Report on the feasibility of implementing stunning prior to slaughter in farmed seabream and seabass

Animal Welfare Education Centre

Barcelona, 2024

Authors: Larrondo, C., Mainau, E. & Llonch, P.

Required citation: Animal Welfare Education Centre (AWEC). 2024. Report on the feasibility of implementing stunning before slaughter in farmed seabream and seabass.

Funding: This report was commissioned by the Animal Welfare Observatory (AWO). The funder has been involved in the report's design and data collection.

CONTENTS

Tables

Figures

Boxes

Glossary

ASC	Aquaculture Stewardship Council
BAP	Best Aquaculture Practices
CO_2	Carbon dioxide
EEG	Electroencephalogram
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
FoS	Friend of the Sea
MAPA	Spanish Ministry of Agriculture, Food and Fisheries (*Ministerio de Agricultura, Pesca y Alimentación de España*)
N_2	Nitrogen
O_2	Oxygen
REV	Social evoked response
SD	Standard deviation
RVO	Vestibulo-ocular reflex
RSPCA	Royal Society for the Protection of Cruelty to Animals
WOAH	World Organisation for Animal Health

Executive summary

There is clear scientific evidence that fish (including seabream and seabass) are conscious and sentient beings, i.e. they are capable of experiencing pain, fear and stress. Despite this, fish lack specific animal welfare legislation at the EU level. The future legislative framework should consider species-specific welfare aspects due to the multiple anatomical, ecological and adaptive differences of the species farmed in aquaculture. Currently, there are recommendations and good practice guidelines focused on improving the welfare of seabream and seabass at different stages of production, with slaughter being one of the most important moments to consider. It has been found that seabream and seabass slaughtered without stunning (direct exsanguination, chilling and asphyxia in air) show behaviours indicative of stress and suffering, taking between 7.5 and 34 min. to lose consciousness depending on the species. Thus, although slaughter in ice water is the most common method in seabream and seabass, it is recommended to apply an effective pre-stun to cause a rapid loss of consciousness, avoiding suffering. The main stunning methods are electrical, mechanical, gas or anaesthetic. Electrical stunning in water or dry stunning has been successfully tested in seabream and seabass farms in the Mediterranean Sea, showing good animal welfare results for both species.

1. INTRODUCTION.

This report analyses the applicability of stunning seabream and seabass, two of the top species reared in Spanish aquaculture, under commercial conditions. It is divided into five parts, each evaluating the applicability of the technologies used to stun them. The first one describes the impact of the timing of slaughter on animal welfare. Fish welfare involves recognising that they are sentient beings. Pre-slaughter stunning is crucial to avoid suffering during the slaughter process, ensuring a rapid loss of consciousness and minimising the fear, pain and stress associated with it.

Next, animal welfare regulations and certifications are discussed. National and international legislation on fish welfare, especially about pre-slaughter stunning, is still under development. The European Union (EU) recognises fish as sentient beings and demands respect for their welfare. At the national level, European regulations are applied, and animal welfare in aquaculture is promoted; however, specific rules on fish welfare remain missing. Also, the main certifications for aquaculture products include requirements for fish humane stunning.

Thirdly, the slaughter process is described, from fasting to the animal's death, mentioning how pre-slaughter phases can significantly influence fish welfare and the final product's quality. Fasting, essential to emptying the digestive system, should not exceed the recommended periods to avoid stress and weight loss. Moreover, high densities in fish farms can increase stress and decrease oxygen, while crowding can be stressful, affecting product quality.

Chapter four explains the different methods of slaughter, stunning and loss-of-consciousness indicators available for farmed fish. Slaughter without pre-stunning is common in the aquaculture sector but is not considered humane, given it causes great suffering before fishes lose consciousness. Slaughter methods include direct exsanguination, chilling in ice water and asphyxia in air. In the case of electrical stunning, it allows for humane killing by inducing unconsciousness quickly.

Finally, the last part of the report identifies technologies applicable in commercial systems that can be used to stun seabream and seabass. To implement them in Spanish farms, the report encourages production companies to carry out pilot tests or adapt the technology to the context of each farm to ensure its proper functioning.

2. ANIMAL WELFARE IN FISH AND THE EFFECT OF STUNNING BEFORE SLAUGHTER.

Sentience, defined as an individual's ability to experience positive and negative emotions, involves some level of consciousness and cognitive abilities, as well as the presence of anatomical structures responsible for these functions (Broom, 2014, 2016a, 2016b). These abilities have been extensively demonstrated in domestic animals (Cornish et al., 2016), although there is still debate about sentience in fish, mainly due to differences in their brain structures (Cottee, 2012; Rose, 2002; Rose et al., 2014).

However, it has been shown that they can experience negative emotions such as fear and pain since they possess the anatomical structures necessary to process them (Broom, 2016b), changing their behaviour and activity at the brain level when confronted with adverse situations (Sneddon, 2003, 2011; Daskalova et al., 2016; Roques et al., 2012). Given this ability to sense, it is necessary to protect fish welfare in production systems. Not only is their physical health, nutrition, and the environment in which they are reared important (Ashley, 2007; Sánchez-Suárez et al., 2020) but also the avoidance of stress and pain arising from inappropriate management practices throughout the production cycle, including the slaughter process (Boaru et al., 2022; Roth et al., 2009).

Therefore, one of the most important steps to promote fish welfare is the application of humane slaughter. It consists of pre-stunning to achieve an immediate or rapid loss of consciousness (<1 second) before death (Figure 1) while avoiding fear, anxiety, pain and stress during the procedure (Brijs et al., 2021; López-Cánovas et al., 2020). Stunning fish prior to slaughter not only prevents suffering but also has positive effects on product quality. Numerous studies have shown that inducing immediate unconsciousness before slaughter improves meat quality in different fish species (Oliveira Filho et al., 2015; Poli et al., 2005).

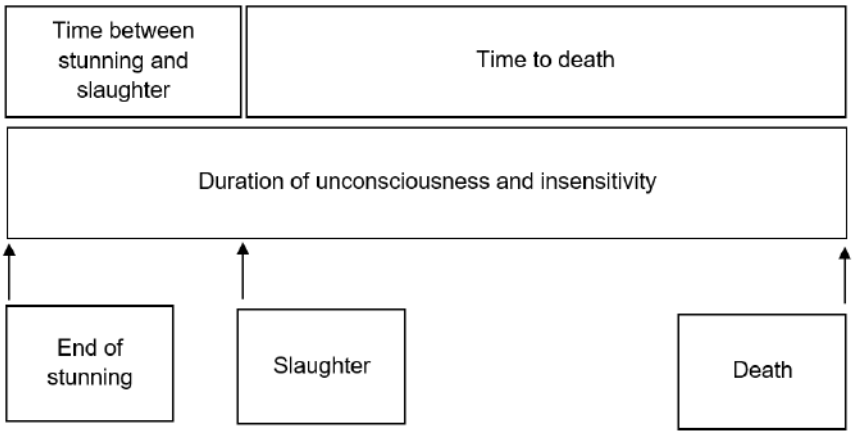


Figure 1. Humane fish slaughter, showing the time between stunning and slaughter and the duration of unconsciousness and death (adapted from EFSA, 2004).

3. LEGAL CONTEXT AND RECOMMENDATIONS IN THE EU AND SPAIN.

3.1. International level

National and international legislation on fish welfare, particularly pre-slaughter stunning, is at an early stage of development, in contrast to that available for terrestrial farmed animals (Policies, 2023). This situation might have been influenced because the demonstration of fish sentience has come later than in other domestic animal species (Brown, 2015). In addition, the high diversity and number of finfish species make it challenging to publish recommendations and/or legislation in this area (Stien et al., 2020). The legal framework is based on voluntary recommendations and general management guidelines, i.e., not species-specific. According to the European Food Safety Authority (EFSA, 2009a, 2009b) experts, specific welfare aspects for each farmed fish species should be considered due to their marked anatomical, ecological, and adaptive differences.

