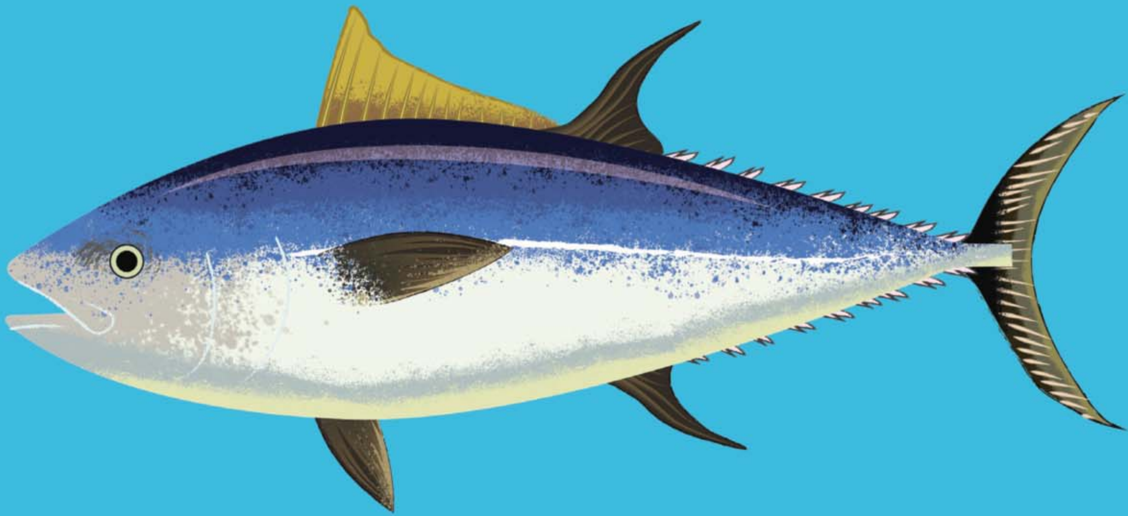
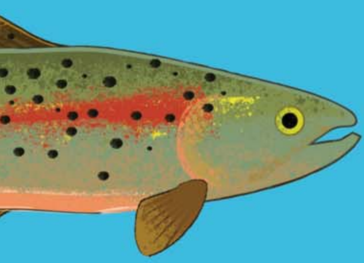
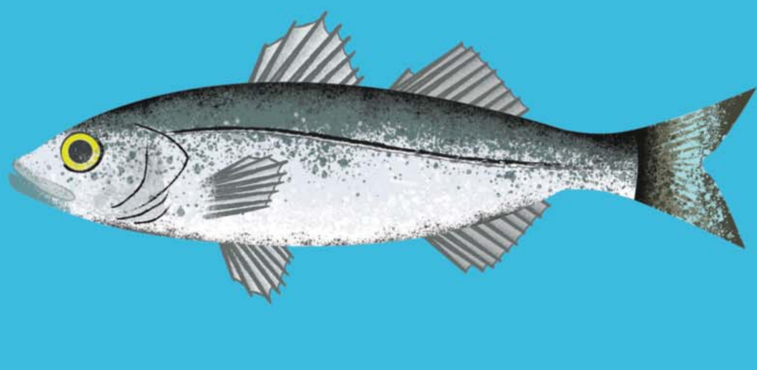




THE WELFARE OF FARMED FISH DURING SLAUGHTER IN THE EUROPEAN UNION



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EXECUTIVE SUMMARY

FISH ARE SENTIENT AND THEIR WELFARE MUST BE PROTECTED

There is a growing body of scientific evidence demonstrating that fish are sentient animals – capable of affective states of pain, fear and psychological stress (Chandroo, Duncan & Moccia, 2004), and examples of impressive cognitive abilities and complex social behaviours are widespread (Brown, 2014). We therefore have a moral obligation to protect their welfare (Brown, 2014). However, fish welfare has been largely overlooked in society, industry and policy. The systems and practices used at slaughter play a crucial role in determining the welfare of farmed animals, and the importance of humane slaughter practices is further magnified when large numbers of animals are involved, as is certainly the case with farmed fish.

In the European Union (EU) alone, an estimated 500 million to 1.7 billion farmed fish were killed for human consumption in 2015[1], comprising a range of species that are slaughtered in a variety of ways. Yet, despite the mounting evidence of fish sentience, and the substantial numbers involved in aquaculture, fish are currently excluded from much of the European Slaughter Regulation (European Union, 2009). The key principle however, that animals "shall be spared any avoidable pain, distress or suffering during their killing and related operations", does apply to fish (European Union, 2009; p.9). Humane slaughter methods should therefore be used, ensuring that fish are effectively stunned prior to killing or killed with a method that guarantees an immediate loss of consciousness.

As with any group of non-human animals, assessing the state of consciousness in fish is challenging. Brain activity, clinical reflexes, behaviours, and responses to noxious stimuli, can all provide insights into the mental state of fish, but studies show that these vary in their reliability and partly depend on the species and context. In addition, some slaughter procedures can leave fish physically immobilised, whereby they appear motionless and unresponsive, but are still conscious and able to feel pain and distress (van de Vis, Abbink, Lambooij, & Bracke, 2014). In these cases, extra caution must be taken when assessing consciousness and the effectiveness of stunning methods.

Each fish species differs in morphology and may therefore respond differently to a given slaughter

[1] As fish are officially recorded by the Food and Agriculture Organization of the United Nations (FAO) in tonnes only, the number of individuals has been estimated using average individual weights, by Mood & Brooke (2015) of fishcount.org.

method, which may also be limited by practicalities in a commercial setting. While essential for good fish welfare, a rapid and humane death can also benefit producers as lower stress is associated with better flesh quality (Poli, 2009). Percussive stunning and electrical stunning can enable humane slaughter for some species when applied correctly. If the systems do not kill, they must be followed by a suitable killing method, such as a gill cut, decapitation or destruction of the brain, which ensures fish are killed before consciousness can recover (OIE, 2010). Shooting and mechanical spiking or coring also have potential to deliver a humane end for some fish species (OIE, 2010). Each of these methods rely on accurate application and minimised handling stress to provide a humane death. When inhumane methods are used, fish are subject to pain and suffering which can last for several hours. Pre-slaughter procedures such as fasting, crowding and transport are also significant sources of stress and must be well managed to minimise suffering at the end of life.

THE STATE OF PLAY FOR KEY FARMED FISH SPECIES

Key farmed fish species produced in the EU include: rainbow trout (130-762 million fish), gilthead sea bream (206-275 million fish), European sea bass (138-172 million fish), common carp (28-142 million fish), Atlantic salmon (22-51 million fish), European eel (6-18 million fish), Atlantic Bluefin tuna (5-17 million fish), North African catfish (5-16 million fish), and turbot (5-14 million fish) (FAOSTAT 2015, calculated according to methods of Mood and Brooke, 2015). For these species, humane slaughter methods are at varying stages of development and implementation, as summarised below.

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RAINBOW TROUT

Denmark, Italy and France are the main producers of rainbow trout in the EU (FAOSTAT, 2015). Trout are killed using several methods, many of which are inhumane and cause considerable suffering. For example, trout that are left to asphyxiate (suffocate) in air or ice slurry, are immersed in water saturated with carbon dioxide gas (CO_2) or those that are decapitated without prior stunning, will suffer poor welfare. However, more humane alternatives are available; there is evidence that electrical stunning can enable humane slaughter when parameters can ensure unconsciousness until death by throat cut or decapitation. Electrical stunning is the predominant method used for small (portion sized; ~400g) trout in the UK. Percussive stunning, more applicable to larger trout, is also an acceptable method when performed accurately and followed by a timely kill method that kills without recovery of consciousness.

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GILTHEAD SEA BREAM

Greece and Spain are the main EU producers of gilthead sea bream (FAOSTAT, 2015). The vast majority of sea bream are killed using the inhumane method of live chilling in ice slurry. Death is eventually caused by asphyxia and there is no stunning effect as unconsciousness is not achieved for several minutes. For example, one study found that sea bream were still active after forty minutes in ice slurry (Huidobro, Mendes, & Nunes, 2001). Percussive stunning or spiking may not be practical due to the relatively small size of sea bream, but electrical stunning prior to chilling in ice slurry is in commercial use and can be humane. This method has been used commercially in some countries, though currently on a very small scale. Further research and development of electrical systems is required but use of ice slurry without prior stunning must be phased out urgently.

EUROPEAN SEA BASS

The main producers of European sea bass in the EU are Greece and Spain (FAOSTAT, 2015). Live chilling in ice slurry, an inhumane method, is the predominant slaughter process and fish slowly lose consciousness and die from asphyxia. This does not constitute a stunning method. As with sea bream, percussive stunning or spiking may not be commercially viable due to the (small) size of sea bass, but electrical stunning prior to chilling in ice slurry can be used and has the potential to be humane. Currently only a small number of farms are using this method commercially and further research and development of systems is needed to ensure effectiveness of stunning. However, the use of ice slurry without prior stunning must be phased out urgently.

COMMON CARP

Half of the common carp in the EU are produced by the Czech Republic and Poland (FAOSTAT, 2015). Humane killing of this species is not straightforward. Carp can survive for several hours out of water, often require several percussive blows to kill, and can recover consciousness after electrical stunning. Studies show that water saturated with carbon dioxide, and live chilling in ice slurry are also slow to act on carp, and that these conditions are highly aversive (Rahmanifarah et al., 2011). The further challenge in protecting carp welfare is that the majority produced in the EU are killed on market stalls at the point of sale, or sold live to the consumer for killing at home. In this case, a fast and painless slaughter is difficult to deliver and regulate. Percussive stunning followed by a killing method was identified by the EFSA as having the lowest negative welfare impact for carp (EFSA, 2009a). However, multiple blows are often required in practice as loss of consciousness is not instant, and injuries can occur (Retter et al., 2018). Experimental work has shown that carp can be rendered instantly unconscious by application of an electrical current (Daskalova, Pavlov, Kyuchukova, & Daskalov, 2016; Lambooij et al., 2007), however, it is unknown whether commercial systems being used currently are delivering the required parameters for an instant stun, or how long it takes for consciousness to recover. This is particularly concerning when no kill method is applied after stunning and the carp are processed while alive and possibly conscious, e.g. in the Czech Republic (IBF et al. 2017). Further research is urgently needed to find a humane method for slaughtering carp, which may involve electrical stunning followed by a percussive blow and subsequent decapitation or cutting of the gill arches.

ATLANTIC SALMON

The majority of Atlantic salmon in the EU are farmed in the United Kingdom (UK) (FAOSTAT, 2015), where approximately 78% are farmed according to RSPCA Assured standards (IBF et al. 2017). The majority are slaughtered by automated percussive stunning followed by a gill cut – a method which can be humane when accurately performed. Some are electrically stunned, which can also cause instant unconsciousness in salmon. However, the stun is reversible and awareness may be recovered before death by gill cut depending on the electrical parameters used. For electrical stunning to be used as part of a humane system, it may be necessary to follow it with a percussive blow or decapitation, to kill more rapidly.

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EUROPEAN EEL

The Netherlands is the main producer of European eels, farming 38% of the EU total (FAOSTAT, 2015). European eels undergo particularly painful and aversive pre-slaughter practices. In order to remove the outer layer of slime produced by the skin, eels are typically immersed in salt or ammonia, and many are eviscerated (gutted) while they are still conscious. Desliming using salt or an ammonia solution should not be performed while eels are still conscious as this is highly aversive and painful (EFSA, 2009e). Some eels are decapitated or have their necks cut without prior stunning but are able to survive these procedures for surprisingly long periods. Decapitated eel heads have been shown to have some brain function for at least 30 minutes and neck-cuts have proved recoverable in laboratory conditions. It is possible to humanely kill eels by electrical stunning with the correct parameters (van de Vis, pers. comms., 2018). Research suggests that combining electrical stunning with nitrogen exposure may also be an effective method (Lambooij et al. (2002).

ATLANTIC BLUEFIN TUNA

The majority of Atlantic Bluefin tuna farming (fattening of wild-caught tuna) in the EU takes place in Malta (FAOSTAT, 2015). Atlantic Bluefin tuna are high performance swimmers and can generate extremely high lactate levels when active, which decreases product quality. Therefore, there is incentive for tuna producers to minimise stress, and therefore activity levels, during the slaughter process. Large tuna are usually shot, either underwater or from the surface, and smaller tuna are spiked or cored. When effectively performed, these methods can cause a rapid loss of consciousness and death. However, the pre-slaughter crowding and handling – including hoisting tuna out of the water using a gaff (a rod with a metal spike on the end that is stuck into the fish) – can cause severe pain and stress, therefore there is significant room for improvement in the welfare of tuna at slaughter.

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NORTH AFRICAN CATFISH

The Netherlands and Hungary are the main producers of North African catfish (FAOSTAT, 2015), where the typical slaughter method is live chilling in ice slurry. This method is inhumane for all fish species and, for the North African catfish, loss of consciousness may take 20 minutes or more (Lambooij et al., 2006; Lambooij, Kloosterboer, Gerritzen, & van de Vis, 2006). An in-water electrical stun followed by decapitation can be humane for North African catfish when effectively performed (Lambooij et al. 2006b; Sattari et al. 2010).

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TURBOT

Over 70% of EU farmed turbot are produced in Spain (FAOSTAT, 2015) and they are typically killed inhumanely without prior stunning. They are either left to asphyxiate in ice slurry, or are exsanguinated (bled out) by cutting of the gill arches and then left to die either in air or ice. This may take several hours in some cases. Percussive stunning can be humane but requires very careful positioning due to the shape of the head, in order to prevent injury and ineffective stunning (Roth, Imsland, et al., 2007). Experimental work suggests that a two-stage electrical stun followed by immersion in ice slurry could offer a humane alternative (Lambooij et al., 2013).

PROGRESS NEEDED TO IMPROVE FISH WELFARE AT SLAUGHTER

Although some improvements to fish welfare at slaughter are gradually being made, currently the vast majority of fish farmed in the EU are killed using inhumane methods. More welfare-friendly alternatives are available for some species but there is a significant amount of work to be done to achieve widespread industry adoption. For other species humane slaughter methods are still under development and research should be prioritised.

A report written for the European Commission in 2017 described an overall lack of compliance by member states with OIE guidelines on fish slaughter. The report also highlighted a key problem being the lack of official assessment of stunning systems and checking of these in practice. A thorough evaluation process should be in place when developing and implementing commercial slaughter systems in order to ensure these are humane in practice, as well as in a research setting.

First, effective stunning parameters must be established in controlled (laboratory) settings. These parameters should cause immediate (or non-aversive) loss of consciousness without recovery or until death occurs by a subsequent kill method. Second, commercial equipment should be constructed to reliably deliver the necessary stunning parameters in practice. Third, the slaughter system (including handling, stunning and killing) must be properly implemented, with staff training and standard operating procedures for use of stunning equipment. Lastly, there must be ongoing verification of effective stunning in-situ. This should include protocols for performing regular checks for consciousness, collection of data from stunning machines, and appropriate enforcement mechanisms, such as surveillance systems and inspections.

There is considerable scope for improving fish welfare at slaughter based on current knowledge and ongoing research. Collaboration between animal welfare researchers and industry members will facilitate the development of more humane systems, which are a necessity according to the basic principles of European legislation on animal welfare.

1. INTRODUCTION

FISH SLAUGHTER - A MAJOR ANIMAL WELFARE ISSUE

In 2014, for the first time, the global production tonnage from aquaculture was greater than that from fisheries and the number of fish farmed for human consumption continues to increase each year (FAO, 2016). In 2015, 52 million tonnes of farmed fish were produced worldwide, including 671 thousand tonnes in the European Union (FAOSTAT, 2015). The number of individual fish slaughtered is not reported, however based on fish tonnage data reported by the Food and Agriculture Organization of the United Nations (FAO) and average fish slaughter weights, an estimated 0.5-1.7 billion farmed fish were killed for human consumption in the EU during 2015 (Mood & Brooke, 2015). This consists of a wide range of species that were farmed and slaughtered in a variety of ways. However, the vast majority of farmed fish in the EU are currently killed using inhumane methods. This presents a major animal welfare issue, in light of the clear and growing evidence (e.g. Braithwaite & Boulcott, 2007; Broom, 2001; Sneddon, 2003, 2004) that fish are sentient animals, capable of affective states of pain, fear and psychological stress (Chandroo et al., 2004).

LEGAL PROTECTION OF FISH

Fish are legally recognised as sentient beings according to The Treaty on the Functioning of the European Union (2012), and therefore full regard must be paid to their welfare. EU legislation provides general protection for animals at slaughter, and while there are some specific requirements for terrestrial species farmed for food, fish are excluded from the majority of these recommendations (European Union, 2009). As explained in Council Regulation 1099/2009, this is due to differences in physiology and slaughter context, and a less developed understanding of the stunning process for fish. However, it is stated explicitly that the key principle remains applicable to fish, which states that "animals shall be spared any avoidable pain, distress or suffering during their killing and related operations" (European

Union, 2009; p. 9). The enforcement of this key principle is the responsibility of EU member states. More detailed recommendations on fish slaughter are given by the World Organisation for Animal Health (OIE, 2010). In fact, these recommendations were used as a benchmark for the assessment of welfare practices in member states in a recent study conducted for the European Commission (IBF et al., 2017). All EU countries, as members of the OIE, should be following these recommendations in the absence of species-specific EU legislation. The key findings of the study were firstly that non-compliant systems (e.g. live chilling in ice slurry, exposure to carbon dioxide in water) are still in wide use, despite the advice from the European Food Safety Authority (EFSA) to move away from these in 2009. Secondly, that stunning equipment is being developed and implemented that has not been verified and documented. This means that systems are being used which may have the potential to stun fish and provide a humane death, but the actual effectiveness of these is largely unknown.

AIM OF THIS REPORT

In this report, we summarise the current understanding and research conducted to date, into the slaughter practices[2] of the main farmed species in the EU. As the ability to assess the state of consciousness is essential for determining the effectiveness of animal stunning methods, the indicators used for fish are described here, along with the associated challenges. The main commercial methods for stunning and killing fish are then evaluated. Lastly, we outline the steps needed to develop humane slaughter systems in order to improve the welfare of farmed fish at slaughter in the EU. Future work will require collaboration between industry and welfare scientists and significant research and development of slaughter systems. Legislative change and effective enforcement of better practice will also be needed in the coming years.

[2] We acknowledge that pre-slaughter handling is an important part of humane slaughter, however it was beyond the scope of this report. Broadly, reducing stress before slaughter involves reducing the time and intensity of crowding, handling and time out of water. For example, moving fish from rearing tanks to the location for slaughter is a cause of stress, but it may be lowered by carefully pumping fish in water from one place to another instead of using nets to transfer them out of water.

2. STUNNING FISH EFFECTIVELY

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2.1. WHAT IS HUMANE SLAUGHTER?

