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Food Safety considerations concerning the species-specific welfare aspects of the main systems of stunning and killing of farmed fish.[1]

Scientific Opinion of the Panel on Biological Hazards

(Question No EFSA-Q-2008-770)

Adopted on 9 July 2009

Relating to Opinions of the Scientific Panel on Animal Health and Welfare:

Species-specific welfare aspects of the main systems of stunning and killing of farmed Atlantic salmon. EFSA-Q-2008-437. Adopted 20 March 2009[2].

Species-specific welfare aspects of the main systems of stunning and killing of farmed fish: rainbow trout. EFSA-Q-2008-438. Adopted 20 March 2009[3].

Species-specific welfare aspects of the main systems of stunning and killing of farmed carp. EFSA-Q-2008-439. Adopted 20 March 2009[4].

Species-specific welfare aspects of the main systems of stunning and killing of farmed eels *(Anguilla Anguilla)*. EFSA-Q-2008-440. Adopted 20 March 2009[5].

Species-specific welfare aspects of the main systems of stunning and killing of farmed seabass and seabream. EFSA-Q-2008-441. Adopted 20 March 2009[6].

Species-specific welfare aspects of the main systems of stunning and killing of farmed turbot. EFSA-Q-2008-442. Adopted 30 April 2009[7].

Species-specific welfare aspects of the main systems of stunning and killing of farmed tuna. EFSA-Q-2008-443. Adopted 30 April 2009[8].

PANEL MEMBERS

Olivier Andreoletti, Herbert Budka, Sava Buncic, John D Collins, John Griffin, Tine Hald, Arie Havelaar, James Hope, Günter Klein, James McLauchlin, Winy Messens, Christine Muller-Graf, Christophe Nguyen-The, Birgit Noerrung, Luisa Peixe, Miguel Prieto Maradona, Antonia Ricci, John Sofos, Eric John Threlfall, Ivar Vågsholm, Emmanuel Vanopdenbosch

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[3] www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902441012.htm

[4] www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902496686.htm

[5] www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902441076.htm

[6] www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902441174.htm

[7] www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902524256.htm

[8] www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902516857.htm

SUMMARY

The European Food Safety Authority (EFSA) asked its Panel on Biological Hazards to deliver a scientific opinion on: Food Safety considerations concerning the species-specific welfare aspects of the main systems of stunning and killing of farmed fish. The Animal Health and Welfare Panel (AHAW) has addressed animal welfare aspects of the main systems for stunning and killing for eight fish species in form of seven separate scientific opinions. In this opinion, the BIOHAZ Panel has focused on the food safety relevance of the stunning and killing factors relating to fish welfare, in a single opinion. Safety of farmed fish and fish products is influenced by farming conditions, pre-slaughtering practices and stunning/killing operations. A former BIOHAZ opinion concluded that fish farmed in Europe have a good record of safety with respect to biological hazards, and that the food safety risk associated with aquaculture products is very low. Post-slaughtering and processing stages have not been considered in this opinion, but they also exert an important influence on the safety of fish and fish products. In this opinion, only biological risks have been assessed.

It is generally considered that use of farming systems based on good hygienic practices including provision of optimal animal welfare increases the animals' resistance to infections and leads to a reduction of the food safety risks associated with the resulting foods of animal origin. In other words, in principle, on-farm animal welfare assurance contributes to the resulting food safety assurance. After slaughtering the biochemistry of the muscle *post-mortem* is influenced by the method used in pre-slaughter handling and stunning/killing of fish and this may have an influence on the microflora. It is also well known for other animal species that any slaughter-related operation that involve penetration of the skin, such as stunning with captive bolt or exsanguinations, carry a risk of introducing pathogenic bacteria from the skin onto/into edible parts of the animal directly or via blood circulation.

The Scientific Panel on Biological Hazards concludes that: based on general principles of food hygiene, some fish stunning & killing related factors (e.g. microbial contamination of water, increased handling, invasive stunning and exsanguination methods) could lead to increasing the risk of microbial contamination of fish. As the scientific information on specific welfare hazards of the stunning and killing practices that could compromise safety of fish products is very limited, a definitive assessment of the food safety risks associated with different stunning and killing methods for fish is not possible at this time. Measures intended to preserve fish welfare by avoiding stress during stunning & killing and improving environmental conditions, are expected to have a positive impact on the safety of the food product.

