SEACASE SUSTAINABLE EXTENSIVE AND SEMI-INTENSIVE COASTAL AQUACULTURE IN SOUTHERN EUROPE

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OPTIMISING FISH SLAUGHTERING PROCEDURES (TECHNICAL MANUAL)

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The deliverable 11 was produced at the mid-term of the project and consists in a technical manual describing the state of the art and main recommendations about slaughtering procedures in European fish farms. The main target of this manual is composed by European farmers.

Optimising Fish Slaughtering Procedures

(Technical Manual)

INDEX

Technical Manual on

FARMED FISH SLAUGHTERING AND WELFARE

1 - Introduction

1.1 General information

The aquaculture sector started growing since the past half-century (around 1970) and continues to grow more rapidly than all other animal food-producing sectors. (FAO, 2007a).

The impact on environment and animal welfare are recent concerns in the aquaculture field. Thus, issues in relation to fish welfare are increasingly coming into view on social and political agendas (Arlinghaus et al., 2007) as a consequence of public concern about how animals are raised and treated (Fraser, 1999, Huntingford et al., 2006). Consequently, discussions and literature concerning this subject are each day more common.

The most important point is that producers now know that adequate welfare procedures during the growing period and at the harvesting phase result in better final products and additionally a better acceptance by the consumer.

This manual has the objective of reviewing the slaughter methods, as well as factors (such as pre-harvesting and harvesting) that influence quality of the final product.

↓ stress, ↑ welfare, ↑ fish quality

1.2 Some useful definitions

1.2.1 Harvesting

From some of the most common definitions of *harvest* or *harvesting* in aquaculture, we can conclude that authors seem all to agree in a broad definition, starting with the catching of the animals but being extended until the final obtaining of a food product. Two examples are:

Harvest is the process in aquaculture whereby a living animal is processed into an edible product. (Lambooij et al., 2002).

Harvesting means those operations which start with taking the fish from the water and end with the transport of live or fresh fish for human consumption to the market (FAO, 1998).

1.2.2 Stunning

When employed before slaughter, *stunning* is defined as a technical process to induce immediate unconsciousness and insensibility in animals, so that slaughter can be performed without avoidable fear, anxiety, pain, suffering and distress (EFSA, 2004). Sometimes it is difficult to distinguish between stunning and slaughtering, as they can occur together or be mixed. For example, a stunning method like a blow in the head can be used to immobilize the animal and then it will be placed in ice, where it will die by lack of oxygen. But, if applied more violently, fish will die immediately and the same blow is not anymore considered as a stunning method, but as a killing method.

fish is alive → <u>fish is unconscious</u> → fish is dead

stunning killing

1.2.3 Slaughtering

Slaughtering is any procedure which causes the death of an animal, normally by bleeding (FAO, 2007b). In case of fish, the most used methods for slaughtering are stunning/killing processes that do not rely on bleeding to cause death. Fish are normally much smaller that terrestrial animals used in human food, in which bleeding is helped by the normal heart beating; bleeding small fish is rarely done, and the objective is different: mainly it is done to avoid blood staining of the fish white muscle that surrounds the column.

1.2.4 Stress

Stress is a condition in which an animal is unable to maintain a normal physiologic state because of various factors adversely affecting its well-being (Francis-Floyd, 2002)

Several factors can affect stress, some of them as indirect as the tank load or the incidence of light in the water. We are more concerned here with the direct factors affecting fish stress, as handling or time before stunning or death.

1.2.5 Welfare (well-being)

Animal welfare has mainly to do with the quality of life of animals (Braastad et al, 2006). But *welfare* is a concept difficult to define and to measure (Poli et al., 2005). Spruijt et al. (2001) suggested a definition: *welfare* is the balance between positive (reward, satisfaction) and negative (stress) experiences or affective states.

It is a controversial and an unresolved subject owing to non-human animals exposed to adverse experiences, such as physical injury or confinement experience (Huntingford et al., 2006), can experience the human feeling of suffer. Stress is common and normal in nature (in many situations, stress is higher in nature than in captivity; good examples are the danger of predation and the uncertainty of food, both essential to survival). Pain is also a very useful biological response, and the lack of pain feeling is a very severe illness. Finally, suffering is a mixture of psychological and physical sensations.

stress ≠ pain ≠ suffering

Five freedoms were defined by the Farm Animal Welfare Council in 1993 as a core framework to help evaluating the welfare of animals whether on farm, in transit, at market or at the place of slaughter (Webster, 2001; FAWC, 2005), and can be applied to aquatic animals as well (figure at next page).