In animal welfare terms, slaughter is humane if the animal dies without suffering. Humane slaughter usually involves a two-stage process: stunning and killing. Stunning is defined by the European Commission as "any intentionally induced process which causes loss of consciousness and sensibility without pain, including any process resulting in instantaneous death" (EC1099/2009). In order to determine whether a slaughter method is humane, it is essential therefore to be able to assess the state of consciousness of the animal. This is important during both the development of slaughter systems, and throughout their use at slaughterhouses.

Consciousness can be considered as "awareness of the world around and of the own body, where unconsciousness means unarousable unresponsiveness" (Lambooij et al., 2010, p. 107) during which "the brain is unable to process sensory input (e.g. during (deep) sleep, anaesthesia or due to temporary or permanent damage to brain function)" (IBF et al., 2017, p. 49). Animals should be made unconscious before any killing method that would otherwise cause pain and suffering. Ideally, consciousness is lost immediately (i.e. within one second) (EFSA, 2004). Where this is not the case, the induction of unconsciousness should be non-aversive and should not cause anxiety, pain, distress, or suffering (EFSA, 2004). If the stunning method is reversible, another procedure must be used to kill the animal and prevent it recovering consciousness (OIE, 2014).

2.2. MEASURING FISH CONSCIOUSNESS

Consciousness cannot be directly studied for any species (Bradshaw, 1998) however brain activity can be measured and used to infer consciousness.

Currently, we can only measure brain activity in laboratory settings, so in less controlled settings (i.e. the slaughterhouse) we must rely on pre-established animal-based indicators of consciousness (Retter et al., 2018). These are behaviours and clinical reflexes that are known to be associated with certain brain activity. Different indicators provide information on different aspects of brain function, and using multiple indicators in combination provides a more robust assessment than any single measure alone (Terlouw, Bourguet, & Deiss, 2016).

Several methods are used to assess fish consciousness and research shows that these vary in their reliability, which partly depends on the species and slaughter method (see Table 1).

Kestin, van de Vis, & Robb (2002) anaesthetised fish and found that during the process of losing consciousness, they first stopped performing normal behaviours (e.g. swimming and maintaining equilibrium), then stopped responding to stimuli (e.g. handling, pin prick to the lip, 6V electric shock), and lastly lost clinical reflexes mediated by the brainstem (e.g. breathing and normal movement of the eyes upon rotation of the body). The loss of clinical reflexes tended to occur at the same time as, or later than, the loss of visually-evoked responses (VERs) in brain activity, which is considered a good indicator of profound brain failure (Robb et al., 2000). Thus, in general, when assessing the effectiveness of stunning, the absence of clinical reflexes mediated by the brainstem (in addition to loss of behaviours and stimulus responses) should be verified.

2.3. PHYSICAL IMMOBILISATION

One major challenge in assessing the effectiveness of stunning is that some methods (both those that are inherently unsuitable and those that are applied ineffectively) can cause physical immobilisation, whereby fish can appear motionless and unresponsive to handling and stimuli, but maintain brain function and may still be conscious (van de Vis, Abbink, Lambooij, & Bracke, 2014). For example, when electrical stunning is effective, fish are rendered instantaneously (i.e. < 1 second) unconscious (e.g. Lambooij et al., 2010). However, if insufficient electrical parameters are used immobilisation can occur, whereby the muscles are paralysed but brain activity indicates that fish are still conscious and able to experience pain and suffering (van de Vis et al., 2003). Hormonal responses (e.g. elevated plasma cortisol levels) that are discordant with what otherwise appears to be an unconscious fish can help identify when physical immobilisation alone has occurred (EFSA, 2004). This means that behavioural measures of unconsciousness are often insufficient for assessing the efficacy of a stunning system (e.g. Lambooij et al., 2010; Retter, 2014). Fish may also be conscious yet motionless due to exhaustion or tonic immobility (feigning death) (van de Vis, Abbink, Lambooij, & Bracke, 2014).

2.4. AVERSIVE CONDITIONS

When a stunning method does not bring about immediate unconsciousness the process should not be aversive, in order for it to be considered a humane method. Attempts to escape and abnormal behaviours (e.g. gasping at the surface of the water, or keeping the mouth and operculum shut) suggest that conditions are aversive, although their absence does not mean than conditions are not aversive. Other measures that can indicate aversive conditions include changes in heart rate (Lambooij, van de Vis, Kloosterboer, & Pieterse, 2002) and elevated levels of plasma cortisol in blood samples taken post slaughter (Barton, 2002). Haematological variables and plasma ion concentrations can also provide some indication of the stress experienced by fish before slaughter, for example due to crowding and handling (Gräns et al., 2016).

2.5. RECOVERY OF CONSCIOUSNESS

Often, unconsciousness resulting from a stunning method is not permanent. The length of a stunning period can vary depending on the stunning parameters (e.g. in electrical stunning: current, voltage, how long the electric field is applied, etc.) or the fish (e.g. size, health status, age). In order to prevent suffering, it is crucial that fish are killed before they recover consciousness (Robb, Wotton, McKinstry, Sørensen, & Kestin, 2000). This means that the state of consciousness should be checked multiple times after stunning to ensure that the process is humane.

For slaughter to be humane:

1. A killing method must be instant or preceded with a stunning method which causes instant unconsciousness. Alternatively, loss of consciousness can be gradual but the method must be non-aversive and painless.

2. Unconsciousness must last until death.

Essential to ensuring 1. and 2. is therefore the ability to:

- Determine the state of consciousness of fish during stunning and until death
- Distinguish between physical immobilisation and unconsciousness due to effective stunning

TABLE 1
Methods used to assess the state of consciousness of fish

Measurement of brain activity - *currently only feasible in laboratory conditions*			
METHOD	**OBSERVATIONS**	**CONCLUSIONS**	**RELIABILITY**
Measure waveform, amplitude and frequency of the spontaneous electroencephalograph (EEG)	Patterns of brain activity	State of brain function	Good indicator of state of consciousness (Lambooij et al., 2015)
Measure (via EEG) visually-evoked responses (VERs) in brain activity triggered by intermittent light flashes	Presence of VERs Absence of VERs	The fish is likely to be conscious The fish is likely to be unconscious	Good indicator of state of consciousness (Robb et al., 2000)
Measure (via EEG) somatosensory-evoked responses (SERs) in brain activity, e.g. responses to pain stimuli	Presence of SERs Absence of SERs	The fish is likely to be conscious The fish is likely to be unconscious	Good indicator of state of consciousness (van de Vis et al., 2003)

Assessing clinical reflexes mediated by the brainstem – *feasible in commercial conditions*			
METHOD	**OBSERVATIONS**	**CONCLUSIONS**	**RELIABILITY**
Test for the vestibulo-ocular reflex (VOR), known as "eye roll" by rotating the fish and observing any eye movements. **In an unconscious fish the eye is fixed in the skull when the fish is rocked from side to side. In a fish retaining some brain function, the eye rotates dorso-ventrally when the fish is rocked (EFSA, 2004)**	Presence of eye roll Absence of eye roll	The fish is likely to be conscious The fish is likely to be unconscious	Good indicator of state of consciousness for many species as this is one of the last things to be lost during anaesthesia and one of the first to appear upon recovery; lost at similar time to loss of VERs. (Kestin et al., 2002). Caution: unreliable for fish that have been live chilled (EFSA, 2004). Also, unsuitable for tuna as the VOR has not been observed in these species.
Assess opercular movement (breathing)	Presence of opercular movement Absence of opercular movement	The fish is likely to be conscious The fish is likely to be unconscious	Good indicator of state of consciousness in some species; lost at similar time to loss of VERs (Kestin et al., 2002). Caution: cannot be used for ram ventilators, e.g. tuna, who do not make opercular movements (EFSA, 2009c). May be more difficult/take longer to assess in some species e.g. flat fish, red gurnard (Kestin et al., 2002).

Assess response to noxious or aversive stimuli – *feasible in commercial conditions*			
METHOD	**OBSERVATIONS**	**CONCLUSIONS**	**RELIABILITY**
Observe behavioural response to prick with a needle, electric shock (e.g. application of 6V) or handling	Presence of a response, e.g. movement / escape behaviour Absence of a response	The fish is likely to be conscious Conclusion not possible	Good indicator of consciousness for some species. Caution: response to needle prick unreliable for dab, gurnard, plaice, sole; response to 6V electric shock unreliable for sea bream, salmon, cod and plaice (Kestin et al., 2002). Caution: absence of this behaviour does not necessarily indicate unconsciousness, particularly after stunning methods that can cause narcosis or paralysis (Kestin et al., 2002)

Assess self-initiated behaviour – *feasible in commercial conditions*			
METHOD	**OBSERVATIONS**	**CONCLUSIONS**	**RELIABILITY**
Observe coordinated behaviour such as swimming or attempts to escape	Presence of coordinated behaviour Absence of a response	The fish is likely to be conscious Conclusion not possible	Good indicator of consciousness. Caution: absence of this behaviour does not reliably indicate unconsciousness, particularly after stunning methods that can cause narcosis or paralysis (Kestin et al., 2002).
Test ability to achieve equilibrium after being inverted	Able to achieve equilibrium Unable to achieve equilibrium	The fish is likely to be conscious Conclusion not possible	Good indicator of consciousness, reliable in many species (Kestin et al., 2002). Caution: absence of this behaviour does not necessarily indicate unconsciousness, particularly after stunning methods that can cause narcosis or paralysis (Kestin et al., 2002).

3. SLAUGHTER METHODS USED FOR FARMED FISH IN THE EU

There are numerous methods used in the EU to slaughter fish. Based on the scientific evidence available, the OIE have concluded that percussive and electrical stunning (followed by gill cut), shooting, and mechanical spiking and coring are acceptable slaughter methods for some fish species, with the *potential* to enable a humane death. This is dependent on the methods being performed accurately and effectively, resulting in minimised pain and stress before death. Other methods are inhumane and should not be used (Table 2). In this section, the main commercial slaughter methods are described.

TABLE 2
Slaughter methods used to kill farmed fish

Methods with the potential to be humane	
1.	Percussive blow (followed by gill cut)[a]
2.	Application of electrical current (followed by gill cut)[a]
3.	Shooting[a]
4.	Mechanical spiking, coring[a]
Inhumane methods	
5.	Live chilling in ice slurry[b]
6.	Exposure to air[b]
7.	Carbon dioxide in holding water[b]
8.	Chilling with ice and carbon dioxide in holding water[b]
9.	Salt or ammonia bath[b]
10.	Gill cut[b]
11.	Decapitation
12.	Manual spiking

[a] Described by OIE, 2010 as enabling humane killing for certain fish groups; [b] described as 'shown to result in poor fish welfare' by OIE, 2010.

3.1. POTENTIALLY HUMANE METHOD:

Percussive blow

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

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

3.2. POTENTIALLY HUMANE METHOD:

Application of an electrical current

According to the OIE (2010), electrical stunning can enable humane killing for some fish groups, e.g. carp, catfish, eel, salmonids and tilapia, providing that death occurs without fish regaining consciousness. A separate kill method is often required after electrical stunning and the combination and timing of these two procedures will determine whether the overall slaughter method is humane.

Generally, electrical stunning works by stimulation of the higher nerve centres in order to “cause their dysfunction, either by induction of epileptiform activity or by complete cessation of function” (Robb, O’Callaghan, Lines, & Kestin, 2002). A general epileptiform insult (i.e a ‘grand mal’ or seizure-like state) involves changing the waveforms in the brain, causing a period of reduced electrical activity which is often associated with a state of unconsciousness (Lambooij & Hindle, 2017). It is characterised by rapid and extreme depolarisation of the membrane potential (Lambooij, Kloosterboer, Gerritzen, & van de Vis, 2006). During this time, the brain is in a stimulated condition and unable to respond to stimuli (Lopes da Silva, 1983 in Lambooij & Hindle, 2017). Commonly, an epileptiform insult involves a ‘tonic’ (body is rigid), ‘clonic’ (uncontrolled activity, e.g. jerking movements) and ‘exhaustion’ phase (rhythmic breathing restarts and awareness recovers) (Robb, O’Callaghan, Lines, & Kestin, 2002). During these phases in a human, for example, the individual is unconscious (Robb, O’Callaghan, Lines, & Kestin, 2002). Similarly, in a study of the brain activity of rainbow trout, immediately after a sufficient electrical current was applied, fish became rigid with some muscular twitches, and showed disrupted brain activity indicative of unconsciousness (Robb et al., 2002). Following this, opercular movements resumed and brain activity indicated a return to consciousness (Robb et al., 2002). Longer durations of unconsciousness (or death) can be achieved by increasing the magnitude of the current, increasing the duration of the applied current and/or decreasing the frequency of the current (Robb et al., 2002). Electrical stunning should be followed by a separate killing method such as gill cutting, percussive blow or decapitation.

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

- Head-only electrical stunning: fish are removed from their holding water and placed head-first into a stunner which delivers an electric current to the head.
- Head-to-body, dry electrical stunning: fish are removed from water and passed over a conveyor belt which acts as one of the electrodes, with a chain of plate electrodes (steel flaps) hanging above to complete the electrical circuit (see figure 1a-b). A variation on this system is what is often referred to as a 'semi-dry' system, which is as above but fish are sprayed with water before passing over the conveyor belt.
- In-water electrical stunning: fish are exposed to an electric current in water, e.g. while pumped through a pipe containing two plate electrodes (continuous flow system) (see figure 1c-d) or in a tank (batch system).

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

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

Insufficient electrical current, voltage or duration can lead to unsuccessful stunning which can be very painful and cause injuries to conscious fish (van de Vis et al., 2003). Alternatively it can mean fish regain consciousness after, for example, having their gills cut, and will experience significant pain and suffering. Ineffective electrical stunning can also lead to immobilisation, where the body is motionless and unresponsive in reflex tests but brain activity shows that the fish remains conscious and likely to be sensible to pain (Kestin, van de Vis, & Robb, 2002; Kestin, Wotton, & Adams, 1995; Retter, 2014; Robb & Kestin, 2002). Therefore, behavioural measures alone are not reliable for assessing electrical stun efficacy.

For commercial applicability, the effect of electrical stunning parameters on product quality will also be considered. Applying an electric current to a fish stimulates the muscles and causes them to contract. This can lead to damage to the spine, dorsal aorta or veins, causing haematomas in the fillet (Hauck, 1949). The current direction (i.e. alternating or direct), field strength and frequency will determine the risk of injury to the fish and subsequent damage to the fillet (Lines & Kestin, 2005; Roth, Moeller, & Slinde, 2004). For industry adoption, electrical parameters must be strong enough to stun effectively, while minimising any negative effects on quality.

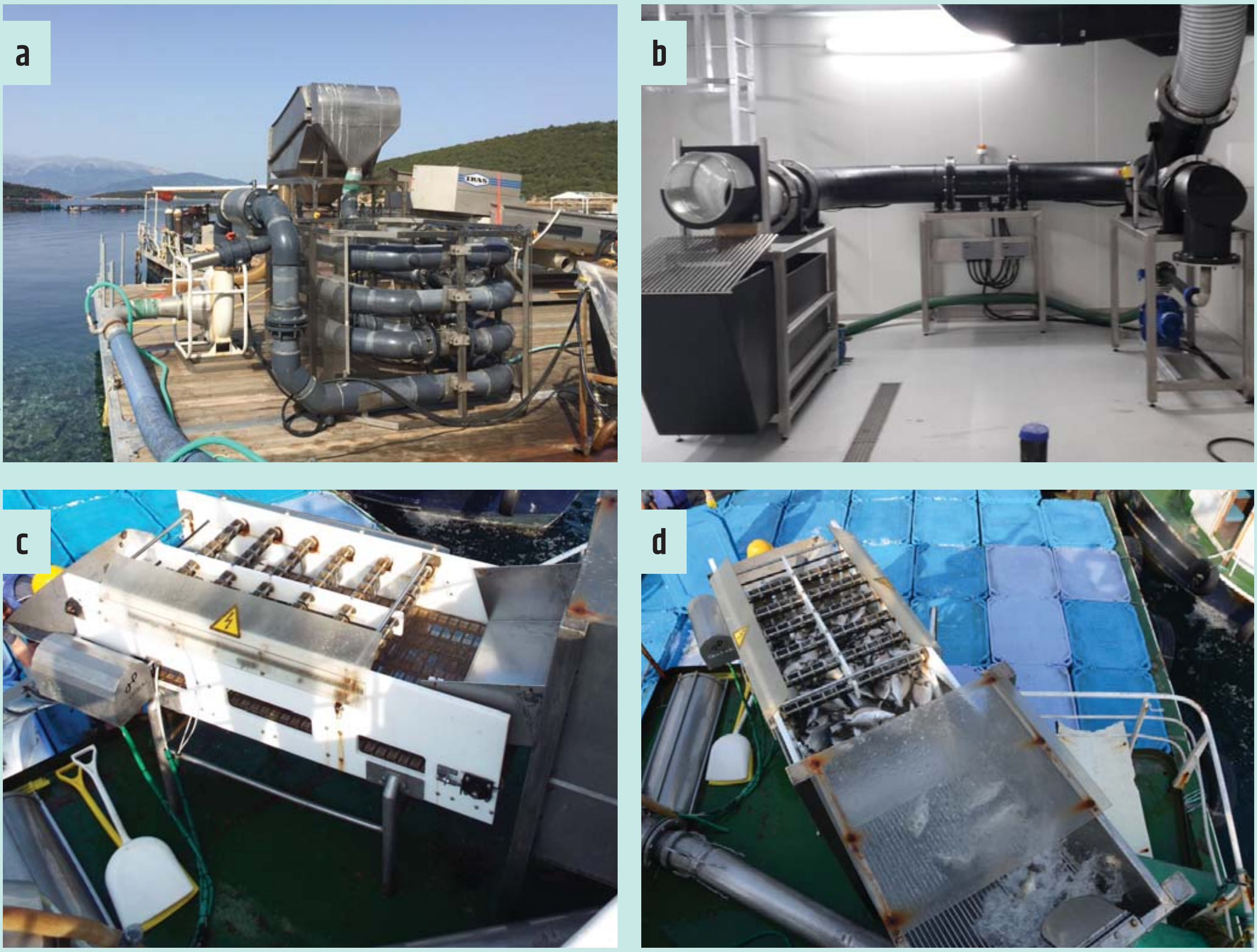

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FIGURE 1
Commercial electrical stunning machines used to stun fish in-water (a and b) or after dewatering (c and d).

3.3. **POTENTIALLY HUMANE METHOD:**

Shooting

Shooting is commonly used to kill large (over 50kg) farmed tuna. Around 70-80% of large tuna farmed in the EU are shot by divers underwater (referred to as 'lupara'), with the remaining 20-30% being shot with a rifle from the surface (EFSA, 2009c).

Using the lupara method, tuna are first crowded in the pen (using nets) then marksmen enter the water and shoot the fish using a power-head (a device which resembles a short gun barrel and contains a single-shot cartridge) fitted to the end of a 2-3 metre-long stick (EFSA, 2009c). This is jabbed into the head of the tuna, which causes the bullet to fire, capable of causing immediate death via destruction of the brain (EFSA, 2009c). The tuna are then hoisted up out of the water and on board the boat where the lateral arteries, and sometimes the gills, are severed using a sharp pointed knife (EFSA, 2009c). Lupara can be a humane method because live tuna are not removed from the water and a single shot is usually sufficient to cause immediate death (Benetti, Partridge, & Buentello, 2015).

