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# SCIENTIFIC OPINION

## Species-specific welfare aspects of the main systems of stunning and killing of farmed seabass and seabream[1]

## Scientific Opinion of the Panel on Animal Health and Welfare

**(Question N° EFSA-Q-2008-441)**

**Adopted on 20th March 2009**



1 For citation purposes: 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 seabass and seabream. The EFSA Journal (2009) 1010, 1-52

## 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 European seabass *(Dicentrarchus labrax)* and gilthead seabream *(Sparus aurata)* in the EU.

A semi-quantitative risk assessment approach was used to rank the risks of poor welfare associated with the different commercially applied stunning / killing methods for seabass and seabream and to identify areas of concern, as well as to provide guidance for future research. The risk assessment was based on expert opinion, due to the limited amount of quantitative data and published peer reviewed data on the effects of the hazards associated with the killing of seabass and seabream. Pre-slaughter stages, immediately before killing, which had a direct impact on welfare were included in the risk assessment. Stunning and killing methods such as electrical stunning and the use of gas mixtures that are not commercially used in Europe were also described but not included in the risk assessment. The three methods assessed were: asphyxia in air; live chilling on ice; and live chilling in ice water slurry, the latter being the most commonly used method in the EU.

The pre-slaughter stages considered were common to all killing methods: i) Feed withdrawal; ii) Crowding; and iii) Removal from water. Hazards associated with pre-slaughter procedures lead to high adverse effects on fish welfare. The most important hazards in the pre-slaughter phase were: i) Very high density in the crowding; ii) Long period of crowding; and iii) exposure to air. High magnitude of the adverse effects was observed for repeated catching as the effect on residual fish that were not killed was considered to be very severe.

Pre-slaughter management practices which keep crowding duration short and ensure a low crowding density level help to reduce distress induced by crowding. Crowding should be synchronised with subsequent slaughter stages so that fish are not crowded for longer than necessary. Measures such as oxygenation or water renewal should be used to minimize deterioration of water quality. Exposure to air should be reduced to the minimum possible time. Research is needed to develop pre-slaughter and slaughter methods that avoid air exposure. Management and marketing practices should be implemented to avoid repeated exposure of the fish population to crowding, netting and prolonged feed withdrawal, and a recovery period should be allowed. According to the farming system, location and species appropriate pre-slaughter procedures and equipments should be identified.

All the commercially used methods of slaughter, including the procedures involving chilling, included a prolonged period of consciousness (several minutes) during which indications of poor welfare were apparent (physiological and behavioural responses). Alternative methods such as carbon dioxide, nitrogen and electrical have only been used experimentally. Carbon dioxide is strongly aversive to seabass and seabream and the fish remain conscious for several minutes. Only electrical stunning can induce an immediate loss of consciousness with recovery being prevented by subsequent chilling of the stunned fish. The Animal Health and Welfare panel recommends the urgent development of commercial stunning methods to induce immediate (or rapid) unconsciousness in seabass and seabream. Methods used in other fish species other than those described in this Opinion may also be applicable to seabass and seabream and the opportunity to develop new methods for slaughtering these species is considerable and should be encouraged.

To the experts' knowledge depopulation for disease control has not occurred. If a disease outbreak would require culling of seabass and seabream on farm, there is no obvious method of choice. Appropriate methods for emergency killing on-farm also need to be developed.

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 developed.

**Key words:** Fish, European Seabass, *Dicentrarchus labrax*, Gilthead Seabream, *Sparus aurata*, animal welfare, risk assessment, pre-slaughter, slaughter, stunning, killing.

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## BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Directive 93/119/EC[2] 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[3], 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, this EFSA report[4] highlighted that different methods for stunning and killing of farmed fish must be developed and optimised according to the species specific different needs and welfare aspects:

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

## TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

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

The following species should be considered:

- Atlantic salmon *(Salmo salar)*
- rainbow trout *(Oncorhynchus mykiss)*
- European eel *(Anguilla anguilla)*
- gilthead seabream *(Sparus auratus)*
- European seabass (Dicentrarchus labrax)
- European turbot *(Psetta maxima)*
- common carp *(Cyprinus carpio)*
- farmed tuna *(Thunnus* spp*)*

2 OJ L 340, 31.12.1993, p. 21–34

3 http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/opinion_ahaw_02_ej45_stunning_en.pdf?ssbinary=true

4 http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/opinion_ahaw_02_ej45_stunning_report_v2_en1,1.pdf?ssbinary=true

## ACKNOWLEDGEMENTS

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

## Scope and objectives of the scientific opinion

The scope of this report is the animal welfare aspects of the stunning and killing of farmed European seabass (*Dicentrarchus labrax)* and gilthead seabream *(Sparus aurata).*

Pre-slaughter procedures were only considered if evidence exists for a direct impact on welfare at stunning and killing. Where fish welfare immediately before and during killing is affected, it is also considered as part of the process. Therefore, the welfare aspects of the farming phase of these species as well as their transport were not included in this report.

The impact on meat quality is not part of this assessment however, references are provided in the text that could be used and evaluated for further socio-economic studies on stunning and killing methods for seabass and seabream.

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.

Food safety issues are addressed by the BIOHAZ panel.

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.

## 1. Introduction

European seabass *Dicentrarchus labrax* and Gilthead seabream *Sparus aurata* are different species belonging to different taxonomic families and their susceptibility to stress and behavioural and physiological responses to stressful situations are known to be different (EFSA 2008).

Sea bass and sea bream are often farmed simultaneously using various types of ongrowing systems, depending on location, procedures and equipment. According to EFSA (2008), it was estimated that the most common intensive growing system for seabass and seabream in Europe is floating net cages, followed by land-based flow-through systems (10%). Only a few farms use (1%) land based recirculation systems for the growing phase. Semi-intensive (ponds) and extensive farms (in coastal lagoons) account for less than 3%. Market weight for seabream and seabass is on average 350g but considerable size range can be observed and nowadays it is common to have 3 size categories, 'portion' (250-350 g) this category represents 90% of the market share, 'medium' (351-600 g) and 'large' (>601g). Some variation is seen between countries and farming system.

Different growing systems apply different farm management and slaughter procedures. However, in all the systems in which the pre-slaughter and slaughter processes are under the farmer's direct control, general procedures are adopted in preparation for the slaughtering period when the fish have reached the required commercial size.

Figure1 illustrates the most common steps that occur in the killing process of seabass and seabream in Europe. Only the steps that appear in the pre-slaughter and the slaughter section which have an impact on the welfare of the fish were considered to be within the terms of references and will be described in the following chapters. Stunning and killing methods that are not commercially used in the EU Member States at present are described in sections 4.4. (gas mixtures) and 4.5. (electrical stunning). These methods were not included in the risk assessment.

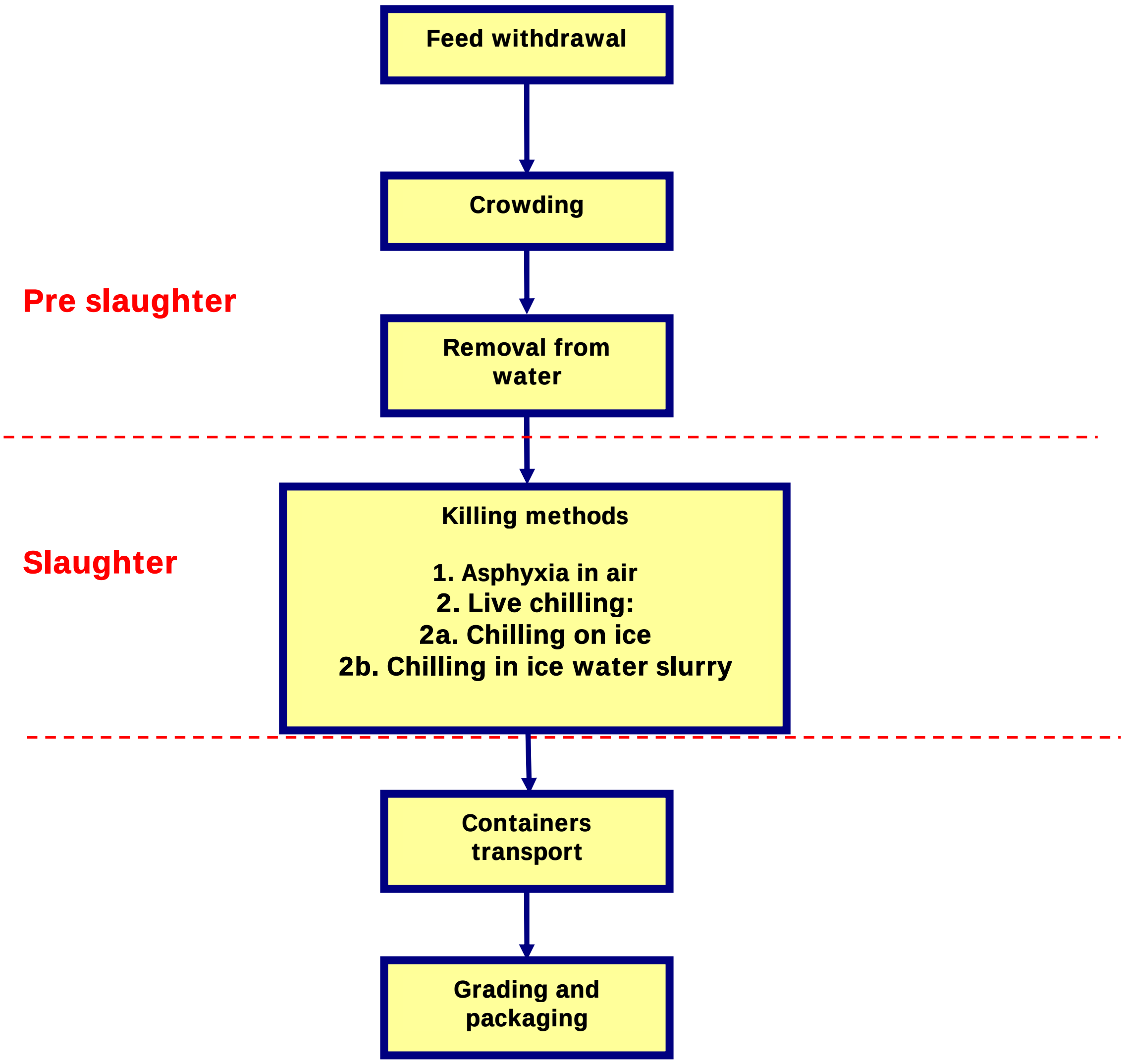



Figure 1: **Seabass – seabream pre-slaughter and slaughter processes**

## 2. Pre-slaughter process

Pre-slaughter procedures are among the most critical points in fish farming management (Wall 2000) and they may affect fish welfare and influence the quality of the final product during storage (Poli et al. 2005; Poli, 2008).

Pre-slaughter processes and procedures include: feed withdrawal, handling and crowding, netting or pumping, loading and unloading, capture and transport at high density, novel environmental conditions and changes in social structure for the fish. All such procedures, no matter how gently they are carried out, may cause adverse effects and physiological reactions (Sigholt et al. 1997 Erikson 1997; Poli et al. 2005). During crowding and transport, stress may also be caused by low water exchange, low oxygen levels and accumulation of fish waste products, i.e. ammonia and carbon dioxide. Some of these stressful procedures are repeated (multiple stressors) several times. The inability of fish to avoid or escape from stressful conditions may cause further distress (Portz et al. 2006, Ashley 2007).

In the following paragraph, pre-slaughter procedures and killing methods used for seabass and seabream are summarised. The main hazards encountered during pre-slaughter are listed and available data on adverse effect arising from the hazards are presented as a function of their intensity, duration and probability of occurrence.

