

SPECIES-SPECIFIC WELFARE ASPECTS OF THE MAIN SYSTEMS OF STUNNING AND KILLING OF FARMED ATLANTIC SALMON

Scientific Opinion of the Panel on Animal Health and Welfare

(Question N° EFSA-Q-2006-437)

Adopted on 20th of March 2009

PANEL MEMBERS

Bo Algers, Harry J. Blokhuis, Anette Bøtner, Donald M. Broom, Patrizia Costa, Mariano Domingo, Mathias Greiner, Jörg Hartung, Frank Koenen, Christine Müller-Graf, David B. Morton, Albert Osterhaus, Dirk U. Pfeiffer, Mohan Raj, Ronald Roberts, Moez Sanaa, Mo Salman, J. Michael Sharp, Philippe Vannier and Martin Wierup.

SUMMARY

Following a request from the European Commission, the Panel on Animal Health and Welfare was asked to deliver a scientific opinion on welfare aspect of the main systems of stunning and killing of farmed Atlantic salmon (*Salmo salar*) in the EU.

Harvesting and processing of farmed Atlantic salmon are the same as for sea-farmed rainbow trout (*Oncorhynchus mykiss*); therefore this scientific opinion and its conclusions can also be applied to the sea farmed trout¹ production.

A semi-quantitative risk assessment approach was used to rank the risks of poor welfare associated with the different commercially applied stunning and killing methods for Atlantic salmon. The risk assessment was also used to identify other areas of concern, as well as to provide guidance for future research. The risk assessment was mainly based on expert opinion, due to the limited amount of quantitative data and published peer-reviewed data on many effects of hazards associated with killing of Atlantic salmon. Pre-slaughter stages which have a direct impact on the welfare immediately before and during killing were included in the risk assessment. Stunning and killing methods that are not commercially used in Europe (e.g. carbon monoxide) were described but not included in the risk assessment. The opportunity to develop new methods for slaughtering Atlantic salmon is considerable and should be encouraged.

¹ Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on welfare aspect of the main systems of stunning and killing of farmed Atlantic salmon. The EFSA Journal (2009) 1012, 1-77

The five stunning and killing methods assessed were: 1. Percussive stunning; 2. Electrical stunning; 3. Carbon dioxide; 4. Live chilling; and 5. Asphyxia in ice slurry. All methods are followed by exsanguination.

The most important hazards in the pre-slaughter phase were associated with crowding and transfer by pumping. Excessive crowding will result in poor welfare. There is a high risk that salmon are subjected to metabolic stress, handling stress and poor welfare (exhaustion) prior to slaughter. Exposing salmon to air causes a major negative impact on their welfare and should be avoided. Crowding of fish should not be performed to a level that they show signs of distress. Indicators for distress are; colour change, escape behaviour and air gulping. Fish should be monitored when exiting the pumping system where the presence of fresh injuries and exhaustion are indicators of poor welfare. After pumping, there should be visual checks for wounds and injuries and any causes of these rectified.

Two to three days of fasting are needed to reduce the metabolic rate and thus the physical activity of the fish which may reduce distress associated with transport. Too short or too long transport and resting period may be an issue in association with the duration of the fasting period. Food deprivation can result in the utilisation of body fat reserves and even functional tissue which is associated with poor welfare.

There will always be a certain risk of poor welfare involved when fish are transported live to slaughter. In closed systems, there are a number of issues that need to be addressed to ensure good fish welfare at slaughter such as to ensure good water quality, e.g. adequate levels of dissolved oxygen. The effect of elevated levels of carbon dioxide, ammonium and total organic carbon, as well as low pH on the welfare of the fish needs to be addressed. If fish are transported under good conditions then the fish may recover from crowding and handling during the transport and thus, transport will not affect fish welfare at slaughter.

Regarding the stunning and killing methods, percussive methods and electrical stunning were assessed to reliably cause unconsciousness in the vast majority of salmon.

In hand held manually fed percussive systems the hazard causing the highest risk for poor welfare is asphyxia. For automated percussive stunning the main hazard is variation of size within the population causing a mis-stun in some fish, e.g. hitting the snout on larger fish. Machines for stunning and killing salmon should not be used if fish may be injured, not stunned or not rapidly killed because of their size or orientation in the machine. For percussive machines, size adjustment of the machines should be done by skilled personnel as it is crucial for stunning efficiency. Percussive systems should have a separate air supply or alternatively have security valves to block the system if the pressure is reduced below a certain threshold.

For electrical stunning the hazard is using too low electrical currents causing paralysis and insufficient stunning. In electrical dry stunners intended for head only application, fish entering tail first will consciously feel the electricity for a few seconds before the head reaches the stunner and thus welfare is poor. There is some risk of poor welfare when applying electrical stunning in water (batch) systems mainly due to mis-stuns or exhaustion due to exposure to electrical current. For electric stunning minimum requirements of the electric field or current should be sufficient to cause an immediate loss of consciousness, i.e. within 1 second. Moreover, after electrical or percussive stunning fish should not recover consciousness before being killed by exsanguination or maceration

Severance of all gill arches on both sides of the fish, or the isthmus, or piercing the heart directly, appears to be the best methods for killing by bleeding out unconscious fish. Exsanguination should be carried immediately after stunning and in every case before

recovery from stunning occurs. It is essential that a sharp knife is used to cut the vessels. Exsanguination without prior stunning is not humane and should not be used.

All stunning systems should have an appropriate backup system to enable an immediate correction from a mis-stun.

Carbon dioxide, asphyxia on ice and asphyxia are the methods resulting in the poorest welfare. Carbon dioxide has the highest risk score because not only was it judged that exposure to the gas causes a strong adverse reaction but it does not reliably result in unconsciousness, thus salmon may be bled or eviscerated when conscious. Killing salmon by asphyxia is judged to be a severe hazard.

Disease control methods used are: pharmacological (overdose of anaesthetics), electrical and maceration all of which should be considered as part of contingency plans. In some cases, slaughter may be performed by normal stunning and killing procedures. In order for an overdose of anaesthetic to be a reliable and humane killing method for salmon more knowledge is needed before being able to recommend minimum dosage and exposure times for specific life stages, body size and water temperature. Such information would help to ensure a minimum time to loss of consciousness and minimum induction of stress. Fish should be stunned or be killed before using mills for maceration.

Some indicators of poor welfare may be used to assess welfare of salmon slaughter under commercial conditions. Standard operating procedures to improve the control of the slaughter process to prevent impaired welfare should be introduced and validated, robust and practically feasible welfare indicators should be further developed.

Key words: fish, animal welfare, risk assessment, pre-slaughter, stunning, killing, slaughter, disease control, Atlantic salmon, *Salmo salar*, rainbow trout, *Oncorhynchus mykiss*

TABLE OF CONTENTS

Panel Members	1
Summary	1
Table of Contents	4
List of Tables	5
List of Figures	6
Background	8
Terms of Reference	8
Acknowledgements	9
Assessment	10
1. Scope and objectives of the scientific opinion	10
2. Pre-slaughter process with direct implications for stunning	10
2.1. Pre-stunning procedures having an impact on stunning and killing	10
2.2. Crowding before pumping	11
2.3. Transfer of fish to the stunning unit	11
3. Stunning and killing systems for farmed salmon	13
3.1. Recognition of consciousness, unconsciousness and death	13
3.1.1. Field methods for determining unconsciousness and insensibility in fish	13
3.1.2. Assessment of aversion to a stunning or killing method	15
3.2. Purpose of slaughter	17
3.2.1. Fish for human consumption	17
3.2.2. Fish not for direct human consumption	17
3.2.3. Emergency slaughter	17
3.2.4. Killing of Broodstock	18
3.3. Specific stunning and killing methods for farmed salmon	18
3.3.1. Carbon dioxide (CO ₂)	18
3.3.1.1. Principle of the method	18
3.3.1.2. Carbon monoxide (CO) killing	19
3.3.2. Live chilling	19
3.3.2.1. Live chilling without the effect of carbon dioxide	20
3.3.3. Percussive stunning	21
3.3.3.1. Principles of percussive stunning	21
3.3.3.2. Percussive stunning in relation to mass slaughter	21
3.3.4. Electric stunning or Stunning and Killing systems	22
3.3.5. Bleeding out / Exsanguination	23
3.3.5.1. Principles of the method	23
3.3.5.2. Exsanguination in relation to mass slaughter	23
3.3.6. Pharmacological methods	24
3.3.7. Maceration	25
3.4. Exposure to procedures at pre-slaughter and slaughter (Questionnaire)	25
4. Application of the risk assessment approach to stunning and killing of Atlantic salmon	25
4.1. Pre-slaughter hazards	26
4.1.1. Salmon slaughtered directly on arrival at the abattoir	26
4.1.2. Salmon in holding cages at the abattoir	27
4.2. Slaughter and stunning hazards	29
4.3. Discussion and conclusions	33
5. Reference to welfare indicators	34
Conclusions and recommendations	36
References	40
Appendix A: Pre-slaughter steps	45
Appendix B: Risk assessment approach	50
Appendix C: Description of hazards related to salmon stunning and killing	57
Appendix D: Parameters used in producing risk and magnitude scores for welfare hazards	62

Appendix E: Risk scores and magnitude of adverse welfare effects associated with stun/kill methods . 69
 Appendix F: Relevant data from the questionnaire..... 74
 Glossary and abbreviations 76

LIST OF TABLES

Table 1. Method for assessing the state of consciousness of fish at slaughter (Kestin et al., 2002) 14

Table 2. Risk and magnitude scores for welfare hazards associated with pre-slaughter management in Atlantic salmon in Europe, in situations where the fish are directly processed as they arrive at the abattoir..... 26

Table 3. Risk and magnitude scores for welfare hazards associated with preslaughter management in Atlantic salmon in Europe, where the fish are in holding for an average of 2 days (48 hours) before they are processed further. 28

Table 4. Risk and magnitude scores for welfare hazards associated with the main stunning/killing methods for Atlantic salmon (*Salmo salar*) in Europe. 31

Table 5. Risk and magnitude scores for welfare hazards associated with the main stunning/killing methods for Atlantic salmon (*Salmo salar*) in Europe, cont'd..... 31

Table 6. Operational indicators (to be used under field conditions) of poor welfare for critical monitoring points. 34

Table 7. Intensity categories for adverse effects arising from hazards associated with pre-slaughter / slaughter operations in Atlantic salmon. 52

Table 8. Duration categories for adverse effects arising from hazards associated with pre-slaughter operations in Atlantic salmon..... 53

Table 9. Duration categories for adverse effects arising from hazards associated with slaughter of Atlantic salmon 53

Table 10. Scoring system for total uncertainty in severity and duration of effect 54

Table 11. Description of hazards related to pre-slaughter management in Atlantic salmon (*Salmo salar*) in Europe. 57

Table 12. Description of hazards related to slaughter management of Atlantic salmon (*Salmo salar*) in Europe. 58

Table 13. Parameters used in producing risk and magnitude scores for welfare hazards associated with preslaughter management in Atlantic salmon (*Salmo salar*) in Europe, where the fish are directly processed as they arrive at the abattoir. 62

Table 14. Parameters used in producing risk and magnitude scores for welfare hazards associated with preslaughter management in Atlantic salmon (*Salmo salar*) in Europe,

where the fish are in holding for an average of 3 days before they are processed further.
 63

Table 15. Parameters used in producing risk and magnitude scores for welfare hazards associated with slaughter methods applied to Atlantic salmon (*Salmo salar*) in Europe.
 64

LIST OF FIGURES

Figure 1. Pathways for pre-slaughter steps from the rearing cage to the processing lines. Dots represent events occurring on a time line from left to right. 13

Figure 2. Time course of the physiological stress response in fish. 16

Figure 3. Risk score and magnitude of adverse welfare effect for individual hazards associated with pre-slaughter management in salmon in Europe, where the fish are directly processed as they arrive at the abattoir. Hazards are ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard. 27

Figure 4. Risk score and magnitude of adverse welfare effect for individual hazards associated with pre-slaughter management in salmon in Europe, where the fish are held for an average of 2 days (48 hours) before they are processed further. Hazards are ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard. 29

Figure 5. Sum of risk scores and magnitudes of the adverse welfare effect for main slaughter methods applied to salmon in Europe, ranked by the sum of the risk score. 33

Figure 6. Time to unconsciousness (insensibility) following stunning / killing (horizontal grey line indicates consciousness threshold above which killing takes place without an adverse effect). 51

Figure 7. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of fully automatic percussive stunning (swim-in) systems (method A) in Atlantic salmon, ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard. 69

Figure 8. Risk score and magnitude of adverse welfare effect for individual hazards associated with hand fed percussive stunning systems with automatic cut (Method B) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard. 69

Figure 9. Risk score and magnitude of adverse welfare effect for individual hazards associated with hand fed percussive stunning systems with manual cut (method C) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated

minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard..... 70

Figure 10. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of live chilling combined with carbon dioxide (Method D) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard..... 70

Figure 11. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of carbon dioxide only (method E) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard..... 71

Figure 12. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of electrical stunning - in-water (batch) systems (method F) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard..... 71

Figure 13. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of electrical stunning - dry systems (method G) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard. 72

Figure 14. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of electrical stunning – pipe line systems (method H) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard. 72

Figure 15. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of metocaine or benzocaine (two pharmacological preparations) (method I) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard. 73

BACKGROUND

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 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 from 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

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 auratus*), European seabass (*Dicentrarchus labrax*), European turbot (*Scophthalmus maximus*), common carp (*Cyprinus carpio*), and farmed tuna (*Thunnus* spp.).

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion: Bo Algers (Chairman), Ann Lindberg (Risk Assessor), Ulf Erikson, Anders Kiessling, and Bjørn Roth.

Mohan Raj (AHAW Panel Member) is also acknowledged for his contribution to Chapter 3.

Jeff Lines is gratefully acknowledged for providing useful technical information and comments on the scientific report.

The AHAW Panel also would like to thank the Member States and stakeholders organisations for the valuable comments which were evaluated by the WG Members and when considered pertinent included in the Scientific Opinion.

The scientific coordination for this Scientific Opinion has been undertaken by the EFSA AHAW Panel Scientific Officers Oriol Ribó, Sofie Dhollander, Ana Afonsa, Tomasz Grudnik, and particularly Jordi Tarrés-Call, Ingfrid Slaatto Naess, and Franck Berthe.

ASSESSMENT

1. SCOPE AND OBJECTIVES OF THE SCIENTIFIC OPINION

The scope of this report is the welfare aspects of the killing of farmed Atlantic salmon, *Salmo salar*.

The objective is to briefly describe the current salmon slaughter practices, to identify welfare hazards and to assess welfare risks associated with those practices through a risk assessment approach. In addition, the aim is to identify suitable welfare indicators at slaughter where they exist.

The pre-slaughter process is only considered where evidence exists for a direct impact on welfare at stunning and killing. Where fish welfare, immediately before and during killing or stunning and slaughter, is affected, it has also been considered as part of the slaughter process. Therefore, the welfare aspects of the farming phase as well as the transport of salmon are not included in this report. Hence, the pre-slaughter period is briefly reviewed in the Appendix A.

Emergency killing for disease control or other reasons is included in the report. However, humane killing of individual fish, in the course of farming operations (i.e. sorting, grading, or background morbidity) is not included.

Much of what has been written about salmon in this report is also relevant for large rainbow trout² production in sea water. Their physiological stress response is similar but is reported to be expressed more vigorously in rainbow trout.

The meat quality is not part of the assessment although, references are provided in the text that could be used and evaluated for further socio-economic studies on slaughtering methods for salmon.

Meat quality and safety are not part of the assessment. Food safety issues are addressed by the BIOHAZ panel of the EFSA.

In drafting this Scientific Opinion, the panel did not take into consideration any ethical, socio-economic, human safety, cultural or religious or management issues, the emphasis has been to look at the scientific evidence and to interpret that in the light of the terms of reference. Nevertheless, it is acknowledged that such aspects can have an important impact on animal welfare.

2. PRE-SLAUGHTER PROCESS WITH DIRECT IMPLICATIONS FOR STUNNING

2.1. Pre-stunning procedures having an impact on stunning and killing

Killing and pre-slaughter treatment of salmon comprises several operations that can have a considerable impact on the welfare of the fish. If they are routinely fasted (1-2 weeks) and transported by well-boat using an open system (good water exchange), there are no particular reasons to assume their welfare at stunning and killing is affected (Erikson et al., 1997; Erikson, 2001; Farrell, 2006). Closed systems for transport (re-circulated water) may

² For citation purposes: Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on Species-specific welfare aspects of the main systems of stunning and killing of farmed carp. The EFSA Journal (2009) 1013, 1-77

facilitate slaughter but may also pose other welfare problems during transport (poor water quality issues) (Erikson, 2001).

It is clear that pre-stunning procedures (crowding and pumping) are often very stressful to the fish meaning they will often arrive at the stunning or killing units in a more or less exhausted state (Erikson, 2008; Mejdell et al., 2009). The major steps from farm cage to the slaughter plant are described in Appendix A.

For optimal operation, some of the new stunning or killing units require that the fish entering the unit are not stressed, otherwise proper operation will be difficult to achieve, and the welfare of the animal may be compromised.

2.2. Crowding before pumping

When the fish are to be slaughtered, they are pumped, either directly from the well-boat, or from the holding cage, typically located near the plant quayside. Where salmon are slaughtered on site, they are brailled or pumped directly to the stunner. New well boat technology using movable bulkheads can probably provide better fish welfare during unloading (see Appendix A). In fish cages, batches of salmon are collected using a sweep net to increase fish density. Today, pressure-vacuum pumps have largely replaced lift nets for this operation and salmon are sucked into a funnel-shaped entry to the hose (35-38 cm in diameter) leading to the pump. During one shift (7 hours), it is common to process fish from at least two cages and up to more than 150 metric tonnes can be processed per shift. For example, with an average fish size of 4 kg, this means that approximately 5000 fish are slaughtered every hour, or 1-2 fish every second.

Typically, fish density is often increased as the volume of the cage or other container is gradually decreased. Fish are typically exposed to this potentially stressful incident for a few minutes up to a few hours. If care is not exercised, the fish can be exposed to air, i.e. by lifting the net too close to the water surface and severe crowding can occur. The skill of the personnel is considered an essential factor in order to minimize handling stress during this operation but personnel should also realise that good fish welfare and convenience may not always go together. It is well-known that fish can change their skin colour as a response to a stressor and in fact, skin colour changes have been suggested as welfare indicators in aquaculture (Iger et al., 2001; Pavlidis et al., 2006). Often, as the salmon are subjected to crowding in the cage and pumping, their dorsal skin colour changes from grey/black to blue/green indicating a stress response.

Furthermore, it has been shown that white muscle pH tends to decrease as the fish density increases towards the end of the pumping operation, indicating the last batches of fish are more stressed as they are pumped on to stunning and killing operations (unpublished field observations). Crowding of salmon prior to live chilling and slaughter has been shown to significantly increase cortisol, glucose, lactate and osmolarity in blood plasma (Skjervold et al., 2001) and a loss of scales (probably also involving loss of mucus) is often seen.

Control points include: monitoring of fish behaviour (e.g. video), checking levels of dissolved oxygen, avoiding exposing fish to air and, if possible, avoiding excessive crowding.

2.3. Transfer of fish to the stunning unit

Salmon will respond to a current by actively swimming against it. This behaviour is an advantage in fish can be moved voluntarily by applying a water current, but it can also constitute a welfare issue. Pumping fish is a commonly used method to move fish by water

current. In situations where metal pipe in the pump have been replaced by Plexiglas tubes, counter current swimming behaviour is commonly observed (Roth. pers. com.). In theory a large salmon would be able to hold position against a current below 0.8 m/s indefinitely, for four hours against a current of 1-1.5 m/s and for tens of minutes against a current of >2-3 m/s (Hinch and Bratty, 2000). In addition, it is well known that large salmon in the wild will utilize turbulence, i.e. “riding the wave” and, thereby, are able to swim against or hold position in even faster currents (Hinch and Bratty, 2000). Recent research suggests that the salmon on average stay about 1 to 2 minutes in the pump system when transferred from holding pen to stunning area (Mejdell et al., 2009). Most salmon leaving from such vacuum pump systems after crowding are exhausted (Erikson, 2008; Mejdell et al., 2009) though whether it is a result of mental or physical (swimming against the current) stress is not known.

When the well-boat arrives at the processing plant, salmon are either transferred to the processing line or to holding cages by using a pressure-vacuum pump. If the fish are to be transferred to the processing line directly from the vessel, the vessel may stay for some hours at the quayside before the unloading process starts early next morning. In such cases, adequate water circulation is carried out using the vessel’s circulation pumps.

Since the holding cages also are used in a production planning context, fish can be kept there for a few hours up to a few days before they are processed. The fish are not fed during this period. Water quality is important at this stage of the collecting and killing procedure. During the summer season, holding cages are often oxygenated to cope with reduced oxygen solubility and increased fish oxygen demand as the water temperature increases. In sheltered areas, the water temperature can rise to 20 C. Oxygenation in tank environment results in accumulation of ammonia and CO₂ compromising welfare. To which extent it is relevant for cage environment it is not known.

Pressure-vacuum pumps have largely replaced lift nets for transferring fish from well-boat or holding cage to the processing line, and it has become more common to use twin pumps (in parallel) rather than using a single pump. The assumed advantage of using twin pumps is that salmon can be supplied to the processing line in a continuous flow of fish, but when a single pump is used, the flow of fish is temporarily stopped during the cycling between vacuum and pressure. White muscle pH values determined before and after pumping suggest that the use of twin pumps located at the level of holding cage (low lifting height on the suction side) is less stressful for fish than using a single pump, particularly if the lifting height is high (i.e. the pump is placed just outside the plant premises) (Erikson, 2008). Typically, the fish are pumped 100 - 150 m in hoses with a diameter of 35-38 cm. On the pressure side, the lifting height is typically about 5-8 m.

Proper design of the transfer system (hoses, pump, strainer, chutes etc) from holding cage to stunning unit is essential for good fish welfare as faulty constructions result in injuries such as excessive scale loss and bleeding snouts. The extent of such injuries varies considerably from plant to plant but few fish are severely injured or killed (Mejdell et al., 2009). Examples of poor construction leading to injury are the presence of inner pipe flanges, sharp bends and fish at high speed colliding with bulkheads, pipe walls etc, after the water has been drained off. Inadequate attention to clearing pipe lines after transport is also a potential welfare hazard. However, incidences of fish left / trapped in the pump system is likely to be low judging from observations of dead or injured fish being flushed out of the system when work is resumed the following morning.

Welfare related data include fish behaviour (if fish are excited: tail flapping; if fish are exhausted: passive lying on its side) and the presence of fresh external damages (fin, skin, body).

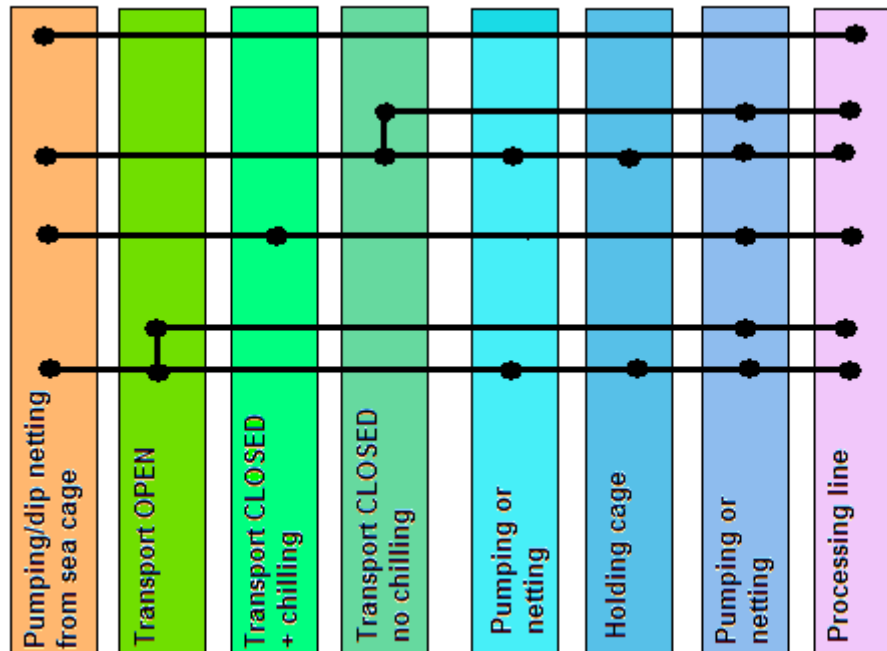


Figure 1. Pathways for pre-slaughter steps from the rearing cage to the processing lines. Dots represent events occurring on a time line from left to right.