An estimated 1-4% of fish must however be shot more than once (the bullet may miss the brain). Accuracy partly depends on the shooter's experience and the sea conditions (EFSA, 2009c). In this case, fish are likely to suffer pain and stress from being shot inaccurately. Furthermore, tuna must be crowded before they are killed by this method, and if this is not done carefully some tuna can become caught in the nets. As tuna are ram ventilators (they must keep swimming in order to pass oxygenated water over their gills), being trapped stationary in the net can cause hypoxia (deprivation of oxygen), suffering and stress until death (EFSA, 2009c).

Shooting with a rifle from the surface is also used for tuna. Fish are either crowded in a smaller slaughtering cage or are kept to one side of the rearing cage using a net (EFSA, 2009c). After being shot, the tuna are bled out in the water then moved onto the deck after death for processing (EFSA, 2009c). Shooting from the surface allows a faster processing speed than lupara so is used when greater numbers of tuna are being killed per day. It can enable humane slaughter when shots are accurate. However it is less precise than the underwater method, with an estimated 7-10% requiring a second shot (EFSA, 2009c). It is also more stressful for fish as it involves more severe crowding (for approximately 15 minutes). For fish that are not shot accurately the first or second time, they may suffer from their injuries for 10 – 15 minutes until they are spiked (see 3.4) in the water at the end of the shooting period (EFSA, 2009c). The process is also stressful for nearby tuna that are not killed, as they are disturbed by the noise of the boat and gun shots and are stressed by blood in the water (EFSA, 2009c).

3.4. POTENTIALLY HUMANE METHOD:

Spiking and coring

Spiking (also known as 'iki jime') and coring are used to stun and kill fish by causing severe and irreversible damage to the brain (FAWC, 2014). The brain is damaged either by pushing a solid, pointed metal rod (spiking) into the head which is then moved around to destroy the brain, or a hollow metal rod (coring) which is usually knocked into the brain with a mallet. These methods are sometimes performed in, and sometimes out of, the water. In aquaculture, these methods are mainly used for large species such as tuna, as the brain is harder to target in smaller fish (EFSA, 2004).

For both methods, accuracy in positioning and delivery of the device is crucial to avoid injury and suffering (FAWC, 2014). The fish is restrained and, in some cases, is percussively stunned with a priest before spiking or coring. If percussive stunning is not used, fish make vigorous attempts to escape during the procedure. This makes it more difficult to perform accurately; the spike may be driven into the head but may miss the brain or cause insufficient damage which does not cause insensibility. There is therefore a risk that fish are injured and disabled but still capable of feeling pain (EFSA, 2004). However, when spiking/coring is correctly and accurately applied it can enable humane slaughter in some species, e.g. salmon (Robb et al., 2000). The EFSA (2004) recommend that manual spiking is "slow to achieve and the technique should not be used", but mechanical methods can be humane. For example, pneumatically operated pistols used to insert the spike make the process more effective. A modification to this method includes captive needle stun/killing systems. These involve pneumatically firing a captive needle into the brain and injecting compressed air, which can cause immediate loss of consciousness in some species (EFSA, 2004).

Although accurate spiking and coring can cause rapid death or loss of sensibility, the associated pre-slaughter handling can be inhumane. When spiking/coring is performed on land, the fish are removed from the water which causes suffering – particularly when hauled out with a 'gaff' (a rod with a hook on the end) which is stabbed into the head of the fish in order to pull it on board the boat. Using a gaff leads to "severe pain and distress" (EFSA, 2009c) associated with this method. Some smaller tuna are spiked in the water which is better for welfare but still involves significant stressors such as crowding (EFSA, 2009c).

After spiking or coring, some fish (e.g. Atlantic Bluefin tuna) are subsequently bled by gill cut and 'pithed'. Pithing consists of inserting a length of rigid monofilament nylon or stainless steel wire into the brain and pushing it as far as possible into the neural canal to destroy the spinal cord. The wire is pushed through the hole made by the spike or, where a coring tool is used, the wire is inserted via the hollow rod. This is supposedly a post-mortem action and isdone for flesh quality purposes: pithing stops muscular activity and the biochemical reactions that contribute to flesh deterioration (EFSA, 2009c).

3.5. INHUMANE METHOD:

Live chilling in ice slurry

Fish are pumped or netted from (ambient) holding water into ice slurry (figure 2). This is a mixture of ice and water in a ratio ranging from 1:2 to 3:1, with typical temperatures of between 0 and 2°C (EFSA, 2009f). Fish eventually die from asphyxiation. This is a low cost method used to kill many fish species and is widespread globally (Oliveira Filho et al., 2015). However, the method is inhumane as it does not stun immediately and is aversive, resulting in "poor fish welfare" (OIE, 2010, p. 3).

The time to unconsciousness and death by ice slurry depends on the species, ice to water ratio, number of fish added, temperature change and other factors (Poli, Parisi, Scappini, & Zampacavallo, 2005), however in no case is it instant, as is required for humane slaughter (see 2.1). Studies show, for example, that it takes anything from five minutes (van de Vis et al., 2003) to 40 minutes (Huidobro, Mendes, & Nunes, 2001) to achieve unconsciousness in gilthead sea bream immersed in ice slurry, which is the predominant method used for this species. As the body temperature of the fish drops rapidly, their metabolic rate, movements and oxygen requirements also decrease; therefore fish generally take longer to die from asphyxia in colder temperatures and the duration of suffering is prolonged (Ikasari & Suryaningrum, 2014).

Immersion in ice slurry is also highly aversive. In commercial practice, large numbers of fish are packed into totes of ice slurry, which may make it difficult for some of the fish to breathe as their opercula become compressed under the weight of fish above (Kestin et al., 1991). Researchers observe

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FIGURE 2
Fish are transferred from their pen using a braille net and immersed in ice slurry without pre-stunning - an inhumane method of slaughter.

"vigorous movements" of fish placed in ice slurry (e.g. Lambooij et al., 2002; van de Vis et al., 2003). This activity tends to slow then cease after a few minutes, but does not necessarily indicate the fish are unconscious, given that rapid cooling of the body can cause muscle paralysis and immobilisation (Kestin, Wotton, & Gregory, 1991; van de Vis et al., 2003). The effects of cold temperatures on fish physiology and behaviour can therefore often be confounding for welfare assessment and may mislead operators relying on fish behaviour/activity to judge unconsciousness. For example in one study, sea bass became motionless after three minutes in ice slurry, yet still responded to external stimuli after 11 minutes (indicating some level of consciousness) (Zampacavallo et al., 2015). Indeed, the EFSA describe live chilling as "an immobilisation method and not a stunning method since it does not induce unconsciousness" (EFSA, 2009d, p. 2).

3.6. INHUMANE METHOD:

Exposure to air

Fish are removed from water and left to suffocate in air. This causes the fine filaments of the gills, no longer supported by the density of water, to collapse and lie on top of each other. This reduces, and eventually prevents, oxygen exchange with the environment (Robb, O'Callaghan, Lines, & Kestin, 2002). There is no stunning effect with this method and suffering before death is prolonged (Poli et al., 2005). Vigorous movements and escape behaviours are commonly observed until the fish become exhausted (Robb & Kestin, 2002; Rahmanifarah, Shabanpour, & Sattari, 2011). The time to loss of consciousness due to asphyxia in air differs between species, with some surviving for very long periods. For example, under certain conditions (low temperature and high humidity), common carp can survive for several hours out of water (EFSA, 2009a). Time to unconsciousness is temperature dependent. For example, in a study by Kestin et al. (1991) rainbow trout lost brain function at 2.6 minutes at 20°C, 3 minutes at 14°C and 9.6 minutes at 2°C. In any case, leaving fish to die by asphyxiation in air results in poor welfare and should not be carried out (OIE, 2010).

3.7. INHUMANE METHOD:

Carbon dioxide exposure in water

Carbon dioxide (CO_2) is bubbled into a tank of water (which is sometimes chilled) until the desired levels are obtained. For rainbow trout, for example, carbon dioxide levels of 200 - 450 mg/l are typically used, leading to a pH level of approx. 5.5 - 6.0 (IBF et al., 2017). Fish are transferred to the tank, where the high levels of carbon dioxide disrupt their blood pH, leading to alteration of brain function (Robb et al., 2002). After an exposure time of 2-4 minutes they are removed and bled out by gill cut.

Escape behaviour is evident in fish exposed to carbon dioxide (e.g. Gräns et al., 2016). The gas can also render fish immobile (paralysed) before they lose consciousness (Kestin et al., 2002) and therefore suffering is likely to last longer than it appears based on their activity. For example, Kestin et al. (1995) reported that trout showed obvious aversion to carbon dioxide for 30s, but loss of brain function took an average of 4.7 minutes at 14°C. Similarly, Robb et al. (2000) found that Atlantic salmon showed aversion for up to 2 min, but brain activity indicated consciousness until 6.1 minutes at 6°C.

This method is inhumane because it is very aversive and is slow and unreliable in causing unconsciousness. Therefore fish suffer for several minutes before losing consciousness, or may be bled or eviscerated while conscious. It is used for some Atlantic salmon in Ireland, and some rainbow trout in France (IBF et al., 2017). The Norwegian Food Control Authority has prohibited the use of CO_2 for fish (Anonymous (2006) cited in IBF et al., 2017). However, CO_2 can still be used in Norway when in combination with live chilling, despite that this method is also inhumane and is stressful for salmon (Erikson, 2008).

3.8. INHUMANE METHOD:

Ammonia or salt bath followed by evisceration

These killing and processing methods are used for eels only. Salt or ammonia solutions are used to remove the slime from the skin of eels, and to immobilise them for easier evisceration (EFSA, 2009e). These treatments do not cause effective stunning and involve "severe pain and distress" (EFSA, 2009e).

The 'salt bath' method involves removing eels from water and putting them into a dry tank, then adding salt (NaCl) or a mixture of salt and aqueous sodium carbonate (Na_2CO_3) (van de Vis et al., 2003). As the eels are piled on top of each other in a dry tank, those at the bottom are subject to the weight of those above which is likely to be painful and distressing. They are left like this for approximately 20 minutes (EFSA, 2009e). Subsequently, to remove the clotted slime and salt, eels are moved to a mixing machine, where they are washed for around 10 minutes in water. After this they are eviscerated (EFSA, 2009e).

Salt is very aversive to eels and it is likely that they are subsequently gutted while still conscious (Morzel & van de Vis, 2003). van de Vis et al. (2003) reported "vigorous attempts to escape...for at least 3 min" after application of salt (p. 215). The salt application causes denaturation of the eels' mucus proteins, which results in clotting and damage to the upper layer of skin (EFSA, 2009e). The salt also damages the eyes and causes them to become opaque (EFSA, 2009e). This is likely to cause extreme pain (EFSA, 2009e).

Based on measurements of brain activity, loss of consciousness may take more than 10 minutes (van de Vis et al., 2001 in EFSA, 2009e) but other evidence (behaviour and responses to stimulation) suggests it may take more than 25 minutes (EFSA, 2009e). Eels are reported to sometimes still be active during the washing process, with movement eventually stopping due to muscular exhaustion (EFSA, 2009e). Some die from osmotic shock (rapid changes in the movement of water across the cell membranes, caused by a sudden change in the solute concentration around the cells), however the majority of eels are believed to still be alive when they are eviscerated (Verheijen & Flight, 1997). Indeed, Verheijen & Flight (1997) observed that when eels were not killed after salt exposure, they remained alive for up to 18 h after the procedure.

An 'ammonia bath' consists of removing eels from water and putting them into a dry container, then adding an ammonia (NH_3) solution (usually a 25% ammonia solution at the ratio of 100kg dry eels: 100ml ammonia solution) (EFSA, 2009e). After around 4 minutes the container is filled with water and the eels are left for approximately 20 minutes (EFSA, 2009e). The container is then emptied and the slime is washed off the eels (either using a tumbler or by replacing the water) which are then eviscerated and processed (EFSA, 2009e).

During this process, the eels bleed from their gill openings. As with the salt application, the mucus layer on the skin is loosened by the ammonia solution as the proteins are denatured. The solution also causes damage to the upper layer of skin and the eyes, which become opaque or white (EFSA, 2009e). This is extremely painful and aversive, and eels also experience exhaustion, dehydration and intoxication with ammonia (EFSA, 2009e). Eels make very vigorous attempts to escape from ammonia and their activity suggests they are still alive for up to 15 minutes, or longer, in ammonia (Kuhlmann & Münkner, 1996 in EFSA, 2009e). Ammonia baths are likely to kill eels before evisceration commences (EFSA, 2009e).

Both the salt and ammonia methods have been prohibited based on welfare grounds in Germany since 1999 (EFSA, 2009e). In the Netherlands the Animal Welfare Council (an advisory body to the Government) proposed to ban these methods (EFSA, 2009e). New Zealand's Code of Welfare on Commercial Slaughter (2010) also forbids any method that de-slimes eels while they are still conscious because of the poor welfare caused.

3.9. INHUMANE METHOD:

Killing without stunning

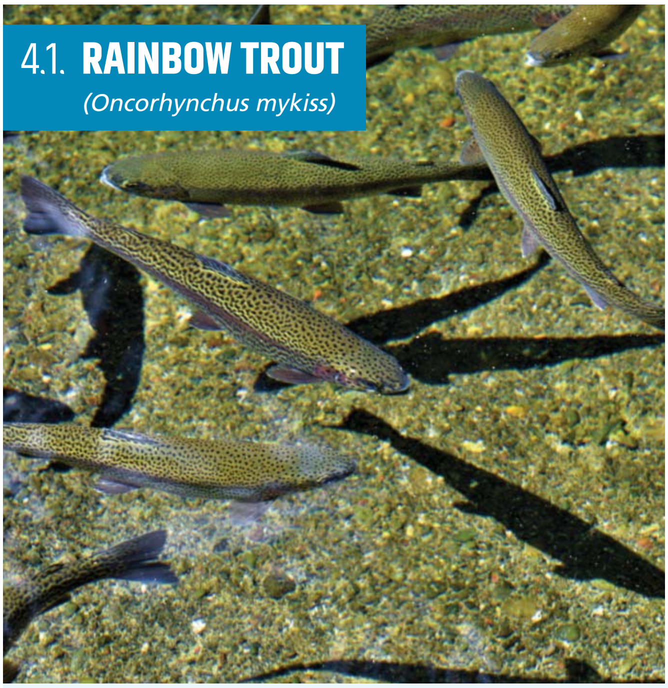
For slaughter to be humane, fish must be stunned before any painful procedure to kill. Therefore gill cut, decapitation, skinning or evisceration without prior effective stunning is not humane and should not be performed. However, many fish are killed commercially without stunning.

4. HOW EFFECTIVE ARE SLAUGHTER METHODS USED FOR EACH SPECIES?

The slaughter methods used vary for different fish species. The physiology and morphology of each species determines how suitable a method may be for causing a humane death, and affects the parameters required to stun/kill. Pressure on the industry to use certain methods for welfare or product quality reasons also determines which methods are adopted and how widely. In this section, the current methods used for each of the key farmed species in the EU are described, including a summary of the research into their effectiveness conducted to date. For an overview of these methods see table 3 (page 42).

4.1. RAINBOW TROUT

(Oncorhynchus mykiss)



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Over 206 thousand tonnes of rainbow trout were produced by 26 countries in the EU in 2015, with Denmark, Italy and France being the top producers (FAOSTAT, 2015; FEAP, 2016; figure 3). This equates to somewhere between 130 and 762 million trout (calculated according to methods of Mood & Brooke, 2015). A range of slaughter methods are currently used for farmed rainbow trout: electrical stunning, percussive stunning, live chilling in ice slurry, carbon dioxide exposure, and decapitation, with some of these methods being used in combination (IBF et al., 2017).

According to the report prepared for the European Commission (2017), 30% of rainbow trout in Denmark are left to asphyxiate in ice and 70% are electrically stunned in water, followed by a throat cut. In France, in-water electrical stunning is also used, but carbon dioxide exposure in water followed by a gill cut is used for large trout.

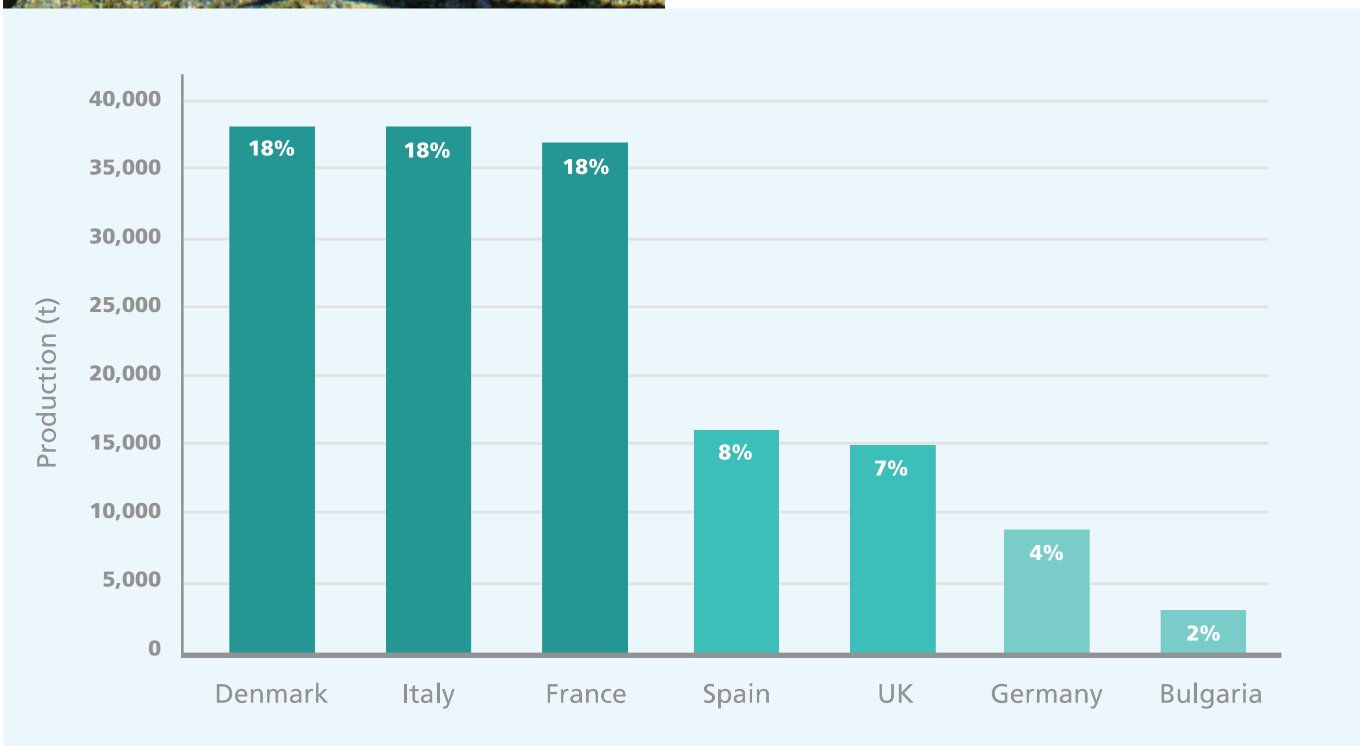

FIGURE 3

Production (tonnes) of EU countries with more than 10 tonnes annual rainbow trout production during 2015 and their percentage contribution to total EU production.