Regardless of the farming system and killing method used, the following operations are performed:

- Pre – slaughter feed withdrawal
- Crowding
- Removal of fish from the water

## 2.1. Pre slaughter feed withdrawal

Feed withdrawal before slaughter is commonly performed to empty the gut and to reduce the probability of fish being contaminated with feed and faeces during the subsequent slaughter procedure and processing (Beveridge 1996). Feed withdrawal also has an important role in pre-slaughter management because it induces a reduction in ammonia excretion by the fish, thus reducing the water quality deterioration occurring during, crowding and transport to slaughter facilities. Under these conditions, the toxicity of ammonia is also increased in seabass (Tudor et al. 1994) and in seabream (Wajsbrot et al. 1993) due to low dissolved oxygen levels. There are different opinions as to what the optimum duration of feed withdrawal should be, and only little evidence comes from research. Current code of practices for salmon farming and research suggest that 72 hours is adequate for the complete clearing of the fish gut, at the same time minimizing adverse effects on fish welfare (Robb, 2008). However, no published papers are available on the effect of feed withdrawal time before pre-slaughter for seabass and seabream. The effect of season (day light intensity /duration and water temperature effects) has not been studied.

The pre-slaughter feed withdrawal period in seabass and seabream usually lasts a few days (in commercial practice 1-3 days are often used), depending on the water temperature and feeding rate, but can be extended up to seven days according to the harvesting period (hazard 1). In recirculation systems longer fasting (up to a week) is required in order to remove unpleasant flesh flavours. At 25°C, 24h starvation is considered an appropriate period to empty the gut if fish are fed properly (Caggiano 2001). Long starvation periods (months) were previously used on the assumption that increasing the starvation period would reduce fat at the muscular level (Einen and Thomassen 1998; Rasmussen et al. 2000), improve the sensory texture of the fish (Einen et al. 1999) and eliminate unpleasant flavour, mainly due to the type of feed used. However, no evidence exists for possible additional benefits from fasting fish longer than 72 hours (Robb, 2008), whereas there is some evidence that long term starvation may induce a reduction in available energy and weight loss. The cumulative stress caused by prolonged feed withdrawal periods and repeated feed withdrawal will lead to immune depression, which will make fish more susceptible to stress mediated diseases during the pre-slaughter period..

In intensive flow-through tanks and cage systems, fish capture may take several days or even weeks (hazard 2) depending on the market strategy of the farms and on the fish return in extensive coastal lagoon systems, such as valliculture. Consequently fish are not starved for the same period and the last fish may experience longer fasting periods.

Seabass and seabream live in temperate regions and are subject to seasonal cycles and alternate periods of feeding and fasting in response to several factors (e.g. feed availability, temperature, spawning, migration, reproduction). Under farming conditions fish do not usually experience feed deprivation, unless certain conditions are imposed, such as essential husbandry operations or stormy weather in the case of fish farmed in net cages (EFSA, 2008). Under experimental conditions, it has been shown that starved fish reduce their metabolic rate and mobilize their energy reserves: during the first three months of starvation lipid catabolism is stimulated and to a lesser extent muscle protein catabolism (Echevarria et al. 1995, Echevarria et al., 1997). However, data are not consistent and show that different species under starvation conditions utilize different metabolic adjustments depending on other factors, such as fish age/weight, and nutritional state (Navarro and Gutiérrez, 1995). A period of starvation in seabass up to 21 days induces an intense mobilisation of hepatic glycogen and triglycerides, with a parallel reduction of the hepatosomatic index and muscle triglycerides (Zammit and Newsholme, 1979). Pérez-Jiménez et al. 2007) demonstrated that growing seabass experience rapid metabolic adjustments to both short term starvation (9 days) and re-feeding, and that diet composition significantly influences the metabolic responses to these nutritional challenges. Seabass fed prior to starvation on a diet containing 50% protein showed higher total amino acid levels, lower triglycerides, total lipid levels and liver glycogen than fish fed on an iso-energetic diet at 42% protein. Liver enzymes (FBPase, GPT and GOT) activities were lower in fish at lower protein levels, probably because the lower gluconeogenesis associated with the higher carbohydrate and lower protein intakes. Starvation for 9 days induced a decrease in plasma metabolites and liver glycogen in both groups, but major changes in GOT, GPT and GDH were only found in seabass fed at 50% protein. After 12 days of re-feeding most metabolic measures returned to pre-starvation levels, except for enzymes involved in lipogenesis, and glucose and cholesterol metabolism in fish fed a low-protein diet. These variables may need to be taken into account when assessing the effect of feed withdrawal.

Long starvation periods in seabass (4 weeks) induce an almost 20% decrease in the length of intestinal microvilli and change in the permeability of intestinal mucosa to amino acids (Avella et al. 1992). Longer fasting periods (50 days) induce loss of weight and condition, loss of intestinal fats and plasma protein, together with a precocious involution of gonad tissue, without any variation in the chemical composition of muscle (Echevarria et al, 1997).

Studies in mammals show that starvation induces the production of reactive oxygen species (ROS), which cannot be removed. The increase in ROS caused pro-oxidant effects, resulting in aging, lipid peroxidation and other body damage (Di Simplicio et al., 1997; Bhat et al., 2007). Although, studies regarding the influence of fasting on antioxidant defences in fish are scarce, both seabass and seabream have shown variations in plasma oxidant and antioxidant status under certain stressful conditions (Di Marco et al. 2007; Bagni et al. 2007).

The effect of prolonged starvation (5 weeks) on antioxidant status and some metabolic-related parameters has been investigated in several species of the sparidae family. Results show that in *Dentex dentex* (Morales et al. 2004) fasting significantly increases lipid peroxidation and leads to a pro-oxidant situation and oxidative stress, despite the activation of antioxidant defence mechanisms (i.e. inhibition of Glucose-6-Phospahte DeHydrogenase activity). Oxidative stress during starvation has been observed also in *Sparus macrocephalus* (Xiao-dong et al, 2007)

Seabream seems to be able to use its energy reserves, namely perivisceral fat, in order to counterbalance the nutrient shortage arising from feed withdrawal for up to 13 days (Ferreira Pinto et al., 2007). Longer feed withdrawal (5 weeks) induces a consequent use of energy, with liver glycogen being utilized early, which is consistent with findings in other species (Navarro et al. 1992; Collins and Anderson 1995), including seabass (Power et al. 2001). Mesenteric fat is gradually utilized and then lipid and protein in the white muscle, which is responsible for the reduction in body weight (16%) when early reserves begin to deplete (Ibarz et al. 2007). However, seabream is able to recover when fed after 5 weeks of feed withdrawal, and the main changes observed in liver glycogen and muscle lipid content revert to normal at the end of re-feeding period. The level of most fatty acids were recovered, but not plasma non-esterified fatty acids (NEFA) and liver protein content.

## 2.2. Crowding

In intensive aquaculture systems, juvenile fish are stocked in tanks, cages or ponds and, depending on the production strategy, may be exposed or not to confinement and short-term crowding (i.e. for grading, vaccination, medication) during all stages of the farming cycle. However, during pre-slaughter handling operations, crowding and confinement represent unavoidable practices, required to rapidly remove fish from rearing units.

Fish crowding can be achieved using different techniques, depending on the farming systems and type of rearing units (ponds, cage, tank, raceways). In tanks and raceways, fish are concentrated at a high density by dropping the water level, confining them to the bottom and then inducing them to move into a section by using nets or barriers. In circular cages and ponds, a sweep net (a small seine net) is often used to crowd all or some of the fish. The sweep net has a corked line that keeps the top edge of the net on the surface and the remainder of the net hangs down through the water (Robb, 2008). The net is pulled slowly to allow fish on the edges to escape and to reduce stress (hazard 3). In square cages, the cage net can also be lifted and rolled slowly, and then a part of population split off by lifting the cage across the middle (Robb, 2008). In small cages, the main cage nets can also be raised, thus crowding the whole population.

The nets used for crowding have to be soft in order to not damage the fish during confinement (hazard 4). The colour of the net is also important. Generally, black nets are not easily recognized by the fish, thus increasing the risk of damage from abrasion through contact. Moreover, operators have a hard job to pick out the fish against the black net and this may lead to the need to repeat the confinement and crowding operations several times. White nets are not used either, but for the opposite reason, namely that they are too easily identified by the fish which thus tend to escape. Operations must therefore be repeated several times, wasting time and causing repeated exposure to stress. No data exist on this topic, but the effect of net colour is worth investigating.

It is well known that crowding acts as a stressor in fish and a stress response has been reported in several papers (Pickering et al. 1981; Mazur and Iwama 1993; Van Raji et al. 1996; Rotlant and Tort, 1997). Both seabass and seabream show the classical pattern of the physiological stress response reported for other reared fishes following exposure to physical confinement and crowding, i.e. increase in plasma cortisol, glucose and lactate levels (EFSA, 2008). Compared with other fish, including seabream, seabass show a more rapid and higher peak of plasma cortisol concentration, higher plasma glucose content (Marino et al. 2001; Di Marco et al.

2008), higher cortisol content of head-kidney homogenates and higher basal unstimulated release of cortisol from the head-kidney (Rotland et al., 2001; Rotllant et al. 2003; Fanouraki et al., 2008). Fish density and duration of crowding are believed to affect the degree of stress experienced by the fish, and environmental factors such as temperature can also modulate the stress response. In Atlantic salmon, Robb et al (2008) reported that crowding duration and the number of sweeps strongly influence stress response. The degree of stress, measured using plasma cortisol concentration as an indicator, is higher in Atlantic salmon crowded for 4h and slaughtered in one large sweep than in those slaughtered in two consecutive sweeps. In addition, the level of crowding influences the stress response in Atlantic salmon: plasma cortisol, osmolality and lactate were found to be significantly higher in fish transported by a well boat from the fish farm to the slaughter house at 300 kg/$m^3$ than in fish transported and handled in the same way at 50kg/$m^3$ (Skjervold et al. 1999). Similar data is not available for seabream and seabass. However in a study on European seabass, aimed to assess the influence on blood and tissue stress parameters of the level of oxygen in the rearing water, the high density of fish in the hour prior to death (400 kg/$m^3$) and the effect of repeated capture in the same tank 24 h after the first capture, Parisi et al. (2003) confirmed the pronounced stress effect of the high stocking density before death and of the repeated capture (hazard 7). In commercial practice the stocking density used for crowding of seabass and seabream is about 250 kg/m3.

As a consequence of crowding, water quality (hazard 6) and especially oxygen availability may rapidly decrease. When fish are crowded at high densities, the concentration of dissolved oxygen will decrease because fish are stressed and more active. The concentration of ammonia and other waste products also increase as less water is available per fish biomass. Some management procedures can be used to reduce the rate at which the water quality deteriorates. Ammonia concentration can be managed by extending the feed withdrawal period to some extent. In tanks and ponds, oxygen level can be increased by using liquid oxygen or bubbling gaseous oxygen through the water. In cages, water renewal can be increased by ensuring that nets are free from fouling and by pumping seawater into the cage. The addition of water also acts as an attraction for the fish and facilitates the operation of capture as well as reducing time of crowding and confinement.

Increased water renewal during crowding, however, may increase the physical activity of fish before slaughter, which in turn may act as an additional stressor (Wood 2001). In both Atlantic salmon and rainbow trout, increased physical activity proved to be less stressful than the reduction of water volume and crowding, but the combination of both (physical exercise and crowding) resulted in the most stressful condition for both species (Thomas et al. 1999), affecting the physiological stress response (i.e. plasma cortisol and lactate), muscle pH and the onset and resolution of rigor mortis.

