrical stunning methods, risks of pre-stun electrical shocks are non-existent or almost non-existent as long as the parameters being used are adequate. For other methods, those risks exist but are limited to a small proportion of individuals and can be prevented or heavily reduced with good system design. Finally, some electrical stunning methods involve a systematic risk of pre-stun electrical shocks: this risk can be seen as being intrinsic to how those methods work.

Possible marks for this hazard:
A: the risk of pre-stun electrical shocks is completely absent (especially if the considered method does not involve electricity) or almost completely absent for the considered method if the electrical parameters being used are adequate

B: The considered method involves a significant risk that fish may consciously endure pre-stun electrical shocks potentially associated with suffering

This welfare hazard has been considered to be disqualifying, due to the potential suffering of fish. Therefore, any method obtaining the mark "B" for this hazard is rejected.

2.11 Thermal shock

Fish are both poikilotherms and ectothermic: they do not have a body temperature regulation system and consequently, their metabolism is regulated depending on the available thermal energy in their environment as it directly impacts the speed of enzymatic reactions. Some species like tuna developed partial thermal regulation: they can increase their body temperature of a few degrees for short term intense physical activities like hunting or fleeing. Depending on the regions where they are found, fish species can tolerate various temperature ranges. Outside of these ranges, fish endure thermal stress which can lead to death due to hypothermia or overly high heat and related organ failures.

Fish are very sensitive to variations in water temperature. Rapid exposure to a temperature gap of 10°C and beyond is considered an intense thermal shock that can impair metabolism and cause gills to collapse which limits gas exchanges and oxygen intake. Cold thermal shock could potentially be perceived as a "cold burn" sensation by certain species on addition to causing physiological stress. For rainbow trout however, the thermo-nociceptors that have been investigated showed that they reacted to heat but not cold (Sneddon 2003). Distress in response to thermal shock may be expressed through aversive behavioural reactions like agitation, rapid swimming, or escape attempts.

Cold water species like rainbow trout and Atlantic salmon can tolerate low temperatures. For instance, the tolerance range after hatching is comprised between 1°C and 20°C for rainbow trout, and between 5°C and 18°C for Atlantic salmon, with some differences depending on life stage (Noble & al. 2018, Noble & al. 2020). RSPCA standards for farmed trout recommend a water temperature comprised between 1°C and 12 °C for fry and fingerlings, and between 1°C and 16°C during on-growing.

The lethal temperature for trout and salmon is below – 0.75 ° C (EFSA 2009a). Therefore, submitting salmonids to low temperatures in order to render them unconscious may not be very effective or may require a long duration. In contrast, warm-water species like sea bass and sea bream are used to live in environments with a water temperature above 12 °C (EFSA 2009c). Thus, submitting them to low temperatures may have a stronger impact regarding the loss of consciousness.

Thermal stress due to cold is likely to be stronger for warm water species like sea bass and sea bream than for cold-water rainbow trout and Atlantic salmon.

Possible marks for this hazard:
A: The considered method does not involve thermal stress
B: The considered method causes a significant thermal shock causing stress and potentially pain

This welfare hazard has been considered to be disqualifying, due to the potential suffering of fish. Therefore, any method obtaining the mark “B” for this hazard is rejected.

2.12 Reliance on workers’ skills

The efficacy of certain methods can depend upon the manual precision and the level of experience of workers. The stunning failure rate can be significantly impacted by the lack of experience of workers, or a decrease of the quality of their work due to fatigue, inadequate slaughter line pace or bad equipment maintenance.

The degree of technicality involved has an impact on the repeatability of the stunning performances depending on workers and their working conditions. A decrease in stunning performance means that fish may require a second stun after having endured a potentially painful mis-stun. It could also result in some fish being immobilised without losing consciousness before being killed.

This hazard can also be relevant to some killing methods (with or without prior stunning) – particularly haemorrhagic methods – because the duration before the loss of consciousness and/or death may be prolonged if the gesture is not executed correctly by workers.

Furthermore, the management and maintenance of complex machines can require certain skills from workers. The same can be said regarding the adequate calibration of stunning parameters (electrical parameters, mechanical pressure parameters for percussive stunning, dosing for chemical stunning) for certain methods.

When observations have been made that workers often do not respect adequate procedures to correctly execute a method (in particular regarding the duration of immersion before retrieval for gas exposure methods), we considered that the method has some level of reliance on workers’ skills.

Possible marks for this hazard:
A: The efficacy of the considered method does not heavily rely on workers’ skills and experience
B: The efficacy of the considered method relies moderately on workers’ skills and experience, in particular regarding technical manual gestures and the management and maintenance of machines
C: The efficacy of the considered method relies highly on workers’ skill and experience, in particular regarding technical manual gestures, the management and maintenance of machines, and adequate calibration of parameters or precise dosing

This hazard has not been considered to be disqualifying.

3. Summary of welfare hazards

In red: marks for which a welfare hazard becomes disqualifying

Disqualifying	Welfare hazards	A	B	C	D
YES	Exposure to noxious chemicals	Absent	Phy : Physiological stress response Phy-Bh : Physiological stress response and behavioural reactions		
YES	Immediateness of the loss of consciousness	<1sec	> 1 sec		
YES	Stunning failure rate	Excellent ≤ 1%	Acceptable >1% ≤ 5%	Unacceptable > 5%	
NO	Risk of recovery of consciousness (reversibility)	Irreversible method	reversible method		
YES	Air exposure	Irrelevant / Absent	≤ 15 sec	> 15sec - ≤ 4 min	> 4 min
YES	High or extreme densities	Irrelevant / Absent	High density	Extreme density & compressions	
NO	Handling	Irrelevant / Absent	Present		
NO	Poor water quality	Irrelevant / Good water quality	Poor water quality	Very poor water quality	
YES	Tissue damage	Irrelevant / Absent	Present		
YES	Pre-stun electrical shock	Irrelevant / Absent or low	Present		
YES	Thermal shock	Irrelevant / Absent	Present		
NO	Reliance on workers' skills	Absent or low	Moderate	High	

III. Current slaughter methods

Currently available slaughter methods are diverse. For each group of methods, the mode of operation as well as current uses of the methods are presented, followed by an analysis of the relevant welfare hazards in order to determine Welfarm's position and recommendations. Killing methods without prior stunning are presented first, followed by stunning methods. For each group of methods, a table summarises the marks obtained by each method through the welfare hazard analysis. A final table synthetises the results of the welfare hazard analysis for all considered methods. Lastly, economic aspects of the different methods in terms of price, stunning line pace and transition costs are discussed.

1. Killing methods without prior stunning

1.1. Haemorrhagic methods

1.1.1. Method description and current use

- **Description**

Haemorrhagic methods are divided into 3 sub-groups:

-**Evisceration** which consists in opening the abdomen and completely removing the viscera.

- **Exsanguination**: which can be done by a **gill cut** (cutting the gill arteries), by slitting the throat up to the heart or, to a lesser extent, by cutting the caudal artery.

-**Decapitation**: by cutting the head off at the junction of the skull and the first vertebra

Animals are usually bled following these killing techniques. For this purpose, they are usually immersed in ice slurry tanks for several minutes to several hours, so that the blood can diffuse out into the water. This improves the visual quality of the meat (Roth & al. 2007) due to a drop in temperature and the evacuation of the blood. Death is the result of the lack of blood renewal leading to the cessation of oxygen supply to the brain (hypoxia and ischaemia).

- **Current use**

Haemorrhagic methods are typically used commercially following a stunning method in order to induce death. Gill cutting can improve the flesh quality (EFSA 2009a). It is therefore commonly practised in salmon and large trout. It can be carried out manually or by using an automated process. The automatic device can be easily integrated into a slaughter line, allowing for a high slaughter line pace.

Portion size trout are usually eviscerated following a stunning method (EFSA 2009a). However, bleeding or evisceration immediately after killing/stunning is not systematically part of the production process for rainbow trout, especially for small trout (personal communication with an organic rainbow trout farmer).

Decapitation is likely to be less used or even not used at all on our target species given that presenting fish with their head can be interpreted as a sign of freshness from the consumer point of view. In addition, visual examination of fish eyes can be used as part of quality assessment indexes (FAO 1999). Decapitation is mainly used to kill eels that are very difficult to kill otherwise.

Unlike practices in wild capture fisheries, haemorrhagic methods are rarely used without prior stunning in European commercial slaughter systems for farmed fish (European Commission 2017, 2018, Eurogroup for Animals 2021). However, a literature review of slaughter practices for tropical and subtropical species stated that evisceration without prior stunning is still practised on farmed fish in some sites around the world (Bowman and Gräns 2019). In France in 2018, in an official survey conducted by the French ministry of agriculture, 32 companies producing salmonids (out of 365 companies surveyed) declared bleeding their fish (Agreste 2020). Regarding mariculture, 6 companies (out of 28 companies surveyed) declared that they bled their fish (Agreste 2020). For companies running extensive pond fish farms, 2 companies (out of 211 companies surveyed) declared bleeding their fish (Agreste 2020). This survey does not specify whether bleeding is carried out with or without prior stunning.

An undercover investigation from the NGO Animal Equality (UK, 2021) revealed that sometimes operators do carry out gill cut on fully conscious fish because of stunning failure rates and overly high slaughter line pace requirements (INVESTIGATION: Fish killed while fully conscious in Scottish Salmon slaughterhouse, Animal Equality UK, 2021; https://www.youtube.com/watch?v=u2uyP74I1EU).

1.1.2. Welfare hazards, Welfarm's position and recommendations:

In addition to the **severe tissue damage** (which is a disqualifying hazard) inherent to these techniques as reported by the EFSA, there are other aspects of haemorrhagic methods that negatively impact fish welfare.

- **Immediateness of the loss of consciousness**

Robb & al. 2000 showed that almost 7 minutes were necessary to cause the loss of consciousness in Atlantic salmon following a gill cut. Thus, gill cutting does not induce an instantaneous loss of consciousness.

According to the same authors, it seems that the time required for the loss of consciousness following exsanguination by gill cutting is greater when the external environment temperature is low, probably due to the lower oxygen requirement of a slowed down metabolism (Robb & al. 2000).

According to Van de Vis (unpublished results 1998 cited by Robb and Kestin 2002), it can take up to 20 minutes for pelagic fish to lose consciousness following evisceration.

Decapitation appears to be the quickest alternative to induce the loss of consciousness. However, according to Stoskopf and Posner (2008), the loss of consciousness following beheading may not be instantaneous due to relative tolerance to hypoxia in fish.

Haemorrhagic methods obtain the mark "B" for this hazard. According to our scoring system, this hazard is disqualifying. Therefore, haemorrhagic methods are unacceptable in this aspect.

- **Risk of recovery of consciousness (reversibility)**

In theory, there should not be a return of consciousness following haemorrhagic methods if they are performed correctly. The loss of consciousness is irreversible.

Haemorrhagic methods obtain the mark "A" for this hazard.

- **Failure rate**

Haemorrhagic methods cannot be described as stunning methods, they are only considered to be killing methods.

The main risks of killing failure are the following:

-If the number of gill arteries being cut is insufficient, bleeding is less effective. A minimum of 3 to 4 gills should be cut for Atlantic salmon. In comparison, 5 gills should be removed for turbot (EFSA 2009bf).

- During gill cutting, the knife may not be placed in the right position or may be poorly serviced.

- The caudal artery may not be cut properly if the incision is not deep enough.

- The throat slit may not be deep enough to reach the heart. This technique requires precision, as the heart needs to be reached without cutting the fish's central bone.

- The evisceration may be incomplete, sparing the inner blood vessels.

- The head may be incompletely sliced during decapitation, partially sparing the arteries. The head may not be separated from the rest of the body in this case. However, incomplete detachment of the head from the rest of the body is easy to detect.

These risks have not been quantified. Therefore, haemorrhagic methods obtain the mark “?” for this hazard.

- **Tissue damage**

Incision of tissues without prior stunning gives rise to pain. It also triggers vigorous escape reactions in fish. Robb & al. 2000 reported that fish display this kind of aversive behavioural reactions during 30 seconds following a gill cut without prior stunning.

Haemorrhagic methods obtain the mark “B” for this hazard. According to our scoring system, this hazard is disqualifying. Therefore, haemorrhagic methods are unacceptable in this aspect.

- **Air exposure**

Haemorrhagic methods require to remove fish out of the water. If the fish are not stunned beforehand, they will be exposed to air while conscious and endure asphyxia. For the manual percussion stunning method, the EFSA 2009ab estimated that fish were exposed to air for 30 seconds. Although this is an entirely different process, a similar panel of gestures can also be observed during haemorrhagic methods (animals are grabbed, held firmly and then hit or cut). We assume that the approximate air exposure duration for haemorrhagic methods is likely to be about the same as what is required for manual percussion i.e. 30 seconds. Following incision, fish are usually returned to water or ice slurry to allow blood spreads.

Haemorrhagic methods obtain the mark “C” for this hazard. According to our scoring system, this hazard is disqualifying when it reaches the level “C” or “D”. Therefore, haemorrhagic methods are unacceptable in this aspect.

- **Handling**

Haemorrhagic methods require animal handling. If fish are not stunned beforehand, they will panic and struggle throughout handling.

During gill cutting, operators keep animals immobilised and then remove the gills using a knife. We assume that the approximate handling time for haemorrhagic methods is likely to be approximately the same as for manual percussion, i.e. 30 seconds (EFSA 2009ab).

Haemorrhagic methods obtain the mark “B” for this hazard.

- **Reliance on workers' skills (low, moderate, high)**

Haemorrhagic methods require the intervention of an operator in numerous steps throughout killing: when catching the fish, during the immobilisation period and at the moment of cutting. Workers must perform very precise gestures. Therefore, the accuracy of gestures depends on workers' experience. The incision must be made in the right place, taking into account the fish morphology and size. The incision should also be deep enough. Indeed, a poorly performed incision can lead to unnecessary and avoidable additional suffering.

Repetitive movements in a short period of time implies biomechanical stresses and fatigue. Uncontrolled biomechanical stresses can constitute a factor of drudgery, according to a report from ANACT (the National Agency for the Improvement of Working Conditions, 2015). It can be assumed that tired workers are less precise in their gestures and may potentially perform more incorrect technical gestures.

If fish are previously stunned, there is no need for workers to immobilise fish. Following fish stunning, manual gill cutting seems to be more efficient compared to cases where an automatic gill cutting device is included in the stunning machine (EFSA 2009ab). This difference could be due to cases of incorrect positioning or orientation of the fish into the machine resulting in inaccurate incisions. Fish batches that are heterogenous in terms of size and weight may also be unsuited to the rigid calibration of gill cutting machines.

Haemorrhagic methods obtain the mark "C" for this hazard.

WELFARM's position and recommendations:

Haemorrhagic methods applied without prior stunning do not meet our animal welfare requirements. Indeed, fish are injured while fully conscious. The occurrence of death is not immediate and these methods come with a high risk of failure as they involve handling conscious and mobile animals while requiring precises gestures. This is even more true if workers are insufficiently trained or experienced. Insufficient precision of gestures increases the risks that the incision may not result in quick and profuse bleeding, increasing the time before the occurrence of death. Finally, the extraction of fish out of water adds additional stress. In sum, stress and pain are omnipresent throughout those killing methods. Haemorrhagic methods without prior stunning are ethically unacceptable.

WELFARM strongly rejects the use of these techniques when they are not preceded by a correct and effective stunning method.

Ideally, manual gill cutting is to be preferred over automated gill cutting following fish stunning. This improves the accuracy of the cut, and it allows workers to double-check the state of consciousness and possibly to carry out emergency stunning if necessary. Operators should ensure that enough gills are cut (at least 4 gills) to limit the risk of regaining consciousness. Gills from both sides should be cut. The more gills that are cut, the more blood will flow out and the faster death will occur (Robb & al. 2000). The blade must be sharp, well honed and well serviced to ensure correct incision. Workers must be properly trained to carry out technical procedures.

The same workers should be assigned to complex tasks (like fish bleeding) in order to guarantee a good efficiency. At the same time, the repetitiveness of gestures should be minimized to avoid damaging workers' health and to maintain a high level of efficacy.

Batches of fish should be uniform in size and morphology to avoid cutting defects. Workers should check that fish are unconscious before inserting them into the gill cutting machine, which implies that the gill-cutting machines should be separate from the rest of the stunning equipment. General maintenance should be carried out regularly, i.e. the blades should be regularly checked, sharpened and changed if necessary according to an internal protocol. Operating instructions should be available and easily accessible to all workers and if possible, in several languages.

Among the haemorrhagic methods described, decapitation should be preferred as it results in a faster death. Evisceration, alone or in combination with gill cutting, is probably also faster than gill cutting alone. Given the risks mentioned by Stoskopf and Posner (2008) unconsciousness may not be completely instantaneous during decapitation. Therefore, ideally the remaining brain parts should be destroyed. Consumers could change their habits and rely on other quality indicators proposed by the aquaculture sector (labels, date of slaughter etc.). The development of more efficient tools could make it possible to integrate beheading into current slaughter lines, while ensuring good flesh quality.

Methods	Exposure to noxious chemicals	Immediateness of the loss of consciousness	Failure rate	Risk of recovery of consciousness (reversibility)	Air exposure	High or extreme densities	Handling	Poor water quality	Tissue damage	Pre-stun electrical shock	Thermal shock	Reliance on workers's skills
Decapitation	A	**B**	**?**	A	**C**	A	B	A	**B**	A	A	C
Evisceration	A	**B**	**?**	A	**C**	A	B	A	**B**	A	A	C
Gills cut	A	**B**	**?**	A	**C**	A	B	A	**B**	A	A	C

Red = disqualifying

1.2 Asphyxia

1.2.1 Method description and current use

- **Description**

Asphyxia in air consists in removing fish from water and simply letting them die in ambient air. Usually, fish are placed in draining tanks that retain them while the water is drained out of the tank. This is a killing method.

IMAGE FROM PIXABAY

- **Current use**

Asphyxia is the most common practice worldwide used to kill farmed fish according to Robb and Kestin 2002. It is reported to be used on species with lower market value such as trout (Robb and Kestin 2002), sea bass, sea bream or carp (EFSA 2009cd). This method seems to be rarely used, or perhaps not used at all for Atlantic salmon.

1.2.2 Welfare hazards, Welfarm's position and recommendations:

In addition to the **air exposure** risk factor (which is a disqualifying welfare hazard), which is inherent to this killing technique, there are other aspects that negatively impact fish welfare.

- **Immediateness of the loss of consciousness**

Asphyxia is one of the most stressful methods and according to the EFSA, among the longest to induce effective death in fish. Animals show intense aversive reactions for several minutes following exposure to ambient air according to the EFSA. Poli & al. 2004 observed that fish killed by air exposure had higher lactate levels and a lower pH compared to fish stun-killed by spiking or percussive stunning methods.

Some species are particularly sensitive to asphyxiation, as they have a higher metabolism and therefore a higher oxygen consumption. Other species can tolerate low oxygen levels because of their lifestyle and develop a high resistance to hypoxia. This is the case for some fish species living inside pond for instance where oxygen rate depends on seasonality, this is also the case for some marine species such as the Senegal Sole for example (Ribas & al. 2007). Among our 4 target species, sea bass has the most developed asphyxia resistance. Sea bass can resist several hours before losing consciousness. Gimenez & al. 2020 and Acerete & al. 2009 reported a tolerance to asphyxia lasting about 2 hours before unconsciousness. Poli & al. 2004 reported a period of 70 +/- 27.6 minutes before the occurrence of death (cited in EFSA 2009c).

According to Poli & al. 2005, trout can resist asphyxia for nearly 15 minutes before losing consciousness. Other results indicate 2,6 to 9,6 minutes depending on temperature (Kestin & al. 1991). In the study by Poli & al. 2005, sea breams showed a significantly higher resistance, needing approximately 25 minutes before losing consciousness.

Asphyxia obtains the mark "B" for this hazard. According to our scoring system, this hazard is disqualifying. Therefore, asphyxia in air is unacceptable in this aspect.

- **Risks of recovery of consciousness (reversibility)**

The effectiveness of asphyxia is complete and irreversible if fish are exposed to air for a sufficiently long time. Asphyxia obtains the mark "A" for this hazard.

There is no suitable reversible stunning technique able to induce a long enough stun to cover for the particularly long delay before the loss of consciousness normally associated with asphyxiation. This is all the more true as it is likely that fish stunned before asphyxia have lower oxygen needs, meaning the duration before the occurrence of death may be even longer. Therefore, if asphyxia is used as a killing method after a reversible stun, recovery of consciousness is likely to occur.

- **Failure rate**

Asphyxiation is a killing method. If the duration of air exposure is long enough, this method has no reason to fail.

However, as the failure rate has not been quantified, asphyxia obtains the mark "?" for this method.

- **High or extreme densities**

Fish are usually dumped and stacked on top of each other within draining tanks. They are kept in extreme densities which likely induces pain and stress. In draining tanks, fish suffer from an intense compression.

Asphyxia obtains the mark "C" for this hazard. According to our scoring system, this hazard is disqualifying when it reaches the level "C". Therefore, asphyxia in air is unacceptable in this aspect.

WELFARM's position and recommendations:

Killing by asphyxia does not meet our requirements in terms of animal welfare. Indeed, this method requires a significant period of time before the loss of consciousness. This duration depends on the specie tolerance to hypoxia. Suffering is omnipresent throughout this killing process. Animals are subjected to constant stress until death occurs.

Therefore, this method is considered ethically unacceptable and is totally rejected by WELFARM.

We have not identified any possible improvements for this killing technique.

Method	Exposure to noxious chemicals	Immediateness of the loss of consciousness	Failure rate	Risk of recovery of consciousness (reversibility)	Air exposure	High or extreme densities	Handling	Poor water quality	Tissue damage	Pre-stun Electrical shock	Thermal shock	Reliance on workers's skills
Asphyxia	A	**B**	?	A	**D**	**C**	A	A	A	A	A	A

Red = disqualifying

2. Stunning methods

2.1. Hypothermia

2.1.1. Method description and current use

- **Description**

There are a variety of killing methods involving hypothermia[2], some of them also involve the occurrence of asphyxia.

IMAGE FROM PIXABAY

- The method of solid ice asphyxia is similar to the usual asphyxia method. However, while fish are exposed to ambient air they are placed on a frozen surface. This method is irreversible.

- The ice slurry method: Fish are immersed in a mixture of water and ice flakes where oxygen is depleted due to the non-renewal of the water and the high density of fish. Sometimes, this method is combined with exposure to CO_2. This method is irreversible but could be reversible if animals are withdrawn from the ice slurry too early. The mixture is commonly drained and only the ice remains in the container. Warm-water fish such as sea bass or sea bream die from hypothermia as the temperature differential between the ice slurry and their living environment is really high. The thermal shock is more violent for them and induces a loss of brain functions. Cold water fish such as salmonids suffer less from hypothermia but rather die from the lack of oxygen.

- Hypothermia in icy water: This technique will not be considered as it has only been used for experimental purposes and little information is available on it.

- **Current use**

The ice slurry technique is the most common killing method used in commercial systems to kill sea bass and sea bream (EFSA 2009c). These methods can also be applied to salmonids, although it appears to be uncommon. In particular, immersion in ice slurry is used commercially on rainbow trout in Spain (Bermejo-Poza & al. 2021). In France, In the official 2018 survey by the French ministry of agriculture, 3 companies producing salmonids (out of 365 surveyed companies), 15 marine fish farming companies (out of 28 surveyed companies), and 3 extensive pond fish farming companies (out of 211 surveyed companies) declared their slaughter method to be a “thermal shock” (Agreste 2020).

Solid ice asphyxiation is mostly used on sea bass, sea bream and rainbow trout.

[2] Concerning zebrafish raised for scientific purposes, several authors recommend to use flash freezing or rapid cooling for euthanasia (Kölher & al. 2017, Valentim & al. 2016, Strykowski & al. 2015). Those techniques consist in a brief exposure to extremely low temperatures. The loss of consciousness is supposed to be instantaneous in this species. However, it is not known whether the loss of consciousness would be instantaneous if applied to larger and/or cold-water fish like some of our target species, which are likely to be more resistant. Flash freezing is also practised on fishing vessels in wild capture fisheries, but the consequences on animal welfare are not yet studied. This method is not used in aquaculture.

2.1.2. Welfare hazards, Welfarm's position and recommendations:

In addition to **air exposure** (which is a disqualifying hazard), which is inherent to asphyxia on solid ice and which sometimes happens with the of ice slurry method too, there are other aspects that negatively impact fish welfare.

- **Thermal shock**

Thermal shock is intrinsic to hypothermic methods and can cause aversive behavioural reactions as well as physiological stress. The temperature difference between the original water and the ice slurry is usually around 10°C causing intense thermal stress in fish. The ice slurry water is usually between 0° and 2°, so for sea bass and sea bream who normally do not live in water below 12°C, the shock is significant. Even in cold water species, it has been shown that thermal shock generates stress. In trout, a shock of 8-9°c to 1-3°c is sufficient to trigger stress, which is reflected in plasma cortisol elevation (Ribas & al. 2007, Barton & Peter 1982).

Sea bass and sea bream:

According to Zampacavallo & al. 2008 (mentioned by EFSA 2009c), when fish were submerged into cold water at 0-2°C, the thermal shock triggered violent aversive reactions. When sea bass and sea bream were immersed in ice slurry, they started to swim frantically during 3 to 4 minutes before their movements slowed down. Thereafter, they lied on their sides or on their backs. Sea bass and sea bream struggle for a longer time when asphyxiated in ambient air (by +65% and +25% respectively) compared to those killed by asphyxia in ice slurry (Bagni & al. 2002 cited in EFSA 2009c).

Rainbow trout and Atlantic salmon:

Salmonids experience stress when introduced in ice slurry and become intensely agitated for several minutes. In salmon, immersion in ice slurry causes an increase in plasma cortisol, glucose and osmolarity (Skjervold & al. 2001). After some time, fish progressively become immobilised due to the intense cold, which does not necessarily mean they no longer suffer. Paralysis makes it harder to evaluate the effectiveness of the loss of consciousness. In this situation, fish can potentially be bled or eviscerated while they are still conscious.

In cold water species, lethal temperatures are not necessarily reached during hypothermic techniques (0-2°C in ice slurry). For instance, the lethal temperature for trout is below -0.75° (Fletcher & al. 1988; EFSA 2009a). Salmonids do not necessarily die because of hypothermia in ice slurry. Rather, ice slurry conditions lead to poor water quality which causes death by asphyxiation: oxygen in the water drops and a deterioration of the respiratory functions occurs due to opercula paralysis.

Hypothermic methods obtain the mark "B" for this hazard. According to our scoring system, this hazard is disqualifying. Therefore, hypothermic methods are unacceptable in this aspect.

- **Immediateness of the loss of consciousness**

Loss of consciousness is not instantaneous for any of the hypothermic techniques. According to the EFSA hypothermic methods are among the longest and most stressful slaughter methods (EFSA 2009abc). Concerning solid ice asphyxiation, the description of this factor is the same as for ambient air asphyxiation, however, the time to induce unconsciousness is longer during asphyxiation on ice

(Kestin & al. 1991, Robb & Kestin 2002, Lines & al. 2003). Oxygen needs are lower when the metabolism is slowed down, which increases tolerance to hypoxia.

Sea bass and sea bream:

As mentioned above, sea bass and sea bream introduced in ice slurry first display a few minutes of very high agitation before becoming paralysed by the cold. Compared to cold water species, death is likely to be caused by hypothermia per se rather than hypoxia for warm water species (De la Rosa et al. 2021). A study assessing the time elapsed before the loss of consciousness through EEG analysis reported a 5 minutes duration for sea bream immersed in ice slurry (Van de Vis & al. 2003). One study based on behavioural indicators reported a 3.8 min duration for sea bream (Vardanis et al. 2017). However, most other studies based on a panel of behavioural indicators of consciousness reported much longer durations for both sea bass and sea bream. Zampacavallo & al. 2015 mentioned : 23-30 minutes for sea bass, Bagni & al. 2007 reported 20 – 45 minutes for sea bass and sea bream, Huidobro & al. 2001 described 40 minutes for sea bream, Simitzis & al. 2014 reported 10 – 20 minutes for sea bass, Acerete et al. 2009 mentioned 34 minutes for sea bass, Roque & al. 2021 described 52 minutes for sea bream, and Poli & al. 2004 and Zampacavallo & al. 2008 (quoted by EFSA 2009c), reported 23,5 ±5 minutes for sea bass. Low external temperatures increase the time period needed before the loss of consciousness (Zampacavallo & al. 2008). This means that the time needed to lose consciousness appears to be higher during winter than during summer (EFSA 2009c). Overall, most results indicate that the loss of consciousness is not immediate and can take anywhere from a few minutes to several tens of minutes.

Atlantic salmon and rainbow trout:

In rainbow trout, if the body temperature is 2°C and if they are taken out of water and left on solid ice to asphyxiate, the time needed to lose consciousness (loss of VEPs) is on average 9.6 minutes, and can last up to 12.6 minutes (Kestin & al. 1991). Other authors mention up to 14-15 minutes in similar conditions (Lines & al. 2003, Poli & al. 2005). But if both their body temperature and the external temperature are higher, the loss of consciousness takes less time: 3 minutes at 14°C, and 2.6 minutes at 20°C (Kestin & al. 1991).

Olsen & al. 2006 showed that when salmon accustomed to living in 8°C water were placed in an ice slurry at 1°C for 45-60 minutes, fish remained vigorous and difficult to handle when trying to carry out a gill cut. The fish remained fully aware. Under such conditions, the ice slurry method for Atlantic salmon is often associated with another technique such as solid ice asphyxia (the water in the tanks is removed and the animals remain between layers of ice), bleeding, or exposure to carbon dioxide.

Hypothermic methods obtain the mark “B” for this hazard. According to our scoring system, this hazard is disqualifying. Therefore, hypothermic methods are unacceptable in this aspect.

- **Risks of recovery of consciousness (reversibility)**

Directly after the loss of the VER signal, if salmonids are removed from the ice slurry and then transferred into ambient temperature water, they quickly regain brain functions and muscle movement (Robb and Kestin 2002, EFSA 2009ab). Thus, if salmonids are not left in ice slurry for long enough, the risk of recovery of consciousness is important. Warm water species are less likely to regain consciousness as they are more sensitive to hypothermic shocks (EFSA 2009c, Stunfishfirst).
However, if the exposure time is sufficient, this method is irreversible.

In theory, this method can potentially be reversible for all species is the duration of application is very short. Nonetheless, in practice, risks appear to be low because producers do not really have incentives to retrieve fish from the ice slurry very early on and to immediately put them in water with suitable parameters in which they could recover consciousness. Immersion in ice slurry obtains the mark “A” for sea bass and sea bream for which the risks of recovery of consciousness are lower, and it obtains the mark “A/B” for salmonids. Asphyxia on solid ice obtains the mark “A” for this hazard.

- **Failure rate**

If the exposure time is sufficient, these methods likely do not have a high failure rate. If hypothermia fails to induce unconsciousness, it will be induced by asphyxia.

However, as the potential failure rates of these methods have not been quantified, hypothermic methods obtain the mark “?” for this hazard.

- **High or extreme densities**

Fish are crammed together into tanks to the point that some fish on top are not even fully submerged (see the following videos : Allevamenti intensive de pesci: prima indagina in Europa ; https://youtu.be/wImDWAA_ALc?t=106; Fish farming cruel secret https://youtu.be/f8W2C9SD-wk?t=91). Many fish are not properly in contact with the ice which slows down the process (this concerns 40% of the batch and prolongs the loss of consciousness by 10 minutes according to EFSA 2009c). Fish on the bottom are under significant pressure due to the weight of those on top of them.

Hypothermic methods obtain the mark “C” for this hazard. According to our scoring system, this hazard is disqualifying when it reaches the level “C”. Therefore, hypothermic methods are unacceptable in this aspect.

- **Poor water quality**

The water quality used in ice slurry is rather poor. The high density of fish and the non-renewal of water makes breathing complicated. Panic increases the consumption of oxygen. Lost scales and spines also contribute to making the water unsuitable for fish. The water quality is very degraded.

Asphyxia on solid ice obtains the mark “A” for this hazard. Immersion in ice slurry obtains the mark “C” for this hazard.

