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SCIENTIFIC OPINION

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

Scientific Opinion of the Panel on Animal Health and Welfare

(Question N° EFSA-Q-2008-443)

Adopted on 30 April 2009

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the Panel on AHAW was asked to deliver a scientific opinion on the species-specific welfare aspects of the main systems of stunning and killing of farmed tuna 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 tuna, and to identify areas of concern, as well as to provide guidance for future research. The risk assessment was mainly based on expert opinion, due to the limited amount of quantitative data on many effects of hazards associated with stunning and killing of tuna. Pre-slaughter handling which have a direct impact on the welfare immediately before and during stunning and killing were included in the risk assessment.

This Scientific Opinion on killing of farmed tuna evaluated the methods currently used in Europe. There are 3 methods currently practised in the EU: 1) underwater shooting (lupara), 2) shooting from the surface, and 3) coring and spiking. The size of the fish and their market destination are key factors in slaughter options. Different pre-slaughter and slaughter methods led to identifying 6 different scenarios.

Crowding of tuna was the most important hazard during the pre-slaughter period.

Based on the risk assessment, underwater shooting (lupara) caused fewer welfare problems for the slaughter of large tuna compared with shooting from the surface. Shooting from the surface induces poor welfare because of severe crowding and a high percentage of fish having to be killed by a second shot. For lupara, the assessment showed a major difference when a back-up diver was present as without one the magnitude of poor welfare increased.

For smaller fish, spiking underwater gave the least poor welfare. However, this method needs to be improved as hoisting or gaffing the tuna before coring or spiking led to poor welfare and involves severe pain and distress.

Some of the methods used in other fish species other than those described in this Opinion may also be applicable to tuna. The opportunity to develop new methods for slaughtering tuna is considerable and should be encouraged.

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

Standard operating procedures to improve the control of the slaughter process to prevent impaired welfare should be introduced and approved by the industry. Valid, robust and practically feasible welfare indicators should be developed.

Key words: fish, welfare, risk assessment, pre-slaughter, stunning, killing, slaughter, disease control, aquaculture, tuna, *Thunnus thynnus*

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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, including farmed fish. As regards farmed fish, this opinion concluded that: "*Many existing commercial killing methods expose fish to substantial suffering over a prolonged period of time.*" Furthermore, "*for many species, there is not a commercially acceptable method that can kill fish humanely.*" Moreover, the respective EFSA report highlighted that different methods for stunning and killing of farmed fish must be developed and optimised according to the species specific different needs and welfare aspects.

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

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

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

The following species should be considered: Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), European eel (*Anguilla anguilla*), gilthead seabream (*Sparus auratus*), European seabass (*Dicentrarchus labrax*), European turbot (*Psetta maxima*), Common carp (*Cyprinus carpio*), and farmed tuna (*Thunnus* spp.).

2 OJ L 340, 31.12.1993, p. 21–34

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ASSESSMENT

1. SCOPE AND OBJECTIVES

The scope of this Scientific Opinion is the welfare aspects of stunning and killing methods applied to farmed tuna, *Thunnus* spp. The Atlantic bluefin tuna (*Thunnus thynnus* L. 1758) is the only tuna species farmed in Europe. Consequently 'tuna' hereafter refers to the Atlantic bluefin tuna.

Throughout the Mediterranean, wild Atlantic bluefin tuna have traditionally been caught using traps consisting of long net walls of several kilometres, designed and positioned to guide tuna migrating along the coast into an enclosed area of net (the device is also known as *Almadraba* or *Tonnara*). The trapped tuna are then brought to the surface by lifting the net of the trap, and are caught by gaffing having been confined in a smaller net where, in the experts' opinion, they experience fear, distress and pain before dying of asphyxia. This Scientific Opinion does not apply to the welfare aspects of stunning and killing methods of tuna captured in the Almadrava or Tonnara. It only applies to farmed tuna.

The objective of this Scientific Opinion is to identify welfare hazards and to assess welfare risks associated with the practices of stunning and killing of farmed tuna. The aim is also to identify, as far as possible, suitable welfare indicators at slaughter where they may exist.

The pre-slaughter process was only considered where evidence exists for a direct impact on welfare at stunning and killing. In this Scientific Opinion, the welfare aspects of the farming of tuna, their transport and killing for humane reasons are not included, but emergency killing at production units for disease control purpose has been considered.

Meat quality is not part of the assessment although, references are provided in the text that could be used further for socio-economic study on slaughter methods for tuna. 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.

2. INTRODUCTION

This opinion briefly describes the current practices for tuna slaughter, including pre-slaughter handling. An overview of tuna farming is provided in Appendix I.

The Atlantic bluefin tuna fattening industry in the Mediterranean Sea has developed in order to achieve: (a) a greater fat percentage in the muscle, which is considered desirable in the sushi and sashimi markets in Japan; and (b) a better price by not flooding the market in the brief fishing period (June to July). When fish flesh is consumed raw, as with sushi and sashimi, the quality requirements are much stricter than when the fish is cooked. The lactic acid accumulation from the anaerobic metabolism of the muscle during struggling on a hook or when fish are seined and moved on deck to die of suffocation (as practised for Atlantic bluefin tuna that are caught by the long-line and set trap fishing industries) can be tasted in fish consumed raw, but not when cooked. Tuna with a high concentration of lactic acid in their flesh (referred to as "yake" in Japanese) are well recognized in the Japanese wholesale market and such fish either fetch a much lower price, or are not considered suitable for sushi or sashimi. When Atlantic bluefin tuna struggle to escape before dying they produce considerable amounts of lactic acid, which may be higher when the fish are not allowed to swim (movement restriction by intense crowding *e.g.* by lifting the nets to the water surface), and they cannot obtain adequate oxygen. Furthermore, due to their ability to conserve metabolic heat produced by the muscles (Graham and Dickson, 2001), their body temperature can increase tremendously during struggling (core temperatures reaching 28-32 C), resulting in severe degradation of the flesh and, again, unsuitable for the sushi and sashimi market.

There are currently no national regulation or industry guidelines specifically addressing the welfare aspects of stunning and killing procedures for tuna. Welfare, meat quality and taste are very much influenced by the treatment of tuna prior to death and there is a strong incentive to kill fish rapidly in order to gain the best prices. In this way welfare and slaughter processes are closely linked. Since more than 90% of the farmed Atlantic bluefin tuna are destined for the Japanese sushi and sashimi market (Buentello *et al.*, 2008) the underlying criterion for all slaughtering methods employed by the fattening industry in the Mediterranean Sea is the maintenance of optimal flesh quality, and at the same time this means that death must occur as quickly as possible, without prior stressing or exhausting the fish (see Section 5).

The development of full aquaculture of the species (i.e., domestication) with production of eggs and juveniles in captivity will ensure that: (a) no fish are collected from the wild (thus helping conservation); and (b) allow for the development of tuna stocks that are better accustomed to farming management operations. For example, the welfare of domesticated tuna is expected to be affected less by noise, the presence of service boats and divers, the use of netting, crowding and other activities and parameters that are involved in the process of killing fish. Therefore, research towards the development of methods for the reproduction, larval rearing and on-growing of fish in captivity are expected to facilitate the development and/or application of killing methods with less negative effect on the welfare of the fish.

3. PRE-SLAUGHTER

Pre-slaughter practices frequently include capture methods that can be very stressful for the fish because of the struggling and crowding that occur during most protocols. Fish make strong escape attempts that, if prolonged, will lead to poor welfare than if they are killed quickly under less stressful conditions. During the pre-slaughter period, fish entering the slaughtering space are exposed to a range of stimuli including: crowding, handling, increased human contact, noise, transport, novel/unfamiliar environments, food deprivation, changes in social structure and changes in environmental conditions such as water contaminated by blood (Gregory, 2008). These changes lead to attempts to adapt and fish experience fear, homeostasis imbalance, increased physical activity, fatigue, physical injury and psychological distress (Conte, 2004; Portz *et al.*, 2006; Ashley, 2007; Gregory, 2008). Behavioural responses and the evaluation of some physiological changes that may indicate stress (*e.g.* increase of catecholamines, cortisol, glucose, lactate, haematocrit) are usually used to indicate when the fish may have suffered fear, distress and pain during the pre-slaughter and slaughter phases (Portz *et al.*, 2006). These measures could be used as welfare indicators to help to improve the pre-slaughter and slaughter practices.

The literature related to stress in reared bluefin tuna, mainly refers to the evaluation of physiological measures related to repeated sampling disturbance (Thomas *et al.*, 2003), confinement (Dyer *et al.*, 2004), stocking density and crowding stress (Percin & Konyalioglu, 2008). There are also some papers on the evaluation of levels of stress determined by different slaughtering methods and its effect on meat quality (Soto *et al*., 2006; Messina & Santulli, 2007, 2008) (see Section 5).

For farmed tuna, the pre-slaughter process varies with the different slaughter options (see Figure 1) but nearly all involve crowding of the fish to varying degrees. According to expert opinion, crowding for gathering the fish in preparation for killing would be in the range of 0.15 fish / m^3 or 20 kg / m^3 (300 fish of 150 kg in 2000 m^3). This crowding would also apply to the coring/spiking options for small tuna. The crowding for killing with the lupara is lower and would be in the range of 0.07 fish / m^3 or 10 kg / m^3 (20 fish of 150 kg in 300 m^3). The crowding for shooting from above is higher; in the range of 4 fish / m^3 or 150 kg / m^3 (40 fish of 150 kg in 40 m^3).

These scenarios give rise to different severities and durations of poor welfare during the time immediately preceding slaughter in relation to crowding, duration of confinement, poor water quality, human contact and noise. A detailed description of the critical points of both phases is reported, for each slaughtering method, in Section 4.

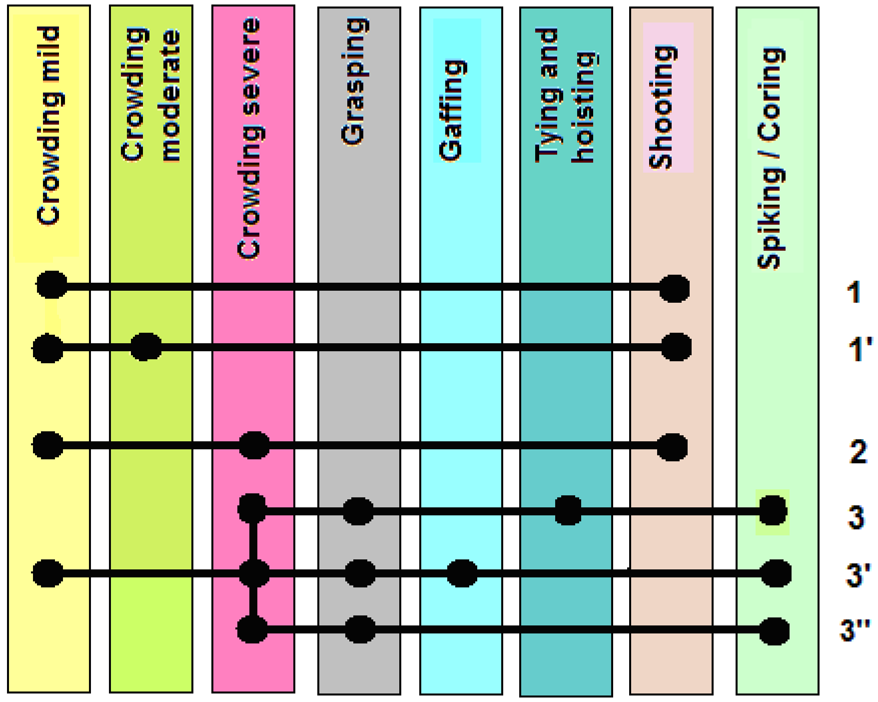

Figure 1: Pre-slaughter and slaughter scenarios for farmed tuna. Dots represent events occurring on a time line from left to right. Pathways 1 & 1’ represent underwater shooting (*i.e.* lupara) involving different levels of crowding; pathway 2 represents shooting from the surface; pathways 3, 3’ and 3’’ respectively represent hoisting or gaffing and spiking or coring options.

4. METHODS FOR SLAUGHTER

This section describes the different methods used for the slaughter of farmed tuna.