In seabass and seabream, there are few studies on the effects of crowding and increased physical activity during pre-slaughter operations. High levels of activity and strenuous exercise are reported in crowded seabass, with important effects on flesh quality. The vigorous swimming during crowding implies an intense use of white muscle. Anaerobic glycolysis increases lactic acid production, causes lower muscle pH and changes the time to onset and the resolution of rigor mortis (Poli et al. 2005, Panebianco et al. 2006, Bagni et al. 2007)

It is well known by those in the industry that when fish are crowded too densely and too rapidly, they show escape behaviour, splashing and gasping. A good crowding is signified by fish that are calm, is rapidly carried out, without fish ending up by lying on top of one another, and with only the occasional fin breaking the surface (HSA, 2008). The only data available on the effect of crowding at slaughter on the welfare of seabass and seabream, is reported by Parisi et al. (2003) and Bagni et al. (2007) and it confirms the practical experience of farmers.

### 2.3. Removal from water

Fish are usually removed from water using nets and pumps. Hand nets are used in extensive and semi-intensive systems to collect fish already confined and crowded at the bottom of the rearing unit, and to remove overstressed or moribund fish during slaughter. Nowadays mechanised brail nets are usually used both in cages and in tanks for commercial slaughter. The brail net comprises a metal hoop, about 1 m in diameter, with a net tube hanging from it and with the free end attached to a rope, which allows the opening and the closing of the net bottom (Robb, 2008). The net is suspended from a small crane, which drags the net through the crowded fish. The suspended net is dropped to the bottom of the tank/cage and is then rapidly winched to the water surface. Some nets are equipped with a device to allow a certain volume of water to be retained in the brail net, thereby reducing the risks of abrasion (hazard 8). It has been suggested that fish should not be held out of water for longer than 10s as after that they will show aversive behaviour to the lack of oxygen and become progressively difficult to handle.

Air lift pumps are used for moving fish over short distances. They are equipped with a compressor that blows bubbles into a pipe and sucks out the fish, and as the fish are always in contact with the water, the risk of physical damage is reduced. Another pumping system is that using venturi pumps which force large volumes of water at a high speed through a pipe and draw away the fish and water from the rearing units. Venturi pumps have to be equipped with a good water drainage system which allows the fish to be released into special containers. Pumps creating a strong suction, if not well managed, can cause physical damage and external haemorrhage (Robb, 2008). Moreover, vacuum pumps do not deliver a continuous water flow and fish can stay in the pipe for long time, with increasing risk of oxygen depletion and struggling at high fish density. Pumps and other equipment used to move fish should be regularly checked and maintained. Pipes should be kept as short and straight as possible and should be flushed at the end of each slaughter (HSA, 2008). Pumps are not commonly used in seabass and seabream farming.

All the methods for removing fish from water cause some stress as it will cause asphyxia. Netting fish at high density by using brail nets tends to increase the risk of damage and abrasion (hazard 10). If the removal of fish out of water occurs in several sweeps, it takes a long time and fish may be exposed to severe stress conditions. In general, the efficiency of hand netting depends on the operator skill and appears to be less stressful although slower (hazard 9).

### 2.4. Repeated catching, effects on residual fish

Repeated exposure to catching adversely affects welfare of fish in several ways: cumulative stress leading to exhaustion, increased chances of abrasion against the net and other fish, and physical damage including loss of scales and fin damage. If fish are not killed immediately, stress mediated immune suppression will occur. (Parisi et al. 2003) reported the effects of exposure to repeated catching on plasma stress indicators, i.e cortisol and haematocrit values in seabass, which significantly increased when fish were caught and removed more than once. Secondary bacterial diseases and other pathological conditions are often observed in residual fish exposed to repeated catching indicating the possibility of a depressed immune state (hazard 11). In these situations, therapeutic measures such as antibiotics are not used due to legal restrictions (in respect of withdrawal periods and maximum residue levels in fish carcasses) resulting in severe adverse effects for fish welfare.

### 2.5. Fish transport

Seabream and seabass are normally removed from the water, and transported to the processing plant in containers with water and ice using boats and/or trucks. Since seabream and seabass are killed by the chilling in water and ice, transport is not considered to be a part of the pre-slaughter process or procedures.

## 3. Killing methods

EFSA launched a questionnaire to all Member States requesting information about the methods in use for the slaughter of seabass and seabream,. Live chilling in ice/water slurry was reported as the more common method and only one country indicated asphyxia (exposure to air) as a rarely used method (EFSA Questionnaire, 2009). Electrical stunning and exposure to water saturated with gas mixtures (carbon dioxide and nitrogen alone or as mixtures) have been tested experimentally on fish farms, but commercial scale trials have yet to be conducted.

### 3.1. Recognition of consciousness, unconsciousness and death

Stunning methods are required to induce immediate or rapid (less than 1 second) unconsciousness. If the induction of unconsciousness is not immediate then it should occur without causing avoidable pain and suffering. 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. In seabass and seabream, field recognition for unconsciousness or death includes absence of breathing and opercular movement, eyes fixed, absence of response to painful stimuli (pin-prick) and loss of balance. In experimental conditions, EEG including visual evoked responses (VER) may also be used (Kestin et al. 2002).

### 3.2. Asphyxia in air

Seabream and seabass are killed by this method simply by removing them from water and leaving them to die in air. Asphyxia is usually achieved by netting from the water or pumping fish and placing them in free draining bins or boxes (hazard 13). No special equipment is required. In most cases, violent attempts to escape are made and maximal stress responses are initiated (Robb and Kestin 2002). When spontaneous movement has ceased the fish are processed.

Seabream removed from water at 23 C and let to die by asphyxia in air, showed an average time to loss of self-initiated behaviour of 4 min and loss of VERs at 5.5 min (van de Vis et al. 2003). Higher ambient temperatures generally result in a faster death. The time necessary to kill seabass by asphyxia was reported to be 70±27.6 min (mean $\pm$ standard error) by Poli et al. (2004) and up to 128 min (Acerete et al. 2009). Seabass and seabream asphyxiated in air struggle longer (about + 65% and + 25%) than those killed in ice water slurry (Bagni et al. 2002) (hazard 12).

From a comparison of commercial and experimental killing methods in seabass, asphyxia was confirmed to be the most stressful killing method, showing the most prolonged period prior to death and a remarkable physical activity (Table 1). Fish killed by asphyxia had higher lactate and lower pH, ATP and adenylate energy charge (AEC) in tissues, and an earlier rigor onset

(Table 3) in comparison with spiking and stunning by percussion (Poli et al. 2004). The study by Poli et al. 2004 also reported a higher haematocrit and plasma cortisol, glucose and lactate values for fish killed by asphyxia in air in comparison with other killing methods, but it is difficult to interpret these values due to the small sample size (n=5) and high variations between individuals.

Table 1: **Sea bass behaviour and stun/killing time** (Poli et al., 2004; Zampacavallo et al., 2008)

| **Method** | **Fish Behaviour** | **No reaction to external stimuli** Average time after application of stunning method (min) |
|---|---|---|
| Ice/water slurry 1:2 | Rapid swimming for the first 3-4 min. | 23.0±5.5 |
| Electricity (24V 50Hz A.C.) | Not evaluated | 2 |
| Asphyxia | Prolonged and violent activity | 70.0±27.6 |
| Percussion | Violent body movements | 0 |
| Spiking | Violent body movements | 0 |
| $CO_2$ 100% | Violent activity in the 1st minute | 7.0±1.4 |
| Ice/water with 100% $CO_2$ | Violent activity in the first 30 seconds | 4.2±0.5 |
| Ice/water with 40%$N_2$ / 60%$CO_2$ | Violent activity in the first 30 seconds | 13.0±2.0 |
| Ice/water with 60%$N_2$ / 40%$CO_2$ | Violent activity in the first 30 seconds | 14.0± 2.0 |
| Ice/water with 70%$N_2$ / 30%$CO_2$ | Violent activity in the first 30 seconds | 14.0± 2.0 |
| Ice/water with 100% $N_2$ | Rapid swimming for the first 3-4 min. | 16.0± 2.0 |

Table 2: **Biochemical tissue parameters which may serve as stress indicators in sea bass** (Poli et al., 2004)

| *Tissue parameter* | *Trial* | **Ice/water slurry 1:2** | **Asphyxia** | **Percussion** | **Spiking** | **Carbon dioxide** | **Electricity 24V 50Hz** | *rsd* | *Sign* |
|---|---|---|---|---|---|---|---|---|---|
| Rigor Index 3 h after death (%) | 2 | 45.3**c** | 100.0**a** | 85.1**ab** | 60.4**bc** | 97.0**a** | 85.1**ab** | 25.1 | ** |
| | 3 | 83.7 | 100.0 | | 94.1 | 100.0 | 100.0 | 8.9 | **n.s.** |
| Muscle pH | 3 | 6.95**a** | 6.65**c** | | 6.84**ab** | 6.88**ab** | 6.73**bc** | 0.13 | * |
| Eye pH | 3 | 7.42**a** | 6.88**c** | | 7.34**a** | 7.16**b** | 7.30**a** | 0.09 | *** |
| Muscle lactate (µmol/g) | 3 | 28.23**c** | 42.41**a** | | 32.58**bc** | 34.67**b** | 36.33**ab** | 4.60 | ** |
| ATP (µmol/g) | 3 | 1.39 | 0.26 | | 2.14 | 2.06 | 0.41 | 0.45 | **n.s.** |
| AEC (µmol/g) | 3 | 0.47 | 0.37**b** | | 0.65**a** | 0.64**a** | 0.40**b** | 0.15 | * |

* for $0.01<p\leq0.05$; ** for $0.001<p\leq0.01$; *** for $p\leq0.001$; a,b,c for $p \leq 0.05$

### 3.3. Live chilling

Live chilling means a transfer from water at ambient temperature into i) ice flakes (solid ice), ii) liquid ice (also known as super cold water at a temperature ranging from -2.3 to-2.8°C ) or iii) ice water slurry (ice flakes and water in a ratio ranging from 1:2 to 3:1). The most commonly used method is the ice water slurry at a significantly lower water temperature, fluctuating from 0 to 2 °C and a temperature differential usually of at least 10 °C (hazard 14), between the water temperature when crowded in tank or cage with ice/slurry. Taking into account that seabass and seabream are Mediterranean fish species, generally living at temperature above 12°C, they may be killed using ice/water slurry. Liquid ice is used only for experimental trials.

Fish are netted and added to a container with ice flakes or ice water slurry. The aim is to simultaneously chill and kill the fish. Usually 200 - 250 kg fish by container of approximately 600 l volume. The quantity of fish placed on the container and their initial temperature (rearing water temperature) will affect the chilling efficiency (hazard 15) (hazard 18). The killed fish are then put into boxes covered by flaked ice generally in an ice/fish ratio of 1:3.

Killing in ice does not result in immediate unconsciousness. Seabream transferred to an ice slurry mixture at 0.5 C from water at 23 C had a average time to loss of brain function of 5 min (van de Vis et al. 2003). Experiments with Atlantic salmon (Skjervold et al., 2001), Atlantic cod (Claireaux et al, 1995), common carp (Van den Burg, 2002), eel (Lambooij et al. 2002), and African catfish (Lambooij et al, 2006)) all showed that exposure to a sudden temperature drop is stressful. Such studies are not available for seabass and seabream, but the onset of unconsciousness being stressful to seabass and seabream cannot be ruled out. The time to loss of consciousness will determine the duration of distress caused to fish. The time to loss of consciousness has been determined using visually evoked responses (VERs) recorded by EEG in seabream (van de Vis et al 2003) but not in seabass. Kestin et al. (2002) compared the effects of asphyxia, chilling and electrical stunning on the times to loss of self-initiated behaviours (swimming and maintaining equilibrium), response to external stimuli (handling, pricking and electric shock with 6V), physical reflexes (eye roll, breathing), and VERs in seabream (Table 3). There is, however, no direct evidence (e.g. from EEG or brain chemistry measured using microdialysis) to indicate that loss of consciousness occurs before immobilisation or muscle paralysis (e.g. measured using electromyogramme, EMG) due to chilling. The time to loss of VERs is quicker than the time to loss of responses to painful stimuli such as pricking and electric shock and reflexes such as eye roll and breathing. Kestin et al. suggested that these positive responses and reflexes occurring in the absence of VERs in the brain could be due to local muscular activity rather than due to conscious responses.