Sometimes, electrical stunning is preceded with exposure to ice slurry, and percussive stunning is also used. In-water electrical stunning is reportedly used in Italy. The effectiveness of the electrical stunning used in these countries is unknown, as the stunning machines have probably not been purchased from major manufacturers (IBF et al., 2017). In Poland, rainbow trout are not stunned before slaughter. Instead, trout are either killed by exposure to ice or ice slurry, often during transport, left to asphyxiate in air, or (large trout) are decapitated without stunning.

In the UK, percussive stunning is the main method used for large trout (FAWC, 2014), and electrical stunning is the main method for smaller trout. Around 80% of trout production is represented by the British Trout Association, and these farms now use electric stunning (Lines, n.d.). Additionally, some UK farms are certified by the RSPCA Assured scheme, and must use either a percussive blow, in-water electrical stunning followed by bleeding, or electrocution (electric current to kill) (RSPCA, 2018).

Percussive blow
When applied correctly, percussive stunning can be effective in causing immediate unconsciousness in trout and may kill by brain haemorrhage (Kestin, Wotton, & Adams, 1995; Robb, Wotton, McKinstry, Sørensen, & Kestin, 2000 in EFSA, 2009b). This is usually followed by evisceration for small trout, or gill cut then evisceration for larger trout (EFSA, 2009b).

Application of an electrical current
Rainbow trout can be instantly stunned by a sufficient electrical current (Robb et al., 2002). Electrical stunning may require a kill method (e.g. throat cut) although with certain parameters it can be used to kill; Robb et al., (2002) found that increasing the stun duration caused an increase in the number of fish that died due to the electrical current. Either is acceptable for welfare if the stun prevents recovery though the number needs to increase to 100%.

Electrical parameters required to effectively stun fish depend on a number of factors and may differ between each set up. However, several studies provide examples of effective parameters. Lines and Kestin (2004) found that, for portion sized rainbow trout (250-400g), a field strength between 3 and 6 V/cm was required for 30 to 60 sec, in order to achieve permanent insensibility.

Testing in-water electric stunning of rainbow trout, Lines (n.d.) found that the duration of unconsciousness increased with electric field strength and stun duration, and decreased with increasing frequency. Haemorrhages were found to be independent of stun duration but decreased with increasing electrical frequency. Lines recommends that a 1000 Hz sinusoidal electric field of 2.5 V/cm rms for 60 seconds results in portion sized rainbow trout being stunned beyond recovery, but without causing carcass damage.

A minimum current of 100 mA at 50 Hz across the head was required for 1 second to stun rainbow trout in a study by Robb et al. (2002). In the same study, researchers found that in a water bath a current density of at least 8.3 A m^{-2} at 50 Hz, for at least 5 seconds, was needed for an effective stun. When applied for at least 30 seconds it was possible to kill all fish with the electricity.

Some rainbow trout (e.g. France) are exposed to live chilling before electrical stunning. Live chilling is aversive and does not induce unconsciousness for several minutes (see below) therefore this is not a humane slaughter method even when effective electrical parameters are used.

Live chilling in ice slurry
Live chilling in ice slurry is inhumane for rainbow trout. It is usually followed by evisceration (for portion sized trout) or exsanguination and evisceration (for large trout) (EFSA, 2009b). In a study by Robb and Kestin (2002, in EFSA, 2009), it took 9.5 minutes (on average) in ice slurry (at 2°C) for rainbow trout to lose consciousness, as indicated by measures of brain activity (loss of visually-evoked responses (VERs)).

Exposure to air
Leaving fish out of water to asphyxiate in air is inhumane and causes prolonged suffering in rainbow trout, the length of which is partly dependent on temperature. For example brain function is only lost after 2.6 minutes at 20°C, 3 minutes at 14°C and 9.6 minutes at 2°C (Kestin et al., 1991). This method should not be used (OIE, 2010).

Carbon dioxide exposure in water
Exposure to carbon dioxide in water is an inhumane method and, for rainbow trout, results in several minutes of suffering before unconsciousness (IBF et al., 2017). It is aversive but also results in physical immobilisation before insensibility; Kestin et al. (1995) reported that trout showed obvious aversive behaviour for 30 seconds in water saturated with carbon dioxide, but brain activity indicated that loss of consciousness took an average of 4.7 minutes at 14°C and 6.1 minutes at 6°C. Longer periods of aversive behaviour have been found, for example over 3 minutes aversion was reported in a study by Marx et al., 1997 (cited in Robb et al., 2002). Carbon dioxide exposure is sometimes combined with chilling in ice slurry. Loss of physical activity is reportedly faster at lower temperatures, but still continues for around a minute (Robb, pers. comms cited in EFSA, 2009b).

4.2. GILTHEAD SEA BREAM

(Sparus aurata)

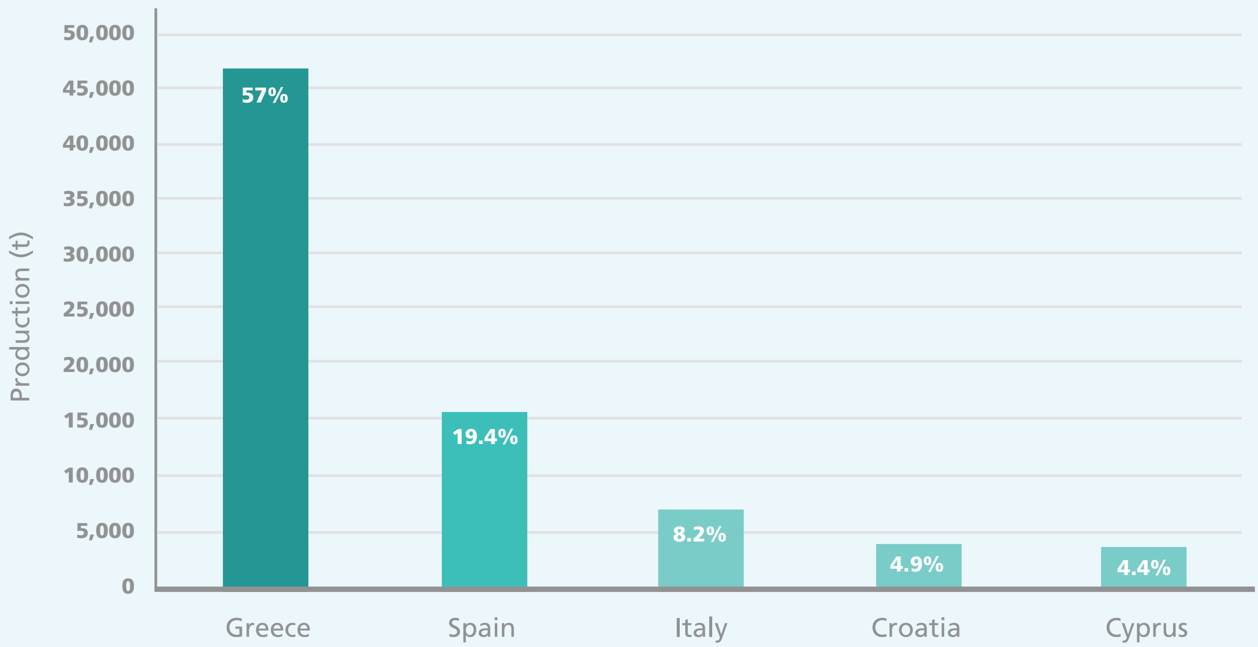

FIGURE 4

Production (tonnes) of the main five EU countries producing gilthead sea bream during 2015 and their percentage contribution to total EU production.

During 2015, over 82 thousand tonnes of gilthead sea bream were produced in the EU, which equates to between 206 and 275 million fish (calculated according to methods of Mood & Brooke, 2015). The majority were produced by Greece, with Spain being the second major producer, responsible for around one fifth of the EU total (FAOSTAT, 2015; figure 4). Currently, gilthead sea bream are killed under commercial conditions predominantly by asphyxia in ice slurry. Asphyxia in air is also sometimes practised (EFSA, 2009f), and electrical stunning before live chilling is being used in a few cases. The EFSA (2009f) assessed asphyxia in air or ice/ice slurry and determined that these methods "included a prolonged period of consciousness (several minutes) during which indications of poor welfare were apparent (physiological and behavioural responses)." Likewise, the OIE advise that these methods should not be used if it is feasible to use alternatives such as percussive or electrical stunning (OIE, 2010). In 2009, the Animal Health and Welfare panel (EFSA) recommended the "urgent development of commercial stunning methods to induce immediate (or rapid) unconsciousness in… seabream" (EFSA, 2009f, p. 2). The most promising method appears to be electrical stunning followed by immersion ice slurry, however more research is needed to confirm stun parameters are capable of inducing unconsciousness for a sufficient period to prevent recovery.

Live chilling in ice slurry

Slaughter by chilling in ice slurry does not meet requirements for humane slaughter, as it is slow to cause unconsciousness and is aversive to sea bream. Studies report times of five minutes (van de Vis et al., 2003) to as long as 40 minutes (Huidobro, Mendes, & Nunes, 2001) to unconsciousness. Vardanis, Divanach, & Pavlidis (2017, p. 3) describe the "great aversion and attempt[s] to escape, by jumping and swimming violently" of sea bream immersed in ice slurry. Measures of stress hormones also support that this process is aversive. In a study by Vardanis et al. (2017) plasma glucose levels were significantly higher in sea bream killed by ice slurry compared with those killed by spiking.

Exposure to air

Some sea bream are killed by removal from water, however this is a very stressful killing method, with a very prolonged time until unconsciousness and death, and significant physical activity (EFSA, 2009f).

Typically, fish make violent attempts to escape and "maximal stress responses are initiated" (Robb & Kestin, 2002 in EFSA, 2009f). The time to loss of consciousness and death is temperature dependent, with higher ambient temperatures leading to faster death (EFSA, 2009f). In a study by van de Vis and colleagues (2003) sea bream left to asphyxiate in air (at 23 °C) did not lose self-initiated behaviours until 4 minutes and lost VERs at 5.5 min, on average. Sea bream asphyxiated in air struggle longer (around 25% longer) than those killed in ice water slurry (Bagni et al. 2002 cited in EFSA, 2009f). But note, this could be due to immobilisation in ice slurry, not loss of consciousness.

Application of an electrical current

It is possible to cause immediate and lasting unconsciousness in sea bream with an electrical current, though further research is needed to identify ideal parameters that are reliable for all fish. van de Vis et al. (2003) experimented with head-only stunning of sea bream, using an alternating current of 50Hz and 80V for 10 seconds. This led to nine of the 10 fish being stunned immediately. Three fish recovered VERs within 16 seconds of the stun (indicating recovery of consciousness) and six appeared to remain unconscious for the next 10 minutes, at which the authors concluded that fish had died. The individual variation in how fish responded post-stun is likely related to the variation in currents achieved for each fish. The study concluded that "seabream appear to require more than 200mA across the head to be stunned. The exact minimum current to achieve stunning in all fishes remains to be determined" (van de Vis et al., 2003, p. 214).

Head-to-body dry electrical stunning (figure 5) is now being used commercially on a small number (currently) of sea bream farms in Europe.

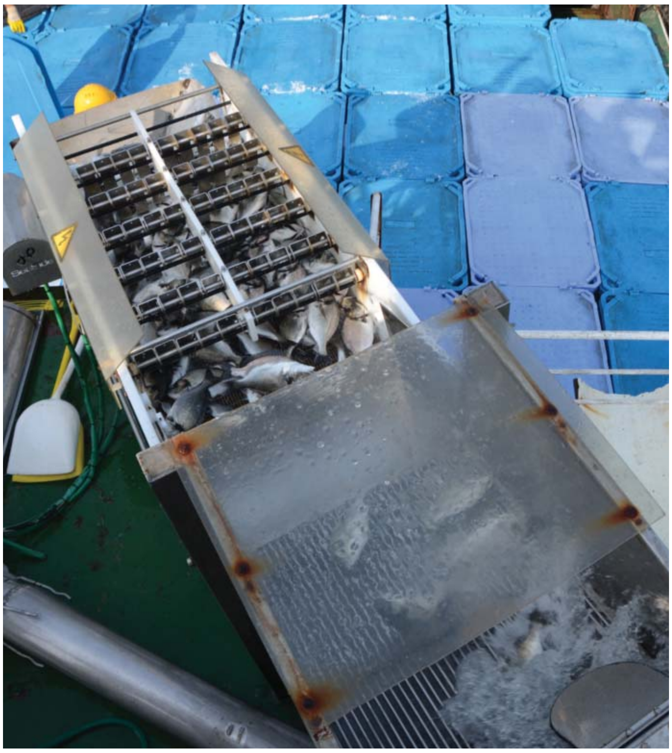

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FIGURE 5

A dry electrical stunning system in commercial use for gilthead sea bream. Fish are pumped from the sea cage to the stunner, and after dewatering, land on the conveyor belt where they receive the electric current.

4.3. EUROPEAN SEA BASS
(Dicentrarchus labrax)

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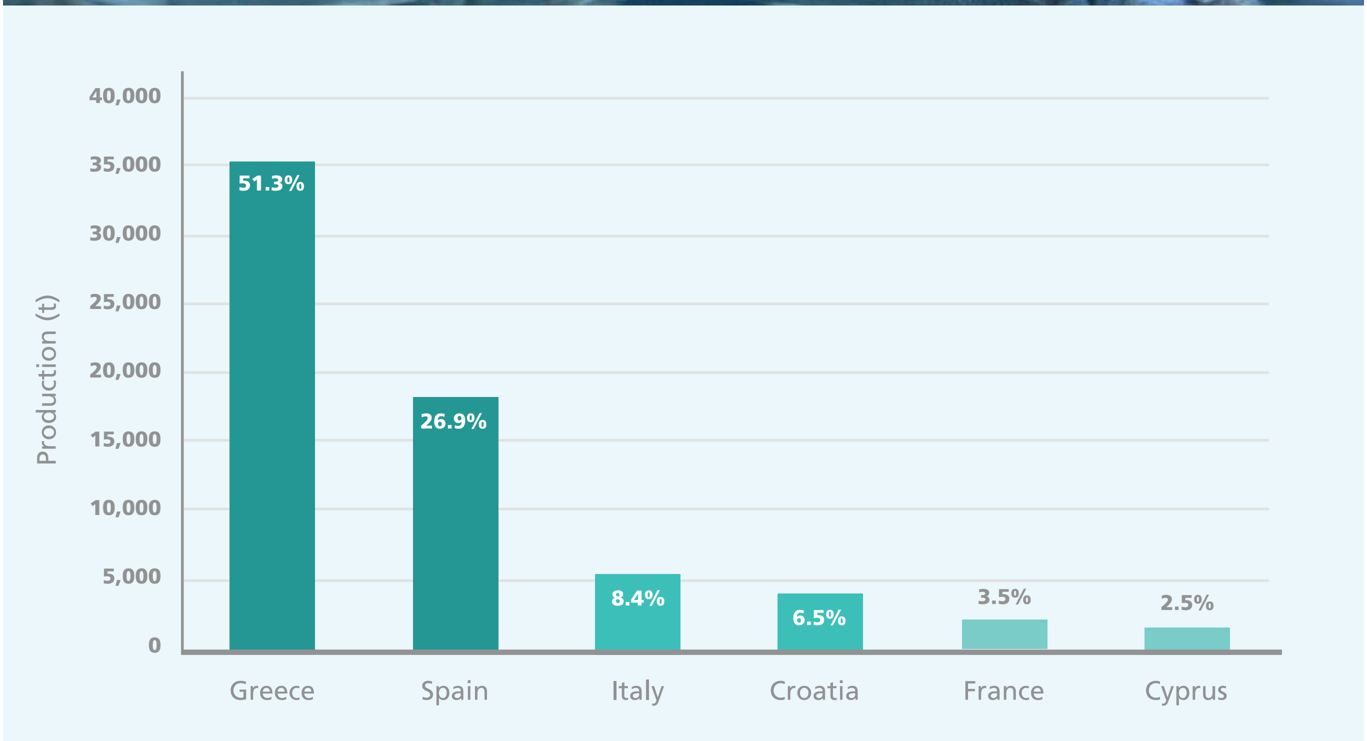

FIGURE 6
Production (tonnes) of the main six EU countries producing European sea bass during 2015 and their percentage contribution to total EU production.

During 2015, the EU produced over 69 thousand tonnes of European sea bass, consisting of approx. 138-172 million individual fish (calculated according to methods of Mood & Brooke, 2015). Over half were produced by Greece, with Spain being the second major producer, responsible for over a quarter of the EU total (FAOSTAT, 2015; figure 6). Currently, sea bass are predominantly killed under commercial conditions by live chilling in ice slurry. Asphyxia in air is also sometimes practised (EFSA, 2009f), and electrical stunning before live chilling is being used in some cases. The EFSA assessed asphyxia in air or ice/ice slurry and determined that the methods "included a prolonged period of consciousness (several minutes) during which indications of poor welfare were apparent (physiological and behavioural responses)" (EFSA, 2009f, p. 2). Likewise, the OIE advise that these methods should not be used if it is feasible to use alternatives such as percussive or electrical stunning (OIE, 2010). In 2009, the Animal Health and Welfare panel (EFSA) recommended the "urgent development of commercial stunning methods to induce immediate (or rapid) unconsciousness in seabass" (EFSA, 2009f, p. 2). The most promising method appears to be electrical stunning followed by immersion ice slurry, however more research is needed to confirm stun parameters are capable of inducing unconsciousness for a sufficient period preventing recovery.

Live chilling in ice slurry

Slaughter by chilling in ice slurry does not meet requirements for humane slaughter, as it is slow to cause unconsciousness and is aversive to sea bass. Research studies report a range of times that sea bass remain conscious after being immersed in ice slurry, however all demonstrate that fish suffer for several minutes. For example consciousness was not lost until 10 minutes (Simitzis et al., 2013), 11 minutes (Zampacavallo et al., 2015), 20 minutes (Zampacavallo et al., 2003), 23 minutes (Poli et al. (2004) and Zampacavallo et al. (2008), cited in EFSA, 2009f), and around 40 minutes in chilled water (Bagni et al., 2007).

Sea bass react with vigorous activity and aversion behaviour in ice slurry. Measures of stress hormones also support that this process is aversive. For example, Marino et al. (2009) (cited in EFSA, 2009f) found that blood cortisol levels were higher in sea bass killed by ice slurry than those percussively stunned, despite that lowered body temperature may restrict the production of cortisol (EFSA, 2009f).

