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Thesis

Welfare Evaluation of Stunning Practices for Farmed Fish in the European Union

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Abstract

An optimal method for stunning animals before slaughter should result in instantaneous and irreversible insensibility. Today, there are various stunning and slaughter practices used around the world for farmed fish. With aquaculture being a growing food sector, the welfare of the animals used has become increasingly important in the consciousness of consumers, researchers and regulatory bodies. With growing research into the subject matter, an overview to summarize and examine how these practices impact on the welfare of the fish, and how well they conform to animal welfare legislation, is warranted to minimize the suffering of farmed fish. Stunning practices used in aquaculture include methods such as electrical and percussive stunning, carbon dioxide and asphyxiation. Each with its own level of effectiveness in terms of how fast the method results in loss of consciousness, whether or not the effect is reversible and how the welfare of the fish is affected as determined by behavioural and physiological stress responses. It was concluded that there is no unambiguous answer as to which stunning method is optimal in regard to animal welfare in modern day aquaculture. The optimal method for a given facility is influenced by factors like practicalities relating to each individual method as well as legislation on EU and national level. Suggestions were made for future research.

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Keywords: farmed fish, stunning methods, fish welfare, welfare indicators, stress responses, aquaculture

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Abbreviations

ATP	adenosine triphosphate
ECG	electrocardiogram
EEA	European Economic Area
EEG	electroencephalogram
EU	European Union
pH	a quantitative measure of the acidity or alkalinity of a solution
ppm	parts per million
SERs	somatosensory evoked responses
VERs	visually evoked responses
VOR	vestibuloocular reflex

1 Introduction

Aquaculture has been a rapidly expanding sector in later years and has almost doubled over the past decade (Lines & Spence, 2014). The industry has been described as helping to combat climate change, preserve ecosystems and contribute to more circular resource management whilst at the same time providing jobs and economic development in coastal and rural communities (European Commission, 2021). Fish has become our most common production animal (Kiessling *et al.*, 2013). The global production of aquaculture and fisheries in 2003 was estimated to be about 132.2 million tonnes (Ashley, 2007). Rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) make up 83% of the EU freshwater aquaculture production (EUMOFA, 2021). Other noteworthy species include European eel (*Anguilla anguilla*), North-African catfish (*Clarias gariepinus*) and various carp, sturgeon and trout species (EUMOFA, 2021). Species such as Atlantic bluefin tuna (*Thunnus thynnus*), Atlantic salmon (*Salmo salar*) and European sea bass (*Dicentrarchus labrax*) are the most common fishes in EU marine aquaculture (Giménez-Candela *et al.,* 2020). Alongside the rapid expansion of the sector, the welfare of the fish has become increasingly important in the consciousness of consumers, researchers and regulatory bodies. Given this growing attention, research into the welfare of farmed fish is essential to help determine optimal practices and to serve as a foundation for future legislation (Ashley, 2007). However, defining and measuring welfare is no simple task (Ashley, 2007; Poli *et al*., 2005). Animal welfare is to a large extent about the feelings experienced by an animal, either by an absence of negative emotions or presence of positive ones (Duncan, 2005). Measuring states of suffering and pleasure in animals is challenging as feelings are subjective and the methods used to measure them are affected by the conditions under which the animals are kept and the care and handling they are subjected to (Duncan, 2005). Farmed fish are generally killed with little or no regard for their welfare with prolonged fasting, stressful transports and inhumane slaughter methods (Lines & Spence, 2014). Many of the stunning methods used today subject fish to considerable suffering over a lengthy period of time (EFSA, 2004). This is due to the practices being designed for commercial efficiency as opposed to welfare considerations. In cases where methods are capable of achieving the desired outcome in a humane manner, the operators lack the knowledge to evaluate them (EFSA, 2004). It is essential that operators are mindful of the welfare of the animals, are well trained and that their equipment is in good condition and carefully maintained (EFSA, 2004). Slaughter methods can be deemed humane if they result in instantaneous insensibility without causing fear or pain (EFSA, 2009). The method should also be irreversible to ensure unconsciousness until death due to exsanguination (Erikson, 2011).