These *five freedoms* do not pretend to avoid/eliminate stress in animals but to prevent their suffering (Webster, 2001).

THE ANIMAL FIVE FREEDOMS (ideal states, not standards)	
1) Freedom from hunger and thirst	Animals should have free access to fresh water and a diet to preserve full health and vigour;
2) Freedom from discomfort	Animals should have a suitable environment including shelter and a comfortable resting area either indoor or outdoor.
3) Freedom from pain, injury and disease	Prevention or rapid diagnosis and treatment should be provided in order to avoid pain, injury and disease of animals.
4) Freedom to express normal behaviour	Sufficient space, proper facilities and company of the animal's own kind should be provided to animals.
5) Freedom from fear and distress	Conditions and treatment should be ensured to avoid mental suffering (anxiety, fear, distress, boredom, sickness, pain, thirst, hunger and so should be minimized) of animals.

Note that, for example for freedom 1), aquaculture typical starving period before harvesting can be considered a period of stress, and is probably a source of some pain and suffering, but in nature also occurs, probably much longer than in farms. This doesn't mean they are desirable; they should be avoided anyway.

1.3 Common indicators used to measure efficacy and final results

industry purposes ← measuring welfare → science purposes

behaviour almost exclusively　　　　　　　　behaviour and many others

1.3.1 Behavioural indicators

Behavioural indicators are non-invasive indicators of fish welfare (FSBI, 2002; Poli et al., 2005) and they may provide a good macroscopic indication (Poli et al., 2005) of stress produced during growing, pre-slaughter handling and slaughter time. These indicators are more practical for use everyday in intensive farms since they are not time consuming and they do not need a complex technology as it is needed to gather data on fish physiology, biochemistry and behaviour for scientific research purposes (FSBI, 2002). Therefore, the most used behavioural indicators for stress at pre-slaughter and slaughter time are (Poli et al., 2005):

- *Swimming motility*;
- *Gill movement*;
- *Capability to maintain the equilibrium* when fish is turned upside down;
- *Movement of the eye*: eyes remain on the same plane as the fish is rotated;
- *Reaction to needle puncture* on the head or tail (pricking);
- *Handling along the lateral line*;
- *Electricity application of low voltage electricity*.

All these "indications" must be based on a previous knowledge about normal behaviour of each species and for specific conditions.

1.3.2 Brain function indicators

The brain function indicators are reliable indicators of the presence of fish sensibility and consciousness. The technologies used to measure the brain activity are electrocardiogram (ECG), electroencephalogram (EEG), visually evoked responses (VER) from photic stimulation, i.e., flashes of light directed to the eyes, and somatosensory evoked responses (Poli et al., 2005). These technologies are technically demanding and time consuming and EEG, ECR and VER are likewise invasive because of the need to implant electrodes (Lambooij et al., 2002; Poli et al., 2005) to perform measurements. For that reason, this technology is more appropriate to be used as a tool in studies done at laboratory conditions than in field and ship conditions (Kestin et al., 2002).

1.3.3 Haematic (physiological) indicators

Fish, as other vertebrates, have physiologic responses to “stress inducers”, “stressors” or factors that induce stress (e.g. handling and transport) to preserve its normal or homeostatic state, that is, to cope with the stress (Barton, 2002, Iwama et al, 2004). This response comprise a complex of physiologic and behavioural adaptive processes that contribute to maintain the specific ranges of temperature, pH and solute concentration so important to keep the normal physiological functions up (Poli et al., 2005).

As a primary effect (primary response) to respond against stress, fish has a neuroendocrine response that consists in releasing of catecholamines, mainly adrenaline and noradrenaline, from chromaffin tissue into the blood stream (Mazeaud et al., 1977; Wendalaar Bonga, 1997; Poli et al., 2005) as well as corticosteroids (cortisol) from the interregnal cells after stimulation of the hypothalamic-pituitary-interrenal (HPI) axis (Wendalaar Bonga, 1997; Mommsen et al., 1999). Afterwards, secondary response occurs caused directly or not by biochemical and physiological effect of the hormone actions firstly released into the blood circulation (Barton et al., 2002). As a result, there is an alteration in blood constituents such as increase of plasma glucose concentration (Mazeaud et al., 1977; Mommsen et al., 1999; Barton et al., 2002; Poli et al., 2005), changes in plasma lactate and ions concentration and a decrease or an increase of plasmatic free fatty acids, depending on the species (Mazeaud et al., 1977, Barton et al., 2002). Other changes observed (Poli et al., 2005) are an increase of heart-beat and of oxygen uptake that increase the hematocrit value. The raise of this value is due to an increase of the number of circulating erythrocytes.