The World Organisation for Animal Health (WOAH) provides international recommendations for farmed fish welfare, contained in its Aquatic Animal Health Code (WOAH, 2022). Chapter 7.3 of the Code details welfare recommendations during stunning and slaughter for those fish destined for human consumption, mentioning that they should be stunned prior to slaughter and that the method of stunning should ensure immediate and irreversible loss of consciousness. It also stresses the regular maintenance of the equipment used, as well as the need for additional stunning equipment, as an alternative to an eventual malfunction of the main one. Furthermore, it points out that all staff involved in these operations must be competent, experienced and knowledgeable about animal behaviour. It is, therefore, essential that they are trained in fish welfare and occupational safety aspects when applying stunning and slaughter methods.

Additionally, article 7.3.6 of the Code addresses fish stunning and slaughter methods, recommending mechanical or electrical stunning depending on the species. Slaughter methods without prior stunning, such as the use of CO_2 in water, salt or ammonium baths, asphyxia, direct exsanguination, among others, are not recommended by WOAH, due to the possibility of causing suffering before loss of consciousness.

3.2. EU legislation and recommendations

EU legislation recognises animals (including fish) as sentient beings, stressing the need to protect their welfare. Thus, in aquaculture, animal welfare requirements must be met while respecting the legal, administrative and customary and/or moral differences between Member States (EU, 2010). The European Commission (EC) Directive 98/58/EC mentions that *"the owner or keeper must ensure that animals are not subjected to pain, suffering or unnecessary harm"*.

EFSA assists the Commission by providing scientific evidence for the drafting of regulations, e.g. the published scientific opinion on slaughter and stunning methods for animals intended for human consumption, including fish. This report argues that there is a lack of humane slaughter methods for a considerable number of farmed fish species, and that several of the available ones are not considered humane because they cause suffering to the animals (EFSA, 2004).

Subsequently, another scientific opinion related to welfare during stunning and slaughter of seabream (*Sparus aurata*) and seabass (*Dicentrarchus labrax*) recognises the need to address welfare in both species independently and ensure it for each life cycle stage. Furthermore, it describes how all slaughter methods used (i.e., suffocation, chilling in ice and chilling in a mixture of ice and water) impact negatively on fish welfare, because handling prior to and/or during slaughter involves exposing them to air for long periods of time. It concludes that there is an urgent need to implement commercially effective, science-based stunning methods that achieve unconsciousness without pain and suffering at the time of slaughter (EFSA, 2009a, 2009b; de la Rosa et al., 2021).

In 2017, the EC established the Animal Welfare Platform, a group of key stakeholders in production animal welfare, aiming to implement and enforce animal welfare legislation by fostering dialogues between competent authorities, businesses, scientists and intergovernmental organisations (EC, 2020). At the legislative level, the Council of Europe (2005) adopted a series of recommendations to protect farmed fish welfare, considering scientific and practical evidence, with the objective of avoiding unnecessary pain, suffering and injury to them. In 2010, the Recommendation on farmed fish (EU, 2010) recognised them as sentient beings capable of experiencing pain and stress, suggesting to *"rear fish without detrimental effects on their living conditions, including their health, and taking into account scientific knowledge of their biological characteristics, available practical experience and the rearing systems"*.

More specifically, EU Council Regulation N0 1099/2009 on the protection of animals at the time of slaughter mentions that *"any person involved in animal slaughter must take the necessary measures to avoid pain and minimise distress and suffering of animals during the process"*. Furthermore, it states that it is a matter of public interest and may affect consumers' perceptions. It also states that improvements in this procedure positively impact meat quality, which is in line with the Farm to Fork Strategy. However, the regulation mentioned above states that research in fish stunning is still under development, so the standard only partially applies to these aquatic animals (Council, 2009).

In 2021, the Guidelines on water quality and management for the welfare of farmed vertebrate fish mentioned relevant aspects for fish welfare, such as minimising exposure to air during pre-handling and slaughter. However, these general

aquaculture guidelines fail to include specific information for seabass and seabream (Marinou, 2020; Policies, 2023).

3.3. Spanish legislation and recommendations

Spain, as a Member State of the EU, is bound by the strict regulations established at the European level. Regarding animal protection, it adhered in 1988 to the European Convention for the protection of animals kept for farming purposes. Regarding aquaculture, there is no specific welfare framework, beyond that established at the EU. The rules already in place regulate diverse aspects, e.g., management of fishing and aquaculture activities, access to water, control of farms, and animal health and welfare. In this last aspect, most of the legislation focuses on health aspects, particularly on the prevention of diseases and management of treatments. A legislative example is Regulation (EU) 2016/429, which establishes animal health requirements for the prevention and control of aquatic animal diseases, transposed by Royal Decree 1590/2009. Related to health requirements during transport, Royal Decree 1082/2009 regulates animal transportation, including those from inland aquaculture facilities.

In terms of fish transportation, Council Regulation N^0 1/2005 states that all movements must be properly authorised by official veterinarians. Operators must complete animal welfare training courses prior to the journey, vehicles for fish transportation must be specifically designed for this function, and the route must be planned to avoid delays to their final destination. This regulation has been applied in Spain since 2016 through Royal Decree 542/2016, listing the minimum standards for animal protection during transportation. It specifically regulates the authorisation and registration of transporters, means of transport and containers, documentation required during transport, obligations of operators, and their training.

In the case of slaughter, the above-mentioned Regulation N^0 1099/2009 ensures that during slaughter and related activities animals do not suffer avoidable pain, distress or suffering. Although it does not detail specific methods for fish slaughter, it is clear that any method used must not cause suffering to them. While the amount of scientific literature was still in its infancy 10 years ago, there is now sufficient evidence to carry out effective stunning, making its application in commercial operations feasible. Given the diversity of species reared in aquaculture, it is challenging to draw definitive conclusions for each one, so current regulations focus on the fundamental principle of avoiding unnecessary suffering of these aquatic animals.

Moreover, EFSA is working on reports to make possible amendments concerning fish slaughter. To date, it has produced nine scientific opinions on actions that the EU should take to improve the welfare of various farmed finfish species, e.g., Atlantic salmon, carp, eel, rainbow trout, seabass, seabream, tuna, and turbot, as well as one

focused on fish slaughter in general. At the Spanish level, aquaculture falls under the Autonomous Communities' competencies, compiled on the Spanish Ministry of Agriculture, Fisheries and Food (MAPA)'s website. These sets of rules cover the necessary requirements for the authorisation, monitoring and control of aquaculture establishments, including official health controls and animal welfare.

Additionally, other tools like good practice guides provide recommendations to improve welfare in aquaculture, e.g., the Guide on Fish Welfare in Spanish Aquaculture, a collaborative work between the Spanish Aquaculture Business Association (APROMAR), MAPA, scientists and animal welfare organisations. Focused on the top farmed fish species in Spain, there are currently three volumes. The first one deals with the concepts and generalities of fish welfare, while the following two develop specific recommendations for farmed seabass and seabream, respectively.

One of the crucial aspects included in these guides is precisely to ensure animal welfare during slaughter, which must be humane and free of pain and suffering. The step-by-step slaughter procedure is outlined, starting with fasting, transportation using pumps or nets, and ending with slaughter. While slaughter in ice water without previous stunning is common, it is encouraged to stun the fish effectively to induce a quick loss of consciousness. Stunning techniques include electrocution and percussion, depending on the species, size and production system.

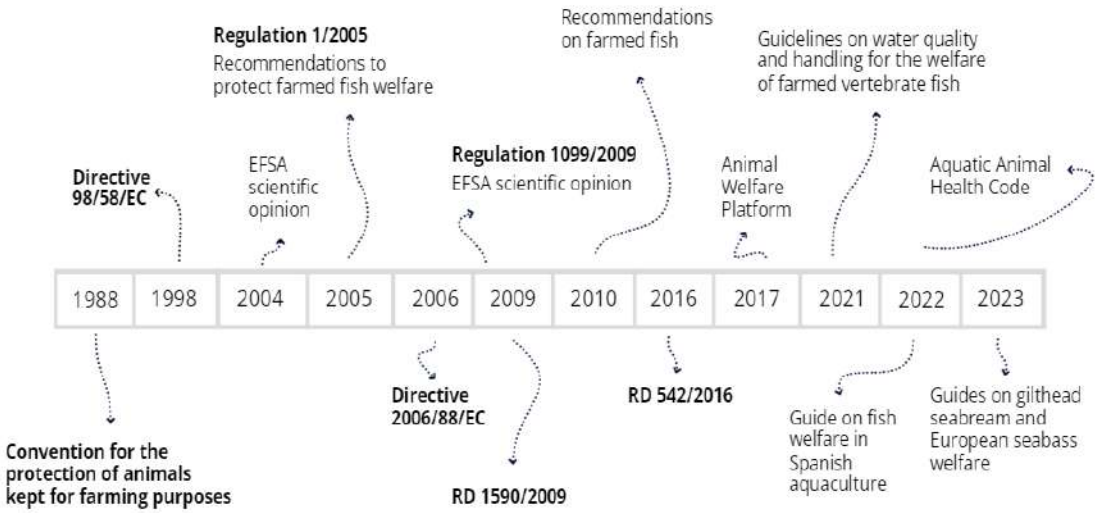


Figure 2. Timeline of the EU, international and Spanish legal frameworks on fish welfare, showing in bold those legally-binding (AWEC, 2024).