4.1. Recognition of consciousness, unconsciousness and death

In tuna, the existence of the Vestibulo-Ocular Reflex (VOR) has not been observed and opercular movements cannot be used to monitor breathing as tuna are ram ventilators (Block and Stevens, 2001), and so the absence of opercular movements is not a sign of unconsciousness. If a tuna shows any co-ordinated activity or responds to a potentially painful stimulation, it is conscious. Tuna can change the colour of their skin, giving a striped appearance, seen in males during courtship, but skin colour has not been validated as a reliable sign of stress.

4.2. Slaughter of Atlantic bluefin tuna

EFSA sent a questionnaire to all Member States to evaluate what methods are in use for the killing and stunning of tuna (see Appendix VI).

There are no stunning methods commercially applied to farmed Atlantic bluefin tuna. The three methods for killing tuna reared in fattening operations are: (1) shooting on the head underwater using a power-head (referred to as lupara) (70-80 % of large tuna); (2) shooting on the head from above out of the water using a shot-gun (20-30% of large tuna); and (3) hitting on the head with a metal spike or coring the brain (100% of small tuna).

The weight of tuna at slaughter varies between countries, as does their classification as ‘small’ or ‘large’ (see Figure 4). Small can mean between 40 and 80 kg and large can mean more than 50 - 80kg; tuna can grow to 600 kg. For the purposes of this opinion, the risk assessment applies to 2 categories of fish: large (above 50 kg) or small tuna (under 50 kg).

The method of slaughter is determined by:

1) The size of tuna:

Small tuna are slaughtered by spiking and fish can be sold either fresh or frozen depending on the market;

Large tuna are slaughtered by either lupara (commonly viewed as the best method to obtain fish for the fresh market) or by shotgun (fish killed by this method are usually frozen before going for consumption raw).

2) The commercial destination of the fish: either to be sold fresh or frozen.

Fish over 50 kg are not easy to handle and can be dangerous for the workers, so they shoot (lupara or shotgun) before establishing direct contact with them.

The method of using an electric harpoon (Soto *et al.*, 2006), which was evaluated in the early 2000s, is no longer employed in the EU.

The percentage of tuna requiring extra shots for lupara is 1-4 % (2nd shot) and 1 % (3rd shot). With shotguns, 7-10 % of fish would be killed by a second shot. The time between the 2 shots is estimated to be in the range of 1-30 seconds. Bleeding is performed on board for lupara, but in the water for the shotgun method. For both methods, all tuna are pithed to ensure meat quality.

Tuna can be very heavy (up to 400-600 kg) and long (1-3 m) and so are not easily handled. Mechanical hoists and cranes are used to lift tuna, by tying a rope just in front of the tail fin, on-board a processing boat or on a special boat that processes and freezes them and takes them to Japan.

Bleeding is carried out by severing the lateral arteries and sometimes the gills with a pointed sharp knife.

Coring is the process whereby a hollow metal rod (about 2-3 cm diameter) is driven into the head of the tuna into the brain through the skull in the area that is covered by a soft translucent tissue over the pineal gland in the frontal part of the cranium (pineal window).

Spiking is similar to coring except the metal rod is solid with a point.

Pithing (Tanigushi method) is a post-mortem action. To pith is to completely destroy the spinal cord in the neural canal. As well as stopping muscular activity, pithing stops the biochemical reactions that contribute to flesh deterioration. Pithing produces a higher-quality tuna. It is carried out with a long flexible metal or rigid nylon wire introduced through the exposed vertebral canal after decapitation of the dead fish with a chain saw. If tuna was stunned and killed by coring, the pithing wire is introduced through the exposed hole in the head.

4.2.1. Shooting underwater using a power head (Lupara)

Shooting underwater is the most commonly employed method for killing large tuna. With this method, the fish are killed with an underwater shot to the head, one-by-one by a diver in the water using a power-head, referred to as lupara. A lupara is a device which resembles a short gun barrel (Figure 5), inside which a single-shot cartridge - commonly used for hunting wild boar- is placed. The lupara is fitted at the end of a stick, that can be between 2-3 metres long (Figure 5) and which, when jabbed into the head of the tuna, fires the bullet. The bullet expands on impact and so damages the head and brain.

The ammunition used for killing in the lupara is the same as the one used in the shot guns for shooting from above the surface: a cartridge for hunting large wild animals, but with lower amount of powder in order to cause a smaller injury and prevent the bullet from going leaving the body of the fish, and to achieve effective killing with just one shot. The cartridge is a classical one with single bullet, normally made of lead. When the bullet hits the head, it deforms and causes a bigger lesion, but at the same time it ensures an instantaneous death. The only difference with the lupara is that the cartridge is sealed with silicone in order to keep the powder dry.

With a proper shot, the fish becomes immediately immobile and dead. Depending on the initial trajectory and inertia, the carcass continues to move with final tendency to sink. The injury is usually small, and there is not much bleeding if the tuna is properly shot. If the tuna is mis-shot, it may be left unable to swim, showing uncoordinated tail flapping. It then receives a second shot. Depending on the company, one or two divers may be present in the water at the same time; this was considered to distinguish the two variants for the lupara method. When the tuna is able to continue swimming, a diver has to follow it for a second shot.

The tuna are crowded by a mild to moderate reduction in the available volume by lifting the bottom or one of the walls of the cage, or by using a seine (referred to as culling net) to isolate the number of fish that will be killed that day from the rest of the population. Crowding is far less than in the other two killing methods (see below). The fish are allowed adequate space to swim around, and are killed one-by-one with a shot to the head. Alternatively, a group of fish from the population of the rearing cage or culling net may be herded into a smaller “slaughtering cage” (300-400 m^2 surface area) that is connected to the rearing cage or to the culling net. Again, the isolated fish are shot with the power-head (lupara), one-by-one. Once a fish is killed, a rope is tied just below the tail fin (caudal peduncle) by another diver and the tuna lifted onto the deck of a service boat using a mechanical hoist. There, it is bled, by slitting the lateral arteries and gills, cored and pithed (Figure 6). In most companies, bleeding is done in the water prior to hoisting the fish on deck; and instead of coring the head, the dead fish is decapitated with a chainsaw to allow pithing.

The lupara method allows for the selection of individual tuna to be killed. It is considered to be the best method from the point of view of flesh quality, as it results in instantaneous death of fish swimming in their rearing cage under mild or moderate crowding conditions in a slaughtering cage. However, the method is time consuming and inefficient for killing large numbers of fish in a day. Thus it is not suitable for small tuna or when a large number of fish must be killed and processed.

For the lupara to be effective and instantaneously kill the fish, the tuna must be shot directly in or in the immediate vicinity of the brain, thus destroying or severely damaging the brain. The tuna usually swim faster and do not approach to the divers. In the case of a missed shot (*i.e.* not directly on the brain), and the tuna is not killed instantly, this is obvious from the continued swimming (though erratic) of the fish. Severe bleeding also takes place as usually missed shots hit the fish on the lower part of the head, thus hitting the gills (see Figure 4).

4.2.1.1.Monitoring points

Monitoring points include loss of body movement and an absence of reaction during handling and hoisting out of the water.

4.2.1.2.Hazards identified with lupara

Pre-slaughter hazards involve crowding and mis-crowding (*e.g.* mild crowding in the rearing cage or moderate crowding in a slaughtering cage) if not done slowly. This may result in some fish being caught in the nets, suffering stress and hypoxia –since they cannot swim backwards - until they die or are killed. The presence of blood in the slaughtering area depends on sea currents and quality of shots.

Slaughter hazards include missed shots. The accuracy of the shot may be affected by: the shooter's experience; sea conditions (including bad weather, strong currents in the area); and the number of fish that need to be slaughtered. The only hazard is a missed shot that may either completely miss or hit the head but not the brain. In the latter case the fish will require another shot and then it will be moved rapidly on to the deck where it is cored, pithed and bled.

4.2.2. Shooting to the head from outside the water

Shooting on the head from above outside the water is the second most commonly employed method for large tuna, as it allows the killing of a large number of fish in a short time. A group of fish destined to be killed (30-70 fish, depending on size) are separated from the main population, and herded into one side of the rearing cage using a seine net. Alternatively, a group of fish from the population in the rearing cage may be herded into a smaller slaughtering cage (called *copo*) that is connected to the rearing cage. The isolated fish are then brought rapidly (maximum 1-3 min) to the surface in order to restrict their movement, and the bottom of the cage is lifted rapidly using hydraulic winches. Trained marksmen standing on the service boat or a platform next to the slaughter cage, shoot the fish on the head using a shotgun loaded with single-bullet cartridges. Usually a single shot is enough to cause immediate death, and sometimes a second shot is given (according to the questionnaire, 7-10% of the tuna would be killed by a second shot). Once all the tuna in a group are thought to have been killed, divers enter the cage, spike any fish that are still alive, and bleed the fish by severing the lateral arteries and gills. The tuna are hauled onto the deck of the service boat using a hoist where the dead fish are decapitated or cored, and then pithed. This process of batch slaughter is repeated multiple times, until the working day is over or the required number of tuna has been killed.

The tuna are killed within 15 minutes approximately after exposure to very stressful conditions (*i.e.* severe movement restriction in the slaughter cage or seine net, bloody water, noise) but it allows processing a large number of tuna per day. However, it is mainly applied to large fish (>50 kg). If the number of fish in a batch is too many it reduces the accuracy of the kill.

When tuna are slaughtered by shotgun from above the surface, they are usually contained inside the slaughtering cage (*copo*); usually this stresses the fish. More blood goes to the water than when using the lupara and this makes the tuna more stressed, but they calm down when the blood disappears. The noise of the supporting boat, human contact and gun detonation causes sudden escape attempts in the other fish in the nets. At the end of the first slaughtering phase of the day, tuna remaining in the cage stop feeding. However, the detonations do not seem to cause a long period of stress to the tuna.

4.2.2.1.Monitoring points

Monitoring points include loss of body movement and absence of reaction after shooting (*e.g.* during hoisting).

4.2.2.2.Hazards identified with shooting from the surface

Pre-slaughter hazards include crowding: a variable number of fish (usually 30-70) are enclosed in a seine, or they are moved to a slaughter cage. In both cases, fish are crowded when the net is hauled in by hand or by a crane. This causes severe crowding as tuna are struggling and cannot swim away.

A slaughter hazard is a missed shot when the bullet does not hit the brain (approximately 10 % of the fish). A second shot would be given. After shooting, mis-shot fish are spiked in the water at the end of the shooting period (10 – 15 min). This is influenced by the shooters' experience the number of shooters (more shooters reduce the time for slaughter), bad sea weather (including strong currents in the area), and the number of fish to be slaughtered.

If tuna smaller than 50 kg are present in the slaughter area, they are slaughtered by spiking or coring in the water after the shooting period for the bigger fish (see section 4.2.3).

In general, to avoid the effect of the blood on the rest of the stock, boats and nets are placed to leeward of the stock cage but the noise of the gun detonation affects the stock fish

4.2.3. Coring or spiking

This method involves a manual coring or spiking of the head ("iki jime" in Japanese). There are three different sub-types of coring/spiking used in different countries.

1. In Croatia, coring is the method employed exclusively for small bluefin tuna, and also in some Mediterranean countries when only a few small fish are present in a caged population of larger individuals. Fish to be killed are crowded close to the surface by lifting the bottom of the cage or using a seine to gather them into one side of the rearing cage. A single tuna is captured by divers by holding it by the opercula and it is then gaffed under the opercula by two people from a service boat and pulled up a slide and onto the deck. While being moved on-deck fish are cored, bled and pithed. Because 2-3 people are managing the fish simultaneously, bleeding takes place during coring, or just after.

2. Tuna are confined in a small seine or a cage net, and several divers (9-10) grasp them, haul them on-board, and then spike and bleed them. In this method gaffing is not used. The method is used where there are only small tuna of less than 50 kg.

3. Where large Atlantic bluefin tuna are farmed, this method is used if any small fish are present in a population after the larger tuna have been killed either by lupara or shot from above. In this case, fish are grasped, flipped dorsally, and hoisted alive on-board by a rope below their tail fin. They are not gaffed but they are cored, bled and pithed on-board.