In conclusion, levels of cortisol, glucose and related increase in plasma lactate can be used as stress indicators (Poli et al., 2005). Furthermore, hematrocrit raise is a good stress index as well as an easy test to process. It is also important to bear in mind that secondary response to pre-slaughter stress can not be achieved in case of rapid death and thereby it is vital to take in consideration the stress undergone at pre-slaughter when physiologic indicators are being evaluated (Poli et al., 2005).

1.3.4 Tissue indicators

Fish, after being slaughtered, will suffer a set of post mortem biochemical process that comprises anaerobic glycolysis and ATP degradation as key events (Haard, 2002; Poli et al., 2005). Those biochemical changes in the muscle are highly influenced by the antemortem history, such as species characteristics, exercise, diet and nutritive status, fasting and stress, of the fish and the method of slaughter prior to circulatory stoppage (temperature of the carcass, processing, handling) (Haard, 2002). Thus, fish freshness is likewise extremely related with pre-slaughter and slaughter management and indicators such as plasma cortisol, lactate and glucose might be very useful as tools to measure stress undergone by fish. Other indicators important as well are the tissue indicators that result from the biochemical and physical changes of muscle during *rigor mortis* process. These indicators can be divided in biochemical and physical indicators. The biochemical indicators can be grouped in three categories (Venugopal, 2006): a) protein changes such as viscosity and hydrophobicity; b) changes in enzymatic activity such as ATPase and c) accumulation of metabolic products such as TMA and free fatty acids. The physical ones are water holding capacity (WHC), texture measurement and muscular pH.

As it was mentioned before, all those tissue indicators are highly influenced by stress factors at pre-slaughter (e.g struggling) and slaughter time (e.g. method of killing). Muscle phosphocreatine, ATP/ADP/AMP and glycogen level can be used as early stress indices since they are considered energy fuel and they influence the rigor mortis (contractile process that occurs in post-mortem muscle) onset, i.e, their early lack are related to the early rigor mortis onset, thus, lack of ATP represents a very important macroscopic stress index (Poli et al., 2005).

Inosine monophosphate (IMP), inosine (HxP) and hypoxanthine (Hx) are products of ATP, ADP and AMP degradation that influence the quality of fish products. It is known that IMP contribute to the fresh fish flavour due to its flavour enhancing properties and its loss by the bacterial nucleoside phosphorylase breakdown brings about loss of flavour of fish flesh (Venugopal, 2006).

1.3.5 Fish quality indicators

The quality changes suffered by the fish owing to conditions at slaughter time (e.g. stress) and during storage (handling and storage temperatures) can be pointed out as follows (Poli et al., 2005):

a) Fish and fillet appearance (physical injuries, flesh gaping and colour);
b) Technological properties of the fish and fillet (*rigor* evolution, texture (firmness, cohesiveness, elasticity), water holding capacity and fillet shrinkage), *rigor mortis* onset and texture (extremely significant for flesh processing);
c) Freshness indicators (*e. g.* dielectric properties or impedance and k-value), spoilage indicators (*e. g.* biogenic amines) and lipid oxidation products (e.g. malonaldehyde);
d) Sensory qualities of raw fish (*e. g.* appearance of skin, rigor status, eye, gills colour, smell, mucus, condition of flesh), shelf-life and differences in some sensory traits of cooked fillets (*e. g.* texture, taste, flavour and odour), despite not being so used.

Those indicators, as it was mentioned, are highly influenced by all factors and conditions at pre-slaughter and slaughter time. However, there are other parameters, such as fat content, also important to the consumers, that are already characterised at death time (Poli et al., 2005). For instance, fish that show an excessive lipid level are not well accepted by the consumer and consequently their value might decrease in the market (Poli et al., 2005).

2 - Pre-harvesting

Handling fish, as other animals, is a very delicate step and it might bring about high stress.