Table 3: **Time (minutes) to loss of responses and reflexes in seabream** (Kestin et al. 2002)

| **Method** | Self-mediated behaviour | Response to stimuli | Reflexes | VERs |
|---|---|---|---|---|
| Asphyxia | 4 | 7.5 | 14 | 5.5 |
| Chilling in ice slurry | 5 | 10 | 10 | 5 |
| Electrical | 0 | 0 | 0 | 0 |

Seabass (Zampacavallo et al. 2008) and seabream (Kestin et al. 2002) move around before slowing and becoming immobilised as their muscles cool. 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 (hazard 16). Gilthead seabream show vigorous attempts to escape on introduction to the ice slurry (van de Vis et al. 2003). The efficiency of hypothermia for killing fish by chilling may depend on the temperature of the culture water and be higher in species acclimated to relatively warm water, such as Mediterranean fish (Acerete et al. 2004). When the differential between the ambient temperature of the fish and the ice slurry is relatively great, thermal shock may shorten the time to loss of brain function (Robb and Kestin 2002). 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 VERs or SERs to water at normal temperatures recovered brain function and subsequently muscular movement quickly (Robb and Kestin, 2002).
There is the possibility that cold stress sensibility of warm water species like seabream and seabass could differ. However the seabream and seabass behaviour in ice-water slurry is generally similar, both actively swimming within the first 3-4 minutes, then slowing movement

and positioning on a side or belly upside-down, mainly on the bottom of the tank (Poli, personal communication; Zampacavallo et al. 2008).

The time necessary to kill seabream in an ice-water slurry varied from 20 to 35 min (Bagni et al., 2002); in seabass it was reported to be 23±5 min (Poli et al., 2004; Zampacavallo et al. 2008) (see Table 1) or 34±0.36 min (Acerete et al. 2009). The time interval depends also on the season and the rearing water temperature, being longer in winter (Zampacavallo et al., 2008; Poli, personal communication).

In seabass stun/killing by asphyxia or electrical stunning resulted in higher average values for physiological stress indicators, such as haematocrit and plasma cortisol, glucose and lactate values than when killing by ice water slurry (Poli et al. 2004). In the same study asphyxia in air and live chilling in ice slurry resulted in cortisol levels that were not statistically different. However these values are difficult to interpret due to the small sample size (n=5) and high variations between individuals. Seabass electrically stunned showed a shorter and anticipated *rigor mortis,* lower muscle pH and ATP at death in comparison with seabass stun/killed in ice/water slurry (Knowles et al. 2007; Zampacavallo et al. 2008). *Rigor mortis* onset was generally delayed or similar to spiking (Table 3) (Poli et al., 2004).

Chilling of seabass in ice and water slurry (+1 C to - 2°C) induces hypothermia and elicits a mild physiological stress response after 6 and 20 min of exposure (Di Marco et al. 2007). The effects of ice and water slurry and several anaesthetics on stress physiology were recently compared by Marino et al (2009) using seabass killed by percussion as control group (water temperature 20.5 C). Results of muscle biochemistry indicate that hypothermia showed a very good capacity to inhibit stress response (Table 4) compared with anaesthetics, during both short and long exposure times (6 and 20 min). Chilling in ice water slurry induced elevated blood cortisol levels in comparison with percussion stunning that continued to increase up to 20 minutes post-exposure, indicating prolonged stress. Differences in osmolality and sodium levels between ice/water slurry and percussion are due to the time spend in air in this latter group. Chemical anaesthetics showed a different capacity to block activation of the HPI-axis and to reduce cortisol stress response: e.g. MS222 (100 mg/l) and 2PHE (300 mg/l) were the less effective (cortisol levels: 143.8 and 156.3 ng/ml at 6 min and 326.9 and 340.0 ng/ml after 20 min, respectively). Clove oil at 100 mg/l is the most effective anaesthetic, however, a marked cortisol increase occurred during prolonged clove oil anaesthesia (Table 4), suggesting that this chemical is less effective in inhibiting the HPI-axis than hypothermia. It is also possible that lowering temperature may slow down the metabolism and production of cortisol and other metabolites, thus reducing the ability to respond to stress.

Table 4: **Physiological stress response in sea bass killed by percussion, hypothermia and an overdose of anaesthetic at 6 and 20 minute (n=30 per treatment)**

| **Blood parameter** | **Percussion (Control)** | **ICE/Water slurry** | | **Anaesthetic (CLOVE OIL 100mg/l)** | |
|---|---|---|---|---|---|
| | | 6 min exp | 20 min exp | 6 min exp | 20 min exp |
| Cortisol (ng/dl) | 15.5 ± 4.2 | 44.4 ± 6.9 **a** | 63.5 ± 12.9 | 34.2 ± 6.2 **a** | 145.9 ± 32 **b** |
| Glucose (mg/dl) | 91.4 ± 5.9 | 105.2 ± 12.8 | 96.6 ± 5.4 | 107.9 ± 11.4 | 244.1 ± 19.4 **b** |
| Osm (mosm/kg) | 364.5 ± 3 | 377.8 ± 2.6 **a** | 377.6 ± 3.2 | 399.8 ± 6.3 **a** | 420.6 ± 4 **b** |
| Lactate (mg/dl) | 21.9 ± 1.7 | 30.3 ± 2.6 **a** | 39.5 ± 1.3 **b** | 44.3 ± 1.1 **a** | 52.6 ± 4 |
| Na (meq/l) | 167.1 ± 1.7 | 177.8 ± 2.5 **a** | 183.3 ± 3.7 | 181.1 ± 2.8 **a** | 190.6 ± 3.6 |
| K (meq/l) | 4.4 ± 0.2 | 4.5 ± 0.2 | 5.5 ± 0.2 **b** | 2.8 ± 0.3 **a** | 3.6 ± 0.5 |

**a** vs control; **b** 20

### 3.4. Gas mixtures

The gas method by $CO_2$ was industrially used in Norway for salmon but it was recognised as being aversive (EFSA 2004). The $CO_2$ method has been suggested in case of need of emergency killing of a batch of fish .

$CO_2$ narcosis is aversive to the fish, including seabass (Table 2) and more than likely to seabream as well, as clearly indicated by its quick and violent reaction, such as repeated swimming around, attempts to escape and abnormal activity before stunning. Immobility is reached within 2-4 min, but fish would experience pain and distress even if unable to demonstrate it behaviourally (Kestin et al. 2002). It was demonstrated that fish remain conscious for varying times according to the fish species (2 min salmon; 3 min trout; 9 min carp; 109 min eel; 7-10 min seabass) (Marx et al. 1997; Robb et al. 2000; Kestin et al. 1995) as demonstrated by complete cessation of rhythmic respiratory movements and heartbeat, absence of VOR and pin-prick response. Acerete et al.(2009) reported 16 min to the time to death for seabass, but with no reference to the what was being observed.

A combination of live chilling in ice slurry dissolved with moderate (60% Nitrogen with 40% $CO_2$ mixture) levels of carbon dioxide in a tank was considered to less aversive than the traditional carbon dioxide narcosis using very high levels of $CO_2$ (Poli et al., 2005; Erikson et al., 2006).

Although gas mixtures are not used commercially for seabass and seabream, $CO_2$ and $N_2$ or combinations of these gases have been tested for stun/killing of seabream and seabass (Poli et al., 2004, 2005; Zampacavallo et al., 2008; Acerete et al., 2009). The concentration of gases, temperature of the water (rearing water and of the stunning bath) and the duration of exposure are key parameters that determine the efficiency of these methods. In general, exposure to carbon dioxide leads to hypercapnia (elevated levels of $CO_2$ in the blood), exposure to nitrogen leads to hypoxia (reduced blood oxygen levels) and exposure to a mixture of $CO_2$ and nitrogen leads to hypercapnic hypoxia (elevated $CO_2$ and reduced oxygen levels). The magnitude of changes in the blood gases would depend upon the levels of these gases in the water. The use of gas and combination of gases in ice/water slurry results in shortening of the time to onset of unconsciousness or death when compared with chilling in ice slurry (see Table 1).

Seabass are relatively more tolerant to hypercapnia compared with other species, in fact major physiological effects are not observed until 50 mg/l $CO_2$ (Marino, 2006), although long term exposure (45 days) to environmental CO2 concentrations above 30 mg/l can induce a stress response (Petochi et al. 2009, submitted). Blood $PCO_2$, [$tCO_2$], blood bicarbonate ions and pH were significantly increased in seabass exposed to 50 mg/l $CO_2$ while blood $PO_2$ was significantly decreased in seabass exposed to 15mg/l $CO_2$ or more. Serum cortisol and glucose levels increased significantly during acute exposure to $CO_2$ at different concentration, but these levels did not change during the chronic phase. Grottum and Sigholt, found that 115.5 - 104.8 mg/l $CO_2$ were the Lethal Concentrations that killed 50% of sea bass exposed for 48 and 120 hours respectively to hypercapnia.

The use of gases other than carbon dioxide has been tried. Wills et al. (2006) reported that the strong reaction emerged for carbon dioxide narcosis was not observed when nitrogen stunning was used to stun rainbow trout, even if the time for loss of consciousness was relatively longer with nitrogen. Similar studies are needed in seabass and seabream.

Mixtures of nitrogen and $CO_2$ gases in ice water slurry have been tried in order to reduce the violent reaction caused by $CO_2$ saturated water as well as the time to stun the fish (see Table 1). The use of gases during live chilling has to be considered as a stun/ killing method and has been shown to shorten the time required to stun seabass, without showing any large difference in muscle stress indicators (Poli et al., 2004; 2005; Zampacavallo et al., 2008) (see Table 5). Muscle biochemistry may not be a reliable stress indicator as due to the cold the metabolic rate is lowered and such changes cannot occur. Ice-water saturated with 60-70% nitrogen (with 40 – 30% carbon dioxide) in particular resulted in the most promising mixture and showed about a 40% stunning/slaughtering time reduction (Poli et al. 2005, Zampacavallo et al., 2008) (Table 1).