3.4. Certifications

Certifications aim to guarantee the animal products' quality through traceability and assurance of ethical and sustainable production practices. In this sense, there are

several aquaculture certifications, including environmental, food quality, and animal welfare. Those that include pre-slaughter stunning standards in fish and that operate in Spain are the Aquaculture Stewardship Council (ASC), Best Aquaculture Practices (BAP), Friend of the Sea, Global G.A.P. and the Royal Society for the Protection of Cruelty to Animals (RSPCA). According to APROMAR's Sustainability Reports, published in 2021 and 2022, around 87% of the volume produced by its companies has some certification seal in the environmental field, while 99% has it in food quality. However, doubts arise about whether these certifications include specific stunning standards before slaughter.

The certifications' standards mention that fish stunning must be effective, taking into account their welfare. They include recommendations to improve fish welfare, such as eliminating the use of inhumane slaughter methods (e.g., ice or suffocation) and replacing them with alternatives (e.g., electrical or mechanical stunning). In addition, they specify that farm staff must be trained adequately (Table 1).

It applies to seabream and seabass.	✓		✓	✓	
Mandatory effective and fast (<1s) stunning before slaughter. Minimal pain and stress.	✓	✓	✓	✓	✓
Staff trained in animal welfare, slaughter process and handling.	✓	✓	✓	✓	✓
Written procedure for stunning and slaughter.	✓		✓		
Responsible for fish welfare during the slaughter process.	✓		✓		✓
The use of ice or asphyxiation is discouraged if there is an effective method for the species.				✓	
Dry electrical stunning is forbidden.					✓

Table 1. Aquaculture fish certification standards for stunning, fish species to which they apply and measures put in place to protect welfare during slaughter (ASC, BAP, Friend of the Sea, Global G.A.P., RSPCA, 2024).

4. STAGES PREVIOUS TO SLAUGHTER: KEY POINTS THAT MAY AFFECT ANIMAL WELFARE AND PRODUCT'S QUALITY

Pre-slaughter stages can have a major impact on fish welfare and product's final quality (Bagni et al., 2007). It is essential to minimise stress during pre-slaughter handling, as fish are subjected to factors that can alter their welfare, such as fasting

for varying lengths of time, crowding at high densities and prolonged times, and harvesting. In this regard, the fish farm staff must be trained, and the steps must be correctly timed with each other and with the subsequent slaughter of the fish, to avoid possible delays throughout the entire process (Ashley et al., 2007; EFSA, 2009b; Lines & Spence, 2014).

1) FASTING

- It is performed to empty the fish's digestive system before slaughter, reducing faeces in tanks and cages and the amount of oxygen the animals use. It depends on the water temperature and the species concerned and is not recommended to last more than five days (Poli et al., 2005; EFSA, 2009b; Lines & Spence, 2014).
- Prolonging the fasting time is of no benefit to the fish, as it increases stress, affects their immune system and increases the likelihood of them becoming ill during this period. Similarly, prolonged fasting leads to the fish using its energy reserves, causing weight losses that affect the final product (Ashley et al., 2007).

2) CROWDING

- It can be a risk factor for fish welfare, mainly when carried out at very high densities. In seabream and seabass, high surface concentration (e.g. 400 kg/m^3) reduces the amount of oxygen in water, increasing stress physiological indicators like cortisol, glucose and lactate and promoting escape behaviours, which intensify as crowding time prolongs.
- Reported crowding densities in seabream and seabass farms are estimated to be around 250 kg/m^3. In both species, low densities during crowding favour a quick slaughter, in contrast to high densities (Poli et al., 2005; Bagni et al., 2007; EFSA 2009b).

3) HARVESTING

- It is one of the most stressful stages for fish before slaughter, as they are usually caught with nets or pumps, which increase the density and contact between them. Removal from the water causes suffocation, increasing vigorous swimming behaviour and escape attempts.
- Sometimes, recapture is necessary, which involves prolonged exposure to air and using the animals' energy reserves, which may affect the final product's quality. At this stage, fish injuries can also occur due to poor net handling, striking each other due to escape behaviours, or inadequate pump pressure. However, pumps are not commonly used in seabream and seabass (EFSA, 2009b; Papaharisis et al., 2019).

5. STUNNING AND SLAUGHTER METHODS

Fish represent the largest group of animals slaughtered for human consumption. According to FAOSTAT (2020), 200 million chickens, 4 million pigs and 900,000 cows are slaughtered daily. Regarding fish, estimates are much higher, amounting to 246,000 tonnes daily (no data is available on individuals), translating into billions of

them slaughtered daily. Of this vast number of animals, only 3-4% of farmed fish are stunned before slaughter globally, which is not mandatory for companies (Loeb, 2019). In other words, most of the slaughter methods used on fish worldwide are considered unacceptable and inhumane because they expose them to suffering for prolonged periods (EFSA, 2004).

The most commonly used slaughter methods without stunning include: a) direct exsanguination, b) immersion in ice water, c) liquid ice, d) CO_2 and e) asphyxia in air. They do not induce an immediate and irreversible loss of consciousness in fish, as behaviours associated with stress and pain may be observed (Lines & Spence, 2014; Rucinque et al., 2021; WOAH, 2022). The longer it takes for fish to lose consciousness, the more stress the animals may experience. Therefore, the time between stunning and slaughter should be minimised for those reversible stunning methods to avoid recovery of sensibility/consciousness (EFSA, 2004, 2009b).

5.1. Slaughter methods without stunning

The main slaughter methods, described in sections 5.1.1 to 5.1.4, are presented below (Table 2).

	EXSANGUINATION	CHILLING	ASPHYXIA
Behaviour 	Vigorous head and tail movements and escape behaviours in both species.	Negative escape behaviours appear for 30 seconds in both species after immersion in ice and water.	Vigorous movements, escape and fighting behaviour in both species.
Loss of consciousness 	No data.	**Seabream**: 15-20 min. **Seabass**: 10-20 min.	**Seabream**: 7,5 min. **Seabass**: death occurs at 34 min.
Fillet quality 	No data.	**Seabream**: change in eye colour (i.e., cloudy eyes), reducing their commercial value to consumers.	**Seabream**: no data. **Seabass**: decreased freshness and alterations in appearance, colour and odour.

Table 2. Main slaughter methods used commercially in seabream and seabass, showing their effect on behaviour, time to loss of consciousness or death and fillet quality.

5.1.1. Direct exsanguination

It consists of removing fish from the water, immobilising them individually, and inserting a sharp knife to cut the gill arches, ventral aorta or dorsal aorta, and/or caudal vein. This method affects their welfare, as they die from lack of oxygen due to blood loss. Its effectiveness also depends on staff training, as incorrect sectioning of all gill arches results in delayed loss of consciousness.

Moreover, fish may be out of water for varying lengths of time, a situation that can significantly increase their suffering from asphyxia. During this period, fish exhibit vigorous head and tail movements and escape behaviours. In Atlantic salmon (*Salmo salar*), these movements can last up to 4 minutes. Therefore, it is crucial to understand the severity of the situation and ensure that exsanguination is only recommended after the fish have been effectively stunned (Robb et al., 2000; Olsen et al., 2014; WOAH, 2022).