1.1 Welfare indicators

Stunning methods renders an animal unconscious by disrupting the neurons or neurotransmitter regulatory mechanisms in the brain (EFSA, 2004). The duration of unconsciousness varies depending on method and species, but should last long enough to assure death through exsanguination. A stunning method should only have to be applied once to get the desired outcome (EFSA, 2004). If the stun proves unsuccessful, it should instantly be followed up by a suitable killing method. The consciousness of the animal must be reliably assessed in order to verify the effectiveness of a given stunning method. The presence or absence of visual indicators is often used to determine insensibility (Bowman *et al.*, 2020). However, a fish could experience suffering without the ability to express it if left paralyzed without anesthesia (Kestin *et al.*, 2002). In a study conducted by Kestin *et al.* (2002), average time for responses and reflexes to be lost was measured in fish killed with various stunning methods. All responses and reflexes were instantly lost when the fish was killed or de facto stunned, whereas these things were gradually lost when a given method did not result in immediate unconsciousness. It was concluded by the authors that a fish is insensible when brainstem-mediated reflexes such as the corneal reflex and rhythmic breathing are no longer present. Objective tests to register sensibility and consciousness include the use of electroencephalogram (EEG), electrocardiogram (ECG), somatosensory evoked responses (SERs) and visually evoked responses (VERs) from flashes of light directed towards the eyes (Poli *et al.,* 2005). EEG measures the degree of openness of a brain pathway in response to flashing light by the use of electrodes (Robb *et al.*, 2000). When this patency cease, VERs are no longer present and the animal will no longer respond to external stimuli (Robb *et al.*, 2000). Since these responses are the last to be lost before death, using EEG is a good method to denote brain death (Robb *et al.*, 2000). The brain activity data provided by EEG is reliable, but the expertise required to carry out the test restricts its use (Poli *et al.,* 2005).

An emphasis on physiological stressors, physical health and satisfaction of biological needs is merely one school of thought regarding welfare in the research community. The other school of thought suggests that welfare is about psychological health and the feelings of the animal (Duncan, 2005). The benefit of the former is that it is based on biological functions that are relatively easy to measure. Conversely, emotions are ill-defined and difficult to measure (Duncan, 2005). Unlike biological functions, emotions cannot be measured in a way that ensures objectivity since it is a subjective state (Duncan, 2005). This is further complicated by the fact that species are different from each other and that species other than humans lack a language to communicate what they feel to us. Changes in behaviour can be an early sign of potential welfare issues due to behaviour being a response to the perceived environment (Martins *et al.*, 2012). The issue with using behaviour as a welfare indicator is that it changes over time, it is difficult to interpret and measure and there are individual differences from one animal to another. It is also a challenge to differentiate behavioural reactions that can be considered beneficial coping strategies from reactions that are detrimental to the welfare of the fish (Martins *et al.*, 2012). Fish producers are not as well acquainted with causes and effects of behavioural changes caused by stress as they are with physiological ones (Conte, 2004). A good understanding of species-specific behaviour is crucial to ensure the welfare of the fish since changes in behaviour can both result in stress and be caused by it (Conte, 2004). Wendelaar Bonga (1997) defined stress as a threat to or disturbance of an animal's homeostasis due to the effects of intrinsic or extrinsic stimuli. These stimuli are often referred to as stressors and they induce physiological and behavioural responses to overcome a perceived threat through compensation or adaptation. If these responses turn chronic, they may lead to reduced resistance to pathogens as well as reproductive issues and growth inhibition (Wendelaar Bonga, 1997). It is likely that stress can affect the welfare of fish in a similar way to other vertebrates based on the structure of neurons and neuronal biochemistry (Lambooij *et al.*, 2002). The experience of pain is also relevant to fish based on anatomical, behavioural and physiological studies (Kestin, 1994). Vocalization during stunning is an indication of pain or suffering, however the absence of it does not mean that the animal is not experiencing pain or suffering (EFSA, 2004). Coping strategies towards stressors may differ from individual to individual within the same species (Martins *et al.,* 2012). As few individuals may not be representative of a group when evaluating welfare, there is a need for behavioural welfare indicators to better asses the welfare of both individuals and entire groups (Martins *et al.,* 2012).