3. STUNNING AND KILLING SYSTEMS FOR FARMED SALMON

3.1. Recognition of consciousness, unconsciousness and death

Stunning methods are supposed to induce immediate or rapid (less than 1 second) unconsciousness, and it is important for people involved in fish slaughtering operations to be able to recognise whether a stunning operation has rendered a fish rapidly unconscious. Such criteria have been published (Kestin et al., 2002).

For salmon, under practical field conditions, signs for the recognition for consciousness include respiratory movements (operculum and jaw) and other coordinated swimming movements (Kestin et al., 2002). Under field research conditions also VOR and response to touch / pain may be used; and in laboratory research EEG responses including visual evoked responses (VER) could be used.

3.1.1. Field methods for determining unconsciousness and insensibility in fish

The following section has been based on research mainly carried out on salmon and trout and so can be considered typical for salmon.

Following the application of a stunning or killing method, if a fish retains the capacity to quickly regain equilibrium when inverted (turned on its back while in water) or exhibits co-ordinated species specific swimming responses, or escape behaviour, or reacts to painful

stimulation with a needle, fin pinch or when handled, it cannot be considered to be unconscious (swimming is a sustained rhythmic motor activity, whereas escape is a ‘one-shot’ response that is much more rapid and forceful than swimming). Absence of these behaviours or responses is considered to be indicative of unconscious or death. But caution need to be exercised as some stunning methods may induce immobilization or paralysis, i.e. a loss of muscular coordination and / or spontaneous physical activity, without unconsciousness. Fish that are simply immobilized or paralysed would experience pain and suffering but are unable to show that behaviourally. Exposure to dissolved carbon dioxide has been reported to cause such an effect in fish (Kestin, Van de Vis and Robb, 2002). As in mammals and birds, certain reflexes mediated by the brain stem such as rhythmic breathing or corneal reflexes can be used to ascertain the effectiveness of stunning or return of consciousness following stunning. In fish, the vestibulo-ocular reflex (VOR) (commonly called eye roll) and breathing reflexes have been used as indicators of brain function (Kestin, Van de Vis and Robb, 2002). In the case of eye roll (VOR), the movement of the eye is observed when the fish is rocked from side to side. In a dead or unconscious fish, the eye remains fixed in the skull. In a fish retaining some brain function, the eye rotates dorso-ventrally when the fish is rocked. Similarly, in the case of ‘breathing’, the operculum and lower jaw of the fish is observed when the fish is either placed in water or kept in air. In a dead or unconscious fish, the operculum and lower jaw do not show any rhythmic movements but vibrations can be seen. Data concerning the abolition and return of VEPs following stunning and positive correlations between the presence or absence of the reflexes support the interpretation that these reflexes are lost during the period of unconscious. Thus, it can be concluded that if the eye roll/VOR reflexes and breathing are absent, a fish is probably dead or unconscious. At present there are no known or reported behavioural indicators that could be used for differentiating fish that are immobilized or paralysed (but still conscious) from those that are unconscious. Both unconscious and immobilized or paralysed fish exhibit positive VOR and respiratory movements. Therefore, to avoid pain and suffering, it is necessary to assume that a fish exhibiting these reflexes is conscious.

Table 1. Method for assessing the state of consciousness of fish at slaughter (Kestin et al., 2002)

Name	Self initiated behaviour		Response to Stimuli			Clinical reflexes	
	Swimming	Equilibrium	Handling	Pin prick	6V shock	Eye roll	opercula movement
Behaviour/reflex	Swimming behaviour	Righting ability	Response to handling	Response to prick on lip	Response to stimulation on lip	Vestibulo-ocular reflex (VOR)	Rhythmic opercular activity
Observation place	In water	In water	In water or air	In air or water	In air	In air	In water or air
Procedure	Observe spontaneous swimming behaviour	Invert fish, observe righting response	Attempt to catch by tail and administer tail pinch, observe response	Prick lightly on lip with enough pressure to cause pricking sensation to human, observe	Stimulate carefully on lip with 6V DC, observe response	Observe eye movement when fish is rolled from side to side through the vertical	Observe opercula for rhythmic movement (similar to breathing in mammals and birds)

			response				
Sequence of observation	1	2	3	5	6	7	4
Score 0*	No swimming	Unable to right	No response	No response	No response	Eyes fixed relative to head	No opercula movement
Score 1*	Slow or abnormal swimming e.g. upside down	Slow to right	Only slow or feeble response after tail pinch(s)	Slow and reduced response	Slow and reduced response	Partial VOR or one eye shows VOR	Slow or irregular movement
Score 2*	Normal swimming	Quickly rights	Immediate vigorous escape attempt on first touch/pinch	Head shake or escape attempt	Head shake or escape attempt	Eyes roll relative to the head whilst attempting to remain upright when fish is rolled	Regular opercula movement
			Some species show no response even when fish is fully conscious	1. Direct stimulation of muscles. 2. Some species show no response when fish is fully conscious	Needs careful observation in some species		Needs careful observation in some species

*General comments, possible artefacts: This scoring system is too simplistic, i.e. all the reflexes are either present or absent. Some comments regarding the presence of combinations of reflexes and their interpretation will be helpful.

Operational indicators suggested to be used under field conditions of poor welfare for critical monitoring points are presented in Table 6.

3.1.2. Assessment of aversion to a stunning or killing method

One of the fundamentals of humane slaughter is that death in an animal should be induced without causing pain, fear or distress. Stunning methods should ideally induce immediate unconsciousness. However, salmon show increased physical activity after exposure to carbon dioxide saturated water (Robb et al. 2000b, Roth et al., 2002) and it is assumed that the process is aversive to the fish, although the absence of such activity does not necessarily mean absence of aversion. Some methods induce immobilisation or exhaustion before unconsciousness, for example, rapid live chilling of Atlantic salmon or exposure to low doses of anaesthetics. In such cases, it may be necessary to examine the effect of the process on changes in stress hormone secretion or heart function. For example, cooling fish rapidly (as occurs in 'live chilling') leads to elevated plasma cortisol levels (Donaldson, 1981; Skjervold et al., 2001), indicating that it may be stressful, and over time to a disturbance of plasma osmolarity (Rorvik et al., 2001). Rapid live chilling results in a marked decrease in the muscle pH, that indicates increased muscle activity, and could be a sign of aversive activity (Skjervold et al., 2001). Considering the methodology used in these studies it is difficult to separate between the effect of cooling and the effect of low dose CO₂. The brain

mechanism(s) by which mixtures of ice and water or super cooled water induce unconscious has not been elucidated but, it is well known that they do not induce immediate loss of consciousness. The possibility that induction of unconsciousness with these methods would not occur without causing distress or suffering cannot be ruled out (Roth et al., 2009). In the absence of direct evidence, one has to rely on physiological stress responses (Figure 2, courtesy Dr. Lluís Tort) and their time course to ascertain the impact of these methods on the welfare of fish. Figure 2 indicates that changes, magnitude and time course, in cortisol levels and metabolites in blood and various tissues occur within seconds, minutes and even hours of exposure to a stressor, which should be considered in the evaluation of the humaneness of these methods. In general, the stress response in fish concerns the principal messengers or products of the activation of the hypothalamic-pituitary-inter-renal axis (HPI-axis) and the brain-sympathetic-chromaffin cells axis (BSC-axis), as reviewed by Wendelaar Bonga (1997).

Activation of the HPI-axis starts in neurons in the nucleus pre-opticus in the hypothalamus, which releases CRH (corticotrophin-releasing hormone) in the vicinity of the corticotrophe cells of the distal lobe of the pituitary. When stimulated with CRH, the corticotrophe cells secrete ACTH (adrenocorticotrophic hormone), the principal stimulator of cortisol release from the inter-renal cells of the head kidney, into the blood (Wendelaar Bonga, 1997). It is known that for fish, a time frame of minutes (see Figure 2) is required for cortisol to increase, whereas for catecholamines (Brain-sympathetic-chromaffin cell axis) their release occurs within seconds. A rapid change in temperature would prevent an increase of cortisol in the blood, as well as other metabolic changes in various tissues.

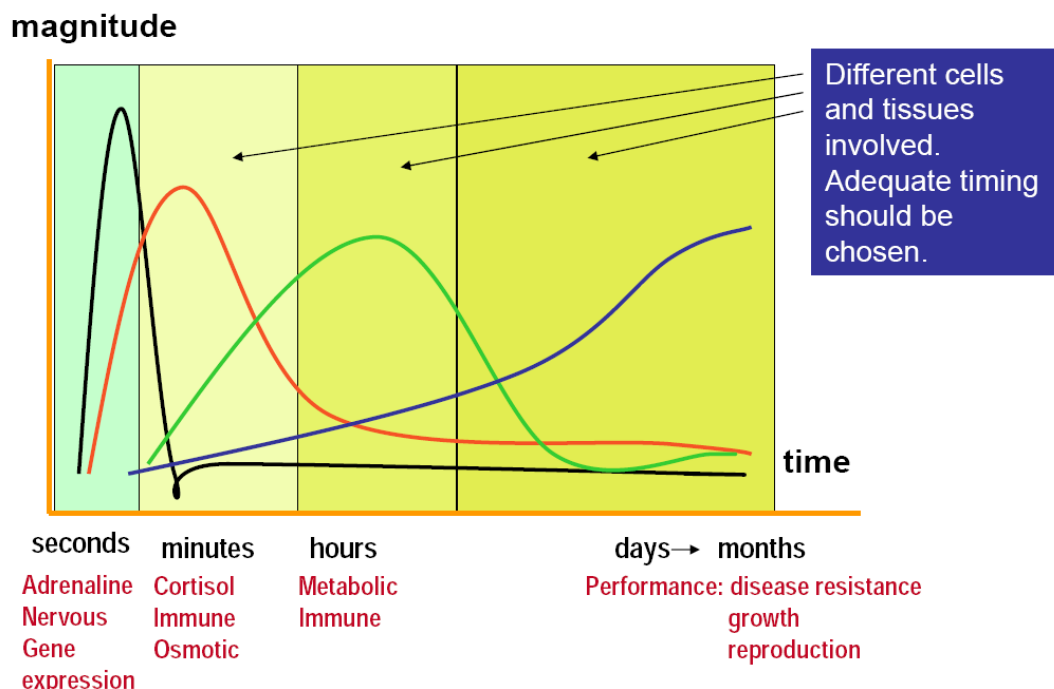


Figure 2. Time course of the physiological stress response in fish.

It should be emphasised that homeostasis is fine-tuned to a particular temperature of rearing (Van den Burg, 2002), and thus a rapid drop in temperature may jeopardize fish welfare. It is possible that changes in cortisol and other parameters induced by thermal shock in fish may not be detected due the time needed for measurable changes. In other words, parameters

measured during or after exposure to a stressor should be measured at times appropriate to the physiological pathways and time courses involved. When responses to a rapid temperature drop in fish needs to be assessed, the BSC axis is considered to be appropriate; the activation of the BSC axis is fast enough to measure responses in fish. Changes in catecholamines (CAs) and their release is controlled by factors from sympathetic nerve terminals, mainly acetylcholine and angiotensin (Wendelaar Bonga, 1997). Thus, the increase of CAs in the blood should be analysed in fish exposed to rapid temperature drop in order to ascertain the impact of chilling methods. It is worth noting that the physiological and biochemical actions of CAs include glucose mobilisation from liver and muscles, enhanced oxygen uptake from the gills, and increased oxygen transfer to the tissues. However, the mobilisation of glucose due to glycogenolysis (enzymic breakdown of glycogen) is slower than the cortisol release in most fish and it is likely that this process is further delayed by a rapid decrease in temperature in fish. Experiments with Atlantic salmon (Skjervold et al., 2001) revealed that exposure to a temperature drop was stressful.

3.2. Purpose of slaughter

3.2.1. Fish for human consumption

For human consumption, the methods used are: percussive stunning, electrical, CO₂, live chilling, ice slurry, all followed by exsanguination. Several combinations of these methods are used.

3.2.2. Fish not for direct human consumption

Salmon that do not fit market criteria will be removed from the production line, often after stunning and killed with the methods refer to in the previous section, but also they may be killed by suffocation in air, and maceration. Degraded fish often includes sexually mature, injured, diseased, low weight and other fish species that are caught in salmon production.

3.2.3. Emergency slaughter

Disease control methods used are: pharmacological, electrical, and maceration all of which should be considered as part of contingency plans. Depending on whether it is a disease outbreak or destruction of a population due to a production error or maturation, emergency slaughter is often carried on site or fish are transported to a designated slaughter facility.

For fish designated for human consumption, emergency slaughter may follow the normal pattern and fish of low quality will be rejected after stunning. Diseased fish not designated for human consumption and not killed on site, are transported in closed well boats. At arrival the fish are either stunned or / and thrown into empty tanks ensuring death by asphyxiation or by overdose of pharmaceuticals before they are macerated and ensiled

In cases where the whole population is unfit for human consumption, emergency slaughter is often carried out at the production site. The choice of methods will vary depending on the amount of fish being killed, or whether it is next to sea-cages, or on land-based tanks for production of fry or smolt. For small numbers of salmon that are easy to handle i.e. land-based systems or broodstock fish, they are killed by hypoxia by reducing water flow to the tanks, or by using an overdose of anaesthetics. The carcasses are placed into closed bins or tanks and transported to a designated processing plant. In cases where large numbers of fish are killed, designated boats for emergency slaughter are used. Due to the toxicity of anaesthetics and the value of fish oil, the latest development is to use electrical stunning in combination with maceration or asphyxiation, but killing by pharmacological methods is also used by adding either metacain or benzocain directly to the water in tanks on board the

transport boat before closed transport to destruction. The electrical stunning devices in these cases include either dry stunning or stunning in seawater using 50 Hz AC.

Stunning should be carried out prior to maceration or exsanguination. Signs of consciousness in fish should be monitored before destruction.

3.2.4. *Killing of Broodstock*

Brood stock (1 tonne of brood stock produces 4000 tonnes of fish) is usually killed by the application of pharmacological methods (See relevant section) before destruction.

3.3. Specific stunning and killing methods for farmed salmon

3.3.1. *Carbon dioxide (CO₂)*

3.3.1.1. Principle of the method

During the 1990s, at least in Norway, rather small CO₂ stunning tanks were largely replaced by bigger live chilling tanks where CO₂ is added at lower levels (Erikson et al., 2006) within the range of 80 – 200 mg l⁻¹ (Erikson, 2008). Some small processing plants may still use small CO₂ tanks. Carbon dioxide is highly soluble in water and has a series of effects including sedation on fish placed in water saturated with the gas. Under commercial slaughter conditions, carbon dioxide is bubbled into a tank filled with seawater and the pH falls as it becomes saturated. Typically, commercial tanks operate at pH levels of about 5.5 - 6.0 corresponding to CO₂ levels of 200 - 450 mg l⁻¹ (Erikson, 2008). Levels of 200 – 500 mg l⁻¹ are necessary to render unconscious large Atlantic salmon (Bell, 1987; Iwama and Ackerman, 1994). Fish are pumped into the water and are left there until struggling stops after 2 – 4 min (Robb, 2001; Wall, 2001; Erikson et al., 2006; Erikson 2008). Subsequently the fish are removed and bled. Time to loss of consciousness in salmon stunned in CO₂ (judged by loss of VERs) is approximately 6 min (Robb et al., 2000a). There is a substantial body of evidence to indicate that fish find immersion in a carbon dioxide saturated environment aversive. On immersion in the CO₂ saturated water, salmon show vigorous aversive reactions, swimming very rapidly and making escape attempts (Wall, 2001; Robb et al., 2000a; Roth et al., 2002). These aversive reactions cause injury and scale loss (Akse and Midling, 1999; Robb et al., 2002; Roth et al., 2002). There is no evidence to show that carbon dioxide has any analgesic or anaesthetic effect, just sedation which does not imply any reduction in pain or fear. Since killing facilities do not usually change the water between batches, it is likely that fish are also exposed to hypoxia and this has been proposed to be the main aversive effect. However, similar behavioural reactions have been reported in fish exposed to high levels of carbon dioxide in a hyperoxic environment (Bernier and Randall, 1998). Based on these observations, fish would appear to find immersion in a bath of CO₂ saturated water very aversive. The high activity in the carbon dioxide stunning bath routinely results in gill haemorrhage, loss of mucus, high metabolic activity and stress (Robb and Kestin, pers. comm.), which may also be aversive for the fish.

Because fish become immobile before losing consciousness (Robb et al., 2000a), there is a risk that fish could be exsanguinated or gutted whilst still being conscious. Industry codes recommend that the fish should be left in CO₂ saturated water for at least 4 to 5 min before exsanguination (Anon, 1995), but observations indicate that fish are often removed when all carcass movements stop after 2 to 3 min (Robb, pers. comm.). In practice, the fish are not rendered unconscious by the process and are killed by subsequent exsanguination, (Robb, pers. comm.). Failure to exsanguinate the fish effectively (which also routinely occurs) results in fish being eviscerated when conscious (Robb, pers. comm.). Moreover, it has been shown

that salmon removed from the CO₂ bath before all respiratory movements have been lost, usually before the fish has lost brain responsiveness, can recover if placed in well-oxygenated water. However if fish are left in the saturated CO₂ solution for a prolonged period, it leads to death.

In summary, exposure to high levels of carbon dioxide is potentially a killing method but in commercial practice it is usually only a sedation method.

This method does not allow good welfare during killing and it is therefore difficult to prescribe conditions that would reduce suffering.

3.3.1.2. Carbon monoxide (CO) killing

The principle behind the use of carbon monoxide (CO) is that CO binds to the haem-iron proteins preventing neuroglobin, myoglobin and haemoglobin binding oxygen, and an animal will lose consciousness and die of anoxia. Since a respiratory regulation of fish is associated with oxygen (O₂) levels and not carbon dioxide (CO₂), there is uncertainty whether exposure to CO followed by oxygen deficiency will cause a euphoric reaction, due to reaction with neuroglobin, rather than asphyxia. This method is not yet used commercially. Preliminary studies carried out by Slinde et al. (2008) show that Atlantic salmon exposed to seawater saturated with CO causes no flight reaction or any reaction associated with stress or discomfort. Within minutes of exposure the salmon start to lose equilibrium followed by impaired swimming reaction, and its ability to respond to tactile stimuli is gradually lost. Within 20 min of exposure the animal can be unconscious since basic reflexes such as eye roll are lost. Although the animals do not show any aversive reactions towards CO saturated water, there is a high risk that the animals will at the later stages during the stunning process display shorter periods with convulsions. At this point the animals reach an irreversible stage where death is inevitable.

3.3.2. Live chilling

When live chilling first introduced into the industry (late nineteen eighties to early nineties) they were placed in front of the much smaller (3 m³) CO₂ tanks and no gases were added to the live chilling tank.

Typical observations were that during the day as salmon slaughter was in progress, the fish passing through the tank gradually became more sedated. It was said that the chilling of live salmon was an effective method to sedate the fish. In hindsight however, this does not appear to be the case. The temperatures in the tanks are in most cases -1 to 3 C where seawater temperatures vary between 5 -19 C. In comparatively large tanks, most of the water must be re-circulated to maintain constant, low temperatures. Consequently, there is a gradual build-up of waste products as large numbers of fish are passing through the tank during the day. Metabolically produced carbon dioxide accumulates to levels of for example 6 - 100 mg l⁻¹, apparently sufficient for light sedation. When these apparently sedated fish were transferred to the subsequent carbon dioxide stunning tank, they seemed to regain consciousness and struggle intensively for 2-4 min until they became quiet. Gradually the use of carbon dioxide tanks were abandoned by the industry and instead lower amounts of carbon dioxide, along with oxygen gas, were added directly to the live chilling tanks. Even though salmon were abruptly chilled from 19 C to 0.4 C in the tank, it still took 2 - 4 min before the fish were sedated (Erikson, 2008). Moreover, Olsen et al. (2006) reported that when salmon were live chilled at 1 C for 45-60 min without addition of carbon dioxide (water pH 8.0), the fish were still very lively and difficult to handle during gill cutting. Taken together, this clearly shows that the live chilling method is basically equivalent to the traditional carbon dioxide stunning

method with similar fish welfare concerns. Also, live chilling of salmon is known to produce elevated levels of plasma cortisol, glucose, lactate and increased osmolality (Skjervold et al., 2001).

In addition to accumulated carbon dioxide, water quality in general is gradually deteriorating in the live chilling tanks during slaughter. From a number of observations of commercial tanks, it is clear that the fish show much aversive behaviour as they are pumped into the tanks. This is largely due to the elevated levels of carbon dioxide, but other factors such as elevated levels of total organic carbon (TOC) clogging the gills may also have an effect (Erikson et al., 2006, Erikson, 2008). For other possible hazards related to water quality in recirculated (closed) systems, refer to the section dealing with live fish transport using closed systems in Annex I.

3.3.2.1. Live chilling without the effect of carbon dioxide

The aim is to simultaneously chill, sedate and kill the fish by suffocation. Chilling of fish prior to killing by another method like exsanguination or carbon dioxide narcosis followed by exsanguination is also practised as a pre-slaughter handling step to sedate or condition fish. ‘Slow chilling’ refers to the gradual lowering of the temperature of the water by refrigeration (at the rate of approximately 1.5 C per hour), whilst the fish are supplied with sufficient oxygen to maintain consciousness. The aim in this application is to chill and sedate the fish whilst maintaining them conscious and alive.

Fish can be killed by ‘rapid chilling’ by first cooling them rapidly and then depriving them of oxygen. Fish are netted or pumped through a de-watering unit and within seconds added to a relatively small tank or bin of chilled brine or ice/water slurry. If added to an ice / water slurry, the water is sometimes drained off after a period, leaving the fish surrounded by ice. The aim is that by depriving the fish of oxygen, either by draining the water or because the quality of the melting ice / water is sufficiently low, the fish will succumb to hypoxia. Temperate species of fish take longer to lose brain function when killed in ice than air. In situations where the ambient temperature is low and the fish are already cold adapted, the fish will suffer no effect from the ice slurry but will die by of anoxia.

Asphyxiation in ice does not result in immediate unconsciousness. It has been proposed that when the differential between the ambient temperature of the fish and the ice slurry is relatively great, thermal shock may shorten time to loss of brain function. When fish are introduced to water at ambient temperature, they continue to swim actively, but when introduced into an ice-slurry, responses can be variable. There is a growing body of evidence that fish find introduction to chilled water stressful. Elevated plasma cortisol levels have been reported (Donaldson, 1981; Kiessling, pers. com.), and over time plasma osmolality is disturbed (Rorvik et al., 2001 Roth et al., 2009). However, because of the progressive muscle paralysis induced by cooling, it is difficult to use behavioural indices to determine whether fish find rapid cooling aversive at later stages of the procedure.

Loss of brain function due to cooling can be reversed if the fish are removed from the cold water too soon. Fish transferred from iced water immediately, after loss of Visual or Sensory Evoked reactions, to water at normal temperatures recovered brain function and subsequently muscular movement quickly (Robb and Kestin, 2002).