- **Tissue damage**

Contact with ice could potentially cause burning sensations (frostbites). However, thermal nociceptors are non-reactive to cold in trout (Sneddon 2003). Cold water species may therefore not be affected by cold burning.

Further studies would be needed to determine whether this contact causes pain and if yes to what extent and in which species.

By precaution, we assume that ice has a negative effect on fish tissue unless new scientific evidence shows otherwise. Furthermore, contact with the sharp ice crystals associated with the compression of individuals on top of each other can potentially lead to injuries.

Hypothermic methods obtain the mark “B” for this hazard. According to our scoring system, this hazard is disqualifying. Therefore, hypothermic methods are unacceptable in this aspect.

WELFARM's position and recommendations:

Killing by asphyxia on solid ice or by immersion in ice slurry does not meet our requirements for animal welfare. Indeed, these methods of killing by hypothermia/asphyxia on ice require a considerable amount of time before the loss of consciousness, which varies depending on the species and the initial water temperature. Suffering is omnipresent due to the extreme densities and thermal shock. Fish are therefore subjected to constant stress until death occurs. Killing is not immediate. Thus, this method is considered ethically unacceptable and is totally rejected by WELFARM.

We have not identified any possible improvements to those killing techniques. It should be noted that asphyxia at ambient air should be preferred over asphyxia on solid ice because death occurs faster.

Methods	Exposure to noxious chemicals	Immediateness of the loss of consciousness	Failure rate	Risk of recovery of consciousness (reversibility)		Air exposure	High or extreme densities	Handling	Poor water quality	Tissue damage	Pre-stun Electrical shock	Thermal shock	Reliance on workers'skills
Ice slurry	A	**B**	?	Sea bass, sea bream A[1]	Trout, salmon A/B[1]	A[2]	**C**	A	C	**B**	A	**B**	A
Solid ice asphyxia	A	**B**	?	A		**D**	**C**	A	A	**B**	A	A	A

Red = disqualifying

1: For sea bass and sea bream the risk of regaining consciousness following immersion in ice slurry is very low. For salmonids which are more resistant to cold, this risk exists if the fish are removed too soon from the ice slurry: they may die afterwards from asphyxiation, especially if they are simply exposed to air.

2: Depending on the practices of the facility, some fish placed at the surface of the water in the tank may be exposed to air if there is not enough water. This video from Esserre Animali illustrates this point: https://youtu.be/jn_owQv-YOk?t=180

Note: Asphyxia on solid ice also incorporates all the issues related to asphyxia in air without solid ice.

2.2 Gas exposure

2.2.1 Method description and current use

- **Description**

Those methods consist in immersing fish in water saturated with certain dissolved gas, in order to cause hypoxia and/or hypercapnia[3]. Those methods are irreversible if they are correctly executed. However, if fish are removed from the gas-saturated water before the complete loss of brain functions, and placed into well-oxygenated water, they can recover consciousness (EFSA 2009b). Under those conditions, the loss of consciousness is reversible.

Most often, the gas involved are the following:

- **Carbon dioxide CO_2** which results in hypercapnia (elevation of blood CO_2 levels) (EFSA 2009abc, Robb & al. 2002). An elevated concentration of CO_2 in the water leads to the apparition of carbonic acid, which entails a drop of the water pH
- **Nitrogen N_2** which induces hypoxia (EFSA 2009abc). This gas can be used alone or in combination with CO_2. The use of such mix results in both hypoxia and hypercapnia.
- **Carbon monoxide CO** which competes with neuroglobin, myoglobin and haemoglobin that normally binds with oxygen, resulting in hypoxia (EFSA 2009b).
- **Noble gas:** tests are being conducted about the use of **argon** for sea bass and sea bream

- **Current use**

Immersion in CO_2 saturated water is used commercially for rainbow trout and Atlantic salmon in some European countries including France (European Commission 2018, L214 2018). In France, in the official 2018 survey from the French ministry of agriculture, 2 salmonids producing companies (out of 365 surveyed companies) declared using immersion in CO_2 saturated water as their slaughter method (Agreste 2020). No marine fish farming nor extensive pond fish farming company declared using this method (Agreste 2020). Those methods are not used for sea bass and sea bream in France (European Commission 2018, EFSA 2009c). Gas-exposure based methods are forbidden as a stunning process for fish in Norway, however, the use of the combination of ice slurry with CO_2 is allowed as a sedation process (Riberolles RSDA 2020).

More generally, the use of CO_2 saturated water baths has been progressively replaced by the use of ice slurry combined with CO_2. Combining gas-exposure to immersion in ice slurry reduces the time needed to achieve loss of consciousness and death compared to ice slurry alone (EFSA 2009abc, Roque & al. 2021).

According to the EFSA (EFSA 2009abc), immersion in CO_2 saturated water is mostly used for small and medium-sized productions with relatively small holding tanks. CO_2 is diffused by bubbling it into the water contained in a closed space. When the pH drops and levels off at around 4,5, the water is considered to be sufficiently saturated. At this point, fish are put into the tank until they no longer move. Then they are bled.

The combination of N_2 and CO_2 has been studied for sea bass and sea bream but is not used commercially for the time being (Zampacavallo & al. 2008, Poli & al. 2004, Roque & al. 2021). Similarly,

[3] Hypercapnia= arterial CO_2 overload

CO and N_2 alone without being combined with CO_2 seem to have only been used as part of experimental settings. The use of CO has been studied for both Atlantic salmon and rainbow trout, but for the latter, only the consequences on flesh quality have been studied. The use of N_2 on its own has been tested with rainbow trout, Atlantic salmon and sea bass but not sea bream.

2.2.2 Welfare hazards, Welfarm's position and recommendations:

- **Exposure to noxious chemicals**

Gas exposure methods involves exposing fish to noxious chemicals that may be perceived as aversive, either directly, or indirectly due to the consequences of the diffusion of the gas on water pH. Aversive reactions can manifest as a physiological stress response and/or through marked behavioural reactions indicating escape attempts.

Physiological stress response

CO_2: The saturation of water with CO_2 leads to the formation of carbonic acid which greatly lowers the water pH. When fish are exposed to this, it causes acidosis and hypercapnia in all studied species which entails a strong physiological stress response (EFSA 2009abc). This physiological response is present in sea bass but to a somewhat lesser extent as this species has a relatively good tolerance to hypercapnia (EFSA 2009c). Increased production of mucus may also be observed which suggests that acidic CO_2 saturated water can be irritating (EFSA 2009a).

CO: The assessment of physiological stress response in relation to immersion in carbon monoxide saturated water is complicated by a particular feature of the mechanism behind the effect of this gas. By interfering with the ability of haemoglobin to transport oxygen in the blood, carbon monoxide is thought to trigger a change in fish metabolism. Fish may rely more heavily on anaerobic metabolic pathways rather than usual aerobic pathways (Bjørlykke & al. 2011, Bjørlykke & al. 2013, Concollato & al. 2016). This involves a particular glycolysis leading to an increase in the production of lactate, lowering blood and muscle pH. Lactate and blood and muscle pH are normally used as secondary stress indicators as increased lactate production is an indirect consequence of the secretion of cortisol and catecholamines. Due to this switch towards increased anaerobic metabolism, increases in lactate observed after immersion in carbon monoxide saturated water can be dissociated from the secretion of cortisol and catecholamines, which makes the interpretation of this indicator complex within the assessment of the physiological stress response (Bjørlykke & al. 2011, Bjørlykke & al. 2013, Concollato & al. 2016).

Rainbow trout

Concollato & al. 2016 compared stress biomarkers in rainbow trout stunned by exposure to CO, electronarcosis or asphyxiated in air. They found that trout exposed to CO had higher plasmatic glucose levels than those slaughtered by asphyxia or submitted to electrical stunning. The levels of lactate were also higher but it could be related to the effect of CO on anaerobic metabolism rather than the effect of elicited stress. Plasmatic cortisol was inferior in trout exposed to CO compared to the other groups, but the difference was not significant. Muscle pH was similar for the three studied methods, but the drop of muscle pH was slower in trout exposed to CO compared to the asphyxiated trout, suggesting that this latter method is more stressful than exposure to CO. Concollato & al. 2020 also compared trout stunned with carbon monoxide with trout submitted to electrical stunning. They observed that energetic ATP and AEC reserves were higher in trout exposed to CO compared to the electrically stunned trout, which suggests a lower stress response, but both groups had similar low-range cortisol levels. Generally speaking, the authors concluded that exposure to carbon monoxide does induce some level of physiological stress in rainbow trout but that this reaction was moderate. Dalle Zotte & al. 2020 studied rainbow trout exposed to CO but focused their analysis on flesh quality and sensory characteristics and not on stress biomarkers, concluding that trout exposed to CO have an acceptable flesh quality.

Wills & al. 2006 studied the effect of immersion of rainbow trout in N_2 saturated water compared to percussive stunning and asphyxia in air. On the basis of post-mortem ATP energy reserves and muscle pH, the authors concluded that this method produces a stronger physiological stress response than what is observed in percussively stunned trout, but a weaker response than what is observed in rainbow trout asphyxiated in air.

<u>Atlantic salmon</u>

The results of Bjørlykke & al. 2011 show that salmon exposed to carbon monoxide have higher lactate levels and a quicker drop in post-mortem muscle pH compared to controls, but those effects could be explained by the effect of carbon monoxide on anaerobic metabolism rather than stress. In fact, no difference was found between the two groups regarding levels of cortisol, glucose, sodium and haematocrits. However, the cortisol levels were rather high in both groups. Bjørlykke & al. 2013 identified a quicker speed of the drop in post-mortem muscle pH and an early onset of rigor mortis in salmon exposed to CO compared to controls, but those effects can also be explained by the anaerobic metabolism and not only stress. In fact, the cortisol levels of fish exposed to CO was compared to two control groups. One control group involved fish left entirely undisturbed. The other control group involved fish exposed to a non-CO saturated water flow in order to distinguish the effect of carbon monoxide from the effect of exposure to the water flow in which CO was dissolved in the treatment group. The results showed that salmon exposed to CO had higher cortisol levels than undisturbed controls, but lower levels than the other controls exposed to a non-CO saturated water flow. According to the authors, this shows that mere exposure to a water flow can be stressful for salmon, and that exposure to CO limited the normal stress response expressed in this situation. The authors therefore consider that CO does not induce marked physiological stress in Atlantic salmon.

Erikson & al. 2011 reported data indicating that immersion in N_2 saturated water induces physiological stress in Atlantic salmon. Observations show a significant decrease of blood pH (7.17 compared to 7.54 for controls) and muscle pH (6.59 compared to 7.31 in controls), and a significant increase in lactate (7.4 mMol/L compared to 2.2 mMol/L in controls) and glucose (4 mMol/M compared to 3.3 mMol/L in controls).

<u>European sea bass</u>

According to the EFSA 2009c, differences in stress biomarkers between sea bass slaughtered by immersion in ice slurry and those slaughtered by ice slurry combined with gas exposure (either N_2 on its own or N_2 mixed with CO_2) are rather small. However, without a comparison with unstressed control fish, it is difficult to interpret this data to assess whether gas exposure induces a physiological stress response. De la Rosa & al. 2021 commented on those same data that although the levels of glucose and lactate were similar across groups, cortisol levels were higher in fish slaughtered by ice slurry alone, potentially due to a longer agony. Zampacavallo & al. 2015 compared sea bass slaughtered in ice slurry with or without associated exposure to either 100% N_2 or a mix of 70% N_2 and 30% CO_2. In both cases, sea bass exposed to gas had a lower muscle pH that those slaughtered by ice slurry alone, suggestion a stronger physiological stress response in gas exposed fish. Similarly, immediately after death, the levels of lactate were similar across conditions, but 5 hours after death, lactate levels were higher in gas exposed fish. ATP energy reserves were inferior in fish exposed to gas suggesting a greater physiological stress response in them. Exposure to carbon monoxide has not been studied in sea bass.

Gilthead sea bream

Roque & al. 2021 carried out trials with two gas mixtures (1= 40% CO_2 + 30% N_2 + 30% O_2 ; 2=30% CO_2 + 70% N_2) on gilthead sea bream and compared the effect of this treatment to that of immersion in ice slurry. The results indicated that levels of cortisol, glucose and lactate were inferior in fish exposed to gas compared to those slaughtered by immersion in ice slurry. This means that gas exposure is associated with less physiological stress than what is observed for the ice slurry method. However, the speed of the drop in post-mortem muscle pH is quicker in gas exposed fish. As there is no comparison with control unstressed fish, it is difficult to assess whether exposure to the gas induces physiological stress. Exposure to carbon monoxide has not been studied for gilthead sea bream.

Aversive behavioural reactions

CO_2: For all species, immersion in CO_2 saturated water causes vigorous aversive reactions (EFSA 2009abc). Fish become very agitated and their behaviour can be interpreted to be an escape attempt, which indicates stress levels of **high intensity** (EFSA 2009abc). After a while, fish become paralysed / immobilised but remain conscious for some time. The time needed before immobilisation seems to be shorter at lower temperatures.

Rainbow trout:

Immersion in CO_2 saturated water triggers intense aversive reactions lasting between 30 seconds to 3 minutes in rainbow trout (EFSA 2009a).

Some tests were conducted about N_2 stunning for rainbow trout (Wills & al. 2006). The authors indicated that rainbow trout do not display aversive reactions in this context.

The use of CO has been tested for rainbow trout but only the impacts on flesh quality and stress biomarkers were studied (Concollato & al. 2016, Concollato & al. 2019, Concollato & al. 2020, Dalle-Zotte & al. 2020). The actual loss of consciousness, the latency before the loss of consciousness, and the potential presence of behavioural aversive reactions have not been studied.

Atlantic salmon:

Immersion in CO_2 saturated water results in aversive reactions lasting between 2 to 4 minutes (EFSA 2009ab) in Atlantic salmon, which then become immobilised. More recent observations reported aversive reactions lasting up to 6 minutes (Erikson 2011).

The use of N_2 is associated with aversive reactions lasting around 3 minutes in Atlantic salmon (Erikson 2011). After 4 to 5 minutes, fish become immobilised and start floating belly-up.

Several experiments were conducted regarding the use of CO. The first results showed no aversive reactions: fish would lose balance in a few minutes and then become unresponsive to stimuli (EFSA 2009b). However, brief convulsions were observed at the end of the stunning process. Another study looked at the use of CO with a progressive elevation of the concentration. There were no aversive reactions, swimming behaviour, responsiveness to stimuli and opercular motions were impaired after

7 to 8 minutes. After 12 minutes, convulsions lasting 3 to 8 seconds were observed (Bjørlykke & al. 2011).

Another publication investigated the use of rapid non-progressive elevation of CO concentration in the water (Bjørlykke & al. 2013). At first, salmon displayed rapid swimming, but this behaviour was also present in the control group. After 2 to 4 minutes, the fish started swimming even more quickly, displaying a behaviour akin to escape attempts, and swam near the surface. The authors interpret this behaviour as related to hypoxia but not necessarily to a painful perception of CO. The loss of balance supervenes after 5 minutes but is followed by another period of intense activity lasting 2 minutes in salmon exposed to high concentrations but not in those exposed to medium concentrations. Finally, fish enter a lethargic state with only a few occasional movements. Those occasional movements can be observed for a longer time (up to 19 minutes) when using medium concentrations.

<u>European sea bass:</u>

Immersion in CO_2 saturated water triggers intense aversive reactions during 1 minute in sea bass (EFSA 2009c). The fish are then immobilised after 2 to 4 minutes, which does not necessarily mean that they are unconscious (EFSA 2009c).

N_2 combined with ice slurry triggers rapid swimming, escape attempts and jumps during 3 to 4 minutes (EFSA 2009c, Zampacavallo & al. 2015). Sea bass immersed in icy water and exposed to a combination of N_2 and CO_2 display vigorous behavioural aversive reactions during the first 30 seconds (EFSA 2009c).

Exposure to carbon monoxide has not been studied in sea bass. Tests are being conducted to study the impact of using a mix of CO_2, N_2 and argon, but the results are yet to be published (Roque & al. 2017, HSA 2018). In fact, argon is known to result in less or no aversive reactions in other species.

<u>Gilthead sea bream:</u>

Immersion in water saturated with CO_2 alone has not been studied in sea bream. However, based on what is known from other species, the EFSA considers that it is very likely that sea breams would also display aversive reactions (EFSA 2009c).

Tests are being conducted to study the impact of using a mix of CO_2, N_2 and argon, but the results are yet to be published (Roque & al. 2017, HSA 2018). Based on what is known for other species, it is possible that this mix could be less aversive than CO_2 alone.

Roque & al. 2021 tested two gas mixtures on sea bream: 1) 40% CO_2 + 30% N_2 + 30% O_2; 2) 30% CO_2 + 70% CO_2). With both mix, fish were calm at first for a duration of 30 to 80 seconds, and then displayed intense but brief aversive reactions for 10 to 12 seconds. Sea breams would then lose balance with their belly oriented towards the surface.

Exposure to carbon monoxide has not been studied in sea bream.

Thus, immersion in CO_2 saturated water induces physiological stress and strong aversive behavioural reactions in all of our target species. The effect of this treatment has been less studied in gilthead sea bream, but by analogy with other species, it is reasonable to think that similar reactions would also take place. For those reasons, exposure to CO_2 obtains that disqualifying mark "B – Phy-Bh". Exposure to nitrogen elicits marked aversive behavioural reactions and physiological stress in Atlantic salmon and sea bass. In gilthead sea bream, exposure to a mix of CO_2 and N_2 results in brief aversive behavioural reactions. For those reasons, exposure to N_2 obtains the disqualifying mark "B –

Phy-Bh" for Atlantic salmon, sea bass and sea bream. Nitrogen does not seem to induce marked aversive behavioural reactions in rainbow trout, but it induces physiological stress. Therefore, this method obtains the non-disqualifying mark "B – Phy" for rainbow trout. Exposure to carbon monoxide has not been studied in sea bass and sea bream, therefore this method obtains the mark "?" for both of those species. Regarding rainbow trout, CO seems to lead to moderate physiological stress but the presence or absence of aversive behavioural reactions has not been studied. Therefore, this method obtains the non-disqualifying mark "B Phy" for this species. For Atlantic salmon, exposure to CO does not seem to cause marked physiological stress if the effects related to anaerobic metabolism are excluded. If the exposure if progressive and not brutal, it seems not to trigger aversive behavioural reactions, but such reactions have been observed in the case of brutal exposure. Therefore, this method obtains the mark "A" for Atlantic salmon.

According to our scoring system, this hazard is disqualifying when it reaches the level "B-Phy-Bh".

- **Immediateness of the loss of consciousness**

Rainbow trout:

After exposure to CO_2, fish become paralysed but remain conscious for a while. The loss of consciousness, estimated through the loss of visually evoked potentials (VEP), happens after 4,7 minutes (Kestin & al. 2002) at 14°C. More recent EEG data estimate the loss of consciousness to take place between 4,5 and 8,5 minutes (Bowman & al. 2020).

During immersion in N_2 saturated water, it is reported that at first, trout are calm, and then start floating belly up after 6 to 8 minutes (Wills & al. 2006). The authors did not conduct a rigorous assessment of the state of consciousness of the fish, be it through EEG or behavioural indicators (opercular movements, vestibulo-ocular reflex). Therefore, the possibility that fish are only paralysed by still conscious cannot be excluded at this stage. Regardless of whether the loss of consciousness will be confirmed or not by later studies, it is not immediate.

Regarding the use of CO on its own, only the impact on flesh quality has been studied. That being said, based on what is known of Atlantic salmon, it seems likely that the loss of consciousness is not immediate.

Atlantic salmon:

The loss of consciousness is estimated by EEG to happen about 6 minutes (Kestin & al. 2002) after immersion in CO_2 saturated water at low temperatures. However, at ambient temperature, salmon were still showing vestibulo-ocular reflex, which is a behavioural indicator of consciousness, after 10 minutes of exposure (Erikson 2011).

After 10 minutes, all salmon continue to show a vestibulo-ocular reflex in the case of immersion in N_2 saturated water.

This same reflex is lost after 20 minutes in the case of CO saturated water (EFSA 2009b).

European sea bass:

Sea basses lose responsiveness to noxious stimuli after 7 to 10 minutes of immersion in CO_2 saturated water (EFSA 2009c).

Regarding immersion in N_2 saturated water, the loss of responsiveness to stimuli happens after 16 minutes (EFSA 2009c). Regarding mixtures of N_2 combined with CO_2, the loss of responsiveness to noxious stimuli happens after 13 to 14 minutes (EFSA 2009c). However, the loss of consciousness has been assessed through EEG.

The use of CO has not been studied for stunning sea bass.

Gilthead sea bream:

Panebianco & al. 2006 consider that the use of CO_2 results in a loss of consciousness (assessed through behavioural indicators) more quickly than immersion in ice slurry. For instance, immersion in ice slurry leads to a loss of consciousness after 20 to 30 minutes in sea bream.

The gas mixtures tested by Roque & al. 2021 (1) 30% CO_2 + 70% N_2 + 30% O_2 ; 2) 30% CO_2 + 70% N_2) are both associated with a duration before the loss of consciousness of 3 minutes estimated through EEG.

Gas exposure methods obtain the mark "B" for this hazard. According to our scoring system, this hazard is disqualifying. Therefore, gas exposure methods are unacceptable in this aspect.

- **Risk of recovery of consciousness (reversibility)**

Normally, if fish are maintained within the gas saturated water for long enough, there is no possibility for consciousness to be recovered. However, if the immersion doesn't last long enough, fish can recover consciousness if they are placed into well oxygenated water.

According to the EFSA (EFSA 2009ab), in order to make sure that fish remain unconscious, it is recommended to expose them to CO_2 for at least 4 to 5 minutes. However, in practice, according to the personal observations made by researcher Robb, fish are routinely retrieved from the water much sooner, after around 2 to 3 minutes, when they are immobilised but still conscious. In that case, death and loss of consciousness are not caused by the gas exposure but as a result of bleeding. Thus, these methods can be irreversible if they are correctly executed and reversible if they are incorrectly executed.

Gas exposure methods obtain the mark "A/B" for this hazard.

- **Failure rate**

As the stunning failure rate has not been quantified, gas exposure methods obtain the mark "?" for this hazard.

- **Poor water quality**

Adding aversive gas into the water deteriorates the water quality and can irritate the gills and skin. In addition, the high densities practiced commercially when using CO_2 stunning can also contribute to the deterioration of water quality due to loss of scales related physical contact between individual fish (especially considering the dorsal spikes of sea bass). If the water is not changed regularly, it will be more deteriorated.

Gas exposure methods obtain the mark "C" for this hazard.

- **High or extreme densities**

As fish display aversive reactions while being held at a high density, it may result in injuries due to physical contact between individuals. Gill bleeding and loss of mucus can happen as a result of escape attempts according to the personal observations of researchers Robb and Kestin (EFSA 2009ab).

The density used with those methods will depend on the practices of each facility. However, the density is likely to be quite high due to the required slaughter line pace.

Gas exposure methods obtain the mark “B” for this hazard.

- **Reliance on workers’ skills**

Normally, gas-exposure based stunning methods do not involve a lot of human intervention in the process. However, the use of inadequate concentration of gas (be it for CO_2, N_2 or CO) may increase the duration required to achieve the loss of consciousness or result in failure to induce unconsciousness. Similarly, if workers do not know or do not respect the required duration necessary to cause the loss of consciousness, it may result in the fish being bled while they are still conscious (due to an early retrieval of the fish from the gas-saturated water). As fish can be paralysed after those methods, it can be difficult for workers to assess whether they have actually lost consciousness, or whether they are only immobilised.

Gas exposure methods obtain the mark “B” for this hazard.

Sidenote: The use of carbon monoxide for stunning can come with safety risks for workers (Berg & al. 2021, Concollato & al. 2020ab). In fact, if a problem happens and the gas is diffused in the air of the room where workers operate instead of through the water, workers would risk asphyxiation. This is all the more dangerous as carbon monoxide is odourless and not irritating which makes it difficult to detect. In some of the studies conducted about this method, a continuous monitoring system of CO levels in the air was in place in order control this risk (Concollato & al. 2020ab). Safety and risk management must be addressed without fail before using this method at a commercial scale.

WELFARM’s position and recommendations:

Regardless of the concentration of CO_2 being used, fish display vigorous behavioural aversive reactions, a marked physiological stress response and the loss of consciousness is not immediate. It does not seem to be possible to significantly improve this method for all considered species. Therefore, **we do not recommend the use of CO_2 stunning**.

N_2 seems not to trigger aversive reactions in rainbow trout but induces some physiological stress, and the loss of consciousness is not fully confirmed - more research would be needed to clarify this aspect. However, N_2 appears to cause behavioural aversive reactions in addition to physiological stress in Atlantic salmon, and a significant proportion of the salmon submitted to this method do not lose consciousness. N_2 combined with CO_2, results in aversive reactions in sea bass, though not as intense as those observed when using CO_2 alone. N_2 used on its own also results in behavioural aversive reactions in sea bass. Mixtures of CO_2 and N_2 are associated with brief aversive reactions and a latency of 2 minutes before the loss of consciousness in sea bream.

Therefore, nitrogen, alone or mixed with CO_2, does not appear to be appropriate for Atlantic salmon, European sea bass and gilthead sea bream. The use of nitrogen alone may have some potential for rainbow trout, but the knowledge base is too uncertain to recommend this method for them.

Exposure to CO seems not to trigger aversive reactions in Atlantic salmon if it is done progressively, however brief convulsions sometimes happen, and escape attempts and oxygen seeking behaviour at the surface can be observed if the elevation of CO concentration is brutal and not progressive. It is difficult to know to what extent those signs may indicate stress or potential suffering.

Exposure to CO seems not to induce marked physiological stress in Atlantic salmon. In rainbow trout, a moderate physiological stress response was identified. The loss of consciousness is not fully confirmed, as there are no available EEG data. And observations on salmon showed that a significant proportion of individuals appear to still be conscious several minutes after treatment.

More studies would be needed, among other things, to investigate the effects of CO on non-salmonids. For the time being, we do not recommend this method.

Mixtures of nitrogen, carbon dioxide and argon will be tested on sea bream but as there are no published results yet, we cannot determine a definite position on this method.

For the time being, gas-exposure based methods are to be rejected, because the loss of consciousness is not immediate and behavioural aversive reactions and physiological stress are often observed. In addition, not enough studies are available about gas mixtures that have the potential to reduce aversive reactions. Furthermore, fish can become paralysed before losing consciousness. This poses a significant risk that some fish may be bled while fully conscious if the workers do not wait long enough before removing them from the gas-saturated water.

Gas-exposure based methods, in their present form, are considered to be either ethically unacceptable (CO_2, N_2 for Atlantic salmon, sea bass and sea bream) or too uncertain (N_2 for rainbow trout, CO) and are therefore rejected by WELFAM.

Further studies are needed regarding gas mixtures. Gas mixtures that do not trigger aversive reactions and allow for an effective loss of consciousness, confirmed though EEG assessment, could be developed. In this case, the loss of consciousness should happen quickly, and the density of fish in the stunning tanks should be limited, which may help maintain better water quality. Lastly, fish should be maintained in the gas-saturated water long enough so that consciousness is lost and cannot be recovered before bleeding, and without causing suffering.

Methods	Exposure to noxious chemicals	Immediateness of the loss of consciousness	Failure rate	Risk of recovery of consciousness (reversibility)	Air exposure	High or extreme densities	Handling	Poor water quality	Tissue damage	Pre-stun electrical shock	Thermal shock	Reliance on workers's skills
CO_2	**B – Phy-Bh**	**B**	**?**	A/B	A	B	A	C	A	A	A	B
N_2	Trout: B - Phy Salmon, sea bass sea bream: **B – Phy-Bh[3]**	**B**	**?**	A/B	A	B	A	C	A	A	A	B
CO	Sea bass, sea bream: **?** Trout: B – Phy[4] Salmon: A[5]	**B**	**?**	A/B	A	B	A	A	A	A	A	B

In red = disqualifying

3: For sea bream, N_2 has only been tested combined with CO_2, which leads to brief behavioural aversive reactions. When combining N_2 and CO_2 sea bass also display behavioural aversive reactions

4: Only physiological parameters have been studied for rainbow trout. Although authors concluded that CO leads to some physiological stress, the stress response seems moderate. The presence or absence of behavioural aversive reactions in response to CO has not been studied in rainbow trout

5: If the effects related to anaerobic metabolism are excluded, CO does not seem to induce marked physiological stress. If the exposure to CO is progressive, it seems like there are no behavioural aversive reactions, but such reactions have been observed in the case of brutal non-progressive exposure

? No information is available about this hazard for our target species

2.3 Mechanical methods

2.3.1 Method description and current use

- **Description**

There is a diversity of mechanical stunning methods which consist in destroying a part of the nervous system by different manner:

- By **frontal non-penetrative percussion**: the shock wave depolarizes the neurons and causes damage to the brain

- By **penetrating percussion**: the piston mechanically destroys parts of the brain

Frontal percussion consists in hitting the fish's skull without penetrating it (EFSA 2009ab), using a flat-shaped weapon with sufficient force to render fish immediately unconscious and insensitive to pain. The kinetic energy generated by the blow is transformed into a shock wave that reaches the entire brain, depolarising neurons and causing damage, thus stunning the fish (Roth & al. 2007). Blood circulation is altered and brain haemorrhage occurs (Roth & al. 2007. EFSA 2009ab). According to Roth & al. 2007, the flat shape of the weapon allows a better accuracy compared to other weapon shapes.

Generally, this method of stunning is irreversible (J.A. Lines and J. Spence, 2014). However, depending on the strength of the blow, the loss of consciousness may be reversible or irreversible (Morzel M & al. 2003).

At this time, three techniques of frontal percussion are distinguished:

- **Manual percussion**: Operators hold fish firmly out of the water in order to deliver a powerful blow to the head. Workers use a club or a hammer. The use of a manual pneumatic percussion device originally developed for small land animals has also been experimentally tested (Robb & al. 2000, Hjelmstedt & al. 2022, e.g : Zephyr-F® Humane Salmon Stunner non penetrative captive bolt, Bock Industries). This type of equipment has the benefit of maintaining a constant striking power (similar to captive-bolt stunners).

Machines have been developed to mechanise and automate percussive stunning: fish enter in a machine which automatically triggers a powerful blow to the fish's skull (PSA, 2012) as soon as they pass through a cylinder equipped with a motion sensor.

- **Semi-automatic percussive stunning a.k.a hand-fed systems** (figure 1) require human intervention (Seafood Innovations SI-7 Generation Flow Through Machines - YouTube) to position fish head first into the cylinder to ensure that they are correctly placed towards the percussive trigger. Therefore, fish are handled by an operator. Following the blow, workers remove fish from the machine and proceed to exsanguination (EFSA 2009ab). It is also possible to integrate an automatic gill-cutting system in the device and in this case, fish are bled automatically within 10 seconds after stunning.

- **Full-automatic percussive stunning a.k.a swim-in systems** (figure 2) have a design that naturally encourages fish to swim towards the cylinder entrance without any operator intervention. In some systems the water flows in the opposite direction to that of the stunning cylinders: this relies on rheotaxis in salmonids which will leads them to swim against the current until they reach the cylinders (Mejdell & al. 2009a, EFSA 2009b, personal communication with researcher Cecilie M Mejdell). The machine will then cut the gills of the fish within 10 seconds after stunning, with an automatic gill-cutting system embedded within the machine (EFSA 2009ab).