Fish can suffer of strong episodes of stress caused by handling since they arrive at the production facilities (hatchery and rearing) till the moment of death at slaughter facilities. This often provokes restrain or confinement for long periods of time outside water and many times held in suboptimal water quality conditions (Schwedler and Johnson, 2000).

Thus, handling fish must be a process done with care and good knowledge of all factors (such as water quality, temperature and densities) that are important for well-being, having in mind not only good production results but also appropriate final product quality.

2.1 Water quality

Water quality is vital for good health, survival and growth of fish either grown in aquaculture systems or grown in wild. As a result, it is important to know, for instance, exactly at what densities, temperatures and pH fish species chosen for production grow better. Furthermore, fish is influenced by water quality through processes such as nitrogen metabolism and respiration (Buttner, 1993) which produce metabolic products such as ammonia and carbon dioxide (Schwedler and Johnson, 1999/2000). According to Buttner (1993), temperature, dissolved oxygen and ammonia are factors more likely involved with fish losses.

2.1.1 Temperature

Fish are poikilothermic (cold-blooded) vertebrate animals and their metabolism is highly influenced by water temperature. They have a relatively limited ability to adjust their body temperature and their metabolic rate by moving into cooler or warmer water. However, each species has a range of temperatures that are optimal to get the best growth. When placed in water with temperatures outside this optimal range, they reduced their growth and quality and, in case of severe extreme temperatures, fish may even die (Buttner 1993).

2.1.2 Dissolved oxygen

The oxygen dissolved in water is likewise a vital factor for fish survival and to get good production results (eg. good growths). Low oxygen level in water is responsible for serious problems on fish health, such as anorexia, respiratory stress and tissue hypoxia (Poli et all, 2005).

Likewise important is the oxygen available at fish harvesting/catch just prior slaughter time. The deficiency in oxygen and stress caused by crowding fish at harvesting imply an intense use of white muscle and consequently anaerobic glycolisis will raise as well as lactic acid production; the muscle pH will decrease (Poli et al, 2005). At slaughter time fish might not have time for recovery of crowding stress. This has implications on *rigor mortis* process, such as pH. Muscle pH will remain high because an early energy sources exhaustion due to an increase in the anaerobic glycolysis rate at pre-slaughter time (e. g. struggling) (Poli et all, 2005) and consequently a decrease of lactic acid production. That is, lactic acid production after death will be shorter and those one produced just before death is gradually cleared from muscle and blood; the lactic acid concentration in muscle after death will not be enough to decrease pH and it will remain high (Schwedler and Johnson, 1999/2000). This is the main cause of faster bacterial growth and protein denaturation, important degradation phenomena.

2.1.3 Ammonia

Ammonia is a very important parameter of water quality and is highly influenced by fish metabolism. The reason for that is because ammonia is one of the end products of protein metabolism in teleost fishes (Van Waarde, 1983). This factor may be very toxic when accumulated in water and its toxicity may be seen by hyperactivity, convulsions, loss of equilibrium, lethargy and coma of fish (Hargreaves, 1998).

In water, ammonia have two forms: NH_3 (ammonia) and NH_4^+ (ammonium ion) that are in equilibrium (Hargreaves, 1998; Crab et al., 2007). The unionized form (NH_3) is more toxic to the fish and at high pH and temperatures is more abundant than NH_4^+ form (Hargreaves, 1998; Crab et al., 2007). However both of them are toxic to fish at certain concentrations depending on species, size, fine suspended solids, surface compounds, metals and nitrites (Colt, 2006). Thus, when fish handling is planned is vital to make restriction of food intake in order to empty fish gut and then reduce the amount of ammonia released through faeces in water as a consequence of handling stress, having in mind that stress levels also increase (but less) with starving.

2.2 Density

The number of fish stocked in a production unit should be enough to reach the production goals and at the same time in a quantity that allows maintaining good health of stock by a proper management (Schwedler and Johnson, 1999/2000). Stock densities are expressed in two ways (Schwedler and Johnson, 1999/2000):

- Effects fish have on environment;
- Effects fish have on each other.

As a result, high fish densities in a production system should be avoided in order to keep good water quality. An excess of waste products will cause higher stress levels.