1.2 Animal welfare legislation and guidelines

The World Organisation for Animal Health (OIE) has been developing welfare standards concerning the stunning and killing of farmed fish since 1995. Issued standards regarding animal welfare are non-binding. The general stipulations published by OIE (2019) in the 22nd edition of the Aquatic Animal Health Code include the following.

I. The choice of method should take account of species-specific information where available.
II. All handling, stunning and killing equipment should be maintained and operated appropriately; it should be tested on a regular basis to ensure that performance is adequate.
III. Effective stunning should be verified by the absence of consciousness.
IV. A backup stunning system is necessary. Any fish mis-stunned, or regaining consciousness before death, should be re-stunned as soon as possible.
V. Stunning should not take place if killing is likely to be delayed such that the fish will recover or partially recover consciousness.
VI. While absence of consciousness may be difficult to recognise, signs of correct stunning include i) loss of body and respiratory movement (loss in opercular activity); ii) loss of visual evoked response (VER); iii) loss of vestibuloocular reflex (VOR, eye rolling).

Fishes are recognized as sentient beings by the EU in Article 13 of the Treaty on the Functioning of the European Union (European Union, 2012). As per Council Regulation (EC) No 1099/2009, animals shall be spared any avoidable pain, distress or suffering during their killing and related operations (European Council, 2009). Furthermore, the loss of consciousness and sensibility shall be maintained until the death of the animal and methods which do not result in instantaneous death shall be followed up as quickly as possible by a procedure to ensure death (European Council, 2009).

Strategic guidelines on sustainable and competitive aquaculture have been published in the EU, containing new guidelines in regard to animal welfare (European Commission, 2021). It states that further action to improve fish welfare is a necessity and emphasized the following objectives.

I. Developing good practices on fish welfare during farming, transport and killing.

II. Setting common, validated, species-specific and auditable fish-welfare indicators throughout the production chain (including in transport and slaughtering).
III. Further research and innovation, in particular on species-specific welfare parameters.
IV. Providing knowledge and skills on fish welfare to aquaculture producers and other operators that handle live farmed fish.

Member States may maintain or adopt national rules regarding the protection of fish at the time of slaughter (European Council, 2009). National legislations and recommendations have been adopted by Member States and EEA countries, but they are less developed compared to those for terrestrial farm animals (European Commission, 2018).

The use of carbon dioxide has been prohibited in Norway to anaesthetize farmed fish since 2006, along with anything else that blocks oxygen uptake, salt, ammonia and other chemicals with a similar effect (Norwegian Ministry of Trade, Industry and Fisheries, 2006). Carbon dioxide is banned in the United Kingdom, albeit with an exception for emergencies (Ashley, 2007). In Sweden it is prohibited to put live farmed fish on ice or in ice slurries (Swedish Board of Agriculture, 2019).

2 Aims of the thesis

I. Investigate and evaluate stunning methods and their impact on the welfare of the fish.
II. Examine welfare indicators and their reliability in determining consciousness and thus susceptibility to pain and distress.
III. Give an account of EU legislation relevant for stunning practices in aquaculture.
IV. Leave recommendations for future research with the aim of bridging gaps in knowledge and to ultimately improve stunning practices in order to protect farmed fish from avoidable suffering.

The overarching ambition of this thesis is to provide an overview of stunning practices for farmed fish and how they impact on animal welfare. The review also encompasses welfare indicators and legislation to provide context and a foundation of knowledge to help contrast and compare different stunning practices from a welfare perspective.

3 Method

This thesis took the form of a narrative review with the objective of producing a comprehensive and critical analysis of current knowledge and practices in regard to the aims of the thesis. Peer-reviewed articles of high relevance were chosen based on the selected keywords featured below, used either individually or in search strings. Names of specific stunning methods were also used to reduce the risk of missing material due to authors' choice of words in titles, keywords and abstracts. Results were limited to articles in English and Swedish for the sake of intelligibility. Exclusion criteria also included articles published earlier than 1994. Sources cited in studies obtained as previously outlined have also been used in cases where they were within the scope of this review. The literature search and selection took place in February and March of 2021. Databases were comprised of Web of Science and Google Scholar. Using more than one database was deemed important due to their internal sorting algorithms, despite the risk of overlapping search results with references being identified multiple times. Article screening was initially restricted to titles and sources in order to eliminate duplicates and to sort out irrelevant articles in a time efficient manner. To determine relevance for remaining articles, a review of abstracts took place. The final screening phase was to read and examine articles in full.