Table 5: **Tissue biochemistry which may serve as stress indicators in sea bass** (Poli et al., 2004)

| *Tissue parameter* | *Trial* | **Ice/water slurry 1:2** | **Spiking** | **Ice/water with 100% $CO_2$** | **Ice/water with 40%$N_2$/ 60%$CO_2$ mixture** | **Ice/water with 60%$N_2$/ 40%$CO_2$ mixture** | **Ice/water with 100% $N_2$** | *rsd* | *Sign* |
|---|---|---|---|---|---|---|---|---|---|
| Rigor Index 3 h after death (%) | 4 | 36.7 | 18.7 | | 64.8 | | 65.2 | 37.9 | **n.s.** |
| | 6 | 78.3 | | 100.0 | 58.7 | 77.3 | | 29.1 | **n.s.** |
| Muscle pH | 6 | 6.83 | | 6.84 | 6.94 | 6.73 | | 0.12 | **n.s.** |
| Eye pH | 4 | 7.40**a** | 7.24 | | 7.13**b** | | 7.22**b** | 0.13 | * |
| | 6 | 7.24**a** | | 7.03**c** | 7.15**b** | 7.17**b** | | 0.04 | *** |
| ATP (µmol/g) | 6 | 2.07 | | 1.48 | 2.86 | 2.32 | | 0.83 | **n.s.** |
| IMP (µmol/g) | 6 | 5.55 | | 5.69 | 5.10 | 5.44 | | 0.89 | **n.s.** |
| AEC (µmol/g) | 6 | 0.75**a** | | 0.66**b** | 0.79**a** | 0.73 | | 0.22 | * |

* for $0.01<p\leq0.05$; *** for $p\leq0.001$; a,b for $p \leq 0.05$

### 3.5. Electrical stunning

Electrical stunning of seabass is still an experimental method, however, equipment manufacturers have produced prototype equipment for large-scale application. For whole body stunning of fish under laboratory conditions, two configurations are available, a batch and a continuous process (e.g. in a trough or pipe). For the whole body electrical stunning method, seabass are placed in a fresh water tank, trough or pipe and an electrical current is passed between two plate electrodes placed on the opposite sides of the tank, trough or pipe. The advantage of a continuous stunner is that in a trough or pipe, smaller electrodes are present which results in saving electric power, compared with a tank of, for instance, 500 l for batch-wise stunning.

Loss of consciousness and sensibility can be induced immediately in seabass in seawater (whole body application) or in air (head-only stunning) (Lambooij et al. 2008)). Stunning of fish in air has been reported for other species. Hence, it is suggested that head-only electrical stunning can be used for seabass as well.

Whole body electrical stunning for 10 s failed to kill seabass and, therefore, an additional method is necessary to kill the fish. For example, seabass can be stunned effectively by electricity and killed by chilling in ice water slurry. EEG recordings revealed that electrically-stunned seabass did not recover during killing by exposure to ice water (Lambooij et al., 2008).

When a 50 Hz sinusoidal alternating current is applied for Atlantic salmon or rainbow trout, this may cause blood spots and broken bones, unlike in eel (Morzel et al. 2003), African catfish (Lambooij et al. 2006), seabass (Lambooij et al. 2008) and tilapia (Lambooij et al., 2008b).

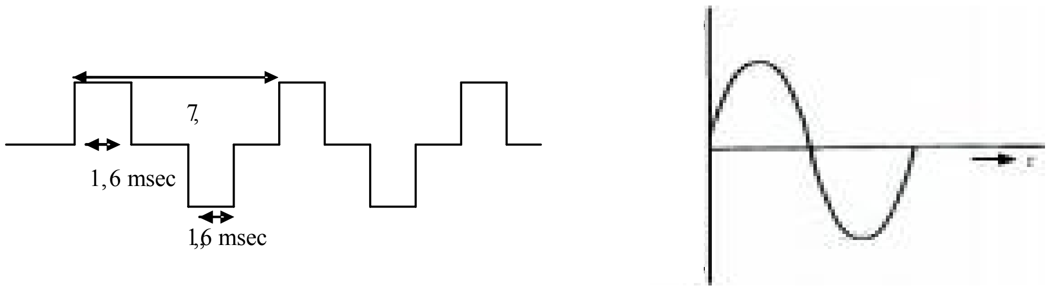
Conditions for experimental electrical stunning of seabass, using two different waveforms, are presented beneath:

a) A 50 Hz sinusoidal current: conditions for stunning

To achieve an immediate stun without recovery, the following conditions should be used for seabass in seawater (50 mS/cm) 50 V (4.4 $A/dm^2$, 50 Hz A.C.) (Fig. 7 B) for 10 s across plate electrodes at 50 cm distance, followed by chilling in ice water. Assessment of product quality of the seabass that were stunned and killed, revealed that carcass downgrading did not occur (Lambooij et al., 2008).

b) A bipolar square wave current (133 Hz, 43% duty cycle: conditions) for stunning

For an immediate and irrecoverable stun in seabass, exposure for 5 s to an overall current density of 3 $A/dm^2$ at an overall field strength of 0.7 Vrms/cm applied head-to-tail in seawater (52 mS/cm) (Figure 2 A) , followed by chilling for 15 min in ice water.




A B

Figure 2: **Bipolar square wave (A) and 50 Hz ac (B)** (Lambooj et al. 2008)

In practice, orientation of the fish in the electric field may not be achievable. When the current is applied side-to-side the overall field strength and current density has to be increased by 35%, as reported for Nile tilapia (Lambooij et al., 2008). For stunning of seabass in seawater in a commercial setting overall field strength of 1.5 Vrms/cm (133 Hz pulsed square wave alternating current (43% duty cycle) is needed to compensate for a side-to-side application of the current. Moreover, the decrease in overall current density due to a high density of seabass in seawater in the tank needs to be compensated (Van de Vis et al., 2008). The latter phenomenon also occurs when another waveform for the electrical current is used. When a tank of 600 l was used and fish were exposed for 20 s, an irrecoverable stun was obtained by adding sufficient flaked ice to the seawater and chilling seabass for 15 min. Assessment of product quality parameters revealed that broken bones and blood spot in the flesh were absent. However, the eyes of the electrically stunned sea bass may be less dark and convex, compared with fish that were killed by live chilling. These conditions have been established under laboratory conditions but more experimental work is needed.

Application of an electric current through the whole body of seabream and seabass is not like electrical stunning of other farm animals and therefore attention has to be given to the relevant differences, for example:

- In fresh water, the electrical conductivity of the water is relatively lower than the conductivity of the fish, and as a result it can be assumed that an applied current will flow through the body (current always flows through the least resistant pathway). In contrast, when sea water is used in the stunning bath, the electrical conductivity of the fish is expected to be lower than that of the water and as a result the amount of current flowing through the fish may not be adequate to induce immediate loss of consciousness. The possibility of a painful exposure to an electric field should be avoided.

- When electrodes are immersed in water, the amount of current generated by a given voltage can vary according to the size of the electrodes and the salinity in the water. However a living fish cannot be described by Ohm's law. A salmon is seawater is exposed to a low voltage the resistance can be in order of magnitude of a 10,000 Ohm, whereas when the animal is exposed to 100 V during electrical stunning in air, the resistance is approx 150 Ohm. It is likely, as has been shown for warm-blooded animals, that the permeability of the skin is a function of the voltage applied. This means that the skin becomes more permeable for the current when the voltage applied to the fish is increased. Thus, Ohms law cannot be used to describe the electrical properties of a live fish.

- The efficacy of stunning is determined by field strength, current density, conductivity of the water, density of fish in seawater, and the waveform of the current

- Any possibility of inducing electro immobilisation in fish should be eliminated.

- The health and safety of operators working in the wet area should be safeguarded.

Electrical stimulation causes the muscle contraction and triggers metabolic processes that may accelerate the progress of rigor ( Poli et al., 2004; 2005; Knowles et al., 2007, Lambooij et al. 2008). The acceleration of *rigor mortis* seemed mostly due to the consumption of metabolic reserves caused by the stress-response activity prior or during the treatment. In seabass, from a comparison of different stunning/killing methods, the evaluation of the stress indicators indicated that electrical stunning gave earlier *rigor* onset and lower pH and ATP values at death than ice slurry (Table3).

## 4. Methods of killing for disease control methods

Emergency killing of fish is necessary in several sets of circumstances. Deformed, moribund and surplus fish that require destruction in fish hatcheries are not part of the current mandate.

Seabass and seabream are not a susceptible species for any of the listed diseases in Annex II CD 2006/88, however the following methods are either in use or could be developed for mass killing on the farm. The present legislation predicts measures to be taken in the case of exotic and non-exotic diseases and also provisions for measures to be taken for emerging diseases. In the case of exotic diseases it is possible that aquaculture animals which have reached commercial size and show no clinical sign of disease may be slaughtered under the supervision of the competent authority for human consumption, or for further processing as long as such procedures are carried out under conditions which prevent the spread of the pathogen responsible for causing the disease. Aquaculture animals which have not reached commercial size or do not show clinical signs of disease shall, in an appropriate timeframe taking into account the type of production and the risk such animals pose for further spread of the disease, be removed and disposed of under the supervision of the competent authority in accordance with Regulation (EC) No 1774/2002, and the contingency plan provided for in Article 47 of the Directive.

The World organization for Animal Health has adopted guidelines concerning welfare aspects of the killing of animals for disease control purposes (http://www.oie.int/eng/normes/mcode/en_chapitre_1.7.6.htm). These guidelines apply to terrestrial animals and so far no guidelines exist in relation to fish. It is reasonable to assume that some principles are common, both in relation to the welfare of animals as well as biosecurity considerations.

Moribund and diseased growing fish can require killing on production farms but these aspects have not been included. Fish can require culling on farms for disease control purposes and emergency killing of illegal imports may be required.

To the experts' knowledge emergency killing for disease control has not occurred in seabream and seabass. If a disease outbreak required culling seabass or seabream, there is no obvious method of choice. Development of an appropriate method for on farm stunning/killing therefore requires additional research (e.g. mobile electrical stunning devices).

The following methods could be used for killing for disease control purpose as they are used for other species but there are no standard operating procedures for the effective use of any of the methods. The choice of methods will vary depending on the amount of fish being killed, and facility equipment. Such methods should be considered as part of contingency plans. Stunning should be carried out prior to killing. Signs of consciousness in fish should be monitored before destruction.

### 4.1. Overdose of anaesthetic

This method cannot be used for commercial seabass and seabream production but in case the product is not destined for human consumption, an overdose of anaesthetic could be considered a suitable way to humanely kill moribund or diseased seabream and seabass. At the moment only MS222 is licensed in EU (EFSA 2008).

### 4.2. Carbon Dioxide and or gas mixture

$CO_2$ narcosis is aversive to seabass and seabream. The gas mixtures used in live chilling with moderate levels of carbon dioxide in combination with another gas such as nitrogen could be used for killing moribund or diseased fish.

The method is under development and has been experimentally tested (see section 4.4)

### 4.3. Maceration

### 4.4. Electrical stunning

The method is under development and has been experimentally tested (see section 4.5)

### 4.5. Live chilling

This method has been described in section 4.2

### 4.6. Asphyxia

This method has been described in section 4.1

## 5. Reference to welfare indicators

Welfare indicators for seabass and seabream have not been satisfactorily assessed and validated so far. Nevertheless, observation of fish responses was taken into account in this opinion and may be used for field monitoring of welfare. Further validation of input and outcome measures is needed.

# 6. Risk Assessment

## 6.1. Application of the risk assessment approach

The Risk assessment method used to assess the risk to welfare of farmed seabream and seabass at the time of killing is described in Appendix A.

Although differences exist between the two species these were mostly related with the duration of the adverse effect and were detailed in the text of the opinion.

The definitions of intensity and the categories for duration of the adverse effect used for the assessment of welfare risks on the killing of seabass and seabream are described in tables .6 , 7 and 8.

Different definitions of intensity for hazards that occurred pre-slaughter and those arising during stunning and slaughter were needed.