For effective use of these coring/spiking methods, the core or spike is rapidly inserted into the brain via the pineal window. In addition, pithing is undertaken immediately after coring or spiking.

4.2.3.1.Monitoring points

Monitoring points include loss of body movement, absence of reaction during bleeding or further processing, check that the core or spike was inserted into the brain via the pineal window.

4.2.3.2.Hazards identified with coring or spiking

Gaffing in the water and coring on-board

<u>The pre-slaughter hazards</u> are: stress of crowding less than one hundred small tuna; pain and distress associated with gaffing and hauling them on-board. Loss of consciousness is normally caused by coring in less than 1 second followed by pithing that takes less than 1 minute.

Crowding can last from 10 min (for the first fish) for up to several hours (for the last fish with an average of more than 30 min). During this period, fish are stressed by the lack of space, the presence of divers, as well as blood in the water. Gaffing (by at least two men) and the consequent tissue damage and hauling is painful and, as the fish is also lifted out of water, lead to asphyxia. Rough handling during the pre-slaughter phase would include chasing the animal for long time, which results in adverse effects such as erratic swimming or exhaustion, mechanical trauma by collision with the net and other fish. There may also be mis-gaffing when a fish drops off the gaffs back into the water and has to be re-gaffed.

<u>Slaughter phase hazards</u> are: cutting of the lateral arteries shortly after gaffing and starting to haul the animal when it is still conscious; coring is usually done through the pineal window and mis-coring may be a hazard when it is in the wrong area and the fish fails to become rapidly unconscious; mis-pithing; bleeding will cause pain if it is done before coring or the fish is not unconscious.

Underwater spiking

<u>Pre-slaughter hazards</u> include: stress due to the crowding, the presence of divers as well as large quantities of blood, since bleeding is done in the water. The possible hazards identified are: crowding, and rough pre-slaughter handling resulting in adverse effects such as erratic swimming and/or exhaustion, and mechanical trauma by collision with the net and other fish.

For <u>slaughter</u>, spiking occurs underwater through the pineal window and tuna are then pithed. Loss of consciousness is achieved in less than 1 second by spiking. The hazard is mis-spiking (wrong area is spiked and the fish may not be made unconsciousn).

In case of mis-spiking, tissue damage includes superficial to deep lacerations of skin and bone layer with haemorrhage, skull fissure or fracture, brain contusion and haemorrhage, depending on the force and angle of the spike.

Grasping and hoisting tuna on board

If small tuna are present in the slaughter cage after the larger tuna have been killed by lupara, they are slaughtered by coring. Divers grasp the small tuna and flip them dorsally, and hoist then on-board where they are bled, cored and pithed. Hazards include crowding, rough handling, and mis-coring.

4.3. Killing for disease control purpose

In Council Directive 2006/88/EC it is stated that the member states shall ensure that fish that show clinical signs of disease are removed and disposed of under the supervision of the competent authority in accordance with Regulation (EC) No 1774/2002. (Art 34). Member states shall take appropriate measures to control an emerging disease situation and prevent that disease from spreading. (Art 41). In the regulation (EC) 1774/2002 fish killed to eradicate an epizootic disease belongs to Category 2 (Article 5) and the method of dealing with the dead fish and their disposal is addressed. But neither in the Directive 2006/88/EC nor in the regulation 1774/2002/EC is there a description of methods for emergency killing and stunning of fish.

To the knowledge of the WG members, stunning and killing for disease control purposes has not occurred in tuna farming. The normal methods of slaughter described above are not appropriate for massive killing for disease control because of the amount of blood and tissue released. In order to avoid such a high risk of further dissemination of pathogens, asphyxia by netting the fish and placing them in a cage on land, or placing them on ice in a container, would accomplish death as well as a suitable container for disposal of the bodies. It takes about 10 to 15 minutes for a tuna to die of asphyxia out of the water. This method would severely affect the welfare of tuna, but has the advantage that no tissues or fluids are released to the aquatic environment and animals can easily be moved off-site for disposal. Theoretically, an overdose of anaesthetic could also be given.

5. Welfare indicators

The assessment of poor welfare requires taking into account the different behavioural, biochemical and physiological processes that might be involved. Changes in measures of brain function, endocrine responses, post-mortem injuries and changes in tissue structure could be studied and, particularly, muscle biochemistry and meat quality (Poli *et al*., 2005, Portz *et al*., 2006; Ashley, 2007).

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

The close interrelationships observed between endocrine acute stress responses and post-mortem biochemical processes suggest that stress indicators such as: levels of cortisol, lactate and glucose, pH, lactate, glycogen, phosphocreatinine, ATP and its catabolites in the blood and body tissues could be used. These parameters are indicators of cellular energy charge and are influenced by the pre-slaughter and slaughter stress conditions (Erikson, 1997; Pottinger, 2001; Tejada *et al*., 2001; Poli, 2005).

Meat quality changes in fish and fillet influenced by stress at slaughter are appearance (physical injuries, flesh gaping and colour), technological properties (rigor evolution, texture, firmness, water holding capacity), freshness indicators and spoilage indicators (biogenic amines, lipid oxidation products), and sensory qualities of raw fish such as taste (Poli, 2005).

The effects of slaughtering methods on some meat parameters in farmed tuna was studied by Garcia *et al*. (2002) and Soto *et al.* (2006) and showing some negative effects of electro-slaughtering on physical injury sufficient to compromise the marketing of the fish. The effect of two different slaughter procedures was evaluated on large tuna in a fattening farm located in western Sicily (Italy). The first one was shooting underwater by lupara without confinement, and the second was shooting on the head from above the water after confinement in a chamber (Messina and Santulli, 2007, 2008). The higher stress was found in tuna killed by the second procedure by biochemical markers in the blood, and in the meat by higher depletion of muscular glycogen causing a reduction in muscle pH and reduced water holding capacity (Messina and Santulli, 2007, 2008). These data support the results of other blood measures of stress confirming that animals killed by shooting on the head from above the water after confinement was more stressful than lupara (Messina and Santulli, 2007, 2008). The greater loss of meat quality related to stress due to confinement at slaughter (lupara compared with shooting from above) was demonstrated by the higher production of total volatile basic nitrogen and malondialdehyde which are markers of nitrogen and lipid degradation (Poli *et al*., 2005; Messina and Santulli, 2007, 2008). These data suggest that, as observed in others farmed fish species (Erikson, 1997; Pottinger, 2001; Tejada *et al.*, 2001; Poli, 2005), the meat quality of farmed bluefin tuna can be related the stress response to slaughter.

6. RISK ASSESSMENT

6.1. Application of the risk assessment approach

Table 1: Intensity categories of adverse effects arising from hazards associated with pre-slaughter and slaughter operations in tuna

Evaluation	Score	In water	In air2
MILD The animal is minimally affected as evidenced by minor changes in behaviour.	1	Signs include rapid swimming away from stimulus and then slowing down.	Not Applicable.
MODERATE	2	Not in the mild or severe category	Not in the mild or severe category
SEVERE The animal is affected greatly, as evidenced by marked changes from normal behaviour	3	Signs might include energetic and purposeful escape behaviour, rapid and erratic swimming, swimming upside down or tilted, colliding with the net, stopping swimming for more than 5 seconds, crowding of fish so they cannot swim, fish having to swim in poor quality, water	Prolonged exposure to air is asphyxia and considered severe. Tail flopping, gill opening, tries to open mouth, frequent gasping movements possibly with exaggerated gill movements, eye position normal, body contractions,

The risk assessment methodology used to assess the risk to welfare of farmed tuna when killed is described in Appendix II. The risk assessment was applied to the slaughter of tuna.

The hazards associated with typical pre-slaughter management were assessed, in relation to their effect on slaughter. The assumption that exposure to the hazard resulted in all the fish suffering the adverse effect held for all hazards. Definitions of intensity of an adverse effect for hazards occurring pre- and post-stunning were defined (Table 1).

Different categorisation for duration of the adverse effect was used for pre-slaughter and slaughter / stunning hazards, as presented in Table 2.

Table 2: Duration categories for adverse effects arising from hazards associated with pre-slaughter and slaughter operations in tuna

Score	Duration for pre-slaughter	Duration for slaughter
1	<1min	<1 sec
2	1-9 min	1-59 sec
3	10-29min	1-2 min
4	>30 min	>2 min

Note: adverse effects with duration of less than one second are not scored

6.2. Risk Assessment Results and Discussion

The review of practical implementation of slaughter methods for farmed tuna has led to the identification of six (6) scenarios. Three scenarios apply to large tuna (2 for lupara, and 1 for shooting); and 3 scenarios were also identified for small tuna coring or spiking, with or without gaffing. The 6 scenarios are presented in Figure 2. Detailed pathways for the various methods of farmed tuna slaughter and a detailed table of the risk assessment are provided in Appendices IV and V.

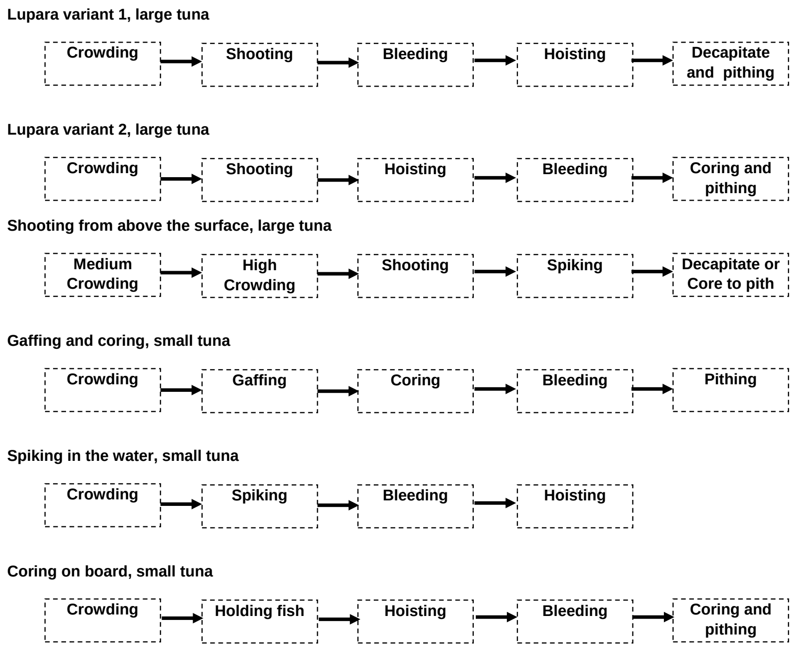

Figure 2 Slaughter scenarios identified for farmed tuna. The pathways are represented as a suite of pre-slaughter and slaughter events as they are applied to large or small tuna in the different Member States.

In the first variant of the lupara method (lupara variant 1), an additional diver is on stand-by in the water and monitors the shooting of the fish and its behaviour afterward, to ensure that the shot resulted in immediate death (*i.e.*, lack of any voluntary movement). If the fish is showing signs that it is not dead, then the second diver shoots it again with a lupara. Bleeding may be done in the water while the fish is being tied in order to be hoisted out of the water on-board the service boat for further processing, which may include decapitation and pithing, or coring and pithing.

In the second variant of the method (lupara variant 2), only a single diver is used and a single shot is delivered to the fish. In the case that the shot misses the brain case and does not result in immediate death (indicated by a lack of any voluntary movement), another diver captures the wounded fish, ties it by the tail and it is hoisted on-board the service boat where it is bled, cored and pithed. In this case, death may occur any time between the lupara shot and coring. Although the percentage of missed shots is very small, it is obviously that in this situation such tuna are exposed to severe negative welfare.

In total, six methods of slaughter were assessed: 2 variants of lupara, shooting from above, gaffing and coring, spiking under the water, and hoisting and coring on board (see detail pathways in Appendix IV). Between four and nine hazards were identified for each method. The risk and magnitude scores for the hazards are given in Table 4 and scoring details are in Appendix V.

As a general principle, the risk assessment and scoring of hazards stop when fish dies or loses consciousness irreversibly in less than one second (*e.g.* successful shot, spiking, or coring; see Table 4). Welfare can only be an issue when fish are conscious. Table 3 provides a synoptic view of overall risk scores for the different methods applied for the slaughter of tuna.