In order to further address the relationship between stunning and killing practices affecting welfare and any food safety hazards, further coordinated research is necessary.

Key words: Fish, stunning / killing, safety, welfare

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Directive 93/119/EC provides conditions for the stunning and killing of farm animals. Fish are legally part of the scope of the EU legislation but no specific provisions were ever adopted.

Following a previous request from the Commission, EFSA issued in 2004 a scientific opinion[9] on the welfare aspects of the principal methods for stunning and killing the main commercial species of animals, including farmed fish. As regards farmed fish, this opinion concluded that "Many existing commercial killing methods expose fish to substantial suffering over a prolonged period of time" Furthermore, 'for many species, there is not a commercially acceptable method that can kill fish humanely". Moreover, the respective EFSA report highlighted that different methods for stunning and killing of farmed fish must be developed and optimised according to the species specific different needs and welfare aspects.

"Fish are often treated as one species when it comes to regulations and legislation governing welfare during farming or at slaughter. But, it is important to realise that a very wide number of species of fish are farmed, with an equally wide variety of ecological adaptations and evolutionary developments. These differences mean that different species fish reacts differently to similar situations. For example, at a given environmental temperature, some species like trout die relatively quickly when removed form water into air, whilst others like eels or marine flatfish can take several hours. Similarly, in electrical stunning situations, eels require a much larger amount of stunning current than trout or salmon to render them unconscious. Species differences need to be taken into account when adopting particular procedures. Processes must be developed and optimised with respect to welfare specifically for each species. For example, it would be as unreasonable to assume that a process developed for killing trout in freshwater would be suitable for killing tuna in the sea as it would be to assume that a system developed for quail would be effective on ostriches."

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

In view of the above, the Commission requests EFSA to issue a scientific opinion on the species-specific welfare aspects of the main systems of stunning and killing of farmed fish. The opinion should assess whether the general conclusions and recommendations of the 2004 opinion apply to the species of fish specified below. Furthermore, the above mentioned conclusions and recommendations should be updated in a species specific approach, integrating where possible reference to welfare indicators and to new scientific developments. Where relevant, the animal health and food safety aspects should be taken into account.

The following species should be considered:

- Atlantic Salmon *(Salmo salar)*
- Rainbow trout (*Oncorhynchus mykiss)*
- European eel *(Anguilla anguilla)*
- Gilthead seabream *(Sparus aurata)*
- European seabass *(Dicentrarchus labrax)*
- European turbot *(Psetta maxima)*
- European Carp *(Cyprinus carpio)*
- Farmed tuna *(Thunnus spp.)*

[9] www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775454.htm

ACKNOWLEDGEMENTS

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ASSESSMENT

1. Introduction

1.1. General relationship between animal welfare and food safety

Existence of a link between animal welfare - particularly as affected by stress and nutrition - and susceptibility to microbial infection, has been recognised for a long time. Food-producing animals, particularly those in intensive farming, are affected by stress that can be due to "natural" or production-related causes. The scientific basis of the link between poor animal welfare and increased susceptibility to infection is complex and relatively poorly understood, but two aspects appear to be particularly relevant: stress-mediated suppression of immune function, and production of neuroendocrine hormones (host- and/or pathogen-related) sensed by pathogens and stimulating their responses including enhanced growth and/or virulence. It is known that a number of farming-related factors e.g. those associated with management practices and husbandry conditions may impose stress on animals. These include handling by humans, inadequate feeding, water temperature and quality, crowding, etc. The response to the stressors is hormone disbalance, osmoregulation disruption, suppression of the inflammatory response (immuno-suppression), and as a result the fish is more susceptible to disease and possibly prone to pathogen carriage and this may have an effect in the safety of fish products (Erikson, U, 2001; Pottinger, TG, 2001).

Fundamental aspects of the relationship between animal welfare and food safety, as indicated in previous BIOHAZ scientific opinions on food safety aspects of different husbandry systems (EFSA, 2007, 2008), are universally applicable to farming of all farm animal species. It is generally considered that use of farming systems based on good hygienic practices including provision of optimal animal welfare increases the animals' resistance to infections and leads to a reduction of the food safety risks associated with the resulting foods of animal origin. In other words, in principle, on-farm animal welfare assurance contributes to the resulting food safety assurance.