The effect of cold temperatures on the physiology and behaviour of sea bass can mislead operators when judging unconsciousness, as fish are unable to outwardly display suffering. For example in a study by Zampacavallo et al. (2015), sea bass became motionless after three minutes in ice slurry, which may be interpreted in the field as unconsciousness. However, the fish were still found to respond to external stimuli until at least 11 minutes, indicating consciousness during this period (Zampacavallo et al., 2015).

Exposure to air

Some sea bass are killed by removal from water, however this is a very stressful killing method, with a very prolonged time until unconsciousness and death, and significant physical activity (Bagni et al., 2007; EFSA, 2009f). Typically, fish make violent attempts to escape and "maximal stress responses are initiated" (Robb & Kestin, 2002 in EFSA, 2009f). The time to loss of consciousness and death is temperature dependent, with higher ambient temperatures leading to faster death (EFSA, 2009f). Sea bass asphyxiated in air struggle longer (around 65% longer) than those killed in ice water slurry (Bagni et al. 2002, in EFSA, 2009f). But note, this could be due to immobilisation in ice slurry, not loss of consciousness. Processing of fish should not begin until after they are dead. Death by asphyxia in air was reported to take 70±27.6 minutes by Poli et al. (2004) and up to 128 minutes in a study by Acerete, Reig, Alvarez, Flos, & Tort (2009).

Application of an electrical current

Immediate loss of consciousness from electrical stunning has been demonstrated in laboratory tests with sea bass, both in seawater (whole body application) and in air (head-only stunning) (Lambooij et al., 2008). An additional killing method is also required as the parameters used to stun are not usually enough to kill. The most suitable electrical stun parameters must be found that can maximise the length of the stun, without causing significant quality issues. Some parameters may cause muscle blood spots and vertebral fractures and cause the mouth and opercula to open wide (Poli, Parisi, Scappini, & Zampacavallo, 2005). However, it is possible to electrically-stun sea bass (lasting until death) then kill by chilling in ice water slurry, which also serves to preserve flesh quality. EEG recordings by Lambooij et al. (2008) suggest that the individual, head-to-tail application of an electrical current of 3.3 Arms/dm2 (sinusoidal 50Hz or pulsed square wave AC, 133Hz, 43% duty cycle) for 1 second, to sea bass is effective in inducing a general epileptiform insult (unconscious and insensible). Combining electrical stunning for 10 seconds with chilling in ice slurry resulted in death of the stunned fish.

4.4. COMMON CARP
(Cyprinus carpio)

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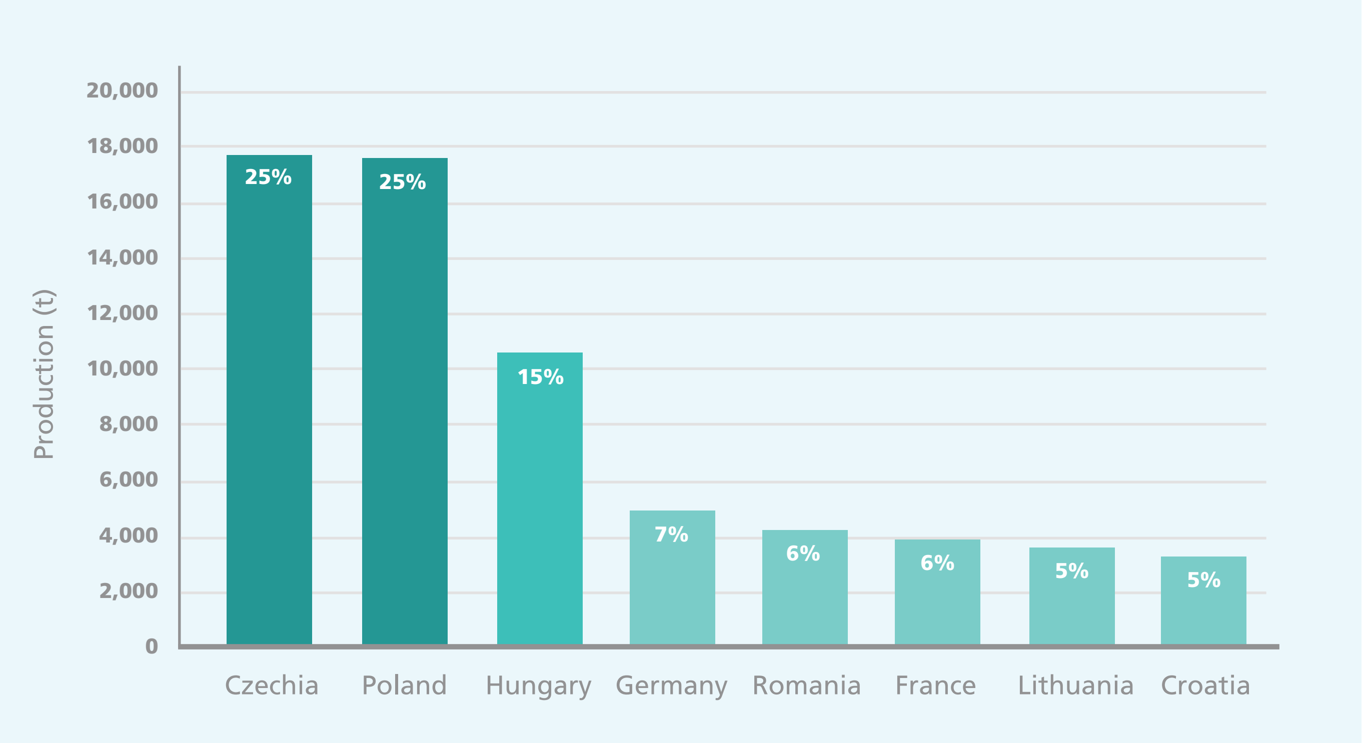

FIGURE 7
Production (tonnes) of the main eight EU countries producing common carp during 2015 and their percentage contribution to total EU production.

Over 71 thousand tonnes of common carp were produced by 17 countries in the EU in 2015, with Poland, the Czech Republic and Hungary being the main producers (FAOSTAT, 2015; figure 7). This equates to somewhere between 28 and 142 million carp (calculated according to methods of Mood & Brooke, 2015).

The majority of carp in the EU are sold to retailers or direct to the consumer while still alive, with an estimated 15% or less slaughtered in commercial processing plants, though this varies by country (EFSA, 2009a). In a 2017 report written for the European Commission, it is stated that 25% of carp in Poland are slaughtered before sale and 75% sold live, while 15% are slaughtered before sale and 85% sold live in the Czech Republic.

Carp slaughtered by members of the public are unlikely to be killed humanely. There is however, no fully verified, effective method in slaughterhouses – though some methods may cause considerably more severe/longer suffering than others. There are several variations of slaughter methods for carp.

In Poland, percussive stunning is often used for carp in slaughterhouses (and may be followed by gill cutting or decapitation) but is preceded by up to 10 minutes of being left out of water, which will cause suffering (IBF et al., 2017). In-water electrical stunning is also used in some slaughterhouses but the effectiveness of these systems has not been verified (IBF et al., 2017).

In the Czech Republic in-water electrical stunning is also used. The Czech Republic Act on the protection of animals against cruelty specifies that "fish in industrial processing may be stunned by a device using 230 V alternating electrical current, carbon dioxide gas or other gas or gas mixture approved according to a special legal regulation" (The Czech National Council, 1992, last amended in 2017). However, no kill method is used subsequent to the electrical stun, according to the EU survey (IBF et al., 2017), so there is a significant risk that consciousness is not lost, or returns, in these fish and they are therefore processed while alive and conscious.

In Germany, electrical stunning (dry stunning, in-water stunning or a combination of both) is the main method, but carp are then killed by gill cut or a percussive blow to the head (IBF et al., 2017; Retter et al., 2018). A percussive blow after electrical stunning may also be responsible for the loss of consciousness (not just the killing) in carp as the electrical stun may be insufficient (Feneis Bernhard, pers.comm. July 2017). Percussion is also used on its own in Germany (IBF et al., 2017; Retter et al., 2018). In a sample of German slaughterhouses, Retter et al. (2018) reported that a killing method (gill cut or destruction of the heart) or evisceration was performed between 30 seconds and 3 minutes after stunning.

According to Daskalova, Pavlov, Kyuchukova, & Daskalov (2016), carp are commonly left to die by asphyxiation in air in Bulgaria.

Currently, an electrical stun, followed by a percussive blow and then a killing method (e.g. decapitation) may be the most humane method for slaughter of carp (Retter et al., 2018); an electrical stun followed directly by decapitation may also be a humane method (IBF et al., 2017). As brain activity may continue after decapitation (Lines & Spence, 2014), it may also be necessary to destroy the brain (e.g. by spiking or maceration of the head)immediately after decapitation to ensure consciousness does not return.

Though it may not be instant, and does have welfare hazards, manual percussive stunning is considered less stressful than CO_2 exposure or live chilling, with blood cortisol measures suggesting that live chilling is the most stressful of these three methods (Varga et al., 2013). Similarly, Daskalova, Pavlov, Kyuchukova, & Daskalov (2016) concluded that percussive stunning was the least stressful for carp tested in their study, as indicated by significantly lower blood glucose levels, compared with electrical stunning and asphyxia in air (Daskalova, Pavlov, Kyuchukova, & Daskalov, 2016).

Live sale to the customer

Carp that are taken home by members of the public may be kept alive for days in make-shift tanks (e.g. bath tubs) before being killed (Lambooij, Pilarczyk, Bialowas, van den Boogaart, & van de Vis, 2007). Live sale also involves additional transport and time out of water, temperature shock, excessive handling and ineffective stunning (EFSA, 2009a). For carp that are slaughtered at home, there is little data on the methods used but they are likely to include death by asphyxia or percussive stun/killing with varying effectiveness (EFSA, 2009a). There is a risk that fish may be processed while still conscious. Carp are also often killed in store by the retailer at the point of sale, usually by a manual percussive blow, followed by immediate gill arch cutting. Alternatively, they cut the spinal cord and blood vessels by decapitation. In either case, carp may be subject to prolonged suffering before slaughter due to stressful handling practices and storing facilities.

Percussive blow

The shape of the skull means carp are particularly difficult to percussively stun effectively, as the brain is well protected (Lines & Spence (2014). Lambooij et al. (2007) tested percussive stunning of common carp. They advise that a manual percussive blow, with a priest (figure 8), is inaccurate and insufficient


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FIGURE 8
A manual percussive blow being delivered to the head of a carp with a wooden priest.

in many cases, and concluded that "since not all carp were unconscious after percussion stunning, it is judged that this method can be used, but there is no certainty for instantaneous loss of consciousness and sensibility". In practice, several blows are often necessary (p. 178).

The efficiency of manual percussive stunning of carp in a sample of German slaughterhouses was evaluated by Retter et al. (2018). They reported that 30.8% (4/13) of carp stunned by percussion showed behavioural signs of consciousness and 23.1% (3/13) showed head injuries indicating mis-hits. These carp were subsequently processed while apparently conscious. Although the majority, 69.2% (9/13), did not show behavioural signs of consciousness after percussive blows, this cannot be taken as confirmation of unconsciousness in carp (Retter et al., 2018).

Application of an electrical current

Some research has been conducted into electrical stunning parameters for carp, showing that it can cause immediate unconsciousness, but only for a limited time (Daskalova, Pavlov, Kyuchukova, & Daskalov, 2016; Lambooij et al., 2007). Lambooij et al. (2007) found that carp can be effectively stunned by a head-only electrical stun using 0.24 ± 0.03 A (~160 V, 50 Hz, a.c.), but the total duration of the stun was 31 ± 14 seconds. An effective stun was also obtained in-water, with a current of 0.14 ± 0.03 A/dm2 (~115 V, 50 Hz, a.c.; electrode distance 16 cm) for 1.2 seconds at a water conductivity of 200 mS. After this stun, some fish responded to pain stimuli as early as 30 seconds later, showed fin movements at around 48 seconds, and returned to normal swimming behaviour at around 2 minutes. This is very unlikely to be long enough to allow bleeding and death before conscious recovery.

However, in the same study Lambooij et al. (2007) found that combining electrical stunning with subsequent chilling in iced water was an effective procedure for slaughter in practice. The application of an electrical current of 0.73 A/dm2 (~411V, 50Hz, a.c.; electrode distance 16 cm) for 5 seconds to individual carp in fresh water, at a conductivity of 330 mS, caused carp to lose consciousness and in the

15 minutes following there were no responses to pain stimuli and heart beats were irregular.

Daskalova et al. (2016) tested a current of 4.7 mA (DC) on commercial-sized carp (average body weight 1213 ± 118 g), applying high voltage (~300 V) for 3 seconds. Some carp required repeat applications of the stun as they still showed signs of consciousness (presence of positive reactions to tactile and visual stimuli), and blood cortisol and glucose concentrations indicated significant levels of stress.

Retter et al. (2018) conducted a laboratory test to see if the behavioural measures typically used to assess consciousness were reliable for carp. The researchers electrically stunned carp then measured opercular movement, righting behaviour and eye roll, while also measuring corresponding brain activity (presence of visually evoked responses (VER) on the EEG). Researchers found that the reoccurrence of behavioural indicators of consciousness was influenced by the duration of exposure to the electrical current (longer exposure was associated with later recovery of behaviours) and in some cases the indicators did not return for several minutes. For example, carp stunned for 5 minutes with 0.09 A/dm2 regained opercular movements after 2.3–9.0 minutes, and those stunned for 5 minutes with 0.14 A/dm2, showed opercular movements after 5-10 minutes. However the VER were recovered by 30 seconds in 31 out of 32 carp. It is important to note that this was the earliest time that recordings could be made post-stun, so the brain activity during stunning and in the 30 seconds post-stun was unknown. The study was therefore not able to demonstrate that carp lost consciousness from the stun, but if it was lost, it was for a relatively short period of time, as the VER indicated consciousness by at least 30 seconds. The authors suggest that the temporary absence of behaviours indicators of consciousness might have been due to exhaustion from the prolonged duration of stunning, rather than an actual loss of consciousness during that time, highlighting the difficulty of recognising unconsciousness in practice. On the other hand "if these behavioural traits can be observed in carp after a stunning operation, these carp have certainly not been stunned" (Retter et al., 2018, p. 9).

The same study also assessed the effectiveness of electrical stunning of carp in a sample of German slaughterhouses. 28% (9/32) of carp displayed behavioural indicators of consciousness after stunning, and 12.5% (4/32) received external injuries during stunning, e.g. from contact with the electrodes during dry electrical stunning.

The lowest percentage of carp showing behavioural indicators of consciousness after stunning was observed in farms that used a combination of electrical stunning followed by a percussive blow; though 7% of carp showed signs that the process was ineffective. However, given that the laboratory test showed that the absence of behavioural measures was not a reliable indicator of loss of consciousness, the percentage of carp that were ineffectively stunned could be higher.

Exposure to air

Carp left out of water to asphyxiate can, when under certain conditions of low temperature and high humidity, survive for several hours (EFSA, 2009a). However these conditions are highly aversive and stressful. For example, in a study by Rahmanifarah, Shabanpour, & Sattari (2011), carp that were removed from water were described to have "agonized and scrambled vigorously", and opercular movements were observed in carp for up to 4 hours and 53 minutes later, which suggests that the fish were likely to be conscious during this time.

Carbon dioxide exposure in water

This is an inhumane method. Rahmanifarah et al. (2011) investigated the responses of carp to carbon dioxide after transferring them to a tank saturated with the gas. Carp reacted with aversive behaviour and "strenuous avoiding reactions" for around 3 minutes (p. 141). They were observed attempting to keep their mouths and operculum closed, and collided with the aquarium wall. After 3 minutes they lost equilibrium but continued to show violent aversive reactions and movements did not stop until around 8 minutes into the experiment. Opercular movements continued until around 15 minutes, suggesting fish were still conscious at this time. Researchers also reported scale diffusion, increased mucus secretion, blackened gills and pale appearance of fish as a result of this method.

In another experiment, elevated blood cortisol measures indicated increased stress in carp exposed to carbon dioxide, compared with those that were percussively stunned (Varga et al., 2013).

Live chilling in ice slurry

Carp that were immersed in ice slurry showed normal swimming behaviour for around 11 minutes, after which they began swimming rapidly and erratically, indicating conditions were aversive (Rahmanifarah et al., 2011). Subsequently, "strong tremors" were observed and at around 16 minutes the carp lost their equilibrium. However opercular movements, indicating consciousness, did not cease until around 48 minutes. Researchers also observed gasping at the surface of water. Increased cortisol levels in the blood of carp that were exposed to ice slurry compared those percussively stunned (Varga et al., 2013) support that carp are stressed by live chilling.

4.5. ATLANTIC SALMON
(Salmo salar)

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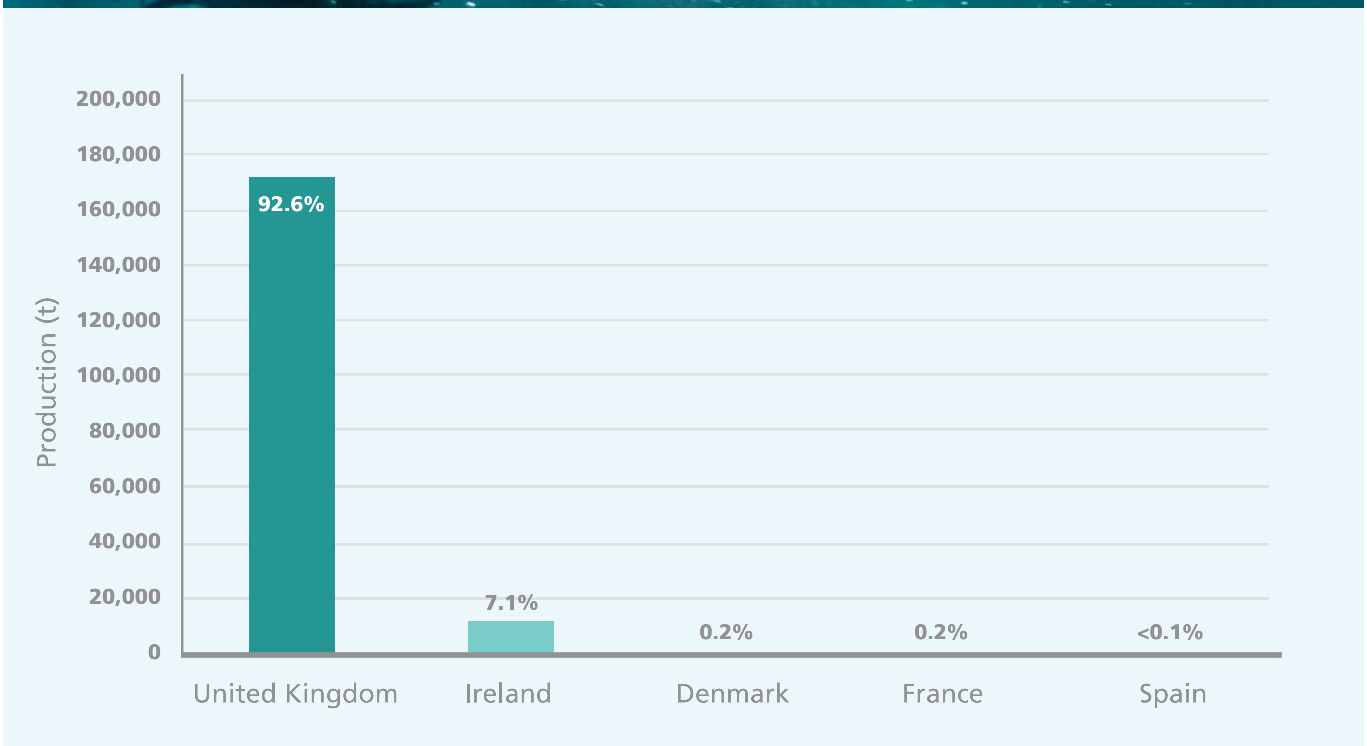

FIGURE 9
Atlantic salmon production in EU member states during 2015 and their percentage contribution to total EU production.