Table 3: Overall risk scores for slaughter methods applied to tuna.

Method	Spiking in the water, small tuna	Lupara variant 2, large tuna.	Lupara variant 1, large tuna.	Gaffing and coring, small tuna.	Shooting from above the surface, large tuna	Coring on board, small tuna
Total	75	89	92	143	152	225

The risk scores range from 75.25 (for spiking of small tuna under the water) to 225 (for coring small tuna on board). For all methods of slaughter crowding was a hazard because this is an unavoidable step in pre-slaughter. Crowding was further distinguished as mild, moderate or severe each having different scores and magnitudes.

Coring small tuna on board had the highest risk score because all fish slaughtered with this method are exposed to handling by divers, hoisting while conscious, exposed to air on the deck of the service boat before they reach unconsciousness. This method had also a maximum magnitude of the adverse effect (225). The method consisting of gaffing and coring of small tuna had a slightly lower total risk score (143.2) – however this method has a significantly higher magnitude. The highest ranked hazard with this system was gaffing.

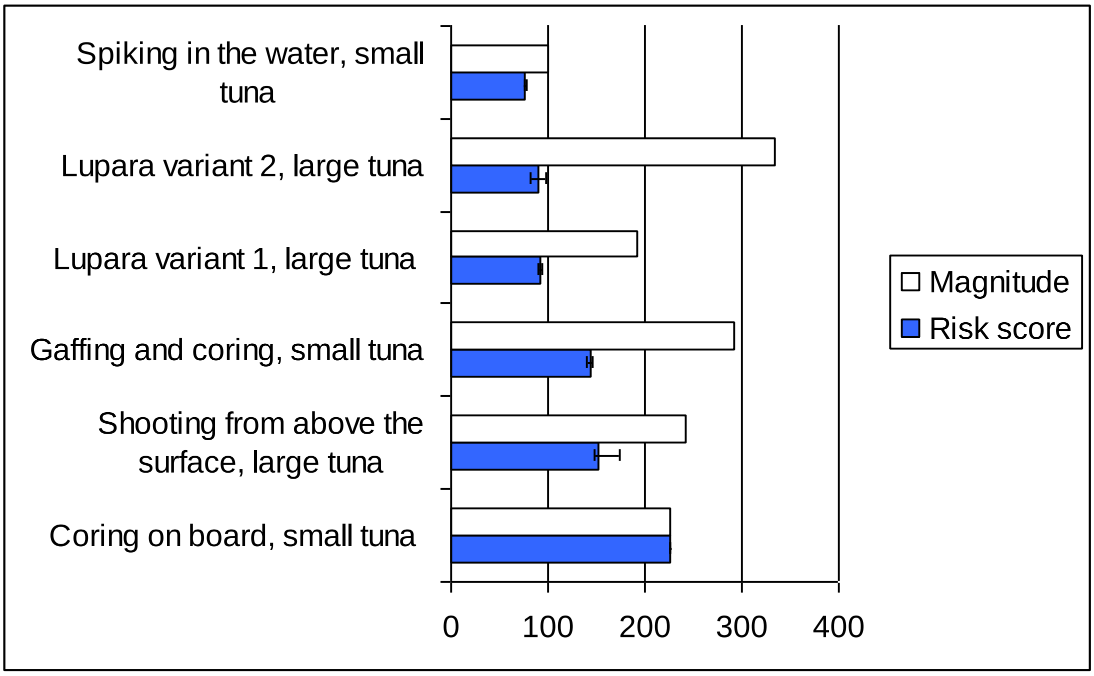

Figure 3: Sum of risk scores and magnitudes of the adverse welfare effect for main slaughter methods applied to tuna in Europe, ranked by the sum of the risk score.

Out of the three methods applied to slaughter small tuna, spiking underwater had the lowest risk score (75.25).

Crowding prior to slaughter always had a significant contribution to the overall risk score.

For large tuna, the slaughter method with the lowest risk scores (89.25 – 91.65) were for the lupara methods. The highest ranked hazards with this method were caused by crowding and mis-shooting. Pre-slaughter may also induce death by asphyxia of tuna inadvertently getting trapped in the net. Having the capacity to shoot a second time in the case of a mis-shot significantly contributed to a reduction in the overall score, but even more to a reduction in the magnitude of poor welfare associated with the method.

Shooting from above the surface of the water with a shot-gun had the highest risk score among methods applicable to large tuna (152). This is because all fish slaughtered with this method are exposed to severe crowding and high rate of mis-shots. This method had also a maximum magnitude of the adverse effect (142).

Variability and uncertainty

Considerable variability was not seen around any of the identified hazards.

From the scoring of uncertainty of intensity and duration it can be judged that for tuna, there was good agreement between the experts about the adverse effects of welfare hazards. However, this knowledge is essentially based on practical experience and expert opinion and not on published papers. Most of the hazards had an uncertainty score of 1.

The question of significant amounts of blood in the water at the time of killing was discussed in terms of welfare and whether it is aversive. Blood is known to have a strong positive effect on the appetite of carnivorous fish such as tuna. However, the presence of blood may be part of an overall deterioration in water quality, although this is difficult to evaluate. It is also possible that fish emit pheromones at the time of the killing that may contribute to distress and fear. The lack of information on this question renders the scoring difficult. Moreover, it was agreed that exposure to bloody waters would be similar in all killing methods (with possible exception for hoisting and spiking on-board).

It is important that persons involved in slaughter of the tuna be trained and skilled in fish handling and welfare, as well as specifically in the handling of tuna. It is also noted that monitoring programmes of slaughter practices would provide valuable data for future risk assessment.

Table 4: Risk and magnitude scores for welfare hazards associated with slaughter of large tuna by shooting from above.

Hazard ID	Hazards	Description of adverse effects	Risk score	Magnitude
Shooting from above the surface			152	
1	Medium density crowding	Some fish being caught in the nets, distress.	67	67
2	High density crowding	More fish caught in the nets, struggling and distress, hypoxia.	75	75
3	Shooting:, 1. time, successful		-	-
4	Shooting:, 1. time, not, successful	Fish probably stunned but may suffer from injury.	10	100
5	Spiking		-	-
Lupara with no back-up diver			89.25	
1	Low density crowding	Few fish being caught in the nets, distress.	30	33.3
2	Die due to asphyxia	Distress and hypoxia until they die or are killed	8	75
3	Medium density crowding	Some fish caught in the nets, struggling and distress, hypoxia.	50	50
4	Shooting: 1. time, successful		-	-
5	Shooting: 1. time, not successful	Fish probably stunned but may suffer from injury.	0.25	25
6	Tied	Distress if conscious.	0.5	50
7	Hoisting on board	Distress if conscious.	0.5	50
8	Bleeding	Distress if conscious.	0.5	50
9	Coring		-	-
Lupara with back-up diver			91.75	
1	Low density crowding	Few fish being caught in the nets, distress.	24.75	25
2	Die due to asphyxia	Distress and hypoxia until they die or are killed	0.5	50
3	Medium density crowding	Some fish caught in the nets, struggling and distress, hypoxia.	66	66.7
4	Shooting: 1. time, successful		-	-
5	Shooting: 1. time, not successful	Fish probably stunned but may suffer from injury.	0.5	50
6	Shooting: 2. Time		-	-
Gaffing and coring			143.2	
1	Medium density crowding	Some fish caught in the nets, struggling and distress, hypoxia.	66.7	66.7
2	Gaffing, 1. time, successful	Distress and pain, possible injuries	73.5	75
3	Gaffing, 1. time, not successful	Distress and pain, possible injuries	1.5	75
4	Gaffing, 2. time	Distress and pain, possible injuries	1.5	75
5	Coring		-	-

Spiking under the water			75.25	
1	Medium density crowding	Some fish caught in the nets, struggling and distress, hypoxia.	75	75
2	Spiking, 1. time, successful		-	-
3	Spiking, 1. time, not successful	Fish probably stunned but may suffer from injury.	0.25	25
4	Spiking, 2. time		-	-
Hoisting and spiking on board			225	
1	Medium density crowding	Some fish caught in the nets, struggling and distress, hypoxia.	50	50
2	Holding fish	Distress and pain rough handling.	50	50
3	Hoisting	Distress and pain, possible injuries	75	75
4	Bleeding	Distress and pain	50	50
5	Coring		-	-

CONCLUSIONS

1. Methods for slaughtering tuna are planned to minimise stress as the quality of meat, and subsequent price on the market, are correlated.
2. No stunning methods are commercially applied to farmed Atlantic bluefin tuna, only methods that kill the fish.
3. The choice of slaughter method to be used is based on the size of the tuna and their market destination, and country based specifications.
4. The three killing methods practised currently by the tuna industry in the EU: 1) underwater shooting (lupara), 2) shooting from the surface with a shot-gun, and 3) coring or spiking. These three methods gave rise to six different slaughter scenarios.
5. The most important hazards during the pre-slaughter phase are associated with crowding of tuna.
6. Based on the risk assessment, underwater shooting (lupara) had the lowest level of suffering for large tuna compared with shooting them from the surface. However, lupara mis-shots are not uncommon (1-5%) but good data for the time taken for a second effective shot are not available.
7. Coring and spiking methods in shallow water caused the lowest level of suffering for the slaughter of small tuna but they could be improved.
8. Gaffing and hoisting the tuna on-board involve severe pain and distress and lead to very poor welfare.
9. To the experts' knowledge depopulation for disease control has not occurred. If a disease outbreak would require culling tuna on a farm, there is no obvious method of choice and appropriate methods for emergency killing on farm need to be developed.
10. There are currently no recognised standard operating procedures available to help prevent impaired welfare for tuna.

RECOMMENDATIONS

1. Standard operating procedures to improve the control of the slaughter process to prevent impaired welfare should be introduced and validated; relevant practical welfare indicators developed.
2. Methods that do not involve gaffing of live conscious tuna should be developed.
3. When lupara is used a back-up diver should be present in case a second shot is needed.
4. 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, for determining improvements over time, and also for benchmarking for those involved in the slaughter of tuna.
5. Valid, robust and practically feasible indicators to evaluate the welfare of tuna during slaughter procedures need to be developed.

6. Methods used in other fish species other than those described in this Opinion may also be applicable to tuna. The opportunity to develop new methods for slaughtering tuna is considerable and should be encouraged.
7. Since the welfare of all farmed fish species studied has been found to be poor when they are killed by being left in air (asphyxia) it should generally not be used.
8. Persons involved in killing of tuna fish should be trained and hence skilled in handling and welfare.
9. Methods applicable to emergency killing of tuna for disease control purpose need to be developed and assessed for their welfare implications.

RECOMMENDATIONS FOR FURTHER RESEARCH

1. Procedures for emergency slaughter of tuna are needed.

2. Domestication of the species has not been achieved; and research on tuna reproduction, physiology and husbandry is needed to develop an aquaculture industry independent of the wild population.

3. Alternative handling techniques that avoid gaffing to move tuna from the water to on-board boat are needed.

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GLOSSARY

Adverse effect	The welfare consequences for an animal in terms of pain and distress when exposed to a hazard.
Almadraba	Spanish word to designate an elaborated and aged-old technique to catch tuna by setting nets in a maze that leads to a central pool called "copo". In Sicily, the method is called *tonnara*
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.
Fork length	A way of measuring some fish, considering the distance between the tip of the jaw or tip of the snout (with closed mouth) to the centre of the fork tail.
Gaffing	A gaff is a large iron hook attached to a pole or handle and used to drag tuna e.g. by the operculum on board a boat.
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
Iki jime	A method for killing tuna destroying the brain
Killing	Any intentionally induced process that causes the death of an animal.
Lairage	Short-term storage of fish in a tank or other facility before slaughter. Fish may be subjected to high stocking densities or materials for short periods.
Lairaging	Holding (keeping) before slaughter.
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.
Pithing	A metal wire inserted into the hole in the skull made by coring and pushed into the spinal cord with the objective of destroying both brain and spinal cord.