5.1.2. Chilling

Fish are immersed in a container or pond with ice. This method is not considered humane, as the fish are immobile but not necessarily unconscious, prolonging the suffering and the time it takes for death to occur. Furthermore, its effectiveness depends on factors such as water temperature, fish density in the rearing unit, and characteristics of the species in question, amongst others. It is not recommended for cold-water fish, with an ongoing debate about its use and implications for subtropical species (EFSA, 2009a, 2009b; Zampacavallo et al., 2014).

Another variant is the use of ice slurry, a suspension of tiny ice crystals in a solution made from salt or seawater (i.e., ice flakes and water in a ratio of 1:2 to 3:1). It is one of the most widely utilised fish slaughter methods in Mediterranean aquaculture, such as the case for farmed seabass and seabream. Provokes a faster loss of consciousness and dead than traditional slaughter in ice water or by asphyxia and affects fish welfare to a lesser extent (Tejada & Huidobro, 2002; de la Rosa et al., 2021; Zampacavallo et al., 2015; Roque et al., 2021).

Despite this, immersion of fish in liquid ice results in death by asphyxia, with seabass and seabream exhibiting negative behaviours around 30 seconds after immersion (Lines & Spence, 2014) until unconsciousness is reached. This time can vary from 10-20 minutes in seabass (Poli et al., 2005; Simitzis et al., 2014) and 15-20 minutes in seabream (Giuffrida et al., 2007). In seabass, brain activity takes up to 5 minutes to lose its standard patterns with this method. Therefore, brain activity is still present after the first minute after immersion, and the animal experiences distress. The immobility does not necessarily reflect that it is dead but rather the inability to move muscles due to the low temperature. The time it takes to lose consciousness will depend on the time it takes for the fish to reach complete immobility, the water temperature and the density of fish in the pond (van De Vis et al., 2003; Zampacavallo et al., 2015).

Liquid ice has improved seabass fillet quality compared to ice immersion alone, reducing the time the fish are entirely immobile (Ntzimani et al., 2023). However, several studies show eye-level problems, such as changes in colouring to a greyish and unclear appearance, a defect known as cloudy eyes. This problem is caused by the rapid drop in fish temperature when immersed in liquid ice, a defect that reduces

the commercial value of seabream and consumer acceptance (Huidobro et al., 2001; Tejada & Huidobro, 2002; Erikson et al., 2019).

5.1.3 Asphyxia

Fish are removed from the water with a net and left to suffocate to death in a tank or on the boat's deck, causing prolonged stress, agony and suffering (Acerete et al., 2009; Poli et al., 2005). Exposure to air for 1-1.5 minutes is stressful in seabream and seabass, resulting in escape behaviours and an increase in plasma cortisol (a hormone whose secretion increases in response to stress). Also, seabass has been categorised as one of the most acutely stress-responsive fish in Mediterranean aquaculture to pre-slaughter handling practices, such as high densities, harvest and exposure to air (Bagni et al., 2007; Fanouraki et al., 2011; Papaharisis et al., 2019).

The time in which death occurs after exposing fish to air varies, depending on each species' degree of resistance to oxygen deprivation. Seabream and seabass can take up to 60 and 25 minutes to die after being out of water, respectively (Poli et al., 2005). Seabass can take up to 34 minutes, affecting their welfare and rapidly deteriorating the meat sensory's quality, e.g., reduced freshness, altered appearance, colour and odour. Thus, this slaughter method is positioned as one of the most stressful and negatively affects the welfare of both species (Bagni et al., 2007).

The alteration of various physiological indicators of stress, such as a 5-fold increase in plasma glucose levels and an 8-fold increase in plasma cortisol levels, has been scientifically documented when asphyxia is used as a method of slaughter in seabass. This evidence supports the conclusion that asphyxia is not an acceptable slaughter method for seabream and seabass, given its detrimental effects on fish welfare and fillet quality (Acerete et al., 2009; EFSA, 2009b; Guida et al., 2016; WOAH, 2022).

5.1.4. Spiking

Brain destruction, also known as pithing, spiking, or ike jime, is done by inserting a sharp object through a fish skull. Usually employed to euthanise fish or other farmed species (i.e., tuna), it is considered a humane technique because it gets a quick death when performed correctly, avoiding stress and suffering. However, it is not recommended for small fish, as trained staff must ensure the procedure is performed safely and effectively.

Lower lactate concentrations have been found in seabream and seabass when using brain destruction compared to electrical stunning. Nevertheless, no differences in stress indicators such as plasma cortisol and glucose concentration were found between the two methods and the species mentioned above. In contrast to slaughter by asphyxia, brain destruction is a method that produces less stress in seabass (Poli et al., 2005; Papaharisis et al., 2019; Boaru et al., 2022).

5.2. Stunning methods

5.2.1. Electrical

Electrical stunning is one of the methods considered humane and recommended for fish by WOAH (2022), particularly for seabream and seabass by EFSA (2009a), because it induces immediate loss of consciousness in less than one second. It can be applied only to the head of the fish or to the whole body, e.g., in a tank with water.

It is recommended for these species because most studies have shown no adverse effects on fillet quality (Lambooij et al., 2008; Simitzis et al., 2014). Electrical stunning improves the tenderness of seabream and seabass meat compared to chilling. Similar results have been obtained in Japanese seabream (*Pagrus major*), where electrical stunning (electric field of 1.8 V/cm and speed of 1.6 m/s) retards fillet degradation, compared to chilling. However, electrical stimulation in seabass has been shown to lead to the earlier onset and faster resolution of rigour mortis (Knowles et al., 2007; Erikson et al., 2012; Zampacavallo et al., 2015; Papaharisis et al., 2019; Angelakopoulos et al., 2022).

Whole body stunning for 5 or 10 seconds, using a 50 Hz sine wave alternating current, causes immediate unconsciousness of seabass, followed by death immersed in seawater with ice. Furthermore, the combination of stunning/slaughter shows that seabass fillet quality is acceptable when assessed by acidity (pH) and colour. Therefore, this alternative is proposed commercially in seabass because of its efficiency in achieving rapid unconsciousness in fish and its advantages from an animal welfare point of view. That said, practical aspects need to be considered for implementation, such as fish density in the tank, electric current intensity, voltage, frequency, duration and water conductivity (Lambooij et al., 2008; EFSA, 2009a; 2009b; Tulli et al., 2015).

5.2.2. Percussive

Fish percussive stunning, whether manual or automatic, is a method that induces immediate unconsciousness and is recognised as humane (Robb et al., 2000; WOAH, 2022). However, manual stunning, when applied individually on a fish-by-fish basis, may be inefficient and impractical at a commercial level on small individuals, such as seabream and seabass (Ashley et al., 2007; de la Rosa et al., 2021; Lines & Spence, 2014).

This method removes fish from the water for varying lengths, significantly generating stress if the handling process is prolonged. Repeatedly removing fish from the water causes an increase in their muscle activity, leading to the use of their energy reserves, impacting fish welfare and potentially negatively affecting fillet quality. Nevertheless, no differences in quality have been found between mechanically stunned fish and

those slaughtered without stunning in ice slurry (Papoutsoglou et al., 1999; van de Vis et al., 2003; Poli et al., 2004; Panagiotis et al., 2014).

5.2.3. Gas

One of the most commonly used gases for stunning or slaughter fish is carbon dioxide (CO_2). However, as evidenced in other terrestrial species (such as pigs) (Llonch et al., 2013), seabream and seabass react with vigorous head and tail behaviours and escape attempts when CO_2 is applied (Poli et al., 2005). In addition, fish can be conscious for several minutes (between 7-10 minutes for seabass) despite being immobile in the first 2-4 minutes after being exposed to the gas, thus requiring the use of an additional slaughter method. Using CO_2 as a method of stunning or slaughter for seabass means that death can take up to 16 ± 0.08 minutes. The effectiveness of CO_2 stunning depends on several factors, such as concentration, water temperature and time of exposure to the gas (Acerete et al., 2009; EFSA, 2009a, 2009b).

It can be used with other gases, showing improvements in fish welfare. In seabream, mixtures composed of 30% CO_2 + 70% N_2 or 40% CO_2 + 30% N_2 + 30% O_2 achieve a faster loss of balance in swimming behaviour and an increase in physiological stress indicators like cortisol, lactate and glucose, significantly lower than when using chilling alone. Similarly, in seabass, the time to reach the correct stun/slaughter is reduced by 40% by adding a mixture of 70% N_2 + 30% CO_2 to the ice water, prolonging its shelf life by 14 days (Roque et al., 2021; Zampacavallo et al., 2015).