In 2015, 185,995 tonnes of Atlantic salmon were produced in the EU, equating to 22-51 million individuals (calculated according to methods of Mood & Brooke, 2015). The vast majority of Atlantic salmon production in the EU takes place in the UK (figure 9). In 2015, over 172 thousand tonnes of Atlantic salmon were produced there, which equates to approx. 20 – 47 million fish (Mood & Brooke, 2015).

Automated percussive stunning followed by gill cutting is the predominant method of slaughter used for Atlantic salmon in the UK – being used for 95% of salmon (IBF et al., 2017). This method can enable humane slaughter. In Ireland, percussive stunning and gill cut is also used for the majority of salmon (92-93%); carbon dioxide exposure (followed by gill cut) is currently used for the remaining 7-8% (IBF et al., 2017). Using carbon dioxide, with or without live chilling, is not a humane method for salmon and causes considerable suffering (Roth, Slinde, & Robb, 2006; Erikson, 2011).

Electrical stunning before slaughter is sometimes used for salmon (e.g. for approximately 50% of salmon in Norway (IBF et al., 2017)). This can be humane when followed by percussion or decapitation, but it may not be humane when followed by gill cutting (IBF et al., 2017). This is because electrical stunning can cause unconsciousness but the length of the stun may not be long enough to prevent suffering before death by gill cut. This method has recently become permitted under RSPCA Assured standards, however these stipulate that "the system must ensure sufficient current is passed through the body of the fish for a sufficient duration to render the fish immediately insensible until death supervenes" (RSPCA, 2018, p. 52).

Electrically stunning fish before delivering a percussive blow may be the most humane method, as electrical stunning may allow for a higher success rate in delivering the percussive blow accurately as fish are immobilised and can be correctly oriented in percussive machines.

Percussive blow

Percussive stunning can cause immediate unconsciousness in salmon and may also be the cause of death if performed with a high enough force to result in cerebral haemorrhage (Lambooij et al., 2010). However it must be applied accurately in order to be effective: hitting the salmon's head in the correct place and with enough force. Tests should be conducted for each model of percussive machine to be sure fish are adequately stunned and for sufficient time to prevent recovery of consciousness.

Generally, unconsciousness from percussive stunning is more likely to occur, and lasts longer, the higher the hammer force (Roth et al., 2007). A study by Lambooij et al., (2010) showed that pressures below 8.1 bars were unsuccessful in causing unconsciousness in Atlantic salmon (live weight 1.5kg). However, percussive stunning with 8.1 to 10 bars caused unconsciousness in the majority of fish (Lambooij et al., 2010). A significant blow causing unconsciousness can be a stun to kill method. In a study by Roth et al. (2007), salmon (of 4-6kg) that were fully unconscious a minute after a percussive blow did not recover consciousness within 10 minutes, and death was the typical outcome.

After salmon are percussively stunned they are typically bled out by cutting of the gill arches. Most percussive machines incorporate an automated gill cut within 10s of the stun being administered. Studies have reported that the time to brain death from a gill cut ranges from 2m 47s to 7m 33s (Robb & Roth, 2003; Hans van de Vis et al., 2003; Robb, Wotton, McKinstry, Sørensen, & Kestin, 2000). Therefore with sufficient force, percussive stunning is able to cause unconsciousness until death, or may itself be the cause of death, and can be considered a humane method.

Injuries from percussive stunning include broken jaws and prolapse, bursting or rupture of the eyes (Roth et al., 2007; Lambooij et al, 2010). Fish will experience severe pain and suffering from these injuries if they are not adequately stunned, or if they recover from the stun before death. In the system investigated by Roth et al. (2007), the more severe eye injuries were associated with percussive blows that caused an efficient stun without recovery. However, slaughterhouses should be aware of this welfare hazard and suitable checks are required to ensure poor welfare is avoided.

Application of an electrical current

When correctly applied, electrical stunning can cause immediate (within one second) unconsciousness in Atlantic salmon (Robb & Roth, 2003; Roth, Imsland, Moeller, & Slinde, 2003). However, the stun is reversible, and there is risk of salmon recovering consciousness before death. Salmon are typically killed after electrical stunning by cutting of the gills (IBF et al., 2017), which may take longer than the period of unconsciousness caused by the stun.

Lambooij et al. (2010) tested electrical stunning of Atlantic salmon followed by gill cutting, and observed on the EEGs that one out of three fish recovered around 3 minutes after the stun. They noted that the vestibulo-ocular reflex in the recovered fish was however absent. Therefore the effectiveness of this method is dependent not only on the stun parameters, but the choice, and speed, of the subsequent kill method.

In a study by Roth et al. (2003) respiration recovered and weak signs of reactivity occurred around 2–4 minutes after electrical stunning. This is concerning, given that brain death from gill cut may take over 7 minutes (Robb & Roth, 2003; Hans van de Vis et al., 2003; Robb, Wotton, McKinstry, Sørensen, & Kestin, 2000).

Roth, Moeller, & Slinde (2004) found that the percentage of salmon stunned by electricity was dependent on the amplitude, frequency, and the duration of the electrical current. However, when electrical parameters are chosen, product quality is also considered; more effective stunning (inciting unconsciousness for longer periods) can cause quality issues (Lambooij et al., 2010). In the study by Roth, Moeller, & Slinde (2004), injuries were most affected by the electrical frequency. The authors recommend "[f]or stunning alone, sinusoidal AC frequencies of 50–80 Hz at 25–50 V/m for 10 s or at 50 V/m for 3–10 s are recommended. However, to minimize the proportion of Atlantic salmon injured by electrical stunning, sinusoidal AC frequencies of 500–1,000 Hz at 50 V/m for 10 s should be used" (p. 215).

Combining electrical and percussive methods, whereby fish are first stunned with electricity (short duration) then percussively stunned, may allow for more accurate and effective percussive stunning as the fish will be motionless. This could result in high welfare standards without compromising carcass quality (Mejdell et al., 2009, cited in EFSA, 2009a). A percussive blow following electrical stunning could instead act as an effective killing method (Lambooij et al., 2010). Decapitation also appears to be an adequate method to prevent recovery of consciousness, as shown by EEG recordings (Roth, Gerritzen, Bracke, Reimert and van de Vis, in preparation, in IBF et al., 2017).

Carbon dioxide exposure in water

Carbon dioxide (CO_2) exposure in water is an inhumane method used to immobilise fish and is used for salmon in some countries. However its market share (at present believed to be 7-8% in Ireland) is decreasing (IBF et al., 2017). In the salmon industry, the levels of carbon dioxide used are typically between 250 and 460 mg/l (Erikson, 2008).

Exposing salmon to carbon dioxide saturated water does not lead to an immediate stun and fish show aversive behaviour for 2-4 minutes (Erikson, 2011) though consciousness is likely to be maintained for even longer. For example, in a study by Robb et al. (2000), Atlantic salmon showed highly vigorous aversion to carbon dioxide for up to 2 minutes (at 6°C), but brain activity indicated consciousness until just over 6 minutes. Fish are usually killed by gill cutting after carbon dioxide. In the study by Robb et al. (2000), after removal from the water, the salmon showed very little movement, however on cutting the gill arches, two of the fish responded with some movement. Subsequently, they made weak head shakes or tail flaps for up to four minutes, with these ending nine minutes after the start of the procedure. Erikson (2011) investigated the potential for lower concentrations of carbon dioxide to stun or anaesthetise salmon without causing stress. However they found that any concentration of CO_2 that is able to cause any immobilisation effects also results in clear indications of stress and compromised welfare.

Another method combines moderate carbon dioxide levels (65-257 mg/l) and oxygen levels of 70-100% saturation with live chilling (0.5 - 3°C) (Erikson et al., 2006 in IBF et al., 2017). Again, fish are not stunned immediately and the process is stressful (FAWC, 2014; IBF et al., 2017). In a study by Erikson (2008), salmon that were live chilled with carbon dioxide were described as swimming sluggishly, they kept their mouth open above the water surface and exhibited a gulping behaviour, and swam with their bodies tilted at an angle between 45–70° relative to the water surface. Roth, Slinde, & Robb (2006) exposed salmon to carbon dioxide in combination with live chilling, and after 40 minutes the authors noted that "The fish were calm after live chilling, but not unconscious, as eye rolling was observed in all individuals" (p.799).

Spiking

Robb et al., (2000) found that spiking using a pneumatic stun gun can cause immediate unconsciousness in Atlantic salmon, however the method as performed in the experiment was not consistent. Inaccurate shots resulted in brain activity (visually-evoked responses (VERs) were maintained) indicating that fish were still conscious for up to 5.6 minutes. Therefore only a system that spikes fish with a high level of accuracy could be a humane slaughter method but that is likely to be difficult to achieve in practice.

Gill cut

Gill cutting without sufficient stunning is painful and inhumane and should not be performed. Salmon take up to 7.5 minutes to lose consciousness after exsanguination without prior stunning (Robb et al., 2000).

4.6. EUROPEAN EEL
(Anguilla anguilla)

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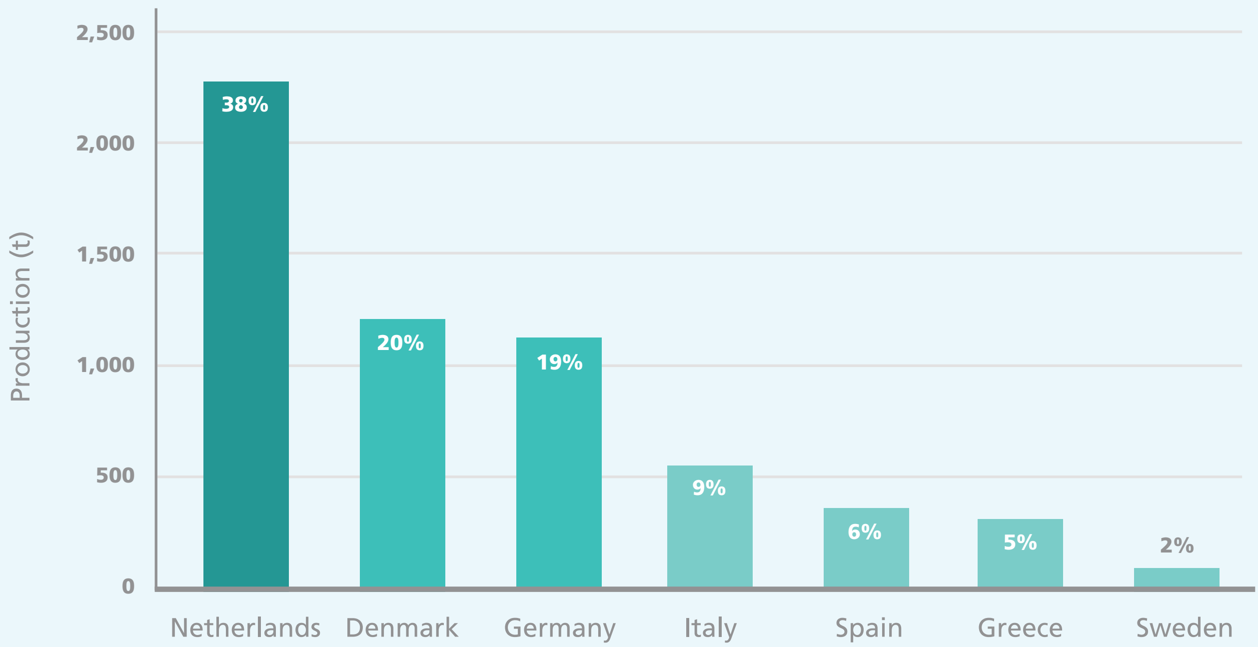

FIGURE 10
Production (tonnes) of the main seven EU countries producing European eel during 2015 and their percentage contribution to total EU production.

Almost 40% of EU European eel production takes place in the Netherlands, with Denmark and Germany being the next significant producers (FAOSTAT, 2015; figure 10). Over 6 thousand tonnes were produced in the EU in 2015, which equates to between 6 and 18 million fish (calculated according to methods of Mood & Brooke, 2015).

Eels are very robust, being able to survive out of water for several days (EFSA, 2004), and are relatively difficult to kill (Lines & Spence, 2014). As part of the slaughter process, the mucous produced by the skin is removed and the processes used also cause significant damage to the skin and eyes which causes suffering for eels. The slaughter and processing methods used for European eels include: salt bath and evisceration; ammonia, washing and evisceration, and; immobilisation by exposure to ice (and salt), washing and evisceration (EFSA, 2009e). These methods have been identified as inhumane (EFSA, 2009e) and are banned by law in Germany (Bundesqesetzblatt, 1993, cited in Lines & Spence, 2014). New Zealand's Code of Welfare on Commercial Slaughter (2010) also forbids any method that deslimes eels while they are still conscious because of the poor welfare caused.

Electrical stunning followed by a killing method appears to be able to deliver a humane death when applied correctly. However further research and better application is needed as, currently, “commercial electrical stunning systems do not guarantee an immediate loss of consciousness for a sufficiently long period for all eels” (EFSA, 2009e, p. 2). The use of a captive needle gun to mechanically stun eels also has potential for humane slaughter, although further development of the method, including better restraint devices, would be needed for use in practice (Lambooij, van de Vis, Kloosterboer, & Pieterse, 2002).

Salt or ammonia bath and evisceration
See section 3.9.

Application of an electrical current
This method is now used in the Netherlands and Germany (Lines & Spence, 2014). Parameters for the electrical stunning of eels have been laid down in German and Austrian legislation. They stipulate a current density, depending on the electrical conductivity of the water: <250 μS/cm, 0.10 A/dm2; 250-500 μS/cm, 0.13 A/dm2; 500-750 μS/cm, 0.16 A/dm2; 750-1000 μS/cm, 0.19 A/dm2 (EFSA, 2009e). A study by Kuhlmann, Münkner, van de Vis, Oehlenschläger, & Koch, 2000 (cited in EFSA, 2009e) showed, using electroencephalograph (EEG) and electrocardiogram (ECG) measurements, that these do not effectively stun all eels. Electrical currents that do not cause effective stuns can instead cause painful shocks and discomfort, and can lead to temporary body imbalance, muscular exhaustion and immobilisation (Lambooij, van de Vis, et al., 2002).

However, effective electrical stunning of eels does appear feasible. Lambooij, van de Vis, Kloosterboer, & Pieterse (2002) experimented with different electrical parameters to increase the length of the period of unconsciousness. They found that in-water electrical stunning of eels can be humane, causing unconsciousness instantaneously and until death. In this study they stunned eels in freshwater using 200V (50 Hz AC current) for 1 second, then immediately followed this with 50 V for 5 minutes and at the same time flushed the water with nitrogen in order to suffocate the eels. The cause of death is in fact presumed to be due to the lack of heart and muscle activity and prevention of oxygen exchange across the skin. This process was effective, as there was no brain activity (EEG) or responses to pain stimuli after stunning. One eel, one hour after stunning, showed partial recovery (some ability to right itself but no response to pain stimuli) however the majority of the eels showed no signs of recovery. The last pain stimulus test was conducted 4 hours after stunning, and eels showed no response; the authors consider the unconsciousness and insensibility to be irreversible. Researchers at the Institute for Marine Resources and Ecosystem Studies (IMARES) have tested commercial equipment for electrically stunning eels and found it could deliver an effective stun (van de Vis, pers. comms., 2018).

Desliming of eels after stunning is possible in commercial conditions (Kuhlmann & Münkner, 1996 cited in Lambooij, van de Vis, Kloosterboer, & Pieterse (2002). Providing eels are eviscerated in a timely manner following effective stunning and subsequent desliming, this could be a humane method of slaughter for this species.

Live chilling with salt
Ice slurry, either on its own or more commonly in combination with high levels of salt, is used to immobilise eels whilst keeping them alive (EFSA, 2009e). Eels are left in a tank of ice slurry overnight and are slaughtered the following day by evisceration while conscious. This presents a number of significant welfare issues. Eels are subject to mechanical pressure from the weight of the ice and other eels; the conditions are aversive and eels attempt to escape; salt damages the upper layer of skin which is likely to be very painful (EFSA, 2009e); conscious animals are then eviscerated and suffer the pain and distress of this process.

Lambooij, van de Vis, Kloosterboer, & Pieterse (2002) investigated the effects of killing eels with cold temperatures. They used chilled water to reduce the body temperature of 19 eels to 5°C before moving them to brine (high concentration

of salt) at -18°C for 15 minutes. Behaviour observations indicated that the eels were stressed in the ice water. As their body temperature dropped, eels generally exhibited four phases of behaviour: 1) exploration of the box (176±89s), 2) escape attempts, 3) abnormal behaviour consisting of pressing their heads strongly to the wall or corner, while showing clonic muscle cramps from head to tail for 183±109s, 4) ceasing movement and settling at the bottom of the box in an abnormal posture but continuing to breathe. Irregular heart rates also indicate that eels were stressed in the chilled water. In seven of the eels, researchers observed a lack of brain activity after 12+/-6 minutes in the iced water, and responses to pain stimuli ceased after 12±5 minutes for 15 eels. However, three individuals were still able to respond when they were placed in the brine at -18°C around 19 minutes from the start of the experiment. Rapid and extreme depolarisation of the membranes, as shown on the EEG, began after 27±17 seconds in the brine and for six eels decreased to low or zero electrical activity. After 15 minutes in the cold brine, none of the eels recovered.

Decapitation/neck cut and evisceration

Some eels may be decapitated or have their neck cut to make for easier processing (the spinal cord is cut just behind the head) without any form of stunning (EFSA, 2009e). Decapitation leads to death through anoxia, however eels have been shown to survive a neck cut when left to recover (i.e. under lab conditions) (Flight & Verheijen, 1993). Brain function in eels can persist for a surprisingly long time after these procedures. Verheijen & Flight (1997) found that loss of reaction in severed eel heads did not occur until 30 minutes after decapitation, and they showed signs of life for up to 8 hours. Similarly, a study by van de Vis et al. (2003) showed that brain function indicating consciousness was not lost until 13 minutes (on average) after decapitation, based on EEG measurements.