Keywords: farmed fish, stunning methods, fish welfare, welfare indicators, stress responses, aquaculture

4 Stunning methods

4.1 Gas stunning

4.1.1 Carbon dioxide

Carbon dioxide narcosis consists of placing fish in water saturated by carbon dioxide that dissolves, creating carbonic acid, bicarbonate and hydron. This has a toxic effect on the brain of the fish due to a lowered blood pH (Poli *et al.,* 2005). It is a commonly used commercial method (Robb *et al*., 2000). It is the predominant method used in Sweden due to its relatively low cost and low toxicity for humans (Kiessling *et al.*, 2013). The benefit of the method is its capability of stunning a large number of fish simultaneously (Poli *et al.,* 2005). However, carbon dioxide narcosis is very aversive to fish (EFSA, 2004). It has been shown to impact negatively on fish welfare and should only be used if it is not feasible to use other methods (OIE, 2019). This conclusion was echoed by Gräns *et al.* (2016) who stated that carbon dioxide should not be used as it causes avoidable suffering.

Erikson (2011) exposed Atlantic salmon (*Salmo salar*) to three different levels of carbon dioxide to test whether there might be a level of carbon dioxide at which fish can be anaesthetized without causing avoidable stress and aversive reactions. High and medium levels of carbon dioxide did not render the salmon insensible whilst also causing stress and triggering aversive behavioural reactions. Low levels of carbon dioxide resulted in a less aversive response, but the sedative effect was too weak to facilitate handling of the fish. Robb *et al*. (2000) compared VERs of Atlantic salmon (*Salmo salar*) prior to and following immersion in carbon dioxide saturated water for 4 minutes. Immersion was followed by vigorous movements by the fish for 2 minutes before subsiding. Aversive reactions to carbon dioxide were corroborated by Gräns *et al.* (2016) and Bowman *et al*. (2020) in Arctic char (*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss*), respectively. In these studies, immersion triggered fierce aversive reactions such as escape attempts and ceaseless swimming behaviour for several minutes before immobilizing the fish. Two of the salmon submerged by Robb *et al.* (2000) displayed VERs at the point of the subsequent gill cut and made tail flaps and feeble head shakes up to 4 minutes after it. The presence of VERs was a clear indication that the fish had not been rendered unconscious, thus being able to experience pain and distress during the process.

But even without the presence of visual indicators, animals may still be conscious. Bowman *et al*. (2020) looked at the reliability of visual indicators like equilibrium, eye-roll reflex and ventilation for assessing consciousness during carbon dioxide narcosis. Loss of ventilation was the last visual indicator to be observed and it happened 4.5 minutes after submersion. However, EEG suggested loss of consciousness occurred between 4.5 to 8.5 minutes after submersion. The authors thus concluded that visual indicators may be an insufficient method for determining insensibility. Furthermore, the time it took to lose visual indicators following stunning was strongly correlated with water temperature. Loss of ventilation took place after 4.5 minutes in 14°C but increased to over 11 minutes in 2°C, suggesting an exacerbation of an already slow stunning method in lower water temperatures. The authors deemed carbon dioxide an inhumane stunning method as it can lead to significant welfare problems given the following.

I. It does not immediately result in loss of consciousness.
II. It triggers aversive behavioural reactions before immobilization.
III. It can result in fish becoming paralyzed whilst remaining conscious due to visual indicators being insufficient in determining insensibility.
IV. It is even less effective in lower water temperatures where the time to lose consciousness is prolonged.

4.1.2 Nitrogen

In a study by Wills *et al*. (2006), rainbow trout (*Oncorhynchus mykiss*) were exposed to nitrogen gas. It was effective after 6 to 8 minutes in 15°C and was found to cause no aversive reactions. Nitrogen gas as a stunning method for Atlantic salmon (*Salmo salar)* was evaluated by Erikson (2011) in relation to the following three welfare criteria.