Pre-slaughter	Anything happening just before stunning, killing or slaughter.
Ram ventilation	Production of respiratory flow in some fish species. The mouth is opened during swimming and water flows through the mouth and across the gills. In ram ventilator fish, perpetual swimming is required to maintain ventilation.
Related operations	Operations such as handling, lairaging, restraining, stunning and bleeding of animals taking place in the context of slaughter and at the location where they are to be killed.
Restraint	A procedure designed to restrict fish movements in order to facilitate effective slaughter.
Rete-mirabile	A complex of arteries and veins lying very close to each other, found in some vertebrates. The rete mirabile utilizes counter-current blood flow within the net (blood flowing in opposite directions) that facilitates an exchange of heat, ions, or gases between the vessel walls so that the two bloodstreams within the rete to maintain a gradient with respect to temperature, or concentration of gases or solutes.
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 magnitude 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.
Tonnara	Italian word to designate an elaborated and aged-old technique to catch tuna by setting nets in a maze that leads to a central pool called “copo”. In Spain, the method is called *Almadraba*
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.

APPENDIX I: BIOLOGY MIGRATION, FISHERIES AND CAPTURE BASED AQUACULTURE OF TUNA

All tuna species are excellent swimmers and their bodies are designed for high performance at both sustainable and burst swimming speeds (Altringham and Shadwich, 2001). Their swimming performance, high metabolic and other physiological function rates, cardiovascular system and capacity to conserve metabolic heat in their red muscle and other essential organs distinguish them from almost all other fish species (Graham and Dickson, 2004). The tuna heart is approximately 10 times the size of other fish species, relative to their body weight, and their blood pressure and pumping rate about three times higher. One of their most amazing adaptations is their exceptional capacity for heat retention and partial endothermy. This is achieved by the "*rete mirabile*", a counter-current heat exchange system in the blood circulation, which allows retention of metabolic heat in the swimming muscles, viscera, brain and eyes (Graham and Dickson, 2001). These specializations enable some tuna, such as the Atlantic (or Northern) bluefin tuna (*Thunnus thynnus*) to maintain sustained, fast swimming and to exhibit an amazing thermal tolerance (~1.8 to 30 C) (Block and Stevens, 2001; Marcinek *et al.*, 2001; Kitagawa *et al.*, 2007), reflecting its ability to expand into high latitude, sub-polar waters, as well as great ocean depths (Graham and Dickson, 2004; Block *et al.*, 2005).

Sexual maturity in Atlantic bluefin tuna is achieved at 3-5 years-of-age in the Eastern Atlantic stock (Abascal *et al.*, 2004; Corriero *et al.*, 2005), much earlier than in the Western Atlantic stock, which occurs at 5-8 years-of-age (Baglin, 1981; Schaefer, 2001). The median size at sexual maturity of females of the Eastern Atlantic and Mediterranean Sea stock is 103.6 cm in fork length, whereas 100% of the fish are mature above 135 cm (Corriero *et al.*, 2005), which corresponds to a body weight of 30-35 Kg. Spawning of Atlantic bluefin tuna of the Western Atlantic stock takes place in the Gulf of Mexico, from April to June (Teo *et al.*, 2007), whereas spawning of the Eastern Atlantic and Mediterranean Sea stock takes place in the Mediterranean Sea from May to July (Corriero *et al.*, 2003; Karakulak *et al.*, 2004). In the Mediterranean Sea, the major spawning areas have been identified around the Balearic Islands (Spain), Malta, the South Tyrhenian Sea (Italy) and the Levantine Sea between Turkey and Cyprus (Nishida *et al.*, 1997; Medina *et al.*, 2002; Corriero *et al.*, 2003; Karakulak *et al.*, 2004; Heinisch *et al.*, 2008). Bluefin tuna have an asynchronous ovarian development (Corriero *et al.*, 2003 & 2007) and spawn multiple times during the reproductive season. The spawning frequency has been estimated to be 1.2 days (*i.e.*, each female spawns every 1.2 days, on average) and producing a relative batch fecundity of ~100,000 kg^{-1} body weight (Medina *et al.*, 2002). Spawning takes place at the water surface in the early evening hours at water temperatures >23 C and the pelagophil eggs hatch in about 28 h. The hatched larvae feed on zooplankton, small crustaceans, as well as on larvae from other fishes, molluscs and jellyfish. The larvae grow at a very fast rate and become juveniles within 30 d after hatching. Juveniles and adults are top predators feeding on pelagic fishes and squid. By the end of the first year, bluefin tuna can weigh 5 kg while their lifespan is about 15 years, with a maximum of 20 years.

The Atlantic bluefin tuna is the species of the highest commercial interest for fisheries. This species is supporting the capture-based tuna aquaculture (or fattening) in the Mediterranean Sea. It is one of three species of bluefin tuna, the other found in the Pacific Ocean (Pacific bluefin, *Thunnus orientalis*) and in the Southern Seas (Southern bluefin tuna, *Thunnus maccoyi*). The Atlantic bluefin tuna is one of the most wide-ranging of fishes, and can be found in the Western Atlantic, from Labrador (Canada) to the coasts of Brazil and in the

Eastern Atlantic from the Lofoten Islands (North of Norway) to the Canary Islands (Spain), as well as throughout the Mediterranean Sea (ICCAT, 2008). The Atlantic bluefin tuna is one of the largest fish species, reaching a body size of up to 700 kg, can swim at speeds of 90 km per hour and can travel on long trans-oceanic migrations (Cort and Liorzou, 1991; Safina, 1995).

The Atlantic bluefin tuna migrate seasonally over long distances between the temperate waters of the Atlantic Ocean where they feed, and the warmer subtropical waters of the Gulf of Mexico and the Mediterranean Sea, where they spawn. Based on their separate spawning areas, the Western Atlantic and the Eastern Atlantic and Mediterranean Sea stocks are considered different stocks by the International Commission for the Conservation of Atlantic Tuna (ICCAT), the international body responsible for the management of and allocation of fishing quotas for the Atlantic bluefin tuna (Schaefer, 2001; Block *et al.*, 2005; ICCAT, 2005). The two stocks established in 1982 by ICCAT are separated by the 45W meridian and are managed independently. As a result of severe over-fishing, restrictive catch limits have been in place since 1982 in the Western Atlantic stock and since 1998 in the Eastern Atlantic and Mediterranean Sea stocks. As of 2004, the Eastern Atlantic and Mediterranean Sea stock accounts for 95% of the global catch (FAO, 2006). Currently, the annual catches in the Mediterranean Sea are estimated to be around 43,000 metric tons per year, although this number exceeds greatly the one reported officially (ICCAT, 2008). Based on the last ICCAT meeting, the quota for 2009 was reduced to 22,000 metric tons (ICCAT 2008) in an effort to reduce the pressure on the wild population and allow recovery of stocks. Development of an aquaculture alternative would be opportune.

Fishing of Atlantic bluefin tuna is done using hand trawling, baitboat, longlines, set traps ("Almadrabas", Spain; "Tonnaras", Italy) and purse seines (ICCAT, 2008). In the Eastern Atlantic and Mediterranean Sea, the major fishing gear for Atlantic bluefin tuna has traditionally been the set traps. However, since the mid-1990s purse seines have become the most prevalent fishing method by far (85% of reported catches), since this method captures the fish alive and supplies the tuna fattening industry (Ottolenghi, 2008). Hand trawling and longlines are allowed to operate throughout the year, whereas set traps are operating during the reproductive migration period (April-September). Purse seines are restricted during the reproductive season (May to 15 June) for fish >130 cm fork length (30 kg body weight – see Figure 4), which are the reproductively mature fish supporting the tuna fattening industry throughout the Mediterranean Sea, with the exception of Croatia. Croatian tuna farmers are the only ones allowed to harvest fish that are smaller than the above limit (but not more than7% can be less than 8 Kg), and their fishing activities extend from 15 April to 15 June.

During their spawning migrations in the Mediterranean Sea, Atlantic bluefin tuna form large schools, which can be detected by their swimming and feeding activities on the surface. Schools may be homogeneous or heterogeneous regarding age and body sizes (Ottolenghi, 2008). In addition, young tuna schools are often associated with smaller tunids, such as skipjack (*Katsuwonis pelamis*) or bonito (*Sarda sarda*). The purse seiners used for the Atlantic bluefin tuna in the Mediterranean Sea are highly efficient, modern fishing vessels equipped with fish-finding sonars and often supported by spotter airplanes (though now prohibited by ICCAT). Once a tuna school is located, a long (>1 km) and deep (200 m) seine is deployed from the mother ship by an accessory boat, which encircles the fish. Along the bottom of the net there are metal rings connected with a cable, which once tensioned from the mother ship close the bottom of the seine creating a "purse" where the fish are trapped. In the past, the fish would have been slaughtered on the spot and transferred to processing-freezing ships for transportation to the markets. One important problem created by the fact that fish are not immediately harvested, but instead are transferred to the fattening industry, is that there is no effective method to determine the fish biomass or size- and age-structure of the population captured by the purse seines. As a result, it has become exceedingly difficult to obtain the necessary data for appropriate stock assessment analysis to be undertaken (FAO, 2005; ICCAT, 2008). Furthermore, as the growth in body weight attained in culture can not be ascertained, the calculation of annual fishery catches based on the reported biomass of tuna harvested by the fattening industry is not considered reliable.

Farm fattening systems for tuna

The bluefin tuna culture is basically an attempt to increase the amount of fat in the fish meat by feeding tuna caught in the wild and placed into floating cages during a limited period of time.

Tuna fattening is a seasonal activity and it involves the capture of fish from the wild and their rearing in sea cages for periods ranging between 3 months to 2 years. Rearing is done to achieve (a) a greater fat percentage in the muscle, which is desirable by the sushi and sashimi markets in Japan, (b) a better price by not flooding the market in the brief fishing period of June-July and (c) a certain amount of growth over the captured fish.

Two very different tuna fattening approaches are practised today. The historically first one, starting in the early 1990s, is the smallest activity in terms of fish biomass and ICCAT allows this only for Croatia. In this industry, the Atlantic bluefin tuna captured are immature (8-20 kg in body weight below 130 cm in fork length – see Annex II). These juvenile tuna are reared in captivity for up to 2 years reaching a harvest size between 30 and 50 kg. At this size they do not obtain the highest price in the Japanese market, which favours fish around 150 kg in body weight, but maintaining the fish longer increases the risk and is not practised. The second tuna fattening approach is followed by all other countries in the Mediterranean Sea and it accounts for the vast majority of the production. In this industry, the migrating mature spawners (>30 kg in body weight and 130 cm in fork length) are captured and reared in sea cages for periods not exceeding 7 months, with the majority of fish harvested in November-December. Nowadays tuna is caught with purse seine (Miyake *et al.*, 2003) in the spawning season when they join together in some areas in the Mediterranean, having special characteristics of temperature, salinity, food.

As mentioned above, the vast majority of Atlantic bluefin tuna captured today by the purse seine fleets is destined for the fattening industry. After capture, a transport cage (30 to 50 m in diameter, 20 m deep) is placed near the purse seine and it is connected with the purse seine by sewing the two nets together. An opening is created and the fish are herded from the purse seine into the towing cage. The transport cage is then towed by a tug-boat at a speed of 1-1.5 knots to the fattening site, which may take between days to weeks. During this long towing period, the fish are fed using frozen fish such as Pacific mackerel, Atlantic mackerel, sardine and herring. Less is known about the mortality during the towing. According to Norita (2003) due to the technical improvement of growing tuna mortality at towing from the fishing ground to the rearing facilities decreased from 20.8% in 1995 to 3.9% in 2000 in Spain. Oray and Karakulak (2003) have reported about 10% in Turkey. In Croatia during cage transfer from fishing ground to the farm, mortalities are around 10-15%.

Upon arrival to the fattening site, the transport cage is placed next to the rearing cage and the fish are transferred inside it in a similar way used earlier for their transfer from the purse seine into the transport cage. The tuna are placed in their definitive fattening units, usually cages of 50 – 120 m of diameter and 25 - 35 m depth (Belmonte y de la Gándara, 2008), but also rectangular (Hattour, 2005). After the towing and because of the transport stress, it is usually necessary a period of 10-15 days to calm the fish down, before starting the feeding

(Hattour, 2005). According to Farwell (2003), the density of tuna inside the cage ranges between 5 and 6.2 Kg/m^3 but usually is lower.