Captive needle gun

Experimental work shows that a captive needle gun can be used to stun eels, by driving a hollow needle into the head to inject pressurised air in the brain and the spinal cord (Lambooij, van de Vis, Kloosterboer, & Pieterse, 2002). In the study by Lambooij and colleagues, after restraining eels using tyribs, the captive needle gun was used on each individual with a shooting pressure of 8 bar and an air injection of 3 bar during 1.5 seconds. EEG recordings were used to assess the state of consciousness of the fish. Out of the 42 eels for which there was a reliable EEG recording, nine showed no brain activity after stunning, while the rest showed theta and delta waves tending to an iso-electric line i.e. no brain activity on the EEG (at an average of 11±8 seconds). There were some slow muscle cramps after stunning for more than one hour in a few eels. In five eels there were severe clonic seizures, which may have been due to incorrect positioning of the pistol, and researchers subsequently stunned these animals twice. No responses to pain stimuli on the EEG or with respect to behaviour were observed.

Stunning eels with a captive needle gun appears to be a promising method for development, however injections must be accurately positioned for it to be effective. In commercial practice, applying this to individual eels may be challenging, but development of a suitable restraining device may make this method feasible.

4.7. ATLANTIC BLUEFIN TUNA

(Thunnus thynnus)

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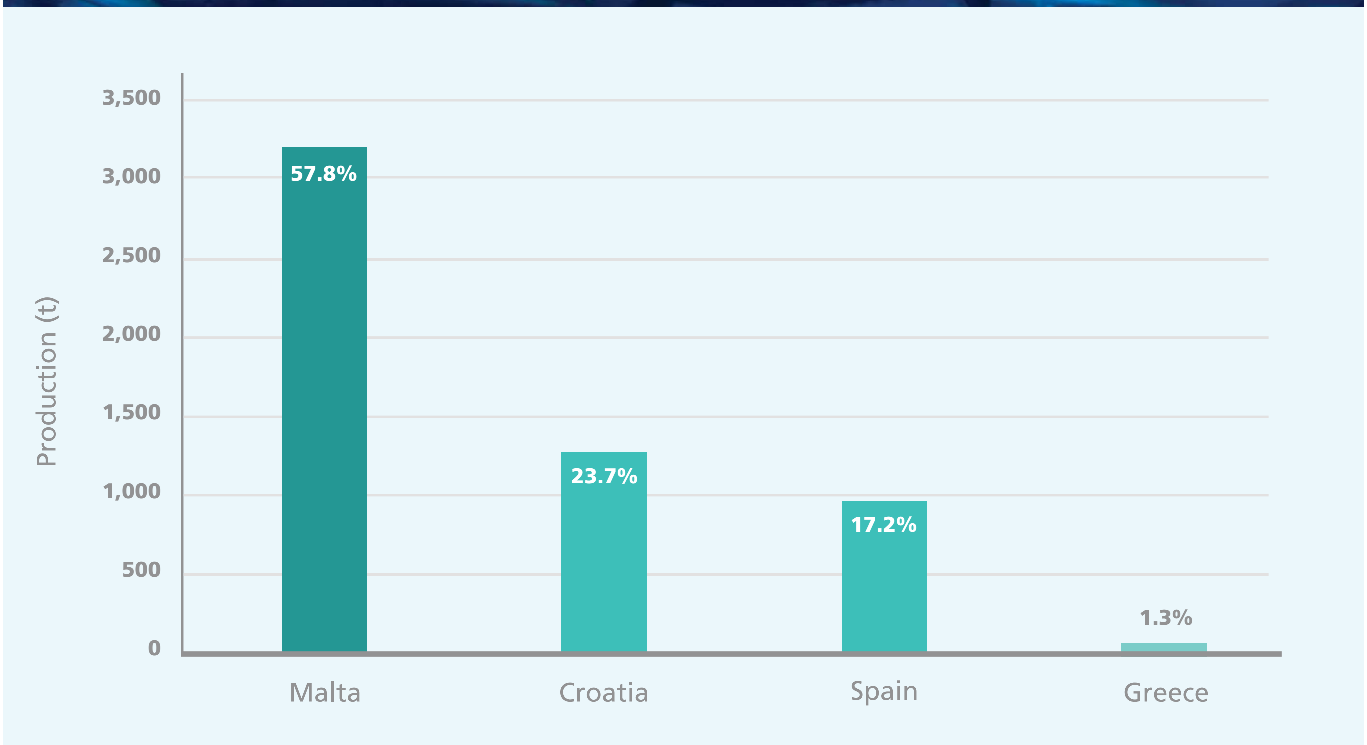

FIGURE 11

Production (tonnes) of Atlantic Bluefin tuna in the EU during 2015 and the percentage contribution of each country to total EU production.

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The EU produced around 5.4 thousand tonnes of Atlantic Bluefin tuna in 2015 (FAOSTAT). Over half of these were farmed in Malta (FAOSTAT; figure 11). Bluefin tuna 'farming' consists of catching wild tuna from the sea and fattening them in cages for periods ranging from 3 months to 2 years (EFSA, 2009c).

Tuna have extremely high lactate dehydrogense activity (Guppy & Hochachka, 1978), which means stressed fish can accumulate extremely high lactate levels during slaughter, which decreases flesh quality (Benetti, Partridge, & Buentello, 2015). Therefore there is strong motivation to minimise stress before slaughter and ensure death is fast, in addition to safeguarding fish welfare, particularly for high value fish. The three methods currently practised in the EU are: underwater shooting (70-80% of large, over 50kg, tuna), shooting from the surface (20-30 % of large tuna), and coring or spiking (100% of small, under 50kg, tuna) (EFSA, 2009c).

Shooting

See section 3.4.

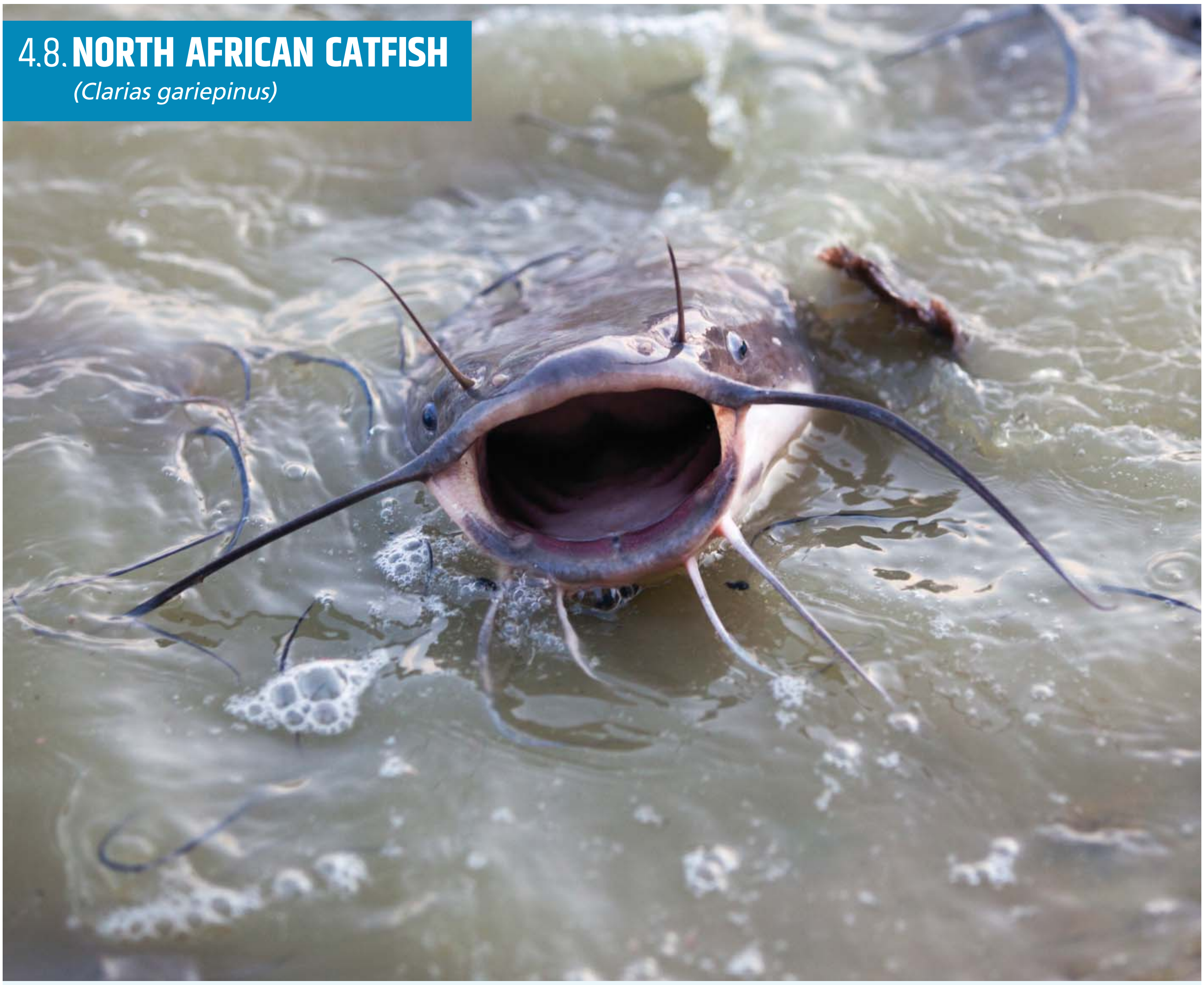
Spiking or coring

Small (<50kg) tuna are typically spiked and/or cored. When performed accurately, spiking can lead to unconsciousness within a second. However there is risk of inaccurate positioning leading to damage to the fish without loss of consciousness which will be painful (EFSA, 2009c).

This method often leads to poor welfare due to the pre-slaughter handling procedures. Tuna are crowded in their pen which is stressful, and in some cases can last several hours. They are chased and corralled, with the risk of contact with the net/other fish and exhaustion. The presence of divers and blood in the water also leads to increased stress (EFSA, 2009c). Tuna may be spiked in the water or removed from the water before spiking. Those that are removed they will suffer "severe pain and distress" and tissue damage from being gaffed and hoisted out of the water; they will also experience asphyxia (EFSA, 2009c). Spiking or coring underwater does not involve the painful gaffing and exposure of fish to air, but stressful pre-slaughter handling is not avoided.

4.8. NORTH AFRICAN CATFISH

(Clarias gariepinus)



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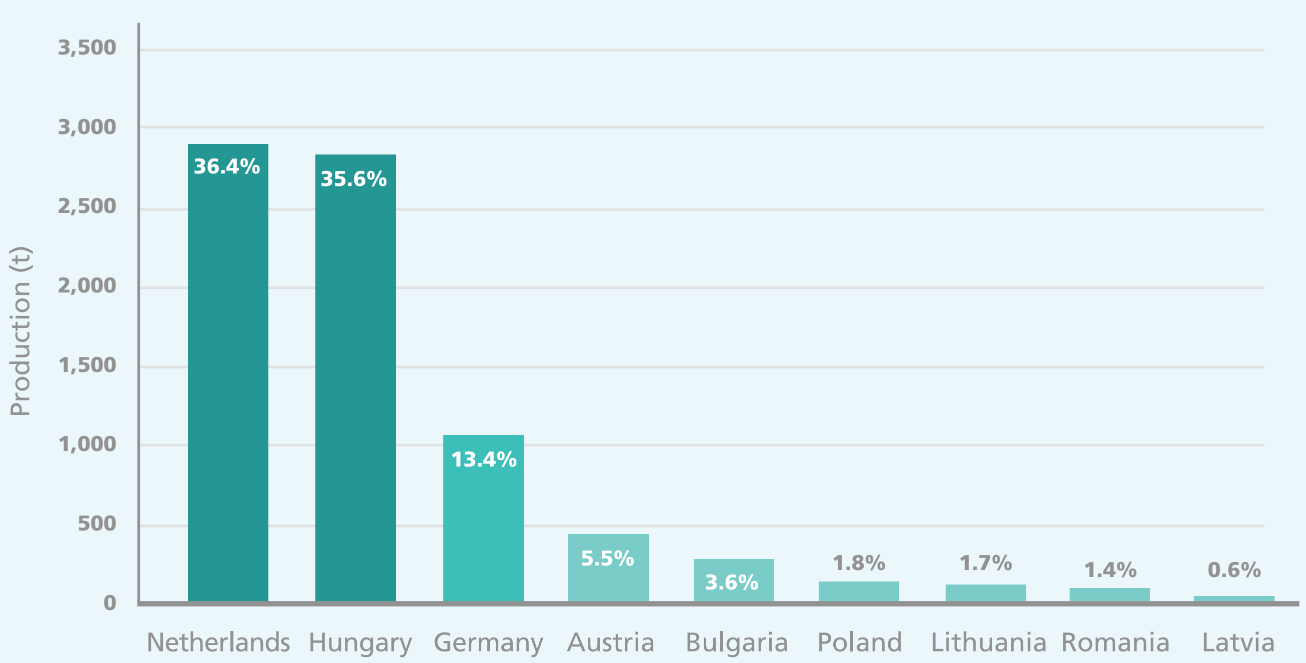

FIGURE 12

Production (tonnes) of North African catfish in the EU during 2015 and percentage contribution of each country to total EU production.

The Netherlands and Hungary are the two primary producers of North African catfish in the EU, together accounting for over 70% of total production (FAOSTAT 2015; figure 12). Over 7.9 thousand tonnes of North African catfish were slaughtered in 2015, which equates to somewhere in the region of 5 to 16 million fish (calculated according to methods of Mood & Brooke, 2015). Ice slurry slaughter is the main method used in the Netherlands and this is inhumane for catfish (Sattari et al., 2010). The skull morphology of African catfish presents difficulties for effective percussive stunning (van de Vis et al., 2003; Lines & Spence, 2014). They are also very resilient to being removed from water, and providing the skin is kept wet, they can stay alive for several days out of water. However electrical stunning appears to be a potentially humane method for this species.

Live chilling in ice slurry

In a 2010 study by Sattari et al. live chilling (before decapitation and evisceration) is reported to be the typical pre-slaughter procedure used for farmed fish in the Netherlands. However, this method has been described as resulting in poor welfare for fish by the OIE (2010). Indeed in African catfish, ice slurry is shown to be a slow method, taking between 5 and 20 minutes to the onset of unconsciousness, and also inducing muscle cramps and tachycardia (Lambooij et al., 2006; Lambooij, Kloosterboer, Gerritzen, & van de Vis, 2006).

Application of an electrical current

Lambooij et al. (2006b) demonstrated that electrical in-water stunning can be effective in inducing unconsciousness in North African catfish within 1 second. The researchers used an average current of 1.60±0.11 A/dm2 (50 Hz, sinusoidal, a.c.) at a conductivity of 876 S of the water. Succeeding this stun with decapitation resulted in minimal brain activity until death meaning that a humane death was likely achieved. Furthermore, Sattari et al. (2010) dry-stunned North African catfish for 9.1±0.4 s using a measured current of 0.91±0.18 A (150 V, AC+DC) followed immediately by decapitation. They used noxious stimuli (clipping of barbels) to test for consciousness, and found only 1 in 10 fish tested showed movement in response to clipping (indicating that this fish may not have been unconscious). However, the lack of response to clipping cannot be taken as reliable confirmation of lack of consciousness. The authors suggest that this may be useful method for slaughter of these fish industrially, however more research would be required to develop it and ensure reliable parameters were used to achieve a higher rate of effectiveness. Researchers at the Institute for Marine Resources and Ecosystem Studies (IMARES) have tested commercial equipment for electrically stunning catfish and found it could deliver an effective stun (van de Vis, pers. comms., 2018).

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4.9. TURBOT
(Scophthalmus maximus)

Over 10 thousand tonnes of turbot were farmed in the EU in 2015, with Spain being the predominant producer (~73% of production), followed by Portugal (~23%) (FAOSTAT, 2015; figure 13). Therefore, between 5 and 14 million turbot (approx.) were farmed (calculated according to methods of Mood & Brooke, 2015).

The EFSA reported in 2009 that turbot are not stunned prior to slaughter under commercial farming conditions (EFSA, 2009g). Instead, they are typically killed by asphyxia in ice slurry (most common method) or exsanguinated (the gill arches are cut on one side) and left to asphyxiate, either in air, on ice or in an ice slurry mixture. This involves very prolonged periods of stress and results in poor welfare (EFSA, 2009g). Indeed, flatfish are particularly resilient – they are less sensitive to oxygen deprivation than salmonids (Morzel, Sohier, & van de Vis, 2003) and turbot can survive out of water for several days (EFSA, 2004). In 2009, the EFSA considered it "a matter of urgency" that commercially viable, humane alternatives (such as electrical stunning followed by chilling or percussive methods) are developed (EFSA, 2009g, p. 20).

Live chilling in ice slurry

Leaving turbot to die by asphyxiation in ice slurry is an inhumane slaughter method but it is the most commonly used method in the EU (EFSA, 2009g). The fish are left in ice water slurry for around 30-60 minutes before the water is drained and they are left to die by asphyxia (on ice). Exposure to ice water slurry is aversive to turbot who show escape behaviour (Roth, Imsland, Gunnarsson, Foss, & Schelvis-Smit, 2007). Furthermore, turbot that are at the bottom of the ice slurry bins will be subjected to considerable pressure from the weight of the ice and fish above, experiencing pain and distress until unconsciousness occurs (EFSA, 2009g).

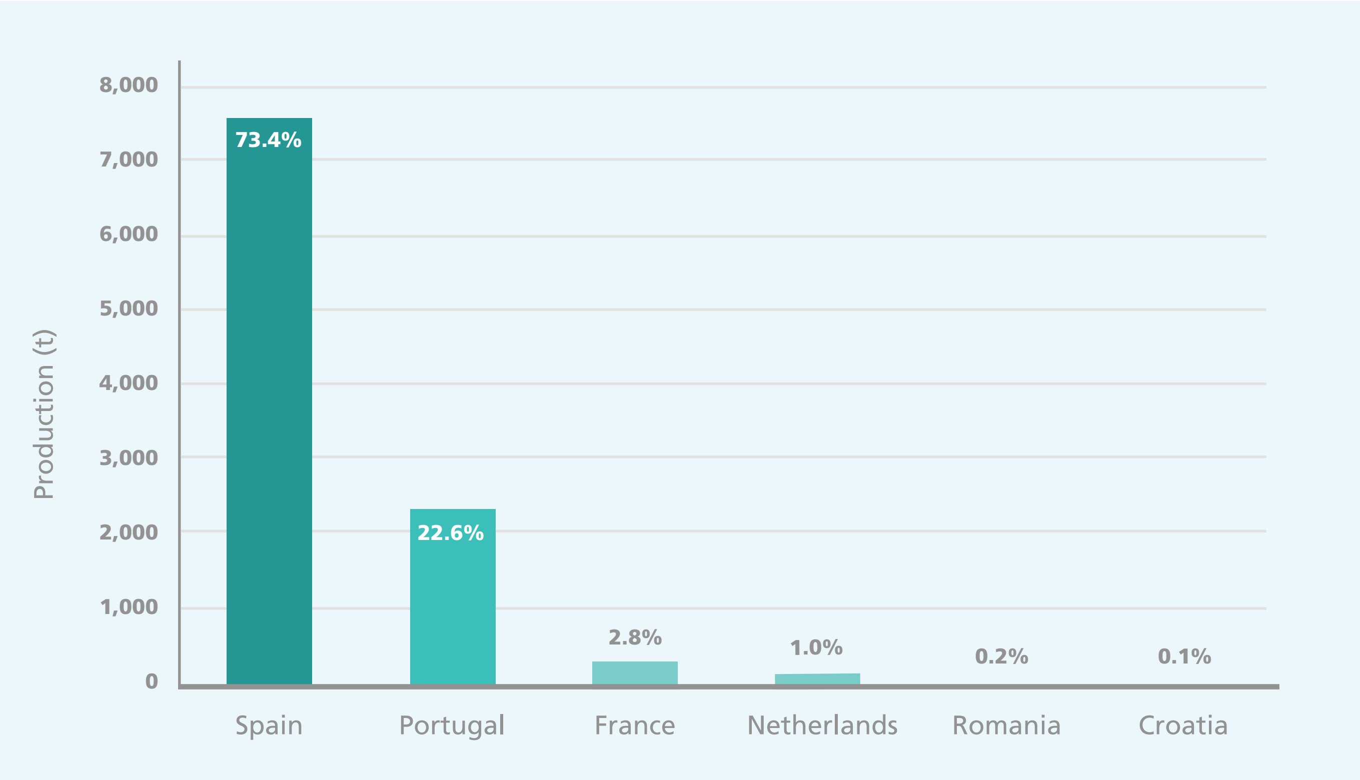

FIGURE 13

Production (tonnes) of turbot in the EU during 2015 and the percentage contribution of each country to total EU production.