1.2. Scope of this opinion

The Animal Health and Welfare Panel (AHAW) has addressed animal welfare aspects of the main systems for stunning and killing for eight species (Atlantic salmon, gilthead sea bream/sea bass, rainbow trout, carp, European eel, tuna and turbot) in form of seven separate scientific opinions (EFSA, 2009a, b, c, d, e, f, g)[2,3,4,5,6,7,8]. In this opinion, the BIOHAZ Panel has focused on the food safety relevance of the stunning and killing factors relating to fish welfare, in an individual opinion incorporating all aspects addressed by the AHAW Panel opinions. There is very limited or unavailable evidence of a quantifiable and directly applicable relationship between animal welfare-relevant factors (at slaughter) and food safety hazards (at consumption). Therefore, only brief considerations based on general principles are presented in this opinion. The terms of reference of the mandate indicate that the focus of the risk assessment should be with the stunning and killing systems and conditions.

Pre-slaughtering practices (fasting, harvesting, transport, etc) will not be considered in detail in this opinion, but they could also influence the safety of fish products. Microbiota initially present in harvested fish depends on the surrounding environment at the time of harvest. Indigenous contamination in the aquatic environment where the fish is caught is influenced by temperature, salinity, oxygen content, presence of organic matter, phyto- and zooplankton, amongst others; also environmental contamination sources (e.g. run-off or sewage waters) may account for part of the microbiota initially present in fish (EFSA, 2008; Howgate, P, 1998; Huss, HH *et al.*, 2003[10]; WHO, 1999[11]).

Post-slaughtering and processing stages will not be considered in this opinion but they are an important additional source of microbial contamination. The experts acknowledge that some biohazards, such as *Listeria monocytogenes*, would be of greater importance during post-harvest stages. Intrinsic and extrinsic conditions (pH, tissue composition, storage temperature and atmosphere, etc.) during these stages affect to a large degree the evolution of microflora during shelf-life of fish, in particular in skin surfaces and gills.

In this opinion, only biological risks have been assessed. As consideration of the occurrence and principles of control of chemical residues (including mycotoxins, and marine toxins) in farm animals is outside the mandate of the BIOHAZ panel, these issues will not be dealt with in this opinion.

2. Main biological food safety hazards associated with fish stunning and killing

Specific biological hazards associated with stunning and killing include those which are indigenous to the aquatic environment where these operations are carried out (*Vibrio, Aeromonas*, *Plesiomonas*, non-proteolytic *Clostridium botulinum*) or occurring as result of faecal contamination, such as farms located in polluted areas, use of excreta as fertilizers, faecal effluents from human sewage, animal farms or wild animals (*Salmonella, Shigella,* pathogenic *Escherichia coli*, *Yersinia*). Other pathogens (such as *Listeria monocytogenes,* proteolytic types of *C. botulinum*, etc.), are ubiquitous bacteria and can have access to fish tissues (cross-contamination) when penetrative mechanical methods or exsanguination is used in stunning / killing.

All these bacteria may be present in sites where the fish is handled and harvested before or during stunning/killing and therefore may reach internal parts of the fish when incisions or wounds are made as a result of stunning/killing operations. There is also a chance that biochemical *post-mortem* changes in tissues can provide favourable conditions for bacterial growth when inadequate *ante-mortem* handling or stunning generates a stress response.

A comprehensive list of hazards identified in aquaculture production systems, and a qualitative estimation of their associated risk, based on published literature has been recently covered in the BIOHAZ Opinion EFSA-Q-2008-297 (EFSA, 2008). The former opinion concluded that fish farmed in Europe have a good record of safety with respect to biological hazards, and that the food safety risk associated with aquaculture products is frequently very low.

3. *Post-mortem* chemical changes in fish

Immediately after fish death a series of changes develop in muscle. *Post-mortem* chemical changes involve glycolysis, enzymatic activity (proteolytic and lipolytic) on various substrates, nucleotide catabolism, and their overall consequences in pH drop and increase in concentration of free non-protein nitrogen (NPN) compounds. As a consequence fish tissues may contain high levels of NPN which are readily available to support bacterial growth. Levels of nucleotides in fish tissues have a direct relationship to quality and spoilage. Lipids can undergo hydrolysis and oxidation. These reactions are either non-enzymatic or enzyme-mediated (microbial or own fish enzymes) (Poli, BM, 1999).