3.3.3. Percussive stunning

3.3.3.1. Principles of percussive stunning

The principle of percussive stunning is that the head is struck with an object with a force sufficient enough to stun or kill instantaneously or the animal dies due to haemorrhaging in the brain (Kestin et al., 2002; Robb and Kestin, 2002; Roth et al., 2007; Lambooij et al., unpublished). The force required to stun or kill is mainly dependent on the structure of the object, and a flat hammer is more efficient than a round or cone headed hammer (Roth et al., 2007). If a percussive hammer correctly hits the head, irreversible death will result (Roth et al., 2007). Recent research by Lambooij et al. (unpublished), showed that when salmon is hit on the head with sufficient force, the EEG is instantaneously lost. However, they also noted that some animals showed no basic reflexes, despite having a conscious state EEG recording. This indicates that a percussive hit can paralyse the animal until consciousness is lost due to haemorrhaging in the brain.

Although commonly practised, there exists little information on optimum conditions for transferring kinetic energy into a shock wave or shaking the brain. In principle, two approaches are used: either a heavy bolt at lower velocity, or a lighter bolt hitting the head at a high velocity. This can also be seen in relations to possible side effects from percussive stunning such as injuries to the eyes, causing eye dislocation (proptosis), eye bursting or rupture, or haemorrhaging (Roth et al., 2007). Other methods such as penetrating bolt may be just as efficient, but require much more accuracy both to avoid convulsions or ineffective stunning (Robb et al., 2000 b; Lambooij et al., 2002 c; Robb and Kestin, 2002).

3.3.3.2. Percussive stunning in relation to mass slaughter

The major advantage of a percussive or captive stun is that it usually kills or renders the fish unconscious instantaneously. One of the major challenges with percussive stunning machines is to get live fish into the machines and a correct hit to the skull. Restraining live fish, out of the water will cause panic and flight reactions both affecting the welfare of the animal and the capacity for loading the machines. In streamlined systems dealing with relative low numbers of fish, manual feeding of live fish can work well. In these systems the fish exit from the pump, and slides towards the percussive station where an operator feeds it into the percussive machine before the fish slides to the next station for manual cutting of the gills. As the salmon industry is moving towards fewer and larger slaughtering facilities different strategies for loading the machines is required to meet capacity using limited manpower, including automatic exsanguination. This can either be done automatically where the fish swims into the system or semi-automatically where the fish is either stunned or sedated prior to manual loading into the machine. For automated systems the challenge is not only that all fish must swim against the current on entering the machine, be in the correct position and be aligned correctly, but the stunning efficiency also depends on the distribution of size within the fish population. A successful stun is first dependent on hitting the skull at the correct position, and there is a risk that a large or mature salmon could be hit on the snout and small salmon on the neck, hence failing to stun the salmon. In order to deal with this back up systems are commonly put in place, where actively moving fish exiting the automatic systems are manually stunned.

For manual or semi-automatic systems the operator has the opportunity to place the fish into the different machines adjusted to two or more ranges size. Although these systems can cover a wider size range than automatic systems, extremes of small or large fish may still be manually stunned before exsanguination.

3.3.4. *Electric stunning or Stunning and Killing systems*

3.3.4.1 Principles of electric stunning

The use of electricity to stun animals before exsanguination is well established for a range of farm animals. As with these other farmed species, farmed fish can be stunned by applying a sufficient electrical potential across the brain to disrupt normal neural activity. This depolarizes the neurons and probably, as in mammals, causes functional change followed by the release of the neuro-inhibitor GABA which results in an extended period of unconsciousness (Cook et al., 1995).

Electric stunning systems for fish are designed to ensure that the fish being stunned lose consciousness immediately, are not exposed to pre-stun shocks, and are unable to recover consciousness before death supervenes.

For Atlantic salmon, several studies have shown that if the fish are exposed to sufficient electric fields in seawater, the animals can be stunned unconscious within one second (Robb and Roth, 2003; Roth et al., 2003). Newer studies with electric dry stunning using coupled 100 Hz AC+DC shows that Atlantic salmon can be stunned unconscious within 0.5 s, having an average current flow through head at 667 mA (Lamboij et al., pers. com.). The frequency of the alternating current electricity affects the voltage or field strength required to stun fish. In general frequencies between 50 and 150 Hz appear to have the greatest effect (Roth et al., 2004; Robb et al., 2002).

Although salmonids are stunned unconscious within one second, a prolonged electric exposure is required in order to secure a sufficient stun lasting beyond one minute (Roth et al., 2003; Robb et al., 2002). For Atlantic salmon a prolonged unconscious condition can result in death as the animal will not regain opercular ventilation in time to prevent the animal from undergoing severe hypoxia (Robb and Roth, 2003). This is a crucial factor during exsanguination, where the fish can recovery. Newer studies by Lamboij et al. (personal communication) show that Atlantic salmon exposed to 5 s of electricity can recover for a short period of time within 3 min post-stun and during exsanguination. There is very little knowledge on the current issue.

3.3.4.2 Electrical stunning in relation to mass slaughter

The most common difficulty with dry stunning is to ensure that the fish are not exposed to pre-stun shocks caused, for example, by entering the machine tail first or because spasms of the fish cause it to lose contact with the electrodes. With in-water stunning it is important to ensure that the electric field in the water is homogeneous and that it is matched both to the fish species and to the water conductivity (Lines and Kestin, 2004). In tank stunning system it is also important to ensure that the batch sizes are small enough to ensure the fish are not stressed as they are loaded into the tank before application of the electricity and that, if they are to be bled, they can all be bled within an acceptable time of removal from the electric field. With continuous flow tube systems it is important to ensure that the residence time in the tube is long enough even for the fastest flowing fish.

A challenge with the development of electrical stunning systems for salmon is to avoid carcass damage. Such damage may appear as bleeding in the flesh along the spinal column due to rupture of the dorsal aorta and veins. The use of high frequency electrical current (500-1000 Hz) appears reduce such damage (Roth et al., 2004; Robb, 2001). The use of coupled

AC+DC current may also be of interest in this respect (Mejdell et al., 2009; Roth et al., 2009b).

A second quality problem that may occur is the early onset of rigor which can be a problem where salmon are to be processed before rigor. Early onset of rigor is probably related to the electro-stimulation of the muscles during the stun and may be reduced by increasing the electrical frequency and minimising the duration of the electrical application (Roth et al., 2006; Roth et al., 2009b).

Where salmon unwanted for human consumption are being killed, death can be caused by prolonged exposure to an electric current without further intervention (Robb et al., 2002). Salmon of a very wide size range and of very variable morphologies can be humanely killed by this method. This prolonged exposure is associated with drop in muscle pH, early onset of rigor mortis, gaping and a softer texture (Roth et al., 2002, 2006, and 2008) however this is of little consequence for these fish.

A novel approach to electric stunning which may result in high welfare standards without compromising carcass quality is to apply an electric stun for a short time, resulting in only a short duration of insensibility, and during this period, while the fish are not struggling, to percussively stun them (Mejdell et al., 2009).

Another electrical approach which can occasionally be observed is to use voltages that are too low to result in immediate insensibility. This may be followed by a voltage that stuns the fish once the muscles have been exhausted (Robb and Kestin, 2002). Further consideration of this approach is not necessary since it inflicts severe and extended periods of pain and hence poor welfare.

3.3.5. *Bleeding out / Exsanguination*

3.3.5.1. Principles of the method

Exsanguination is regarded as a part of the slaughter procedure preceded by stunning. It is the main killing method for salmon intended for human consumption. Exsanguination of fish after stunning and before recovery prevents fish regaining consciousness. Exsanguination (by gill cutting) without stunning is a relatively slow method for killing fish, taking at least 4.5 to 6 min to lose VERs (Robb et al., 2000; van de Vis et al., 2003). If less than three to four gills are cut or torn, the time to unconsciousness would be considerably longer. The fish were reported to show clear signs of aversive behaviour for the first 30 sec whilst bleeding. The time for the fish to die by exsanguination appears to be temperature related with salmon at lower temperatures taking longer to die (Robb et al., 2000a). Recommendation: no fish should be active in the bleeding tank.

3.3.5.2. Exsanguination in relation to mass slaughter

Atlantic salmon are commonly exsanguinated after stunning or killing to improve carcass quality. To achieve exsanguination, three or four gills are cut either manually or automatically with machines to bleed for a period of 10 to 30 min (Wardle, 1997). For manual cutting a knife is used to cut the gill arches before the knife is angled and the gill arches are cut whilst pulling the knife out. Manual gill cutting is apparently the safest method to exsanguinate the animals since the operator aims for the gills regardless of fish size and species. A failure to exsanguinate the animal properly can occur because the knife is not in the correct position, or because a blunt knife fails to cut the gill arches. Compared with manual exsanguination, machines are far less efficient in bleeding the animal properly. Like percussive machines, a successful cut is dependent on the size and orientation of the fish. For

automatic systems fish can enter the machines in a wrong position resulting in a mis-cut and failing to exsanguinate the animal. The accuracy of the machines depends on fish size. Fish outside of the expected range would therefore result in mis-cuts depending on the size distribution of the population. Also exsanguination of mature males, due to their different morphology, may result in a mis-cut either in the jaw or in the mouth cavity.

Aversive behaviour in gill cut salmon after live chilling with CO₂ has been reported (Roth et al., 2006) causing muscle pH to drop during exsanguination. Furthermore, they reported that approximately 1 % of salmon showed signs of consciousness prior to evisceration, 30 min after bleeding due to a mis-cut

More recent studies using EEG showed that after electrical stunning (100 Hz, 110 V, PDC for 5 s) approximately 20 % of Atlantic salmon regained consciousness for a short period of time during exsanguination (Lambooij et al., unpublished).

3.3.6. *Pharmacological methods*

Humane killing by anaesthetics in commercial salmon farming is applied during the juvenile freshwater stage and for brood stock and emergency slaughter where fish are not intended for human consumption. In New Zealand and Chile, isoeugenol is used for stunning in combination with exsanguination for food fish. This anaesthetic is prohibited for such use in the EU. The mechanism of effect of isoeugenol in relation to stress is described by Zahl et al. (2009b). A large selection of anaesthetic agents is being used in fish, but only metacaine (MS-222) and benzocaine are used for euthanasia in salmon at the same level (Havbruktstjensten, pers. com.). Fish are immersed in these agents to produce general anaesthesia but their mode of action is not fully understood (Hara and Sata 2007; Ueta et al., 2007). Robb and Kestin (2002) found that brain activity could be detected for more than 15 minutes in salmon after exposure to either of MS-222 or benzocaine.

Subjecting the fish to handling and confinement prior to immersion is likely to elicit a stress response. Factors as crowding, netting, pumping low water quality, low oxygen, pH etc in addition to concentration of anaesthetic and exposure time, fish size, life stage, water temperature and salinity are all factors that are known to affect both induction time and the stress response (see Zahl et al., 2009b - submitted). As a general rule induction time seems to increase with body weight, while increased water temperature and stress seems to reduce it (see Ross and Ross, 2008 and Zahl et al., 2009a - submitted) but time to loss of consciousness may vary. Exhausted fish most likely have a longer induction time because of malfunctioning gill exchange Also exhausted fish could easily be mistaken for an unconscious fish.

The slow induction of unconsciousness may provide time for the fish to detect the agents due to their very distinctive chemical properties. They may be sensed through taste and smell and may also act as irritants to the skin. Furthermore, as the anaesthetic starts to take its effect, loss of balance may also elicit a stress response. So the length of time needed to induce anaesthesia is of importance. Finally, the mode of action of the compounds is likely to affect the stress response. Anaesthetic agents may affect the endocrine system and themselves induce elevations in plasma cortisol (Oyama, 1973; Oyama and Wakayama, 1988; Kiessling et al., 2009). In a study by Zahl et al. (2009b) earlier reports of Kiessling et al. (2009) were confirmed in that MS 222 exposure causes a much faster and quantitatively larger plasma cortisol peak than exposure to benzocaine in salmon. On the other hand benzocaine leads to a much longer and a bimodal plasma cortisol peak compared with MS-222, indicating that the physiological stress response of salmon differed markedly according to the chemicals used. Furthermore, in both human and veterinary medicine, anaesthesia is often preceded by

administering a sedative in order to calm the patient and reduce stress caused by the anaesthetic or the anaesthetic procedure. Such pre-anaesthesia sedation has been tested in several fish species including salmon with good results in order to reduce stress (see Zahl et al., 2009a; b).

3.3.7. *Maceration*

Maceration is some times used to dispose of degraded fish following stunning. Where only a part of the population is to be discarded (sexually mature, PD infected, other fish species that follows the salmon), fish are transported as usual to the processing plant. There, the fish to be discarded either follows normal routines (stunning and bleeding) or they may not be stunned or bled (small fish species, sexually mature). Stunned and not stunned fish are placed in tubs without water. Most will die from asphyxia, but some may not before they are macerated. There are few studies on maceration of live animals using mill type devices, but different types of maceration equipment have been tested for the killing of day old chicks. Homogenizing and meat mill type equipment has been used (Hillbrich, 1975; Hilbrich & van Mickwitz, 1977; Jaksch & Mitterlehner, 1979) and technical recommendations have been made.

3.4. Exposure to procedures at pre-slaughter and slaughter (Questionnaire)

An enquiry regarding stunning and killing methods of farmed fish was sent out to organizations and competent authorities in 22 EU and EC countries. EFSA received 6 answers from 4 countries concerning the stunning and killing of salmon (Norway, United Kingdom, Iceland and Greece, see Appendix F).

Pumping is by far the most common way of transferring the fish to the processing line.

Transportation by well boat is done differently in different countries. Iceland uses a closed system with no chilling whereas the UK uses a mainly closed system with chilling. Norway uses mainly open systems.

The methods of stunning vary between countries: Iceland uses mainly ice slurry without CO₂ (75%) and some percussive stunning (25%). United Kingdom uses only percussive stunning. Live chilling with CO₂ is the most common method in Norway (51%). Other methods used in Norway are exposure to CO₂ (20%), and percussive stunning (14%), electric stunning (7%), ice slurry without CO₂ (6%), and combinations of methods (3%).

All killing for salmon is reported to be exsanguination

The salmon industry is subject to changes in legislation as well as in technical developments, so these figures are likely to change over time. There are also reasons to believe that there are some uncertainties about some of the received responses.

4. APPLICATION OF THE RISK ASSESSMENT APPROACH TO STUNNING AND KILLING OF ATLANTIC SALMON

The risk assessment method used to assess the risk to welfare of farmed Atlantic salmon when stunned and killed is described in Appendix B.

The risk assessment was applied to the stunning and slaughter of Atlantic salmon. Salmon are either i) taken directly to slaughter on arrival at an abattoir or ii) kept at the abattoir for up to two days (lairage). The hazards associated with both approaches were assessed, in relation to their effect on stunning and killing in general. The parameters used in producing risk and magnitude scores for welfare hazards are presented in Appendix D.

The assumption that exposure to the hazard resulted in all the fish suffering the adverse effect held for all hazards.

Definitions of intensity of an adverse effect for hazards occurring pre- and post-stunning were defined (Table 7).

Different categorisation for duration of the adverse effect was used for pre-slaughter and slaughter / stunning hazards (Table 8 and Table 9).

4.1. Pre-slaughter hazards

4.1.1. Salmon slaughtered directly on arrival at the abattoir

Nine hazards were identified (Table 2) (details in Appendix C) for salmon which are slaughtered directly on arrival at an abattoir, of which one (brailing) was considered at two different magnitudes. The risk scores ranged from 0.13 to 6.67. The highest ranking risk was to be in metabolic stress after transport (with no time to recover). This hazard had a higher score in comparison with the rest, because the duration of the adverse effect is long, on average 180 minutes. The second highest risk score was 2.50, seen for a hazard associated with pumping with poor pipe design. Hazards ranking high on the magnitude scores (indicating a severe impact on the fish that were actually affected) were; injuries obtained during transport, fish in metabolic stress post-transport and being exposed to shallow water/air during crowding (Figure 3).

The sum of the risk scores for all the hazards was 13.6.

Variability and uncertainty

Variability is captured by estimates of the minimum and maximum values of the probability of exposure to the hazard. The estimates of the minimum and maximum values of the probability of exposure produced fairly narrow ranges, partly dependent on the fact that the point estimates were quite small. The uncertainty did not affect the ranking of the hazards.

For most hazards the score regarding the uncertainty of the adverse effect was one, indicating that there is substantial evidence/high level of consensus within the scientific community about these effects. The exceptions were seen for all hazards associated with pumping, where limited data exist.

Table 2. Risk and magnitude scores for welfare hazards associated with pre-slaughter management in Atlantic salmon in Europe, in situations where the fish are directly processed as they arrive at the abattoir.

Hazard ID	Pre-slaughter hazards	Description of adverse effects	Risk score	Magnitude
1	post-transport status Fish is in metabolic stress (e.g. after a not-well performed closed transport)	stress	6.67	67
2	Fish is injured during transport	Pain associated with the injury, distress	0.50	100
3	crowding Fish exposed to shallow water and air	gill irritation, distress, exhaustion	1.00	50
4	Water oxygen levels low (due to poor supervision)	distress, escape behaviour	0.25	25
5a	Dry brailing	abrasion, exhaustion	0.03	25
5b	Wet brailing	distress	0.04	8

	pumping			
6	Poor pipe design	trauma, injuries, pain	2.50	25
7	Delay in pipe due to slow water flow (crowding, low oxygen)	stress, exhaustion	0.83	8
8	Delay in pipe due to poor system logistics	stress, exhaustion	1.67	33
9	Getting stuck in vacuum pressure valve	stress, pain, associated with trauma	0.13	25

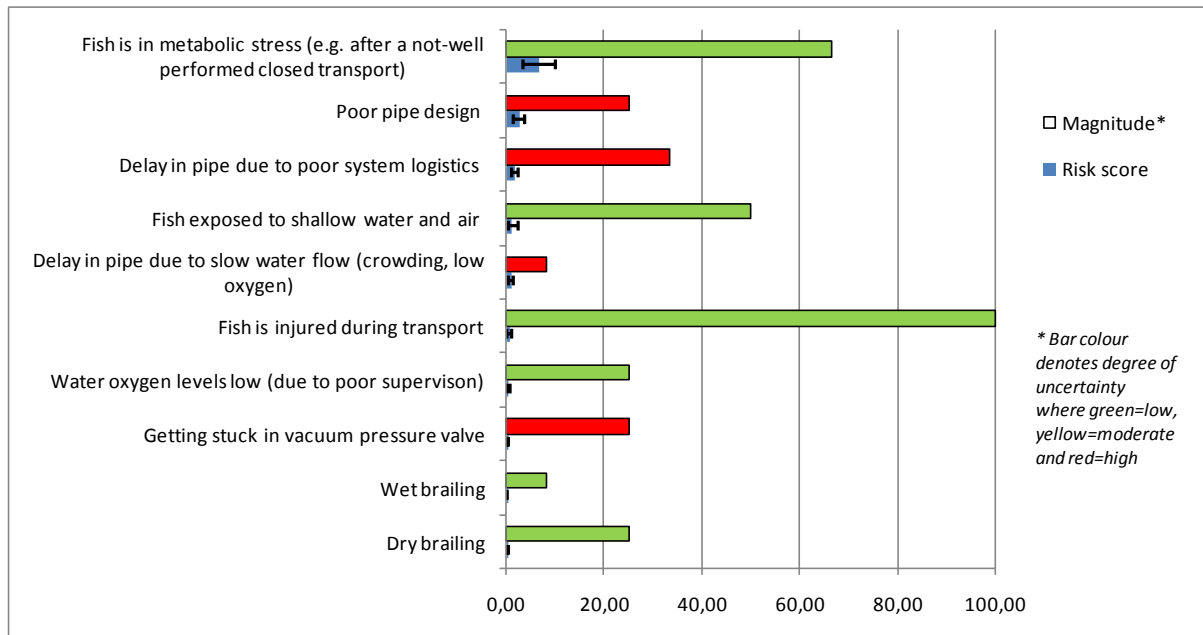


Figure 3. Risk score and magnitude of adverse welfare effect for individual hazards associated with pre-slaughter management in salmon in Europe, where the fish are directly processed as they arrive at the abattoir. Hazards are ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard.

4.1.2. Salmon in holding cages at the abattoir

Salmon that are held at the abattoir prior to slaughter experience the same number of hazards (n=9) as salmon that are slaughtered directly upon arrival (Table 3), with two differences. It is assumed that salmon have time to recover from the transport and so the risk of being affected by post-transport metabolic stress at the time of slaughter is regarded as negligible. However, instead they are potentially exposed to poor water quality during lairage. The risk scores had a more narrow range; from 0.13 to 2.50, with poor pipe design being ranked the highest. Similar hazards (as for direct slaughter) ranked highest on the magnitude scores, i.e. injuries obtained during transport, and being exposed to shallow water/air during crowding (Figure 4). Transport injuries and poor water quality both had the highest duration score, based on an average of 36 hours with the adverse effect before stunning and slaughter starts.

The sum of the risk scores was 7.11.

Table 3. Risk and magnitude scores for welfare hazards associated with preslaughter management in Atlantic salmon in Europe, where the fish are in holding for an average of 2 days (48 hours) before they are processed further.

Hazard ID	Pre-slaughter hazards	Description of adverse effects	Risk score	Magnitude
	post-transport status			
10	Fish is injured during transport	Pain due to injuries, distress	0.50	100
	lairage			
11	Poor water quality (pH, DO, water temp)	distress	0.17	33
	crowding			
12	Fish exposed to shallow water and air	gill irritation, distress, exhaustion	1.00	50
13	Water oxygen levels low (due to poor supervision)	distress, escape behaviour	0.25	25
14a	Dry brailing	abrasion, exhaustion	0.04	25
14b	Wet brailing	distress	0.03	8
	pumping			
15	Poor pipe design	trauma, injuries, pain	2.50	25
16	Delay in pipe due to slow water flow (crowding, low oxygen)	distress, exhaustion	0.83	8
17	Delay in pipe due to poor system logistics	distress, exhaustion	1.67	33
18	Getting stuck in vacuum pressure valve	distress, pain, associated with trauma	0.13	25

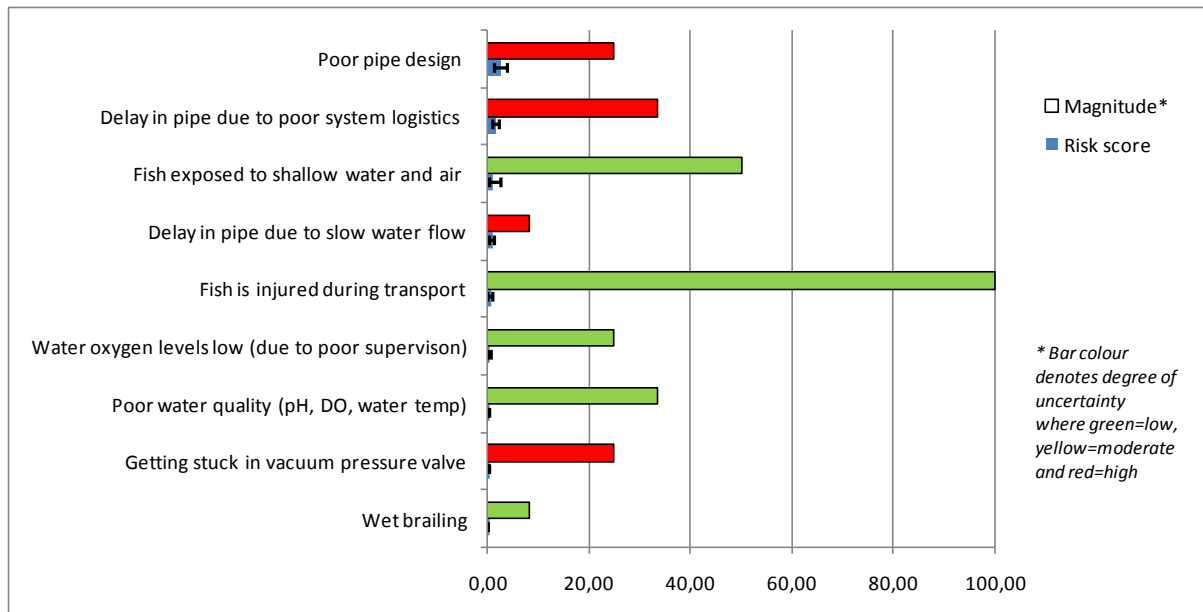


Figure 4. Risk score and magnitude of adverse welfare effect for individual hazards associated with pre-slaughter management in salmon in Europe, where the fish are held for an average of 2 days (48 hours) before they are processed further. Hazards are ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard.