Table 6: **Intensity categories for adverse effects arising from hazards associated with pre-slaughter / slaughter in seabass /seabream**

| Evaluation | Score | Pre slaughter | Slaughter |
|---|---|---|---|
| **MILD**<br>The animal is minimally affected as evidenced by minor changes in behaviour and physiological stress responses.(a) | 1 | Signs might include rapid swimming away from stimulus, eye position normal. Minor loss of scales and minor physical injuries in some fish, some foam begins to appear in the water, change in colour and pattern. | Signs might include escaping behaviour such as rapid swimming away from stimulus, eye position normal. Minor loss of scales and minor physical injuries in some fish, change in colour and pattern Infrequent gasping. |
| **MODERATE** | 2 | Not in the mild or severe category | Not in the mild or severe category |
| **SEVERE**<br>The animal is affected greatly, as evidenced by marked changes from normal behaviour, physiological stress responses and the presence of physical injury.(a) | 3 | Signs might include: energetic and purposeful escape behaviour, rapid and erratic swimming, Crush injuries and abrasion lesions from net or from contact with other fish , severe loss of scales, damages by fin reflex (seabass), excessive foaming in the water, excessive mucus production darkening in colour or change of pattern, splashing, gasping, flapping. | Signs might include: energetic and purposeful escape behaviour, tail flapping, splashing, gill opening, frequent gasping movements possibly with exaggerated gill movements, positive VOR, crush injuries and abrasion lesions from contact with other fish , severe loss of scales, damages by fin reflex (seabass), excessive mucus secretion, darkening in colour or change of pattern. Change of normal course of rigor mortis. |

Table 7: **Duration categories for adverse effects arising from hazards associated with pre-slaughter operations in seabass/ seabream**

| Duration (minutes)[1] | Score |
|---|---|
| < 5 | 1 |
| 5 – 30 | 2 |
| >30 – 60 | 3 |
| > 60 | 4 |

[1] The duration of the adverse effect consequential to exposure to feed deprivation was 7 and 30 days.

Table 8: **Duration categories for adverse effects arising from hazards associated with slaughter of seabass/ seabream**

| Duration (minutes) | Score |
|---|---|
| < 2* | 1 |
| 2 - 5 | 2 |
| >5 – 25 | 3 |
| > 25 | 4 |

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

The assumption that exposure to the hazard resulted in all the fish suffering the adverse effect held for the hazards related to slaughter but only to exposure to air during the pre-slaughter. For all other hazards in the pre- slaughter stage, the probability that the exposure to the hazard leads to the adverse effect (P adverse effect) was also assessed and estimates of the most likely maximum and minimum for the proportion of the population exposed to the hazard were calculated.

All four factors (probability of exposure to the hazard and of population exposed that suffered the adverse effect; 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.

Risk score = [(*I_adverse_effect* /3) * (*D_adverse_effect* / 4)* (*P_hazard*) * *(P adverse effect)] *100*

Where the following are defined:

- the intensity of the adverse effect (*I_adverse_effect*)
- the duration of the adverse effect (*D_adverse_effect*)
- the probability of exposure to the hazard (*P_hazard*)
- the probability that the exposure to the hazard leads to the adverse effect*(P adverse effect)*

The minimum, most likely and maximum values for *P_hazard* and *(P adverse effect)* were used to generate minimum, most likely and maximum estimates of the risk score.

## 6.2. Risk assessment: discussion and results

### 6.2.1. *Hazards associated with pre-slaughter*

Eleven hazards were identified (Table 9) (details in Appendix A) that may occur in pre-slaughter. The risk score ranged from 0.3 to 25. The highest ranking risks were very high crowding density and exposure to air during netting, the first had a very severe and prolonged effect and the second a severe effect in all fish.

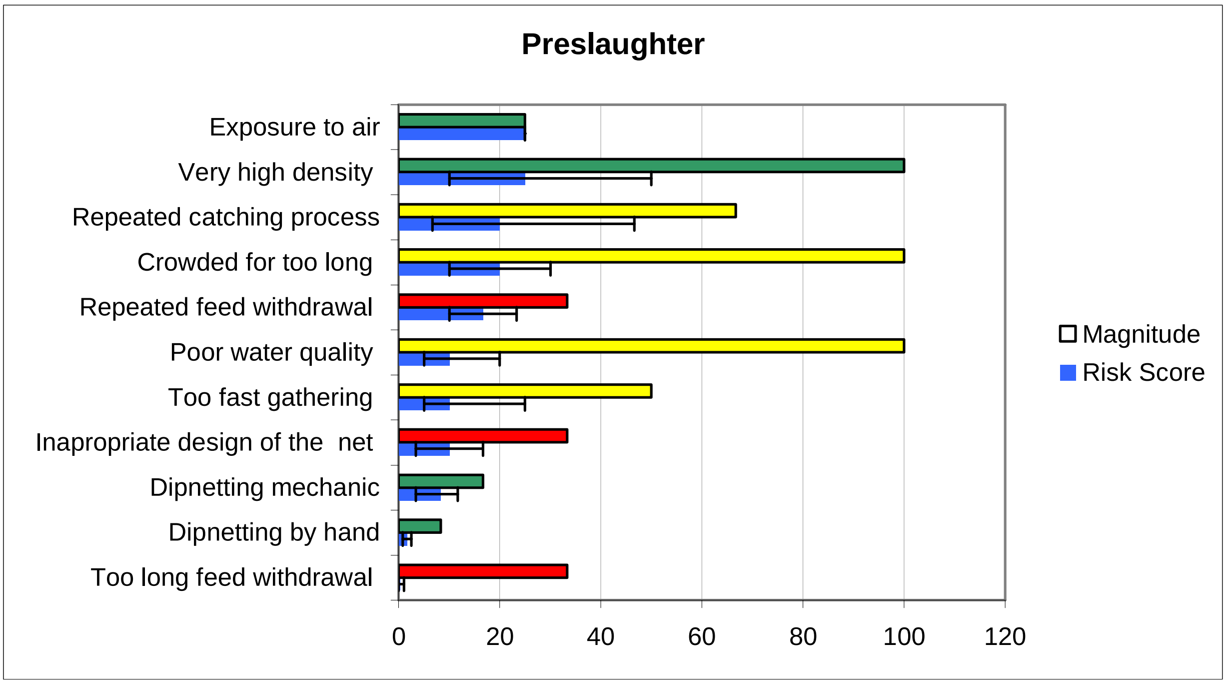
The sum of the risk scores of all the hazards was 147.

Table 9: **Risk and magnitude scores for hazards associated with pre-slaughter**

| Hazard ID | Pre-slaughter hazards | Description of adverse effects | Risk score | Magnitude |
|---|---|---|---|---|
| | **Feed withdrawal** | | | |
| 1 | Too long feed withdrawal | Hunger, immune depression, early onset of rigor mortis, relative changes in physiological stress indicators | 0.3 | 33.3 |
| 2 | Repeated feed withdrawal for residual fish | | 16.7 | 33.3 |
| | **Crowding** | | | |
| 3 | Inappropriate design of the net | Crush injury, loss of scales, foaming in the water, damage by fin reflex (seabass), escaping behaviour, splashing, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress indicators. | 10.0 | 33.3 |
| 4 | Too fast gathering (1st step) | | 10.0 | 50.0 |
| 5 | Crowded for too long (in the crowding net) | | 20.0 | 100.0 |
| 6 | Poor water quality (pH, DO, water temp, NH4) | | 10.0 | 100.0 |
| 7 | Very high density (>250 kg/ m3) | | 25.0 | 100.0 |
| | **Removal from water** | | | |
| 8 | Exposure to air | Escape behaviour abrasion from net, contact abrasion between fish, crush injury, loss of scales, excessive mucus production, damage by fin reflex (seabass), flapping, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress indicators. | 25.0 | 25.0 |
| 9 | Dipnetting by hand | | 1.7 | 8.3 |
| 10 | Dipnetting mechanical | | 8.3 | 16.7 |
| 11 | Repeated catching process | | 20.0 | 66.7 |
| | | | 147a | |

a sum of risk scores for the hazards of pre- slaughter

Figure 3: **Risk score and magnitude of adverse welfare effect for individual hazards associated with pre-slaughter**




Hazards are ranked by risk score. Error bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard. The uncertainty associated to magnitude of the adverse effect was represented by: red =High, yellow=Medium and green =Low.

#### 6.2.1.1. Variability and uncertainty

Variability is captured by estimates of the minimum and maximum values of the probability of exposure to the hazard and also for the minimum and maximum values of the probability that exposure to the hazard leads to the adverse effect. The uncertainty scores varied from 1 to 3. There was good consensus amongst the experts about the choice of estimates, however, there is little supporting published data. The uncertainty score of 1was given on the basis of consistent observations in commercial practice.

### *6.2.2. Hazards associated with killing*

Three killing methods were assessed (details in Appendix A). For each method of slaughter the risk scores for the hazards were summed (Table 10**)**. It should be noted that different definitions for intensity of the adverse effect were used for slaughter and stunning hazards compared with the pre-slaughter hazards.

The risk scores range from 42.5 for ice slurry chilling to 137.5 for asphyxia. The highest risk score was obtained by asphyxia in air (137.5) as a killing method followed by chilling on ice (132.5). The lower overall risk score was obtained by the chilling in ice slurry (42.5) which is the more commonly used method in commercial seabass and seabream farming in Europe.

In the method chilling in ice flakes, death due to asphyxia constituted the highest risk hazard followed by compression by other fish and ice. Chilling in ice slurry had the lower overall risk score. The major hazard was high density in the chilling containers followed by death due to asphyxia after recovery from chilling

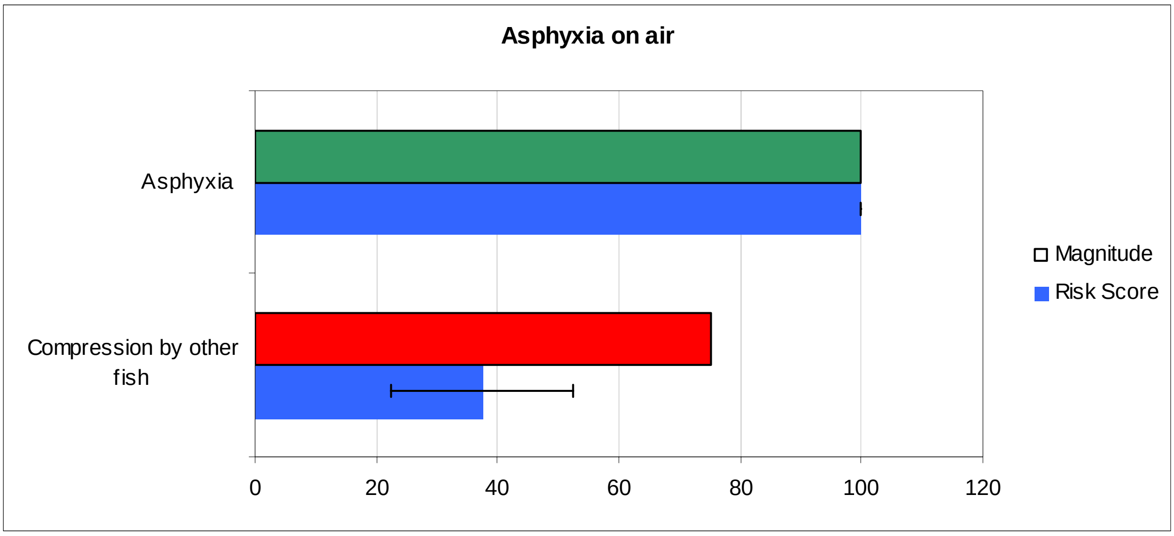
Table 10: **Risk and magnitude of adverse effect scores for the hazards associated with each killing method**