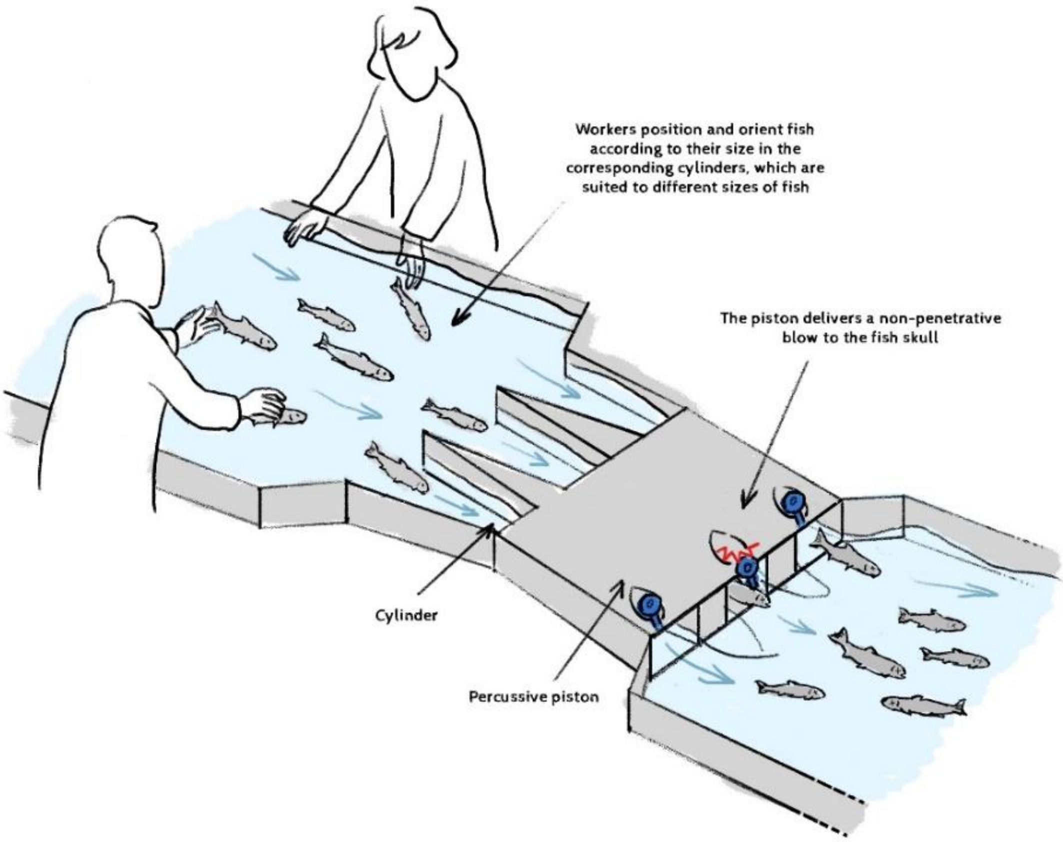


Figure 1: Semi-automatic percussive stunning (picture : Welfarm ©)

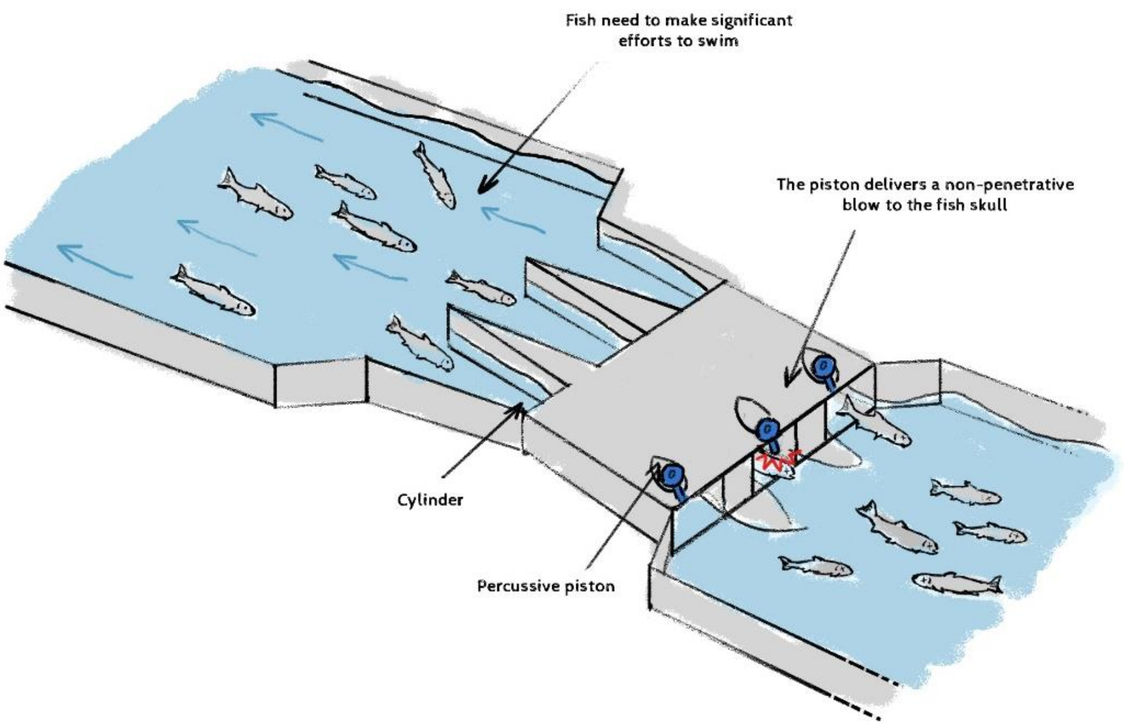


Figure 2: FULL-AUTOMATIC PERCUSSIVE STUNNING (picture : Welfarm ©)

Spiking involves penetrating the skull of a fish with a sharp weapon and then fiddling it inside the brain to cause severe damage, rendering the fish unconscious and unresponsive (CIWF 2018; OIE, 2010). This technique, if managed correctly, causes destruction of the cerebellum or medulla oblongata and induces immediate and irreversible unconsciousness (Roth & al. 2007; Robb & al. 2000; Robb and Kestin 2002, Poli & al. 2005). The success of this method is highly dependent on the experience of the operator (Poli & al. 2005). It requires a high degree of precision and dexterity to target the correct area to induce unconsciousness. Incorrect handling can cause suffering to fish. Spiking can be carried out manually or with the help of an automated machine that enables decerebration (FranceAgriMer (2019)).

- **Current use**

Frontal percussion:

According to the European Commission reports (2017, 2018), manual percussion is not used a lot in France. Manual percussion only allows for a slow slaughter line pace. In a study conducted in 18 different rainbow trout slaughter plants in Germany, manual percussion was used in 38% of cases (Jung-Schroers & al. 2020). In France, in the 2018 official survey by the French ministry of agriculture, 76 salmonid producing companies (out of 365 surveyed companies), 4 marine fish farming companies (out of 28 surveyed companies), and 4 extensive pond fish farming companies (out of 211 surveyed companies) declared using percussive stunning methods (Agreste 2020). This survey does not indicate which sub-type of percussive stunning companies are using.

Semi-automatic and full-automatic percussive stunning are used for large rainbow trout and Atlantic salmon (EFSA 2009ab). These methods are followed by either a manual or an automatic exsanguination or evisceration (EFSA 2009a). Semi-automatic and full-automatic percussive stunning machines are not suited for rainbow trout under 1 Kg (portion sized trout). These techniques allow for a high slaughter line pace.

These two methods are commonly used in England (FAWC 2014, RSPCA 2020, EFSA 2009ab). It is also the case in Scotland, as 70% to 80% of Scottish salmonid farms committed to follow the RSPCA assured scheme standards, which require either percussive or electrical stunning (Rodgers 2017). These two methods are also used a lot for Atlantic salmon. In 2009, they were used on 25% of Atlantic salmon in Iceland, and 14% of salmon in Norway (EFSA 2009ab). Some French companies use Baader's full-automatic percussive stunning systems on large rainbow trout (personal communication with a stunning equipment supplier company).

Finally, sea bass and sea bream are not commonly stunned by percussive stunning in the industry. Semi-automatic and full-automatic percussive stunning machines have not been developed for these species.

Penetrating percussion, also known as spiking

This technique is not really used commercially for farmed fish. However, according to Poli & al. 2005, it has been used in an experimental context. This method is considered unsuitable for small fish (such as portion-sized tout). Automatic spiking has only been used experimentally (Roth & al. 2007). Manual spiking requires skills and precise gestures.

Spiking followed by demedullation is referred to as “ikejime”. Demedullation consists in driving a rod through the fish spine. This is a traditional Japanese technique. This method is mainly used on high commercial value wild capture fish such as tuna (FranceAgriMer ikejime report). Ikejime is not commonly used on slaughter plants. There is a limited use of this method in high-end restaurants in

France, mostly for wild caught fish which are sometimes kept alive in the restaurant to be slaughtered in front of customers. The aim of this practice is to guarantee the freshness of the product to consumers to offer superior quality products (FranceAgriMer 2019). Fish slaughtered this way are brain dead but maintain an enzymatic activity for some time.

2.3.2 Welfare hazards:

- **Immediateness of the loss of consciousness**

The loss of consciousness is instantaneous if parameters are correctly set up. Mechanical methods obtain the mark "A" for this hazard.

- **Risk of recovery of consciousness (reversibility)**

For percussive methods, the loss of consciousness is irreversible if the parameters are correctly set up but may be reversible if the power of the blow is insufficient (see part 2.3.3). Spiking leads to an irreversible loss of consciousness.

Percussive methods obtain the mark "A/B" for this hazard. Spiking obtains the mark "A" for this hazard.

- **Failure rate**

Full-automatic percussive stunning/swim-in system:

According to the EFSA 2009ab, full-automatic percussive stunning has a failure rate of 10%, which is the highest failure rate of all frontal percussion methods. It can be explained in part by the lack of operator control upstream, at the entrance of the stunning cylinders. A proportion of the fish can arrive in front of the piston in an unsuited position (tail first or belly up), and endure a mechanical shock without losing consciousness (personal communication with researcher Cecilia Mejdell). Thus, the blow does not hit the fish in the appropriate location. Moreover, unlike semi-automatic percussive stunning, fish are not sorted out by size upstream to be led into cylinders specifically suited for their size. This increases the likelihood that the piston may hit fish at the back of their head or on the tip of the rostrum, leaving the brain intact (EFSA 2009ab, personal communication with a stunning equipment producer company). According to the report of the Norvegian Veterinary Institute (Mejdell & al. 2009a), a lot of fish require an emergency stunning when this method is used. This report mentions that it was difficult for operators to correctly assess whether fish were in need of emergency stunning as a lot of them exhibited temporary convulsions.

In addition, according to one stunning equipment supplier, the level of agitation of the fish as they arrive in the system has an impact on failure rates. This observation comes from their experience with full-automatic percussive stunning systems but can likely be extended to semi-automatic percussive stunning systems as well.

Semi-automatic percussive stunning/hand-fed systems

Atlantic salmon:

Regarding semi-automatic percussive stunning, the failure rate varies depending on whether or not an automated gill cutting system is integrated (EFSA 2009b). Generally, the failure rate of such systems is inferior to that of full-automatic percussive stunning (EFSA 2009b). Instances of failure can be explained by an incorrect positioning of fish by operators in front of the stunning area.

The failure rate is estimated to be of 5% when an automatic gill-cutting system is incorporated within the machine (EFSA 2009b). Without this automatic gill-cutting device, the failure rate is estimated to be 2% (EFSA 2009b). It is not possible for operators to check the consciousness status of fish before bleeding in systems integrating an automatic gill-cutting device (in which case the gill cut is performed within 10 seconds after stunning). If fish are not positioned correctly when they enter the automatic gill cutting system, or if their size is not suited to the system, the incision of arteries may be incorrectly performed. In contrast, in systems without automatic gill-cutting, operators can observe fish directly after stunning to assess their consciousness status before bleeding and perform an emergency stunning if needed.

<u>*Rainbow trout*</u>

The use of semi-automatic percussive stunning followed by a manual gill cut is associated with a failure of 5% for rainbow trout according to the EFSA 2009a, which is 3% higher than what is reported for salmon. As rainbow trout are smaller than Atlantic salmon, they may be a bit more difficult to hit precisely. Further studies would be needed to understand this difference of failure rates between rainbow trout and Atlantic salmon.

Manual percussions:

Regarding manual percussion, the failure rate is 5% for rainbow trout (EFSA 2009a), and there are no estimates for our other target species. This failure rate estimate is equivalent to that of semi-automatic percussive stunning. More recent empirical data based on behavioural indicators of consciousness estimate the failure rate of manual percussion for rainbow trout to be 8,7% based on a study of 8 sites using this method in Germany (Jung-Schroers & al. 2020). There were some variations across sites, failure rates ranging from 0% and up to 20%. As those data are more recent, we decided to use this figure to attribute the disqualifying mark “C” to manual percussion regarding failure rate. The use of manual percussion in commercial systems seems difficult at large scales in terms workforce needs and slaughter line pace.

Instances of failure can be explained by an insufficient pressure (i.e below 8 bars) exerted by the weapon of the operator or by a bad positioning of the fish which does not allow the optimal adjustment of the blow.

Regarding pneumatic manual non penetrating percussive stunning, a recent study found a 0% failure rate on rainbow trout (Hjelmstedt & al. 2022). However, this study has only been conducted in a laboratory environment on 10 individual fish. Therefore, those data cannot really be used to assess the stunning failure rate in a commercial setting.

Penetrating percussion, also known as spiking:

The study by Roth & al. 2007 showed that stunning by automatic spiking results in a poor stun, despite the penetration of the skull and considerable physical damage. In case of failure, there is a high risk of pain and suffering (Tobiassen & Sørensen 1999; Robb & al. 2000; Van der Vis & al. 2001; Etude sur le poisson vendu vivant et le poisson ikejime en France – Frane AgriMer 2019). This method requires a lot of precision to destroy the small sized brains of salmonids. In order to stun fish, the tip must penetrate the cerebellum and/or medulla oblongata (Roth & al. 2007; Robb & al. 2000; Robb & Kestin 2002), which requires high precision (Poli & al. 2005). The study by Roth & al. 2007 showed that the use of a flat non-penetrating weapon resulted in a better stun than the use of a sharp weapon or a conical weapon despite the latter weapons penetrating into the cranial cavity.

Sidenote: Sexually mature fish have an elongated rostrum which prevents semi-automatic and full-automatic percussive stunning machines to efficiently reach the brain. In addition, these fish require a stronger blow than immature fish to be correctly stunned (Roth & al. 2007). The presence of those fish at the slaughterhouse is related to deficiencies of prior controls because sexually mature fish are usually not meant to be slaughtered and commercialised (EFSA 2009b). This is a common problem in commercial farms (Roth & al. 2007). Sexually mature fish are sometimes grinded (maceration) without prior stunning or bleeding (EFSA 2009b).

Generally speaking, mechanical methods involve significant risks of suffering if they are not correctly executed. If the blow is not well adjusted or not powerful enough, haemorrhage, asphyxia, paralysis without loss of consciousness, dislocation or bursting of the eyes while the fish are conscious can happen (Roth & al. 2007).

Full-automatic percussion and manual percussion obtain the mark “C” for this hazard. According to our scoring system, this hazard is disqualifying when it reaches the level “ C ”. Therefore, full-automatic percussive stunning and manual percussive stunning are not satisfying in this aspect. Semi-automatic percussive stunning obtains the mark “B” for this hazard. As the failure rates of spiking have not been quantified, this method obtains the mark “?” for this hazard.

- **Air exposure:**

Fish are exposed to air during the handling required to deliver the blow for manual percussion. The duration of air exposure during handling prior to manual percussion was estimated to be about 30 seconds by the EFSA 2009a. The duration of air exposure for spiking has not been estimated, however, given the precision required to perform the gestures, we hypothesise that the time required to perform spiking is somewhat greater than that required to deliver a blow during manual percussion, which requires less precision.

For semi-automatic percussive stunning, the EFSA observed that fish were exposed to air at several stages of the process (when they arrive in the system and if there is not enough water, when they are handled to be positioned at the entrance of stunning cylinders) for a prolonged period of time.

Atlantic salmon:

The duration of air exposure was estimated to be 1 minute for salmon when using the semi-automatic percussive system, according to EFSA 2009b.

Rainbow trout:

The duration of air exposure was estimated to be 2 minutes and 30 seconds for trout when using the semi-automatic percussive system, according to EFSA 2009a.

We have no explanation for this difference in air exposure duration between salmon and trout.

More recent semi-automatic percussive stunning machines have been developed. Those systems seem to involve shorter durations of air exposure. On more recent available videos of those systems, the duration of air exposure appears to last from less than 15 seconds to about 1 minute (it is difficult to precisely assess this duration on videos) (Seafood Innovations SI-7 Generation Flow Through Machines, https://www.youtube.com/watch?v=Dg1YrLGc2yw; Seafood Innovations

Autofeed Fish Stunner, https://www.youtube.com/watch?v=7zrSP3LJ2Yk). When exchanging with one stunning equipment supplier, we were told that the corridors where fish are kept as the move towards the stunning cylinders are not necessarily sold with the machines. Rather, they are designed for each specific site. According to one stunning equipment supplier, it is possible to design systems so that fish remain within water and air exposure only last a few seconds, be it for full-automatic or semi-automatic percussive stunning systems. This is also the opinion of Cecilie Mejdell from the Norwegian Veterinary Institute (personal communication). In those corridors, fish can move in water (although the density is high) and operators can handle fish within water. We decided to consider the most recent systems to assess this hazard.

Full-automatic percussive systems do not induce air exposure during the process leading to fish stunning. However, once fish are inside the stunning cylinders, water is flushed away, and fish are exposed to air during a few seconds (personal communication with a stunning equipment producer company).

According to our scoring system, this hazard is disqualifying when it reaches the levels “C” or “D”. Manual percussion and spiking obtain the mark “C”. Therefore, those methods are unacceptable in this regard. Full-automatic percussive stunning obtains the mark “A”. Semi-automatic percussive stunning obtains the mark “B/C” depending on the design of the machine. This method can either be accepted or disqualified depending on whether the design of the system allows or does not allow for air exposure to be limited to a maximum of 15 seconds.

- **High or extreme densities**

In semi-automatic and full-automatic percussive systems, fish are held at a high density. This is especially true for full-automatic percussive systems. Indeed, the report from the Norwegian Veterinary Institute (2009) points out that a high density promotes the movement of fish towards the cylinders entrance, in full-automatic percussive systems. Available videos of semi-automatic percussive stunning machines also show that fish are subjected to high density in the holding tank before being introduced into the cylinders entrance.

Manual percussion and spiking obtain the mark “A” for this hazard. Full-automatic and semi-automatic percussive stunning obtain the mark “B” for this hazard.

- **Handling**

Handling induces significant stress in fish (Gabriel & al. 2011). Full-automatic percussive systems are not affected by this problem, but manual percussion, spiking and semi-automatic percussive system are.

Manual percussion requires workers to handle fish and exert pressure on them to keep them still. Fish may slip or even fall off if the gesture is not carried out correctly. The EFSA 2009ab estimates the duration of handling to be around 30 seconds during manual percussion. No such information is available for the spiking method, however, given the precision required to perform a correct spiking, we hypothesise that the time required to perform spiking is greater than the time required to deliver a blow during manual percussion.

The EFSA 2009ab estimates the duration of handling to be about 1 minute during semi-automatic percussive stunning. More recent videos discussed above show designs of semi-automatic percussive machines which require very little handling.

Manual percussion, spiking and semi-automatic percussive stunning obtain the mark “B” for this hazard. Full-automatic percussive stunning obtains the mark “A” for this hazard.

- **Tissue damage**

No mechanical percussion method is affected by this hazard. The flesh is not penetrated by the piston, although a few mis-stunned fish may consciously experience being hit by the piston. On the other hand, tissue damage is intrinsic to spiking. In theory the stun is immediate, however, in cases of failure (inaccuracy of the gesture) fish may suffer from severe pain due to tissue damage.

Spiking obtains the mark “B” for this hazard, in relation to consequences in case of stunning failure. According to our scoring system, this hazard is disqualifying. Therefore, spiking is not satisfying in this aspect. The other mechanical methods obtain the mark “A” for this hazard.

- **Reliance on workers skills (low, moderate, high)**

Manual percussion: The experience and fatigue of operators are important factors impacting the success of manual percussion (J. A.Lines, 2014). The efficiency of operators decreases after 30 minutes of activity (Escudero 2018).

Spiking: This method is highly dependent on workers’s skills as it requires particularly precise gestures to be effective (report on ikejime, FranceAgriMer).

Semi-automatic percussive stunning: When placing fish into semi-automatic percussive systems, the skills of operators to sort fish according to their size and to position them at the entrance of the cylinders can affect the efficacy of the stunning process.

In addition, operators' lack of knowledge about the correct pressure settings to apply and inappropriate cadence speeds can negatively impact animal welfare, as shown in this investigation from the NGO Animal Equality: INVESTIGATION: Fish Killed While Fully Conscious in Scottish Salmon Slaughterhouse, https://www.youtube.com/watch?v=u2uyP74I1EU).

Full-automatic percussive stunning: by definition this method does not require human intervention, therefore it is not really dependant on worker’s skills.

Manual percussion and spiking obtain the mark “C” for this hazard. Semi-automatic percussive stunning obtains the mark “B” for this hazard. Full-automatic percussive stunning obtains the mark “A” for this hazard.

- **Fish exhaustion: another aspect to consider**

This problem is only relevant to full-automatic percussive systems. The design of the access paths towards the entrance of the cylinders requires fish to make some swimming efforts, which can cause moderate to severe fatigue (2-10 min of effort) (EFSA 2009ab). This fatigue can be a source of stress. This swimming effort is more challenging if fish have already been challenged and exhausted by transportation and transfers. The intensity of fish exhaustion depends on the design of the machine.

IMAGE FROM PIXABAY

However, it would be necessary to compare the duration reported by the EFSA in 2009 with observations in more recent systems.

In the wild, salmonids can swim rivers upstream and therefore sometimes make significant swimming efforts. This is not the case for sea bass and sea bream.

The report from Mejdell & al 2009a, showed that the rainbow trout sometimes lose their balance and begin to float upside down in full-automatic percussive systems. They then regain their balance a few minutes later. Rainbow trout appeared to be somewhat more affected by exhaustion than Atlantic salmon in this type of system.

As this factor is specific to full-automatic percussive systems, we decided to exclude it from the systematic welfare hazard assessment.

2.3.3 Calibration of parameters for frontal percussive stunning, Welfarm's position and recommendations

Frontal percussion: stunning performance VS flesh quality

The 2 studies cited below were carried out on Atlantic salmon, but the results may be relevant for rainbow trout as well which is also a salmonid.

The study by Roth & al. 2007 showed that a pressure below 7.2 bar is associated with instances of stunning failure involving either an absence of loss of consciousness or a recovery of consciousness occurring after an average of 3.6 minutes. The more powerful the blow, the more likely it is that the loss of consciousness is immediate, long-lasting, or irreversible.

The same study also showed that after a well-adjusted blow, there are no external damage to the skin or head. However, a momentary change of the colour of the head area was observed, with a dark spot at the location of the blow and a lighter halo around this spot. After 10 minutes, this phenomenon faded and then disappeared. There was no prolapsus of the eyes (nictitating gland) but the authors commonly observed haemorrhages under the cornea and sometimes even eye bursting, which was the most severe damage reported. Damages were observed more frequently with more powerful blows. Although less frequent, severe damages also occurred when the blow was below the 7.2 bar threshold.

A more recent study by Lambooij & al. 2010, using a commercial semi-automatic percussive system showed that at a pressure of 8.1 bar, there could still be some risk of recovery of consciousness. Between **8.1 bar and 10 bar** the stunning success rate was better, and haemorrhages were observed inside the brain cavity, eventually causing death.

In the same study, it was observed that one of the fish showing signs of consciousness on the EEG following the blow did not show a behavioural response to harmful stimulation. Therefore, the risk of immobilisation should also be considered when using this method.

A recent study on 700-800g rainbow trout showed that manual pneumatic percussive stunning with a pressure of **8,6 bars** can induce immediate and permanent unconsciousness. This study used EEG analysis of VEPs to confirm unconsciousness (Hjelmstedt et al. 2022).

The study by Roth & al. 2007 showed that the proportion of carcass damage seems to increase with the force exerted on the fish skull. Broken upper and lower jaws as well as eye bursts were observed. No haemorrhages were found on the surface of the fish fillet. **The most severe eye damage appears to occur at pressure values corresponding to the settings most likely to result in an effective irreversible stun (Figure 3).**

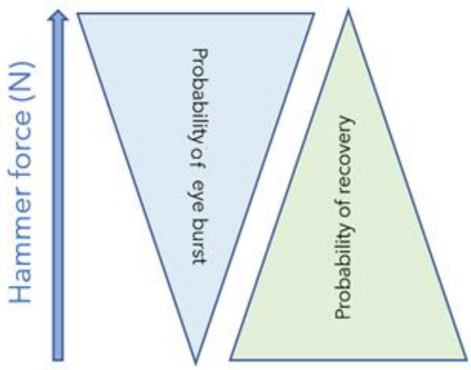


FIGURE 3: PROBABILITY OF EYE BURST AND RECOVERY OF CONSCIOUSNESS WITHIN 10 MINUTES AFTER PERCUSSION IN RELATION TO HAMMER FORCE. INSPIRED BY ROTH & AL. 2007

According to the EFSA 2009a, the effectiveness of percussive stunning depends on the force of pressure being applied, on the velocity and on the weight and shape of the weapon used. Roth & al. 2007 also indicated that the transfer of energy from the shock of the blow is dependent on the flexibility of the cranial bone structure. Optimising these parameters could improve the efficacy of stunning while maintaining acceptable flesh quality according to Roth & al. 2007. Further studies are needed on this topic.

For sea bass and sea bream, as far as we know, only manual percussion has been studied. Automated percussive stunning systems do not currently exist for these species. Nevertheless, semi-automatic percussive stunning may be an interesting option to effectively stun these fish. However, scientific research regarding the appropriate pressures necessary to achieve an acceptable stunning success rate for this method would be required to develop the method for sea bass and sea bream. Indeed, fish species can be more or less suited to percussive stunning in relation to their particular bone structure. For example, catfish, common carp, tilapia, pangasius and pikes are not suited for this type of method (J. A. Lines, 2014, PSA, 2012). Furthermore, the use of frontal percussion on fish species which have both of their eyes on only one side of their body, or with a very flat shape is also questionable (J. A. Lines, 2014).

Carcass damage may be an issue with frontal percussion. Few studies exist on this topic for our target species (Di Marco & al. 2007 in Zampacavallo & al. 2015). A study on turbot showed (abstract: Morzel & al. 2003) an increase in pH and water retention observed post-mortem with a delay in the onset of rigor mortis, after frontal percussion. Conversely, a low pH has been observed in sea bass following percussive stunning (Di Marco & al. 2007). According to Van de vis & al. 2003, the flesh quality of sea bass stunned by frontal percussion is similar to what is observed after immersion in ice slurry (which is commonly used on this species). Further research is needed to better assess the impact of percussive stunning on fish flesh quality.

WELFARM's position and recommendations

Given the issues previously discussed, semi-automatic percussive stunning (hand fed systems) followed by manual gill cutting (i.e. the gill cutting system is not integrated within the machine) appears to be the mechanical method involving the least amount of welfare hazards.

In fact, this technique allows for a low failure rate (2% to 5%) thanks to the controls carried out by operators before and after stunning. These controls allow fish to be sorted according to their size before entering the cylinders thus decreasing stunning failures. Controls also allow an assessment of the state of consciousness downstream of the percussive system so that emergency stunning can be performed before gill cutting if necessary.

Similarly, manual gill cutting allows operators to treat the fish one by one ensuring that the exsanguination has been carried out correctly (reducing the risk of recovery of consciousness). However, the design and management of machines must be carried out in a manner allowing to keep the duration of air exposure under 15 seconds.

We recommend semi-automatic percussive stunning/hand fed systems as this method seems to have better fish welfare outcomes in comparison with other methods currently available on the market. This method can be widely used on salmonids above 1 kg. If commercial machines are developed in the future, theoretically, they could be used on sea bass and sea bream but studies are needed to determine the appropriate pressure to be applied for those species.

Full-automatic percussive stunning has relatively few disadvantages. However, it does require swimming efforts, and its stunning failure rate is high - which is the most important issue. Therefore, we do not recommend the use of full-automatic percussive stunning at this point.

Spiking and manual percussion involve too much air exposure to be recommended, and failure rates may be high. Moreover, spiking involves tissue damage which can give rise to pain in case of failure. Thus, we do not recommend those methods.

If **we only consider more recent machines that meet our requirements, semi-automatic percussive stunning is considered to be acceptable in terms of animal welfare by WELFARM. However, it is not the best method among acceptable methods.**

Batches of fish should have uniform size and shapes to avoid stunning failures. An operator should be positioned upstream and downstream of the slaughter line to ensure that the slaughter process is carried out correctly.

For all mechanical methods, fish should be kept under circulating water with a flow suitable for the species, and properly oxygenated. Wherever possible (i.e. without compromising the proper functioning of the device), the density should be reduced as much as possible to avoid causing air exposure due to a lack of space in the tank.

A possible solution, although more expensive, would be to previously stun fish by electronarcosis so that they are unconscious during handling and air exposure (see section 2.6). However, this combination is incompatible with full-automatic percussive systems as fish need to be conscious to be able to properly swim forward to reach the entrance of stunning cylinders.

Although manual percussion and spiking are disqualified, if those methods are used, fish should be kept hydrated. Moreover, manual percussion should be preferred over spiking as the gestures require less precision, and it does not involve tissue damage.

Manual percussion with a pneumatic device should be preferred over manual percussion with a priest. However, it is important to make sure that the distance between the animal and the pneumatic device is short, for a more precise blow. The pressure should be adequate. The pneumatic percussion device should be regularly calibrated, in particular if operators observe a high stunning failure rate as illustrated in this video: Jarvis France étourdisseur à gaz HPS1, https://youtu.be/dROW4TyzSBY.

Operators should be properly trained for their tasks. Operators should be appointed to the same tasks so that they can develop an expert gesture allowing for a better efficacy. At the same time, the repetitiveness of gestures should be regulated in order to safeguard the health of workers to maintain a high level of precision.

Manual gill cutting, or the use of an automated gill cutting machine separate from the stunning device (so that operators can check consciousness status before bleeding), should be preferred.

There should be a pressure control system integrated within the percussive device, to ensure a sufficiently powerful blow to stun fish (EFSA 2009ab). Current knowledge indicates that to ensure effective stunning on Atlantic salmon, the pressure should be of **8.1 bars** or higher. Regarding rainbow trout, a pressure of **8.6 bars** or higher has been proven to be effective. There is currently a knowledge gap on pressure levels required for sea bream and sea bass.

Regarding full-automatic percussive stunning, in addition to its high stunning failure rate, attention should be given to the following aspects. Due to the high swimming efforts needed to reach the cylinders, this technique may not be suitable for sea bass and sea bream, which are not accustomed to intense swimming exercise in the wild. Furthermore, Mejdell & al 2009a advises to light up the entrance of the cylinders in order to facilitate fish swimming. In addition, it seems that a high density is required to encourage fish to swim forward. Some stunning equipment suppliers advise to cover up the water corridor leading to the entrance of the cylinders to reduce the agitation of fish.

Reducing the agitation of fish is thought to reduce stunning failure rates during full-automatic and semi-automatic percussive stunning (personal communication with a stunning equipment producer company). Prior sedation, either chemical or done by a progressive reduction of water temperature, may be considered for this purpose under certain conditions (see part IV.2.1.2 for more details).

Full-automatic and semi-automatic percussive systems are not suited for oversized fish, as they impose pressure on the machine which vibrates. Equipment must be suited to the size of the fish introduced in the device.

As it has been reported that some fish end up floating belly up in the water corridor leading to the cylinders in full-automatic percussive systems, there should be an operator controlling that fish arrive correctly into the system, even in the case of full-automatic systems.

It is important for operators to be able to perform emergency stunning with a manual pneumatic percussive device when necessary.

Potentially, the integration of a grading machine sorting fish according to their size before they enter the stunning cylinders may be explored to reduce the failure rate of full-automatic percussive systems. However, the consequences of such a grading machine on animal welfare would need to be considered, and rigorous observations should be carried out to study whether adding such machines can really reduce failure rates. Caution is required as current grading machines often involve air exposure and fish often bump into the metal surface of the machine as can be seen on the following videos : Grading sea bass 40g to 60g https://www.youtube.com/watch?v=g2ajKrKZjEc&t=184s; 46 Fish pump and grading machine for sea bream https://www.youtube.com/watch?v=H1u3kQG6_XE).