Variability and uncertainty

When looking at the variability/uncertainty for pre-slaughter hazards involving a holding period, they produced a picture very similar to what was seen for direct slaughter. For all hazards, the estimates of the minimum and maximum values of the probability of exposure produced fairly narrow ranges, and the uncertainty did not in itself put doubt on how the hazards were ranked according to the risk score.

For most hazards the score regarding the uncertainty of the adverse effect was one indicating that there is substantial evidence/high level of consensus within the scientific community about these effects. The exceptions were seen for all hazards associated with pumping, where limited data exist to support the estimates.

4.2. Slaughter and stunning hazards

Nine methods of stunning and slaughter were assessed (details in Appendix E). Between five and nine hazards were identified for each method. The risk and magnitude scores for the hazards were summed by method (Table 4 and Table 5).

The risk scores range from 36.3 (for manually fed percussive stunning systems with manual cut) to 293.4 (for live chilling in combination with carbon dioxide (CO₂)). For six of the methods of stunning / slaughter evisceration was a hazard, because the event of a mis-stun followed by a mis-cut was regarded as possible.

Live chilling in combination with CO₂ (method D) had the highest risk score because all salmon slaughtered with this method are exposed to CO₂ at levels where it is regarded highly unlikely that they reach unconsciousness before evisceration. In addition, the CO₂ tank provides an environment where the water quality will be poor for most of the fish and all of them are subjected to a temperature shock during the live chilling process. CO₂ exposure,

exsanguination and mis-cuts were all hazards with a maximum magnitude of the adverse effect (100). The method of using CO₂ only (without live chilling) had a slightly lower total risk score based only on the fact that there was no hazard from temperature shock – however this method was in every other respect regarded as being associated with the same welfare hazards as CO₂ combined with live chilling.

Most other methods had risk scores in the range between 40 and 90. The method ranked in fourth place was electrical stunning using in-water (batch) systems. All fish slaughtered by this method are crowded in a tank, and electrically exhausted at sub-stun voltage levels of electricity after which they are stunned at appropriate levels; however it will take a few seconds before they become unconscious. The differences seen in risk score between the electrical stunning systems were mostly due to different hazards pre-stunning, where the batch system involves crowding, and the dry system involves being in air and a potential delay in stunning due to the position of the fish on the conveyor. The pipe system has neither of these but does have a hazard associated with poor pipe design. For all electrical stunning methods, it is the exposure to different levels of electric current that produces significant welfare risks as it will take more than 1 second before fish become unconscious and consequently, they will experience electrical shocks while conscious. The hazards with the highest magnitude scores for all three electrical stunning methods were potential mis-cuts, with delayed unconsciousness from a slow bleed-out, or dying from asphyxia, both with high severity and long duration. However, these hazards had low risk scores, indicating that they are unlikely events.

The two pharmacological methods assessed (metocaine and benzocaine) were judged as being equivalent, and so they are presented together. They had a summary risk score comparable with electrical stunning methods and percussive stun/kill methods. Their highest welfare hazards arise from the distress caused by exposure to pharmaceuticals, as all fish slaughtered by this method will experience this effect. Netting prior to application of the preparations had the second largest risk score. All hazards associated with pharmacological methods had the highest score for duration, based on adverse effects lasting between 3 and 10 minutes. Two hazards; dying from asphyxia or dying as silage, had maximum magnitude of the adverse effect, but were both regarded as unlikely events.

The slaughter method with the lowest risk score (36.3) was seen for one of the percussive stun/kill methods - the hand-fed system with manual cut. The highest ranked hazards with this system were caused by fish being handled manually and being out of water prior to stunning. The magnitude of the adverse effect for these hazards were, however, quite low (17 for both). Still there were two hazards out of five identified that had maximum magnitude of the adverse effect – being mis-cut or being eviscerated while conscious. The probability of exposure to a mis-stun, and subsequent hazards like being conscious at cutting and at exsanguination, was judged as slightly higher for the hand-fed percussive stunning system with an automatic cut compared with manual cutting.

Table 4. Risk and magnitude scores for welfare hazards associated with the main stunning/killing methods for Atlantic salmon (*Salmo salar*) in Europe.

Hazard ID	Slaughter hazards	Description of adverse effects	Risk score	Magnitude		
A	percussive stunning - swim-in system, fully automatic exhaustion (swimming into the system)	distress	41.76	400		
1a ¹			23.75	25		
1b ¹	severe exhaustion (swimming into the system)	distress	3.00	100		
2	mis-stun	pain, stress, trauma	5.00	50		
3	mis-cut; if conscious	pain, trauma, stress	1.00	100		
4	exsanguination; if conscious	pain, trauma, stress	9.00	100		
5	evisceration; if conscious	pain, trauma, stress	0.01	25		
B	percussive stunning - hand-fed system, automatic cut being handled manually	distress	40.83	283		
6			16.67	17		
7			16.67	17		
8			2.50	50		
9			2.50	100		
10			2.50	100		
C	percussive stunning - hand-fed system, manual cut being handled manually	distress	36.33	317		
11			16.67	17		
12			16.67	50		
13			1.00	50		
14			0.10	100		
15			1.90	100		
D	live chilling + carbon dioxide temperature shock	distress, exhaustion	293.35	317		
16			33.33	33		
17			100.00	100		
18			60.00	67		
19			99.90	100		
20			0.10	100		
21	exsanguination (proper) (fish are regarded as being conscious)	pain, trauma, stress	0.01	25		
E			carbon dioxide only exposure to high levels of CO2	distress, exhaustion	260.01	392
22					100.00	100
23					60.00	67
24					99.90	100
25					0.10	100
26	0.01	25				

¹ a and b takes into account that this hazard has different levels of magnitude

Table 5. Risk and magnitude scores for welfare hazards associated with the main stunning/killing methods for Atlantic salmon (*Salmo salar*) in Europe, cont'd.

Hazard ID	Slaughter hazards	Description of adverse effects	Risk score	Magnitude
F	electrical stunning - in-water (batch) system		81.83	367
27	crowding prior to stunning	stress	16.67	17
28	electrical pre-treatment	escape behaviour, pain, distress, exhaustion	50.00	50
29	exsanguination; if conscious	pain, trauma, stress	15.00	75
30	mis-cut; if conscious	pain, trauma, stress	0.10	100
31	evisceration; if conscious	pain, trauma, stress	0.01	25
32	asphyxia; if conscious	distress, pain	0.05	100
G	electrical stunning - dry system		40.00	433
33	asphyxia (out of water)	distress	8.33	8
34	fish enter tail first	escape behaviour, pain, stress	7.50	25
35a ¹	experiencing electricity while conscious ; low voltage system (<50 V)	escape behaviour, pain, distress, exhaustion	2.50	50
35b ¹	experiencing electricity while conscious ; medium voltage system (50-110 V)	escape behaviour, pain, distress, exhaustion	6.00	25
35c ¹	experiencing electricity while conscious ; high voltage system (>110 V)	pain, trauma, distress	0.50	25
36	exsanguination; if conscious	pain, trauma, stress	15.00	75
37	mis-cut; if conscious	pain, trauma, stress	0.10	100
38	evisceration; if conscious	pain, trauma, stress	0.01	25
39	asphyxia; if conscious	distress, pain	0.05	100
H	electrical stunning - pipe line system		47.66	325
42	poor pipe design	trauma, injuries, pain	5.00	50
43	mis-stun homogeneous electric fields	pain, trauma, stress	25.00	25
44	exsanguination; if conscious	pain, trauma, stress	22.50	75
45	mis-cut; if conscious	pain, trauma, stress	0.10	100
46	evisceration; if conscious	pain, trauma, stress	0.01	25
47	asphyxia; if conscious	distress, pain	0.05	100
I	pharmacological methods (metocaine, benzocaine)		86.84	467
48	netting	abrasion, exhaustion	13.33	67
49	low water quality	distress, gill irritation	3.33	33
50	crowding, incl. too low water levels	distress	3.33	33
51	exposure to pharmaceuticals	escape behaviour, distress	66.67	67
52	insufficient levels of anaesthetics => prolonged exposure time	distress, respiratory collapse	0.03	33
53	mis-stun (insufficient time of exposure to anaesthetics)	stress	0.03	33
54	asphyxia; if conscious	distress, pain	0.10	100
55	silage	stress, pain	0.01	100

¹ a-c takes into account that this hazard has different levels of magnitude, related to different designs of dry electrical stunning systems

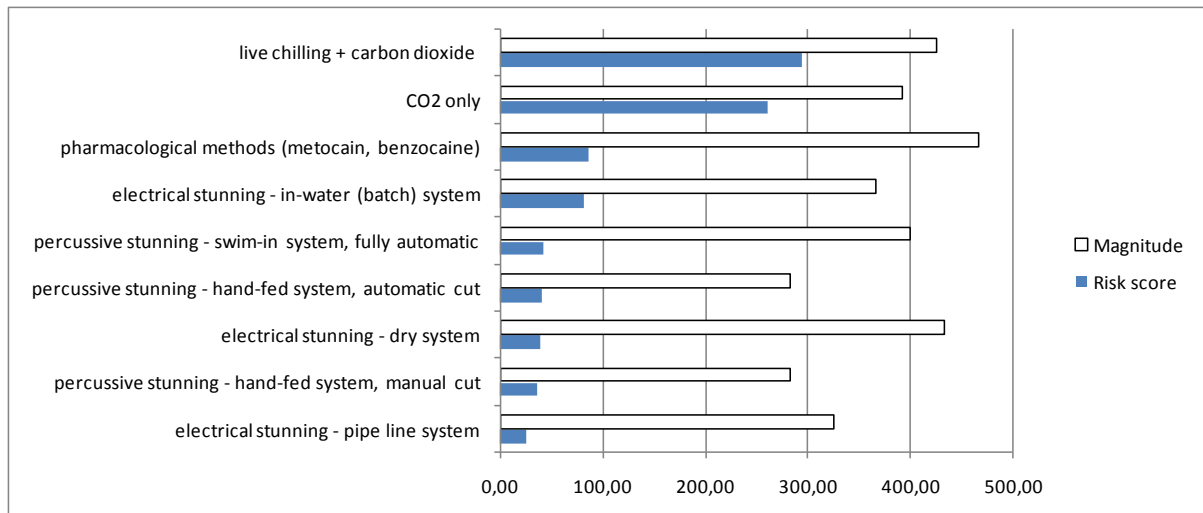


Figure 5. Sum of risk scores and magnitudes of the adverse welfare effect for main slaughter methods applied to salmon in Europe, ranked by the sum of the risk score.

Variability and uncertainty

Considerable variability was not seen around any of the 52 hazards (of which one had two magnitude levels, and one had three). The only methods for which the variability could indicate a different rank in risk score were the electrical stunning systems (dry and pipe line). For several hazards the entire population was exposed hence most likely, minimum and maximum values for the probability of exposure to the hazard were equal to one.

From the scoring of uncertainty of severity and duration it can be judged that for salmon, there is some knowledge about adverse effects of welfare hazards available. Approximately 50% of the hazards had an uncertainty score of 1. The methods with the highest uncertainty scores were the electrical stunning methods.

4.3. Discussion and conclusions

Lairage at an abattoir provides an opportunity for salmon to recover from transport prior to slaughter.

Some of the hazards associated with unloading and moving salmon at the abattoir could be mitigated through better management.

Methods involving exposure to CO₂ involve high welfare risks. The methods that are most robust from a welfare point of view appear to be percussive stun/kill methods and some electrical stunning methods. However, with some electrical stunning methods, there are inherent welfare risks (affecting all fish slaughtered by the method) associated with the use of low voltage to exhaust fish prior to stunning.

5. REFERENCE TO WELFARE INDICATORS

Some indicators of poor welfare may be used to assess welfare of fish slaughter under commercial conditions. However, welfare indicators have not been satisfactorily assessed and validated so far. Observation of fish response was taken into account in this approach.

Table 6 provides indication on possible operational indicators for Atlantic salmon.

Table 6. Operational indicators (to be used under field conditions) of poor welfare for critical monitoring points.

Methods	Operational indicators (in field conditions) of poor welfare for critical control points
Transport at arrival to abattoir	<i>Control points include:</i> Control at unloading. <i>Indicators of poor welfare:</i> Trauma.
Holding cage	<i>Control points include:</i> Same as for fish rearing <i>Indicators of poor welfare:</i> Same as for fish rearing
Crowding (in well boat and in holding pens)	<i>Control points include:</i> Monitoring of fish behaviour (e.g. video) during crowding procedure <i>Indicators of poor welfare:</i> Fish dorsal skin colour changes from grey/black to blue/green. Dissolved oxygen levels should not be below 70 % saturation. Burst swimming close to the surface. Swimming on their side. Swimming with their belly up, Fish at surface gulping. Fish exposed to air is an indicator of poor welfare. Exhausted fish.
Pumping	<i>Control points include:</i> Fish exiting the pump system. <i>Indicators of poor welfare:</i> Tail flapping (during air exposure), Presence of fresh external damages (fin, skin, body), Fish remaining in pump system after cessation of pumping (fish coming out exhausted or dead when pumping is resumed).
Carbon dioxide	<i>Control points include:</i> Behaviour at entering and leaving the tank. <i>Indicators of poor welfare:</i> Escape behaviour. Indicators of consciousness as fish leave the tank.
Live chilling with carbon dioxide	<i>Control points include:</i> Behaviour on entering and leaving the tank. <i>Indicators of poor welfare:</i> Escape behaviour. Indicators of consciousness as fish leave the tank. Oxygen level not lower than 70%.
Live chilling without carbon dioxide	<i>Control points include:</i> Behaviour at entering and leaving the tank. <i>Indicators of poor welfare:</i> Signs of consciousness.
Percussive stunning/killing	<i>Control points include:</i> Monitoring of fish immediately before and after stunning. Back-up system (manual stunning) present. <i>Indicators of poor welfare:</i> In water drained, the indicator is excessive tail flapping. In swimming in systems it is ability to swim appropriately. After stunning, recognition of consciousness.
Electrical stunning/killing: Dry stunning	<i>Control points include:</i> Monitoring of fish immediately before and after stunning. Manual back up system (manual stunning) present. Duration of exposure to air (after water drained off) before stunning should be as short as possible. <i>Indicators of poor welfare:</i> In water drained, the indicator is excessive tail flapping and orientation of fish. After stunning, recognition of consciousness.
Electrical stunning/killing:	<i>Control points include:</i> Monitoring of fish immediately before and after stunning. Manual back up system (manual stunning) present. Duration of exposure to air (after

<p>In water stunning (batch stunning)</p>	<p>water drained off) before stunning should be as short as possible. Control of water quality before onset of electricity.</p> <p><i>Indicators of poor welfare:</i> In swimming in systems it is ability to swim appropriately. After stunning, recognition of consciousness. Oxygen to be above 70% in the stunning tank. Monitoring of fish before electric stunning.</p>
<p>Electrical stunning/killing: In-water stunning: Continuous flow tube</p>	<p><i>Control points include:</i> After stunning. Presence of the back up system.</p> <p>Indicators of poor welfare: signs of consciousness.</p>
<p>Bleeding out/exsanguination</p>	<p><i>Control points include:</i> Fish in the bleeding tank. If automatic system used, monitoring entering the machine. Effectiveness of the cut.</p> <p><i>Indicators of poor welfare:</i> Orientation (in automatic systems). Signs of consciousness.</p>
<p>Pharmacological methods</p>	<p><i>Control points include:</i> Levels of anaesthetics, water quality. Behaviour during induction</p> <p><i>Indicators of poor welfare:</i> signs of consciousness after normal induction time (1 to 3 minutes). Oxygen below 70% saturation. Escape behaviour.</p>
<p>Maceration</p>	<p><i>Control points include:</i> Stunning before maceration in all fish. Machine adapted to size of fish. Using mills for maceration of fish should ensure that all fish are instantaneously killed when put in the macerator machine.</p> <p><i>Indicators of poor welfare:</i> Signs of consciousness before maceration.</p>

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. This Scientific Opinion on stunning and killing of Atlantic salmon evaluated the methods currently used in farmed Atlantic salmon in Europe. Methods used in other fish species other than those described in this Opinion may also be applicable to Atlantic salmon.
2. Some indicators of poor welfare may be used to assess welfare of fish slaughter under commercial conditions.
3. If fish do not show the Eye roll and 'breathing' reflexes, then they can be considered unconscious. Paralysis or exhaustion may influence these responses.
4. If a fish shows Eye roll and Breathing reflexes but no co-ordinated activity or response to painful stimulation, it may be unconscious or just paralysed. It should then be given the benefit of the doubt and considered conscious.
5. If a fish shows any co-ordinated activity or responds to handling or painful stimulation, it is conscious.
6. Exposing salmonids to air causes a major negative impact on their welfare, including a maximal hypothalamic pituitary-interrenal axis response, and should be avoided.
7. Excessive crowding will result in poor welfare.
8. Two to three days of fasting are needed to reduce the metabolic rate and thus the physical activity of the fish which may reduce stress associated with transport. Too short or too long transport and resting period may be an issue in association with the duration of the fasting period. Food deprivation can result in the utilisation of body fat reserves and then functional tissue which is associated with poor welfare.
9. The effect of pumping in the welfare of salmon has not yet been satisfactory evaluated.
10. Crowding and pumping will subject the fish to metabolic and handling stress, all of them resulting in poor welfare. Poor pump line design may cause severe physical trauma and thus poor welfare.
11. There will always be a certain risk of poor welfare involved when live fish are transported to slaughter. With the information presently available, there are no particular reasons to assume that transport with open valves (flow-through water) represent a situation where good fish welfare at slaughter is challenged. However, in closed systems, there are a number of issues that need to be addressed to ensure good fish welfare at slaughter such as to ensure good water quality, e.g. adequate levels of dissolved oxygen. The effect of elevated levels of CO₂, NH₄⁺ and TOC, as well as low pH on the welfare of the fish needs to be addressed.
12. If fish are transported under good conditions (open transport) then the fish may recover from crowding and handling during the transport and thus, the transport will not affect the fish welfare at slaughter.
13. As the fish are supplied to the stunning or killing unit operation, in terms of struggling (muscle pH) they can be anything from little exposed to handling stress to absolutely exhausted. There is a high risk that salmon is subjected to metabolic stress, handling

- stress and poor welfare (exhaustion) prior to slaughter. Moreover, loss of mucus and scales, altered skin colour, and sometimes external injury, are observed as the fish are ready to be stunned.
14. There is a high risk of poor welfare when using live chilling and CO₂ or only CO₂ as method of slaughter, in particular because high concentrations of CO₂ are aversive and the fish may recover before killing.
 15. Carbon dioxide (CO₂) stunning does not allow good welfare during killing and it is therefore difficult to prescribe conditions that would reduce suffering.
 16. Live chilling without CO₂ does not cause immediate unconsciousness and the method appears to be aversive to fish.
 17. Methods of killing fish by chilling the fish often involve exposure to increased carbon dioxide concentrations, low oxygen concentrations and increased gill contact with organic matter.
 18. In hand held manually fed percussive systems the hazard causing the highest risk for poor welfare is asphyxia.
 19. For automated percussive stunning the main hazard is variation of size within the population causing a mis-stun in some fish either hitting the snout on the outermost size ranges.
 20. For electrical stunning the hazard is using too low electrical currents causing paralysis and insufficient stunning.
 21. For fish entering the electrical dry stunner, intended for head only application, with the tail first will consciously feel the electricity for a few seconds before reaching the head.
 22. There is some risk of poor welfare when applying electrical stunning in water (batch) system mainly due to mis-stun or electrical exhaustion.
 23. Severance of all gill arches on both sides of the fish, or the isthmus, or piercing the heart directly, appears to be the best method for killing by bleeding out unconscious fish.
 24. There is a high risk of poor welfare when benzocaine and metacaine are used in seawater for killing salmon.
 25. In order for an overdose of anaesthetic to be a reliable and humane killing method for salmon more knowledge is needed before being able to recommend minimum dosage and exposure times for specific life stages, body size and water temperature. Such information would help to ensure minimum time to loss of consciousness and minimum induction of stress.
 26. Using mills for maceration, fish should be previously stunned, and fish should then be instantaneously killed.

Recommendations

1. Standard operating procedures to improve the control of the slaughter process to prevent impaired welfare should be introduced and relevant practical welfare indicators developed.

2. Since the welfare of all farmed fish species studied has been found to be poor when they are killed by being left in air (asphyxia) or when they are exposed to carbon dioxide in water, these methods should generally not be used for any species as alternative methods are available.
3. A surveillance (monitoring) programme should be initiated so that data is available in the future for an improved risk assessment and for determining improvements over time and also for benchmarking for those involved in the slaughter of fish.
4. The opportunity to develop new methods for slaughtering salmon is considerable and should be encouraged.
5. Valid, robust and practically feasible indicators to evaluate the welfare of salmon during slaughter procedures need to be developed
6. Persons involved in killing fish should be trained and hence skilled in handling and welfare.
7. Taking current knowledge into account, it seems reasonable to suggest that the fasting period should not exceed one week if the welfare of the animal is highlighted.
8. Crowding of fish should not be performed to the level that they show distress. Indicators for distress are: colour change, escape behaviour and air gulping.
9. Fish should be monitored when exiting the pumping system where presence of fresh injuries and excessive exhaustion are indicators.
10. After pumping, there should be visual checks for wounds and injuries.
11. No salmon should be killed by being left in air.
12. Carbon dioxide should not be used for stunning and killing salmon. Chilling of live fish is not at present a humane method of killing fish so should not be used, either alone or in combination with the use of carbon dioxide.
13. Machines for stunning and killing salmon should not be used if fish may be injured, not stunned or not rapidly killed because the size or orientation in the machine. Unless a back-up system exists for rapid re-stunning.
14. For percussive machines, size adjustment of the machines should be done by skilled personnel as it is crucial for stunning efficiency. All percussive stunning systems should have a back-up system.
15. The percussive systems should have a separate air supply or alternatively have security valves blocking the system once the pressure is reduced below to a certain threshold.
16. All stunning systems should have an appropriate backup system to correct from mis-stun.
17. For electric stunning minimum requirements of the electric field or current should be sufficient to stun fish to unconsciousness within 1 second.
18. Combining electrical stunning with percussive stunning or maceration, the minimum requirements of the current duration should prevent fish from recovering prior these events.
19. Combining electrical stunning with exsanguination, the minimum requirements for the current duration should prevent fish recovering during bleed-out.
20. Exsanguination without prior stunning is not humane and should not be used.

21. When exsanguination is performed after effective stunning, major vessels, for example 3-4 gill arch vessels on at least one side of the fish or the ventral aorta, should be cut to ensure rapid bleed out.
22. Effective cutting of the gill arches at least on one side of the fish.
23. It is essential that a sharp knife is used to cut the vessels.
24. Exsanguination should be carried immediately after stunning and in every case before recovery from stunning occurs.