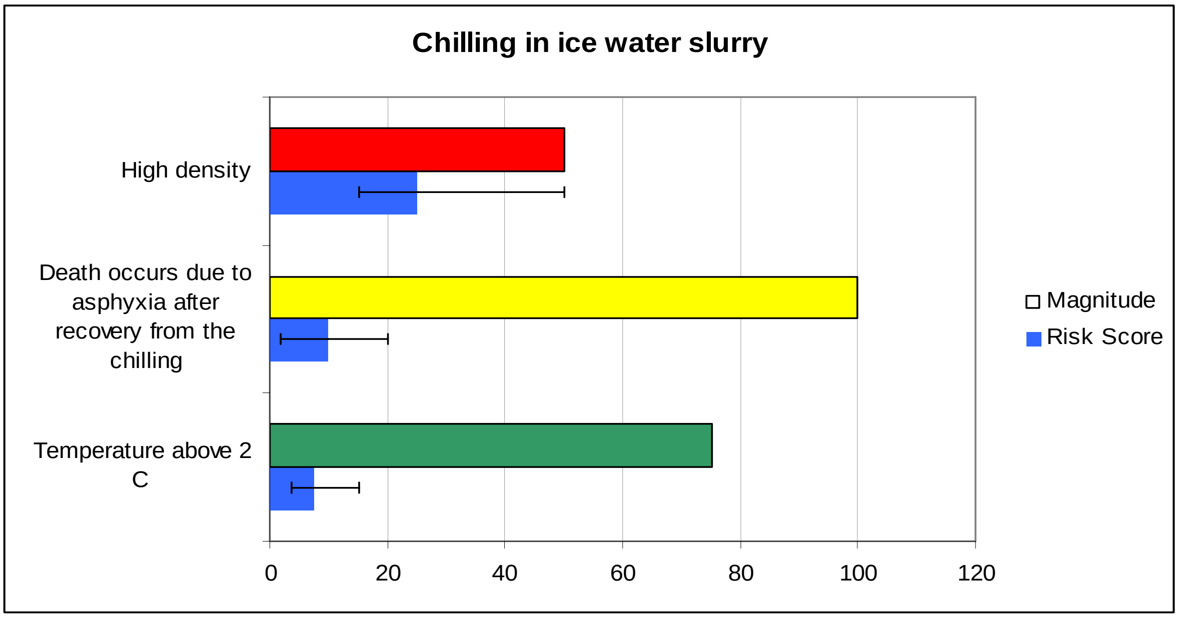
| Hazard ID | Slaughter hazards | Description of adverse effects | Risk score | Magnitude |
|---|---|---|---|---|
| | **Asphyxia in air** | | | |
| 12 | Asphyxia in air | Escape behaviour, contact abrasion between fish, crush injury, loss of scales, excessive mucus production, damage by fin reflex (seabass), flapping, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress | 100.0 | 100.0 |
| 13 | Compression by other fish | Crush injury, loss of scales, damage by fin reflex (seabass), escaping behaviour, splashing, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress indicators. | 37.5 | 75.0 |
| | | | **137.5a** | |
| | **Chilling in ice water slurry** | | | |
| 14 | Temperature above 2 C | Escape behaviour, attempt to keep head and tail out of water trying to escape, contact abrasion between fish, crush injury, loss of scales, excessive mucus production, damage by fin reflex (seabass), flapping, gasping, early onset of rigor mortis, relative changes in physiological stress indicators. | 7.5 | 75.0 |
| 15 | High density, crowding | Escape behaviour, contact abrasion between fish, crush injury, loss of scales, excessive mucus production, damage by fin reflex (seabass), flapping, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress. | 25.0 | 50.0 |
| 16 | Death occurs due to asphyxia after recovery from the chilling | Escape behaviour, contact abrasion between fish, crush injury, loss of scales, excessive mucus production, damage by fin reflex (seabass), flapping, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress indicators | 10 | 100.0 |
| | | | **42.5a** | |
| | **Chilling on ice** | | | |
| 17 | Asphyxia | Escape behaviour, attempt to keep head and tail out of water trying to escape, contact abrasion between fish, crush injury, loss of scales, excessive mucus production, damage by fin reflex (seabass), flapping, gasping, early onset of rigor mortis, relative changes in physiological stress indicators | 75.0 | 75.0 |

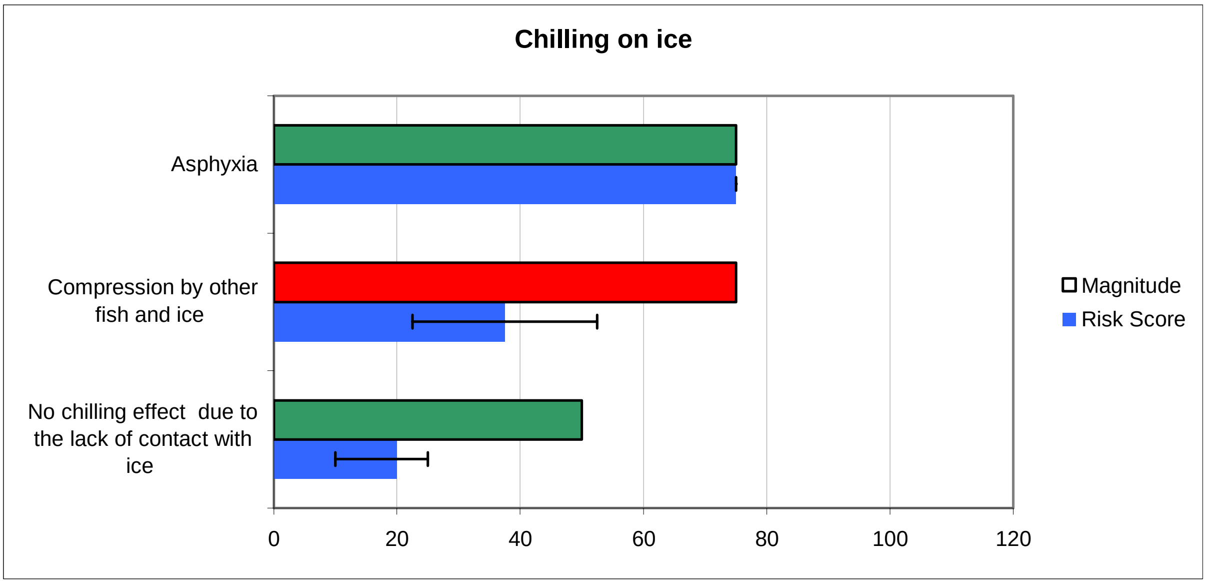
| | | | | |
|---|---|---|---|---|
| 18 | No chilling effect due to the lack of contact with ice | Escape behaviour, attempt to keep head and tail out of water trying to escape, contact abrasion between fish, crush injury, loss of scales, excessive mucus production, damage by fin reflex (seabass), flapping, gasping, early onset of rigor mortis, relative changes in physiological stress indicators | 20.0 | 50.0 |
| 19 | Compression by other fish and ice | Crush injury, loss of scales, damage by fin reflex (seabass), escaping behaviour, splashing, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress indicators. | 37.5 | 75.0 |
| | | | **132.5a** | |

a sum of risk scores for the hazards of that slaughter / stun method;

Figure 4: **Risk and magnitude of adverse effect scores for the hazards associated with each slaughter method**






Hazards are ranked by risk score. Error bars show the estimated minimum and maximum values for the risk score, reflecting the uncertainty about the probability of exposure to the hazard. The uncertainty associated to magnitude of the adverse effect was represented by: red =High, yellow=Medium and green =Low.

##### 6.2.2.1. Variability and uncertainty

The variability for the slaughter stage represents the probability that the fish killed by the particular method was exposed to a certain hazard. Once exposed to the hazard an adverse effect was always observed hence most likely, minimum and maximum values for the probability that exposure to the hazard leads to adverse effect were equal to one (Appendix A). The uncertainty score was from one to three. Field observations are consistent and clear however, there are often no published data supporting the choice of parameters.

### *6.2.3. Overall comparison of slaughter methods*

The total scores for the summed pre-slaughter and slaughter hazards are given in Table 12. The scores for the pre-slaughter hazards do not vary with the slaughter method.

Table 11: **Ranking of methods for seabass/seabream killing**

| **Method** | **Asphyxia in air** | **Chilling in ice** | **Chilling in ice water slurry** |
|---|---|---|---|
| Pre-slaughter score | | 147 | |
| Slaughter score | 137.5 | 132.5 | 42.5 |
| **Total** | **284.5** | **279.5** | **149.5** |

## CONCLUSIONS

1. Hazards associated with pre-slaughter procedures lead to high adverse effects on fish welfare.
2. The most critical hazards are: i) Very high density (>250 kg/ $m^3$) in the crowding; ii) Long period of crowding; and iii) exposure to air. High magnitude was observed for repeated catching process where the effect on residual fish was considered very severe.
3. Crowding is an unavoidable step in the pre-slaughter process. Crowding leads to deterioration of water quality which constitutes a hazard of high magnitude.
4. All the killing methods used commercially involve exposure to air during pre-slaughter which represents a severe risk for fish welfare.
5. There are different welfare implications associated with pre-slaughter procedures and the application of best practices during the several phases of pre-slaughter process could help to reduce the impact on fish welfare.
6. At present there are no validated and robust indicators available to evaluate in practice the welfare of seabass and seabream associated with slaughter procedures.
7. Three methods are used at present to kill seabass and seabream under commercial conditions: (a) asphyxia in air; (b) live chilling on ice; and (c) live chilling in ice slurry.
8. All of these commercial methods include a prolonged period of consciousness (several minutes) during which indications of poor welfare are apparent (physiological and behavioural responses).
9. Alternative methods such as carbon dioxide, nitrogen and electrical have been used only experimentally.
10. Carbon dioxide is strongly aversive to seabass and seabream and the fish remain conscious for several minutes.
11. Of these methods, only electrical stunning can induce immediate loss of consciousness and recovery is prevented by subsequent chilling of stunned fish.

## RECOMMENDATIONS

1. Pre slaughter management practices which keep crowding duration short and ensure a low crowding density level help to reduce stress induced by crowding at pre-slaughter. Crowding should be synchronised with the subsequent slaughter processes so that the fish are not crowded for longer than is necessary. Measures such as oxygenation or water renewal should be used to minimize deterioration of water quality.
2. Exposure to air should be reduced to the minimum possible time and research employed to develop pre-slaughter and slaughter methods that avoid air exposure.
3. Management and marketing practices should be implemented to avoid repeated exposure of the fish population to crowding, netting and prolonged feed withdrawal. A recovery period should be allowed.

4. According to the farming system, location, species etc, appropriate pre-slaughter procedures and equipments should be identified. Training of the operators is of great importance to be sure that best practices are applied.
5. This Scientific Opinion on seabass and seabream stunning and killing evaluated the methods currently used in farmed seabass and seabream in Europe. Methods used in other fish species other than those described in this Opinion may also be applicable to carp.
6. Standard operating procedures to improve the control of the slaughter process to prevent impaired welfare should be introduced..
7. A surveillance (monitoring) programme should be initiated for all the fish species 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.
8. The opportunity to develop new methods for slaughtering fish is considerable and should be encouraged.
9. Valid, robust and practically feasible indicators to evaluate the welfare of seabass and seabream during slaughter procedures need to be developed.
10. Persons involved in killing fish should be trained and hence skilled in handling and welfare.
11. Development of commercial stunning methods to induce immediate (or rapid) unconsciousness in seabass and seabream is urgently required.
12. Objective welfare indicators that can be applied in the field are required, e.g. the use of dorsal fin reflex occurring in seabass in response to stress.