I. It should minimize aversive reactions following exposure.
II. It should result in unconsciousness.
III. It should not allow for recovery from the effects during 10 minutes post-stunning.

Unlike aforementioned study by Wills *et al*. (2006), fish exposed to nitrogen gas exhibited aversive reactions and even higher levels of stress compared with fish exposed to high levels of carbon dioxide. Nitrogen did not meet any of the welfare criteria and was deemed an inappropriate stunning method.

4.2 Electrical stunning

Electrical stunning can result in instantaneous loss of consciousness and insensibility (EFSA, 2004). However, this effect does not last a sufficiently long time to ensure death before the fish regain consciousness (EFSA, 2004; Lines & Spence, 2014). The duration of insensibility is influenced by electrical parameters, the duration of application and measures taken post-stun (Lines & Spence, 2014). If the electric current is optimized for the species and applied correctly, the method can result in quick immobilization and insensibility (Poli *et al.,* 2005). However, if applied for too long, electrical stunning can cause a violent physical response ranging from wide-open mouths to vertebral fractures (Poli *et al.,* 2005).

Stunning with electricity can be achieved both in and out of water, sometimes referred to as wet and dry stunning (Gräns *et al.,* 2016; Lines & Spence, 2014). Electrical stunning in water has the capability of stunning a large number of fish of various sizes simultaneously, without having to handle or restrain the fish in any way (Lines & Spence, 2014). The voltage and current needed to achieve the desired outcome are affected by water conductivity as well as the species and orientation of the fish. In commercial settings it is not uncommon to load fish onto a metal grate before running an electrical current through it after the water has been drained from the surface (Conte, 2004). The advantage of dry stunning is that a higher current density can be achieved, which is beneficial when attempting to stun resilient species such as iridescent shark catfish (*Pangasianodon hypophthalmus*). However, there is a risk of fish receiving pre-stun shocks if they exempli gratia are lying on top of each other or if their tails make initial contact with the electrodes as opposed to their heads (Lines & Spence, 2014).

In a study by Brijs *et al*. (2021), VERs of African sharptooth catfish (*Clarias gariepinus)* were monitored in response to various stunning methods. It was noted that an increase in stun duration resulted in a significantly increased duration of insensibility following electrical stunning. All fish recovered VERs within 30 seconds of a 2 second stun, a time that increased to 1.7 to 4.9 minutes when the duration of the stunning was increased to 10 seconds. Still, the duration following a 10 second stun was not sufficiently long to ensure the fish died from exsanguination before regaining consciousness. It was noted that the issue of recovering sensibility could be remedied by subsequently placing the electrically stunned fish in an ice slurry. Using this combination of methods, none of the fish regained consciousness. Lines & Spence (2014) noted that some species, such as Atlantic salmon (*Salmo salar*), European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*), will die from hypoxia before regaining consciousness if placed in still water or ice while stunned. This is to prevent any flow of oxygenated water across the gills, something that occurs if placed in moving water which can result in a potential recovery.

In aforementioned study by Gräns *et al.* (2016), behavioural and physiological stress indicators in Arctic char (*Salvelinus alpinus*) were assessed after being stunned with carbon dioxide and electricity. Unlike carbon dioxide, electrical stunning was almost instantaneous and did not induce any aversive behavioural reactions. However, fish stunned with electricity had plasma levels of cortisol twice as high compared with those exposed to carbon dioxide. A possible explanation for this result, given the aversive response to carbon dioxide, was that the fish were not actually stunned. Immobilization of fish after electrical stunning is no guarantee of unconsciousness (Poli *et al.,* 2005). The fish could have been electro-immobilized and thus still conscious and susceptible to pain and anxiety. On average the fish recovered after 5 minutes, which constitutes a significant risk of regaining consciousness before dying from exsanguination (Gräns *et al.,* 2016).

4.3 Mechanical stunning

4.3.1 Percussive

Percussive stunning is accomplished by a sufficiently strong blow to the head with the objective to cause brain damage (OIE, 2019; Poli *et al.,* 2005). It is a method commonly used for stunning fish such as salmon and trout (Poli *et al.,* 2005). If the blow is sufficiently powerful, it will result in massive brain disruption, instantaneous loss of consciousness and possibly death (Poli *et al.,* 2005). If correctly applied, the ensuing loss of consciousness is typically irreversible (OIE, 2019).