Recommendations for further research

1. There is a need for further research on the effect of different pumping systems on the welfare of live salmonids. There is an uncertainty of effective functioning if fish pass through the system in the same order as entering it, or if some take longer than others depending on individual swimming capacity and physiological state. Little is known in this regard to different pumping systems, e.g. single pumps may create a variations in flow enabling some fish to resist the current for longer periods than systems providing a more even flow, or the effects of bends etc leading to turbulence.
2. In addition there is a need for research to determine critical control points including crowding stress, water quality, pH, organic matter and controls to ensure that the loss of consciousness is irreversible before handling or any other procedure. Finally administration of pre-anaesthesia sedation in the normal holding tank before either netting or administration of full anaesthesia should be evaluated as a way to ensure humane euthanasia.
3. More research into behavioural indications of insensibility, establishing a statistical basis for interpreting behaviours.
4. Studies should be carried out to study the capability of immediate killing of fish using mills for maceration.
5. The effects of the pre-slaughter fasting period on subsequent fish welfare at slaughter should be studied more in depth.
6. Colour change is indicative of stress, but how it can become an indicator of poor welfare needs further research.
7. As there is no acceptable method for the use of currently available pharmaceuticals for euthanasia, more research is needed in this area.
8. Establishing proper control points, welfare indicators and protocols require research.
9. Improvements are needed in the transfer methods for live fish.
10. Systems should be considered to avoid pumping or transfer of fish (e.g. placing stunners at the cage) so that only dead fish is transported to the processing plant.
11. More research is required to prevent recovery during exsanguinations, with electric stunning systems, while minimizing muscle stimulation.
12. Principles for percussive stunning should be investigated to determine optimum ways for transferring kinetic energy into a shock wave, to concuss the brain.

REFERENCES

- Akse and Midling, 1999 L. Akse and K.Ø. Midling, Oppdrettskveite: Håndteringstress, Bedøving og Bløgging/Sløying: Effekt på rigor mortis, Utblødning og Kvalitet, Farmed Halibut: Handlingstress, Stunning and Exsanguination/Gutting. Effect on Rigor Mortis, Bleeding and Quality, Rapport Fiskeriforskningen i Tromsø, Norway (1999) No. 10, 33 pp.
- Anon, 1995. Operating manual for the product certification schemes for Scottish quality farmed salmon and smoked Scottish salmon. Scottish Quality Salmon Ltd. Inverness Scotland, UK.
- Bell GR (1987) An outline of anesthetics and anesthesia for salmonids, a guide for fish culturists in British Columbia. Canadian Technological Report of Fisheries and Aquatic Sciences, vol 1534. Department of Fisheries and Oceans, Nanaimo, BC. 16p.
- Bernier, N.J. and Randall, D.J., 1998. Carbon dioxide anesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anesthetic treatment. *J. Fish Biol.* 52, pp. 621–637
- Claireaux G, Webber D, Kerr S, Boutilier R. 1995. Physiology and behaviour of free-swimming Atlantic cod (*Gadus morhua*) facing fluctuating temperature conditions *J Exp Biol.*;198(Pt 1):49-60.
- Cook, C.J., Devine, C.E., Gilbert, K.V., Smith, D.D. and Maasland, S.A., 1995. The effect of electrical head-only stun duration on electroencephalographic measured seizure and brain amino acid neurotransmitter release. *Meat Science* 40, pp. 137–147
- Donaldson, E.M., 1981. The pituitary-interrenal axis as an indicator of stress in fish. In Pickering A.D. (ed) *Stress and Fish*. 11-48 Academic press, London, UK.
- Einen O, Thomassen, MS (1998) Starvation prior to slaughter in Atlantic salmon (*Salmo salar*). II. White muscle composition and evaluation of freshness, texture and colour characteristics in raw and cooked fillets. *Aquaculture* 169, 37-53.
- Einen O, Waagan B, Thomassen, MS (1998) Starvation prior to slaughter in Atlantic salmon (*Salmo salar*). I. Effects on weight loss, body shape, slaughter- and fillet yield, proximate and fatty acid composition. *Aquaculture* 169, 37-53.
- Erikson U (2001) Potential effects of preslaughter fasting, handling and transport. In: *Farmed Fish Quality* (S. Kestin and P. Wariss, Eds.), pp 202-219, Blackwell Science, Oxford
- Erikson U (2008) Live chilling and carbon dioxide sedation at slaughter of farmed Atlantic salmon: A description of a number of commercial case studies. *J. Appl. Aquaculture* 20, 38-61.
- Erikson U, Hultmann L, Steen JE (2006). Live chilling of Atlantic salmon (*Salmo salar*) combined with mild carbon dioxide anaesthesia. I. - Establishing a method for large-scale processing of farmed fish. *Aquaculture* 252, 183-198.
- Erikson U, Sigholt T, Seland A (1997) Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*). *Aquaculture* 149, 243-252.
- Falconer, D.D., 1964. Practical trout transport techniques. *Prog. Fish-Cult.* 26:51-58
- Farrell AP (2006) Bulk oxygen uptake measured with over 60 000 kg of adult salmon during live haul transport at sea. *Aquaculture* 254, 646-652.

- Gerritzen, M., Lambooi, B and Van de Vis, H., 2008. Inline registration of heart rate and body temperature of free swimming eel. Oral presentation at a conference in Maastricht, The Netherlands.
- Hara, K. and T. Sata (2007). "The effects of the local anesthetics lidocaine and procaine on glycine and gamma-aminobutyric acid receptors expressed in *Xenopus* oocytes." *Anesthesia and Analgesia* 104(6): 1434-1439.
- Hilbrich, P. 1975. Tierschutzgerechtes Töten von Eintagsküken. *Arch. Tierärztl. Fortblid.* 3: 25-31.
- Hilbrich, P., von Mickwitz, G. 1977. Tierschutzgerechtes Töten aussortierter Eintagshänchen und nicht schlupffähiger Küken im Brutei. *Berliner und Münchener Tierärztl. Wschr.* 90: 355-358.
- Hinch, S.G., and Bratty, J. 2000. Effects of swim speed and activity pattern on success of adult sockeye salmon migration through an area of difficult passage. *N. Amer. J. Fish. Manag.* 129:604-612.
- Hobe H, Wood CM, Wheatly MG (1984) The mechanisms of acid-base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. I. Extra- and intracellular acid-base status. *Respiration Physiology* 55, 139 – 155.
- Iger Y, Abraham M, Zhang L, Stoumboudi M, Alexis M, Tsangaris K, Wendelaar Bonga S, van Ham E (2001) Fish skin alterations as indicators for stress in fresh- and seawater aquaculture. *Eur Aquaculture Soc*, Special publication No. 29:109-110.
- Iversen M, Finstad B, McKinley RS, Eliassen RA, Carlsen KT, Evjen T (2005) Stress responses in Atlantic salmon (*Salmo salar* L.) smolts during commercial well boat transports, and effects on survival after transfer to sea. *Aquaculture* 243, 373-382.
- Iwama GK, Ackerman PA (1994) Anaesthetics. In: Hochacka PW, Mommsen TP (Eds), *Biochemistry and Molecular Biology of Fishes*, vol 3. Elsevier Science BV, pp 1-15.
- Jaksch, W., Mitterlehner, A. 1979. Euthanasie von Eintagsküken in der Massentierhaltung. *Wien. Tierärztl. Mschr.* 66, 37-46, 145-149.
- Kestin SC, van de Vis JW and Robb DHF 2002 Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them. *Veterinary Record* 150: 302-307.
- Kiessling, A., Johansson, D., Zahl, I.H. and Samuelson, O.B. 2009. Pharmacokinetics, plasma cortisol and effectiveness of benzocaine, MS-222 and isoeugenol measured in individual dorsal aorta cannulated Atlantic salmon (*Salmo salar*) following bath administration. *Aquaculture* 286: 301-308.
- Lambooi, E., Kloosterboer, R.J., Gerritzen, M.A., Van de Vis, J.W (2006). Assessment of electrical stunning of farmed African catfish (*Clarias gariepinus*.) and chilling in ice water for loss of consciousness and sensibility. *Aquaculture*, 254, 388-395.
- Lambooi, E., van de Vis, J.W., Kloosterboer, R.J., Pieterse, C. 2002, Welfare aspects of live chilling and freezing of farmed eel (*Anguilla anguilla* L.): neurological and behavioural assessment, *Aquaculture* 210, pp. 159–169.
- Lines, J. and Kestin, S. 2004. Electrical stunning of fish: the relationship between the electric field strength and water conductivity *Aquaculture* 241(1-4): 219-234.

- Mejdell CM, Midling KØ, Erikson U, Evensen T.H. Slinde E (2009) Evaluering av slaktesystemer for laksefisk i 2008– fiskevelferd og kvalitet. Veterinaerinstittutets rapportserie 01-2009. Oslo: Veterinaerinstittutet
- Midling KØ, Mejdell C, Olsen SH, Tobiassen T, Aas-Hansen Ø, Aas K, Harris S, Oppedal K, Femsteinevik Å (2008) Slakting av oppdrettslaks på båt, direkte fra oppdrettsmerd, pp.59. Nofima Marin-rapport Nr 6, Tromsø, Norway
- Noble C, Kadri S, Mitchell DF, Huntingford FA (2008) Growth, production and fin damage in cage-held 0+ Atlantic salmon pre-smolts (*Salmo salar* L) fed either a) on-demand, or b) to a fixed satiation-restriction regime: Data from a commercial farm. *Aquaculture* 275, 163-168.
- Norwegian Scientific Committee for Food Safety (2008) Transportation of fish within a closed system - Opinion of the panel on animal health and welfare of the Norwegian Scientific Committee for Food Safety. 63 pp (ISBN 978-82-8082-242-0).
- Olsen SH, Sørensen NK, Stormo SK, Elvevoll E (2006) Effect of slaughter methods on blood spotting and residual blood in fillets of Atlantic salmon (*Salmo salar*). *Aquaculture* 258, 462-469.
- Oyama, T. (1973). "Endocrine responses to anesthetic agents." *British Journal of Anaesthesia* 45(3): 276-281.
- Oyama, T. and S. Wakayama (1988). "The endocrine responses to general anesthesia." *International Anesthesiology Clinics* 26(3): 176-181.
- Pavlidis M, Papandroulakis N, Divanach P. (2006) A method for the comparison of chromaticity parameters in fish skin: Preliminary results for coloration pattern of red skin Sparidae. *Aquaculture* 258, 211-219.
- Robb DHF (2001) The relationship between killing methods and quality. In: *Farmed Fish Quality* (S. Kestin and P. Wariss, Eds.), pp 220-233, Blackwell Science, Oxford
- Robb DHF and Kestin SC 2002 Methods used to kill fish: Field observations and literature reviewed. *Animal Welfare* 11: 269-282.
- Robb, D. H., Wotton, S. B. and van de Vis, J. W. 2002. Preslaughter electrical stunning of eels. *Aquaculture Research*, 33: 37-42.
- Robb, D.H.F. and Roth, B. 2003. Brain activity of Atlantic salmon (*Salmo salar*) following electrical stunning using various field strengths and pulse durations *Aquaculture* 216(1-4): 363-369.
- Robb, D.H.F., O'Callaghan, M., Lines, J.A., and Kestin, S.C. 2002. Electrical stunning of rainbow trout (*Oncorhynchus mykiss*): factors that affect stun duration *Aquaculture* 205(3-4): 359-371.
- Robb, DHF, Kestin SC and Warriss PD, 2000a. Muscle activity at slaughter: I. Changes in flesh colour and gaping in rainbow trout. *Aquaculture* 182, Issues 3-4, 15 February, Pages 261-269
- Robb, D.H.F., Wotton, S.B. McKinstry, J.L., Sørensen N.K and Kestin S.C., 2000b, Commercial slaughter methods used on Atlantic salmon: determination of the onset of brain failure by electroencephalography. *Vet. Rec.* 147, pp. 298–303
- Rørvik, K.A. Skjervold, P.O Fjæra, S. O. Mørkøre, T/ and. Steien, S.H. 2001. Body temperature and seawater adaption in farmed Atlantic salmon and rainbow trout during prolonged chilling, *J. Fish Biol.* 59 (2001), pp. 330–337.

- Ross, L. G. and B. Ross (2008). Anaesthetic and sedative techniques for aquatic animals. Oxford, Blackwell Publishing.
- Roth B, Birkeland S, Oyarzun, F. 2009b. Stunning, pre slaughter and filleting conditions of Atlantic salmon and subsequent effect on flesh quality on fresh and smoked fillets. Aquaculture. In press.
- Roth B, Moeller D, Veland J.O, Imstrand A, Slinde E. 2002. The effect of stunning methods on rigor mortis and texture properties of Atlantic salmon (*Salmo salar*). J. Food Sci. 67: 1462-1466
- Roth B, Slinde E and Robb DHF 2007 Percussive stunning of Atlantic salmon (*Salmo salar*) and the relation between force and stunning. Aquacultural Engineering 36: 192-197.
- Roth B., AK Imstrand and Foss A. 2009a, "Live chilling of turbot and subsequent effect on behaviour, muscle stiffness, muscle quality, blood gases and chemistry". Animal Welfare 2009, 18: 33-41
- Roth, B., Imstrand, A., Moeller, D., and Slinde, E. 2003. Effect of electric field strength and current duration on stunning and injuries in market-sized Atlantic salmon held in seawater North American Journal of Aquaculture 65(1): 8-13.
- Roth, B., Moeller, D., and Slinde, E. 2004. Ability of electric field strength, frequency, and current duration to stun farmed Atlantic salmon and pollock and relations to observed injuries using sinusoidal and square wave alternating current North American Journal of Aquaculture 66(3): 208-216.
- Roth, B., Oines, S., Rotabakk, B.T., and Birkeland, S. 2008. Using electricity as a tool in quality studies of Atlantic salmon European Food Research and Technology 227(2): 571-577.
- Roth, B., Slinde, E., and Arildsen, J. 2006. Pre or post mortem muscle activity in Atlantic salmon (*Salmo salar*). The effect on rigor mortis and the physical properties of flesh Aquaculture 257(1-4): 504-510.
- Skjervold PO, Fjæra SO, Østby PB, Einen O (2001) Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 192, 265-280.
- Tang S, C.J. Brauner, A.P. Farewell (2009) Using bulk oxygen uptake to assess the welfare of adult Atlantic salmon, *Salmo salar*, during the commercial live-haul transport. Aquaculture 286, 318-323.
- Ueta, K., T. Suzuki, et al. (2007). "Local anesthetics have different mechanisms and sites of action at recombinant 5-HT₃ receptors." Regional Anesthesia and Pain Medicine 32(6): 462-470.
- Van de Vis, J.W., Kestin, S.C., Robb, D.F.H., Oehlenschläger, J., Lambooi, E., Münkner, W., Kuhlmann H., Kloosterboer, R.J. Tejada, M. Huidobro, A. Otterå, H. Roth, B., Sørensen, N.K Akse, L. Byrne H. and Nesvadba, P. 2003. Is humane slaughter of fish possible for industry?, Aquaculture Research 34, pp. 211–220.
- Van den Burg, E.H. (2002): Neuroendocrine control of temperature acclimation in teleost fish. PhD thesis Radboud University Nijmegen.
- Wall AJ (2001) Ethical considerations in the handling and slaughter of farmed fish. In: *Farmed Fish Quality* (S. Kestin and P. Wariss, Eds.), pp 108-115, Blackwell Science, Oxford

- Wardle, C., 1997. Welfare of Farmed Salmon and Impact on Post Harvest Quality. In: Robb D. (Ed.) Minutes of workshop: Welfare of Fish at Slaughter. University of Bristol, U.K., 4th March 1997
- Wedemeyer G.A (1996) Transportation and handling. In: *Principles of Salmonid Culture* (eds W. Pennel & B.A. Barton), pp.727-758. Elsevier, Amsterdam.
- Wendelaar Bonga, S.E., 1997. The stress response in fish. *Physiol. Rev.* 77, 591-625.
- Westers H (1984) *Principles of Intensive Fish Culture* 109 pp. Michigan Department of Natural Resources, Lansing, MI.
- Wood CM, Turner JD, Graham MS (1983) Why do fish die after exercise? *J Fish Biol* 22, 189-201.
- Zahl, I.H., Kiessling, A, Samuelsen, O. and Hansen, T, (a) Anaesthesia of Atlantic cod (*Gadus morhua*) – effect of pre-sedation, and importance of body weight, temperature and acute stress. Accepted with revisions in *Aquaculture*.
- Zahl, I.H., Kiessling, A.K., Samuelsen, O.B. and Olsen, R.E. (b). Anaesthesia induces stress in Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*). Submitted to *Fish Biochem. Physiol.*

APPENDIX A: PRE-SLAUGHTER STEPS**Fasting before harvesting**

In Norway, salmon are typically fasted in the production cage for 1 – 2 weeks before the fish are collected by well boat. Often, the duration of fasting period is based on the seasonal changes in seawater (SW) temperature, i.e. for about one week during summer and about two weeks during winter. The seasonal changes in SW temperature can be large (3 to 20 C) in the fjords where the fish farms are located. Obviously, the temperature range depends on the geographical location of a particular farm.

There are several reasons for fasting the fish before harvesting. Firstly, it is done to reduce the metabolic rate (oxygen demand and excretion of waste products, Westers 1984) and the physical activity of the fish before handling and live transport. To effectively reduce salmonid metabolic rates, a fasting period of 2-3 days is required (Falconer 1964, Wedemeyer, 1996).

Secondly, the digestive tracts should be emptied to reduce water fouling (undigested feed, faeces and microorganisms) during transport, and to avoid cross-contamination (residual feed, gut enzymes and bacteria) of the flesh when the fish are gutted and processed further. However, anecdotal information from the industry suggests that a fasting time of 2 – 3 days may be a little on the short side to ensure sufficient clearance of digestive tracts.

In a review of the effect of fasting on flesh quality, it was concluded that several weeks would be required to significantly change composition or other quality parameters (Erikson 2001). In fact, long-term fasting can cause economic losses as Einen et al. (1998) reported that Atlantic salmon lose weight and condition during fasting, stabilizing after 30 days. Moreover, Einen and Thomassen (1998) concluded that fasting is a rather weak tool for changing salmon fillet quality. Thus, it seems reasonable to conclude that there is no weight quality or economic justification for prolonged periods of fasting of this species.

From a fish welfare point of view, little information is available on the effect of the duration of starvation period. One factor is that food deprivation can lead to aggressive behaviour among the fish within the cage. When fish are fed less than on-demand, this will increase the incidence of dorsal fin erosion (Noble et al. 2008). This suggests that the period of feed withdrawal should be kept as short as possible on welfare grounds.

Harvesting procedures at production cage

Traditionally, the fish are collected from the sea-cages in a batch-wise manner by well boats. Before the well boat arrives at the site, the portion of the biomass in the sea-cage to be harvested is collected in a sweep net. This is done to increase fish density to facilitate transfer of fish to the well boat hold. In the seventies, it was common that loading was carried out by using large lift nets. The method was later on improved by using lining on the net so that the fish could be transferred without drainage of water. Since the early nineties, it has been gradually become more common to make use of the siphon principle to load the fish. This method, thought to be gentle to the fish, is more or less the standard method in use. Firstly, the water level of the well boat hold is lowered below the sea surface. Then, the suction of water and fish is initiated by starting the pressure-vacuum pump mounted on the vessel's deck. As the flow is established, the vacuum pump is turned off and the collected number of fish in the sweep net will gradually fill the hold. The hose diameter is typically 14-15 inches. As observed visually, this operation seems to be very gentle to the fish. Assessment of white muscle pH before and after transfer showed no statistical difference showing that the fish did not attempt any vigorous escape reactions (unpublished field observations).

As mentioned above, the fish density is increased before loading and crowding will occur at some point, particularly towards the end of the loading operation. This can be stressful to the fish and fish welfare issues should therefore be paid attention to. Crowding is discussed below in connection with the use of holding cages.

An alternative harvesting procedure is just emerging in the aquaculture industry. Here, the fish are pumped to percussion stunning machines located on a specially designed harvesting vessel. After stunning, the fish are bled and transferred to refrigerated seawater (RSW) tanks on board. Then, the fish are transported to the processing plant for gutting and further processing. From a fish welfare point of view only, this slaughter method seems to be very attractive since repeated handling, crowding, pumping of live fish is avoided. Trials have shown that salmon slaughtered on such a vessel can exhibit high initial muscle pH and very long pre-rigor times, showing the fish were exposed too little ante-mortem handling stress (Midling et al. 2008). It remains to be seen to what extent this harvesting method will be adopted by salmon industry. Since the various operations for slaughter of the fish are in principle similar to those occurring within the confines of the land-based processing plants, these operations are described above in connection with crowding in holding cage, transfer of fish to processing line, and percussion stunning.

Transport to processing plant

In Norway, practically all farmed salmonids are routinely transported by well boats to the processing plant to be slaughtered and processed. In 2008, there were 97 vessels approved by the Norwegian Food Safety Authority for transport of live fish and many of those were well boats used by the salmon industry. The carrying capacity of well boats ranges from about 50 (older vessels) to 2250 m³ (newer vessels).

In principle, two transport strategies could be chosen, either using an open system or a closed system. Since most vessels are equipped with a RSW system, chilling the live fish on board is also possible. Due to the high water exchange rates required, the use of the RSW system is in practice associated with closed (or semi-closed) systems only.

The most modern vessels have video systems for monitoring fish behaviour in the holds. Water quality (dissolved oxygen, pH, temperature, and sometimes carbon dioxide) is logged throughout the entire haul. In many cases, a fish counter is used during loading to provide information of the total biomass taken on board as well as data on fish size distribution.

In a commercial setting, the transport economy is of course dependent on the biomass being transported. Thus, the maximum wanted biomass to transport under the given conditions must be balanced with what is physically possible in terms of stress, mortality and reduced fish quality. For example, at high SW temperatures during summer, the holds are often oxygenated to be able to keep fish densities reasonably high. If SW temperatures in the sea-cages approaches about 18-20 C, the well boat crew can be rather reluctant to actuate transports at all since Atlantic salmon cannot endure much handling and stress under such conditions.

Open system

Practically all transports are carried out using an open system, meaning that fresh SW is constantly circulated at high rates through the valves as long as the vessel is en route. Based on oxygen uptake rates, it has been concluded that Atlantic salmon quickly recover from loading stress (Farrell 2006). Due to the high SW exchange rates, the water quality and fish welfare is good in the holds (Erikson 1997, 2001, Farrell 2006, Tang et al. 2009). Similarly, when Atlantic salmon smolts are transported by well boat, it has been shown that the loading process (including crowding and vacuum-pumping from the sea-cage) is more stressful than

the actual live transport. In fact, based on data from 5 well boat transports, the plasma cortisol, glucose and lactate values showed that the fish recovered from stress during transport. The fish density, SW temperature and transport duration ranged from 17 - 42 kg m⁻³, 7.9 - 9.6 C, and 4 - 40 h, respectively (Iversen et al., 2005). Moreover, under normal transport conditions it has been shown that the white muscle stress indicators (pH and high-energy phosphates) as well as fillet quality are not affected by transport. The transports from cage to processing plant were carried out under the following conditions (range): dissolved oxygen 60 -120 % saturation, transport duration 1.5 - 5 h, SW temperature 6-15 C, fish density 119-177 kg m⁻³ (Erikson 1997, 2001).

A survey of 150 commercial transports of salmon with 9 different well boats revealed that no adverse effects on fish quality were reported under the following, typical conditions: oxygen was added to the hold in 39 % of the transports, dissolved oxygen 70-120 % saturation, duration of transport 0.3 – 8.5 h, fish density 41 – 255 kg m⁻³, SW temperature 3 -17 C, and time at quay before unloading 0 -13 h (Erikson, 2001).

An 11 h well boat transport with adult salmon at a fish density of about 100 kg m⁻³ and SW temperature of 11-12 C appeared to promote good fish welfare (Farrell, 2006).