When tuna reproduce, they lose a considerable amount of body weight, and this influences the marketable quality of their flesh. To increase this amount of fat tuna are fed with low price small pelagic fish with high quantities of fat (Belmonte *et al.*, 2007). Bluefin tuna are fed mainly with a mixed diet (Vita *et al.*, 2004) composed principally of a variety of small pelagic species (mainly defrosted baitfish, Belmonte *et al.*, 2007) including sardine, pilchard, round sardinella, herring, mackerel, horse mackerel, chub mackerel, bogue and squid. The proportion and volume of the feed varies among the different countries and form farm to farm, with feed composition depending on the availability of the species generally used. Tuna are generally fed 1-3 times a day with a mixture of defrost baitfish (Ottolenghi, 2008). In most countries, a scuba diver remains in the cage during feeding and signals to stop when tuna are satisfied. Another feeding system consists in offering a daily feed input of 2-10 % of the estimated tuna biomass, depending on the water temperature and the fish size composition of the cage.

Without accurate initial length or weight measurements of the fish during cage farming, growth and feed conversion rates (FCR) are only estimates. FCR are usually high, around 15-20:1 (Farwell, 2003; Katavic *et al.*, 2003). For large specimens is sometimes beyond 40:1 and 10-15:1 for smaller fish. Actually the highest FCRs are due to the fact that big tuna practically do not grow; but just increase their body fat. After 8 months of rearing, tuna show a weight increase of 40-50% for smaller fish and 10-30% for the larger ones (Norita, 2003). The daily feeding rate of defrosted fish is approximately 5 - 10% of body biomass (Farwell, 2003; Ottolenghi *et al.*, 2004). Higher feeding rates occurred in summer 1.5 months after stocking (Norita, 2003). It seems that overfeeding is a common practice, obviously because the feeding is at satiety (Aguado and García-García, 2005). At the end of the fattening period, weight of the fish increased by 25 - 35% (Percin and Konyalioglu, 2008).

In general the food is distributed by means of a hose attached to the board of the boat and directed to the centre of the cage. With pumping water, the bait fish on the deck is carried to the cage through the inside of the hose. Tuna have a hierarchic behaviour; so that the biggest ones eat first while the smaller ones wait at the bottom of the cage until the others lose their interest for the food and they can start to eat (Belmonte y de la Gándara, 2008). Another feeding system consists in offering a daily feed input of 2-10 % of the estimated tuna biomass, depending on the water temperature and the fish size composition of the cage (Ottolenghi, 2008).

Katavic (2003) reported that mortality ratio of tuna in farms is about 15 - 20% per fattening season, while during the adaptation period, the stress mortality is 2/3 of the total mortality ratio. Nevertheless (Norita, 2003) pointed out that from 1995 to 2000, the mortality rate of tuna reared in Tuna Farm of Mediterráneo S.L. in Spain, was reduced from 15.8 to 3.7%. Nowadays in Croatia, the same way as in Spain, mortalities during the fattening process are almost negligible. According to the working group experts, mortality can range from 0 to around 5% depending on the farm, season, weather, etc… This is in case when no sharp environmental changes take place. Sometimes and because of accident or natural catastrophes, mortality can be quite higher than that, leading to lose the total of stock.

The average price of fattened tuna in Japan is around 36 Euro/kg, but when the market demand is high and tuna meat is of high quality, the price can occasionally reach 120 Euro/kg (Aguado *et al.*, 2004). Less stressed tuna achieve better prices, confirming the importance of killing tuna correctly both for ethic reasons and to preserve the quality of the flesh (Ugolini *et al.*, 2005).

Croatia. Croatian companies start stocking tuna in May or June but the season lasts until late summer (September). Croatians start their farming with small specimens weighting around 30-50 kg. They use small cages and keep the specimens for a long period.

Mortalities during the fattening process in Croatia are almost negligible, while during cage transfer from fishing ground to the farm, they are around 10-15%. This is the case when no sharp environmental changes take place.

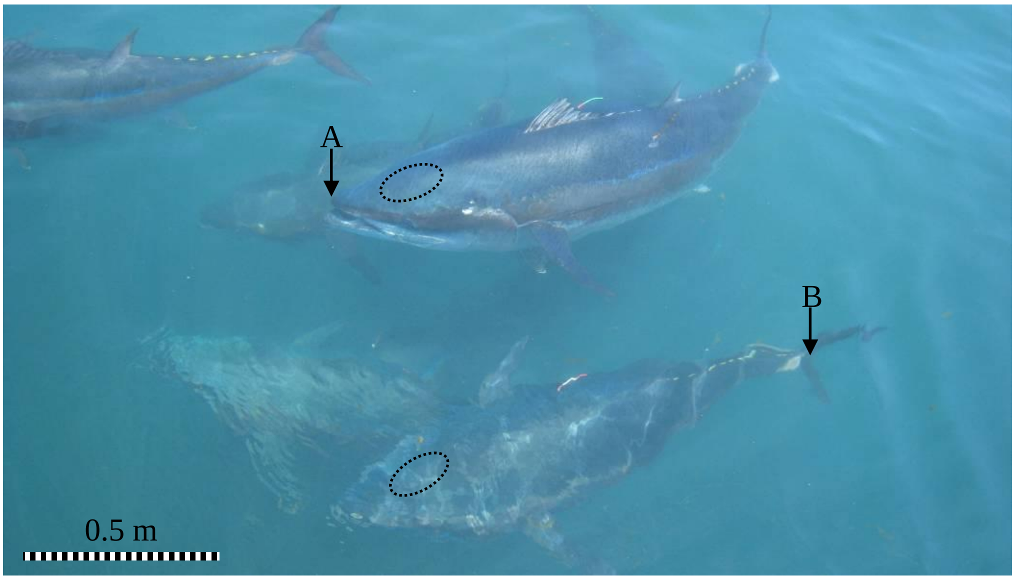
Rest of Mediterranean. The season to start stocking tuna begins usually on May or June and lasts for about 2 months. Generally the weight of the stocked tuna is between 150-200 kg, but countries as Italy, Malta and Spain may even stock big tuna weighting as much as 600 kg. The size of the cages is bigger than the ones used in Croatia, and the time of farming is shorter.

In the Mediterranean there are currently 68 farming facilities for Bluefin tuna culture: 8 are Croatian, 3 Cypriots, 14 Spanish, 2 Greeks, 15 Italians, 8 Maltese, 1 Libyan, 4 Tunisians, and 13 Turkish (www.iccat.int/en/ffb.asp).

In most of Mediterranean countries, the season to start stocking tuna begins usually on May - June and lasts for about 6 - 8 months. Generally the weight of the stocked tuna is between 150-200 kg, but countries as Italy, Malta and Spain may even stock giant tuna weighting as much as 600 kg (Ottolenghi *et al.*, 2008). We shouldn’t forget that usually the big tuna higher than 150 kg obtain the highest prices in Japan. Because of the overfishing of the wild stock normally the mean size of tuna are decreasing year by year.

In Spain and in most of the Mediterranean countries (Peric, 2003; Oray and Karakulak, 2003), tuna are slaughtered from November to January, depending of market demands, and in end of January all the facilities are emptied waiting for the new fattening season in May-June (Belmonte *et al.*, 2007).

APPENDIX II: PHOTOGRAPHIC ILLUSTRATIONS



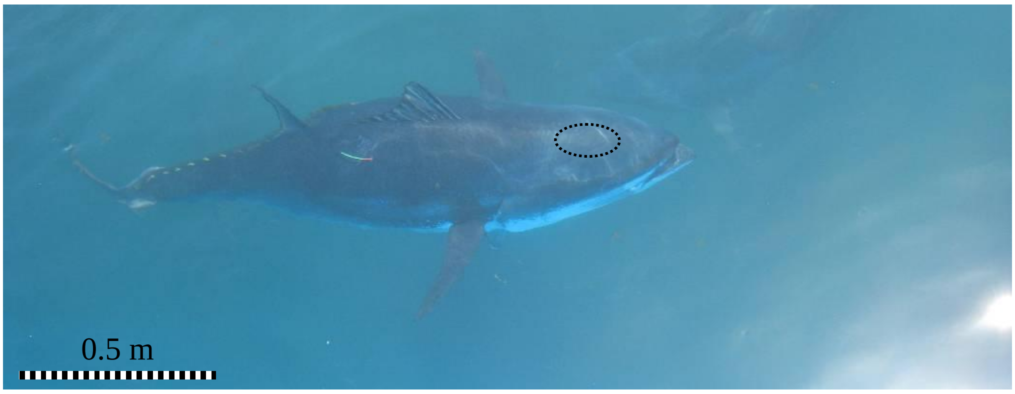


Figure 4: Length measures and brain area in tuna. Points A and B illustrate the points for measuring fork length. The ellipse on the head of the tuna is the target to hit the brain with the power head or with the shoot gun

Figure 5: Shooting underwater using a power head (Lupara). A) Cartridge barrel of the lupara. B) Details of the single shot cartridge; C) Diver using the lupara