The biochemistry of the muscle *post-mortem* and the onset of *rigor* are influenced by the method used in pre-slaughter handling and stunning / killing of fish (Lowe, RM *et al.*, 1993; Proctor, MRM and McLoughlin, JV, 1992; Robb, DHF and Warriss, PD, 1997; Wills, CC *et*

[10] www.fao.org/docrep/006/y4743e/y4743e00.htm
[11] www.who.int/foodsafety/publications/fs_management/aquaculture/en/index.html

al., 2004). Depending on glycogen reserves, *rigor mortis* starts some time after death. This can occur immediately when fish are starved or severely stressed. Severe, prolonged stress at slaughter time can deplete muscular energy reserves, which disrupts lactic acid production and alters final muscular pH. In this situation *rigor mortis* starts immediately or shortly after death. In case of acute stress, more lactic acid will be produced, therefore the pH value will decrease dramatically (Caggiano, M, 2000)[12]. Other additional factors affecting the onset and resolution of *rigor* are the storage temperature, the fish species, and the size of the fish. Stunning and killing methods can have an indirect effect on fish microflora due to *post-mortem* changes (in ***substances***: concentration of carbohydrates, phosphocreatine, ATP, nucleotides, aminoacids; or in ***properties*** and ***parameters***: pH, water holding capacity-WHC, K value[13]), which may accelerate or retard the growth of microflora (including pathogens) during storage.

Stunning and killing by hypothermia has been shown to accelerate the onset of *rigor*, while percussive stunning using a blow on the head gives a delay of up to 18 hours in trout (Azam, K *et al.*, 1989) and in salmon (Proctor, MRM and McLoughlin, JV, 1992). The stress caused by handling and crowding prior to slaughter affects the muscle glycogen content, the evolution of *rigor*, muscle compounds (lactate) and parameters (pH) in salmon, with lactate values significantly higher in crowded fish groups (Skjervold, PO *et al.*, 1999; Skjervold, PO *et al.*, 2001). Several parameters measured in the blood and body tissues have been used as stress indicators in seabream and salmon (levels of cortisol, lactate and glucose, pH, glycogen, phosphocreatine, ATP and its catabolites) and it is known that they are influenced by the pre-slaughter and slaughter stress conditions (Erikson, U *et al.*, 1997; Pottinger, TG, 2001; Tejada, M *et al.*, 2001).

When fish are exposed to stress before death, energy-rich compounds in the muscle are consumed during the anaerobic preslaughter activity, and therefore the muscle pH at slaughter is lower and the drop rate is faster. Stress caused by live exsanguinations and prolonged electrical stimulation gave rise to a rapid drop of muscle pH and onset of *rigor mortis* in turbot (Roth, B *et al.*, 2007) and salmon (Roth, B *et al.*, 2009), as compared to less stressing percussive system. No differences in pH were detected after 48h (salmon) or 72h (turbot). According to Morzel, M *et al.* (2003) flesh from turbot killed by percussive systems had a higher pH and higher water content in the very early stage of *post mortem* storage, but also a much delayed *rigor mortis.* In contrast, turbot killed by electricity entered most rapidly into *rigor mortis*; their flesh was significantly softer throughout the entire storage time and was also redder and darker. According to Ozogul, Y and Ozogul, F (2004) mean *K* values of rainbow trout slaughtered by percussive stunning were significantly lower than those slaughtered by the ice slurry method, which means a better quality and lower concentration of several metabolites (inosine and hipoxantine) which are an indication of fish freshness in muscle. Stunning and killing of eels in water performed with a combination of electricity and oxygen removal led to redder, firmer flesh with a higher pH, with reduced loss of freshness (evaluated by K value), as compared with desliming the fish in dry salt followed by evisceration (Morzel, M and van de Vis, H, 2002). Giuffrida, A *et al.* (2007) investigated the effects of several slaughter methods on the quality of fresh and smoked trout and fresh gilthead seabream during storage at 2ºC. Electrically stunned trout had slower ATP depletion of raw muscle and lower lipid oxidation in smoked product during storage. Gilthead seabream immersed in ice slurry after the harvest showed a more regular ATP depletion than in fish exposed to CO2. Killing tuna by shooting on the head from above the water, after confinement was shown to provoke acute stress and meat had higher depletion of muscular glycogen, causing a reduction of muscular pH and WHC

[12] http://ressources.ciheam.org/om/pdf/c51/00600291.pdf

[13] K value is used as a freshness quality index and it is calculated from ATP degradation products (inosine (Ino) plus hipoxantine (Hx) divided by the sum of ATP, ADP, AMP, IMP, Ino and Hx. In many fish species, ATP, ADP and AMP disappear shortly after death while IMP increases initially and then decreases as Hx and Ino increases. Values up to 0.65 are considered to indicate good quality for raw fish in most species.