Methods	Exposure to noxious chemicals	Immediateness of the loss of consciousness	Failure rate	Risk of recovery of consciousness (reversibility)	Air exposure	High or extreme densities	Handling	Poor water quality	Tissue damage	Pre-stun electrical shock	Thermal shock	Reliance on workers's skills
Manual percussion	A	A	**C**	A/B	**C**	A	B	A	A	A	A	C
Semi-automatic percussive system	A	A	B	A/B	B/**C**[6]	B	B	A	A	A	A	B
Full-automatic percussive system	A	A	**C**	A/B	A	B	A	A	A	A	A	A
Spiking	A	A	?	A	**C**	A	B	A	**B**	A	A	C

Red = disqualifying

6: depends on the design of the machine

?: This factor has not been studies on any of our target species

2.4 Chemical anaesthesia

2.4.1 Method description and current use

- **Method description**

Some anaesthetics can impact the neurological activity of fish. Anaesthetics can reduce stress and consciousness levels and may even lead to a complete loss of consciousness. They can also have an analgesic effect and reduce pain. A given molecule does not always combine all of those aspects.

Several authors came up with standardised grids defining different stages of anaesthesia, which are divided into 3 to 5 stages depending on authors (Michel 2018, Javahery & al. 2012). The following stages of anaesthesia induction were defined by Christian Michel, researcher at the National institute of agricultural and environmental research in France (INRAE), based on his personal observations on salmonids and on the synthesis of other existing grids (Michel 2018). Most publications consider that anaesthesia is effectively induced when fish reach at least stage III - 1.

- 0) Normal state: swimming, balance, opercular rhythm, muscle tone and reactivity to visual and tactile stimuli are normal

- I) Sedation: short and intense excitement phase, progressive decrease of reactivity to visual and vibratory stimuli, cessation of voluntary swimming and progressive fall on the substrate, preservation of balance and muscle tone

- II) Tranquillization: total loss of reactivity to visual and vibratory stimuli, partial loss of balance accompanied by ataxia, decrease in muscle tone, transient increase in opercular rhythm

- III - 1) Anaesthesia: total loss of balance, sharp decrease of muscle tone, little change in opercular beat frequency

- III - 2) Surgical anaesthesia: total loss of reactivity and muscle tone, decrease in the rhythm and amplitude of opercular movements but maintenance of their regularity

- IV) Irreversible anaesthesia: sporadic opercular movements followed by the complete cessation of opercular movements, pronounced bradycardia followed by cardiac arrest, hypoxia, death

The effects of a molecule can vary across species and depend on factors such as the quantity administered, the duration of exposure, density, fish size, stage of development, hormonal activity, sex, physicochemical parameters of the water, in particular salinity and temperature (Schroeder & al. 2021, EFSA 2009b). As salt is chemically active, it can interact with certain molecules. For instance, the EFSA recommends not to use MS-222 (tricaine mesylate) in seawater (EFSA 2009b). The absorption of molecules and their degradation depends directly on the metabolism of fish which is related to water temperature. At higher temperatures, the metabolism is faster: consciousness is lost more quickly but is also recovered more quickly (Javahery 2012, Zahl & al 2011). Furthermore, an increase in temperature leads to hypercapnia and acidaemia which stimulates hyperventilation, which in turn decreases the time needed for of anaesthetics to produce their effect, as well as the time needed to eliminate these anaesthetics via the gills, thus leading to the recovery of consciousness (Neiffer & Stamper 2009). The opposite effect occurs at low temperatures (Neiffer & Stamper, 2009).

Conducting rigorous studies to assess the effects of each molecule for each species, and to determine the doses corresponding to each stage of anaesthesia, is particularly important.

Prior to anaesthesia, fish are generally deprived of food for 12 to 24 hours. Fasting prevents regurgitation which can impact the gills and induce excretion of nitrogenous waste into the environment (Schroeder & al, 2021).

One of the most used fish anaesthetics is called TMS or MS-222 (tricaine mesylate) which belongs to the group of metacains (Kiessling & al. 2009). It induces a rapid and deep anaesthesia (Ackerman & al. 2005) and is used to facilitate handling of fish throughout the rearing cycle, or to kill fish for emergency slaughter in case of sanitary issues, or to kill broodstock fish that are not destined to human consumption (EFSA 2009b). The lethal dose is around 400-500 mg/L (Ackerman & al 2005). Fish slaughtered this way are not fit for human consumption because this product can have harmful consequences on human health (Harvard laboratory safety guidelines MS-222). The half-life of MS-222 is 1.7 minutes in Atlantic salmon (Kiessling & al. 2009). A lower dosage, around 25-100 mg/L can be used to anaesthetise fish for handling, transportation, invasive sample collection or vaccination (Kiessling & al. 2009). If exposure to the molecule is prolonged, it can lead to death (Marking & al. 1967).

AQUI-S, whose active ingredient is **isoeugenol**, is **the only anaesthetic used commercially** to stun fish destined to human consumption to this day. The manufacturer considers that a dose of 15 to 20 mg/L during bathing is sufficient to induce anaesthesia in most fish (AQUI-S New Zealand Ltd).
The effect of isoeugenol is reversible at certain doses but can become irreversible in case of overdosing. Isoeugenol can either sedate or anaesthetise fish depending on what is needed.

AQUI-S is composed of 50% isoeugenol and 50% polysorbate 80 which is an emulsifier (Al-Roumi & al. 2014). AQUI-S should not be confused with AQUI-S 20E, which is another anaesthetic composed of 10% eugenol and 90% excipient (Owens & al. 2017).

Clove oil, on the other hand, is composed of eugenol, isoeugenol and methyl eugenol. Commercially available, clove oil contains about 84% eugenol, and it is possible to find oils with 100% eugenol according to Neiffer & Stamper 2009.

The manufacturer of AQUI-S indicates that its product should only be used in freshwater, while AQUI-S 20E could be used in both fresh water and sea water (AQUI-S tech sheet, AQUI-S 20E Tech sheet, AQUI-S New Zealand Ltd). The EFSA states that water salinity can impact the efficacy of chemical anaesthetics by reacting with the molecule (EFSA 2009b). Nevertheless, some researchers have tested AQUI-S anaesthesia in seawater, against the manufacturer's instructions, sometimes with relatively good results (Erikson 2011).

Ethanol is often associated with the use of eugenol to increase water solubility. A recent study by Barbas & al. 2021 showed that small concentrations of ethanol have no major effect on fish. The study by Sink & al. 2007 reached the same conclusion. Similarly, Mylonas & al. 2005 tested the effect of bathing sea bass and sea bream for 15 minutes with 2.5 ml/L ethanol. The results showed that ethanol exposure did not induce change in behaviour or ventilation. Readman & al. 2013 showed that ethanol is not perceived as aversive by zebrafish either.

Isoeugenol is a derivative of eugenol. These two molecules are structurally very similar and some authors consider that the results of studies on one molecule can be reasonably extended to the other molecule (Berg & al. 2021). Eugenol is the active ingredient and the main compound in clove oil and causes a strong and potentially unpleasant odour for the consumer. Clove oil changes the taste of fish flesh, making it unusable in a commercial system. AQUI-S is supposed to limit this adverse effect compared to clove oil (personal communication with AQUI-S New Zealand Ltd).

Depending on the dosage and the duration of exposition, it can be used to induce light sedation (consciousness is maintained) or different stages of anaesthesia leading to a loss of consciousness. Used as an anaesthetic overdose, it becomes lethal.

Depending on the number of fish considered, anaesthetic products can be delivered in different ways:

- Injection: This method of administration can be used for small numbers of fish in the context of scientific research or for emergency slaughter to a lesser extent.
- Short bathing: after crowding, fish are taken from their holding tank with a net and transferred into a high-concentration solution for some tens of seconds. Workers may then carry out interventions like stripping for egg collection, sorting out malformed individuals, vaccination, or emergency slaughter.
- Long bathing: the anaesthetic is added into the tank where fish are held for a long duration.

Doses vary according to the purpose, low doses can be used for sedation prior to transportation, medium doses can be used for stunning before killing, and high doses can be used to induce death by overdose for slaughter. Some time is needed before effects come into action, up to tens of minutes depending on doses (EFSA 2009b). When used by bathing, the molecule is mostly absorbed by the gills, and sometimes through the skin for some scaleless fish species (Jabahery & al. 2012) and circulates in the bloodstream to reach the targeted nervous pathways.

The thickness of the skin has an influence on the absorption of the anaesthetic. Thus, thinner or loosely scaled skin favours absorption compared to thicker or densely scaled skin (Ferreira & al 1984 cited by Neiffer & Stamper, 2009).

When muscular activity decreases, ventilation decreases too, leading to a reduction of available oxygen for organs, but it can be compensated by good water oxygenation. Molecules are then degraded by the organism and the effect ceases.

Some authors report that isoeugenol works by blocking sodium calcium and potassium channels, inhibiting N-methyl D-aspartate (NMDA) receptors, and potentiating GABAergic receptors (Sneddon 2012).

Anaesthetics can be introduced into the water under different forms, either as a liquid, or as a powder to be dissolved, and is then absorbed by the fish gills (Schroeder & al. 2021).

- **Current use**

Among the previously mentioned anaesthetics, only isoeugenol is used in commercial settings for consumption purposes.

It is produced and developed in New-Zealand under the commercial name “AQUI-S”, for the high-end Atlantic salmon sector to improve animal welfare and flesh quality at slaughter. Unlike eugenol, isoeugenol does not modify the taste of the flesh. The availability of isoeugenol is somewhat limited (Schroeder & al. 2021).

For the time being, the use isoeugenol to stun fish is not allowed within the European union due to uncertainties related to the impact of residues in terms of food safety for consumers. Regulation CE N° 363/2011 of the 13 April 20211 prohibits the sale for consumption of fish containing a residual isoeugenol concentration above 6000 µg/Kg. Requests of authorisation are under way in Europe and Northern America. Manners in which isoeugenol can be used varies depending on countries. In New-Zealand and Australia, the use of AQUI-S is allowed with no limitation. It can be used both throughout rearing (e.g to facilitate handling related to sea lice treatment for Atlantic salmon) and for stunning before killing for fish destined to consumption. In Chili and Viet Nam, its use is restricted to needs related to the rearing phase, with no upper limit. In Norway, Iceland and the Faroe Islands, its use is restricted to the rearing phase, and a regulatory holding period is in place. In Korea, Honduras and Costa-Rica, it can be used for slaughter with no regulatory limitation regarding residues (Bowman & Gräns 2019).

Laboratory safety rules for the use of AQUI-S indicate that it can cause organ damage if repeatedly ingested in high doses, and that it can cause skin and eye irritation (AQUI-S New Zealand Safety Data sheet). In the US, isoeugenol has been classified as "Generally Recognised As Safe" in 1965 (European Medicines Agency 2020). However, more recent studies have shown that isoeugenol may be carcinogenic to male mice which led to the end of attempts to authorise this molecule as an anaesthetic for fish destined to human consumption without any holding period between the last exposure and slaughter (Meinertz & al. 2009). The Center for Veterinary Medicine of the US Food and Drug Administration also reported safety concerns related to the consumption of fish flesh containing eugenol and/or isoeugenol residues (Center for Veterinary Medicine, US FDA, 2007).

In the case of anaesthesia prior to killing or in the case of killing by anaesthetic overdose, the residual molecules (remnants of undegraded anaesthetic molecules and degradation products) are no longer eliminated by the organism because the metabolism has stopped. In rainbow trout, after exposure to AQUI-S at either 14mg/ for 60 minutes or 34 mg/L for 10 minutes, isoeugenol residues in the flesh reached concentrations of 57 300 µg/Kg and 78 800 µg/Kg, i.e. approximately 10 times the limit of 6,000 µg/Kg authorised in the European Union for fish intended for human consumption (Meinertz & al. 2006).

The half-life of isoeugenol is 25 minutes in Atlantic salmon (Kiessling & al. 2009). The half-life of isoeugenol total residues equivalent in rainbow trout fillets has been estimated to be between 0.91 h and 1.73 h (Meinertz & al. 2009). When rainbow trout are exposed to 17 mg/L of AQUI-S for 60 minutes, they metabolise the molecule so that concentrations in tissue fall below the regulatory limit of 6000 µg/Kg 4 hours after exposure. These results imply that the use of isoeugenol for stunning by chemical anaesthesia immediately prior to slaughter in rainbow trout cannot comply with the European regulatory limit for isoeugenol residues in fish flesh. However, under certain conditions, sedation with isoeugenol, if carried out a few hours before slaughter, may potentially be compatible with this European regulatory threshold.

2.4.2 Welfare hazards, Welfarm's position and recommendations:

The welfare hazard analysis is only focused on isoeugenol because it is the only molecule that can potentially be used for anaesthetic stunning of fish destined to human consumption. As isoeugenol is structurally very similar to eugenol, some results from studies that investigated the effects of eugenol were taken into account in this analysis.

- **Exposure to noxious chemicals**

Anaesthetics can sometimes have an aversive nature (EFSA 2009b). This may affect fish during the induction of anaesthesia while they are still conscious. Anaesthesia reduces stress during handling which is a source of stress for fish. However, anaesthesia itself may also cause stress. Stress caused by anaesthetics can manifest as a physiological stress response without any behavioural reactions. For example, MS-222 causes high physiological stress in sea bream at low doses (Molinero & Gonzalez 1995) manifested as an increase of biomarkers like plasma cortisol, glucose and lactate.

In more severe cases, it can result in aversive behavioural reactions (rapid swimming, head shaking, rapid head movements described by some authors as "coughing", escape attempts), which indicate a state of severe stress, marked discomfort and potential irritation of the gills and/or skin caused by the molecule. These reactions can vary according to the dosage and sensitivity of different species. Eugenol, which is an oil, coats the gill epithelium thus blocking respiratory pathways and gas exchanges (Sladky & al 2001 cited by Neiffer & Stamper, 2009). In addition, repeated exposure to low doses of eugenol can result in mild necrosis of the gills (Afifi & al 2001 cited by Neiffer & Stamper, 2009).

Physiological stress

Atlantic salmon

The study by Eriskon (2011) showed that Atlantic salmon anaesthetised by exposure to 17mg/L of AQUI-S for approximately 30 minutes, had plasma lactate values ranging between 4.7 and 6.9 mmol/L compared to 2.2 mmol/L in controls. A slight increase in plasma glucose was also observed (between 3.6 and 5.6 mmol/L compared to 3.3 mmol/L in controls), however blood and muscle pH were relatively similar to that of controls and cortisol levels were not studied. Zahl & al. 2010 also reported that exposure to 5.4 mg/L of isoeugenol for about 5 minutes induced a rise in cortisol, with a peak at 0.23 ng/g/h after one hour, requiring 5 hours to return to basal levels. However, stress biomarkers induced by isoeugenol were less significant than those induced by the other anaesthetics tested in this study, like MS-222. The authors suspect that isoeugenol, as well as the other anaesthetics tested, may be irritating to the fish's skin and could cause damage to their mucus. Schroeder & al. 2021, observed a slight increase in cortisol levels after salmon were exposed to 2mg/l of isoeugenol. When exposed to a higher concentration of isoeugenol (12.5 mg/l), an increase in cortisol was also observed. Thus, isoeugenol appears to induce a physiological stress response in salmon.

Rainbow trout:

Davidson & al. 2000 reported that anaesthesia with 17mg/L of AQUI-S caused a sharp rise in cortisol levels in rainbow trout after 30 minutes, reaching 293 ng/L compared to 13 ng/L in controls. Cortisol levels then decreased back to basal level after four hours, but a second peak was observed 16 hours after exposure. The authors speculate that this second peak could be explained by gill irritation caused by the molecule lasting for several hours. Haematocrit levels also increased significantly (40.9%) after 30 minutes of isoeugenol exposure compared to controls (24.5%). Moreover, during crowding, fish previously exposed to AQUI-S showed higher cortisol levels compared to controls. The authors therefore consider that anaesthesia with 17 mg/L AQUI-S causes significant physiological stress in rainbow trout and does not reduce the stress response induced by crowding. Zahl & al. 2012 also report that elevated plasma catecholamine concentration and haematocrit levels were observed in response to isoeugenol in Chinook salmon - another species of the genus *Oncorhynchus* to which rainbow trout belong. However, Wagner & al. 2002 showed that anaesthesia at a dose of 35-40 mg/L of AQUI-S effectively reduced stress caused by a stripping procedure in trout, but a peak in cortisol levels was observed 20 h after anaesthesia similar to the peak observed 16 h after anaesthesia by Davidson & al. 2000. The authors suspect that this cortisol peak is due to gill irritation caused by the anaesthetic.

Hoskonen & al. 2006 suggest that clove oil limits stress induced by handling in rainbow trout. In a 2018 review including 10 studies about clove oil (eugenol) anaesthesia in rainbow trout, elevation of stress biomarkers was identified in at least 4 of the studies (Priborsky & Velisek 2018). The authors conclude that "the physiological stress response of fish anaesthetised with clove oil could be minimal or significant, depending largely on the duration of anaesthesia and on the dose" (Priborsky & Velisek

2018). The study by Sink & al. 2007 showed that exposure to eugenol led to significantly higher cortisol levels in treated fish compared to controls (about 49-56 ng/ml vs. about 11.8 ng/ml), but the levels were significantly inferior compared to unanaesthetised trout submitted to stressful crowding. The study showed that eugenol reduced stress but was not as effective as MS-222. In contrast, the study by Wagner & al 2003 cited by Sink & al. 2007 showed a better efficacy of eugenol compared to MS-222.

European sea bass:

The physiological response of sea bass to isoeugenol or eugenol anaesthesia has been little studied to our knowledge. Di Marco & al (2007) reported high plasma lactate levels in sea bass anaesthetised with clove oil (eugenol) (100 mg/L) compared to those killed by cranial percussion or ice slurry immersion. Simitzis & al. 2013 found a slightly lower muscle pH in sea bass anaesthetised with clove oil compared to sea bass shot by frontal percussion or immersed in ice slurry.

Gilthead sea bream:

During a simulated transport, sea bream sedated with 2 mg/L of AQUI-S showed a rise of cortisol, plasma glucose and osmolality compared to controls (which also endured a simulated transport but without sedation), indicating a physiological stress response (Jerez-Cepa & al. 2021). Similarly, it was observed that when sedated with clove oil (eugenol) at 2.5 mg/L during transport, blood cortisol levels as well as the expression of genes related to cortisol secretion measured in adrenal tissues in fish were higher than in control (Jerez-Cepa & al. 2019). Karabournioti 2015 reported that a longer duration of exposure to AQUI-S was associated with a rise of plasma glucose, which could indicate a stress response, although no effect was detected regarding haematocrit levels. However, Matos & al. 2010 demonstrated that sea bream anaesthetised with AQUI-S (60 µL/L) had a lower level of physiological stress than sea bream crowded without anaesthesia, based on the evolution of *rigor mortis* and muscle pH. Lopèz-Canovas & al. 2019 and De la Rosa 2021 compared the effect of immersion in ice slurry with or without dissolved eugenol. Their results showed that adding clove oil (eugenol) (5 to 15 mg/Kg of ice) encapsulated with B-cyclodextrin (to make it more water soluble) to the ice slurry decreased stress biomarkers. They also showed that adding eugenol reduces the time before immobilisation in the ice slurry. In blue bream (*Sparidentex hasta*) belonging to the sparidae family (like our target species *Sparus aurata)*, anaesthesia with AQUI-S can decrease cortisol levels (Al-Roumi & al. 2014).

Thus, it appears that exposure to isoeugenol or eugenol can cause a physiological stress response in all of our target species. Rainbow trout seems to be the species for which this physiological stress response to isoeugenol is the strongest. Despite this physiological stress response, there is also evidence that anaesthesia with isoeugenol or eugenol can reduce handling stress under certain conditions, but this is not consistently observed.

Behavioural aversive reactions

Atlantic salmon

The study by Erikson (2011) did not observe behavioural aversive reactions in salmon exposed to 17mg/L AQUI-S for 30 minutes. Zahl & al. 2010 did not observe aversive behavioural reactions in salmon anaesthetised with 5.4 mg/L isoeugenol for 5 minutes either.

Rainbow trout:

Wagner & al. 2002 reported that when rainbow trout were placed in a water bath with an AQUI-S concentration of 80 mg/L, fish reacted by violently shaking their heads. However, they quickly reached stage III anaesthesia at this high concentration. The study by Sink & al. 2007 showed violent head shaking accompanied by a behaviour described as "coughing" following the administration of the isoeugenol. The study by Keene & al 1998 reported that all trout exposed to eugenol exhibited a form of "coughing" for 20 seconds prior to the effect of eugenol. These reactions were observed at all the concentrations that were tested, including low doses: 1 ppm, 2ppm, 5 ppm, 15 ppm and 30 ppm. Other authors who have studied isoeugenol and eugenol in rainbow trout did not report aversive behavioural reactions even in cases where anaesthesia produced a physiological stress response.

European sea bass

No behavioural aversive reaction to isoeugenol or eugenol has been described in the literature for sea bass.

Gilthead sea bream

No behavioural aversive reaction to isoeugenol or eugenol has been reported in the literature in sea bream to our knowledge.

Thus, brief behavioural aversive reactions have been observed in rainbow trout in response to eugenol or isoeugenol exposure. However, this phenomenon has not been observed in Atlantic salmon, sea bass or sea bream to our knowledge but it cannot be excluded that it could be reported in the future. A recent literature review of 18 tropical and subtropical fish species mentioned that no published results showing aversive reactions induced by isoeugenol had been reported in any of the species studied (Bowman & Gräns 2019). Barbas & al. 2021 reported brief behavioural aversive reactions during an exposure to eugenol in the Tambaqui fish (*Colossoma macropomum)* (personal communication with researcher Luis Andre Barbas).

Readman & al. 2013 developed a method to study the potential aversiveness of anaesthetics. Fish are maintained in a tank, half of which contains a dissolved anaesthetic (at 50% of the dose required to induce anaesthesia) while the other half does not. Time spent and distance swum in each of the two area as well as swimming speed are recorded and analysed. When both parts of the tank are filled with anaesthetic-free normal water, fish swim in a similar way in both areas. When one of the two halves of the tank contains dissolved hydrochloric acid, which is an aversive chemical with an extremely low pH, fish swim faster when they are in contact with the molecule, and strongly avoid the area. This method showed that seven out of the nine tested anaesthetics are perceived as aversive by zebrafish. Isoeugenol (used with a concentration of 10 mg/L) was considered the second most aversive anaesthetic among the nine molecules considered in this study. The authors recommend not to use aversive anaesthetic for this species anymore, and call for research on the possibility to sedate fish with non-aversive molecules prior to anaesthetising them with potentially aversive molecules. Such results in zebrafish mean that we need to be cautious about the potential aversiveness of isoeugenol for our target species.

Most anaesthetic studies only measure the time needed to induce a stage III anaesthesia in relation to dosage, and do not necessarily focus on the absence of aversive reactions. Therefore, it cannot be excluded that some of the experiments conducted so far may have triggered behavioural aversive reactions without it being reported by the authors in their results. Ensuring the absence of behavioural aversive reactions is an important aspect that should be taken into account in future studies. A study design similar to that of Readman & al. 2013 on zebrafish should be used to properly assess this aspect in our target species.

Chemical anaesthesia with isoeugenol obtains the mark “B-Phy-Bh” for rainbow trout, meaning that it triggers both physiological and brief behavioural aversive reactions. According to our scoring system, this hazard is disqualifying when it reaches the level “B-Phy-Bh”. Therefore, this method is not satisfying for rainbow trout in this aspect. This method obtains the mark “B-Phy” for Atlantic salmon, sea bass and sea bream, because it can cause the elevation of stress biomarkers even though behavioural aversive reactions have not been reported for these species.

- **Immediateness of the loss of consciousness**

Generally speaking, studies about the loss of consciousness induced by isoeugenol use behavioural indicators which are less reliable than EEG assessment (Bowman & Gräns 2019, Berg & al. 2021). Authors generally limit their observations to reporting that fish reach a stage of anaesthesia corresponding to stage III-1 and/or III-2 of the previously presented grid. In rainbow trout, after the induction of a level of anaesthesia with MS-222 corresponding approximately to the stage III-2 previously mentioned, the loss of consciousness has been confirmed by EEG after approximately 5 minutes of exposure (Bowman & al. 2019). This could potentially mean that the behavioural state corresponding to stages III-1 and III-2 of anaesthesia, when observed in response to other molecules, is probably associated with the loss of consciousness. However, this is an extrapolation. A proper confirmation of the loss of consciousness by EEG assessment during anaesthesia with isoeugenol on our target species is required. Moreover, a study on Tambaqui fish (Colossoma macropomum) showed that during eugenol anaesthesia, brain activity resembling an epileptiform seizure was recorded instead of a progressive decrease in brain activity which is usually observed during anaesthesia (Barbas & al. 2021). The authors think that fish are likely to be unconscious during this epileptiform seizure but are worried about the potential harmful neurological consequences of this seizure on them, if the goal is only to anaesthetise and not to kill (personal communication with researcher Luis Andre Barbas).

Atlantic salmon:

The study by Eriskon (2011) showed that when exposed to a concentration of 17mg/L of AQUI-S, Atlantic salmon showed initial signs of sedation after 2-3 minutes, then, they lost balance and stopped swimming after 4-9 minutes. However, 30-40 minutes were required to induce complete anaesthesia associated with the loss of the vestibulo-ocular reflex. According to Schroeder & al. 2021, the time needed so that exposure to 2.5 mg/l of isoeugenol produces anaesthetic effects is about 5–15 min. During this time, fish swimming activity, oxygen consumption, CO_2 production and anxiety are reduced (Schroeder & al. 2021). However, this dosage is not sufficient to induce a loss of balance in salmon. A concentration of 12.5mg/l induces anaesthesia within 15 minutes.

Rainbow trout:

The study by Wagner & al. 2002 found that the average induction time of anaesthesia is around 133 seconds (and up to 210 seconds) with 40 mg/L of AQUI-S. With 17 mg/L of AQUI-S, Davidson describes that trout are slightly sedated after 2-3 minutes, but the full induction of anaesthesia does not occur until 8-10 minutes. During the 30 minutes of exposure, rainbow trout always retained their opercular beat. Zahl & al. 2012 mentioned a duration of 12 to 15 minutes to induce a proper anaesthesia with a dosage of 7.5 to 10 mg/L of isoeugenol. According to Prisborsky & Velisek 2018, exposure to 40-60 mg/L of clove oil (eugenol) for 3-6 minutes is sufficient to induce surgical anaesthesia. Wagner & al. 2003 reported an average induction time of 126 seconds for one batch, and 68 seconds for another with 60 mg/L clove oil. The study by Keene & al. 1998 showed that following exposure to 5mg/L of eugenol, trout lost their response to stimuli that would normally induce fear, and they also displayed a partial loss of balance. When they were no longer exposed to the chemical, fish recovered their ability to swim normally after the elimination of the molecules. With 40-140 mg/L of eugenol, the complete loss of balance and reactivity only occurred after 30-50 seconds of exposure. Ossen & al. 2017 reported an anaesthesia induction time of 1.9 minutes for fish exposed to 250 mg/L of AQUI-S 20E (corresponding to 25 mg/L of eugenol). Other reports in the literature mention that eugenol can induce the loss of equilibrium (mild sedation) in adult rainbow trout within 3 minutes of exposure (Prince & al. 2000). Exposure to a concentration of 30 mg/L induced a loss of balance, swimming behaviour and weakened opercular movements.

<u>Gilthead sea bream:</u>

Jerez-Cepa & al. 2021 reported that sea bream exposed to 5 mg/L of AQUI-S are slightly sedated after 6.6 minutes and reach deeper sedation only after 25 minutes. At 15 mg/L, deep anaesthesia is achieved in about 10 minutes, at 30 mg/L, a duration of 3.9 minutes is required, and at 60mg/L, anaesthesia is achieved in only 83 seconds. For clove oil (eugenol), Jerez-Cepa & al. 2019 described an anaesthesia induction time of 9 minutes in sea bream at 20mg/L, 3 minutes at 40 mg/L, and about 80 seconds at 60 mg/L. Karabournioti 2015 showed a positive correlation between the weight of sea bream and the time required for the induction of anaesthesia at 50 mg/L of AQUI-S: 4 minutes are required for 50 g fish, and 11 minutes are required for 450 g fish. At 40mg/L of clove oil, less than three minutes are required to induce anaesthesia in sea bream (Mylonas & al. 2005).

<u>European sea bass:</u>

At 40mg/L of clove oil, less than three minutes are required to induce anaesthesia in sea bass (Mylonas & al. 2005). At 105 mg/L of clove oil, the induction of anaesthesia takes about 2 minutes (Simitzis & al. 2013).

Thus, the loss of consciousness is not instantaneous during chemical anaesthesia with isoeugenol or eugenol in all our target species. Depending on the dosage, the first sedative effects occur within a few minutes, and between one minute and several tens of minutes are needed to induce deep anaesthesia. Higher dosages favour a shorter induction time of anaesthesia. Fish size tends to increase the duration of anaesthesia induction (Karabournioti 2015). A higher water temperature favours a faster induction (Javahery & al. 2012).

Fish are likely to be affected by density, water quality and physiological stress (or even aversive behavioural reactions) triggered by the anaesthetic during the time period before reaching unconsciousness. Chemical anaesthesia with isoeugenol obtains the mark “B” for this hazard. According to our scoring system, this hazard is disqualifying. Therefore, chemical anaesthesia with isoeugenol is not satisfying in this. However, it is likely that the progressive sedation leading to unconsciousness limits the negative effects of welfare hazards to some extent. The interpretation of the mark obtained for this hazard should therefore take into account this aspect.

- **<u>Risk of recovery of consciousness (reversibility)</u>**

Chemical anaesthesia can induce either a reversible or an irreversible loss of consciousness depending on dosage and on the duration of exposure. The recovery of consciousness can occur 5 to

10 minutes after putting fish in anaesthetic-free water (Schroeder & al. 2021). In that case, the anaesthetic is excreted through the gills and urine (Schroeder & al. 2021).

Several lethal dosages have been reported in the literature. According to Keene 1998, exposure of rainbow trout to 15 - 30 mg/L eugenol for 96h results in 100% mortality. The lethal concentration of AQUI-S for 50% of individuals is 7.7mg/L after 96h exposure in rainbow trout (AQUI-S safety data sheet, AQUI-S New Zealand ltd). In sea bream, at 55 mg/L of clove oil, if exposure to the molecule is prolonged for 15 minutes after reaching deep anaesthesia, it results in a mortality rate of 83%. Matos & al. 2010 used a concentration of 270 µL/L of AQUI-S to achieve a lethal anaesthetic overdose in seabream. Neiffer & Stamper 2009 reported that, in general, using a dose 5 to 10 times higher than the dose known to induce anaesthesia, and leaving fish immersed in the solution for 5 to 10 minutes after the complete cessation of opercular beats results in death by overdose for various anaesthetic molecules.

In the case of non-lethal dosing, if the dosage and duration of exposure are sufficient, it appears possible to bleed fish without them regaining consciousness, if bleeding takes place quickly after the induction of anaesthesia. However, if the dosage and duration of exposure are insufficient, or if fish are placed for several minutes in anaesthetic-free water after anaesthesia while waiting to be bled, there is a risk of recovery of consciousness. In the case of lethal overdose, there is no risk of regaining consciousness.

Chemical anaesthesia with isoeugenol obtains the mark “A/B” for this hazard.

- **Failure rate**

The stunning failure rate has not been quantified for this method. Therefore, chemical anaesthesia with isoeugenol obtains the mark “?” for this hazard.

- **High or extreme densities**

Fish need to be exposed to anaesthetics for several minutes and it is likely that they will be held in tanks at high density during this time. The anaesthetic may be able to limit the ability of fish to feel frictions between individuals, and more generally could reduce the stress related to high density. That being said, in the study by Erikson & al. 2011, two to three minutes were needed before the onset of the first sedative effects (partial to total of responsiveness to stimuli). During this time, it is likely that fish could experience discomfort related to the density.

Chemical anaesthesia with isoeugenol obtains the mark “B” for this hazard.

- **Poor water quality**

Water quality may not be very good due to the high density, even though fish may not be very agitated thanks to sedation and thus may not produce a lot of waste. Similarly to the issue of density, the anaesthetic could reduce the potential discomfort related to poor water quality, but as the sedative effects only appear after a few minutes (Eriskon & al. 2011), fish risk to experience some stress before the onset of sedation and anaesthesia.