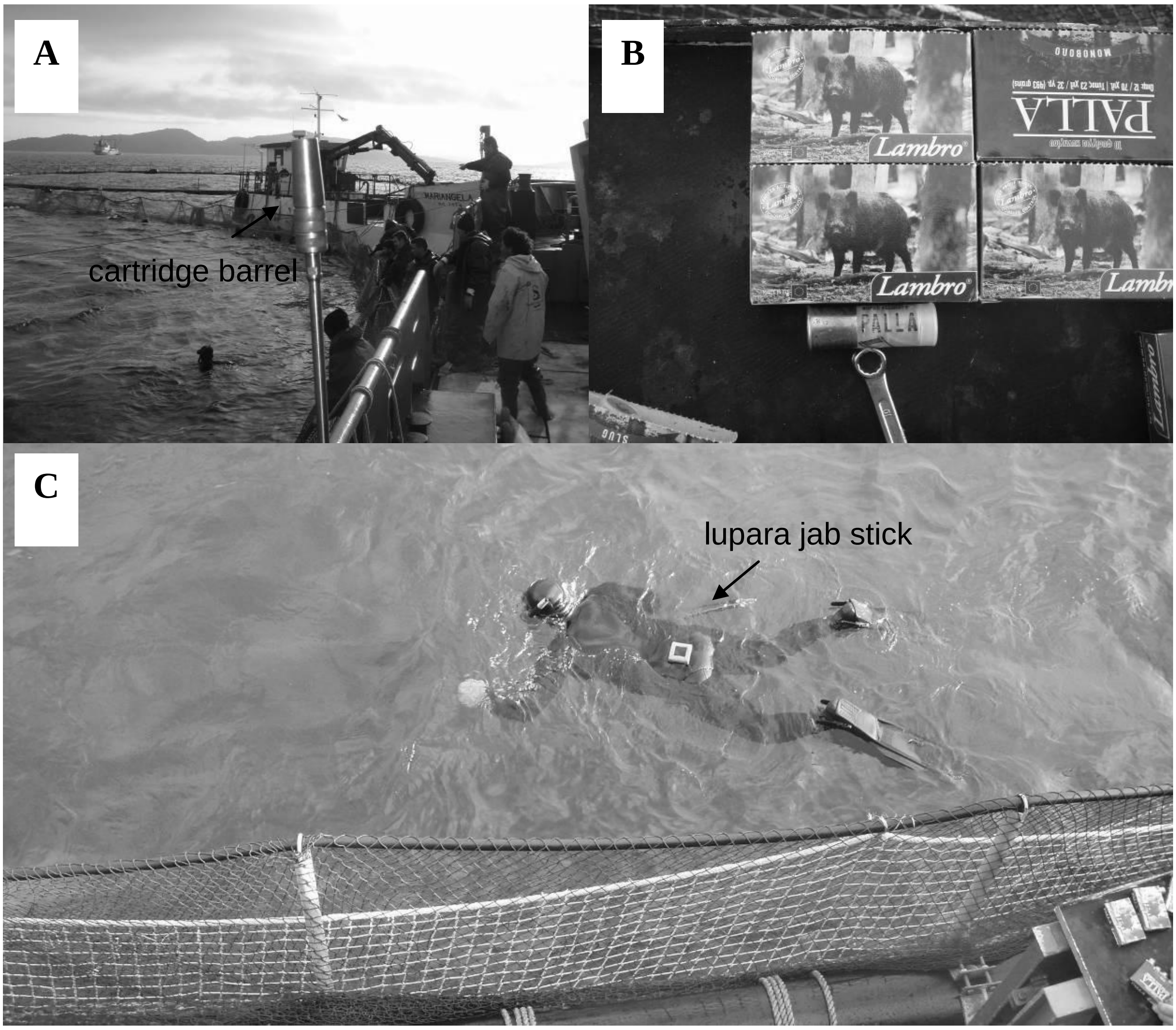
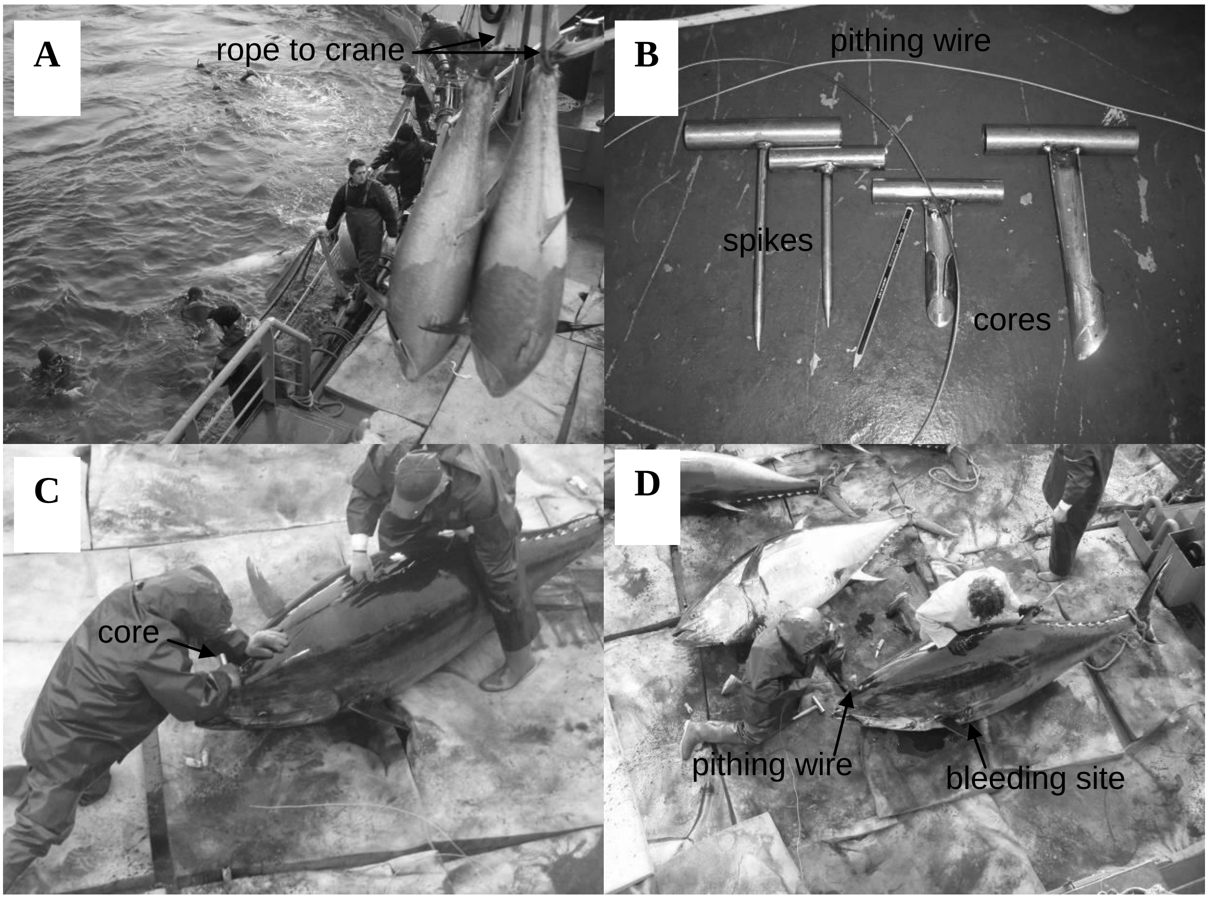


Figure 6: Coring after gasping and hoisting tuna on board. A), Hoisting the tuna with a crane; B) Spikes, cores and pithing wire; C) Coring and bleeding; D) Pithing with the wire



APPENDIX III: 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 and/or 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[3].

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

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

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

The general risk assessment is in line with the approach previously used in the EFSA welfare reports (EFSA, 2007a; EFSA, 2007b; EFSA 2007c; EFSA, 2008a; EFSA, 2008b; EFSA, 2008c; EFSA, 2008d; EFSA, 2008e) with some modifications according to the risk question posed.

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

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).

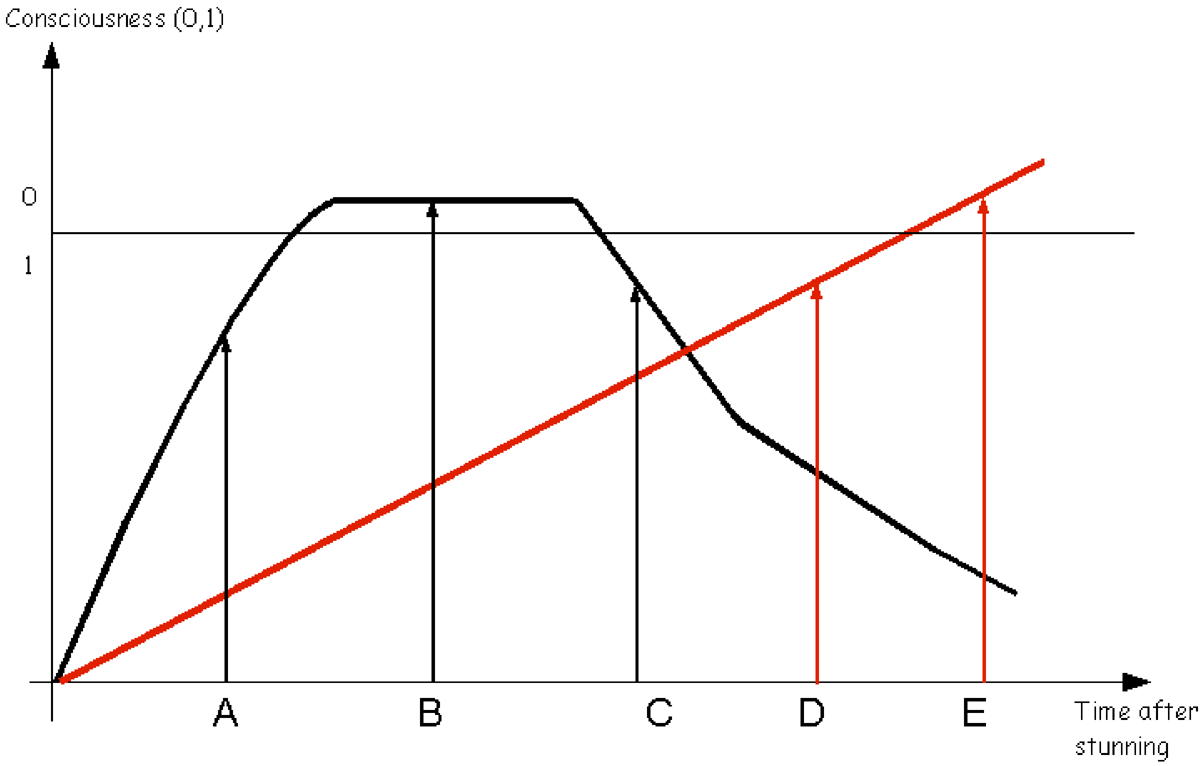


Figure 7 Time to unconsciousness (insensibility) following stunning / killing (horizontal grey line indicates consciousness threshold above which killing takes place without an adverse effect).

The 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

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.

Table 5: 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)

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.

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

The duration of the adverse effect

The time during which an animal will on average experience the adverse effect was estimated in minutes. The duration of an adverse effect can be longer than the duration of the hazard, for example a 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 2).

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 magnitude 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 6).

Table 6: 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 score**.**

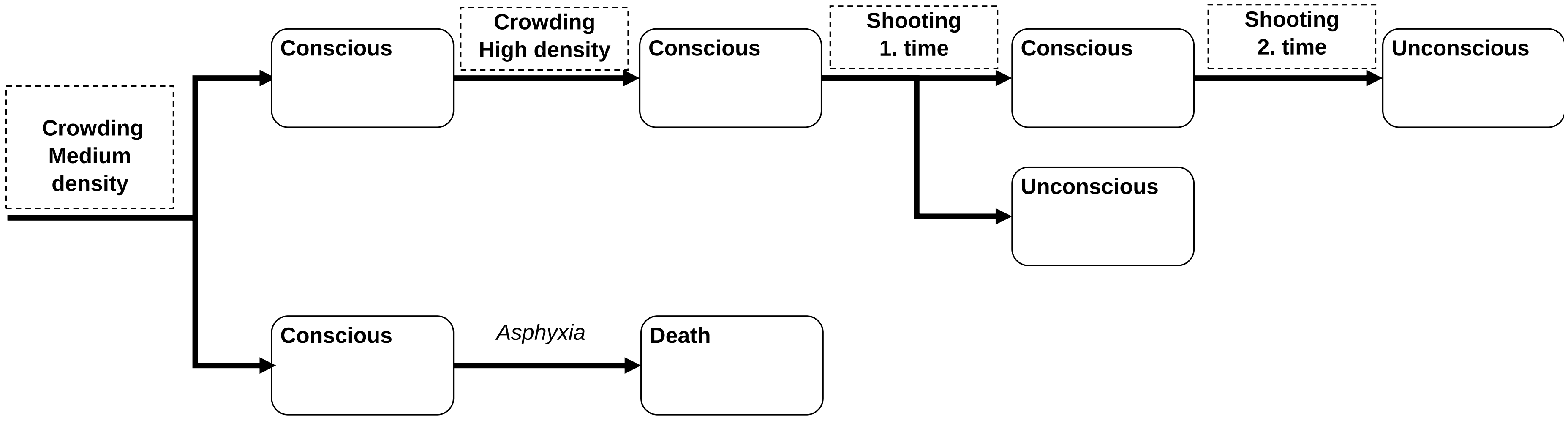
Interpretation of the risk score

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).

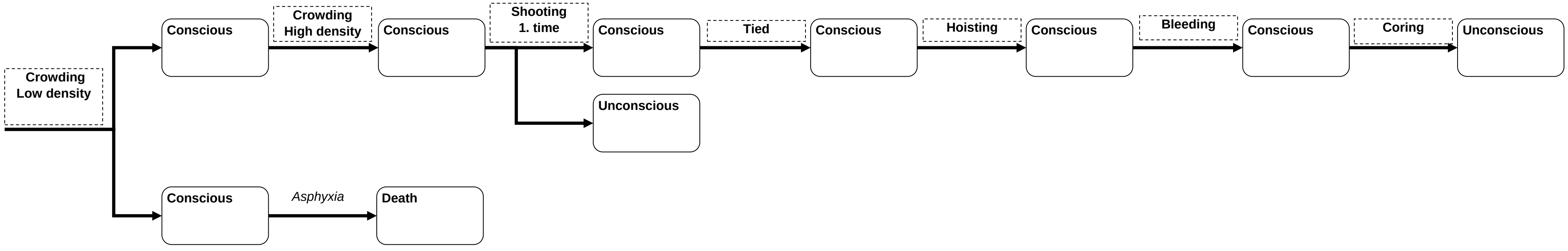
One key objective of this work is to compare different methods of stunning and slaughter within each species. This will be achieved by summing the risk scores for all the hazards arising for each method of stunning and slaughter. This figure will be used to rank and compare the methods. Risk scores are given for the commonly used methods. 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 IV: DETAILED PATHWAYS FOR RISK ASSESSMENT OF TUNA SLAUGHTER