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## GLOSSARY / ABBREVIATIONS

| | |
|---|---|
| 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. |
| Hypercapnia | A condition with a raised level of carbon dioxide in blood. |
| Hyperoxia | A condition with oxygen saturation above 100% of the normal atmospheric equilibrium for a given temperature and salinity. |

| | |
|---|---|
| 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. |
| Magnitude of the adverse effects | A function of intensity and duration of welfare impairment for fish. |
| Percussive stunning | A blow in the head is applied with a club, less often with a spring-loaded or pneumatic device. |
| 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. |
| Slaughter | The killing of animals for human consumption. |
| Slaughterhouse | Any establishment used for slaughtering fish. |
| Starvation | A period of food deprivation such that the animal metabolises tissues that are not food reserves but are functional tissues. |
| 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. |
| Vestibulo-ocular reflex (VOR) | A reflex where eye movement occurs in a conscious fish when rocked from side to side (commonly called eye roll). |
| Visual evoked reflexes (VER) | Evoked EEG activity in the brain with a visual stimulus. |

## APPENDICES

### APPENDIX A

### 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 effect[5].

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.

[5] 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.

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, 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 1).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.

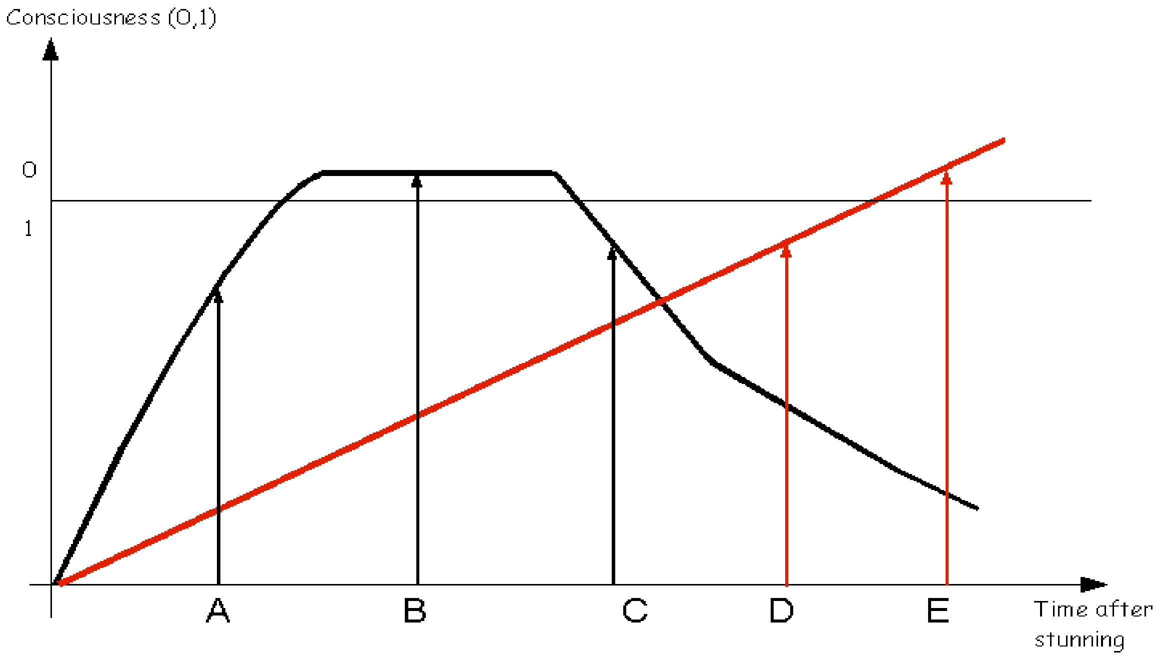


Figure 1. **Time to unconsciousness (insensibility) following stunning / killing** (horizontal grey line indicates consciousness threshold above which killing takes place without an adverse effect).

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

**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 1. 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 12. **Observable signs considered by experts when scoring the intensity of an adverse effect in farmed fish arising from hazards associated with the pre-slaughter or slaughter period**

| Evaluation | Score | Description |
|---|---|---|
| Mild | 1 | The animal is minimally affected as evidenced by minor changes in behaviour (e.g. rapid swimming away from stimulus and then slowing down, eye position normal). |
| Moderate | 2 | The animal is affected as evidenced by behaviour changes which can be considered moderate (more pronounced than minor but not severe). |
| Severe | 3 | The animal is affected greatly, as evidenced by marked changes from normal behaviour (e.g. energetic and purposeful escape behaviour, eyes rolling, rapid and erratic swimming, swimming upside down or tilted, colliding with the net, stopping swimming for more than 5 secs, crowding of fish) |

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.

**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 miss-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 (Table 7 and 8).

## 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 13).

Table 13: **Scoring system for total uncertainty in intensity and duration of effect**

| Evaluation | Score | Description |
|---|---|---|
| low | 1 | 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. |
| medium | 2 | 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 |
| high | 3 | 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.

Risk score = [*(I_adverse_effect /3) * (D_adverse_effect / 4)* (P_hazard)*] * 100

Where the following are defined:

the intensity of the adverse effect *(I_adverse_effect)*

the duration of the adverse effect *(D_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:

Magnitude score = [(*I_adverse_effect* /3) * (*D_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_adverse_effect*) = 3
- score for the duration of the adverse effect (*D_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.

Minimum score = (3/3 * 3/4 *0.01) * 100 = 0.75

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 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 12). 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.

## APPENDIX B

Table 14: **Parameters used in producing risk and magnitude scores for welfare hazards associated with pre slaughter and slaughter of seabass and seabream**

| Hazard ID | | Description of adverse effect | Intensity | Duration | | Uncertainty | Probability of exposure to the hazard | | | Probability that exposure to the hazard leads to the adverse effect | | | Risk score | | | Mag |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | score | min | score | score | most likely | min | max | most likely | min | max | most likely | min | max | |
| | **Feed Withdrawal** | | | | | | | | | | | | | | | |
| **1** | Too long feed withdrawal | Hunger, immune depression, early onset of rigor mortis, relative changes in physiological stress indicators | **1** | 7 days | **4** | **3** | **0.1** | 0 | 0.2 | **0.1** | 0.05 | 0.15 | **0.3** | 0.0 | 1.0 | **33.3** |
| **2** | Repeated feed withdrawal for residual fish | | **1** | 30 days | **4** | **3** | **0.5** | 0.3 | 0.7 | **0.5** | 0.1 | 0.7 | **16.7** | 10.0 | 23.3 | **33.3** |
| | **Crowding** | | | | | | | | | | | | | | | |
| **3** | Not adequate design of the net | Crush injury, loss of scales, foaming in the water, damage by fin reflex (seabass), escaping behaviour, splashing, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress indicators. . | **1** | 120 | **4** | **3** | **0.3** | 0.1 | 0.5 | **0.2** | 0.1 | 0.3 | **10.0** | 3.3 | 16.7 | **33.3** |
| **4** | Too fast gathering (1st step) | | **2** | 30 | **3** | **2** | **0.2** | 0.1 | 0.5 | **0.3** | 0.2 | 0.5 | **10.0** | 5.0 | 25.0 | **50.0** |
| **5** | Crowded for too long (in the crowding net) | | **3** | 60-120 | **4** | **2** | **0.2** | 0.1 | 0.3 | **0.5** | 0.3 | 0.7 | **20.0** | 10.0 | 30.0 | **100.0** |
| **6** | Poor water quality (pH, DO, water temp, NH4) | | **3** | 60 | **4** | **2** | **0.1** | 0.05 | 0.2 | **0.4** | 0.05 | 0.7 | **10.0** | 5.0 | 20.0 | **100.0** |
| **7** | Very high density (>250 kg/ m3) | | **3** | 60 | **4** | **1** | **0.25** | 0.1 | 0.5 | **0.5** | 0.3 | 0.8 | **25.0** | 10.0 | 50.0 | **100.0** |
| | **Removal from water** | | | | | | | | | | | | | | | |
| **8** | Exposure to | Escape behaviour abrasion from | **3** | 2 | **1** | **1** | **1** | 1 | 1 | **1** | 1 | 1 | **25.0** | 25.0 | 25.0 | **25.0** |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | air | net, contact abrasion between fish, crush injury, loss of scales, excessive mucus production, damage by fin reflex (seabass), flapping, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress indicators. | | | | | | | | | | | | | |
| **9** | Dipnetting hand | | **1** | 2 | **1** | **1** | **0.2** | 0.1 | 0.3 | **0.2** | 0.1 | 0.3 | **1.7** | 0.8 | 2.5 | **8.3** |
| **10** | Dipnetting mechanic | | **2** | 3- 5 | **1** | **1** | **0.5** | 0.2 | 0.7 | **0.3** | 0.1 | 0.5 | **8.3** | 3.3 | 11.7 | **16.7** |
| **11** | Repeated catching | | **2** | 120 | **4** | **2** | **0.3** | 0.1 | 0.7 | **0.3** | 0.1 | 0.7 | **20.0** | 6.7 | 46.7 | **66.7** |
| | **Asphyxia** | | | | | | | | | | | | | | | |
| **12** | asphyxia in air | Escape behaviour, contact abrasion between fish, crush injury, loss of scales, excessive mucus production, damage by fin reflex (seabass), flapping, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress indicators | **3** | 20-70 | **4** | **1** | **1** | 1 | 1 | **1** | 1 | 1 | **100.0** | 100.0 | 100.0 | **100.0** |
| **13** | Compression by other fish | Crush injury, loss of scales, foaming in the water, damage by fin reflex (seabass), escaping behaviour, splashing, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress indicators. | **3** | 10 | **3** | **3** | **0.5** | 0.3 | 0.7 | **1** | 1 | 1 | **37.5** | 22.5 | 52.5 | **75.0** |
| | **Chilling in ice slurry** | | | | | | | | | | | | | | | |
| **14** | Temperature above 2 C | Escape behaviour, attempt to keep head and tail out of water trying to escape, contact abrasion between fish, crush injury, loss of scales, excessive mucus production, damage by fin reflex (seabass), flapping, gasping, early onset of rigor mortis, relative change in physiological stress indicators. | **3** | 5 to 25 | **3** | **1** | **0.1** | 0.05 | 0.2 | **1** | 1 | 1 | **7.5** | 3.8 | 15.0 | **75.0** |
| **15** | high density, crowding | | **2** | 5 to 25 | **3** | **3** | **0.5** | 0.3 | 1 | **1** | 1 | 1 | **25.0** | 15.0 | 50.0 | **50.0** |
| **16** | death occurs due to asphyxia after recovery from the chilling | | **3** | 30 to 60 | **4** | **2** | **0.1** | 0.02 | 0.2 | **1** | 1 | 1 | **10.0** | 2.0 | 20.0 | **100.0** |
| | | | | | | | | | | | | | **42.5** | 14.2 | | **225.0** |
| | **Chilling in ice flakes** | | | | | | | | | | | | | | | |
| **17** | Asphyxia | Escape behaviour, attempt to | **3** | 10 | **3** | **1** | **1** | 0 | 0 | **1** | 1 | 1 | **75.0** | 0.0 | 0.0 | **75.0** |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **18** | No chilling effect due to the lack of contact with ice | keep head and tail out of water trying to escape, contact abrasion between fish, crush injury, loss of scales, excessive mucus production, damage by fin reflex (seabass), flapping, gasping, early onset of rigor mortis, relative change in physiological stress indicators. | **2** | 10 | **3** | **1** | **0.4** | 0.2 | 0.5 | **1** | 1 | 1 | **20.0** | 10.0 | 25.0 | **50.0** |
| **19** | Compression by other fish and ice | Crush injury, loss of scales, foaming in the water, damage by fin reflex (seabass), escaping behaviour, splashing, gasping, change in colour or pattern, early onset of rigor mortis, relative changes in physiological stress indicators. | **3** | 10 | **3** | **3** | **0.5** | 0.3 | 0.7 | **1** | 1 | 1 | **37.5** | 22.5 | 52.5 | **75.0** |