After taking the fish from the water and restraining it, blunt trauma to the cranium can be achieved manually by a hand-held captive bolt device or by the use of a club, the latter often referred to as a priest (Conte, 2004; Poli *et al.,* 2005). However, semi-automatic stunning equipment is becoming more prevalent in the industry (EFSA, 2004). The fish are manually inserted head first and upon contact with a trigger inside the stunning apparatus, the fish receives a powerful blow to the head by a pneumatic, flat-headed hammer. This particular method results in irreversible stunning in more than 99% of cases if performed with accuracy (EFSA, 2004).

The welfare of the fish is affected by the time out of water, the positioning inside any automated equipment as well as the precision and power of the blow to the cranium (Lines & Spence, 2014). Mis-hits and damage to the head can occur (EFSA, 2004; European Commission, 2018). The execution of the method is affected by the training and state of fatigue of the operator (EFSA, 2004; Lines & Spence, 2014). Automated equipment largely overcomes these issues, but is not likely to fit a wide range of fish sizes without sorting to remove small, large and deformed fish (Lines & Spence, 2014). Percussive stunning also involves individual handling of fish, which is more labor-intensive and thus not necessarily as economically feasible compared to certain other stunning methods that can be applied to a large number of fish at once (EFSA, 2004).

African sharptooth catfish (*Clarias gariepinus)* stunned manually with a priest were in 64% of cases instantaneously and irreversibly rendered unconscious based on the loss of VERs (Brijs *et al*., 2021). The issues raised by the authors was the difficulty in manually performing the stunning procedure with precision as well as determining the force needed to render a fish insensible. To accomplish these things with live and struggling fish is a challenge, one clearly demonstrated by the fact that 36% of the fish in the study recovered VERs after 2.1 to 11.1 minutes despite the researcher attempting to strike the fish with precision under relatively controlled conditions. To facilitate handling of the fish and ensure its welfare there may be a need to sedate fish prior to the stunning. However, there is currently no anaesthetics permitted for use on fish produced or imported into the EU due to a lack of food safety evaluations (European Parliament, 2001).

4.3.2 Spiking

Spiking is a method whereby a spike is driven through the cranium and deep into the brain of the fish in order to destroy it (EFSA, 2004; Poli *et al*., 2005). This is commonly achieved with a pneumatical pistol and may even result in immediate death (EFSA, 2004). However, manual spiking without any mechanical aids is an existing practice whereby fish are taken out of the water and a spike is driven into the skull (EFSA, 2004). This non-mechanical practice is slow compared with mechanical spiking aids and should not be used (EFSA, 2004). When spiking is executed correctly, the resulting loss of consciousness is immediate (Poli *et al*., 2005).

The method requires restraining of the fish to ensure accuracy. However, it may be difficult to use spiking on smaller species as anatomical markers serve as essential visual aids to target the brain, such as the pineal window in tuna (EFSA, 2004). The method may also fail and cause injury if the fish is making attempts to escape during the procedure or in cases with smaller species where the brain is harder to hit with accuracy. Because of this, spiking should only be done with restrained fish larger than 10 kg (EFSA, 2004).

Robb *et al*. (2000) studied the time taken for Atlantic salmon (*Salmo salar*) to lose VERs after being subjected to various stunning methods. Together with percussive stunning, spiking was the only method to result in an immediate loss of VERs. In contrast to methods such as carbon dioxide, correctly executed spiking did not cause any aversive reactions. However, failed spiking attempts did show aversive responses indicative of pain. If spiking or percussive stunning is carried out with accuracy to ensure an immediate loss of consciousness, both can be considered humane methods (Poli *et al*., 2005; Robb *et al*., 2000).

4.4 Chemical stunning

4.4.1 Isoeugenol

Derived from the flowers and leaves of the clove tree (*Eugenia aromatica*), clove oil is widely used exempli gratia as dental local anaesthetic (Anderson *et al*., 1997).