(Messina, C and Santulli, A, 2007, 2008). Two stunning/slaughtering methods (ice asphyxia and carbon dioxide narcosis) for European sea bass were compared with asphyxia without ice to observe stress response and quality indicators (Acerete, L *et al.*, 2009). Only minor differences in quality were found, and lower pH values were recorded immediately after asphyxia slaughter, but differences were minor (0.2 pH units) after 96h.

Other studies have studied the influence of stunning/killing systems on fish quality. Since fish quality is not included in the assessment, only brief considerations are given below. There is evidence that inadequate handling before/during slaughtering may lead to quality changes (Robb, DHF *et al.*, 2000; Roth, B *et al.*, 2002; Sigholt, T *et al.*, 1997). Sensory changes are seen mostly in appearance, texture and odour. A series of characteristics in the general appearance (such as eye convexity, skin and eye brightness, gills' aspect, colour, mucus, etc.), smell and tissue condition are useful indicators of freshness and good reflection of *post-mortem* changes. Quality defects such as blood spotting (Roth, B *et al.*, 2005) are also attributable to the stunning/killing method.

4. Possible effects of stunning/ killing systems on microbiological safety of fish

A limited number of articles report on the influence of the stunning / killing system on the fish microbiology. Tejada & Huidobro (2002) studied three stunning methods (immersion in ice salt-water slurry, asphyxia in air and percussive stunning followed by immersion in ice plus water) and *post-mortem* handling (gutting) in gilthead seabream. Depending on the slaughter method slight differences in microbial groups investigated (*Enterobacteriaceae* and total viable counts) were found, and no effect was detected on biochemical indices, physicochemical parameters or sensory properties. Onset of *rigor* in fish killed by percussion was delayed. Gutting had a much larger influence on microbiology as compared to non-gutted fish. Ozogul, Y and Ozogul, F (2004) found no significant differences in total viable bacterial count of trout stored in ice and modified atmosphere packaging, regardless of the slaughter methods (percussive stunning and death in ice slurry). Scherer, R *et al.* (2006) compared two slaughter methods (immersion in ice-water slurry and electrical stunning followed by ice slurry asphyxiation) for grass carp (*Ctenopharyngodon idella*). No significant differences in the shelf-life were observed between the methods investigated.

Table 1 summarises the main stunning and killing methods used in farmed fish as evaluated by the AHAW Panel opinions (EFSA, 2009a, b, c, d, e, f, g) and the potential food safety implications. Killing methods for the purpose of disease control are not considered in this assessment.

Table 1. Main stunning and killing methods used in farmed fish and the potential food safety implications

Method	Used in fish species*	Potential negative Food Safety Considerations	Source of information if available (for fish or for other animal species)
Electrical			
Electrical stunning	Salmon, trout, sea bass/bream, turbot, Carp.	Unlikely if done out of water. If performed under immersion microbiological quality of water should be taken into account.	Yes, some inconclusive information for carp (Roth, B *et al.*, 2009; Roth, B *et al.*, 2007)
Whole body electrical stunning in water with desliming and evisceration	Eel	Unlikely. Microbiological quality of water should be taken into account. Immediate evisceration would be beneficial for hygiene of fish.	Not Available.
Experimental electrical stunning	Eel	Unlikely. Microbiological quality of water should be taken into account.	Not Available.