The use of N_2 combined with CO_2 is proposed as a measure to reduce the negative behaviours in fish generated by CO_2 and improve seabream and seabass welfare when gases are used as a stunning method, and be a viable alternative to implement in the aquaculture of Mediterranean species (Polo et al., 2005; EFSA, 2009b; Roque et al., 2021).

5.2.4. Anaesthesia

Clove oil, whose active compound is eugenol (4-allyl-methoxyphenol), is an efficient and effective anaesthetic agent for inducing unconsciousness in seabream and seabass. Fillet quality and welfare improvements have been demonstrated in seabass when fish are stunned with clove oil and slaughtered in water slurry. When both methods are combined, there is less brain activity in fish, compared to only using slaughter in water slurry (Mylonas et al., 2005; Miliou et al., 2011; Simitzis et al., 2014).

Once commercial trials have been conducted, the use of clove oil could present a practical stunning alternative. Its application in cages or tanks requires no special training for staff, making it a user-friendly option. Despite not being officially regulated as an anaesthetic stunning agent for fish destined for human consumption in the EU (EU Regulation 141/2014), it is authorised as an anaesthetic in countries such as

Australia, Chile and New Zealand, further highlighting its potential (Zampacavallo et al., 2015; López-Cánovas et al., 2020).

Another variant of clove oil use is the same product but nanoencapsulated in β-cyclodextrins, which, when applied in water or embedded in ice, has shown improvements in seabass welfare, reducing the stress response, improving the quality of its fresh fillet, and extending its shelf life by up to 4 days (Navarro-Segura et al., 2019; López-Cánovas et al., 2020).

5.3 Fish unconsciousness indicators

Assessing loss of consciousness in fish in aquaculture production systems is difficult (Brijs et al., 2021; WOAH, 2022). For that, behavioural indicators can be used to check the effectiveness of stunning.

Behavioural indicators for assessing unconsciousness in fish after stunning

- Absence of escape behaviours.
- Absence of ventilatory reflexes.
- Body and respiratory movement, loss of opercular activity.
- Inability to maintain balance.
- Loss of vestibulo-ocular reflex (VOR, eyes turned outwards).
- Loss of visual evoked responses (VER).
- No reaction to painful stimuli.
- Uncoordinated swimming movements.

The use of behavioural indicators is a valuable tool to assess unconsciousness. However, on a scientific level and to more accurately establish that stunning methods are effective, it is necessary to complement them with neurophysiological or neurochemical evidence, such as evaluating brain activity by electroencephalogram (EEG). If the stunning method was indeed effective, long-lasting neuronal depolarisation occurs at the brain level, affecting neurotransmission and keeping the animal in a state of insensibility and unconsciousness, time which is variable depending on the method utilised (EFSA, 2004; Brijs et al., 2021).

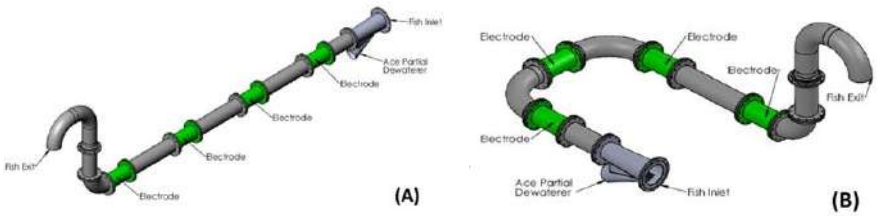
6. STUNNING EQUIPMENTS FOR SEABREAM AND SEABASS

We have contacted and/or obtained information from the leading equipment companies that offer humane slaughter of seabream and seabass (i.e., Ace Aquatec, OPTIMAR, Smile Fish and Baader). In all cases, the companies claim to have viable solutions installed in different countries worldwide. However, for their application in

both species in the Mediterranean Sea (i.e., the Iberian Peninsula area), it would be necessary to carry out previous engineering studies and climatic and maritime conditions to adapt each solution to the specific conditions of the fleets and fish farms. The main solutions available for seabream and seabass stunning are described below.

6.1 Commercially available stunning equipments

6.1.1 In-water electrical stunning

Company	Ace Aquatec
Method's description	It consists of pumping from cages or tanks into a stun tube using a centrifugal pump. The stun tube has electrodes that generate an electric current through the water, stunning the fish electrically in water as they flow through the tube.
Equipment	Humane Stunner Universal (A-HSU) A-HSU in seabream and seabass is equipped with 4 x 250 mm electrodes made of high-density polyethene. The electrical contact between the fish and the water ensures that they are stunned reliably and without needing prior electric shocks, regardless of their size. The electric field in the tube lasts up to 20 seconds. It is designed to ensure that the fish loses consciousness immediately without showing any escape behaviour or suffering, allowing operators to bleed or ice the fish safely and efficiently. The electrode configuration confines the high-voltage energy to the tube's centre, and the ends are grounded (0 voltage). A-HSU is mainly installed on land, but some projects are already installed on ships, where the tube layout changes (Figure 3).  **Figure 3**. Sketch of the in-water electrical stunner tube installed on land (A) and installed on the ship (B) (Ace Aquatec, 2024).
Species	Seabream, seabass, salmon, tilapia and trout.

Despite the lack of scientific studies on the effectiveness of this stunner, according to information provided by the company, the constant flow of fish avoids overcrowding and stress, which are critical factors in achieving an optimal result in terms of welfare and production.

6.1.2 Dry electrical stunning

Developed with the Norwegian Food Research Institute (NOFIMA) and the Norwegian Foundation for Industrial and Technical Research (SINTEF), this stunner complies with European and Norwegian requirements.

Company	OPTIMAR
Method's description	It involves exposing fish out of the water to an electric current.
Equipment	Optimar Electric Stunner™ Specifically, after dehydration, the fish arrive on a conveyor belt that acts as one of the electrodes. At the top is a chain of electrodes, six rows of ten steel fins hanging above. In this way, the circuit is completed when the two sides (i.e., belt and fins) collide. The fish are exposed to the current for 10 seconds to increase the duration of unconsciousness (Figure 4).  **Figure 4**. Dry electrical stunning equipment (Optimar, 2024).
Species	Cod, seabream, seabass, salmon, and trout.

Studies have been conducted under commercial conditions to evaluate the efficiency of Optimar's electric dry stunner on seabream and seabass in three Turkish companies: Sürsan, More and Falschill, jointly with Seachill and Tesco. According to Sürsan, this methodology allowed 97% of fish to show no signs of unconsciousness after stunning, based on behavioural indicators (Compassion in World Farming, 2017). Future research should confirm loss of consciousness based on EEGs and determine whether variability in fish size reduces stunning's efficiency.

For example, preliminary tests in lumpfish (*Cyclopterus lumpus*) determine that fish lose consciousness within 1 second, with both the Optimar dry and Askvik Aqua wet electrical stunner, and that increasing the duration to 10 seconds and cold immersing the fish results in permanent insensibility. Despite this, it is found that, in dry electrical stunning, the electrical potential difference in the brain correlates negatively with fish size, indicating that the solution is more efficient in smaller animals (NOFIMA, 2024).

Company	SmileFish
Method's description	It involves exposing fish out of the water to an electric current.
Equipment	It also manufactures an electric dry stunner designed for fish processing facilities and used in Turkey (Figure 5). According to the business, this stunner speeds up fish processing, makes it more efficient and hygienic, and increases the final product quality.
Species	Seabream, seabass.

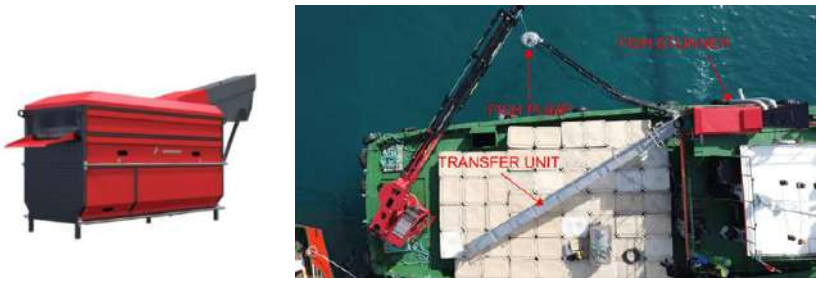


Figure 5. SmileFish stunner (left) and a detail of its installation on the boat (right) (SmileFish, 2024).