Keene *et al*. (1998) studied if eugenol, a derivative of clove oil and the basis for isoeugenol, was an effective anaesthetic for rainbow trout (*Oncorhynchus mykiss*). It was observed that both recovery time and fear responses increased exponentially with exposure time. The authors did note that fish are rarely anaesthetized for longer than 6 to 10 minutes in aquaculture and that low doses thus can be used with a temporal margin of safety. The recommended dose for inducing anaesthesia in juvenile rainbow trout was determined to be exposure to 40 to 60 ppm for a duration of 3 to 6 minutes. Eugenol did not present any long-term negative effects as no mortalities or abnormal behaviours were observed within a period of two weeks post-anaesthesia.

In a study to assess stress-reducing capabilities of stunning methods for European sea bass (*Dicentrarchus labrax*), it was observed that clove oil anaesthesia followed by asphyxia in ice seems to be a humane combination with positive effects on both welfare and flesh quality (Simitzis *et al*., 2013) Clove oil anaesthesia was also deemed practical as it can be easily applied and does not warrant any particular training of personnel.

Isoeugenol can be an appropriate pre-stunning sedative due to the absence of an aversive behavioural response and the resulting ease of handling the fish (Brijs *et al*., 2021). When Atlantic salmon (*Salmo salar*) were exposed to various anaesthetics, Iversen *et al*. (2003) noted that eugenol-based ones had a stress-reducing effect and were effective in low doses. The authors also noted that these anaesthetics are inexpensive and without any adverse effects for people and the environment.

Erikson (2011) assessed isoeugenol alongside carbon dioxide and nitrogen as stunning agents for Atlantic salmon (*Salmo salar*). Four criteria were formulated for a given method to take welfare and product quality into consideration.

I. It should minimize aversive reactions upon exposure to the gases or anaesthetic.
II. It should be able to produce unconsciousness.
III. It should not allow for any recovery during the selected 10 min post stunning.
IV. It should promote 'rested harvest' by minimizing muscle activity.

Isoeugenol was the only method to satisfy all criteria.

A high welfare standard may be possible with isoeugenol as it induces insensibility with few side effects (Lines & Spence, 2014). However, its utilization is being stifled by food safety regulations in the EU (Lines & Spence, 2014).

4.5 Asphyxia

Most fish are killed without stunning in aquaculture through asphyxiation in air or in ice (Lines & Spence, 2014). Asphyxia in air is the oldest method whereby fish is left to die out of the water, a method marked by prolonged suffering for the fish (Poli *et al*., 2005). This can be achieved by a process known as dewatering, which means that the water is drained from the fish container leading to asphyxiation (Conte, 2004). Certain species such as carp and eel have a certain resistance to hypoxia, thus prolonging the time it takes for them to die (Poli *et al*., 2005).

When immersed in ice, the body temperature and metabolic rate rapidly decrease and the fish eventually die of anoxia (Poli *et al*., 2005). Asphyxia in ice is a particularly advantageous method for fish farmers as it eventually kills the fish and at no additional cost or labor cools and preserves them post-mortem (Lines & Spence, 2014).

In a study by Acerete *et al*. (2009), the suitability of asphyxia as a stunning method was compared with carbon dioxide for European sea bass

(*Dicentrarchus labrax*). Asphyxia in air was deemed highly stressful for the fish due to an increase in levels of plasma glucose and cortisol by a factor of 5 and 8, respectively. By comparison, asphyxia in ice and carbon dioxide resulted in a 1.5 to 1.7 times increase of plasma glucose and an increase of cortisol levels by a factor of 5. Studying effects on stress levels in Nile tilapia (*Oreochromis niloticus*) from asphyxia in ice, electricity and carbon dioxide, Olivieira Filho *et al*. (2015) found that asphyxia in ice caused the highest levels of stress based on concentration of adenosine triphosphate (ATP) and increase in plasma cortisol. Acerete *et al*. (2009) also observed that time taken until death from asphyxia in ice and carbon dioxide narcosis differed markedly with death from carbon dioxide taking 16 minutes and asphyxia in ice taking 34 minutes.