Chemical anaesthesia with isoeugenol obtains the mark “B” for this hazard.

- **Reliance on workers’skills**

The technicality involved in dosing anaesthetics can be challenging. Workers need to administer the right quantity of anaesthetic to reach the desired level of anaesthesia. The quantity of fish, the species, the average weight of fish, and the water volume need to be taken into account. In the case of death by lethal anaesthetic overdose, the precision of dosing may be less of an issue as effects can be secured by overdosing within the limits of acceptable food safety thresholds.

Chemical anaesthesia with isoeugenol obtains the mark “C” for this hazard.

WELFARM’s position and recommendations:

For the time being, it is difficult to come up with a definite position on the use of chemical stunning using isoeugenol anaesthesia, although this method has some potential. This method could be of particular interest for on farm euthanasia of fish that are not intended for human consumption, as it is inexpensive and requires few logistics.

The presence of brief aversive reactions described in rainbow trout suggests that this method should not be recommended for this species unless further studies show that it is possible to avoid this effect. In addition, a proper confirmation of the loss of consciousness by EEG and not only by behavioural indicators is needed. The main other drawbacks are the induction of physiological stress in response to exposure to chemicals, and the fact that fish would be held in high density in water of potentially poor quality. The loss of consciousness is not instantaneous which disqualifies this method according to our scoring system. However, even though unconsciousness is not instantaneous, it may be less problematic if the period prior to the loss of consciousness is not associated with significant suffering (when there are no strong behavioural aversive reactions). Initial sedative effects prior to the loss of consciousness may potentially reduce the discomfort of fish during this period. More studies are necessary to assess whether animal welfare can be safeguarded during the period leading to unconsciousness. European regulation on acceptable residue levels in fish flesh is currently a barrier to implementation.

More studies are needed to provide a final position on this subject. So far, most studies have been focusing on reversible anaesthesia before handling. More studies focused on pre-slaughter stunning and lethal overdose would be necessary. Rigorous assessment of the loss of consciousness, risks of recovery of consciousness, failure rates, and of the potentially aversive nature of anaesthetics in terms of physiological and behavioural stress response should be the aims of future studies. In addition, scientific studies about anaesthetic dosages have often been carried on juvenile or on small fish. As Neiffer & Stamper (2009) point it out, caution is required when using these dosages as a reference for adult fish. Studies about the potential aversiveness of polysorbate 80 (soluble emulsifier) which is an excipient of AQUI-S should also be carried out.

In 2009, regarding chemical anaesthesia prior to slaughter, regardless of the molecule being used, the EFSA concluded that "As there is no acceptable method for the use of available pharmaceuticals for euthanasia, more research is needed in this area" (EFSA 2009b). In its opinion published in 2021 on stunning and slaughter methods for salmonids, the animal welfare board of the Swedish University of Agricultural Sciences concluded that “it has not been possible to find satisfactory scientific evidence that anaesthetic methods are acceptable in terms of animal welfare”.

Thus, WELFARM considers that the use of isoeugenol anaesthesia as a method of stunning prior to killing, or as a method of killing by lethal overdose, has some potential. However, the degree of uncertainty seems too high to recommend this method for the time being. If future studies ensure that this method is effective and does not lead to strong behavioural reactions related to aversiveness, it could be reconsidered.

Isoeugenol or similar anaesthetics could also be used for pre-stunning during handling and air exposure related to other slaughter methods (see part III.2 for more details).

The use of anaesthetics requires skills and knowledge regarding adequate dosing, but risks of dosage mistakes are more limited in the case of lethal overdosing.

The manufacturer of AQUI-S recommends a two-step dosing procedure, whereby fish are first exposed to a low concentration to induce sedation, and then the concentration is increased. This way, fish are already sedated or even anaesthetised when the concentration is increased, which could potentially limit aversive effects. This recommendation is based common sense but would need to be rigorously studied to be confirmed.

Administration methods that require the least amount of handling should be preferred. Long bathing i.e exposure for several minutes by dissolving the anaesthetic in holding water seems to be the most relevant pathway.

To limit issues related to poor water quality, it is important to keep the water well oxygenated to avoid stress throughout sedation, anaesthesia, or euthanasia for as long as fish remain conscious. The density at which fish are held during bathing should be minimised within the limitations imposed to maintain a realistic slaughter line pace.

Method	Exposure to noxious chemicals		Immediateness of the loss of consciousness	Failure rate	Risk of recovery of consciousness (reversibility)	Air exposure	High or extreme densities	Handling	Poor water quality	Tissue damage	Pre-stun Electrical shock	Thermal shock	Reliance on workers'skills
Isoeugenol anaesthesia	Trout **B- Phy-Bh**[7]	Salmon, sea bass, sea bream B - Phy	**B**[8]	?	A/B	A	B	A	B	A	A	A	C

7: Behavioural aversive reactions have only been reported in rainbow trout so far, but the possibility that similar reactions could be reported in salmon, sea bass and sea bream in the future cannot be excluded. Behavioural aversive reactions are not systematically reported for rainbow trout, and it may be possible to alleviate them by using a progressive increase of the concentration although it is still uncertain

8: The loss of consciousness is not immediate and supervenes after the induction of some level of physiological stress or even behavioural aversive reactions. However, if future studies are able to establish that chemical anaesthesia can induce unconsciousness without evoking behavioural aversive reactions while limiting physiological stress, the non-instantaneous nature of the loss of consciousness may be less problematic than for other methods.

2.5 Electrical methods

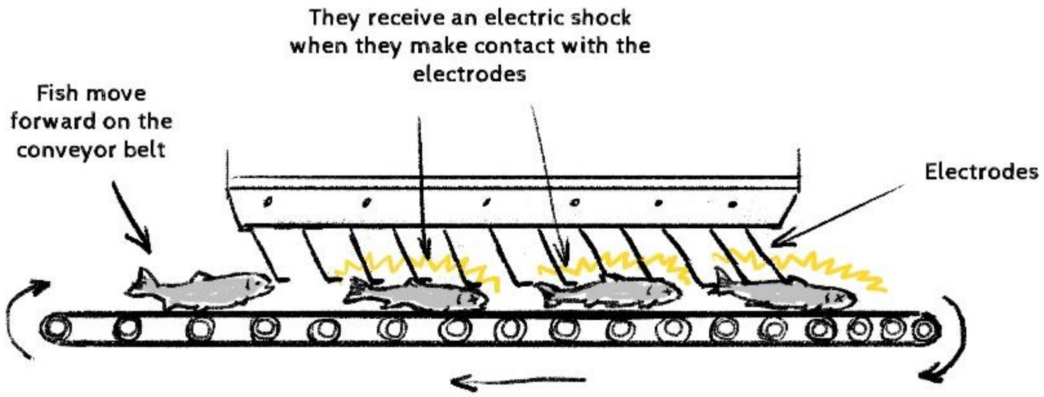
2.5.1 Method description and current use

- **Description**

Electronarcosis consists in stimulating the central nervous system with an electric current to induce a state of immediate unconsciousness and insensibility in fish. The application of electrical current through the brain produces an instantaneous depolarisation of neuron membranes resulting in a massive epileptic seizure (ANSES 2014; CIWF 2018; EFSA 2009abc). During this seizure, fish go through a tonic phase where the whole-body contracts, and a clonic phase characterised by rapid erratic body motions, followed by immobility (EFSA 2009a). The clonic phase is not always present though (Robb & al. 2002).

There are several methods to induce electronarcosis:

- "**Head-only**" electrical stunning is conducted outside of water and consists in applying an electrode directly on the fish head to deliver an electric shock. The fish is positioned head first by a worker.



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- FIGURE 4: HEAD-TO-BODY ELECTRICAL STUNNING (PICTURE : WELFARM ©)

- "**Head-to-body**" electrical stunning (figure 4) involves at least two electrodes: one touching the head, and the other one touching the rest of the body. It works by moving fish forward on a conveyor belt above-which metal strips acting as electrodes are hanging. The metal strips come into contact with fish as the conveyor belt moves them forward into the system. This method can be executed without any water, or while leaving a small quantity of water (though not enough for the fish to swim or breathe) in the system.
- "**Dry batch**" electrical stunning consists in putting fish in an empty metallic tank without water, the sides and/or the bottom of which are electrodes. Once the electrodes are turned on, fish are exposed to an electrical shock, either through the direct contact with the electrodes, or indirectly as the current travels across the bodies of the fish in contact with one another
- "**Dry prod**" electrical stunning consists in putting fish in an empty tank without water. Then, a metallic prod acting as an electrode is lowered into the batch of fish and delivers an electrical shock to the fish making contact with it. The shock may then indirectly reach other fish by traveling across the bodies of the fish in contact with one another. This method can be used with a single prod or with several prods. In some cases, the metallic prod is shaped like a "Y".

Other methods are practiced under water. Electrodes are positioned in the system and electricity circulates between them, by going through the water and the fish who act as electrical conductors. There are four in-water electrical stunning methods (Lines & al. 2003):

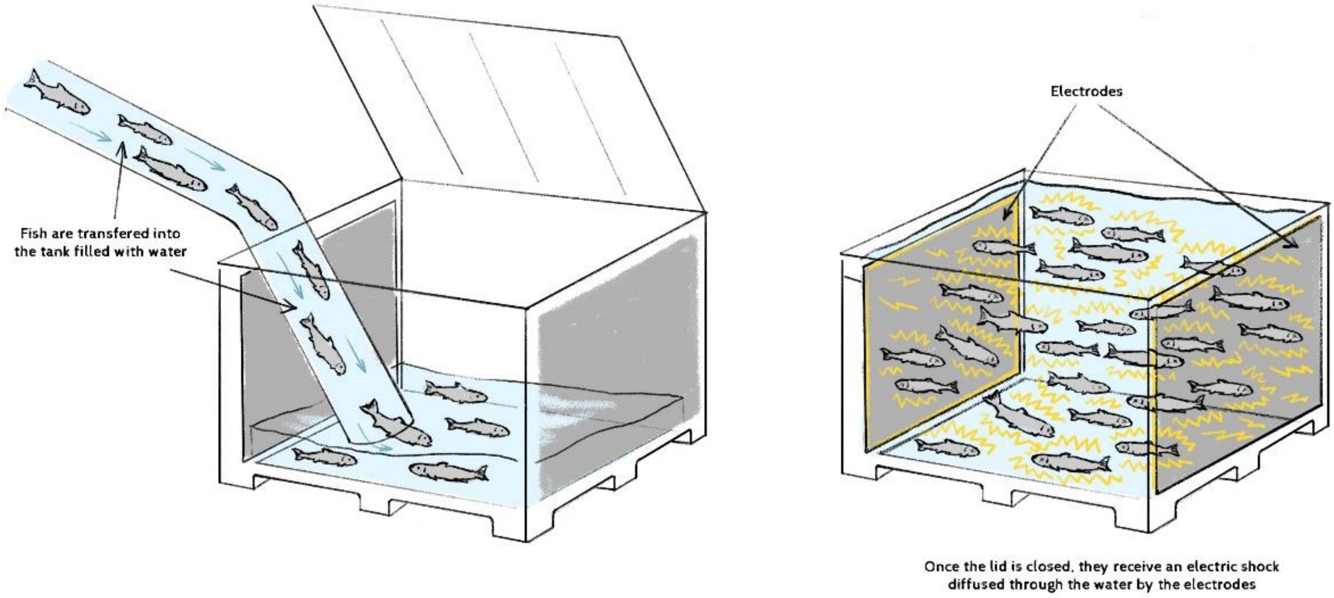

FIGURE 5: IN-WATER BATCH ELECTRICAL STUNNING (PICTURE : WELFARM ©)

- **In-water batch** electrical stunning (figure 5): a batch of fish is put into a water-filled tank, the sides of which are covered with metallic plaques acting as electrodes. Once the fish are inside, the lid is closed and the system is turned on. (videos of such systems can be found here: Fish_electric_slaughter https://www.youtube.com/watch?v=L18fRyZgHh8; FIAP Profiwork Fishstunner is a robust and low-maintenance device https://www.youtube.com/watch?v=Nzz9kfeg9vo).

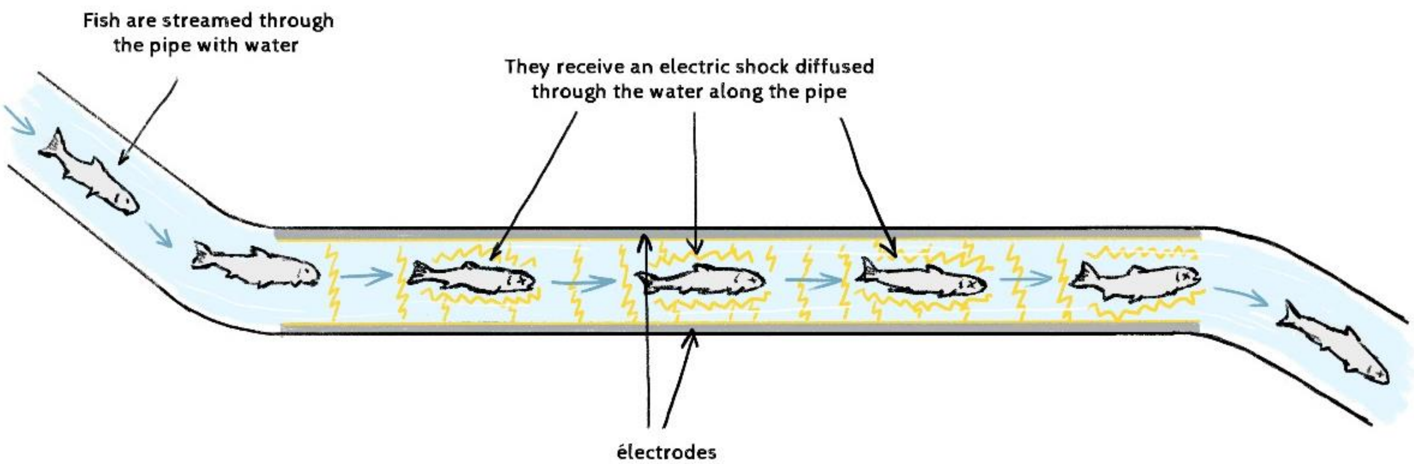

FIGURE 6: IN-WATER PIPELINE ELECTRICAL STUNNING (PICTURE : WELFARM ©)

- **In-water pipeline** electrical stunning (figure 6): this method consists in streaming fish along with water through a pipeline by using a pump. The electrodes are positioned along the pipeline to create an electric field within the pipe during the whole journey of the fish inside the system. The longer the pipeline, the longer fish will be exposed to the electric field.

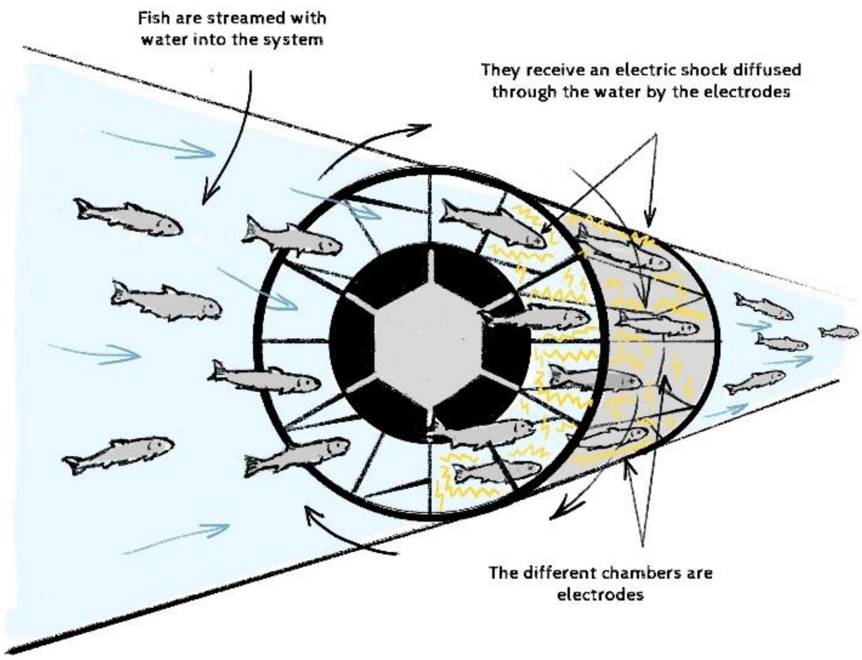

FIGURE 7: IN-WATER ROTATING ELECTRICAL STUNNING (PICTURE : WELFARM ©)

- **In-water rotating** electrical stunning (Lines & al. 2003) (figure 7): this method works with a cylinder divided in several chambers. The chambers are metallic and act as electrodes. Fish arrive into the chambers through a pipe in which water is streamed, and receive an electric shock. The cylinder rotates on its own axis so that fish are maintained in the chambers for a while where they are submitted to electronarcosis. This rotation allows for the duration of exposure to the electrical shock to be long enough to induce unconsciousness for a sufficiently long duration. Once the chamber arrives in front of an opening, the water flow expels the stunned fish out of the system.
- **In-water prod electrical stunning** consists in putting fish in a tank full of water. Then, a metallic prod acting as an electrode is lowered into the tank and delivers an electrical shock. The shock is delivered directly to the fish making contact with the electrodes, and indirectly as the prod creates an electrical field around itself in the water. If several prods are used, it is possible to use one prod as a positive terminal and the other one as a negative terminal to create an electrical field in the water between the two prods. In some cases, the prod may be shaped like a "Y".

- **Current use**

Electrical stunning is used in Denmark, Italy and France for rainbow trout. **In France, the European commission (2018) reported that this method was often used after an immersion in icy water.** In 2021, Aqualande, the French and E.U leading company in rainbow trout farming, announced an investment of 1 million euros to implement in-water electrical stunning on their sites (Aqualande RSE report). In France, in the 2018 official survey by the French ministry of agriculture, 88 salmonid producing companies (out of 365 surveyed companies), 1 marine fish farming company (out of 28 surveyed companies) and 6 extensive pond fish farming companies (out of 211 surveyed companies) declared using electrical stunning (Agreste 2020). The survey does not specify which type of electrical stunning companies are using. In addition, a research project about electrical stunning for sea bream and sea bass is currently being conducted in France, lead by the technical institute of aviculture (ITAVI) as part of the B – ABA project, initiated by the French fish farming interbranch (personal communication with the ITAVI). In the United-Kingdom and in Ireland, electrical stunning is used on Atlantic salmon, but it is not the most commonly used method. A field study investigating 18 slaughter plants for rainbow trout in Germany revealed that 48% of the studied sites were using electrical stunning (Jung-Schroers & al. 2020). Finally, European sea bass and gilthead sea bream are not commonly stunned through electronarcosis according to the European commission (2018) and the EFSA (EFSA 2009c). However, those warm-water species have been the subject of studies about electrical stunning.

It appears that in practice, head-only electrical stunning is not really used in commercial settings and is rather used in research protocols, especially for studies investigating the efficacy of different electrical parameters to stun fish (HSA 2018).

Regarding head-to-body electrical stunning, such machines are available on the market[4] to stun rainbow trout and Atlantic salmon, and marginally for sea bass and sea bream (CIWF Driving innovation in humane fish slaughter). Some Turkish producers own this type of device to stun sea bass and sea bream (European Commission 2018, personal communication with NGO Kaffesiz Türkiye). However, in practice, Turkish producers only use electrical stunning when it is explicitly required by their client, as using it somewhat slows down the slaughter line pace, although this opinion is not shared among all Turkish producers (personal communication with NGO Kaffesiz Türkiye). Head-to-body machines developed in-house by fish farmers themselves or by local electricians hired for this purpose are sometimes used in Germany (Jung-Schroers & al. 2020).

In-water electrical batch stunning is used commercially for rainbow trout and Atlantic salmon (EFSA 2009ab), and studies have been conducted on this type of system for gilthead sea bream (Van de vis & al. 2003) and sea bass (Lambooij & al. 2008, Zampacavallo & al. 2015). In Germany, a field study that looked at the slaughter process of 18 sites producing rainbow trout found that this method was the most common type of electrical stunning used among those sites (Jung-Schroers & al. 2020). This method is also commonly used in Switzerland, in particular with the equipment sold by the company Rundum Fisch (https://rundumfisch.ch/produkt/fischbetaeubung-ruf-100/). In-water electrical batch stunners can either be machines sold by specialised companies or designed in-house by fish farmers by hiring a local electrician (Jung-Schroers & al. 2020, personal communication with a stunning equipment producer company). This method allows for low to moderate slaughter line pace. The project StunFishFirst, which was aimed at developing electrical stunning technologies for fish, mentions in its report that a trial of in-water electrical batch stunning that works with seawater has been conducted for sea bass on a commercial setting by a Turkish producer named Noordzee Su Urünleri (https://www.noordzee.com.tr/). In-water electrical batch stunners marketed by the German company FIAP are also used with sea water by some Thai producers (personal communication with a stunning equipment producer company).

As for in-water pipeline electrical stunning, this method is available for Atlantic salmon, rainbow trout, sea bream and sea bass (EFSA 2009ab, Papaharisis & al. 2019, see Humane Stunner Universal by the Scottish company Ace Aquatec, Elektrobedover Askvik Aqua). It allows for high or very high slaughter line pace.

[4] For instance, the Norwegian company Optimar sells head-to-body electrical stunning machines. The Turkish company Smilefish sells head-to-body systems for sea bream and sea bass

Finally, in-water rotating electrical stunning has only been developed for rainbow trout, and to our knowledge this type of machine is not marketed by any company. The company Test Trout Valley, who was involved in the partnership that led to the development of those systems, still possess 4 prototypes and patented part of the mechanism. However, this company is not an equipment supplier but a rainbow trout producer. According to them, those machines allow for a slaughter line pace of 4 to 6 tonnes per hour. Only a few prototypes of this type of system have ever been produced (Lines & Kestin 2004a).

Dry batch electrical stunning, dry prod electrical stunning as well as in-water prod electrical stunning are used for rainbow trout in Southern European countries like Spain and Italy (https://www.youtube.com/watch?v=Yun0u5ynRoY&t=3s I pesci negli allevamenti europei | nuova INCHIESTA – Essere Animali, https://www.youtube.com/watch?v=AWnMs22tV5k Investigation Reveals Abuse of Sensitive, Intelligent Fish at American Farms – Mercy for Animals). A few years ago, in-water prod electrical stunning was used in Switzerland too (personal communication with a swiss rainbow trout farmer). We do not have proof that those methods are used in France, but it seems quite likely as we were told that some fish farmers carry out electrical stunning with systems designed in-house and crafted by local electricians (personal communication with a stunning equipment producer company). The European commission report and information presented by the statistics department of the French ministry of agriculture indicate that electrical stunning methods are used in France without indicating which sub-categories of electrical stunning methods are used (European commission 2018, Agreste 2020). Therefore, it is possible (but not proven) that those three methods could be used in France.

In-water electrical stunning methods appear to be more frequently used than dry electrical stunning methods (EFSA 2009abc).

2.5.2 Welfare hazards, Welfarm's position and recommendations:

- **Immediateness of the loss of consciousness**

In theory, if the parameters are adequate and if the system is correctly designed, electrical stunning methods can render animals unconscious instantaneously (i.e in less than 1 second, EFSA 2009ab, StunFishFirst, Lamboiij & al. 2008, Hjelmstedt et al. 2022). Different combinations of electrical parameters (voltage, frequency, intensity, duration of application, type of current (AC / DC) etc.) can be effective to induce instantaneous unconsciousness in fish.

However, if the parameters used are inadequate and/or if the electrical current mostly goes through a body part other than the brain, the loss of consciousness may not be instantaneous. In such cases, fish are at risk of consciously enduring pre-stun electrical shocks (see the section about pre-stun electrical shocks) during a few seconds. For reasons presented in the section about pre-stun electrical shocks, it can be assumed that there is a significant risk that the loss of consciousness may not be immediate (or may not even occur at all) for many fish with dry batch electrical stunning, and for both dry and in-water prod electrical stunning.

Electrical methods obtain the mark "A" for this hazard, except for dry batch electrical stunning, dry prod electrical stunning and in-water prod electrical stunning, which all obtain the mark "A/B". According to our scoring system, this hazard is disqualifying. Therefore, these three methods may be unacceptable in this aspect.

- **Risk of recovery of consciousness (reversibility)**

Electrical stunning methods can be reversible or irreversible depending on the parameters being used. Some parameters can also result in intermediate performance i.e leading to both a reversible stun on some individuals and to an irreversible stun on others. In practice, the parameters allowing for irreversible stunning (in that case, the process can be referred to as “electrocution”) are often associated with issues related to flesh quality. For this reason, parameters leading to reversible stunning are more likely to be used in commercial settings.

When using parameters resulting in a reversible stun, if the method is not quickly followed by an adequate killing method, fish will recover consciousness.

Atlantic salmon:

A study by Lambooij & al. 2010 (using dry head-to-body stunning) showed the existence of a risk of recovery of consciousness throughout exsanguination. In fact, despite the gill cut being performed shortly after (within 20 seconds) stunning by electronarcosis, one out of the three fish that were observed in the experiment showed responsiveness to noxious stimuli 3 minutes after stunning, before becoming unconscious again.

Rainbow trout:

After electrical stunning, some authors reported a duration of insensibility of at least 5 minutes in rainbow trout, assessed through behavioural indicators of consciousness (Lines & al. 2003, Lines & Kestin 2005, EFSA 2009ab). As the authors only observed the animals for 5 minutes, it cannot be excluded that some recovery of consciousness may still happen after the end of this 5-minute observation timeframe. Moreover, the authors reported that an unquantified “small proportion” of individuals was showing signs of consciousness after electrical stunning with a rotating system (Lines & al. 2003). A more recent study observed rainbow trout for 20 minutes after electrical stunning and found that almost no fish showed signs of consciousness based on behavioural indicators (Bermejo-Poza & al. 2021). As this duration is quite long, it is likely that the stun was irreversible for most individuals in this study. Similarly, another recent study also showed that it is possible to induce a permanent loss of consciousness with electrical stunning in rainbow trout if adequate settings are used (Hjelmstedt & al. 2022). The authors confirmed the loss of consciousness by EEG analysis (loss of visually evoked potentials (VEPs) of brain functions. They observed an immediate loss of consciousness lasting during the 15 minutes of observation post stunning (Hjelmstedt & al. 2022). The latency before the loss of consciousness in the case of bleeding without prior stunning is estimated to last 6 minutes in rainbow trout (EFSA 2009a), which is inferior to the 15-20 minutes of insensibility reported by Bermejo-Poza & al. 2021 and Hjelmstedt et al. 2022. This suggests that it is possible to bleed rainbow trout before they can recover consciousness after electrical stunning. Nonetheless, the possibility that a certain proportion of individuals could recover consciousness before or during bleeding cannot be entirely ruled out in the case of reversible stunning. In fact, the results of Hjelmstedt & al. 2022 show that when using a setting leading to a reversible stun, depending on the selected parameters, the duration of insensibility before the recovery of consciousness can last 10 to 415 seconds.

European sea bass:

Knowledge about electrical stunning is relatively limited for sea bass. Published results showed that an in-water electrical batch stunning was able to stun all individuals involved (Lambooij & al. 2008, Zampacavallo & al. 2015).

The duration of insensibility produced by reversible electrical stunning in sea bass varies between 30 seconds to 5 minutes (Lamboiij & al. 2008).

Gilthead sea bream:

There is very few available information about electrical stunning in sea bream in terms of reversibility and duration of insensibility. Results reported by Van de vis & al. 2003 mention that for a given set of parameters, out of 10 individuals, 9 were successfully stunned, among which 6 were permanently stunned, and 3 recovered consciousness as soon as 16 seconds after the stun.

As reversibility depends on the parameters, electrical methods obtain the mark "A/B" for this hazard.

- **Failure rate**

If inadequate parameters are used, particularly if the voltage is too low (under 50 V according to the EFSA), fish may consciously experience the electric shock for several seconds and become immobilised while remaining conscious. This phenomenon is called electro-immobilisation (EFSA 2009abc).

Rainbow trout and Atlantic salmon:

The stunning failure rate is estimated to be 3% for in-water batch electrical stunning, and 1% for in-water pipeline electrical stunning by the EFSA with no further precision about the associated parameters (EFSA 2009a). This figure is only available for rainbow trout. Nonetheless, it gives a general idea of the efficacy of those methods, that can reasonably be extended to other salmonids like Atlantic salmon.

For rainbow trout, the results of a field study in Germany showed that for in-water batch electrical stunning, the stunning failure rate was comprised between 0% and 5% in most sites (Jung-Schroers & al. 2020). On three sites, the failure rates were extremely high (30% on one site and 80% on two sites). However, those sites were characterised by a particular (too high or too low) water conductivity, and the use of inadequate stunning parameters (short application duration, low voltage). By modifying the parameters and the water conductivity, the failure rates improved to reach acceptable levels on those sites. Regarding head-to-body electrical stunning, the stunning failure rates of the sites that were investigated were comprised between 0% and 5% (Jung-Schroers & al. 2020). Failure rate has not been quantified for in-water rotating methods, but authors mentioned that only a "small proportion" of fish was showing signs of consciousness, be it due to stunning failure or due to recovery of consciousness (Lines & al. 2003).

European sea bass and gilthead sea bream:

For in-water pipeline electrical stunning for sea bass and sea bream, the equipment supplier company Ace Aquatec which sells such systems considers that 100% of individuals are correctly stunned (personal communication). We do not have scientific information to fact check this claim. However, the researcher Jeff Lines, who has first-hand experience with Ace Aquatec's system, told us that he did not see any sign of stunning failure or of recovery of consciousness in sea bass and sea bream stunned with this machine (personal communication).

As part of a trial conducted about head-to-body electrical stunning in Turkey for sea bream and sea bass in collaboration with CIWF, a failure rate of 3% was reported (CIWF Driving innovation in humane fish slaughter). However, this rate was estimated based on behavioural indicators and not through EEG. Therefore, it cannot be excluded that the true failure rate could be somewhat higher as it is possible that some fish that were deemed to be unconscious may only have been electro-immobilised.

<u>In-water electrical stunning with sea water:</u>

Several researchers in the field (Albin Gräns, Per Hjelmstedt, Dieter Steinhagen) indicated to us that caution is required when it comes to in-water electrical stunning done in sea water (personal communication). The high salinity of sea water gives it a conductivity which is higher than that of fish tissues. Consequently, a large proportion of the electric energy will circulate only through the water rather than through the fish tissues. Therefore, the field strength must be particularly high to reach thresholds allowing for the loss of consciousness in the case of in-water electrical stunning done with sea water, in order to compensate for this misdirection of energy. Field strength depends both on the voltage and on the distance between the electrodes. It appears possible to reach sufficient field strengths even with sea water in the case of pipeline electrical stunning, but it seems difficult to achieve in the case of batch stunners due to the longer distance between the electrodes. That being said, in-water batch electrical stunners are being used with sea water in Thailand and Turkey, and some equipment suppliers (personal communication with two stunning equipment producer companies) consider that it could be possible to adapt those systems for them to be efficient even with sea water. Nonetheless, as a precaution, we prefer not to recommend the use of in-water batch electrical stunning with sea water for the time being, unless evidence of effectiveness is brought in the future. Another option would be to carry out in-water batch electrical stunning with freshwater even for saltwater species, which some researchers are doing in an experimental context (personal communication with researcher Joao Saraiva, CCMAR, Algarve University). However, this option is sub-optimal from an animal welfare perspective as putting saltwater fish into fresh water will cause them to endure an osmotic shock giving rise to significant physiological stress.

<u>Dry batch electrical stunning, dry prod electrical stunning, in-water prod electrical stunning</u>

We do not have quantified estimates of the failure rates for dry batch electrical stunning, dry prod electrical stunning and in-water prod electrical stunning. However, some aspects lead us to think that the failure rate for those three methods is likely very high. It is very likely that the failure rate is well over the threshold of 5%. The reasons why we suspect the failure rate to be high for these three methods are the same reasons related to the risks associated with these methods regarding pre-stun electrical shocks : heterogeneity of the strength of the electrical field for in-water methods, lack of direct contact of the electrodes with the fish head for a large proportion of the fish, reduced shock intensity due to the resistance of the fish bodies for the fish receiving the shock only in an indirect manner as the current travels through the bodies of fish in contact with one another (see the section about pre-stun electrical shocks).