Mechanical			
Percussive stunning	Salmon, trout, turbot, carp	Unlikely	Not available for fish. Yes for similar method for mammals
Captive needle method	Eel	Spread of microbial contamination from wound to tissues is theoretically possible. Also microbial cross-contamination of fish through handling or via the needle is theoretically possible.	Not available for fish. Yes for similar method for cattle and sheep.
Shooting under water with power head, or from outside the water	Tuna	Spread of microbial contamination from wound to tissues is theoretically possible.	Not available for fish. Yes for similar method for cattle and sheep.
Coring or spiking	Tuna	Spread of microbial contamination from wound to tissues is theoretically possible. Also microbial cross-contamination of fish through handling, or via the needle is theoretically possible.	Not available for fish. Yes for similar method for cattle and sheep.
Asphyxia			
Asphyxia in ice / ice slurry	Salmon, trout, sea bass/bream, turbot	Unlikely. Microbiological quality of water should be taken into account. Some biochemical *post-mortem* changes in tissues can result from stress responses.	Yes for fish (Ozogul, Y and Ozogul, F, 2004; Tejada, M and Huidobro, A, 2002)
Asphyxia in air	Sea bass/bream, Carp	Unlikely. Some biochemical *post-mortem* changes in tissues can result from stress responses.	Yes for fish (Acerete, L *et al.*, 2009)
Exsanguination	Salmon, trout, turbot	Microbial cross-contamination of fish through handling, or via utensils is theoretically possible.	Not available for fish. Yes for similar method for other farm animals.
Decapitation / neck cut with evisceration	Eel	Microbial cross-contamination of fish through handling, or via utensils is theoretically possible.	Not available for fish. Yes for similar method for other farm animals.
Pharmacological methods	Salmon, trout	Unlikely microbiological negative effects. Potential chemical residues (outside the scope of this document)	Not Available.
Carbon dioxide	Salmon, trout, sea bass/bream	Unlikely. Potentially beneficial effects in microbiological quality of water, and decrease in pH in blood.	Not available for fish. Yes for similar method for pigs (decrease pH in blood).
Other combined methods			
Live chilling combined with carbon dioxide sedation	Salmon, trout, sea bass/bream	Unlikely. Microbiological quality of water should be taken into account. Some biochemical *post-mortem* changes in tissues can result from stress responses.	Not Available.
Salt bath, desliming and evisceration	Eel	Microbiological quality of water and salt should be taken into account. Possible contamination with halotolerant pathogenic microorganisms	Not available for fish. Yes for shellfish (Martinez-Urtaza, J. and Liebana, E., 2005a; Martinez-Urtaza, J. and Liebana, E. , 2005b).
Ammonia, washing and evisceration	Eel	Unlikely. Potential beneficial effect for microbiological quality of water.	Not Available.

Immobilization by exposure to ice (and salt), washing and evisceration	Eel	Unlikely. Microbiological quality of water should be taken into account. Some biochemical *post-mortem* changes in tissues can result from stress responses.	Not Available.
Chilling and freezing	Eel	Unlikely. Microbiological quality of water should be taken into account. Some biochemical *post-mortem* changes in tissues can result from stress responses.	Not Available.

*According to AHAW Opinions

Potential for microbial cross-contamination associated with salt bath, desliming and evisceration. A study has shown that the use of low quality salt can be a source of *Salmonella* Senftenberg for sea-food processing facilities. This could further contribute as a source of contamination of coastal waters (Martinez Urtaza, J and Liebana, E 2005a,b). Therefore salt could potentially be a vehicle of contamination of eel.

Potential for microbial cross-contamination associated with stunning of fish using invasive (penetrating) mechanical methods. In case of slaughter of mammalian farm animals, it is well known that any slaughter/dressing-related that involve penetration of the microbiologically contaminated skin, such as when using penetrating captive bolt method for stunning, carry a risk of introducing pathogenic bacteria from the skin onto/into edible parts of the animal directly or via blood circulation (Buncic, S *et al.*, 2002). In an early study, marker bacteria had been inoculated on bolt of a penetrative captive bolt pistol and subsequently were found in spleens of stunned animals (Mackey, BM and Derrick, CM, 1979). More recently, when animals (lambs) were inoculated with marker bacteria into the brain through the stun wound immediately after stunning by a cartridge-operated, penetrative captive bolt pistol, these organisms were found in internal organs, lymph nodes and deep muscles (Buncic et al., 2002). Moreover, when the pistol which had been used to stun one brain-inoculated lamb was used to stun consecutive, non-inoculated lambs, the marker organisms were found in stun wounds and blood of the latter. By analogy with mammalian animals' situation, it could be hypothesised that when invasive mechanical stunning methods, involving penetration of skin and brain, are used in fish, similar direct or blood circulation-mediated introduction of pathogenic bacteria from the skin (and the water if the fish is submerged) into fish edible tissues could occur. To date, however, no studies on penetrating stunning-mediated microbial contamination of fish have been published.