During unloading, the vessel's circulation pumps are providing adequate SW exchange of the holds. Pressure-vacuum pumps have largely replaced traditional lift nets for transfer of fish from well boat to holding cage or directly to the fish processing line. Two new concepts are being introduced in the salmon industry. Instead of lowering the water level in the hold, normally necessary for increasing fish density before pumping, the fish are slowly and gently forced to swim out from the hold using a moveable bulkhead. To avoid possible crowding stress, fish behaviour is constantly video monitored as the bulkhead gradually is decreasing the volume of the hold. The other new unloading method is based on pressurizing the hold.

Closed system

Presently, closed system (re-circulated water) transports of adult salmon are not carried out on a regular basis in Norway. However, on occasions, fish with diseases have been transported to processing plants using closed systems. In such cases, the valves are closed from just after the fish are loaded and during the time the vessel passes sheltered areas where other fish farms are located. Out on the open sea, the valves are opened, and as the vessel starts to approach the processing plant, located in sheltered areas, the valves are shut. Smolts are sometimes transported in closed systems from the hatchery to the sea-cages.

The issue of transporting live salmon to the processing plant in closed systems on a routinely basis has been raised several times over the years. The incentive for doing this has been thought to promote better disease control, that is, if the transported fish are infected by pathogens, an open system transport can be risky since the effluent may reach other fish farms along the way to the processing plant. Since control of disease is presently a major issue in the salmon industry in Chile (exporter of farmed salmon to the EU) a shift to closed well boat transport is particularly being examined there.

Since reduced water temperature results in a decrease of metabolic rates (e.g. lower oxygen consumption and excretion of waste products such as carbon dioxide and ammonium) and activity levels, lowering the transport water temperature (RSW) means that a larger biomass can be transported making the transport more cost-effective. Another incentive for using chilled transport is that this makes it possible to deliver pre-chilled, calm fish to the processing plant (in cases where the fish are delivered directly to the processing line).

An RSW-chilled (closed) transport has been evaluated under commercial conditions. A modern well boat with two separate holds (250 m³ each) was loaded with salmon at the sea-

cage. The biomass was divided equally between the holds resulting in a fish density of 90 kg/m³. The SW in one of the holds had been pre-chilled to 1 C (RSW, closed system) whereas the other hold was operating with open valves (as traditionally) at 8 C (SW temperature that day). During the 2-3 h of loading and transport to the plant, only the closed hold was oxygenated (no equipment for purifying the re-circulated water was used). During transport, the fish in the two holds behaved differently. As usual in the open system, the fish distributed themselves reasonably equally in the whole water column. In the closed system on the other hand, the fish tended to gather quite closely together near the bottom of the hold. In both holds, the fish exhibited typical slow, aerobic red muscle-based swimming behaviour although the fish in the closed hold seemed to be even more torpid. The dissolved oxygen levels in both holds varied between 70-110 % saturation. In the open system, the water quality basically resembled clean SW. In the closed system, the carbon dioxide levels increased steadily up to 45 mg l⁻¹ causing a drop in the water pH of 1.3 units from pH 8.0 (start to end of transport). The alkalinity increased from 2.25 to 2.45 μmol l⁻¹ and the salinity was constant at 33.3 ppt. The TAN (NH₄⁺ + NH₃) increased to 2520 μg l⁻¹, but the toxic fraction of this, NH₃, showed only a moderate increase up to 2.0 μg l⁻¹ which is well below the proposed safety level for fish farming (20 μg l⁻¹). Moreover, the water gradually became less transparent and some foaming occurred as was seen on the water surface. Reflecting these changes, the Colour value increased from about 2 to 8 mg Pt l⁻¹ (distilled water = 0 mg Pt l⁻¹) and the total organic carbon (TOC) increased from 1.4 to 4.8 mg l⁻¹. The concentration of Fe³⁺, used as indices of blood haemoglobin (re-circulated water containing live fish has often a reddish tint), increased from 10 to 108 μg l⁻¹. When fish were individually netted from the hold after the transport, they hardly struggled at all. The body temperature was then similar to that of the transport water (1 C). It is possible that the fish were lightly sedated due to the accumulated carbon dioxide. The plasma chloride values before (sea-cage), and after transport of fish from the open and closed systems were 149 ± 8, 141 ± 8, and 155 ± 5 mmol l⁻¹, respectively. This indicated a mild stress response for the chilled fish. The white muscle pH values of fish from both groups were typical of rested fish (pH 7.3 - 7.5). No mortalities were observed in either hold. Summarized, the study showed that calm, pre-chilled fish could be delivered to the processing line without loss of biomass during transport. On the other hand, the water quality in the closed hold gradually deteriorated which lead to elevated levels of plasma chloride and altered fish behaviour (Erikson 2001). From this study alone, it was not clear whether fish welfare was seriously compromised.

In a simulated live fish transport (a similar closed system) experiment at fish densities of 227 – 329 kg m⁻³ (fish size 4 – 5 kg) for 5 h at 15 C and heavy oxygen super-saturation (up to 250 %), firstly lead to sluggish behaviour and reduced gill ventilation rate. After a while, the fish exhibited a gulping and coughing behaviour as they kept their mouths above the water surface. Later, brief burst of activity were observed. When the experiment was terminated after 5 h, most fish tended to stand upright quietly at the bottom of the tank scarcely with any gill movement at all (Erikson 2001). It is well-known that high levels of dissolved oxygen cause reduced ventilation rate, build-up of metabolically produced carbon dioxide, and reduced blood pH (hypercapnia and acidosis) (Hobe et al. 1984). In turn, this may affect brain activity and thus behaviour. Notably, in a parallel experiment with a dissolved oxygen level of 80 % saturation, the fish also exhibited adverse behaviour but less extreme. This probably showed that also other water quality parameters may contribute to the changes in behaviour. The plasma chloride values of fish exposed to poor water quality for 5 h showed a clear stress response at 155 – 170 mmol l⁻¹ whereas control fish (good water quality) exhibited values in the range of 140 – 145 mmol l⁻¹. The white muscle pH values of 7.3 – 7.4 on the other hand, indicated rested fish, in accordance with the fact that no excessive struggling took place during the entire duration of the experiment (Erikson, 2001).

Basically, RSW live chilling tanks, commonly used in connection with sedation and slaughter of salmon, resembles closed transport systems. The holding time of the fish are much shorter though, 20 - 60 min when used in the slaughter line. For more details on how deteriorating water quality might affect the fish, refer to the descriptions of live chilling and carbon dioxide sedation above.

Another factor to consider is delayed mortality. This may occur if the fish are not slaughtered shortly after transport using closed systems. On 5 occasions, adult salmon have been crowded in the sea-cage and then transported for about 2 h in oxygenated closed containers to our laboratory. At arrival, with elevated levels of metabolically produced carbon dioxide (low pH) and oxygen supersaturated transport water, the fish had probably developed acidosis (Erikson, 2001). After transfer of the fish to large holding tanks with excellent water quality, in 3 out of 5 cases the fish recovered within a few hours after transport. However, in the other 2 cases, the fish started to die after some hours. Within a week, all fish had died. The following pattern was observed: fish behaviour was not normal as no shoaling took place and several fish stayed close to the bottom of the tank and swam occasionally around in a random pattern. Even though water exchange was good, water clarity was constantly reduced probably due to loss of mucus and then scales. After a few days, fungi were observed on the skin. Probably, the fish did not recover from the acidosis caused by handling and transport. It has been suggested that intracellular acidosis may play an important role for fish death under severe conditions where a drop in blood pH is observed (Wood et al. 1983).

According to expert observations, when mucus was lost, they probably suffered from a severe iono-regulation failure which might have been another cause of death. These observations show that the fish may appear quite normal just after transport, but in fact they may be so severely stressed that they will not recover and eventually die (unpublished results). Thus, in terms of fish welfare, this is a point to take into consideration.

Transport of fish in closed systems has recently been reviewed by the Norwegian Scientific Committee for Food Safety (2008).

APPENDIX B: RISK ASSESSMENT APPROACH

Introduction

Overall the risk assessment was constrained due to limited scientific data and consequently a semi-quantitative assessment was carried out often based on expert opinion. Because of this lack of data, the Panel on Animal Health and Welfare recommends that a surveillance / monitoring programme should be initiated for all the fish species so that in the future it may be possible to carry out a quantitative risk assessment.

In this section, the risk assessment method used to assess the risk to welfare of farmed fish at the time of killing is described.

Risk assessment is a systematic, scientifically based process to estimate the probability of exposure to a hazard, and the magnitude of the effects (consequences) of that exposure. A hazard in animal welfare risk assessment may be defined as a factor with the potential to cause a negative animal welfare effect (adverse effect). Risk is a function of both the probability that the hazard and the consequences (characterised by the adverse effect) occur.

Three parameters were scored to assess the importance of a hazard; the intensity of the adverse effect that the hazard causes, the duration of the adverse effect and the probability of exposure to the hazard. The population in question is the fish killed in the EU by the selected method of stunning and slaughter.

The probability of exposure to the hazard corresponds to the percentage of all fish exposed to the hazard. Thus if 4% of the all the fish killed by a particular method are exposed to a hazard there is a probability of 0.04 that any randomly selected fish within that population is exposed. The consequence of exposure can be assessed by scoring the intensity and the duration of the adverse effect in the individual. The risk assessment was based on two assumptions;

1. all fish exposed to the hazard experienced the same intensity and duration of the adverse effect.
2. in the absence of any evidence to the contrary, it is assumed that all fish exposed to the hazard experience the adverse effect³.

Factors which adversely affect fish welfare are considered in the risk assessment. In absence of reliable data, the volume of fish slaughtered by each method is not taken into account. Thus the results are not weighted by the volume of fish slaughtered by each method.

The definitions of intensity and the categories for duration of the adverse effect used for the fish species considered in this scientific opinion are in the relevant section in each Scientific Opinion.

In the following paragraphs the risk assessment process for hazard identification and characterization and the probability of exposure to the hazard are described as well as the way they were scored. Finally the risk scoring process is described.

The general risk assessment is in line with the approach previously used in the EFSA welfare reports (EFSA, 2007a; EFSA, 2007b; EFSA 2007c; EFSA, 2008a; EFSA, 2008b; EFSA,

³ if this assumption was not found to be sound for a particular hazard an additional parameter (probability that exposure resulted in the adverse effect) was used.

2008c; EFSA, 2008d; EFSA, 2008e) with some modifications according to the risk question posed.

Hazard identification

The objective of the hazard identification is to identify potential welfare hazards associated with each stunning and killing method. The identification was based on a review of the literature and field observations. The scope of the risk assessment included the period leading up to killing (which may be the time spent in lairage for fish killed in a slaughterhouse). The adverse effect caused by each hazard is described. In order to consistently identify hazards associated with stunning and killing, the relationship between the time from applying a stun method, unconsciousness and the point at which the killing method was applied are illustrated graphically (Figure 6). Various scenarios (A to E) in which hazards may arise were identified as follows:

‘A’ where a fish is killed in some potentially painful way (asphyxia, bleeding out) while it is conscious i.e. before it has been made unconscious; and

‘B’ represents a fish that has been stunned and is killed or it dies after it is unconscious;

‘C’ where a fish has been stunned but it recovers consciousness and is killed in some potentially painful way (asphyxia, bleeding out).

‘D’ represents a fish that, like A is killed in some potentially painful way (asphyxia, bleeding out) while it is conscious but has also suffered from the aversive nature of the stunning method; and

‘E’ represents a fish that has been stunned and is killed or it dies after it is unconscious but has also suffered from the aversive nature of the stunning method.

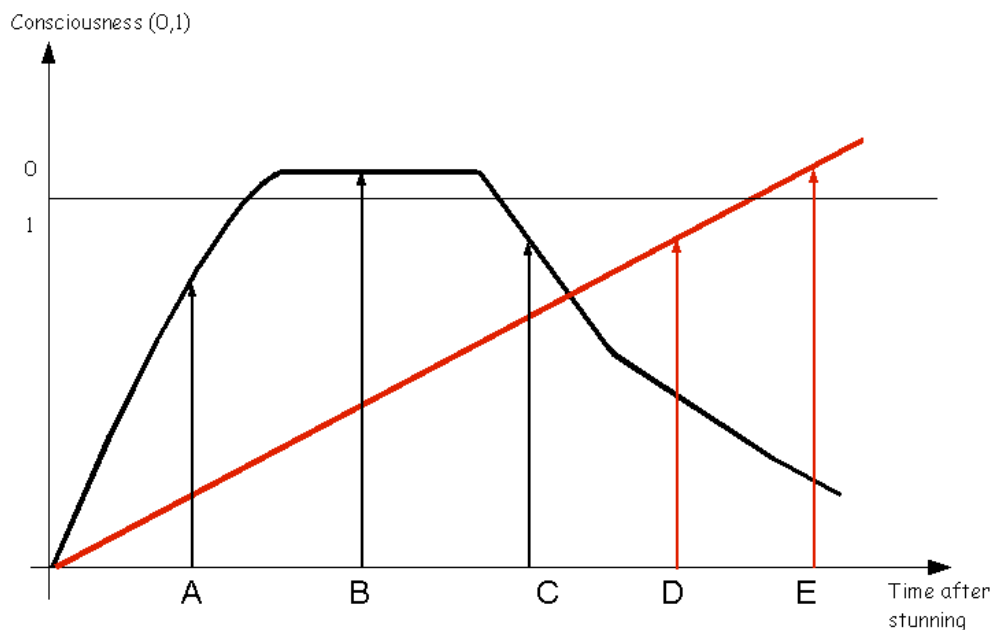


Figure 6. Time to unconsciousness (insensibility) following stunning / killing (horizontal grey line indicates consciousness threshold above which killing takes place without an adverse effect).

The scenarios above do not take into account hazards arising from gathering animals during pre-slaughter or killing without stunning.

Table 7. Intensity categories for adverse effects arising from hazards associated with pre-slaughter / slaughter operations in Atlantic salmon.

Evaluation	Score	In water	In air
MILD The animal is minimally affected as evidenced by minor changes in behaviour	1	<u>Pre-stunning:</u> Signs include rapid swimming away from stimulus and then slowing down. Increased ventilation. Colour change on the back. <u>Post-stunning:</u> Shallow and irregular gill ventilation, single reflexive gasps, weak eye rolling, weak fin movements.	<u>Pre-stunning:</u> Up to 3 seconds is mild for salmon (no notable behavioural changes). <u>Post-stunning:</u> Weak eye rolling, no response to handling, no gill movements, only single reflexive gasps. Not in mild or severe categories.
MODERATE	2	Not in mild or severe categories	Not in mild or severe categories.
SEVERE Marked changes from normal behaviour	3	<u>Pre-stunning:</u> Swimming upside down or tilted, gulping in the surface or lying down on the bottom apathic, full eye roll. Injuries. <u>Post-stunning:</u> Panic flight reaction, response to handling, full eye roll.	<u>Pre-stunning:</u> Panic tail flopping, gasping movements possibly with exaggerated gill movements, full eye roll. Injuries. <u>Post-stunning:</u> Tail flopping and breathing movements.

Hazard characterisation

Intensity

If a fish is unconscious, by definition there is no adverse welfare effect at that time. Therefore, before assessing the intensity of any adverse effects, consideration must be given as to whether the fish is conscious or not; this is a binary judgement (i.e. degrees of un/consciousness are not assessed). There is evidence that signs associated with consciousness and unconsciousness at the time of killing apply to all fish species as they do for general anaesthesia (Kestin et al., 2002). If it is conscious, the appropriate score for the degree of intensity of the adverse effect must be selected: mild, moderate or severe. If unconsciousness is achieved or induced with no suffering, or any pain or distress is for less than one second, then it is assumed that there was no welfare hazard. The issue of consciousness is mainly relevant to hazards associated with the killing method. If unconsciousness was achieved immediately (less than one second) then it is assumed that there was no hazard associated with the proper and effective application of that method and so this was not included in the risk assessment.

Generic guidelines for defining intensity categories for pre-slaughter hazards and slaughter hazards are given in Table 11. The approach taken has been to define only the mild and severe categories; the moderate is defined as being neither mild nor severe. Thus, by default hazards which are considered to have welfare consequences which are not in the severe or mild category fall into the moderate category. This approach was taken as scientists are reasonably confident in recognising the extreme states of intensity but as these states are on a continuum, allocating a distinct moderate banding is more difficult and contentious. Appropriate descriptions for the categories of intensity will vary between species and are given for each species in the Scientific Opinion.

Additionally, different definitions of intensity for the same species may be required for hazards that occur before killing, compared with at the time of killing. The descriptions of intensity for these pre-slaughter adverse effects are given for each species in the Scientific Opinion.

Table 8. Duration categories for adverse effects arising from hazards associated with pre-slaughter operations in Atlantic salmon

Duration (minutes)	Score
< 5 ¹	1
5 – 15	2
>15 – 60	3
> 60	4

¹adverse effects with a duration of less than one second are not scored

Table 9. Duration categories for adverse effects arising from hazards associated with slaughter of Atlantic salmon

Duration (minutes)	Score
< 0.17 (<10 second) ¹	1
0.17 – 1	2
>1 – 2	3
> 2	4

¹adverse effects with a duration of less than one second are not scored

Finally, each hazard was assessed and ranked by magnitude and occurrence independently of other hazards. For some hazards there may be more than one adverse effect. For example, all fish netted will be exposed to air, but in addition they may be injured e.g. skin lesions due to contact with the net or other fish.

The duration of the adverse effect

The time during which an animal will on average experience the adverse effect was estimated in minutes. The duration of an adverse effect can be longer than the duration of the hazard, for example a mis-stun takes a fraction of a second but the adverse effect lasts until the animal is unconscious or dies. Thus the duration of the hazard is included in the duration of the adverse effect.

Different time periods may be used for the adverse effects arising from pre-slaughter hazards compared with the hazards associated with slaughter. The definitions of duration used are given in the relevant section of the Scientific Opinion.

Exposure assessment

The exposure assessment is performed by assessing the proportion of the population of interest (i.e all fish in the EU being killed by the method in question) that is likely to experience the hazard. This proportion is equal to the probability of exposure to the hazard (P_hazard). It is recognised that the proportion of the population exposed to a selected hazard will vary depending on the farm of origin and slaughterhouse. Estimates of the most likely, maximum and minimum values for this proportion are required. The range of values provides an indication of the uncertainty of the estimate (see next section).

Uncertainty and variability

The degree of confidence in the final estimation of risk depends on the uncertainty and variability (Vose, 2000). Uncertainty arises from incomplete knowledge and/or when results are extrapolated from one situation to another (e.g. from experimental to field situations) (Vose, 2000). Uncertainty can be reduced by carrying out further studies to obtain the necessary data, however this may not always be a practical possibility. It can also be appraised by using expert opinion or by simply making a judgment.

Variability is a statistical and biological phenomenon and is not reducible by gathering further information. The frequency and severity of welfare hazards will inevitably vary between farms and countries and over time, and fish will vary individually in their responses. However, it is not always easy to separate variability from uncertainty. Uncertainty combined with variability is generally referred to as total uncertainty (Vose, 2000).

Total uncertainty associated exposure to the hazard was captured by estimates of the maximum and minimum estimates of the most likely value of the proportion of the population exposed to the hazard. For the other parameters (intensity and duration of the adverse effect) total uncertainty was scored on a scale of 1-3 (Table 10).

Table 10. Scoring system for total uncertainty in severity and duration of effect

Score		Description
1	low	Solid and complete data available; strong evidence in multiple references with most authors coming to the same conclusions, or Considerable and consistent experience from field observations.
2	medium	Some or only incomplete data available; evidence provided in small number of references; authors' or experts' conclusions vary, or Limited evidence from field observations, or Solid and complete data available from other species which can be extrapolated to the species being considered
3	high	Scarce or no data available; evidence provided in unpublished reports, or Few observations and personal communications, and/or Authors' or experts' conclusions vary considerably

Risk Characterisation

The scoring process

The scoring was undertaken by the working group in plenary. The estimates were based on current scientific knowledge, published data, field observation and experience (as summarised in this report).

Calculation of the risk score

All three factors (probability of exposure to the hazard; intensity of adverse effect; duration of adverse effect), were included in calculating the final risk score of a hazard. The score for each parameter was standardised by dividing the score by the maximum possible score for that parameter. Thus all parameters have a maximum value of one. The risk score is the product of the standardised scores multiplied by 100 (for ease of comparison) and thus has a maximum value of 100.

$$\text{Risk score} = [(I_{\text{adverse_effect}} / 3) * (D_{\text{adverse_effect}} / 4) * (P_{\text{hazard}})] * 100$$

Where the following are defined:

the intensity of the adverse effect ($I_{\text{adverse_effect}}$)

the duration of the adverse effect ($D_{\text{adverse_effect}}$)

the probability of exposure to the hazard (P_{hazard})

The minimum, most likely and maximum values for P_{hazard} were used to generate minimum, most likely and maximum estimates of the risk score. If only one risk score is given it refers to the most likely. It is also assumed that hazards usually occur independently of each other.

Calculation of magnitude of adverse effect

The magnitude of the adverse effect is the product of the scores for intensity and duration according to the following formula:

$$\text{Magnitude score} = [(I_{\text{adverse_effect}} / 3) * (D_{\text{adverse_effect}} / 4)] * 100$$

It has a maximum score of 100. The magnitude provides an indication of the impact of the hazard on the fish which are exposed to the hazard and experience the adverse effect. Thus a hazard that causes a prolonged and severe adverse effect but which affects only a small proportion of the population will have a low risk score but a high magnitude of severity score.

Worked example – mis-stun

Mis-stun may result when a concussive stunning method is used. This will give rise to an adverse effect. It was estimated that the adverse effect had a intensity score equal to 3. The duration (time from mis-stun to death or re-stun) was judged to last between one and two minutes, hence a score of 3. It was estimated that the probability that the hazard occurs was 0.04 (i.e. 4% of fish suffer a mis-stun), with minimum and maximum estimates of 0.01 and 0.10, respectively. In summary:

- score for the intensity of the adverse effect ($I_{\text{adverse_effect}}$) = 3
- score for the duration of the adverse effect ($D_{\text{adverse_effect}}$) = 3 (between one and two minutes)
- the probability that the hazard occurs (P_{hazard}) = 0.04

(ranging from a minimum estimate of 0.01 to a maximum estimate of 0.10)

Thus the risk score for this example mis-stun is:

$$(3/3 * 3/4 * 0.04) * 100 = (1 * 0.75 * 0.04) * 100 = 3$$

This score has a range that is determined by the minimum and maximum estimates of the probability that the hazard occurs (P_{hazard}), 0.01 and 0.10 respectively.

$$\text{Minimum score} = (3/3 * 3/4 * 0.01) * 100 = 0.75$$

$$\text{Maximum score} = (3/3 * 3/4 * 0.1) * 100 = 7.50$$

The magnitude equals intensity score/3 * duration score/4 * 100; and in this example is 75:

$$(3/3 * 3/4) * 100 = 75$$

Interpretation of the risk score

Due to the limited amount of quantitative data on many effects of hazards on fish stunning and killing, the risk assessment was mainly based on expert opinion. The methodology used does not give a precise numerical estimate of the risk attributed to certain hazards; however the output can be used to rank the problems and designate areas of concern, as well as, guidance for future research. The methodology does not take into account interactions between factors and assumes linearity in the scores. These assumptions cannot be tested. Secondly, the risk scoring is semi-quantitative. Thus the scores allow a ranking but the absolute figures are not on a linear scale (e.g. a risk score of 12 should not be interpreted as being twice as important as a risk score of 6).

One key objective of this work is to compare different methods of stunning and slaughter within each species. This will be achieved by summing the risk scores for all the hazards arising for each method of stunning and slaughter. This figure will be used to rank and compare the methods. Risk scores are given for the commonly used methods (see Table 9). However, it should be noted that insufficient data were available to calculate the overall exposure to the hazard within the European population, i.e. how commonly are those methods actually used within the member states of the EU. For comparison purposes, this calculation is important as it quantifies more precisely the number of fish at risk for that particular method of slaughter. Moreover, a hazard with a small risk score but a high magnitude may still have serious welfare effects for a large number of fish. The converse is also true.