In-water pipeline electrical stunning obtains the mark “A” for this hazard. In-water batch electrical stunning (done in freshwater) and head-to-body electrical stunning obtain the mark “B” for this hazard. As its failure rate has not been quantified, in-water rotating electrical stunning obtains the mark “?” for this hazard. Dry batch electrical stunning, dry prod electrical stunning and in-water prod electrical stunning also obtain the mark “?” for this hazard as their failure rate has not been quantified. However, we believe that the actual failure rate of these three methods is likely to be well over 5%, which would lead to their rejection.

- **Air exposure**

Head-only systems, dry batch systems, dry prod systems and some head-to-body electrical stunning systems require to expose fish in air which is a stressor for them. In head-to-body electrical stunning systems, the duration of this air exposure has been estimated to last around 5 seconds by the EFSA for Atlantic salmon (EFSA 2009b). The EFSA does not provide estimation of this duration for rainbow trout, but it is likely similar to that provided for Atlantic salmon.

Nonetheless, more sophisticated head-to-body stunners that allow for reduced air exposure thanks to a pump that extracts fish from their tank and places them in another water-filled corridor exist. In this case, fish move towards the electrodes on their own (see video Optimar Swim in, Stun, Bleed, https://www.youtube.com/watch?v=hQ3dGwxafkM ; Electrical Stunning for Humane Slaughter of Sea Bass and Sea Bream, https://vimeo.com/394211327). Some companies estimate the duration of air exposure to be only within 1-2 seconds for head-to-body systems (personal communication with a stunning equipment producer company).

As part of a field study in Germany, the authors measured slightly higher stress biomarkers (cortisol, glucose, lactate, sodium) in fish submitted to head-to-body electrical stunning compared to in-water batch electrical stunning. The authors suspect that this difference could be explained by the brief air exposure experienced by the fish in the case of head-to-body electrical stunning (Jung-Schroers & al. 2020). For batch stunners, air exposure can be avoided by using a fish pump during filling.

Undercover investigations (https://www.youtube.com/watch?v=Yun0u5ynRoY&t=3s I pesci negli allevamenti europei | nuova INCHIESTA – Essere Animalie, https://www.youtube.com/watch?v=AWnMs22tV5k Investigation Reveals Abuse of Sensitive, Intelligent Fish at American Farms – Mercy for Animals) regarding dry batch electrical stunning as well as dry prod electrical stunning show that fish are submitted to air exposure for a duration which appears to be well over the 15 second thresholds. When prod electrical stunning is carried out in-water, investigation footage shows that due to high density, the fish at the top of the batch can lie outside of water and endure air exposure.

In-water electrical methods have the advantage of avoiding air exposure. In-water pipeline electrical stunning, in-water batch electrical stunning and in-water rotating electrical stunning obtain the mark “A” for this hazard. Head-to-body electrical stunning obtain the mark “B” for this hazard. Dry batch electrical stunning and dry prod electrical stunning obtain the mark “C” for this hazard. According to our scoring system, this hazard is disqualifying when it reaches the level “C”. Therefore, these two latter methods are unacceptable in this aspect.

- **High or extreme densities**

In in-water and dry batch stunners, while the tank is being filled before activation, fish will be crammed at very high density (EFSA 2009ab). The way fish are transferred into the tank can be more or less brutal. Fish will experience such conditions for a short period before being stunned.

This type of crowding is also present for in-water and dry prod electrical stunning methods, but in-water pipeline electrical systems are supposed to prevent this type of problem.

In-water and dry batch electrical stunning, as well as in-water and dry prod electrical stunning obtains the mark “B” for this hazard. Other electrical methods obtain the mark “A” for this hazard.

- **Poor water quality**

In in-water batch stunners, as well as for in-water prod electrical stunning systems, the water quality may be poor due to the high density of fish in the tank (EFSA 2009ab).

Those two systems obtain the mark “C” for this hazard. Other electrical methods obtain the mark “A” for this hazard.

- **Tissue damage**

In-water rotating electrical stunning system involves the risk that a small proportion of the fish may not enter correctly into the chambers. Those fish could be hit or even torn apart by the paddles if they haven’t fully entered the chamber as the system rotates (Lines & al. 2003). The proportion of individuals who may endure this hazard is not known but is estimated to be negligeable (personal communication with the company who developed and owns 4 prototypes of those systems).

In-water rotating electrical stunning obtains the mark “A/B” for this hazard. According to our scoring system, this hazard is disqualifying. Therefore, this method can be accepted or rejected depending on its performance on this criterion. Other electrical stunning methods obtain the mark “A” for this hazard.

- **Pre-stun electrical shocks**

  All electrical stunning methods involve a potential risk of pre-stun electrical shock if the parameters used are inadequate, leading to a shock too weak to induce immediate unconsciousness. For some methods, the risk of pre-stun electrical shocks is almost non-existent as long as the parameters used are adequate, while for other methods, significant risks of pre-stun electrical shocks are present even when the electrical parameters used are adequate.

  In-water rotating systems, in-water pipeline systems and head-only systems are not associated with significant risks of pre-stun electrical shocks as long as the parameters used are adequate. However, the risk of pre-stun electrical shocks is present at various levels for in-water and dry batch electrical stunning, in-water and dry prod electrical stunning and for head-to-body electrical stunning.

  For in-water batch electrical stunning and for head-to-body electrical stunning, the risk of pre-stun electrical shocks is limited to a small proportion of fish and can be prevented with good system design for the most part.

  For in-water batch electrical stunning, the risk of pre-stun electrical shocks is related to the level of homogeneity of the electrical field strength inside the stunning tank (Lines & Kestin 2004, EFSA 2009ab, HAS 2018, personal communication with researcher Jeff Lines). If the inside of the tank is covered with a series of electrodes laid as thin parallel metallic strips rather than with large plate electrodes spanning the whole width of the tank, the resulting electrical field may be heterogeneous with areas where the field is too weak to induce immediate unconsciousness (HSA 2018, personal communication with researcher Jeff Lines). But if the electrodes do actually cover the complete width of the tank, the risk of pre-stun electrical shocks is mostly controlled (HSA 2018, personal communication with researcher Jeff Lines). However, even if the system does use wide plate electrodes, a significant risk of pre-stun electrical shocks remains if this type of system is used with salt water for reasons related to the setting of electrical parameters in relation to water conductivity (see explanation in the section dedicated to failure rates).

For head-to-body systems, the risk of pre-stun electrical shocks is essentially related to the possibility that fish may arrive tail-first rather than head-first into the system (EFSA 2009ab). In that case, fish may consciously endure a pre-stun electrical shock for a few seconds as their tail is in contact with an electrode until their head finally makes contact with an electrode enabling the current to reach their brain making them unconscious. However, this risk only applies to a minority of fish and can mostly be controlled by implementing preventative measures like the so-called “rectifiers”. Rectifiers are in-water systems positioned in submerged corridors before the entrance of head-to-body stunners. They are designed to reduce the proportion of fish arriving tail-first into head-to-body stunners. They result in 84% to 99% of fish arriving correctly positioned (i.e head-first) into the system (Mejdell & Gismervick 2009b).

Risks of pre-stun electrical shocks are much more significant for dry batch electrical stunning, dry prod electrical stunning, and for in-water prod electrical stunning. Those methods have not been properly assessed in the scientific literature to our knowledge, but a general understanding of how electrical stunning works lead us to think that they come with high risks of pre-stun electrical shocks. In dry batch electrical stunning, only a minority of fish have their head directly in contact with an electrode. Therefore, all the other fish are at high risk of consciously enduring a pre-stun electrical shock. Fish who have one body part other than their head in contact with an electrode are at risk of receiving an electrical shock without it reaching to their brain, hence putting them at risk of enduring a pre-stun electrical shock. Fish which are not in direct contact with an electrode, and which are indirectly receiving an electrical shock as the current travels through the bodies of other fish in contact with one another may also endure pre-stun electrical shocks. As the current first needs to cross the body of one or several fish before reaching them, the strength of the electrical shock will be diminished due to the resistance of those previous fish bodies. If this resistance makes the strength of the final electrical shock insufficient, the loss of consciousness may not be immediate, or may not even occur at all, resulting in pre-stun electrical shocks.

Those issues and mechanisms also apply to dry prod electrical stunning. For in-water prod electrical stunning, fish are at high risks of enduring pre-stun shocks because the shape of the electrode does not allow for a homogeneous electrical field inside the stunning tank. The field strength will be weakened in the areas of the tank the farthest apart from the electrode and will therefore likely be insufficient to immediately stun fish in those areas, putting them at risk of consciously enduring pre-stun electrical shocks.

Head-only systems, in-water pipeline systems, and in-water rotating systems obtain the mark “A” for this hazard. In-water batch electrical stunning and head-to-body electrical stunning obtain the mark “A/B” for this hazard. Those two methods can be tolerated if measures are used to control the risk of pre-stun electrical shocks. Dry batch electrical stunning, dry prod electrical stunning and in-water prod electrical stunning obtain the mark “B” for this hazard. According to our grading system, this hazard is disqualifying. Therefore, those three latter methods are disqualified.

- **Reliance on workers’ skills**

Inadequate parameters calibration can result in stunning failure or recovery of consciousness before bleeding. In addition, if the parameters are inadequate, the result can be electro-immobilisation i.e immobilisation of the fish without rendering them insensitive to pain. In this situation, it is difficult for workers to distinguish correctly stunned fish, and fish that are immobilised but still conscious (Gräns & al. 2015, Berg & al. 2021). In fact, immobilised fish may not respond to consciousness checks, thus behavioural consciousness indicators are not always reliable in this context (CIWF 2018, Berg & al. 2021). The calibration of adequate parameters and the maintenance of electrical stunning machines involves some level of skill.

Electrical methods obtain the mark “B” for this hazard.

WELFARM’s position and recommendations :

Some electrical stunning methods – in-water pipeline systems, in-water rotating systems, in-water batch systems, head-to-body systems – can meet animal welfare requirements. Nonetheless, some of those methods are better than others. In-water methods have the benefit of avoiding air exposure. As it is currently practiced, the moment when fish are being transferred into the tank for batch stunning is likely to be stressful due to high densities and potentially poor water quality.

Rotating electrical stunning comes with the potential risk of causing tissue damage on some fish. However, rotating and pipeline systems have the benefit of avoiding issues related to overly high densities and poor water quality that come with batch stunning.

Thus, in-water pipeline electrical stunning appears to be the electrical method that involves the least amount of welfare hazards throughout the slaughter process. However, some stress may still be involved as fish are driven by a powerful pump. In addition, there is some risk that a few fish could bump against the pipe walls, if there is an angle and if stunning is not correctly executed, but very few individuals are concerned by this risk (EFSA 2009ab).

As for in-water electrical stunning conducted in sea water, it appears that only pipeline systems are able to achieve good stunning performance in this context. Therefore, we only accept the use of in-water batch electrical stunning when it is conducted with fresh water and not with sea water.

Three electrical stunning methods – dry batch electrical stunning, dry prod electrical stunning, in-water prod electrical stunning – appear to be unsatisfying because they are associated with significant risks of pre-stun electrical shocks, and with failures rates which are likely to be high. In addition, dry batch electrical stunning and dry prod electrical stunning come with an extended duration of air exposure.

More studies would be needed to further expand knowledge about electrical stunning especially for in-water pipeline electrical stunning which appears to have the most potential in terms of animal welfare.

If the parameters are calibrated so that the stun is reversible, it implies that the risk of recovery of consciousness before or during killing is not null. This risk can be limited by performing an efficient killing method as soon as possible after stunning.

Therefore, WELFARM considers that electrical stunning can be acceptable if it is carried out correctly, with a preference for in-water pipeline electrical stunning. However, some types of electrical stunning methods – dry batch electrical stunning, dry prod electrical stunning and in-water prod electrical stunning, are unacceptable.

The use of parameters resulting in irreversible stunning, without risks of recovery of consciousness, is preferable even though the chances of carcass damage are higher.

Field observations made by Jung-Schroer & al. 2020 on slaughter plants revealed that the latency between stunning and bleeding was comprised between 2 to 5 minutes for most sites but could last up to 120 minutes on some sites. Such a long latency is unacceptable. Electrical methods used with parameters associated with a reversible stun come with a risk of recovery of consciousness before or during killing after stunning. If they are used, the reversible stunning must be followed by a quick and efficient killing method before fish can regain consciousness.

Haemorrhagic killing methods are usually used after electrical stunning. In order to minimise risks of recovery of consciousness, the methods leading to death in the least amount of time must be preferred. Among haemorrhagic killing methods, decapitation is likely to be the fastest, followed by evisceration combined with a bilateral gill cut, followed by evisceration without any gill cut, which all are quicker than mere gill cutting. Gill cutting will cause death more quickly if the cut is performed on both sides rather than only on one side. Although ideal, decapitation may be difficult to implement by some producers because examining the head of the fish is sometimes done as part of quality assessment processes (FAO 1999).

Otherwise, percussion of the skull can also be used as a killing method to prevent risks of recovery of consciousness after reversible electrical stunning (see part 2.6) (Mejdell & al. 2009a).

For tilapia, turbots and sea bass, it has been shown that immersion in ice slurry after electrical stunning could prevent the recovery of consciousness (StunFishFirst, Lambooij & al. 2008). Thus, out of 10 sea bass that had been stunned with parameters associated with a recovery of consciousness taking place about 73 seconds after stunning in some fish, none recovered consciousness if they were immersed in ice slurry within 20 seconds after stunning (Lambooij & al. 2008). The authors claimed that such fish had reached a state of cerebral death. Therefore, we recommend immersing sea bass in ice slurry after electrical stunning to prevent risks of recovery of consciousness before/during bleeding.

The prevention of recovery of consciousness related to subsequent immersion in ice slurry has not yet been shown for sea bream, rainbow trout and Atlantic salmon. It appears likely that this effect also exists in sea breams as they are a warm-water species. Preliminary yet to be published results show that cardiac arrest is quicker after electrical stunning if sea bream are put in ice slurry following stunning (personal communication Maria J. Cabrera-Alvarez and Sonia M. Antonio Soares, CCMAR, Algarve University). Cardiac arrest stops the circulation of blood, and therefore, the brain blood supply. Thus, it speeds up brain death, which prevents the recovery of consciousness. Immersing sea bream in ice slurry before/during bleeding following electrical stunning is therefore likely to be beneficial for sea bream. For instance, this process was used in the trial conducted in Tukey in partnership with CIWF about head-to-body electrical stunning for sea bream and sea bass (CIWF Driving innovation in humane fish slaughter).

However, the benefit of ice slurry to prevent recovery of consciousness after electrical stunning is more uncertain for salmonids who are cold-water species. In fact, the duration before the loss of consciousness after immersion in ice slurry without prior stunning is estimated to be 9,6 minutes for rainbow trout (Robb & Kestin 2002). Furthermore, some authors reported that in the case of stunning with an in-water rotating electrical system followed by immersion in ice slurry for rainbow trout, an unquantified “small proportion of individuals” displayed signs of consciousness (Lines & al. 2003). Therefore, immersion in ice slurry after electrical stunning does not appear to be particularly beneficial for salmonids at this stage.

For in-water batch electrical stunning, changing water between each session and using oxygen-saturated water may help to reduce the water quality issues. The density in the tanks should be limited but reducing the density may be difficult in terms of maintaining a cost-effective slaughter line pace. Using fish pumps to transfer the fish into the tank avoids submitting them to air exposure and compressions which can happen when using brail nets. Some Swiss producers use brail nets with in-built weighing systems to fill in-water batch electrical stunning tanks (personal communication with a Swiss rainbow trout producer). The need to weight fish or to estimate the quantity of fish taken before slaughter could potentially be made compatible with using fish pumps to fill in-water batch electrical stunning tanks by integrating an automatic fish counter within the pump. Filling tanks with some level of water before starting to transfer the fish reduces the risk for fish to get hurt by bumping abruptly onto the bottom of the tank.

As field strength is dependent upon the distance between the electrodes, the electrodes should be positioned on the sides of the tank with the shortest distance between each other (HSA 2018). Electrodes should be positioned on the sides of the tank and not at the bottom/under the lid, because in that case, electricity may not flow as well if the tank is not filled to the brim and if bubbles/foam appear at the surface underneath the top electrode (HAS 2018). Large plate electrodes spanning the whole surface of the tank should be preferred over the use of several thin metallic strips in order to keep the electric field as homogenous as possible (HSA 2018, personal communication with the researcher Jeff Lines, FIAP website).

In-water pipeline systems should avoid sharp angles and prefer a round design with no small angles.

The risk of collision/ tearing related to the paddles of rotating stunning systems should be thoroughly assessed to identify its prevalence. Technical adjustments in the size of the chambers, the speed of rotation and of the water stream may be considered to reduce this hazard. However, it has been said that this issue was negligeable (personal communication with the company who developed the in-water rotating electrical stunning prototype).

2.5.3 Calibration of parameters for electrical stunning

In commercial settings, the electrical parameters used are usually a compromise between flesh quality requirements and stunning performance.

The parameters that favour optimal stunning performance can induce carcass damage like broken bones along the spine and blood spots of various size in the filet. In contrast, the parameters that favour flesh quality can be associated with stunning failure or quick recovery of consciousness.

Ideally, the use of irreversible stunning is preferable from a welfare perspective. If not possible, a compromise must be found between quality and welfare requirements. It means using parameters that allow for both a long enough stun that provides a sufficient timeframe to perform a quick killing method before recovery of consciousness, and acceptable product quality.

Rainbow trout:

Increasing field strength and the duration of application of electricity results in a longer stun duration and a higher proportion of correctly stunned individuals (Robb & Roth 2003, Hjelmstedt & al. 2022). Reducing the frequency appears to improve the stunning performance, 50 Hz being the lowest frequency tested (Robb & Roth 2003).

According to Halsband 1967, different fish species react differently to the same frequencies (e.g common trout compared to eels and minnows). Nonetheless, the required frequency to stun rainbow trout likely corresponds to those for Atlantic salmon (Roth & al. 2003).

The results from Robb & al. 2002 show that a setting of 50 Hz and 100 mA applied for one second can induce unconsciousness. According to the authors, this intensity is a minimum threshold. A recent study used EEG analysis of brain functions to identify the minimal settings needed to induce an epileptiform insult rendering rainbow trout unconscious for a 1 second-long application (Hjelmstedt & al. 2022). The trial used in-water batch electrical stunning with a water conductivity around 1000 µS cm^{-1} (Hjelmstedt & al. 2022). For a 50 Hz AC sinusoidal shock, the results indicate the field strength needs to be above or equal to 2.08 ± 0.01 Vrms cm^{-1}, and that the current density needs to be above or equal to 0.22 ± 0.003 Arms dm^{-2} (Hjelmstedt & al. 2022). However, if the shock is applied only for 1 second, the stun is reversible, and consciousness quickly returns: EEG measures show that VEPs return within 10 to 415 seconds depending on the parameters (Hjelmstedt & al. 2022). A longer application is necessary to achieve a satisfying duration of insensibility. Even when the shock is only delivered for 1 second, the duration of insensibility increases when field strength and current density are increased (Hejlmstedt & al. 2022).

Low frequencies, high field strengths and long application duration are associated with more carcass damage (Robb & Roth 2003), in particular in terms of blood spotting. However, combining a low frequency (50 Hz) with a low field strength (1 V/cm r.m.s) seems to avoid the quality issues that occur with low frequency when the field strength is elevated (Lines & Kestin 2005). Certain Swiss rainbow trout producers using in-water batch electrical stunning from the company Rundum Fisch do not report any flesh quality downgrading when they are stunned in an irreversible manner with those systems (personal communication with rainbow trout farmers).

On the contrary, the stunning performance deteriorate dramatically as the frequency is increased, particularly when reaching 2000 Hz (Robb & Roth 2003). It seems that from 2 600 Hz and beyond, it is not even possible to stun fish (Robb & Roth 2003).

In the case of in-water electrical stunning, water conductivity has an impact on stunning performance. When the water conductivity is high (but remaining similar or below the conductivity of fish tissues), the field strength necessary to induce unconsciousness is lower. Lines & Kestin 2004 constructed a mathematical model (see figure 8) that can be used to adapt field strength in relation to water conductivity and the duration of application, when using a frequency of 1000 Hz.

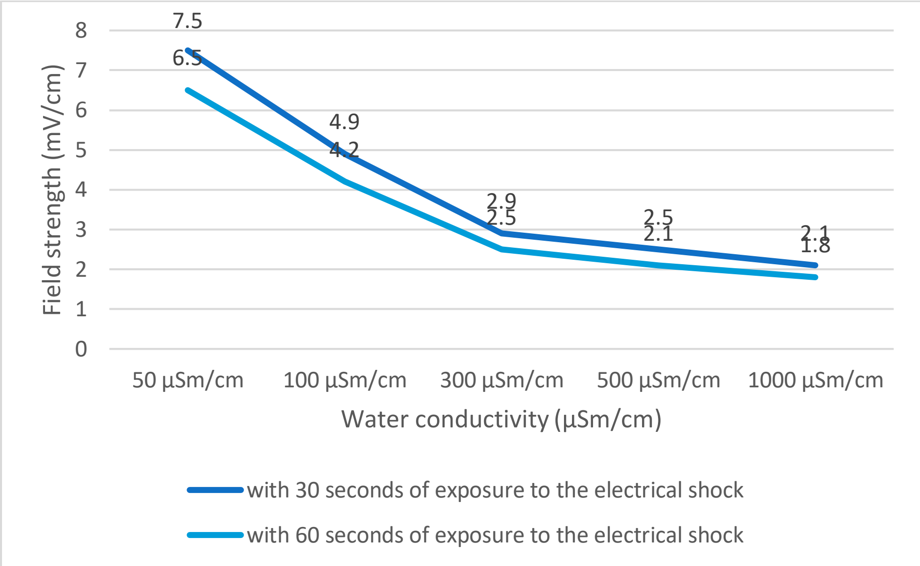

FIGURE 8: REQUIRED FIELD STRENGTH TO INDUCE PERMANENT INSENSIBILITY IN PORTION SIZED RAINBOW TROUT IN RELATION TO WATER CONDUCTIVITY WHEN USING A 1000HZ FREQUENCY. INSPIRED BY LINES & KESTIN 2004. BASED ON A MATHEMATICAL MODEL ASSUMING THAT FISH ARE ORIENTED PERPENDICULAR TO THE DIRECTION OF THE FIELD

The same authors also estimated the minimal field strength that must go through the head of portion sized trout to induce unconsciousness. According to them, it requires 2.6 V/cm at 60 seconds of application, 3.4 V/cm at 30 seconds of application, and 5.5 V/cm at 15 second of application.

The animal welfare NGO Fairfish recommends the use of the settings suggested by Lines & al. 2003 for rainbow trout i.e 1000 Hz, 2,5 V/m, 60 seconds of application, and sinusoidal waveform.

Lines & Kestin 2005 also put forward a calibration based on a two stages process for portion sized rainbow trout. It consists in submitting fish to a first brief electrical shock with a high frequency immediately followed by a second shock at a low frequency but maintained for a longer duration, while using sinusoidal waveform. The first shock is meant to induce unconsciousness instantaneously, and the second shock is meant to prolong the duration of insensibility produced by the stun. This two stages setting doesn't have particular benefits from a welfare point of view compared to the previous settings mentioned. However, it has some benefits in terms of flesh quality and energy use. There is also a benefit in terms of costs because the electronic equipment needed to create a shock at 1000 Hz for several seconds are more costly that those needed for a 50 Hz shock (Lines & kestin 2004a). This type of two-stages setting is used by the companies Ace Aquatec and Rundum Fisch in their electrical stunning equipment.

Bermejo-Poza & al. 2021 used behavioural indicators of consciousness to compare the stunning performance of two settings that differed only by the intensity achieved in rainbow trout with head-only stunning. The tests were conducted with a setting using AC current at 50 Hz for one second and an intensity of either 200 mA rms, or 400 mA rms. Consciousness status was assessed based on the persistence of opercular beats, vestibulo-ocular reflex, responsiveness to noxious stimuli and righting behaviour at 1, 11 and 21 minutes after stunning. For each indicator, a mark (0= the indicator is absent, 1= the indicator is weakly present, 2=the indicator is fully present) was attributed. Results showed an average score inferior to 1 for all indicators at 1 minute after stunning, as well as 11 and 21 minutes after stunning, indicating a loss of consciousness. The consciousness scores were slightly better at 400 mA compared to 200 mA, though the difference was only significant for opercular motion measured at 21 minutes after stunning. Therefore, a higher intensity appears to be beneficial.

Nevertheless, the average scores were not always equal to 0 which means that a small failure rate persists.

A recent German field study showed that out of 189 individuals that went through electrical stunning with 50 V and AC current, only 4 still presented a vestibulo-ocular reflex which indicates good stunning performance (Jung-Schroers & al. 2020). Contrary to previous results, the data from this study did not show any difference in the proportion of fish displaying signs of consciousness between fish treated with 50 Hz, 100 Hz and 1000 Hz, and between fish submitted to 30 seconds vs 60 seconds of application, in the case of 50 V AC current stun.

However, the rate of recovery of consciousness was higher in the case of a 30 seconds application compared to a 60 seconds application when DC current was used, which supports previous results showing an association between longer application duration and better stunning performance. The benefits of a longer application duration are also supported by the fact that in the field, facilities using an application lasting only a few seconds had high failure rates that were reduced by extending the application duration beyond 30 seconds. The authors also recommend achieving a current density above 0.1 A dm^2 to maintain good stunning performance.

In terms of carcass quality, the authors observed haemorrhage in the filet in 25% to 65% of electrically stunned fish in the commercial slaughter plants. Haemorrhages were less frequent and less marked with DC current compared to AC current. When AC current was used, the authors did not find an association between the application duration nor the frequency with the prevalence of haemorrhages.

Regarding conductivity, the authors observed on the field that some facilities using in-water electrical batch stunning had very high failure rates. Those slaughter plants stood out when looking at the conductivity of the water they were working with. The conductivity was either overly low (< 500 µS cm^{-1}) or too high (close to 1000 µS cm^{-1}). A low conductivity will make the diffusion of energy through the water harder. A high water conductivity, if it is close to or above the conductivity of the fish tissues, will make the energy flow mostly throughout the water rather than through the fish tissues (Berg & al. 2021). Therefore, to allow for efficient stunning with high water conductivity, a very high field strength is needed which is difficult to achieve in a batch system due to the distance between the electrodes (personal communication with the researchers Albin Gräns, Per Hjelmstedt and Dieter Steinhagen). The authors observed that adding a small quantity of salt into the water to raise the conductivity above 500 µS cm^{-1} could dramatically decrease the failure rates on the sites working with low conductivity water. The authors recommended to keep water conductivity comprised between 500 µS cm^{-1} and 1000 µS cm^{-1} which approximately corresponds to the conductivity of rainbow trout tissues.

Hjelmstdt & al. 2022 tried to identify the parameters needed to induce irreversible stunning in rainbow trout. Their study was lead with an in-water batch electrical stunning system, with a water conductivity around 1000 µS cm^{-1}, on 800 g – 1 Kg rainbow trout. Consciousness status was assessed by EEG. The brain activity of fish was recorded for 15 minutes after stunning. The results can be used to recommend a setting combining a frequency of 50 Hz, with AC sinusoidal current, with a field strength above or equal to 10,2 V_{rms} cm^{-1} and a current density superior or equal to 0,84 A_{rms} dm^{-2}, applied for 30 to 60 seconds. The duration of the shock appears to be especially important to achieve an irreversible stun. This study did not assess the impact of this setting on flesh quality.

<u>Atlantic salmon:</u>

To achieve high carcass quality with Atlantic salmon, the frequency must be relatively high around 1000 Hz, but beyond this threshold, some deterioration of the carcass can be observed (Roth & al. 2004, tests conducted in a system akin to in-water batch stunning). In contrast, low frequencies can also induce some level of damage to the flesh. For Instance, the study by Roth & al. 2004 showed that at 50 Hz, the fish had a proportion of haematoma found in the filet comprised between 60% to 90%. Above 80 Hz, the proportion of haematoma would diminish to around 20 – 40 % at 120 Hz, and 0 – 10% for 500 Hz and 1000 Hz. The proportion of damage would increase again above 1000 Hz.

Carcass damage observed following electronarcosis are not caused by the epilepsy itself (Roth & al. 2003). Damages seem to be mostly caused by the strong muscle contractions throughout the exposure to the electric shock (Roth & al. 2012).

On another note, the study showed that carcass quality depended on the frequency being used, but not necessarily on the duration of the electric shock nor the voltage. However, considering the small number of studies on this topic, more data would be needed to confirm this observation which contradicts other results.

The study by Roth & al. 2003 showed that for a given set of parameters (single-phase current, 50 Hz, AC, sinusoidal, maximum intensity of 25 A), when varying the voltage and the duration of the shock, the latter factor was the one which had the greatest impact on flesh quality. Under 1,5 seconds of exposure, no carcass damage was observed for voltages of 100 V and 125 V.

To increase stunning performance, the use of a low frequency comprised between 50 Hz / 80 – 100 Hz is recommended (Roth & al. 2004, GrimsbØ & al. 2016). Otherwise, the study by Roth & al. 2004 showed that for a given set of parameters (AC current, 50 V/m, 60 A, 10 second of application), the use of frequencies comprised between 50 Hz and up to 500 Hz succeeded to stun 100 % of the fish, whereas the stunning success rate would decrease when reaching frequencies around 1000 Hz.

A longer application of the shock seems to allow the use of higher frequencies while maintaining a 100 % stunning rate. But further studies would be needed to confirm this.

Independently of the shock duration and voltage used, flesh quality can be preserved at 500 Hz according to Roth & al. 2004. Thus, it appears that when using a 10 second application, at 50 V/m and 500 Hz, there are both good stunning performance (100 % of the fish being stunned) and between 0% and 10% of carcass damage. Further studies would be needed to consolidate this observation.

The study by Roth & al. 2004 brought evidence that stunning success depends mostly on the frequency, the shock duration and the field strength (voltage). The study by Roth & al. 2003 found that the proportion of salmon correctly stunned as well as the duration of the stun depended on the duration of the shock, especially regarding the first 3 minutes of the shock.

The use of AC current seems to be better in terms of stunning performance, while the use of DC current favours flesh quality (Lamboiij & al. 2010).

When using AC current, the use of a sinusoidal waveform allows for better flesh quality compared to a squared waveform (Roth & al. 2004).

<u>European sea bass:</u>

Generally speaking, few data are available on this topic. A 2008 study about in-water batch stunning in sea water suggested to use a current density of 3.3 Arms/dm^2, with either 50 Hz and a sinusoidal waveform, or 133 Hz with pulsed-square wave with a 43% duty cycle for a duration of 10 seconds (Lambooij & al. 2008). Such settings would be both relatively efficient and acceptable in terms of flesh quality.

Those results are based on several experiments. One of them tested the following calibration: 50 Hz, AC current, squared wave, current density of 5 A r.m.s/dm^2 in sea water with a conductivity of 53 mS/cm. The results showed that out of 10 fish, all of them were stunned. Among them, 2 would not recover consciousness, 5 would recover balance at 73 ± 23 seconds, and 8 recovered swimming at 79 ± 64 seconds.

Another experiment tested combining 133 Hz, AC current, squared wave, 33 V r.m.s, current density of 3 A r.m.s/dm^2, pulsed current with a duty cycle of 43%, applied for 1 second only within sea water of a conductivity of 52 mS/cm. All the fish were correctly stunned. Out of 27 individuals, one reached brain death, 13 recovered responsiveness to noxious stimuli after 30 seconds, 9 recovered those reactions after 2 minutes, and 4 other after 5 minutes. Thus, this calibration was able to stun the fish but the duration of insensibility varied greatly. Insensibility was irreversible for one individual and was comprised between 30 seconds to 5 minutes for others. Other results from this study showed that for a given calibration, an application duration of 10 seconds is more efficient than if it only lasts 1 second.