6.1.3 Percussive stunning

Company	Baader
Method's description	It involves inducing immediate unconsciousness by a strong blow (or percussion) to the fish's skull, individually and out of the water.
Equipment	Baader 101 It combines a stunning and percussive system (Figure 6). It is used in different countries worldwide, but it remains to be applied to seabream and seabass due to its limitations for small-size farmed fish at commercial levels (de la Rosa et al., 2021).
Species	Salmon.

Figure 6. Baader 101 percussive stunner for salmon (Baader, 2024).

6.2 Complementary technology to stunners: pumping equipment

Transporting fish out of the water using nets is stressful and causes physical damage due to overcrowding (EFSA, 2009b; Lines & Spence, 2014). For this reason, it is recommended that they be replaced with pumping equipment. A wide variety of pumps can be used to capture fish prior to stunning. They are available on the market for seabream and seabass of all sizes and with a wide range of pumping capacities (from 5 to 80 tonnes/hour).

Some examples of pump manufacturers are FAIVRE, VAKI, Whaspower and GroAqua. In Spain, we contacted Euskan, which offers different pumping equipment configurations for specific needs. According to information provided by the company, its technology (use of vacuum pumps instead of centrifugal ones, Figure 7) offers a constant and stable flow, reducing stress at the suction port and along the pipes. A laminar flow with a smooth transition between vacuum and pressure achieves this.

Figure 7. Suction pumps (Euskan, 2024).

6.3 Implementation of seabream and seabass stunning equipment in Spain

According to sources consulted through a survey distributed to six seabream and seabass-producing companies in different Mediterranean countries (including Spain, Greece and Turkey), the main difficulties with the implementation of stunning under current production conditions are: (1) access to technology and (2) implementing on-farm stunning.

6.3.1 In-water electrical stunning

Ace Aquatec equipment, used by companies like Philosofish (Greece), Scottish Sea Farms (Scotland), and Cedar Crest Trout Farm (Canada), can be utilised on land or on a boat. The length of the tube is adjustable to meet the required throughput, reaching up to 20-30 tonnes/hour (equivalent to 67-100 fish of approximately 5

kg/min), and can be installed either on or below deck. A unique feature is its ability to pass under the boat's deck, freeing up space for fish storage on ice (Figure 8).

Figure 8. Example of an electric tube stunner on deck (Ace Aquatec, 2024).

Although the in-water electric stunner is being used in different countries, there is no evidence of its operation in the Western Mediterranean. Therefore, further studies would be necessary to ensure its correct implementation, including the following situations:

- If it is used on new ships, it would be necessary to ensure that the area's climatic conditions are compatible with the equipment. Ambient temperatures should not be a problem, as A-HSU currently operates in areas with minimum temperatures of -20ºC and a maximum of up to 40ºC (Table 3).

- Regarding waves, the equipment is being used in Nordic countries with waves up to 5-6 metres, according to Ace Aquatec. However, since they are highly variable in space and time, a long-term study of waves at the specific locations of Spanish farms would be required, using, for instance, reliable databases such as that of the European Centre for Medium-Range Weather Forecasts (Karathanasi et al., 2022) or sensors installed directly on buoys, boats or the farms themselves.

- If the stunner is adapted to existing vessels, and in addition to the compatibility of the marine conditions, a prior engineering study would be necessary to adjust it to the Spanish fleet. To ensure its viability, the vessels' dimensions, the stunner's characteristics, such as weight (1,000 kg), and the electrical power required (3 phases of up to 60 KW) must be considered. These are key factors and, in some cases, limiting, especially in older vessels.

		ONSHORE		OFFSHORE
Number of farms with A-HSU		**Indoor**	**Outdoor**	**Outdoor**
		22	8	11
Min. temperature (ºC)	Average ± SD	2,2 ± 7,57	-5,4 ± 5,37	-1,3 ± 7,28
	Minimum value	-20	-15	-10
Max. temperature (ºC)	Average ± SD	18,4 ± 5,85	28,8 ± 5,18	32,1 ± 4,85
	Maximum value	30	40	38

Table 3. Ambient temperature (minimum, maximum, average and standard deviation or SD; ºC) of 41 onshore and offshore fish farms with the A-HSU.

6.3.2 Dry electrical stunning

When applied to dry-slaughtered fish, it could be integrated into current aquaculture operations in the Mediterranean, as it is a further step between net weaning and storage on ice. The technical peculiarities of Optimar's electric dry stunner installation do not seem, a priori, to be a limiting factor in small boats: dimensions of 1.5 m long x 0.8 m wide, weight of 250 kg (plus an additional 60 kg of the electrical cabinet) and single-phase electrical supply. It should be noted that, regarding animal welfare, it is recommended to use electrical dry stunning combined with harvest by pumps rather than nets.

For example, for 400-600 g seabass (5t/hour), Euskan estimates that two 500 l tanks (2.5 m long x 1.5 m wide x 1.5 m high; 400 kg), an 11 KW power unit (1.25 m long x 40 cm wide x 1.5 m high; 440 kg) and a 120 kg water separator would be required. Also, using flexible hoses with a polyurethane interior is recommended. Despite this, ensuring the equipment's size and weight fit Spanish vessels and its compatibility with producers' farm practices in local conditions is necessary. Thus, conducting studies on engineering and marine conditions is advisable to allow the stunner's proper functioning.

Currently, this machinery is being used by a Turkish company in the Aegean and Mediterranean Seas to stun seabream and seabass (Figure 9). This producer concentrates one of the largest fishing fleets in the Mediterranean for both species, with the capacity to process 18,000 tonnes of fish per year, distributed in three locations throughout the country. Pre-slaughter stunning is part of their animal welfare strategy, described on their website. They ensure that 100% of the animals processed at their facilities are stunned, applying their commitment to improving animal welfare throughout the production chain.

Figure 9. Optimar stunner with a dewatering unit (right) used on the Turkish coast to stun seabream and seabass (Optimar, 2024).

Finally, it should be noted that the Spanish aquaculture sector, gathered by APROMAR, launched a tender in February 2024 to validate electric stunning equipment and determine whether it can improve fish welfare compared to the current ice slurry method. According to APROMAR, the technology must be viable for operating on standard aquaculture vessels in Spain and adaptable to different commercial sizes of seabream, seabass, and meagre.

7. CONCLUSIONS

The main conclusions of this report are the following:

1. There is scientific evidence that recognises seabream and seabass as **sentient beings**.

2. **Effective stunning** before slaughter is recommended to achieve a rapid loss of consciousness, avoiding suffering.

3. Slaughter by chilling or asphyxia in the air means that it takes seabream between **7.5-20 minutes** to lose consciousness and between **10-34 minutes** for seabass.

4. Under commercial aquaculture production conditions, **in-water** or **dry electrical stunning** effectively improves animal welfare and product quality.

5. Several producers already use electrical stunning in different countries worldwide, including some operating in the **Eastern Mediterranean**.

6. **Engineering**, **weather** and **marine studies** are needed to adapt commercial solutions to the specific conditions of Spanish fleets and farms.

8. REFERENCES

1. Acerete, L., Reig, L., Alvarez, D., Flos, R., & Tort, L. (2009). Comparison of two stunning/slaughtering methods on stress response and quality indicators of European sea bass (*Dicentrarchus labrax*). *Aquaculture*, **287(1–2)**, 139–144.

2. Angelakopoulos, R., Dimitroglou, A., Papaharisis, L., & Moutou, K. A. (2022). Electrical Stunning Has the Potential to Delay Fillet Degradation Post-Harvest in Red Seabream (*Pagrus major*). *Aquaculture Journal*, **2(4)**, 302–315.

3. Ashley, P. J. (2007). Fish welfare: Current issues in aquaculture. *Applied Animal Behaviour Science*, **104(3-4)**, 199–235.