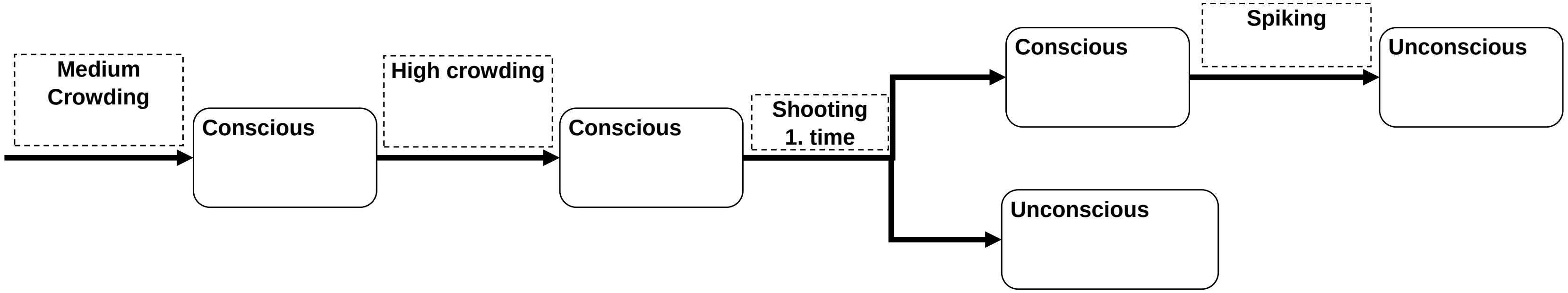
Lupara variant 1, large tuna



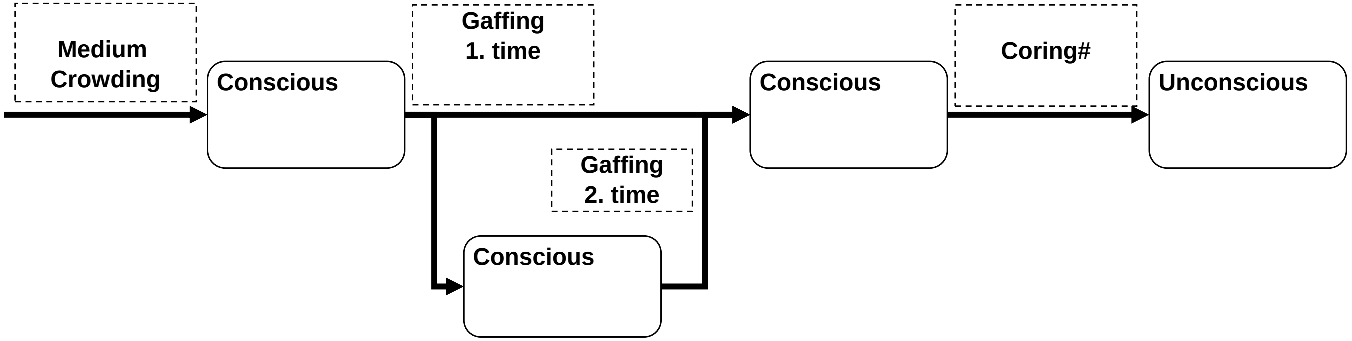
Lupara variant 2, large tuna fish



Shooting from above the surface, large tuna

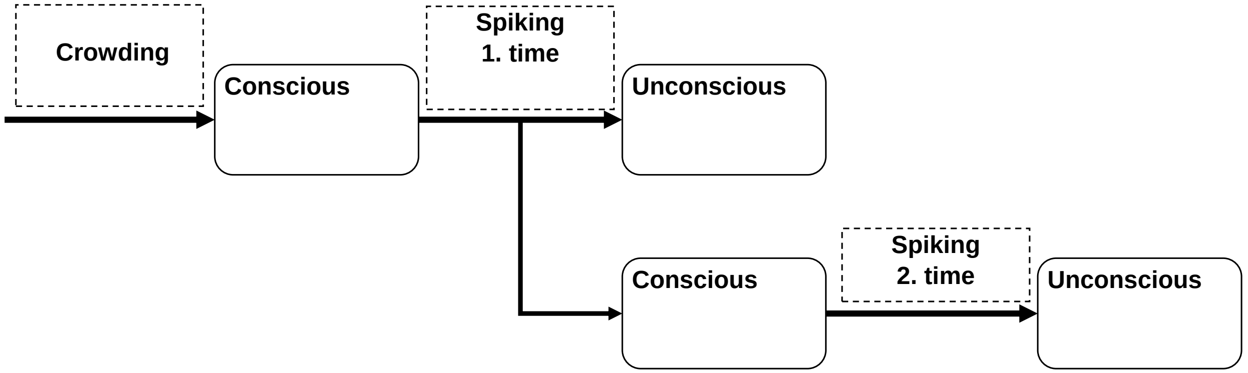


Gaffing and coring, small tuna

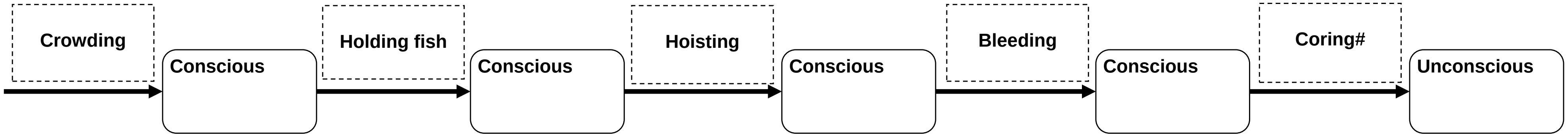


Note # 95% (90,98) of tuna fish are cored 1 time, 5% are cored 2. time. However, all tuna fish are unconscious after 1. coring

Spiking in the water, small tuna



Coring on board, small tuna



Note # 96% (92,98) of tuna fish are cored 1 time, 4% are cored 2. time. However, all tuna fish are unconscious after 1. coring

APPENDIX V: DETAILED TABLE FOR RISK ASSESSMENT OF TUNA SLAUGHTER

Table 7: Scoring for hazards associated with pre-slaughter and slaughter of tuna

Hazard	Probability			Intensity score	Duration Score	Uncertainty	Magnitude	Risk score		
	Min	ML	Max					Most likely	Lower	Upper
Shooting from above the surface, large tuna										
Medium density crowding		1.0		2	4	1	67	67	67	67
High density crowding		1.0		3	3		75	75	75	75
Shooting, 1. time	0.67	0.9	0.94	-	-		-	-	-	-
Shooting, failed	0.06	0.1[4]	0.33	3	4		100	10	6	33
Spiking		1.0		-	-		-	-	-	-
								152	148	175
Lupara (variant 2), large tuna										
Low density crowding	0.8	0.9	0.95	1	4	2	33.3	30	26.7	31.7
Asphyxia	0.05	0.1[5]	0.2	3	3		75	8	3.75	15
Moderate density crowding		1.0		2	3		50	50	50	50
Shooting, 1. time	0.97	0.99	1.0	-	-		-	-	-	-
Shooting, failed	0.0	0.01[6]	0.03	1	3		25	0.25	0	0.75
Tied		1.0		3	2		50	0.5	0.5	0.5
Hoisting on board		1.0		3	2		50	0.5	0.5	0.5

[4] Shooting is successful for 90% of the tuna. However, 10% of tuna fish are conscious after the first shot and a second shot is needed to obtain unconsciousness. It is assumed that the second shot is successful.
[5] The tuna die due to asphyxia
[6] Shooting is successful for 99% of the tuna. However, 1% of the tuna are still conscious when tied, hoisted on board and bled.

Hazard	Probability			Intensity score	Duration Score	Uncertainty	Magnitude	Risk score		
	Min	ML	Max					Most likely	Lower	Upper
Bleeding		1.0		3	2		50	0.5	0.5	0.5
Coring		1.0		-	-		-	-		-
								89.25	81.9	98.9
Lupara (variant 1), large tuna										
Low density crowding	0.96	0.99	1.0	1	3	1	25	24.75	24	25
	0	0.01	0.04[7]	3	2		50	0.5	0	2
Moderate density crowding		1.0		2	4		66.7	66	66	66
Shooting, 1. time	0.97	0.99	1.0	-	-		-	-	-	-
	0	0.01[8]	0.03	3	2		50	0.5	0	1.5
Shooting, 2. time		1.0		-	-		-	-	-	-
								91.75	90	94.5
Small tuna, gaffing and coring										
Medium crowding		1		2	4	1	66.7	66.7	66.7	66.7
Gaffing, 1. time	0.95	0.98	1.0	3	3		75	73.5	71.25	75
	0.0	0.02[9]	0.05	3	3		75	1.5	0	3.75
Gaffing, 2. time		1.0		3	3		75	1.5	1.5	1.5
Coring		1.0		-	-		-	-	-	-
								143.2	139.4	146.9

[7] The tuna die due to asphyxia

[8] Shooting is successful for 99% of the tuna. However, 1% of tuna fish are conscious after the first shot and a second shot is needed to obtain unconsciousness. It is assumed that the second shot is successful.

[9] 2% of tuna are mis-gaffed and gaffing for the second time is needed. It is assumed that the second gaffing is successful.

Hazard	Probability			Intensity score	Duration Score	Uncertainty	Magnitude	Risk score		
	Min	ML	Max					Most likely	Lower	Upper
Small tuna, spiking under the water										
Crowding		1.0		3	3	1	75	75	75	75
Spiking, 1. time	0.9	0.99	1.0	-	-		-	-	-	-
	0.0	0.01[10]	0.1	3	1		25	0.25	0	2.5
Spiking, 2. time		1.0		-	-		-	-	-	-
								75.25	75	77.5
Small tuna, hoisting and coring on board										
Crowding		1.0		2	3	2	50	50	50	50
Holding fish		1.0		3	2		50	50	50	50
Hoisting		1.0		3	3		75	75	75	75
Bleeding		1.0		3	2		50	50	50	50
Coring		1.0		-	-		-	-	-	-
								225	225	225

[10] Spiking is successful for 99% of the tuna. However, 1% of the tuna are conscious after the first spike. A second spike is needed to obtain unconsciousness. It is assumed that the second spiking is successful.

APPENDIX VI: QUESTIONNAIRE ON METHODS USED FOR SLAUGHTER OF TUNA IN EUROPE

The objective of this questionnaire was to collect data on methods used for farmed tuna slaughter in Europe under the scope of the mandate received from the European Commission. The questionnaire was distributed to EFSA's Focal Points network and the Stakeholders.

1. What is the % of fish killed with the following method?

A) Large fish (>80 Kg)?

1.	Lupara	__%
2.	Shot from outside the water	__%
3.	Spike in the head	__%
		100%

B) Small fish (<80 Kg)?

1.	Lupara	__%
2.	Shot from outside the water	__%
3.	Spike in the head	__%
		100%

NB: The spike can be solid if used in the water or hollow (*i.e.* the corer used to drill the fish skull in order to open a duct and insert the wire/rod used to destroy the spinal cord) if used outside the water and on board the harvesting boat.

2. What % of the fish killed with a lupara underwater are shot

1. once __%
2. twice __%
3. three times ___%
4. more than three times ___%

100%

3. What % of the fish killed with a shotgun from above water are shot?

1. once __%
2. twice __%

100%

4. How long does it take between the first and second shots?

1. using the lupara __
2. using the shot from above the water __

5. When fish are killed with the lupara, bleeding is done

1. in the water before the fish are hoisted onto the boat ___ Yes __ _ No

2. after the fish are onboard ___ Yes ___ No

NB: Fish are killed and hoisted straight-away (within 5-10 sec) one by one, therefore the loss of blood in the water from the wound is negligible

6. When fish are killed with a shot from above the water, bleeding is done

1. in the water before the fish are hoisted onto the boat _ __ Yes ___ No

2. after the fish are onboard _ __ Yes ___ No

NB: with shot from above the water the fish are killed in groups and therefore fish remain dead in the water for few minutes and lose some blood in the water from their wound until they are hoisted.

7. Is a metal wire/rod used to destroy the spinal cord in fish killed with:

1. Lupara __ _ Yes ___ No

2. Shot from outside the water __ _ Yes ___ No

3. Spike in the head __ _ Yes ___ No

NB: Destruction of the spinal cord is important for quality reasons and must be operated immediately after killing of the fish irrespective of the method employed, in order to mainly avoid stagnation of blood in the blood vessels and fish flesh (it is found to promote effective bleeding which prevents spoilage).