The tests conducted as part of the StunFishFirst project on sea bass showed through EEG and behavioural assessment that electrical stunning applied for 1 second (1 V rms/cm, 50Hz, AC, 4.3 A/dm^2, 41 000 µS) could induce an instantaneous loss of consciousness quickly followed by a recovery of consciousness (after about 48 seconds). Two other settings (1) 1,6 Vrms, 50 Hz, AC, 0,15 A/dm^2, 1000 µS ; 2) 3,2 V rms/cm, 1000 Hz, AC, 0,2 A/dm^2, 1000 µS) applied for 1 second too seemed to be effective to induce unconsciousness but those parameters were assessed based on behavioural indicators alone (and not by EEG). The authors suggest to perform electrical stunning with a longer application duration in order to prolong the duration of insensibility. In this project, the tests on sea bass concluded that there was no significant flesh quality downgrading after in-water batch stunning and in-water pipeline stunning. However, in the case of batch stunning, the eyes of sea bass were less convex and less dark compared to slaughter by ice slurry. Nonetheless, the producer involved in the trials considered that the fish could still be marketed.

Zampacavallo & al. 2015 also tested out electrical batch stunning, but without water, as the system was not suited to function in sea water. Two electrical settings were tested. The first worked in two steps with a first stage at 400 Hz, 120 V for 1 minute, and a second stage at 50 Hz, 40 V for 3 minutes. The second calibration tested consisted in a shock at 50 Hz and 40 V applied for 4 minutes. Based on behavioural indicators, the authors concluded that no fish had been showing signs of consciousness during the following 20 minutes of observation. However, the authors concluded that both of those calibrations resulted in significant flesh quality downgrading in terms of the earliness of rigor mortis and short shelf life.

Another study looked into head-to-body stunning, also with a two steps calibration (Knowles & al. 2007). The first step used a field strength of 1,2 V/cm for 2 seconds, and the second step used a field strength of 0,6 V/cm for 4 seconds. The other characteristics of the parameters were not specified. This study was solely focused on flesh quality and did not report measures regarding stunning performance except a general observation of immobility after stunning. However, the authors concluded that this setting was associated with acceptable flesh quality outcomes.

Common settings for European sea bass and gilthead sea bream

A head-to-body electrical stunning trial (with a stunning equipment from the company Optimar) has been conducted for sea bass and sea bream in Turkey as part of a collaborative project between CIWF, Tesco and Seachill (Hilton Seafood) (Driving innovation in humane fish slaughter). The fish were submitted to a shock during 8 to 10 seconds. The system used a combination of AC and DC current and worked with the following parameters (see figure 9):

	Start	Mid 1	Mid 2	Mid 3	End
Voltage :	110 V	90 V	50 V	40 V	20 V
Amper :	15 A	15 A	15 A	25 A	15 A
Hertz :	50	50	50	50	50

FIGURE 9: ELECTRICAL PARAMETERS OF THE ELECTRODES OF THE HEAD-TO-BODY ELECTRICAL STUNNING SYSTEM USED IN THE TRIAL CARRIED IN TURKEY BY CIWF, TESCO AND SEACHIILL (HILTON SEAFOOD) (DRIVING INNOVATION IN HUMANE FISH SLAUGHTER)

Based on behavioural indicators, the company reported a stunning failure rate of 3%. After stunning, fish were immersed in ice slurry to prevent risks of recovery of consciousness.

On another note, in-water pipeline electrical stunning is also available commercially for sea bass and sea bream but the parameters used are not reported in the published literature (Papaharisis & al. 2019).

Gilthead sea bream

Knowledge about electrical stunning for sea bream is very limited. Unpublished results mentioned by Kestin & al. 2002 confirmed an instantaneous loss of consciousness in electrically stunned sea breams. In addition to the trial lead by CIWF, only one study has been published to report results about electrical stunning for sea bream (Van de Vis & al. 2003). The study used head-only dry electrical stunning and the loss of consciousness was measured by EEG. Only two tests were carried out. The first relied on the following calibration: AC current, 50 Hz, 80 V applied for 1 second. The measures indicated that intensity was comprised between 27 mA and 200mA for 9 fish out of 10 and rose up to 450 mA for one individual. This calibration was ineffective: out of the 10 fish tested, only one fish (the one for which intensity reached 450 mA) was correctly stunned for 37 seconds. The authors then looked at the efficacy of those same parameters but with an application duration of 10 seconds instead of just 1. The measured intensity rose around 400 mA for all fish. The results were better: out of 10 fish, 9 were correctly stunned. Among those, 3 recovered consciousness in 16 seconds, and the remaining 6 fish did not show signs of consciousness during the 10 minutes of observation, which the authors interpreted as death. On this basis, the authors concluded that an intensity of at least 200 mA was required to stun sea bream. A yet to be published study investigated in-water batch electrical stunning (electrodes positioned at 50 cm from each other) for sea bream in freshwater with a sinusoidal 100V setting, applied for 20 seconds, leading to a field strength of 2 V/cm (personal communication Maria Cabrera-Alvarez, Sonia Antonia Soares, CCMAR, Algarve University). According to the preliminary results, visual indicators of consciousness indicate that this setting appears to be effective, but the authors did not carry an EEG assessment of the state of consciousness. The authors indicate that if the shock is only applied for 10 seconds, many sea bream quickly recover consciousness.

Potential innovation

In the context of electrical stunning for cattle, a particular setting called Single Pulse Ultra-high Current (SPUC) showed promising results (Robins & al. 2014). Research is being conducted to test SPUC electrical stunning on fish with Ace Aquatec's in-water pipeline system (Fish farmer Magazine 2020). This type of calibration could have the benefit to cause electrocution i.e an irreversible loss of consciousness, while preserving carcass quality (personal communication with CIWF staff). However, no results have been published about this yet.

Worker safety

Risks for the safety of workers have been mentioned in relation to electrical stunning. Such risks were evoked by Italian producers in response to Essere Animali's ask to implement head-to-body electrical stunning for sea bass and sea bream at sea (personal communication with NGO Essere Animali). However, the trial carried by CIWF with Tesco's suppliers mentions that the site manager did not report safety issues for workers. Some Eurogroup for Animals staff members indicated to us that they know some producers who used this type of system on boats without major safety concerns. The researcher Jeff Lines also considered safety risks related to in-water batch stunning. Such systems could be dangerous if someone falls into them or if the system is not well electrically isolated and is surrounded with stagnant water on the ground. Putting a safety system like an interlock, so that it is not possible to turn electricity on as long as the lid has not been closed could limit those risks. However, Jeff Lines commented that he was worried that workers may remove the interlock to achieve a faster slaughter line pace. The trial conducted about in-water batch electrical stunning at sea for sea bass as part of the StunFishFirst project, as well as the German field study (Jung-Schroers & al. 2020) about those systems for rainbow trout, did not report particular concerns regarding worker safety. In Switzerland, one producer using in-water batch electrical stunning installed their equipment inside a small enclosure with a gate, which automatically closes when the stunner is activated. With this system, it is not possible for workers to enter the enclosure and to stand nearby the stunning tank while it is delivering the electrical shock (personal communication with a rainbow trout farmer).

WELFARM's recommendations:

Several aspects must be taken into account in order to exploit studies about electrical stunning in a commercial setting. Water temperature, salinity/conductivity, the weight and number of fish, their heterogeneity are all aspects to consider to make sure that recommendations are applicable to all conditions.

Ideally, settings leading to irreversible stunning are preferable to those that result in reversible stunning, but they may be associated with unwanted consequences on product quality.

It should be made sure that all electrical parameters used result in an immediate loss of consciousness in fish. The voltage and current intensity must be sufficient to meet recommendations in the most recent scientific literature to avoid risks of inefficient stunning and electro-immobilisation. Electrical equipment should be regularly calibrated to ensure that adequate parameters are used to stun animals. Regular maintenance of the equipment should be carried out to ensure that it is properly working.

In the case of reversible stunning, the resulting duration of insensibility as well as the duration required for animals to die following a killing method must be taken into account to prevent risks of recovery of consciousness, even after performing a killing method. All killing methods are not suitable for all species. The killing method should be chosen based on the duration of insensibility caused by the prior stun, and the duration needed for the killing method to induce death.

Electrical stunning methods have the potential to negatively impact carcass quality, among other things through blood spotting and bone damage (Poli & al. 2005). The degree of damage depends on the calibration of the parameters. This creates a dilemma between the optimisation of parameters to preserve high flesh quality, and the optimisation of parameters to achieve the best stunning performance possible. Some parameters (in particular low frequency) with the best welfare outcomes (i.e more likely to achieve irreversible stunning for as many fish as possible, and/or ensuring the longest duration of insensibility) are also those with the worst carcass quality outcomes (Lines & al. 2003).

It is difficult to come with explicit precise recommendations of optimal parameters for electrical stunning. Several recommendations can be found in the scientific literature, but they are usually a compromise between animal welfare and product quality requirements. The performance of the studied electrical parameters settings in terms of the proportion of correctly stunned individuals, durations of insensibility and risks of recovery of consciousness, are usually reported for experimental set ups carried out on small numbers of individuals. We have some doubts about the possibility to extend those findings to all sites in commercial settings. In fact, conditions vary site by site regarding fish weight, the quantity of fish that need to be stunned that the same time, and water conductivity among other things.

Those aspects can impact stunning performance. There are some data about adjusting the parameters to take into account water conductivity for rainbow trout, but it is not the case for sea bream and sea bass. The number of fish present in an in-water batch stunner seems to impact stunning performance (Robb & al. 2002). As far as fish weight and size are concerned, available information is partially contradictory. The EFSA (EFSA 2009ab) considers that small fish are less likely to be correctly stunned than large fish in in-water electrical stunning, other authors consider that the weight of fish only has a minor influence (Robb & al. 2002, StunFishFirst). This is also the opinion of one equipment supplier company that it may not be needed to modify electrical parameters in relation to fish weight (personal communication). But another author did report an effect of the size of the fish in Atlantic salmon (Roth & al. 2003).

Therefore, if the recommendations available in the literature can be used as a basis to guide parameter calibration, it is still necessary to ensure the efficacy of electrical stunning equipment and their calibration on the field, site by site. If signs of recovery of consciousness like opercular beat, vestibulo-ocular reflex or responsiveness to noxious stimuli are observed after electrical stunning, the parameters should be adjusted to reduce their occurrence and ensure an efficient stun for all individuals. Knowledge acquired mostly with salmonids shows that increasing the application duration, increasing field strength (for in-water stunning) or voltage (for dry stunning) to reach sufficient intensity / current density, and minimising frequency to be as close as possible to 50 Hz, allows for improved stunning performance (Robb & al. 2002, Hjelmstedt & al. 2022). The benefit of a longer duration of application is also observed for sea bass and sea bream (Lambooj & al. 2008, Van de Vis & al. 2003). The duration of application appears to play an important role on the reversibility or irreversibility of the stun, and on the duration of insensibility. Based on the results obtained for rainbow trout, it is possible to recommend that the shock should be delivered for at least 30 seconds to ensure irreversibility (Hjelmstedt & al. 2022).

This knowledge basis makes it possible to advise in which direction parameters should be modified if issues of ineffective stunning or early recovery of consciousness are observed on sites. However, it is difficult to suggest an optimal setting for each parameter for each species at this point in time. Rainbow trout may be an exception to this rule, as more precise results are available for this species, which allows us to recommend a setting of 50 Hz AC sinusoidal current with a field strength superior or equal to 10.2 Vrms cm^{-1}, and a current density superior or equal to 0.84 Arms dm^{-2}, applied for 30-60 seconds, to achieve an immediate and irreversible loss of consciousness during in-water electrical stunning (in a water with a conductivity around 1000 µS/cm). We also acknowledge that low frequencies, long application duration, and elevated field strengths are associated with more frequent carcass downgrading.

The type of water (freshwater or sea water) should be suited to the considered species. Submitting fish to an abrupt change in salinity involves physiological stress (osmotic shock). In France, rainbow trout are mostly raised in fresh water and sea bass, sea bream and Atlantic salmon are raised in sea water. Salinity, and therefore conductivity, is important for in-water electrical stunning. For rainbow trout, authors have recommended to use water with a conductivity comprised between 500 µS/cm and 1000 µS/cm. If the water has a conductivity below 500 µS/cm, it is possible to add some salt in the water to slightly increase its conductivity. For this reason, facilities using in-water electrical stunning should be equipped with a conductivity-meter to monitor water conductivity. In theory, electrical stunning can work both in freshwater and sea water if the field strength is high enough. That being said, as sea water has a conductivity above that of fish tissue, a greater part of the energy will flow through the water rather than through the fish, which means that higher field strengths are needed in sea water. Field strength partially depends on the distance between the electrodes. It is possible to reach sufficient field strengths in sea water with pipeline systems, but it appears difficult to achieve the same for batch stunning systems as their electrodes are located further apart from each other (personal communication with the researchers Albin Gräns, Per Hjelmstedt and Dieter Steinhagen). For this reason related to electrical parameters, the use of in-water batch electrical stunning only appears acceptable when used with freshwater for freshwater species and not when used with sea water. Potentially, using in-water batch electrical stunning tanks with freshwater for saltwater species as some researchers have tried in an experimental context, could be considered (personal communication with Maria Cabrera-Alvarez & Sonia Antonio Soares, CCMAR, Algarve University). However, this option is sub-optimal from an animal welfare perspective because putting saltwater fish in freshwater causes them to endure an osmotic shock, and therefore physiological stress.

Methods	Exposure to noxious chemicals	Immediateness of the loss of consciousness	Failure rate	Risk of recovery of consciousness (reversibility)	Air exposure	High or extreme densities	Handling	Poor water quality	Tissue damage	Pre-stun Electrical shock	Thermal shock	Reliance on workers'skills
Head-to-body	A	A	B	A/B	B	A	A	A	A	A/**B**[9]	A	B
In-water batch	A	A	B	A/B	A[10]	B	A	B	A	A/**B**[9]	A	B
Dry batch	A	A/**B**[11]	?[12]	A/B	**C**	B	A	A	A	**B**	A	B
In-water pipeline	A	A	A	A/B	A	A	A	A	A	A	A	B
In-water rotating	A	A	?	A/B	A	A	A	A	A/**B**[13]	A	A	B
Dry prod	A	A/**B**[11]	?[12]	A/B	**C**	B	A	A	A	**B**	A	B
In-water prod	A	A/**B**[11]	?[12]	A/B	A	B	A	B	A	**B**	A	B

9: Risks of pre-stun electrical shocks can be reduced or prevented with adequate system design

10: Depending on slaughter plant operating procedures, some fish at the top of the tank may endure air exposure if there is not enough water in the tank

11: The loss of consciousness may be immediate for the fish directly in contact with electrodes, and may not be immediate for the ones which are only indirectly exposed to the electrical shock

12: The failure rate has not been quantified but there are reasons to believe that it is likely to be high (> 5%)

13: The risk of tissue damage is only limited to a small (but unquantified) percentage of fish which may collide with the system's paddles

We decided not to include head-only electrical stunning in this table because this method is not currently used in commercial settings. This method has only been used as part of laboratory experimental set ups, mainly to study the effect of electrical parameters. The use of this method in a commercial setting seems unlikely to us due to slaughter line pace requirements and the difficulty of practical implementation

2.6 Combination of electrical and percussive methods

2.6.1 Method description and current use

- **Description**

Several authors have argued that the combination of electrical stunning followed by percussive stunning could be beneficial. In fact, those methods appear to be complementary and associating them could eliminate some of the welfare hazards that are involved when those methods are used independently. This possibility is mentioned by the EFSA, the Aquaculture Advisory Council, a report from the national veterinary institute of Norway (NVI), the scientific council on animal welfare of the Swedish University of agricultural sciences (SLU) as well as CIWF.

In theory, all electrical stunning methods could be followed by a percussive stunning method (manual or semi-automatic). However, full-automatic percussive stunning cannot be preceded by electrical stunning, because this method requires fish to be conscious to make some efforts to swim in water towards the percussive cylinders.

It is important to make sure that the considered electrical stunning method is compatible with the following percussive method in terms of slaughter line pace. In fact, as electrical stunning can be reversible depending on how the parameters are calibrated, the subsequent percussive stunning must take place as soon as possible after electrical stunning to reduce risks of recovery of consciousness before percussion.

- **Current use**

In practice, the most discussed combination is the association of in-water pipeline electrical stunning followed by semi-automatic percussive stunning (Mejdell & al. 2009a). One Scottish salmon producer already have two of such systems in commercial use for Atlantic salmon, combining Ace Aquatec's in-water electrical pipeline with a semi-automatic percussive system (personal communication with the Scottish company).

A video available on youtube (Electrical and percussive stunning of salmon, https://www.youtube.com/watch?v=SXqUJxURrDg) also shows a combined system made of a head-to-body electrical system followed by a semi-automatic percussive system to stun Atlantic salmon. However, the identity of the company who possesses the system shown on the video is unknown.

A field study in Germany which investigated 21 slaughter process for rainbow trout on 18 different commercial sites, reported that in 14% of cases, a manual percussive stunning was practiced following electrical stunning (either in-water batch stunning or dry head-to-body stunning) (Jung-Schroers & al. 2020).

Currently, systems combining electrical and percussive stunning still seem to be rare. However, the prevalence of such systems could increase in the future due to the benefit of this combination.

As semi-automatic percussive stunning systems are only available for large rainbow trout and Atlantic salmon for the time being, this combination is not available yet for portion sized rainbow trout, sea bream and sea bass.

2.6.2 Welfare hazards, Welfarm's position and recommendations:

- **Immediateness of the loss of consciousness**

In a combined system, the initial loss of consciousness is meant to be caused first by electrical stunning. As previously discussed (see 2.5.2), the loss of consciousness should be instantaneous.

This method obtains the mark "A" for this hazard.

- **Risk of recovery of consciousness**

Electrical stunning, depending on the parameters used, can be reversible, which involves risks of recovery of consciousness. Having a reversible electrical stunning be followed by percussive stunning – which is irreversible – allows to eliminate this welfare hazard. However, the duration between electrical stunning and percussion should be as short as possible to avoid risks or recovery of consciousness before percussion.

This method obtains the mark "A" for this hazard.

- **Failure rate**

Stunning failure rate for electrical stunning of rainbow trout has been estimated to be 1 % for in-water pipeline systems and 3% for batch systems, which can reasonably be extended to Atlantic salmon (EFSA 2009a). For sea bass and sea bream, the stunning failure rate is almost null in in-water pipeline systems according to the observations of the researcher Jeff Lines (personal communication). The stunning failure rate of the head-to-body method was estimated to be 3% for sea bass and sea bream based on behavioural indicators (CIWF Driving innovation in humane fish slaughter). Field observations in Germany reported failure rates comprised between 0% and 5% for in-water batch stunning and head-to-body stunning for rainbow trout in most sites (Jung-Schroers & al. 2020). Semi-automatic percussive stunning has a failure rate estimated to be around 5% by the EFSA.

Taken independently, electrical methods and semi-automatic percussive stunning methods already have relatively low failure rates. It can reasonably be assumed that by combining both methods, the failure rates would be even lower. In particular, as stunned fish are easier to handle, it will be easier for workers to grade and position them correctly in the stunning cylinders, which may help reduce failure rates even further.

This method obtains the mark "A" for this hazard.

- **Air exposure**

As previously discussed, in-water batch and pipeline electrical stunning do not involve air exposure if they are carried out correctly. Head-to-body methods may require a brief air exposure. The same can be said of semi-automatic percussive stunning. However, the short duration (< 15 seconds) of air exposure remains under the acceptability thresholds suggested by the EFSA for both of those methods.

Combining in-water pipeline electrical stunning with semi-automatic percussive stunning renders the fish unconscious when they will be submitted to the air exposure related to semi-automatic percussive stunning. Therefore, this combination allows for the elimination of this welfare hazard.

This method obtains the mark "A" for this hazard.

- **Handling**

Semi-automatic percussive stunning requires brief handling to grade the fish according to their size and to orient them towards the stunning cylinders. With prior electrical stunning, fish will already be unconscious when they will be handled, which results in the elimination of this welfare hazard.

This method obtains the mark “A” for this hazard.

- **Reliance on workers’ skills**

The gestures involved in semi-automatic percussive stunning are not highly technical. Nonetheless, the maintenance and adequate calibration of two complex equipment like in-water electrical pipeline and semi-automatic percussive stunners requires some skills.

This method obtains the mark “B” for this hazard.

WELFARM’s position and recommendations:

The combination of electrical stunning followed by percussive stunning makes it possible to keep the benefits of both of those methods while fixing their main flaws. This is particularly true regarding the combination of in-water pipeline electrical stunning with semi-automatic percussive stunning. This last combination obtains the mark “A” on all our criteria except regarding reliance on workers’ skills in relation to the level of skills involved in the maintenance and adequate calibration of the parameters of the equipment. However, the costs of this method may be a limit for its implementation.

WELFARM considers that the combination of an electrical stunning followed by percussive stunning is an acceptable method. In particular, the combination of in-water electrical pipeline stunning followed by semi-automatic stunning appears to be the best method available for the time being.

The general recommendations previously mentioned regarding electrical and percussive methods are also relevant when both of those methods are combined. To minimize the risks of recovery of consciousness before percussion, the time between electrical stunning and percussion should be as short as possible.

Methods	Exposure to noxious chemicals	Immediateness of the loss of consciousness	Failure rate	Risk of recovery of consciousness (reversibility)	Air exposure	High or extreme densities	Handling	Poor water quality	Tissue damage	Pre-stun electrical shock	Thermal shock	Reliance on workers's skills
In-water electrical pipeline + semi-automatic percussive stunning	A	A	A	A	A	A	A	A	A	A	A	B

3.Synthesis of the welfare hazard analysis of all methods

The table below summarises all the previous synthetic tables for all considered methods. It allows for a global overview of the welfare hazards associated with each method of stunning and killing, and an easier comparison of the methods. Superscript numbering (1 to 13) refer to the comments that can be found under the previous synthetic tables for each category of methods.

Methods	Exposure to noxious chemicals	Immediateness of the loss of consciousness	Failure rate	Risk of recovery of consciousness (reversibility)	Air exposure	High or extreme densities	Handling	Poor water quality	Tissue damage	Pre-stun electrical shock	Thermal shock	Reliance on workers'skills
Decapitation	A	**B**	**?**	A	**C**	A	B	A	**B**	A	A	C
Evisceration	A	**B**	**?**	A	**C**	A	B	A	**B**	A	A	C
Exsanguination	A	**B**	**?**	A	**C**	A	B	A	**B**	A	A	C
Asphyxia	A	**B**	?	A	**D**	**C**	A	A	A	A	A	A
Ice slurry	A	**B**	?	Sea bream, sea bass: A[1]; Trout, salmon: A/B[1]	A[2]	**C**	A	C	**B**	A	**B**	A
Asphyxia on ice	A	**B**	?	A	**D**	**C**	A	A	**B**	A	**B**	A
CO_2	**B.Phy-Bh**	**B**	?	A/B	A	B	A	C	A	A	A	B

Methods	Exposure to noxious chemicals	Immediateness of the loss of consciousness	Failure rate	Risk of recovery of consciousness (reversibility)	Air exposure	High or extreme densities	Handling	Poor water quality	Tissue damage	Pre-stun Electrical shock	Thermal shock	Reliance on workers's skills
N_2	Trout: B.Phy Salmon, sea bass, sea bream: **B.Phy.Bh**[3]	**B**	?	A/B	A	B	A	C	A	A	A	B
CO	Sea bass, sea bream: ? Trout: B.Phy[4] Salmon: A[5]	**B**	?	A/B	A	B	A	C	A	A	A	B
Manual percussive stunning	A	A	**C**	A/B	**C**	A	B	A	A	A	A	C
Semi-automatic percussive stunning	A	A	B	A/B	B/**C**[6]	B	B	A	A	A	A	B
Full-automatic percussive stunning	A	A	**C**	A/B	A	B	A	A	A	A	A	A
Spiking	A	A	?	A	**C**	A	B	A	**B**	A	A	C
Isoeugenol anaesthesia	Trout: **B.Phy-Bh**[7] Salmon, sea bass, sea bream: B.Phy[7]	**B**[8]	?	A/B	A	B	A	B	A	A	A	C

Methods	Exposure to noxious chemicals	Immediateness of the loss of consciousness	Failure rate	Risk of recovery of consciousness (reversibility)	Air exposure	High or extreme densities	Handling	Poor water quality	Tissue damage	Pre-stun Electrical shock	Thermal shock	Reliance on workers's skills
Head-to-body el. stunning	A	A	B	A/B	B	A	A	A	A	A/**B**[9]	A	B
In-water el. Batch stunning	A	A	B	A/B	A[10]	B	A	B	A	A/**B**[9]	A	B
Dry el. batch stunning	A	A/**B**[11]	?[12]	A/B	**C**	B	A	A	A	**B**	A	B
In-water el. pipeline	A	A	A	A/B	A	A	A	A	A	A	A	B
In-water el. rotating	A	A	?	A/B	A	A	A	A	A/**B**[13]	A	A	B
Dry el. prod	A	A/**B**[11]	?[12]	A/B	**C**	B	A	A	A	**B**	A	B
In-water el. prod	A	A/**B**[11]	?[12]	A/B	A	B	A	B	A	**B**	A	B
In-water el. pipeline + semi-automatic percussive stunning	A	A	A	A	A	A	A	A	A	A	A	B

4. Economic aspects of slaughter practices

Different stunning equipment have variable and non-negligeable costs. Here are some indicative prices of equipment obtained through exchanges with equipment suppliers or through the European commission's report. In-water electrical pipeline stunning cost between 60 000 €, 90 000 € and 280 000 € depending on the model (personal communication with several stunning equipment suppliers). Tanks for in-water electrical batch stunning are more affordable. The Italian company Scubla Aquacoltura Engeineering Ecologia offers devices of different sizes for prices of 128 €, 1 467 € (Fishkill EG100), to 2 668 € (Fishkill EG 600, with a carrying capacity of 300 Kg of fish). The German company FIAP offers models within a price range of 889 € to 1790 €. Other more sophisticated models can reach prices of 6 000 €, 8 000 € or 10 000 € (personal communication with equipment suppliers). Simpler tanks can also be designed and produced directly by local electricians hired by fish farmers. In this case, the cost of the material could be around 1 500 € (personal communication with a stunning equipment producer). A company in Turkey sells head-to-body electrical stunners for a price of 80 000 € (personal communication NGO Kaffessiz Türkiye), which is twice as much as the price range mentioned by the European Commission for similar systems in Europe.

Manual pneumatic percussive device designed for poultry but that can likely be used for fish (in particular for emergency stunning) can cost around 65 € only. According to AQUI-S manufacturer, the use of AQUI-S anaesthesia before slaughter implies added costs of 1 to 2 New-Zealand dollar cents (0.6 to 1.2 euro cents) per Kg of fish (AQUI-S New Zealand Ltd).

In the table below are other estimates of the price range and slaughter line pace of commonly used equipment used for slaughter (figure 10).

Equipment	Investment (euros)	Cost per year (euros)
Pump 40-50 ton/hr	200 000	24 000
Pump 20-30 ton/hr	100 000	12 000
Pump 2-3 ton/hr	50 000	6 000
Electro-stunner in-water on harvest boat 5 ton/hr (sea bass, sea bream)	140 000	15 400
Electro-stunner in-water in abattoir (salmon) 20 ton/hr	120 000	13 200
Electro-stunner in-water in abattoir (trout) 20 ton/hr	100 000	11 000
Electro-stunner after dewatering 3-20 ton/hr (salmon, trout)	55 000	8 250
Electro-stunner after dewatering 2-20 ton/hr (sea bass, sea bream)	40 000	6 000
Percussion stunner 5-20 ton/hr (salmon)	60 000	9 000
Decapitation robot	100 000	20 000

FIGURE 10: COST AND SLAUGHTER LINE PACE ESTIMATES FOR FISH SLAUGHTER EQUIPMENT (INSPIRED BY THE EUROPEAN COMMISSION REPORT 2017, P14

The choice of the method will necessarily depend on the ability of producers to invest.

The 2018 report by the European commission estimated the added costs associated with the implementation of OIE standards related to humane slaughter (meaning the uptake of either percussive or electrical stunning). Those estimates vary both in relation to species and the context of the studied countries.

For Atlantic salmon, the commission estimates added costs to be comprised between 0.02 €/Kg in the UK, and 0.09 €/Kg in Ireland. For rainbow trout, added costs are estimated to be 0.04 €/Kg in Denmark and 0.24 €/Kg in France. In Italy, the commission even considers that the transition could allow reduce costs up to 0.06€/Kg due to a decrease in workforce needs.

For sea bream and sea bass, transition costs towards electrical stunning are expected to be 0.04 €/Kg in Spain, 0.05 to 0.06 €/Kg in Greece, and 0.11 to 0.13 €/Kg in Italy. For those latter species, the report indicates that "Although the cost increase is quite modest, even for relatively small volume producers as in Italy, the profit margins on most of these farms might prevent producers from investing." The report claims that from 2009 to 2013, sea bass and sea bream production was not profitable without subsidies in most member states, which can make investment difficult for this sector.

On another note, the choice of relevant methods also depends on the expected slaughter line pace, related to the size of the production. With some systems, it is possible to stun a lot of fish at the same time while others have a more limited carrying capacity. Regarding in-water rotating electrical stunning, the company Test Trout Valley indicated to us that this system allows for a slaughter line pace of 4 to 6 tonnes per hour. For in-water batch electrical stunning, assuming that 5 to 10 minutes are needed per batch, a slaughter pace line of 1.8 to 3.6 tonnes per hour can be expected.

Finally, goals related to the aspect of the final product set by the producer or the retailer may influence the choice of methods (e.g flesh quality matters related to the calibration of electrical and percussive stunning, consumer acceptance of beheaded fish etc…).

It has been said that in practice finding electrical equipment can be harder than finding percussive stunning equipment (HSA 2018).

IV- WELFARM's general recommendations

The welfare hazard analysis can be used to identify three categories of methods: recommended methods, methods that are too uncertain to be recommended yet, and rejected methods. Among acceptable methods, all methods are not on a par: it is possible to come up with a hierarchy of methods based on their degree of compatibility with animal welfare, leading to a recommendation cascade.

Furthermore, it is also possible to come up with cross-cutting recommendations relative to the different phases of the slaughter process and related operations. Our recommendations regarding pre-stunning and arrival to the slaughter plant are presented, followed by cross-cutting recommendations about the stunning process.

1. Slaughter methods: recommendation cascade

Status	Acceptable methods (Ranked based on welfare outcome)	Summary of the marks
1	In-water electrical pipeline followed by semi-automatic percussive stunning (*only available for Atlantic salmon and large trout for the time being*)	*1B*
2	In-water electrical pipeline stunning	2B
3	In-water rotating electrical stunning	3B + “?”
3	Head-to-body electrical stunning	5B
4	Semi-automatic percussive stunning *(only available for Atlantic salmon and large trout for the time being)*	5B + 1C
4	In-water batch electrical stunning (*only in freshwater*)	6B

Status	Methods with some potential but too uncertain to be recommended
?	Isoeugenol anaesthesia
?	Nitrogen (N_2) alone for trout and sea bream
?	Progressive carbon monoxide (CO)

Status	Rejected methods
✗	Decapitation
✗	Evisceration
✗	Exsanguination
✗	Asphyxia
✗	Ice slurry
✗	Asphyxia on ice
✗	Carbon dioxide (CO_2)
✗	Nitrogen (N_2) alone for Atlantic salmon and sea bass
✗	Nitrogen (N_2) mixed with carbon dioxide (CO_2)
✗	Manual percussive stunning
✗	Full-automatic percussive stunning
✗	Spiking
✗	Dry batch electrical stunning
✗	Dry prod electrical stunning
✗	In-water prod electrical stunning

Six methods were considered to be acceptable. Among those, a ranking was constructed based on the marks obtained as the outcome of the welfare hazard analysis. When two marks were possible for one method for a given welfare hazard, the least favourable mark was used for the final grading, with a few exceptions. For in-water rotating electrical stunning, the welfare hazard “tissue damage” would normally be disqualifying if the worse off mark was used for the final grading. Because this hazard is conditional and limited to a small proportion of fish for this method, we decided to give this method a “B” regarding this hazard but not to disqualify it. Therefore, this method was not excluded from the category of acceptable methods. This method obtained the mark “?” regarding failure rates. This mark was treated as equivalent to a « B » within the ranking process, due to Lines & al. 2003 describing the proportion of fish showing signs of consciousness as “small”. Head-to-body and in-water batch electrical stunning methods obtained the mark “A/B” regarding the risks of pre-stun electrical shocks. Using the least favourable of those two marks would normally lead to disqualifying those methods. However, contrary to dry batch electrical stunning, and to in-water and dry prod electrical stunning, it is possible to implement preventative measures to reduce the risk of pre-stun electrical shocks for head-to-body and for in-water batch electrical stunning systems. In addition, if a pre-stun shock does occur in head-to-body systems, it is very brief. For those reasons, we decided not to disqualify them. Likewise, for semi-automatic percussive stunning, the marks B and C are both possible for this method regarding air exposure. If air exposure reaches the level described for the mark C, this method would be disqualified. However, considering that there are more recent models involving shorter durations of air exposure, and because we believe that it is possible to design machines involving less than 15 seconds of air exposure, we decided not to disqualify this method.