References

Vose D (2000) Risk analysis - a quantitative guide. John Wiley & Sons, Chichester

APPENDIX C: DESCRIPTION OF HAZARDS RELATED TO SALMON STUNNING AND KILLING

Table 11. Description of hazards related to pre-slaughter management in Atlantic salmon (*Salmo salar*) in Europe.

Hazard ID	Identification of hazard	Description of the hazard
Pre-slaughter - no lairage <i>post transport status</i>		
1	Fish is in metabolic stress (e.g. after a not-well performed closed transport)	Osmoregulatory imbalance, acidosis; Always fish dying from this step (below 1%)
2	Fish is injured	Scales off is the major injury due to crowding and pumping; minor injuries to the skin; major injuries to the skin and muscle and bones (haemorrhages, oedema, broken backs). Always fish dying from this step (below 1%)
Crowding		
3	Fish exposed to shallow water and air	Primary stress reaction because of loss of water column.
4	Water oxygen levels low (due to poor supervision)	Hypoxia, leading to acidosis, panic and respiratory distress
5	(Dip netting)	Abrasion, exhaustion
Pumping		
6	Poor pipe design	Causing injuries, sharp angles, junctions between pipes may severe the body surface, high drops down to a grid
7	Delay in pipe due to slow water flow (crowding, low oxygen)	Self-explanatory
8	Delay in pipe due to poor system logistics	Stops between pumping sessions (may last from 5 to 30 minutes, e.g. lunch break, shifts etc.): fish gets stuck
9	Getting stuck in vacuum pressure valve	Fish will get heavily injured, cut in half
Pre-slaughter – lairage <i>post transport status</i>		
10	Fish is injured	Same as 2
holding cage		
11	Poor water quality (pH, DO, water temp)	Low oxygen, algae, high fresh water exposure (runoffs of freshwater after rains) causing chronic problems (from half a day to a week), mortality happens (seasonality).
crowding		
12	Fish exposed to shallow water and air	Same as 3
13	Water oxygen levels low (due to poor supervision)	Same as 4
14	(dip netting)	Abrasion, exhaustion
pumping		
15	Poor pipe design	Same as 6
16	Delay in pipe due to slow water flow (crowding, low oxygen)	Same as 7
17	Delay in pipe due to poor system logistics	Same as 8
18	Getting stuck in vacuum pressure valve	Same as 9

Table 12. Description of hazards related to slaughter management of Atlantic salmon (*Salmo salar*) in Europe.

Hazard ID	Description of hazard	Description of the hazard
	Slaughter Percussive stunning - swim-in system, fully automatic	
1a	Exhaustion (swimming into the system)	Fish are exhausted because of being crowded and struggling. Exhausted fish are on the bottom or go to the surface for gulping.
1b	Severe exhaustion (swimming into the system)	Fish are severely exhausted because of being crowded and struggling. Exhausted fish are on the bottom or go to the surface for gulping.
2	Mis-stun	Too low pressure in the pressure chamber (sudden drop below 7-8 bars), hammer missed the correct location on skull, wrong orientation of the fish.
3	Mis-cut; if conscious	Failure to cut any major aorta or vein due to size or orientation. The fish is cut by knives without proper gill slit (knife is either mis-oriented (cut in the head), or on the side or too on front of the jaw).
4	Exsanguination; if conscious	The fish lose gradually consciousness (10 minutes) as it bleeds out in the exsanguination tank
5	Evisceration; if conscious	Failure to kill by percussive blow, or exsanguinate, or asphyxia, prior to evisceration.
	Percussive stunning - hand-fed system, automatic cut	
6	Being handled manually	Distress because of being held in air and handled
7	Asphyxia	Being in air
8	Mis-stun	Same as 2 but only caused by air pressure and placement in the system
9	Mis-cut; if conscious	Failure to cut any major aorta or vein due to size.
10	Exsanguination; if conscious	Same as 4
	Percussive stunning - hand-fed system, manual cut	
11	Being handled manually	Same as 6
12	Asphyxia	Same as 7
13	Mis-stun	Same as 8

14	Mis-cut; if conscious	Failure to cut the gill arch (unsharpened knife, partial cut).
15	Exsanguination; if conscious	Same as 4
	Live chilling + carbon dioxide	
16	Temperature shock	Stress reaction to a drop of temperature. Respiratory failure
17	Exposure to moderate levels of CO ₂	pH in the range of 6.2 and high
18	Low water quality (organic material, low pH, ammonia..)	Stress reaction and gill irritation
19	Exsanguination (proper) (fish are regarded as being conscious)	Same as 4 - duration may be longer due to low temperature.
20	Mis-cut	Same as 14
21	Evisceration; if conscious	Same as 4
	Electrical stunning - in-water (batch) system	
22	Crowding prior to stunning	Poor water quality, high density of fish, exhaustion from crowding
23	Electrical exhaustion (low current or voltage)	Current is too low to stun the fish in less than 1 second. The animal can consciously feel the electricity for a period 30 seconds. Escape behaviour, pain, distress, exhaustion
24	Mis-stun (insufficient current or voltage)	In the second phase, stunning with 50Hz electricity, 70 Volts and stunning should happen within 3 seconds
25	Exsanguination; if conscious	Same as 4 - fish may recover from the stunning (significant # of recovery after 3 minutes - 10% estimate)
26	Mis-cut; if conscious	Same as 14 - fish may recover from electrical stunning.
27	Evisceration; if conscious	Same as 5
28	Asphyxia; if conscious	If mis-cut happens, animals may die from asphyxia due to poor water quality in the exsanguination tank.
	Electrical stunning - dry system	
29	Asphyxia	Fish is exposed to air
30	Fish enter tail first	Up to 50% of fish can enter tail first and then feel electricity for about 2 to 3 seconds before the electrodes reach the head
31	Mis-stun 1 (insufficient current or voltage)	Insufficient current. some systems use low voltage (below 50Volts - human safety, money savings) - expand time before actual stunning (up to 20 seconds). Fish is exhausted and paralysed.

32	Mis-stun 2 (insufficient current or voltage)	High voltage system but insufficient current to the brain - animals will come out stunned eventually due to a 5 seconds to 15 seconds stun duration.
33	Exsanguination; if conscious	Same as 4
34	Evisceration; if conscious	Same as 5
35	Asphyxia; if conscious	Same as asphyxia in exsanguination bath
Electrical stunning - pipe line system		
36	Mis-stun 1 (insufficient current or voltage)	Insufficient voltage or complete failure of the system - no stunning
37	Mis-stun 2 (insufficient current or voltage)	Delayed stunning. some systems use low electric field (stunning after 1 second) or produce heterogenic electric field. Small fish may escape field and not get properly stunned.
38	Poor pipe design	See pre-slaughter steps
39	Exsanguination; if conscious	Same as 4
40	Evisceration; if conscious	Same as 5
41	Asphyxia; if conscious	Same as
Pharmacological methods (isoeugenol)		
42	Low water quality	Same as 18
43	Crowding, incl. too low water levels	Distress
44	Exposure to pharmaceuticals	Escape behaviour, distress
45	insufficient levels of anaesthetics => prolonged exposure time	Distress, respiratory collapse
46	Mis-stun (insufficient time of exposure to anaesthetics)	Stress
47	Asphyxia; if conscious	Distress, pain
Pharmacological methods (benzocaine)		
48	Low water quality	Distress, gill irritation
49	Crowding, incl. too low water levels	Same as 22
50	Exposure to pharmaceuticals	Aversive taste or smell for fish, irritating to skin, mucosa and gill
51	Insufficient levels of anaesthetics => prolonged exposure time	Mis-stun by too low dosing, prolonging 50 and 49
52	Mis-stun (insufficient time of exposure to anaesthetics)	Too short exposure, mis-stunning
53	Asphyxia; if conscious	Same as 7
CO2 only		
54	Exposure to high levels of CO2	Panic, release of catecholamines, low pH (below 5) acid bath, acute irritation, aversive behaviour,
55	Low water quality (organic material, low pH, ammonia..)	Respiratory failure, asphyxia, acidosis, mucous, scales, etc...

56	Exsanguination (proper) (fish are regarded as being conscious)	Same as 4
57	Mis-cut	Same as 3
58	Evisceration; if conscious	Same as 5

APPENDIX D: PARAMETERS USED IN PRODUCING RISK AND MAGNITUDE SCORES FOR WELFARE HAZARDS

Table 13. Parameters used in producing risk and magnitude scores for welfare hazards associated with preslaughter management in Atlantic salmon (*Salmo salar*) in Europe, where the fish are directly processed as they arrive at the abattoir.

Haz. ID	Pre-slaughter hazards	Intensity	Duration (min ¹)	Duration (score ²)	Uncertainty	Probability of exposure			Risk score		
						Most likely	Min	Max	Most likely	Min	Max
post-transport status											
1	Fish is in metabolic stress (e.g. after a not-well performed closed transport)	1	3 hours	4	1	0.1	0.05	0.15	6.67	3.3	10.0
2	Fish is injured during transport	3	3 hours	4	1	0.005	0.001	0.01	0.50	0.1	1.00
crowding											
3	Fish exposed to shallow water and air	2	30	3	1	0.02	0.01	0.05	1.00	0.5	2.50
4	Water oxygen levels low (due to poor supervision)	1	15	3	1	0.01	0.005	0.02	0.25	0.1	0.63
5a	Dry brailing	3	1	1	1	0.001	0.000	0.01	0.03	0.0	0.25
5b	Wet brailing	1	1	1	1	0.005	0.002	0.01	0.04	0.0	0.08
pumping											
6	Poor pipe design	3	3	1	3	0.1	0.05	0.15	2.50	1.2	3.75
7	Delay in pipe due to slow water flow (crowding, low oxygen)	1	4	1	3	0.1	0.05	0.15	0.83	0.4	1.25
8	Delay in pipe due to poor system logistics	2	10	2	3	0.05	0.03	0.07	1.67	1.0	2.33

9	Getting stuck in vacuum pressure valve	3	3	1	3	0.005	0.001	0.01	0.13	0.0 3	0.25
---	--	---	---	---	---	-------	-------	------	------	----------	------

¹ Unless another time unit is indicated

² 1 = <5min, 2 = 5-15min, 3 = 15-60 min, 4 = >60min

Table 14. Parameters used in producing risk and magnitude scores for welfare hazards associated with preslaughter management in Atlantic salmon (*Salmo salar*) in Europe, where the fish are in holding for an average of 3 days before they are processed further.

Haz. ID	Pre-slaughter hazards	Intensity	Duration (min ¹)	Duration (score ²)	Uncertainty	Probability of exposure			Risk score		
						Most likely	Min	Max	Most likely	Min	Max
10	post-transport status Fish is injured during transport	3	3 days	4	1	0.005	0.001	0.01	0.50	0.1 0	1.00
11	lairage Poor water quality (pH, DO, water temp)	1	3 days	4	1	0.005	0.001	0.01	0.17	0.0 3	0.33
12	crowding Fish exposed to shallow water and air	2	30	3	1	0.02	0.01	0.05	1.00	0.5 0	2.50
13	Water oxygen levels low (due to poor supervision)	1	15	3	1	0.01	0.005	0.02 5	0.25	0.1 3	0.63
14a	Dry brailing	3	1	1	1	0.001	0.000 1	0.01	0.03	0.0 0	0.25
14b	Wet brailing	1	1	1	1	0.005	0.002 5	0.01	0.04	0.0 2	0.08
15	pumping Poor pipe design	3	3	1	3	0.1	0.05	0.15	2.50	1.2 5	3.75
16	Delay in pipe due to slow water flow (crowding, low oxygen)	1	4	1	3	0.1	0.05	0.15	0.83	0.4 2	1.25
17	Delay in pipe due to poor system logistics	2	10	2	3	0.05	0.03	0.07	1.67	1.0 0	2.33

18	Getting stuck in vacuum pressure valve	3	3	1	3	0.005	0.001	0.01	0.13	0.0	0.25
										3	

Table 15. Parameters used in producing risk and magnitude scores for welfare hazards associated with slaughter methods applied to Atlantic salmon (*Salmo salar*) in Europe.

Haz. ID	Slaughter hazards	Intensity	Duration (min ¹)	Duration (score ²)	Uncertainty	Probability of exposure			Risk score		
						Most likely	Min	Max	Most likely	Min	Max
A	percussive stunning - swim-in system, fully automatic										
1a	exhaustion (swimming into the system)	1	2	3	1	0.95	0.9	0.99	23.75	22.50	24.75
1b	severe exhaustion (swimming into the system)	3	10	4	2	0.03	0.001	0.05	3.00	0.10	5.00
2	mis-stun	3	10	2	1	0.1	0.05	0.15	5.00	2.50	7.50
			sec								
3	mis-cut; if conscious	3	30	4	2	0.01	0.001	0.020	1.00	0.10	2.00
4	exsanguination; if conscious	3	6	4	1	0.09	0.080	0.099	9.00	8.00	9.90
5	evisceration; if conscious	3	2 sec	1	2	0.0005	0.000	0.001	0.01	0.00	0.03
							1				
B	percussive stunning - hand-fed system, autom. cut										
6	being handled manually	1	1	2	1	1	1	1	16.67	16.67	16.67
7	asphyxia (out of water)	1	1	2	1	1	1	1	16.67	16.67	16.67
8	mis-stun	3	10 sec	2	1	0.05	0.01	0.1	2.50	0.50	5.00
9	mis-cut; if conscious	3	30	4	2	0.025	0.015	0.035	2.50	1.50	3.50
10	exsanguination; if conscious	3	6	4	1	0.025	0.015	0.035	2.50	1.50	3.50
C	percussive stunning - hand-fed system, manual cut										
11	being handled manually	1	1	2	1	1	1	1	16.67	16.67	16.67
12	asphyxia (out of water)	1	1	2	1	1	1	1	16.67	16.67	16.67
13	mis-stun	3	10 sec	2	1	0.02	0.01	0.05	1.00	0.50	2.50
14	mis-cut; if conscious	3	30	4	2	0.001	0.000	0.002	0.10	0.01	0.20
							1				
15	exsanguination; if conscious	3	6	4	1	0.019	0.01	0.048	1.90	1.00	4.80

D	live chilling + carbon dioxide											
16	temperature shock	1	3	4	1	1	1	1	33.33	33.33	33.33	
17	exposure to moderate levels of CO2	3	30	4	1	1	1	1	100.00	100.00	100.00	0
18	low water quality (organic material, low pH, ammonia..)	2	30	4	2	0.9	0.8	0.95	60.00	53.33	63.33	
19	exsanguination (proper) (fish are regarded as being conscious)	3	6	4	1	0.999	0.998	0.999	99.90	99.80	99.95	5
20	mis-cut	3	30	4	2	0.001	0.000	0.002	0.10	0.05	0.20	5
21	evisceration; if conscious	3	2 sec	1	2	0.0005	0.000	0.001	0.01	0.00	0.03	1

¹ Unless another time unit is indicated

² 1 = <0.17 min (10 sec), 2 = 0.17-1 min, 3 = 1-2 min, 4 = >2min

Haz. ID	Slaughter hazards	Intensity	Duration (min ¹)	Duration (score ²)	Uncertainty	Probability of exposure			Risk score			
						Most likely	Min	Max	Most likely	Min	Max	
E	carbon dioxide only											
22	exposure to high levels of CO2	3	6	4	1	1	1	1	100.00	100.00	100.00	0
23	low water quality (organic material, low pH, ammonia..)	2	6	4	2	0.9	0.8	0.95	60.00	53.33	63.33	
24	exsanguination (proper) (fish are regarded as being conscious)	3	6	4	1	0.999	0.998	0.999	99.90	99.80	99.95	5
25	mis-cut	3	6	4	2	0.001	0.000	0.002	0.10	0.05	0.20	5
26	evisceration; if conscious	3	2 sec	1	2	0.0005	0.000	0.001	0.01	0.00	0.03	1
F	electrical stunning - in-water (batch) system											
27	crowding prior to stunning	1	1	2	1	1	1	1	16.67	16.67	16.67	
28	electrical pre-treatment	3	30 sec	2	1	1	1	1	50.00	50.00	50.00	

29	exsanguination; if conscious	3	2	3	3	0.2	0.1	0.3	15.00	7.50	22.50
30	mis-cut; if conscious	3	30	4	2	0.001	0.000	0.002	0.10	0.01	0.20
31	evisceration; if conscious	3	2 sec	1	3	0.0005	0.000	0.001	0.01	0.00	0.03
32	asphyxia; if conscious	3	8	4	2	0.0005	0.000	0.001	0.05	0.01	0.10
G electrical stunning - dry system											
33	asphyxia (out of water)	1	5 sec	1	1	1	1	1	8.33	8.33	8.33
34	fish enter tail first	3	2 sec	1	1	0.3	0.2	0.6	7.50	5.00	15.00
35a	experiencing electricity while conscious ; low voltage system (<50 V)	3	15 sec	2	1	0.05	0.01	0.1	2.50	0.50	5.00
35b	experiencing electricity while conscious ; medium voltage system (50-110 V)	3	2 sec	1	1	0.24	0.1	0.35	6.00	2.50	8.75
35c	experiencing electricity while conscious ; high voltage system (>110 V)	3	2 sec	1	2	0.02	0.001	0.05	0.50	0.03	1.25
36	exsanguination; if conscious	3	2	3	2	0.2	0.1	0.3	15.00	7.50	22.50
37	mis-cut; if conscious	3	30	4	2	0.001	0.000	0.002	0.10	0.01	0.20
38	evisceration; if conscious	3	2 sec	1	3	0.0005	0.000	0.001	0.01	0.00	0.03
39	asphyxia; if conscious	3	8	4	2	0.0005	0.000	0.001	0.05	0.01	0.10

Haz. ID	Slaughter hazards	Intensity	Duration (min ¹)	Duration (score ²)	Uncertainty	Probability of exposure			Risk score		
						Most likely	Min	Max	Most likely	Min	Max
H 40	electrical stunning - pipe line system experiencing electricity while conscious	3	3 sec	1	2	0.4	0.05	0.6	10.00	1.25	15.00
41	exsanguination; if conscious	3	2	3	2	0.2	0.1	0.3	15.00	7.50	22.50
42	mis-cut; if conscious	3	30	4	2	0.001	0.0001	0.002	0.10	0.01	0.20
43	evisceration; if conscious	3	2 sec	1	3	0.0005	0.0001	0.001	0.01	0.00	0.03

44	asphyxia; if conscious	3	8	4	2	0.0005	0.0001	0.001	0.05	0.01	0.10
I	pharmacological methods (metocaine, benzocaine)										
45	netting	2	3	4	1	0.2	0.1	0.3	13.33	6.67	20.00
46	low water quality	1	3	4	1	0.1	0.05	0.15	3.33	1.67	5.00
47	crowding, incl. too low water levels	1	5	4	1	0.1	0.05	0.15	3.33	1.67	5.00
48	exposure to pharmaceuticals	2	3	4	2	1	1	1	66.67	66.67	66.67
49	insufficient levels of anaesthetics => prolonged exposure time	1	10	4	2	0.001	0.0005	0.002	0.03	0.02	0.07
50	mis-stun (insufficient time of exposure to anaesthetics)	1	10	4	2	0.001	0.0005	0.002	0.03	0.02	0.07
51	asphyxia; if conscious	3	6	4	1	0.001	0.0005	0.002	0.10	0.05	0.20
52	silage	3	3	4	1	0.0001	0.0000	0.000	0.01	0.01	0.02
							5	2			

¹ Unless another time unit is indicated

² 1 = <0.17 min (10 sec), 2 = 0.17-1 min, 3 = 1-2 min, 4 = >2min

Haz. ID	Pre-slaughter hazards	Intensity	Duration (time)	Duration (score ¹)	Uncertainty	Probability of (exposure)		
						Most likely	Min	Max
I	pharmacological methods (metocaine)							
48	netting	2	3	4	1	0,2	0,1	0,3
49	low water quality	1	3	4	1	0,1	0,05	0,15
50	crowding, incl. too low water levels	1	5	4	1	0,1	0,05	0,15
51	exposure to pharmaceuticals	2	3	4	2	1	1	1
52	insufficient levels of anaesthetics => prolonged exposure time	1	10	4	2	0,001	0,0005	0,002
53	mis-stun (insufficient time of exposure to anaesthetics)	1	10	4	2	0,001	0,0005	0,002
54	asphyxia; if conscious	3	6	4	1	0,001	0,0005	0,002
55	silage	3	3	4	1	0,0001	0,00005	0,0002
J	pharmacological methods (benzocaine)							
56	netting	2	3	4	1	0,2	0,1	0,3
57	low water quality	1	3	4	1	0,1	0,05	0,15
58	crowding, incl. too low water levels	1	5	4	1	0,1	0,05	0,15

59	exposure to pharmaceuticals	2	3	4	2	1	1	1
60	insufficient levels of anaesthetics => prolonged exposure time	1	10	4	2	0,001	0,0005	0,002
61	mis-stun (insufficient time of exposure to anaesthetics)	1	10	4	2	0,001	0,0005	0,002
62	asphyxia; if conscious	3	6	4	1	0,001	0,0005	0,002
63	silage	3	3	4	1	0,0001	0,00005	0,0002

¹ 1 = <0.17 min (10 sec), 2 = 0.17-1 min, 3 = 1-2 min, 4 = >2min

APPENDIX E: RISK SCORES AND MAGNITUDE OF ADVERSE WELFARE EFFECTS ASSOCIATED WITH STUN/KILL METHODS

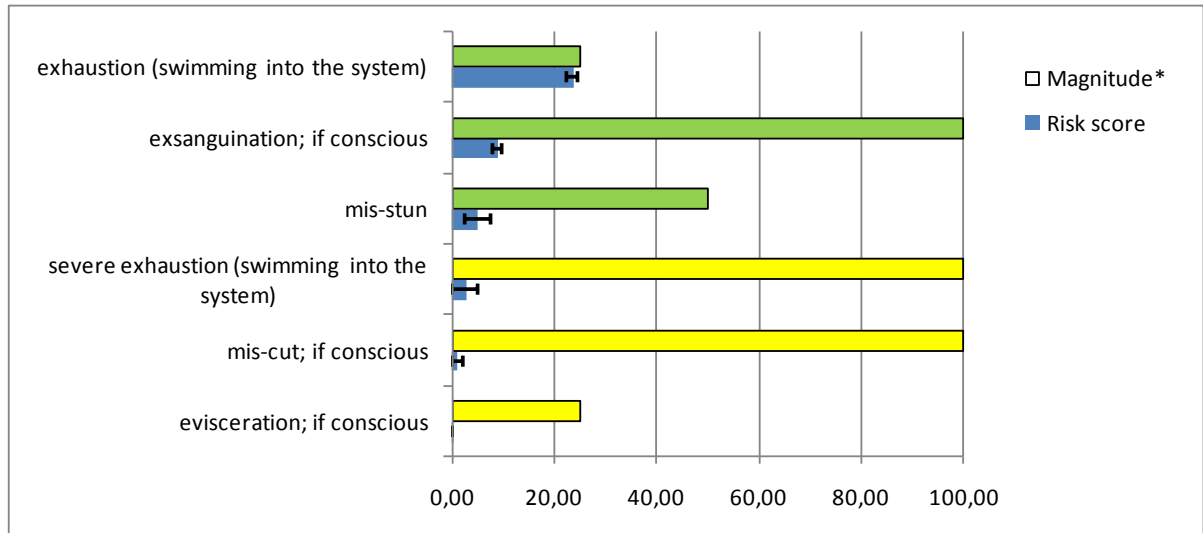


Figure 7. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of fully automatic percussive stunning (swim-in) systems (method A) in Atlantic salmon, ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard.