Potential for microbial cross-contamination associated with exsanguination of fish. In case of slaughter of mammalian farm animals, it has been demonstrated that bacterial contamination of edible tissues, via blood circulation, can occur if contaminated knife is used for sticking (Jensen, LB, 1945; Labadie, J *et al.*, 1977; Mackey, BM and Derrick, CM, 1979). Consequently, sterilization of sticking knives in hot water before sticking has become a regulatory requirement for abattoirs. By analogy with mammalian animals' situation, it could be hypothesised that when non-sterilised knives/tools are used to cut blood vessels in fish (i.e. for exsanguination), similar blood circulation-mediated introduction of pathogenic bacteria from the skin into fish edible tissues could occur. To date, however, no studies on exsanguination-mediated microbial contamination of fish have been published.

The food safety relevance of invasive stunning- and exsanguination-associated microbial cross contamination. The health risks posed by pathogenic bacterial cells present in deep parts of fish flesh (if internally contaminated during penetrating stunning/exsanguination) would be higher than in case of the bacterial cells present only on the skin surface. Namely, the meat surface is normally cooked to higher temperatures than the meat centre (particularly with milder cooking commonly used for fish), so any pathogens in the centre have a better chance to survive (Buncic, S *et al.*, 2002). However, published experimental studies on invasive stunning- and exsanguination-associated microbial cross contamination involved inoculation of related tools with high levels of marker bacteria, but such levels do not necessarily reflect actual (probably lower) levels of bacterial contamination of the tools under commercial conditions. Therefore, those studies provide a proof that microbial meat safety risks associated with penetrating stunning/ exsanguination of mammalian food animals exist, but the results do not enable quantification of these risks (Buncic, S *et al.*, 2002).

Potential effects of fish stunning with carbon dioxide in water on microflora. Carbon dioxide is an acidic gas i.e. when dissolved in water produces carbonic acid so, for example, the stun-tank sea water containing 200-500 mg CO_2 per litre used for salmon stunning has pH values between 5.5 and 6.0 (Erikson, U, 2008). It could be expected that this acidic water is to some extent inhibitory for microorganisms in the water and on fish skin, compared with neutral pH water, but published information on such effects (potentially beneficial from fish hygiene perspective) is lacking. On the other hand, it is known that high fish activity in carbon dioxide stun bath routinely results in haemorrhagic damage of gills (Robb, DHF and Kestin, SC, 2002). It could be expected that this could lead to introduction of waterborne microorganisms into blood circulation of the fish, but no related published information is available. Furthermore, a question could be raised as to whether fish breathing-in the acidic carbon dioxide stunning water leads to any decrease of pH in blood or edible tissues of the fish, which could be beneficial from the meat hygiene/safety perspective, but no related published information is available. In pigs stunned by carbon dioxide, this chemical is carried in the blood in a readily available form and passed to the cerebrospinal fluid that bathes the brain and the spinal cord, where it increases acidity i.e. decreases the pH from 7.4 in normal (conscious) pigs to 7.1 (onset of anaesthesia) or below 6.8 (deep anaesthesia) (Wotton, S, 2006), but there are no indications of associated drop in pH of the meat. Overall, currently available knowledge is insufficient to assess the food safety relevance of carbon dioxide stunning of fish.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. Based on general principles of food hygiene, some fish stunning and killing related factors (e.g. microbial contamination of water, increased handling, invasive stunning and exsanguination methods) could lead to increasing the risk of microbial contamination of fish.
2. As the scientific information on specific welfare hazards of the stunning and killing practices that could compromise safety of fish products is very limited, a definitive assessment of the food safety risks associated with different stunning and killing methods for fish is not possible at this time.
3. Measures intended to preserve fish welfare by avoiding stress during stunning and killing and improving environmental conditions, are expected to have a positive impact on the safety of the food product.

RECOMMENDATIONS

In order to further address the relationship between stunning and killing practices affecting welfare and any food safety hazards, further coordinated research is necessary.

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