Semi-automatic percussive stunning did not obtain any disqualifying mark (except for the risk of long air exposure which was addressed as a special case). This method obtained 7A + **5B** + **1C.** This method obtained the worse marks among acceptable methods.

In-water batch electrical stunning (fresh water only) did not obtain any disqualifying mark (except for the risk of pre-stun electrical shocks which was addressed as a special case). This method obtained 6A + **6B**. This method obtained a score relatively similar to that of semi-automatic percussive stunning.

Head-to-body electrical stunning did not obtain any disqualifying mark (except for the risk of pre-stun electrical shock which was addressed as a specific case). This method obtained 7A + **5B**. This method can be distinguished from previously mentioned methods by its inferior number of B. Therefore, it is tied with in-water rotating systems at the third rank, above semi-automatic percussive stunning, and in-water batch electrical stunning.

In-water rotating electrical stunning did not obtain any disqualifying mark (except for the risk of potential tissue damage which is addressed as a special case). This method obtained 8A + **3B** + **1 “?”.** This method comes with less numerous welfare hazards than previously mentioned methods. Nonetheless, there is a potential risk that some fish could endure “tissue damage” under certain conditions, this hazard being a disqualifying factor. However, considering that this risk is only present for a small proportion of fish it was decided not to disqualify this method. It must also be pointed out that the stunning failure rate has not been quantified for this method. Overall, this method is tied with head-to-body electrical stunning at the third rank, above semi-automatic percussive stunning and in-water batch electrical stunning.

In-water pipeline electrical stunning did not obtain any disqualifying mark. This method obtained: 10A + **2B**. The number of B is somewhat inferior to that of head-to-body and rotating electrical stunning. Nonetheless, those methods can be distinguished on two key factors: pipeline stunning does not involve air exposure and is estimated to have a very low failure rate. Moreover, head-to-body electrical stunning involves a moderate risk or pre-stun electrical shock and rotating electrical stunning involves a moderate risk of tissue damage. Those risks are absent in the pipeline method. Therefore, in-water pipeline electrical stunning was ranked higher in the hierarchy.

Finally, the combination of in-water electrical pipeline stunning followed by semi-automatic percussive stunning allows for the elimination of the risk of reversibility that may come with electrical stunning, which is a significant improvement. Moreover, brief air exposure and handling usually endured by fish in semi-automatic percussive stunning would take place while the fish are already unconscious with this combination. In addition, as stunned fish are easier to handle, prior electrical stunning may help to further reduce the failure rate of semi-automatic percussive stunning. This combination of methods obtained: 11A + **1B**.

The final ranking of methods is based on the summary of marks obtained as the outcome of the welfare hazards analysis but also on more general reasoning and considerations. The methods ranked first and second in the hierarchy clearly stand out from the rest of other acceptable methods. However, the ranking of other acceptable methods involves more uncertainty. In fact, the ranks that were attributed could potentially vary depending on the way marks are attributed, on the relative importance given to the different welfare hazards, on the way uncertainties are addressed, and on whether the best or the worst version of each method is considered.

Three methods are considered to have some potential but to be too uncertain to be recommended for the time being: isoeugenol anaesthesia, immersion in nitrogen-saturated water (when nitrogen is used alone and not mixed with carbon dioxide), and immersion in progressively carbon monoxide-saturated water.

Isoeugenol anaesthesia has some potential, in particular in the context of on-farm slaughter of fish that are not destined to human consumption. This method does not result in an immediate loss of consciousness and some physiological stress, or even behavioural aversive reactions in some cases (rainbow trout) take place before the loss of consciousness. Consequently, the marks obtained disqualifies this method and it should normally be rejected. However, we decided to classify this method within the category of “uncertain” methods because the lack of immediateness of the loss of consciousness may be less problematic than for other methods considering that the distress of fish prior to the loss of consciousness may be limited, and potentially reduced by the first sedative effects of the molecule. Two important aspects warrant caution. First, brief aversive behavioural reactions have been observed in rainbow trout, but those reactions are not reported systematically. It may be possible to reduce this problem by increasing the dosage progressively, but it should be studied empirically. If aversive behavioural reactions have not been reported in other species to our knowledge, the possibility of using this method without eliciting such behavioural reactions should be more thoroughly guaranteed. Furthermore, exposure to the anaesthetic can cause various levels of physiological stress in our target species. Secondly, the loss of consciousness has not been studied with EEG. This type of confirmation is needed to exclude the possibility that fish may be immobilised while remaining conscious. In addition to those two aspects, the main drawbacks of this method are exposure to poor water quality at high density and the reliance on workers’ skills regarding dosing - which requires to take into account several factors to be adequate.

The study which investigated immersion in nitrogen-saturated water for rainbow trout did not carry a thorough assessment of the loss of consciousness, neither through behavioural indicators nor through EEG. The absence of rigorous confirmation of the loss of consciousness leads us to avoid recommending this method for the time being. Moreover, even if the loss of consciousness was to be confirmed, it is not instantaneous. For this reason, this method obtained the mark B for this hazard, which is normally disqualifying. However, as rainbow trout seem not to show behavioural aversive reactions in response to this method, similarly to our reasoning about isoeugenol, we could potentially tolerate the non-immediateness of the loss of consciousness. However, the absence of behavioural aversive reactions needs to be more thoroughly confirmed because only one study was conducted about it on rainbow trout, and it is known that when this method is used for Atlantic salmon, which is also a salmonid, aversive reactions are displayed. Moreover, this method induces physiological stress in rainbow trout. Nitrogen used alone has not been studied for sea bream. If future studies bring convincing evidence that this method does result in a loss of consciousness with a failure rate inferior to 5%, and if the absence of behavioural aversive reactions is confirmed for rainbow trout and sea bream, then this method could potentially be considered acceptable for those species. However, conducting further studies about this method on Atlantic salmon is not relevant considering that this species shows behavioural aversive reactions and physiological stress in response to nitrogen.

Immersion in progressively carbon monoxide-saturated water seems to have some potential for Atlantic salmon as this method seems not to result in aversive reactions. However, the loss of consciousness has not been rigorously confirmed, and behavioural indicators revealed that a large percentage of salmon remained conscious. Similar to nitrogen used alone, carbon monoxide does not result in an immediate loss of consciousness, but potentially, it could still be tolerated if we are relatively certain that the period leading to unconsciousness is not associated with intense suffering. If future studies confirm that this method does not entail aversive reactions, and that it results in a loss of consciousness with a failure rate below 5%, it could potentially be considered as an acceptable method. This method has not been studied for sea bass and sea bream. For rainbow trout, carbon monoxide seems to induce a moderate physiological stress response, but the presence or absence of behavioural aversive reactions has not been studied.

Fifteen methods were considered to be ethically unacceptable and are therefore rejected by Welfarm. These methods all obtained one or several disqualifying marks.

This category comprises all haemorrhagic methods without prior stunning, be it decapitation, evisceration or exsanguination, mostly because they involve tissue damage while the fish are conscious and therefore, considerable pain. Asphyxia in air was also rejected mostly due to the very long and stressful time needed before the loss of consciousness, during which fish are agitated and struggling in air. Methods based on hypothermia (immersion in ice slurry, asphyxia on ice) involve a severe thermal shock resulting in agitation and physiological stress, which leads to their rejection. Most of the gas exposure methods are also rejected. In fact, immersion in CO_2-saturated water is associated with physiological stress and strong behavioural aversive reactions in rainbow trout, Atlantic salmon and sea bass, and by analogy, it is very likely to be the case for sea bream too. When mixing CO_2 with N_2, the same issues are observed although their intensity is decreased compared to the use of CO_2 alone. The use of pure nitrogen has not been studied in sea bream. For this species, nitrogen was only tested in combination with CO_2 which results in aversive reactions. Such results warrant caution regarding the use of pure nitrogen (not combined with CO_2) for sea bream. Immersion in N_2 -saturated water is rejected for Atlantic salmon as it results in strong behavioural aversive reactions and physiological stress. The outcome seems similar for sea bass but the level of evidence is lower as nitrogen was studied in combination with a thermal shock. Manual percussion and spiking are rejected mostly due to the duration of air exposure involved, and because of significant failure rates. However, even if the duration of air exposure was reduced, these two methods are still associated with several other non-disqualifying welfare hazards (especially handling) and therefore still appear inferior to the methods that were considered to be acceptable. Eventually, manual pneumatic percussive stunning could be beneficial only for species for which there are no better alternative (which is not the case of our target species), while better methods are being developed, or for extremely small scale sites, with a very low slaughter line pace. Full-automatic percussive stunning is somewhat special. We chose to reject this method due to its high failure rate. However, if further development leads to progress in this regard, it could potentially become acceptable, although some attention needs to be given to the issue related to risks of exhaustion while the fish swim into the system. Dry batch electrical stunning, dry prod electrical stunning and in-water prod electrical stunning are rejected because they are associated with a systematic risk of pre-stun electrical shocks, and because their failure rates are likely to be high even though they have not been quantified yet. In addition, dry batch electrical stunning and dry prod electrical stunning involve an air exposure of an unsatisfying duration.

2. Cross-cutting recommendations about the different steps of the slaughter process and related operations

2.1 Arrival to the slaughter plant (pre-stunning):

Transportation and transfers from the place of rearing to the slaughter plant can be demanding for animals. There are several modalities of transfer depending on the sector and on countries. Generally speaking, our target species are displaced in the following manner (European commission 2017):

- In Norway, Atlantic salmon are crowded in their sea cages and harvested with fish pumps. Then, they are transported within special “well boats” towards nearby slaughter plants. There, fish are pumped once again and transferred to either lairage tanks or directly to the slaughter line. Sometimes, producers use so called slaughter vessels i.e boats equipped with stunning and slaughter systems to slaughter fish directly aboard next to sea cages (personal communication NGO Norwegian Animal Protection Alliance).
- In Greece, sea bass and sea bream are crowded in sea cages and harvested with brail nets and put into ice slurry bins to slaughter them directly on boats. Slaughter processes of sea bream and sea bass appear to be similar in France
- In France, rainbow trout farms sometimes have their own slaughter unit on site. In the 2018 official survey by the French ministry of agriculture, 49 salmonid producing companies declared having a slaughter plant and 98 declared having a product processing plant, while 221 declared that they did not have any slaughter equipment (out of 365 surveyed companies) (Agreste 2020). Fish are transferred using pumps or nets towards mobile machines nearby the rearing raceways. If the slaughter unit is not mobile and located close to the rearing raceways, fish are transferred to the slaughter line either with fish pumps or using small transportation tanks. If the slaughter plant is further away, fish are pumped into a transportation truck which takes them to the slaughter plant where they are then poured into either a lairage tank or directly into the slaughter line.
- Regarding marine fish farming in France, in the 2018 official survey by the French ministry of agriculture, 6 companies declared having a slaughter facility and 1 company declared having a product

processing plant, while 10 companies declared that they did not have any slaughter equipment, out of 28 surveyed companies (Agreste 2020).

- For extensive pond fish farming in France, in the 2018 official survey by the French ministry of agriculture, 7 companies declared having a slaughter plant, 4 companies declared having a product processing plant and 189 companies declared that they did not have any slaughter equipment out of 211 surveyed companies (Agreste 2020).

Slaughter and transportation processes involve one or several steps of "crowding" during which fish are grouped at high density on one side of the raceway / tank / sea cage to be harvested. One or several transfers, may be carried out with either fish pumps or nets (in some cases brail nets) before reaching a lairage tank or the slaughter plant. Sometimes, transportation in a truck or in a boat is needed. Such handling and transportation procedures are not necessarily specific to slaughter and may be carried out several times throughout the rearing cycle.

WELFARM's recommendations:

IMAGE FROM PIXABAY

Such handling procedures are demanding and stressful for fish. Transportation of live animals should be avoided as much as possible. It is preferable to slaughter fish directly on site in good conditions.

Regarding fish kept in sea cages, slaughter should ideally take place nearby the cage i.e next to the sea cage, on boats (for small productions), on a floating pontoon or on specially designed slaughter-boats for large productions.

In order to access more advanced technologies that are more costly, mutualisation of slaughter equipment that can be displaced on different sites, should be considered. A slaughter-boat or truck could be shared among several sites across a given geographic area.

If it is not possible to humanely slaughter fish directly on site, it is preferable to carry out transportation with good conditions towards a humane slaughter plant, rather than performing inhumane slaughter methods on site to avoid transportation.

During transfers, the use of fish pumps is recommended compared to the use of brail nets which involves compressions and air exposure.

IMAGE FROM PIXABAY

During transfers with fish pumps, shocks at the entrance or the exit of the pumps should be avoided. Fish should not exit fish pumps to arrive onto a solid surface without water. The height between the exit of the pump, and the place where fish are expected to fall should be minimised. The pump should be suited to the considered species, but also to the size and the number of fish being pumped. The flow speed must be adequate and suited to the species.

Fish pumps are stressful for fish, but other methods involve even greater stress.

If fish pumps are not available, nets used for crowding and transfer must have a mesh size suited to the size of the fish and be knotless to reduce abrasion. Nets should not be metallic. More generally, equipment used to transfer fish should be designed in order to avoid injuring fish (e.g with smooth surfaces).

Air exposure should be minimised, and the pressure put on fish should be kept to a minimum to avoid injuries. This is particularly true for species with dorsal spikes like sea bass, for which the use of brail nets should be prohibited.

Crowding should be carried out in steps in order to reduce the duration during which fish are maintained at a high density (E.U Platform on animal welfare 2020). To avoid hypoxia, oxygenating the water in which fish are crowded may be considered through oxygen diffusion or aeration (E.U Platform on animal welfare 2020). Fish scototaxis can be taken advantage of to perform crowding: fish should rather be crowded in a dark and shaded area (Canadian Code of practice for the handling and care of farmed salmonids 2021). The degree of surface agitation must not be too high. If it is, corrective actions must be taken such as oxygenation or loosening of the crowding net to provide more space to fish. The scoring system of the Canadian Code of Practice for the handling and care of farmed salmonids 2021 can be used to assess the level of surface agitation. Carrying crowding by very hot weather should be avoided, as fish are already under thermal stress. Fish should rather be crowded at hours of the day when the temperature is cooler, while taking into account the chronobiology of the considered species.

If a period of lairage within the slaughter plant is necessary, it should be as short as possible. Lighting of the lairage tank should match the photoperiod that fish are used to as much as possible. Within lairage tanks, water quality should be persevered, and density limited as much as possible.

Fish are usually fasted before slaughter to empty their guts, which allows some benefits in terms of food safety and helps preserving water quality throughout transportation. While fasting is necessary, it is often too long, sometimes lasting up to 2 weeks for salmonids (EFSA 2009b). The duration of fasting prior to transportation and slaughter should be kept to the minimum needed to clear the guts (E.U Platform on animal welfare 2020). This duration depends on metabolism and therefore also on water temperature. It also depends on the weight of the fish.

The EFSA 2009ab considers that for rainbow trout, welfare is impaired if starvation lasts for longer than 50 degree-days [5]and recommends not to exceed one week of fasting for Atlantic salmon. More recent publications consider that a maximum duration of 3 days of fasting is sufficient to clear the gut in Atlantic salmon (Lines & Spence 2012, 2014). At 19°C, 24 hours are enough to clear the gut of rainbow trout, and signs of decreased immune function starts appearing after 3 days of starvation (Lines & Spence 2014). Bermejo-Poza & al. 2017 tried to identify an optimal fasting duration and concluded that “a pre-slaughter fast from 17.2 degree-days, to 22.3 degree-days can minimize the stress response in rainbow trout and produce better flesh quality”. The 2021 edition of the Canadian Code of Practice for the handling and care of farmed salmonids provides estimates of the minimum duration needed to ensure gut clearance in farmed salmonids in relation to weight, species and water temperature (Figure 11). Estimates vary between 9.1 degree-days to 15 degree-days for fish between 90 g to 695 g and reach 28 degree-days for Atlantic salmon of 5.6 Kg. Until more specific studies are

[5] Degree-days are a unit of time relative to animal growth in aquaculture. As the metabolism of fish depends on water temperature, in terms of physiology, a duration of one day has a very different meaning depending on whether the water temperature is at 1°C or at 10°C. Degree-days are a unit of time divided by temperature : 10 degree-days correspond to a duration of 10 days at a water temperature of 1°C, a duration of 1 day in a water temperature of 10°C, and half a day in a water temperature of 20°C.

carried out, for salmonids, we recommend to limit fasting to a maximum of 20 degree-days for fish under 500 g of body weight, and to a maximum of 30 degree-days for fish above 500 g.

There are less available data about the minimum time needed for gut clearance and the ideal durations of feed withdrawal periods for sea bream and sea bass. The EFSA classifies prolonged feed withdrawal as a welfare hazard for sea bass and sea bream too and reports current practices to mostly be comprised between 3-7 days of fasting, although longer durations can also be practiced (EFSA 2009c). According to the Hellenic Aquaculture Producers Association guide for good fish welfare practices, sea breams appear to be more sensitive to feed withdrawal than sea bass: they exhibit more stress, lose weight more quickly and take longer to recover their original weight after fasting (Pavlidis & Samaras 2020). However, the Hellenic guide of good fish welfare practices highlight feed withdrawal as a welfare risk but does not specify a recommended maximum feed withdrawal duration.

Compassion In World Farming recommends not to exceed 72 h of feed withdrawal for sea bream and sea bass, based on a general recommendation originally developed for Atlantic salmon. The “Label Rouge” specifications for sea bream and sea bass sets a required minimal feed withdrawal period of 48 h if the water temperature is below 16°C and of 36 h if the water temperature is above 16°C, and a maximal feed withdrawal period of 2 weeks. The minimal values mentioned by the Label Rouge equates to roughly 30 – 32 degree-days.

The MedAid project, listing operational welfare indicators for sea bream, came up with a grading system to assess the welfare impact of feed withdrawal on sea bream. The authors considered that feed withdrawal periods of up to 55 degree-days did not negatively impact fish welfare, that feed withdrawal periods lasting 55 to 110 degree-days were a mild stressor, and that feed withdrawal periods exceeding 110 degree-days were a severe stressor. Although those figures were designed for sea bream, the authors used references from studies carried out on sea bream, but also on sea bass and on salmonids. In addition, considering that sea bream are believed to be more sensitive to fasting than sea bass, extending the MedAid number from sea bream to sea bass is probably acceptable. The fact that the MedAid 55 degree-day number exceeds the required minimal fasting duration mentioned in the Label Rouge specifications (i.e around 30-32 degree-days) for both sea bream and sea bass is also reassuring to extend this number to sea bass, and to expect it to be workable on farms. Until more precise species-specific studies are carried out, Welfarm recommends a maximum feed withdrawal duration of 55 degree-days for both sea bream and sea bass.

Species	Weight (g)	Temperature (°C)	Duration (hours)	Degree-days (°D)
Atlantic salmon	5 600	4	168	28
	695	7.1	48	14.2
	150 - 200	9	30	11.3
	900 - 1450	13.4	24	13.4
Rainbow trout	142	10	28	11.7
	91	15	24	15
	140-145	18	14	10.5
Brown trout	90-300	5.2	42	9.1
	90-300	9.8	27	11
	90-300	15	15	9.4

FIGURE 11: ESTIMATES OF THE MINIMUM DURATION NEEDED FOR GUT CLEARANCE IN SALMONIDS IN RELATION TO SPECIES, WEIGHT AND WATER TEMPERATURE (INSPIRED BY THE CANADIAN CODE OF PRACTICE FOR THE HANDLING AND CARE OF FARMED SALMONIDS 2021)

2.2 Throughout the stunning and slaughter process:

- **Handling**

Handling fish should be minimised. As much as possible, handling should be carried out under water. Equipment used to handle fish should be wet before coming into contact with fish.

During handling, fish must not be hit. They should not be handled in a way that may cause pain or suffering. In particular, fish should not be lifted by the tail, the gills or by other body parts that may give rise to pain.

- **Water quality**

Water parameters must be suited to the considered species. These parameters must be controlled regularly (O_2, pH, temperature, CO_2 etc.). The temperature must be within the species comfort range.

O_2 levels must be optimised. Alarm systems should be in place to signal insufficient oxygen levels. Water should be regularly renewed. The available volume of water should be sufficient. The flow rate should be controlled to avoid stressing fish.

- **Density**

Stocking density should be kept as low as possible. Periods when fish are maintained at high density should be as short as possible. The density should allow fish to swim normally and should not result in injuries.

- **Reliance on workers' skills :**

Staff in contact with the fish should be continuously trained to safeguard animal welfare. Workers must be able to recognise the signs indicating the loss of consciousness and the recovery of consciousness of the considered species. They must be trained in live fish handling.

The tasks requiring a high level of precision in gestures that will impact animal welfare (e.g spiking), should be given to the same workers to optimise efficiency and to safeguard animal welfare. That being said, the repetitiveness of gestures should be regulated to avoid deteriorating the health of workers and to maintain the efficacy of gestures. In fact, according to the National agency for improving working conditions (ANACT), repetitiveness allows workers to acquire operational expertise and to improve the precision of technical gestures. However, those tasks should not reach overly high levels of drudgery due to repetitiveness as defined by ANACT's 2015 report: "The execution of repeated gestures requiring the same joints or body segments conducted under time constraints and at high speed during most of the working time".

- **Pre-stunning sedation: an option to be explored**

Even stunning methods that we consider to be acceptable involve some level of stress for fish. In particular, a brief air exposure is often required in the case of semi-automatic percussive stunning and head-to-body electrical stunning. Semi-automatic percussive stunning also involves brief handling. In-water batch electrical stunning involves a short period of time when fish are kept at high density in water which may be of sub-optimal quality before losing consciousness. Furthermore, transportation towards the slaughter plant, and transfers by fish pumps within the slaughter plant are stressful.

For those reasons, sedating fish prior to stunning could be considered to limit stress. For instance, this option is recommended by the British Veterinary Association (recommendation n°22, BVA 2020). In addition, pre-stunning sedation may render fish easier to handle in the case of semi-automatic percussive stunning. This would make the task of workers easier and perhaps could help to reduce stunning failure rates. In fact, according to one stunning equipment supplier, the degree of fish agitation before stunning has an impact on stunning failure rates in the case of percussive stunning, both for semi-automatic and full-automatic percussive stunning (personal communication). Sedation is not the same as deep anaesthesia which can lead to the loss of consciousness. It solely consists in diminishing of the level of arousal of the fish to limit its stress, without going all the way to deep anaesthesia and a loss of consciousness.

IMAGE FROM PIXABAY

Two options could be explored. Isoeugenol (AQUI-S) may be used for this purpose with a lower dosage than when this molecule is used to induce lethal anaesthesia overdose. However, the current E.U regulation about residual concentrations of iseugenol in flesh of fish destined to human consumption must be respected. Therefore, the time needed to metabolise the molecule for the considered species must be studied. The benefits of sedation must be rigorously studied and demonstrated before the uptake of this process. In fact, for sea bream, sedation with 2 mg/L of AQUI-S did not reduce stress during transportation but increased cortisol levels (Jerez-Cepa & al. 2021). Similarly, caution is particularly required regarding rainbow trout which can sometimes exhibit brief aversive reactions to isoeugenol and eugenol.

For cold-water species like salmonids, sedation through progressive cooling may be considered, as cooler temperatures entail a decrease in metabolism. One stunning equipment supplier told us that some of their clients used this type of sedation before full-automatic percussive stunning, mostly because reducing fish agitation can help to reduce stunning failure rates (personal communication). Thus, the EFSA reports that a progressive decrease in the temperature, with a speed of 1.5° C per hour, in well oxygenated water, can induce sedation in Atlantic salmon (EFSA 2009b). The report requested by the European parliament about the welfare of aquatic animals during transportation also supports this recommendation and considers that it can be extended to rainbow trout as well (Saraiva & al. 2021). In addition, the authors of the report point out that in case of sedation by cooling, the temperature should not decrease below 6°C (Saraiva & al. 2021). This procedure could potentially be practiced in cases where fish are to spend several hours in lairage at the slaughter plant before slaughter. The progressivity of the decrease in temperature is very important: this type of sedation should not be performed if time constraints do not allow for the respect of the rule of 1.5 °C per hour. Unless new studies demonstrate that it is not an issue, sedation by cooling should only be done for cold-water species and not for warm-water species. For the latter, there is a possibility that cooling could induce significant physiological stress and potential suffering.

- **Stunning**

Stunning equipment should be designed to avoid injuries, suffering or arousal of the fish. The device characteristics should be suited to the size and the species of the fish.

Fish should be introduced into the stunning equipment only once workers are ready. Fish should not be placed into stunning equipment needlessly for a prolonged time (e.g during work breaks).

Irreversible stunning is preferable as it limits risks of recovery of consciousness. Stunning parameters should match what is specified by the equipment user guide.

User guides of stunning equipment should be available and translated in the languages of the workers. Maintenance should be carried out regularly in accordance with instructions from the equipment supplier. Equipment failure must be corrected as soon as possible.

Stunning equipment should come with a system to display stunning parameters so that workers in charge of stunning can easily access this information.

Ideally, the equipment should give off a visual or sound cue if the electrical parameters are insufficient, or if the pressure is insufficient for percussive stunning. Such signal should come with the possibility for workers to take corrective measures to avoid causing injures and suffering to fish.

A back-up stunning system must be quickly available in case of initial stunning failure. Manual pneumatic percussion can be used as a back-up stunning method.

Emergency procedures must be established for the stunning unit. Standardised Operating Procedures (SOP) must be complete and updated.

- **Checking the state of consciousness**

Consciousness status must be checked after stunning before performing a killing method. A second check of the consciousness status should be performed after application of the killing methods, in particular during bleeding after reversible electrical stunning. Scientific studies determine unconsciousness visually by the absence of opercular motions, the loss of the vestibulo-ocular reflex, and the absence of responsiveness to noxious stimuli. A recent study supported the validity of those indicators through EEG in the case of electrical stunning for rainbow trout (Jung-Schroers & al. 2020). This publication also concludes that shaking and irregular muscular contractions can be present in unconscious fish. However, other studies found that behavioural indicators are not as reliable as previously thought. Thus, one study showed that in the case of stunning by immersion in CO_2 saturated water at low temperatures, brain activity (and sensitivity), assessed by the presence of visually evoked potentials, could persist up to 3.5 minutes after the cessation of opercular motions, and up to 6.5 minutes after the loss of balance for some individuals (Bowman & al. 2020). Another recent study showed that visually evoked potentials (VEPs), a conservative indicator of consciousness, can return way earlier than opercular beat after a reversible electrical stunning in rainbow trout (Hjelmstedt & al. 2022). In this study, the authors observed that one individual had recovered its brain functions as soon as 10 seconds after stunning, while its opercular beat rate only resumed 194 seconds after stunning. Therefore, behavioural indicators of consciousness are not perfect, because they sometimes are absent in conscious fish (Berg & al. 2021, Hjelmstedt & al. 2022). Despite those limitations, behavioural indicators are the best available indicators that can be used by workers on sites.

Indicators of consciousness	
Those signs must be absent in a stunned fish. If present, the fish must receive emergency stunning.	
Breathing	**Presence of opercular motions**
Eyes	**Presence of the vestibulo-ocular reflex**
Coordinated movements	**Presence of swimming or escape attempts**
Responsiveness to noxious stimuli	**Presence of reactions after contact (e.g tail pinching)**
Balance	**The fish keeps its balance (swims belly down)**

If fish show signs of recovery of consciousness, they must be stunned again with a back-up system (manual pneumatic percussion) which must be quickly available.

- **Bleeding**

Before bleeding, consciousness status must be checked to ensure that fish are unconscious. Bleeding should be carried out as soon as possible after stunning. Fish must not recover consciousness before nor during or after bleeding. Thus, gestures should be precise and allow for complete and profuse bleeding. As much as possible, methods leading to death in the least amount of time are to be preferred so as to reduce risks of recovery of consciousness. Decapitation is ideal. If not possible, evisceration may be practiced, ideally in association with a gill cut. If not possible, gill cutting alone without evisceration or decapitation may be performed. Gill cutting should be conducted on both sides and not just one side. Emergency procedures should be established for the bleeding unit.

Conclusion

All stunning and killing methods currently available have both strengths and weaknesses in regard to animal welfare. However, it is already possible to identify some ethically unacceptable methods which must be phased out. Haemorrhagic methods (decapitation, evisceration, exsanguination) without prior stunning, asphyxia in air, asphyxia on ice, immersion in ice slurry, manual percussive stunning, spiking, immersion in CO_2 saturated water, dry batch electrical stunning, dry prod electrical stunning as well as in-water electrical stunning are all methods which must be rejected. The situation for other gas exposure methods (immersion in N_2 saturated water, immersion in CO saturated water, other gas mixtures) is somewhat more complex. Some must be rejected for certain species (pure nitrogen for Atlantic salmon and sea bass, nitrogen combined with CO_2). For other methods and species (nitrogen for rainbow trout and sea bream, immersion in progressively carbon monoxide - saturated water, gas mixtures involving argon), uncertainty is too high for them to be recommended for the time being. Full-automatic percussive stunning should be rejected due to its overly high stunning failure rate but could potentially become acceptable if this problem is to be resolved in the future, though caution is required regarding the issue of potential exhaustion as the fish swim into the system.

Other available methods can be considered acceptable, but they are not on a par. The combination of in-water pipeline electrical stunning followed by semi-automatic percussive stunning appears to be the best available method so far. The use of in-water pipeline electrical stunning quickly followed by an efficient killing method ranks second. Head-to-body electrical stunning and in-water rotating electrical stunning come next. Semi-automatic percussive stunning and in-water batch electrical stunning (only in freshwater) can also be acceptable. Regarding semi-automatic percussive stunning, caution is required: this method is only acceptable if the design is adequate and guarantees that air exposure is kept under 15 seconds. Isoeugenol anaesthesia is too uncertain (especially regarding its potential aversiveness) to be recommended for the time being but could have some potential for the on-farm slaughter of fish not destined to human consumption. The combination of in-water pipeline electrical stunning with semi-automatic percussive stunning, or the use of in-water pipeline electrical stunning alone clearly stands out as the best available methods. However, the hierarchy of other acceptable methods comes with more uncertainty and subjectivity. It is possible that our assessment of methods can evolve in the future in relation to scientific and technological progress.

The slaughter practices of the fish farming sector need to evolve towards higher welfare methods. This is what consumers want: 89% of them claim that humane slaughter is an important animal welfare criterion for farmed fish, according to a 2018 survey conducted with more than 9 000 citizens of the European Union by the ComRes Institute for Eurogroup for Animals.

In France, the Interbranch committee of aquaculture products (CIPA) created a platform (the B. ABA project) to exchange with researchers to improve their slaughter practices (n°104 Droit Ethique et Sciences – LFDA). The European commission demonstrated interest in this topic through the reports it published (European commission 2017, 2018). Moreover, the commission announced that as part of the “Farm to Fork strategy”, animal welfare regulations on slaughter and transportation will be revised. This revision can and must be an opportunity to include requirements about fish slaughter that can be built upon OIE standards.

Other issues about aquatic animal slaughter are also emerging. Slaughter practices of farmed decapod crustaceans (in particular live boiling) and cephalopods must also evolve towards better animal welfare (BVA 2020). Furthermore, conditions in which fish are captured and slaughtered in wild capture fisheries must also be addressed in the future (Mood 2010, Eurogroup for Animals 2021). Knowledge transfer from aquaculture practices will be essential to achieve progress in this field.

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