4. Bagni, M., Civitareale, C., Priori, A., Ballerini, A., Finoia, M., Brambilla, G., & Marino, G. (2007). Pre-slaughter crowding stress and killing procedures affecting quality and welfare in seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*). *Aquaculture*, **263(1-4)**, 52–60.

5. Boaru, A., Struți, D., & Georgescu, B. (2022). Guidelines and Implications of Fish Slaughtering in the Ensuring of Welfare and Product Quality. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. *Animal Science and Biotechnologies*, **1(79)**, 1.

6. Brijs, J., Sundell, E., Hjelmstedt, P., Berg, C., Senčić, I., Sandblom, E., Axelsson, M., Lines, J., Bouwsema, J., Ellis, M., Saxer, A., & Gräns, A. (2021). Humane slaughter of African sharptooth catfish (*Clarias gariepinus*): Effects of various stunning methods on brain function. *Aquaculture*, **531** (September 2020).

7. Broom, D.M. (2014). Sentience and Animal Welfare. CABI PUBLISHING, England.

8. Broom, D.M. (2016a). Sentience and animal welfare: New thoughts and controversies*. Animal Sentience*, **5(11)**.

9. Broom, D.M. (2016b). Fish brains and behaviour indicate capacity for feeling pain. *Animal Sentience*, **3(4)**.

10. Brown, C. (2015). Fish intelligence, sentience and ethics. *Animal cognition*, **18(1)**, 1-17.

11. Commission, T. H. E. E. (2020). *31.1.2017*. *2015* (November 2015), 61-66.

12. Compassion in World Farming, 2017. Driving Innovation in Humane Fish Slaughter: Developing an electrical stunning system for European sea bass and gilthead seabream.

13. Consejo, P. E. y del. (2009). Reglamento (CE) nº 1099/2009 del Consejo de 24 de septiembre de 2009 relativo a la protección de los animales en el momento de la matanza. *Diario Oficial de La Unión Europea*, **L 303**, 1-30.

14. Cornish, A., Raubenheimer, D., & McGreevy, P. (2016). What we know about the public's level of concern for farm animal welfare in food production in developed countries. *Animals*, ***6*(11)**, 1-15.

15. Cottee, S. Y. (2012). Are fish the victims of "speciesism"? A discussion about fear, pain and animal consciousness. *Fish Physiology and Biochemistry*, **38(1)**, 5-15.

16. Council of Europe (2005). Recommendation concerning farmed fish adopted by the Standing Committee of the European Convention for the Protection of Animals Kept for Farming Purposes (T-AP) on 5 December 2005. Council of Europe. Available at: https://www.coe.int/t/e/legal_affairs/legal_co-operation/biological_safety_and_use_of_animals/Farming/Rec%20fish%20E.asp

17. Daskalova, A. H., Bracke, M. B. M., van de Vis, J. W., Roth, B., Reimert, H. G. M., Burggraaf, D., & Lambooij, E. (2016). Effectiveness of tail-first dry electrical stunning, followed by immersion in ice water as a slaughter (killing) procedure for turbot (*Scophthalmus maximus*) and common sole (*Solea solea*). *Aquaculture*, **455**, 22-31.

18. de la Rosa, I., Castro, P. L., & Ginés, R. (2021). Twenty years of research in seabass and seabream welfare during slaughter. *Animals*, **11(8)**, 1-9.

19. EFSA. (2004). Opinion of the scientific panel on animal health and welfare on a request from the commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. EFSA Journal, **45**, 1-29.

20. EFSA. (2009a). Food Safety considerations concerning the species-specific welfare aspects of the main systems of stunning and killing of farmed fish. *EFSA Journal*, **1.190**, 1-16.

21. EFSA. (2009b). Species-specific welfare aspects of the main systems of stunning and killing of farmed Seabass and Seabream. *EFSA Journal*, **7**, 1010.

22. EU Platform on Animal Welfare Own Initiative Group on Fish (2022). Guidelines on Water Quality and Handling for the Welfare of Farmed Vertebrate Fish. Text available at: https://food.ec.europa.eu/system/files/2022-07/aw_platform_plat-conc_guide_farmed-fish_en.pdf.

23. Erikson, U., Lambooij, B., Digre, H., Reimert, H. G. M., Bondø, M., & van der Vis, H. (2012). Conditions for instant electrical stunning of farmed Atlantic cod after de-watering, maintenance of unconsciousness, effects of stress, and fillet quality - A comparison with AQUI-S^{TM}. *Aquaculture*, **324-325**, 135-144.

24. Erikson, U., Truong, H. T. M., Le, D. V., Pham, P. D., Svennevig, N., & Phan, V. T. (2019). Harvesting procedures, welfare and shelf life of ungutted and gutted shortfin pompano (*Trachinotus falcatus*) stored in ice. *Aquaculture*, **498**(November 2017), 236-245.

25. European Union. (1998). Directiva 98/58/CE del Consejo de 20 de julio de 1998 relativa a la protección de los animales de las explotaciones ganaderas. *Official Journal of the European Union*, 23-27.

26. Fanouraki, E., Mylonas, C. C., Papandroulakis, N., & Pavlidis, M. (2011). Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture. *General and Comparative Endocrinology*, **173(2)**, 313-322.

27. Giuffrida, A., Pennisi, L., Ziino, G., Fortino, L., Valvo, G., Marino, S., & Panebianco, A. (2007). Influence of slaughtering method on some aspects of quality of gilthead seabream and smoked rainbow trout. *Veterinary Research Communications*, **31(4)**, 437-446.

28. Guida, L., Walker, T. I., & Reina, R. D. (2016). The adenylate energy charge as a new and useful indicator of capture stress in chondrichthyans. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, **186(2)**, 193-204.

29. Huidobro, A., Mendes, R., & Nunes, M. L. (2001). Slaughtering of gilthead seabream (*sparus aurata*) in liquid ice: Influence on fish quality. *European Food Research and Technology*, **213(4-5)**, 267-272.

30. Karathanasi, F.E., Soukissian, T.H., Hayes, D.R. (2022). Wave Analysis for Offshore Aquaculture Projects: A Case Study for the Eastern Mediterranean Sea. Climate 10, 2.

31. Knowles, T. G., Brown, S. N., Warriss, P. D., Lines, J., Tinarwo, A., Bravo, A., Carvalho, H., & Gonçalves, A. (2007). Effect of electrical stunning at slaughter on the carcass, flesh and eating quality of farmed sea bass (*Dicentrarchus labrax*). *Aquaculture Research*, **38(16)**, 1732–1741.

32. Lambooij, B., Gerritzen, M. A., Reimert, H., Burggraaf, D., André, G., & Van De Vis, H. (2008). Evaluation of electrical stunning of sea bass (Dicentrarchus labrax)

in seawater and killing by chilling: Welfare aspects, product quality and possibilities for implementation. *Aquaculture Research*, **39(1)**, 50–58.

33. Lines, J. A., & Spence, J. (2014). Humane harvesting and slaughter of farmed fish. *OIE Revue Scientifique et Technique*, **33(1)**, 255–264.

34. Loeb, J. (2019). '3 to 4% of farmed fish stunned globally'. *Veterinary Record*, **185**, 70-70.

35. López-Cánovas, A. E., Cabas, I., Chaves-Pozo, E., Ros-Chumillas, M., Navarro-Segura, L., López-Gómez, A., Fernandes, J. M. O., Galindo-Villegas, J., & García-Ayala, A. (2020). Nanoencapsulated clove oil applied as an anesthetic at slaughtering decreases stress, extends the freshness, and lengthens shelf life of cultured fish. *Foods*, **9(12)**, 1-19.

36. Llonch, P., Rodríguez, P., Jospin, M., Dalmau, A., Manteca, X., & Velarde, A. (2013). Assessment of unconsciousness in pigs during exposure to nitrogen and carbon dioxide mixtures. *Animal*, **7(3)**, 492–498.

37. Marinou, E. (2020). Guidelines on Water Quality and Handling for the Welfare of Farmed Vertebrate Fish. *EU Platform on Animal Welfare Own Initiative Group on Fish*, **1**, 20.

38. Miliou H., Tsopelakos, A., Dalla, C., & Papadopoulou-Daif- oti Z. (2011). Effects of pre-slaughter and slaugther methods to the brain monoaminergic activities and plasma glucose levels in sea bass, Dicentrarchus labrax. In: *Abstracts of Official Congress of European Aquaculture Society*, Rhodos, Athens, p. 711-712.