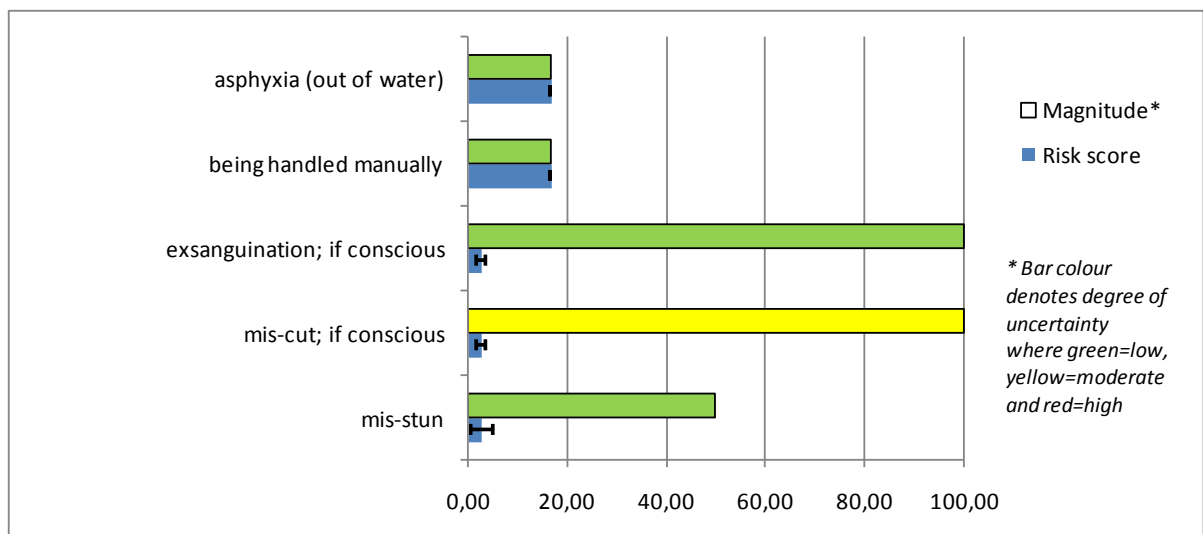


Figure 8. Risk score and magnitude of adverse welfare effect for individual hazards associated with hand fed percussive stunning systems with automatic cut (Method B) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated

minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard.

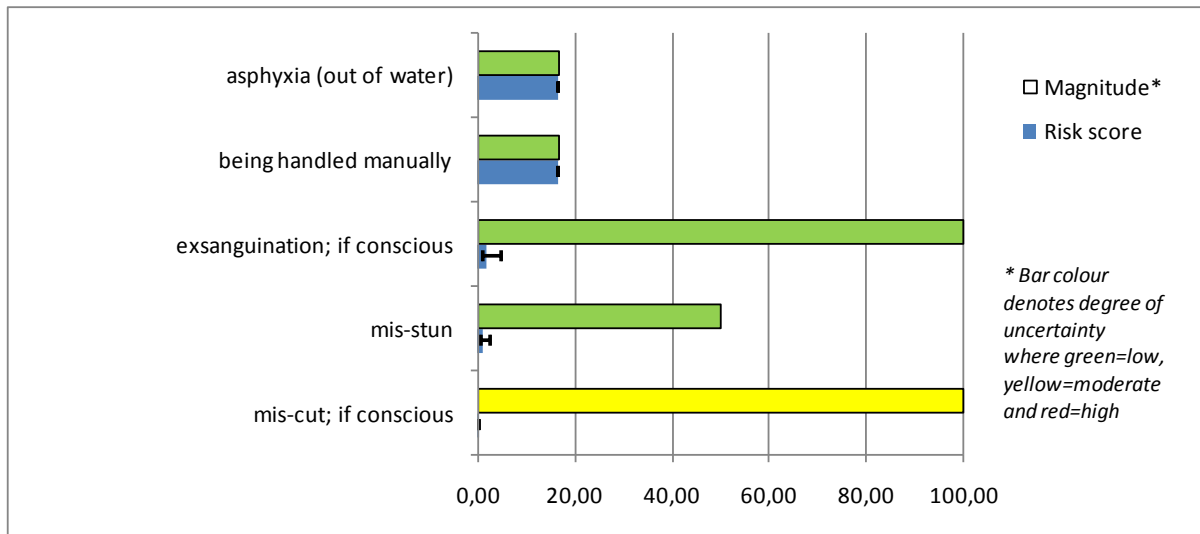


Figure 9. Risk score and magnitude of adverse welfare effect for individual hazards associated with hand fed percussive stunning systems with manual cut (method C) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard.

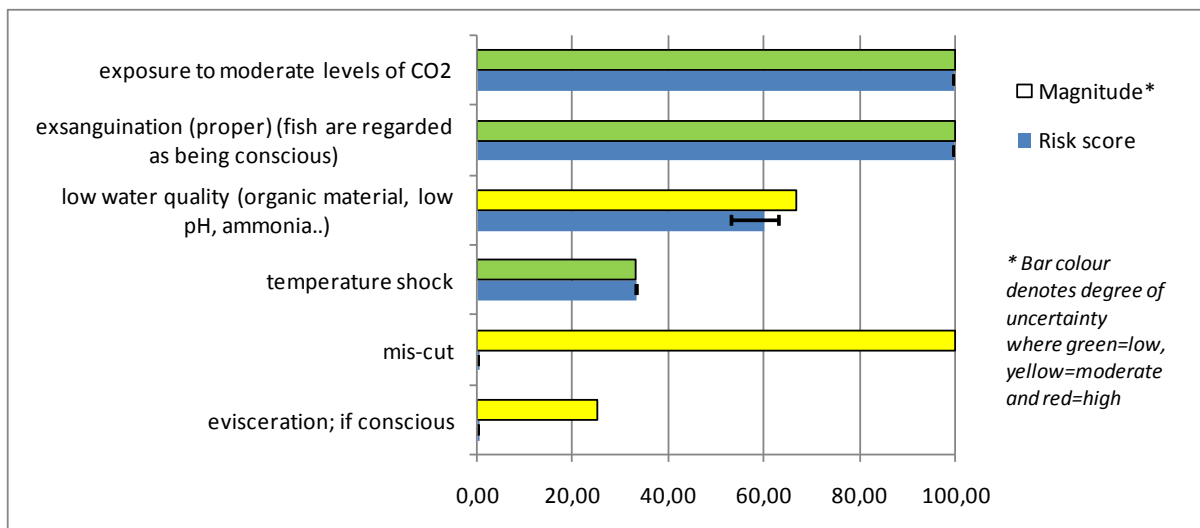


Figure 10. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of live chilling combined with carbon dioxide (Method D) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard.

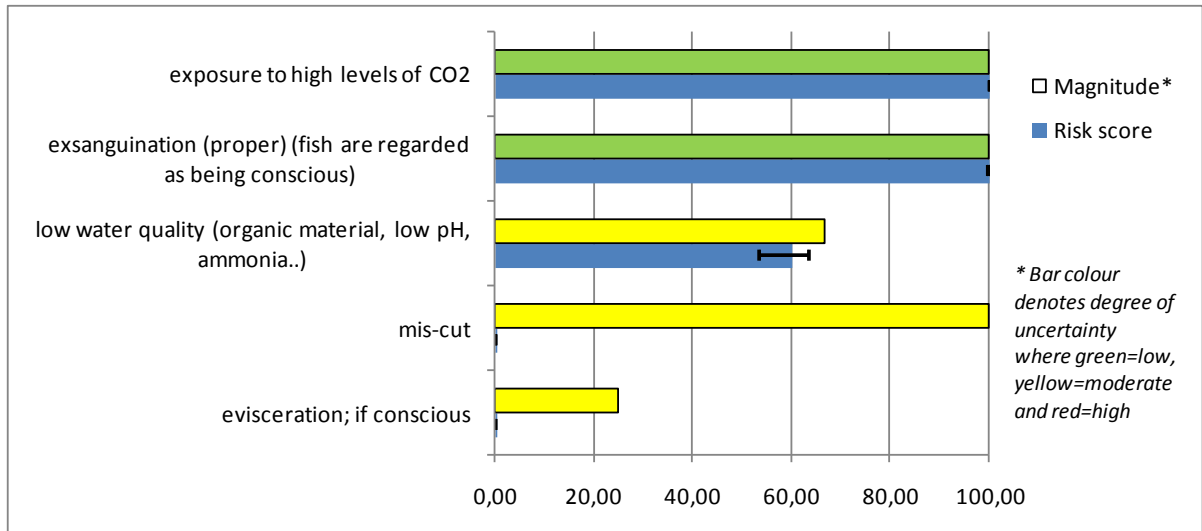


Figure 11. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of carbon dioxide only (method E) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard.

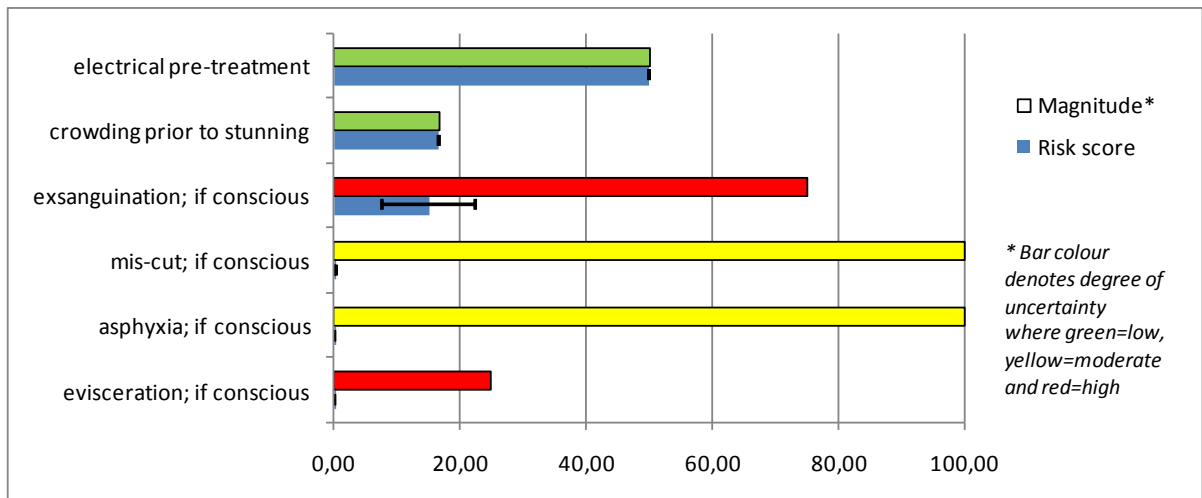


Figure 12. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of electrical stunning - in-water (batch) systems (method F) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard.

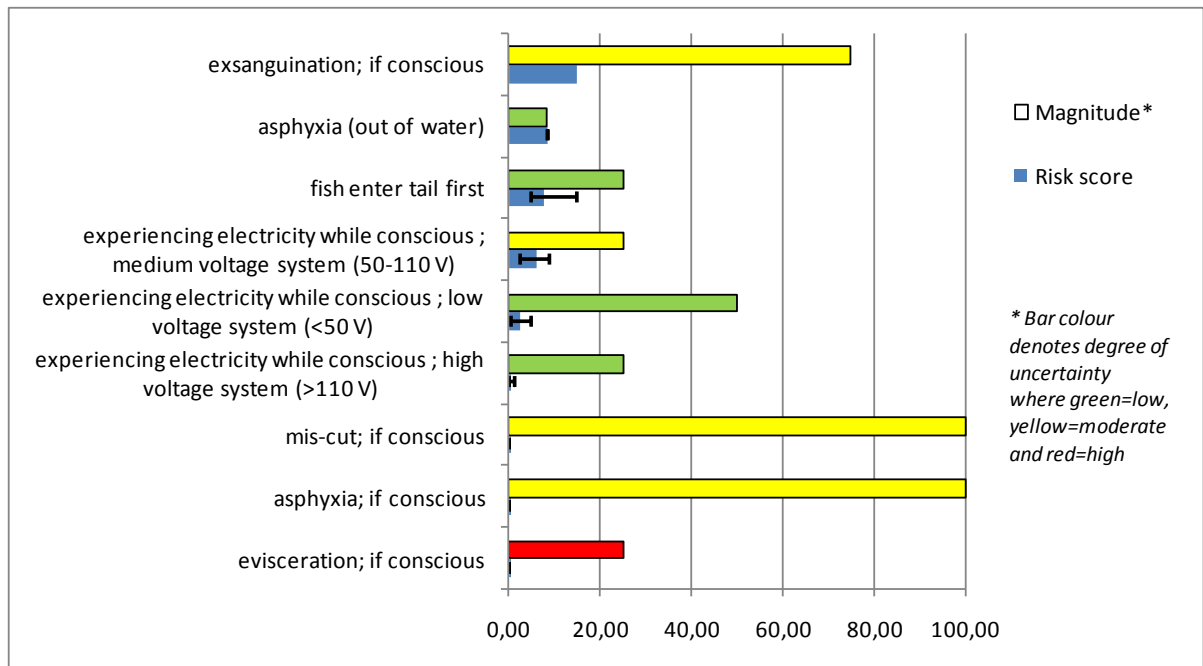


Figure 13. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of electrical stunning - dry systems (method G) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard.

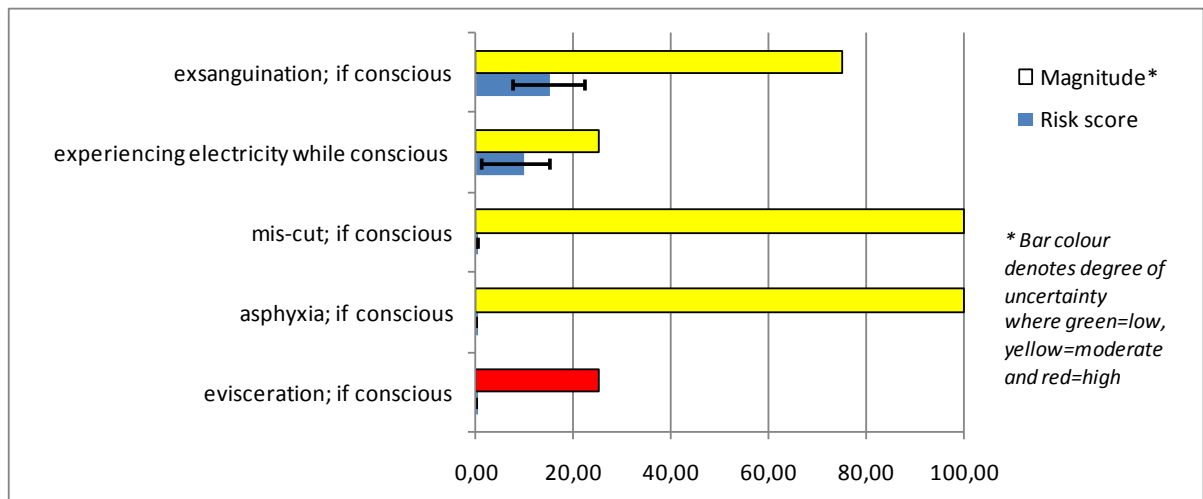


Figure 14. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of electrical stunning – pipe line systems (method H) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard.

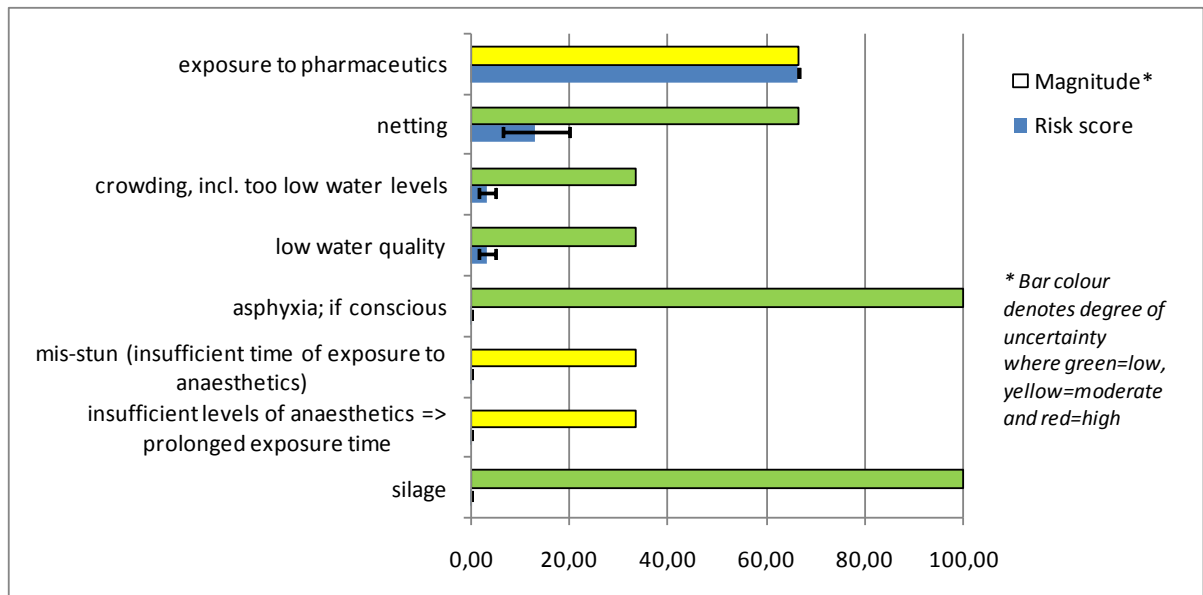


Figure 15. Risk score and magnitude of adverse welfare effect for individual hazards associated with the use of metocaine or benzocaine (two pharmacological preparations) (method I) in Atlantic salmon (*Salmo salar*), ranked by risk score. Black bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard.

APPENDIX F: RELEVANT DATA FROM THE QUESTIONNAIRE

	ICELAND	UK	UK	Norway	Norway	Greece
		SSPO	RSPCA	NSF		
	MS	SK	SK	SK	MS	MS
Total production of Atlantic salmon (<i>Salmo salar</i>) in metric tones?	2008- 292, 2006 - 7000	136 755		855 000	2007 - 744 222	2007- 11
For what percentage of the total production is crowding...					it is legal requirement	
..supervised?	100%	100%	100%		100%	
..not supervised?						
..method not known?				x		
Method of loading from cages during harvesting (transportation or direct slaughter at cage), by percentage of the total production					* see comment	
Pumping	90%	95%	100%	100%	99%	common
Dip netting	10%	5%			1%	Very common
Not known						
Live transportation by well-boat, by percentage of the total production						
Open holds		0%			99%	
Closed system with RSW chilling		70%	80%		few boats	
Closed system (no chilling)	100%	15%			1%	
No live transportation (slaughter at cage)		15%		0	1 boat	rarely
Not known				100 (wellboat)		
What percentage of the total production is transferred to holding cages before slaughter?		15%	?	90%	95%	none
Method of unloading from well-boat for further processing (holding cage or direct slaughter), by percentage of the part of production that is transported by boat?			see comment			
Pumping	50%	70%		100%	98%	common
Dip netting	30%	0%			occurs	Very common
Moveable bulkhead	20%	30%			4 - 5 %	
Not known						
Method of transfer to processing line (from holding cage), by percentage of the total production?						
Pumping	100%	70%	90%	100%	100%	
Dip netting		30%			occurs	
Not known						
Methods for stunning, by percentage of the total production?						

Exposure to CO ₂ (without live chilling)		0%			19.50%	
Ice slurry, without CO ₂	75%	0%			5.50%	
Live chilling with CO ₂		0%			51.40%	
Percussive stunning	25%	100%	100%		13.90%	common
Electric stunning		0			6.90%	rarely
Other					2.8% **	
Not known						
Primary method for killing, by percentage of the total production?						
Exsanguination	100%	see comment	100%		100 % ***	
Percussive		see comment				
Not known						
Comments		Percussion and exsanguination together form two stages of slaughter procedure 100%			* Siphoning is also used to a great degree and in these cases it will not be necessary to pump mechanically as the fish are moved passively through the flexible tube ** electrical and percussive stunning together ***Exsanguination is mandatory	

GLOSSARY AND ABBREVIATIONS

Glossary

Adverse effect	The welfare consequences for an animal in terms of pain and distress when exposed to a hazard.
Asphyxia	A process where fish die from hypoxia. This may happen in some species by: taking them out of water; by partially bleeding animals out; by preventing gill movements e.g. crushing; and by reducing oxygen content of the water.
Crowding	Keeping animals at stocking densities that are high or that reduce swimming volume e.g. by hoisting a net.
Depopulation (Emergency killing for disease control)	A process of killing animals for public health, animal health, animal welfare or environmental reasons, sometimes under the supervision of the competent authority.
Dip-net	A net used to dip into a tank or cage to catch fish for the purpose of transfer of fish to another pond or facility or to market or for slaughter.
Duration	Specifically used with 'intensity' in the context of evaluating the magnitude of the adverse effect.
Emergency killing	The killing of animals that are injured or have a disease associated with severe pain or suffering and where there is no other practical possibility to alleviate this pain or suffering.
Exposure Assessment	The quantitative and qualitative evaluation of the likelihood of hazards to welfare occurring in a given fish population.
Hazard	Any factor with the potential to cause an adverse welfare effect on fish.
Hazard characterisation	The qualitative and quantitative evaluation of the nature of the adverse effects associated with the hazard.
Hazard Identification	The identification of any factor capable of causing adverse effects on fish welfare.
Hypoxia	A condition with low oxygen saturation in the water or a condition with low oxygen saturation in the water (blood).
Intensity	The quality of pain or distress per unit time
Killing	Any intentionally induced process that causes the death of an animal.
Lairage	Short-term storage of fish in a tank or other facility before slaughter. Fish may be subjected to high stocking densities or materials for short periods.
Magnitude of the adverse effects	A function of intensity and duration of welfare impairment for fish.
Pre-slaughter	Anything happening just before stunning, killing or slaughter.
Risk	A function of the probability of an adverse effect and the magnitude of that effect, consequent to a hazard for fish.
Risk Assessment	A scientifically based process consisting of the following steps: i) hazard identification, ii) hazard characterisation, iii) exposure assessment and iv) risk characterisation.
Risk Characterisation	The process of determining the qualitative or quantitative estimation, including attendant

	uncertainties, of the probability of occurrence and severity of known or potential adverse effects on welfare in a given fish population based on hazard identification, hazard characterisation, and exposure assessment.
Severity	Sometimes used to denote intensity.
Size-grading	Sorting the fish according to size
Slaughter	The killing of animals for human consumption.
Slaughterhouse	Any establishment used for slaughtering fish.
Stocking density:	Number of fish in a defined volume of water.
Stunning	Any intentionally induced process that causes loss of consciousness and sensibility without pain, including any process resulting in instantaneous death.
Uncertainty Analysis	Uncertainty refers to the extent to which data are supported by published evidence. A method used to estimate the uncertainty associated with model inputs, assumptions and structure/form. This includes also uncertainty, due to the lack of reliable publications, uncertainty in the scientific results etc.
Variability	The natural biological variation that occurs in a population of animals. Not to be confused with uncertainty as it cannot be reduced by simply decreasing uncertainty.
Visual evoked reflexes (VER)	Evoked EEG activity in the brain with a visual stimulus.
Abbreviations	
A	Ampere
ACTH	Adrenocorticotrophin hormone
AHAW	Animal Health and Welfare
BSC axis	Brain sympathetic – chromaffin cells axis
CAs	Catecholamines
D_adverse	effect the duration of the adverse effect
EFSA	European Food Safety Authority
EEG	Electro-encephalogram
EC	European Commission
ECG	Electro-cardiogram
EU	European Union
HPI axis	hypothalamic Pituitary interregional axis
mA	milli-Ampere
mV	milli-Volts
MS	Member States
μS	micro-Siemens
P_hazard	L the probability that the hazard occurs
SER	Somato-sensory evoked reflex
SS_adverse	effect the intensity of the adverse effect
TOC	Total organic carbon
V	Volts
VER	Visual evoked reflexes
VOR	Vestibulo-ocular reflex