Likewise prior to slaughter, fish may suffer of strong episodes of stress caused by fish crowding. Harvesting is needed in this phase, and producers have to group the fish. This process make them struggling and swimming vigorously which can be very traumatic if it lasts too long (Poli et al., 2005). Anaerobic glycolysis will increase as well as lactid acid production and lowering of muscle pH brought about by the intensive use of white muscle during vigorous swimming (Poli et al., 2005). Consequently, negative effects on fillet quality may be observed, for instances, textural quality (Poli et al., 2005). Another important consequence of too high fish densities is the decrease of dissolved oxygen concentration available in water and the increase risk of abrasion damage through contact with nets or other fish (Humane Slaughter Association).

To sum up, fish at this point should be handled in a relaxed way by bringing up and crowding them slowly so as to avoid gasping, splashing, struggling and abrasion. Moreover, fish should be kept crowded for a maximum of two hours (as recommended by HAS, no date).

2.3 Starving period

Before slaughter, fish should be kept without food for a maximum of 72 h to avoid depletion of glycogen sources. The length of this period is influenced by temperature and feeding rate, for instances, at 25º C, 24 h without food will be enough, in case of fish have been fed properly (Caggiano, 1999). This will induce fish to empty gut prior to slaughter and to lowering problems of microbial contaminations with spoilage and other organisms (Caggiano, 1999) as well as to keep water quality in good conditions at the moment of transport and crowding prior to slaughter (Poli et al., 2005).

3 - Harvesting

Fish harvesting is a vital step in getting fish out of production facilities in good condition (Jensen and Bruson, 1992), not only for management handling/transport but also for slaughtering fish. The equipment choice depends of several factors such as volume and size of fish harvest, the frequency of use, the size of the operation and the capital available (Jensen and Bruson, 1992). Therefore, at this point is pretended to make a brief review of harvesting methods as well as stress provoked by each of them.

3.1 Lowering water level

This is a method very frequent in water ponds of considerable dimensions, namely in non-intensive farming systems. Main problems are time consumption and the stress induced in all fish from the pond, so it is preferably applied when all stock is to be removed at the same time. It also induces some fish crowding and stress, but in certain circumstances it can be quick and effective. Sometimes it is the only possible method, but even in these cases farmers complain about the mud at the bottomn, that makes very difficult the final collection of fish, even with almost no water at the bottom.

3.2 Nets

This is the most common and simple method to catch fish. It can be applied to cages (by simply pulling the nets to the surface), to race-ways and other tanks with rigid walls, or even used to catch fish in water-lowering methods. From hand nets (small basket nets with a rigid mouth) to long rectangular nets, all sizes and shapes are used.

In long tanks (*e. g.* race-ways) a rectangular net, with an upper floating cable and a loaded bottom cable, can easily be used by two operators, that start each at each side of the tank and use the net to concentrate the fish in the other extremity, taking some or all fish with a hand-held basket net or other final system. The method is somewhat stress-inducing, but it can be rapid and effective, at a very low cost, reasons that support its wide use.

Many ponds of non-intensive systems have bottoms and walls that do not allow the proper use of nets, because they are naturally irregular, they have thick layers of bottom mud or they were protected against robbery with bottom structures that are precisely installed to make the use of nets impossible, but they also have this effect when the farmer wants to catch their own fish.

3.3 Mechanical harvesting

In some circumstances, fish can be pumped out of the water. This possibility is normally confined to some species (due to skin and body resistance) and depends on the final destination of the fish, as well as a previous concentration method near the suction structure or tube. It is typical of the more advanced fisheries and intensive aquaculture, thus relatively rare in non-intensive systems.

3.4 Other methods

During Seacase project, other possible methods will be searched and will be added to this short list, but these are the most widely used and others, if they exist, are used only occasionally, probably showing a very low economical relevance.

4. Stunning (inducing unconsciousness)

Nowadays stunning an animal prior to slaughter is a key and mandatory step, not only to be in accordance with welfare legislation but also to improve quality of final product. There are several possible methods to perform stunning, that can be divided in “slow methods”, the ones that do not cause immediate loss of consciousness such as thermal shock/ice slurry, CO_2 narcosis and chemical anesthesia and “fast methods”, which cause immediate loss of consciousness such as cerebral concussion and electrical shock (Robb and Kestin, 2002)

MAIN STUNNING METHODS	
Slow methods	thermal shock
	CO_2 narcosis
	chemical anesthesia
Fast methods	cerebral concussion (blow in the head)
	electrical shock
	brain spiking

4.1 Cerebral concussion

Stunning by cerebral concussion is a widely used commercial method, mainly on salmon and large fish (Wall, 2001). This method consists of a hit on the head with a fast moving, mostly manually applied club (Wall, 2001, Hans van de Vis et al., 2003), even though automatic percussive stunning are become available (Robb and Kestin, 2002).