Monitoring the brain activity of turbot immersed in ice slurry shows that it does not induce immediate unconsciousness, and instead causes a reduction in brain activity over time (Lambooij et al., 2015). In the study by Lambooij et al. (2015), chilling turbot in ice slurry was initially associated with elevated heart rates (suggesting the cooling was stressful for the fish), and some turbot responded to needle scratches for as long as 75 minutes indicating consciousness was still present. Gill movements decreased over time but were still detectable up to 75 minutes after chilling in ice slurry. The time to loss of gill movements, brain activity and responses to noxious stimuli was not fully tested – the experiment ended at 75 minutes so it is possible that the fish would be alive and conscious for longer than this.

Exsanguination without stunning

Some turbot are killed by being exsanguinated (usually by cutting of gill arches on one side) after removal from their holding water, or after a period of chilling in ice slurry (EFSA, 2009g). Cutting the gills of a conscious turbot (i.e. without stunning) causes pain and distress (EFSA, 2009g) and they suffer for long periods before death (Morzel et al., 2003). Morzel et al. (2003) found that turbot that were exsanguinated by gill cut and left to bleed out in water (15°C) maintained behavioural responses indicative of consciousness for at least 30 minutes. Others were exsanguinated by cutting of the caudal vein, and some of these fish showed signs of consciousness as long as 90 minutes later (the last time point tested).

For turbot left to bleed out in ice slurry (1-3°C), Morzel et al. (2003) report that behavioural responses were lost by 30 minutes, although some breathing was observed. However in another study, Roth, Imsland, et al. (2007) demonstrated even longer periods of consciousness after exsanguination and immersion in ice slurry; turbot showed escape behaviour and other responses to physical handling and were still reactive after an hour of bleeding, upon which they were killed by percussive blows.

Application of an electrical current

Electrical stunning appears to be a promising method for enabling humane slaughter of turbot (Morzel et al., 2003). A study by Lambooij et al. (2013) found that an electrical stun followed by immersion in ice slurry could be a humane method for turbot. In the study, after a 1 second head to tail, in–water stun (with a current density of 3.2 + 0.3 A/dm2), turbot were rendered immediately unconscious. However when tested 30 seconds later, 11 out of the 17 turbot tested were found to have brain activity (shown on EEG) and 3 showed physical signs of consciousness. However, when stunned for a longer period (for 5 seconds) followed by chilling in ice water for 15 minutes, all turbot showed only minimal brain activity and did not display brain or behavioural responses to pain stimuli. The authors concluded that this method was sufficient to prevent recovery of the fish.

A more recent study by Daskalova et al. (2016) determined that it is possible to effectively stun turbot in a dry electrical stunner, inducing immediate unconsciousness. However, the study highlighted the importance of a longer stun to maintain unconsciousness for long enough to prevent recovery. They were able to cause instant (within 1 second) unconsciousness using a current of 2.39±0.91Arms (by applying 125.5±0.6Vrms (100Hz)) in 25 out of 26 turbot tested (shown by EEG patterns). However, a longer current was required to prevent recovery: after a head-first stun for 20 seconds (passing 1.27±0.40Arms for 1 s and 0.65±0.21Arms through the fish) they observed no recovery during chilling in ice slurry for any of the 13 turbot tested. EEG measures recorded for 5 minutes following stunning suggested the turbot did not regain consciousness. The authors assume that turbot were killed by asphyxia in the ice slurry within 15 minutes after the stun due to the lack of ventilation by the stunned fish. However they did not test this explicitly in the study so further confirmation is needed.

Percussive blow

Percussive stunning is commonly used for the slaughter of other flatfish species such as halibut (EFSA, 2009g). Morzel, Sohier, & van de Vis (2003) report an immediate loss of consciousness using an air gun (8 bars of pressure), and conclude that percussive stunning appears to be a suitably humane method for the slaughter of portion-sized farmed turbot. Due to the shape of the head however, percussive stunning of turbot can be difficult and often results in eye prolapse due to positioning of the eyes (Roth, Imsland, et al., 2007) which would be extremely painful if the stun was not effective. This means manual application would be needed rather than automated systems which may be less suitable for large scale commercial operations.

In a study by Roth, Imsland, Gunnarsson, Foss, & Schelvis-Smit (2007), percussive blows were used to kill fish that were still alive and active after being exsanguinated and left to bleed out for an hour. For many of the fish, more than one blow was required to kill, which led to "substantial damages to the head and eyes" therefore further pain may have been felt by fish before death. This is also likely to be unacceptable for quality reasons as turbot are usually sold whole to the consumer (EFSA, 2009g).

TABLE 3

Summary of the main slaughter methods used for the key fish species farmed in the EU, with reference to their capacity to kill fish humanely.

Species	Slaughter methods used that are can be humane when carried out properly	Slaughter methods (in commercial use or experimental) that could be humane but require further refining and/or verification	Slaughter methods used that are inhumane
RAINBOW TROUT	**Large trout** Electrical current then decapitation Percussive blow then gill cut or decapitation **Small trout** Electrical current then chilling in ice slurry Percussive blow		Exposure to air Live chilling in ice slurry Live chilling in ice slurry then electrical current Carbon dioxide in water Carbon dioxide in chilled water Decapitation Evisceration
GILTHEAD SEA BREAM		Electrical current then chilling in ice slurry	Exposure to air Live chilling in ice slurry
EUROPEAN SEA BASS		Electrical current then chilling in ice slurry	Exposure to air Live chilling in ice slurry
COMMON CARP		Electrical current followed by a percussive blow and then decapitation Electrical current then decapitation Percussive blow then decapitation	Sold alive for home slaughter Exposure to air Live chilling in ice slurry Carbon dioxide in water or ice slurry
ATLANTIC SALMON	Percussive blow then gill cut or decapitation Electrical current then decapitation Electrical current then percussive blow then gill cut or decapitation		Live chilling in ice slurry Carbon dioxide in water or ice slurry
EUROPEAN EEL	Electrical current followed by chilling in ice slurry and salt	Electrical current and nitrogen exposure in water Captive needle gun	Salt or ammonia bath Live chilling with salt Decapitation or neck cut
ATLANTIC BLUEFIN TUNA	Spiking Coring Shooting		
NORTH AFRICAN CATFISH	Electrical current followed by decapitation		Live chilling in ice slurry
TURBOT		Electrical current followed by chilling in ice slurry Percussive blow then gill cut	Live chilling in ice slurry Exposure to air

N.B. These lists are not exhaustive; other combinations of stun and kill methods are used in practice. Categorisation in this table does not take associated pre-slaughter handling methods into account.

5. DEVELOPING HUMANE SLAUGHTER SYSTEMS

Many of the main species farmed in the EU are slaughtered using inhumane methods, while others are slaughtered by means that have the potential to be humane.

A thorough protocol for developing humane slaughter systems is needed, which ensures that fish are stunned effectively. This is needed for any new systems to be implemented, but also may be required to confirm that existing commercial systems are working effectively.

The verification protocol must include steps to check that consciousness is not being recovered after stunning, and should account for the potential difficulties in interpreting this due to physical immobilisation.

The information required to carry out this process for each fish species, and the results from any testing of equipment, should be collated for continual improvement and collaboration between member states. A reference centre set up by the EU or OIE as part of its Animal Welfare strategy may be a good way to coordinate this process.

The following four steps are suggested for development of humane slaughter systems:

1. ESTABLISH STUNNING PARAMETERS IN THEORY:

Parameters required for effective stunning of each species must be established in controlled laboratory settings. For example with percussive stunning, the required parameters to achieve immediate loss of consciousness without recovery, would include the force, shape and size of the hammer in relation to the specific species and size of fish. For electrical stunning, this would include the voltage and current required, at specific water conductivities, in relation to the species and size of fish. Onset of unconsciousness, and maintenance of this, should be determined using multiple methods including electroencephalograph (EEG) and electrocardiogram (ECG) measurements. These measures should also be used to test whether effective stunning can be verified in the field using behavioural observations, as the latter are not reliable in many cases. The stunning parameters (combined with the kill method, where used) must be sufficient to ensure that fish do not recover consciousness before death occurs; tests should determine the time until death by each method. Information on the variability, or consistency, based on size, age, etc., should be reported. The results should be published in peer-reviewed journals for wide dissemination.

2. DEVELOP EQUIPMENT TO DELIVER AN EFFECTIVE STUN:

After parameters are established, commercial stunners should then be developed. It is crucial to test that:

Fish are fully stunned within one second – fish should be checked for signs of consciousness after one second, even if the stunning duration is longer than one second in practice. For example, even when the fish are due to be stunned for 5 minutes during normal commercial practice, for verification purposes the electricity should be applied for 1 second only and then fish should be checked for consciousness. This would determine whether the 5 minute stun is causing a gradual (which would be unacceptable) or instant loss of consciousness.

Fish do not recover after stunning – when systems do not kill instantly, and a secondary kill method (e.g. gill cut or immersion in ice slurry) is used, the length of the stun should be checked. The routine stun and kill process should be performed but fish should be checked for recovery of consciousness until death can be confirmed. In addition, with another batch of fish, the stun should be administered as per routine, but no kill method performed and the fish should then be returned to holding water to see how long, if at all, it takes for them to regain consciousness.

Testing of equipment should be carried out in collaboration with research institutes and the results of such testing should be documented and made publically available. Equipment should be designed to facilitate monitoring and verification in practice, i.e. designed for easy observation of the fish throughout the process, and automatic recording of stunning parameters delivered. An established approval system for stunning equipment is required to ensure that that stunners on the market meet the requirements of EU legislation and deliver humane stunning in practice.

Although options for testing unconsciousness are more limited on farm (e.g. EEG cannot be measured in this setting), it is essential to verify that the stunning parameters (established in step 1) are delivered reliably in practice, and that there are no overt signs of fish recovering consciousness before death occurs.

a. For electrical stunning this includes ensuring adequate: current density, voltage, waveform of the electrical current, duration of exposure to the electricity, water conductivity, etc. whether in water or after dewatering. The method of transferring the fish into the stunner should be assessed and the maximum time interval between fish leaving the stunner and the application of a killing method should be measured.

b. For percussive stunning these specifications include whether fish enter the equipment head first and whether the air pressure that drives the bolt for percussion is sufficiently high and accurately performed.

3. IMPLEMENTATION OF THE STUNNING SYSTEM:

Equipment that has been verified by step 2 should be installed at slaughterhouses. Equipment should be set up/ laid out with the required monitoring of the system in mind, e.g. it should allow for staff to observe the process.

To be used effectively by staff, the implementation process should include:

a. Standard operating procedures for use of stunning equipment, including instructions for monitoring (see 4a-c)

b. Guidelines for the whole process, including pre-slaughter fasting and handling, to optimise welfare

c. Staff training and assessment of competence.

4. VERIFICATION OF EFFECTIVE STUNNING IN-SITU:

"Everyone is responsible" for ensuring the welfare of fish farmed in the EU is protected at slaughter. Various measures could be taken to ensure that systems are working reliably and effective stunning is not only achieved at implementation but is maintained over time, including responsibilities on three levels:

a. **Manufacturer:** design system to allow general monitoring (including CCTV) and automatic recording of parameters delivered by the equipment. There should be clearly visible and audible warnings made by machines if the parameters fall outside what is required for effective stunning.

b. **Slaughterhouse:** Staff should monitor the stunning and killing process as it is occurring to ensure that correct parameters are being delivered, and that behaviour and appearance of all fish is consistent with effective stunning. A selection of fish should be carefully checked for any signs of consciousness by staff during each processing session, using methods appropriate to the species. Management should review data (ideally automatically recorded) from stunning machines, e.g. in electrical stunning systems, the electric field delivered to each batch of fish, to check that correct parameters are being delivered at all times.

c. **External bodies:** Overview of the whole process and wider collection and evaluation of the systems, in order to work on continual improvement of the systems. In addition to routine monitoring, including observations and data automatically recorded, it may be appropriate to carry out spot checks to ensure that fish are stunned effectively, using indicators of consciousness/unconsciousness, e.g. behavioural indicators identified in step 1.

This is carried out by:

i. Third party certification schemes

ii. Veterinary authorities

iii. EU audits or inspections carried out by DG SANTE to ensure the national authorities are fulfilling their legal obligations. This can involve audits on site, or by desk based exercises or collation of Member States data. The audit focuses on the control system rather than individual premises and it culminates in a written report.

Welfare at slaughter cannot be separated from the welfare requirements which lead up to it. This includes requirements for minimising pre-slaughter and transport fasting times, handling and transport. Handling systems may need to be adjusted to fit in with systems of effective stunning.

6. CONCLUSION

European Union law requires that fish "shall be spared any avoidable pain, distress or suffering during their killing and related operations" (European Union, 2009, p. 9). The European Commission assesses compliance with these requirements based on OIE guidelines which identify slaughter systems which can be humane if applied properly, with appropriate parameters, and those which cannot.

Systems which can be humane include electrical and percussive stunning followed, where necessary, with a suitable killing method (OIE, 2014). Species-specific parameters are required to ensure that stunning, whether by percussive blow or electrical current, is effective. In many cases unconsciousness caused by these stunning methods, though near-instantly achieved, is recoverable after some time. Therefore the speed at which death can be achieved by the killing method – and hence the choice of killing method – is crucial in determining if slaughter is humane. The OIE also approves killing with a free bullet and spiking or coring for some species (OIE, 2014).

The OIE has also stated that "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" have been shown to cause poor welfare and should not be used where it is feasible to use humane methods (OIE, 2014, p. 128).

For certain fish species in some countries, methods recommended by the OIE have been widely adopted and applied using verified equipment. There is also use of methods which could in principle be humane, but using equipment and parameters which have not been independently and thoroughly verified. Lastly, inhumane methods, such as exposure to carbon dioxide or killing in ice-slurry without prior stunning, continue to be used in many member states. These cause severe and prolonged suffering for hundreds of millions of fish across the EU each year.

As such, there is an urgent need to co-ordinate further research into effective stunning parameters and to document the verification of stunning equipment. A reference centre dedicated to developing humane fish slaughter, set up by the EU or the OIE, could greatly facilitate this process. Meanwhile, member states must adopt more humane methods where they are available in order to comply with requirements of EU legislation on the killing of animals, to spare fish avoidable pain, distress and suffering.

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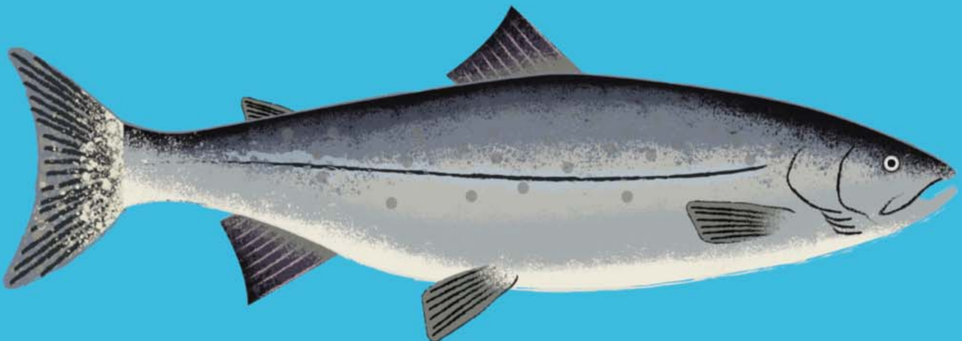

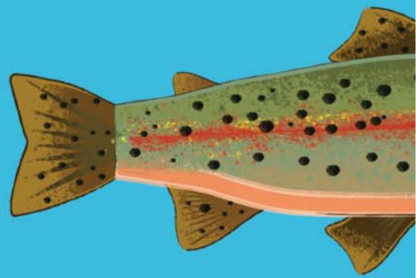

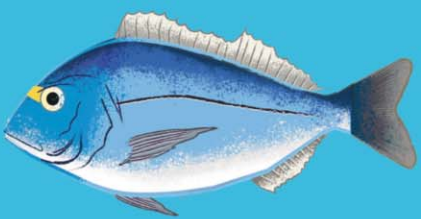

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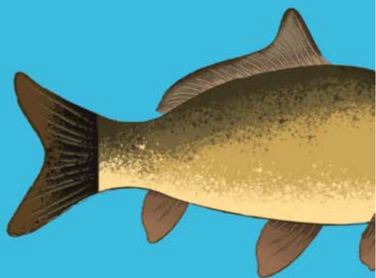

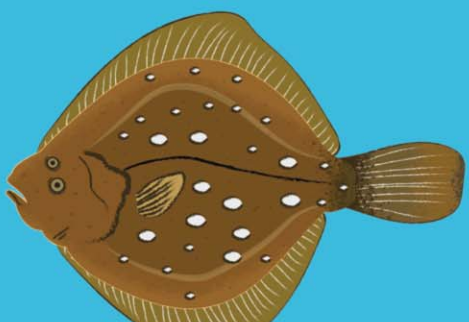

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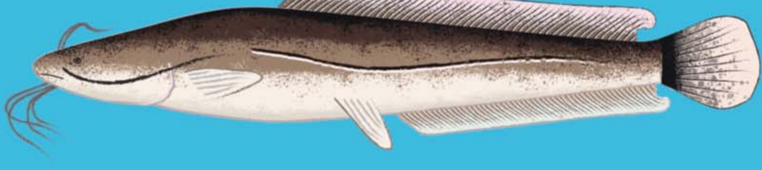

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