39. Mylonas, C. C., Cardinaletti, G., Sigelaki, I., & Polzonetti-Magni, A. (2005). Comparative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures. *Aquaculture*, **246(1-4)**, 467-481.

40. Navarro-Segura, L., Ros-Chumillas, M., López-Cánovas, A. E., García-Ayala, A., & López-Gómez, A. (2019). Nanoencapsulated essential oils embedded in ice improve the quality and shelf life of fresh whole seabream stored on ice. *Heliyon*, **5(6)**.

41. Ntzimani, A., Angelakopoulos, R., Semenoglou, I., Dermesonlouoglou, E., Tsironi, T., Moutou, K., & Taoukis, P. (2023). Slurry ice as an alternative cooling medium for fish harvesting and transportation: Study of the effect on seabass flesh quality and shelf life. *Aquaculture and Fisheries*, **8(4)**, 385–392.

42. Oliveira Filho, P. R. C. D., Oliveira, C. A. F. D., Sobral, P. J. D. A., Balieiro, J. C. D. C., Natori, M. M., & Viegas, E. M. M. (2015). How stunning methods affect the quality of Nile tilapia meat. *CyTA-Journal of Food*, **13(1)**, 56-62.

43. Olsen, S. H., Joensen, S., Tobiassen, T., Heia, K., Akse, L., & Nilsen, H. (2014). Quality consequences of bleeding fish after capture. *Fisheries Research*, **153**, 103–107.

44. Organización Mundial de Sanidad Animal (OMSA). (2022). Código Sanitario para los Animales Acuáticos. In: Animal, O.M.d.S. (Ed.) OMSA, 300.

45. Papaharisis, L., Tsironi, T., Dimitroglou, A., Taoukis, P., & Pavlidis, M. (2019). Stress assessment, quality indicators and shelf life of three aquaculture important marine fish, in relation to harvest practices, water temperature and slaughter method. *Aquaculture Research*, **50(9)**, 2608–2620.

46. Papoutsoglou, S. E., Miliou, H., Chadio, S., Karakatsouli, N., & Zarkada, A. (1999). Studies on stress responses and recovery from removal in gilthead sea bream *Sparus aurata* (L.) using recirculated seawater system. *Aquacultural Engineering*, **21(1)**, 19–32.

47. Poli, B. M., Scappini, F., Parisi, G., Zampacavallo, G., Mecatti, M., Lupi, P., Mosconi, G., Giorgi, G., & Vigiani, V. (2004). Traditional and innovative stunning/slaughtering methods for European sea bass compared by the complex of the assessed behavioural, plasmatic and tissue stress and quality indexes at death and during shelf life. In *Proceedings of 34th WEFTA Conference*, Lubeck, Germany. pp. 58-63.

48. Poli, B. M., Parisi, G., Scappini, F., & Zampacavallo, G. (2005). Fish welfare and quality as affected by pre-slaughter and slaughter management. *Aquaculture International*, **13(1-2)**, 29-49. 49. Policies, C. (2023). *Animal welfare of farmed fish Policy Department for Structural and Cohesion Policies Directorate-General for Internal Policies PE*. *June*.

50. Robb, D. H. F., Wotton, S. B., Mckinstry, J. L., Sørensen, N. K., & Kestin, S. C. (2000). Commercial slaughter methods used on Atlantic salmon: Determination of the onset of brain failure by electroencephalography. *Veterinary Record*, **147(11)**, 298-303.

51. Roque, A., Gras, N., Rey-Planellas, S., Fatsini, E., Pallisera, J., Duncan, N., Muñoz, I., Velarde, A., & Hernandez, M. D. (2021). The feasibility of using gas mixture to stun seabream (*Sparus aurata*) before slaughtering in aquaculture production. *Aquaculture*, **545** (July).

52. Roques, J. A. C., Abbink, W., Chereau, G., Fourneyron, A., Spanings, T., Burggraaf, D., van de Bos, R., van de Vis, H., & Flik, G. (2012). Physiological and behavioral responses to an electrical stimulus in Mozambique tilapia (*Oreochromis mossambicus*). *Fish Physiology and Biochemistry*, **38(4)**, 1019–1028.

53. Rose, J. D. (2002). The Neurobehavioral Nature of Fishes and the Question of Awareness and Pain. *Reviews in Fisheries Science*, **10(1)**, 1-38.

54. Rose, J. D., Arlinghaus, R., Cooke, S. J., Diggles, B. K., Sawynok, W., Stevens, E. D., & Wynne, C. D. L. (2014). Can fish really feel pain? *Fish and Fisheries*, **15(1)**, 97–133.

55. Roth, B., Birkeland, S., & Oyarzun, F. (2009). Stunning, pre slaughter and filleting conditions of Atlantic salmon and subsequent effect on flesh quality on fresh and smoked fillets. *Aquaculture*, **289(3-4)**, 350-356.

56. Rucinque, D. S., Ferreira, P. F., Leme, P. R. P., Lapa-Guimarães, J., & Viegas, E. M. M. (2021). Ocimum americanum and Lippia alba essential oils as anaesthetics for Nile tilapia: Induction, recovery of apparent unconsciousness and sensory analysis of fillets. *Aquaculture*, **531** (August 2020).

57. Sánchez-Suárez, W., Franks, B., & Torgerson-White, L. (2020). From land to water: Taking fish welfare seriously. *Animals*, **10(9)**, 1–11.

58. Simitzis, P. E., Tsopelakos, A., Charismiadou, M. A., Batzina, A., Deligeorgis, S. G., & Miliou, H. (2014). Comparison of the effects of six stunning/killing procedures on flesh quality of sea bass (*Dicentrarchus labrax*, Linnaeus 1758) and evaluation of clove oil anaesthesia followed by chilling on ice/water slurry for potential implementation in aquaculture. *Aquaculture Research*, **45(11)**, 1759–1770.

59. Sneddon, L. U. (2003). The evidence for pain in fish: The use of morphine as an analgesic. *Applied Animal Behaviour Science*, **83(2)**, 153–162.

60. Sneddon, L. U. (2011). Pain perception in fish: Evidence and implications for the use of fish. *Journal of Consciousness Studies*, **18(9–10)**, 209–229.

61. Stien, L. H., Bracke, M., Noble, C., & Kristiansen, T. S. (2020). *Assessing Fish Welfare in Aquaculture*.

62. Tejada, M., & Huidobro, A. (2002). Quality of farmed gilthead seabream [*Sparus aurata*] during ice storage related to the slaughter method and gutting. *European Food Research and Technology*, **215(1)**, 1–7.

63. Tulli, F., Fabbro, A., D'Agaro, E., Messina, M., Bongiorno, T., Venir, E., Lippe, G., Tibaldi, E., & Stecchini, M. L. (2015). The effect of slaughtering methods on actin degradation and on muscle quality attributes of farmed European sea bass (*Dicentrarchus labrax*). *Journal of Food Science and Technology*, **52(11)**, 7182–7190.

64. Unión Europea. (2010). Tratado de Funcionamiento de la Unión Europea. Diario Oficial de La Unión Europea, 83/195.

65. Van De Vis, H., Kestin, S., Robb, D., Oehlenschläger, J., Lambooij, B., Münkner, W., Kuhlmann, H., Kloosterboer, K., Tejada, M., Huidobro, A., Otterå, H., Roth, B., Sørensen, N. K., Akse, L., Byrne, H., & Nesvadba, P. (2003). Is humane slaughter of fish possible for industry? *Aquaculture Research*, **34(3)**, 211–220.

66. van de Vis, H., Reimert, H., Grimsbø, E., Gerritzen, M., Foss, A., Roth, B. (2024). Electrical stunning and killing of Lumpfish with commercial waterbath and dry stunners. NOFIMA. Final report 7/2024.

67. Zampacavallo, G., Parisi, G., Mecatti, M., Lupi, P., Giorgi, G., & Poli, B. M. (2015). Evaluation of different methods of stunning/killing sea bass (*Dicentrarchus labrax*) by tissue stress/quality indicators. *Journal of Food Science and Technology*, **52(5)**, 2585–2597.