Movement of African sharptooth catfish (*Clarias gariepinus)* ceased 1.9 to 6.3 minutes after immersion in an ice slurry (Brijs *et al*., 2021). The fish spent a majority of this time making energetic attempts to escape. Movement ceased before losing VERs, meaning that fish exposed to this method were immobilized before losing consciousness. According to the authors, this represents a significant welfare risk. If taken out of the ice slurry after up to 30 minutes and placed in water at 19.6 °C, the fish recovered VERs after 3.4 to 11.9 minutes and body movement and rhythmic ventilation after an additional 5.4 to 10.2 minutes. The method was concluded to be inhumane. An assessment shared by Gräns *et al*. (2016) who noted that the method results in avoidable suffering before death and should not be used. However, Brijs *et al*. (2021) did suggest that ice slurry immersion could possibly be used in conjunction with a faster stunning method as loss of consciousness is sustained until death, assuming the fish remains in the ice slurry longer than 40 minutes.

5 Conclusion

There appears to be no unambiguous answer as to which stunning method is optimal in regard to animal welfare. The optimal method for a given facility may be situational and differ based on practicalities relating to each individual method. There is also legislation on EU and national level that may impact on the alternatives available to the farmer.

A humane stunning method should result in instantaneous and irreversible loss of consciousness for the animal without causing suffering. The effectiveness of the method must be verified by the use of neurophysiological indicators as absence of visual or behavioural indicators cannot reliably determine insensibility. Generally speaking, mechanical methods such as percussive stunning and spiking are considered to be highly effective and reliable when executed with accuracy. These methods do come with practical limitations as they are less suitable for large numbers of fish and may require investments in both equipment and training of personnel. Electrical stunning appears to be an effective albeit unreliable method, being reversible as well as carrying an inherent risk of immobilizing the fish with no loss of consciousness. However, electrical stunning could be followed by a mechanical stunning method to prevent the stun from being reversed. Carbon dioxide as well as asphyxia have both been criticized for causing significant welfare issues, with several authors strongly advising against their use. Both methods are deemed highly stressful, does not immediately result in loss of consciousness and triggers aversive behavioural responses. Furthermore, under certain conditions fish may recover from the effects of asphyxia whereas carbon dioxide can result in fish becoming paralyzed whilst remaining conscious. The research concerning possible effects of using nitrogen gas as a stunning agent for farmed fish is limited and the results are conflicting in terms of how the gas affects the fish. Whereas one study observed no issues, another found nitrogen to cause aversive behavioural responses and high levels of stress. The use of isoeugenol seems promising, particularly when used in combination with other methods. This can be done to prevent a desired effect from reversing itself, to facilitate handling of the fish to apply a method with greater accuracy or to mitigate the effects of a prolonged and stress-inducing method. These effects may make electrical stunning as well as asphyxia in ice more viable in the future when combined with isoeugenol.

Concomitantly with the welfare evaluation of stunning practices, it is essential to also consider how well they adhere to EU legislation. Given the issues associated with certain stunning methods, as outlined above, it is questionable as to whether methods such as asphyxia, carbon dioxide and electricity actually comply with the letter and spirit of the rules laid down in Council Regulation (EC) No 1099/2009, which amongst other things stipulates that animals shall be spared any avoidable pain, distress or suffering during their killing and that the loss of consciousness and sensibility shall be maintained until the death of the animal. Furthermore, there are exceedingly few countries that has prohibited the use of dubious stunning methods for farmed fish on a national level. It seems like the legislative process is lagging behind the knowledge accrued from research conducted in the field, which points toward a need to bridge this chasm and better take the welfare of the animals used into consideration when drafting legislation and developing or adopting new practices.

The results from the reviewed studies present several opportunities for new research directions. A transition to more humane stunning practices would be greatly facilitated if it resulted in competitive advantages by satisfying a demand for better animal welfare amongst consumers or if it could be achieved without substantial investments in new equipment. Further studies should aim at investigating the economic impact of implementing acceptable stunning practices in regard to animal welfare. These considerations have a great potential impact moving forward for the industry in adopting more welfare-friendly stunning practices. Further studies should also be undertaken investigating the effects of stunning methods for different species of fish as well as determining whether stunning methods are sufficiently effective and reliable when used in conjunction with each other without compromising the welfare of the fish.

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