In practice, the stun is often not immediate and fish are hit more than once (Wall, 2001).

STUNNING POSSIBLE EFFECTS		
Too weak	→	fish immediately or rapidly recovers (second blow is needed)
Ideal	→	fish loose conscience and never recovers before killing phase
Too strong	→	fish is immediately killed

Depending on the strength applied the concussion can be irrecoverable (Hans van de Vis et al., 2003) and kill the animal instantly. In case of the operator is tired and not rotated, inaccurate blows might happen which can lead to eye damage, bruising and some down-grading (Wall, 2001). Flat-fish are a group of fish which shape makes percussive stunning difficult to achieve (Wall, 2001). Damaging eyes and down-grading quality are two possible examples of flat-fish quality damage (Wall, 2001). Likewise percussive stunning is not considered a suitable method for others species such as sea bream and eels (van de Vis, 2001) Despite troubles of execution, percussion is considered a fast and humane stunning method (Wall, 2001, Hans van de Vis et al., 2003) when correctly applied. Then fish bleeding should be done to assure death of fish and to improve quality of flesh (Poli et al., 2005).

4.2 Electrocussion

Electrocussion, electrical stunning or electrical shocking is considered as humane and it is mostly used to stun salmon, trout and eels (Giuffrida, 2003), despite eels shown to be more resistant to electrical current and therefore require higher electrical currents to achieve reasonable periods of insensibility (van de Vis et al., 2001). The fish unconsciousness obtained highly depends on the intensity and length of the current applied until fish death (Poli et al., 2005). When the voltage applied is not correct, this method has negative side effects to the carcass quality as well as to fish welfare (Wall, 2001). Muscle bruising (haemorrhages) and spinal fractures are consequences that may be observed (Wall, 2001, Poli et al., 2005). Another important drawback of its employment is concerning the operator safety in the case of using old equipment (Wall, 2001) since water has high conductivity power.

4.3 CO_2 narcosis

This method is widely used commercially (van de Vis et al., 2003), mostly with salmon and trout (Poli et al., 2005). It consists in placing fish in a bath of water containing dissolved carbon dioxide (CO_2) (Wall, 2001). The high concentrations of CO_2 in water induce a decrease on blood pH which drop brings about disruption of processes in the brain and eventual death (Robb, 2001). CO_2 dissolved in water forms an acid and saturated water with this gas has a pH of roughly 5.0 (Anon, 1995, Poli et al., 2005). Therefore, when fish are added to this water the blood takes up the carbon dioxide and blood pH decreases (Robb, 2001).

Carbone dioxide narcosis is efficient and fast in narcotizing a large number of fish and is valid for several species and for all sizes (Wall, 2001; Poli et al., 2005). 2-4 min is the time spent until fish are immobilized (Robb, 2001; Poli et al., 2005). However, CO_2 narcosis provokes a large number of aversive reactions in fish during the first minutes after fish have been put in that water (Robb, 2001; van de Vis et al., 2003; Poli et al., 2005), such as repeated swimming around the tank and attempts to escape (Robb, 2001; Poli et al., 2005). Other drawback is that fish remain conscious for a certain time, depending on species, after its immobilisation (Robb, 2001; Poli et al., 2005). Hence, carbon narcosis is not a stun method considered humane (van de Vis et al., 2003). Much more studies concerning CO_2 narcosis method need to be done in the future in order to find out a controlled carbon dioxide deliver system or the use of inert gases to promote anoxia to convert CO_2 narcosis in a more humane method (Wall, 2001).

4.4 Thermal shock

Ice slurry stunning is used in Mediterranean Countries for small sized species, such as sea bream and sea bass, and in UK for rainbow trout since it is considered a quick and easy process (Poli et al., 2005). This method consists in adding fish to an ice water/slurry and then water is drained off leaving the fish mixed in the ice (Robb, 2001, Robb and Kestin, 2002) or in packing live in flake ice (Robb and Kestin, 2002). This rapidly decreases fish body temperature with a similar reduction in the metabolic rate, resulting in much slower movements (Robb, 2001, Wall, 2001, Poli et al., 2005). In consequence, fish oxygen requirement are minimal paired with the capability of fish to tolerate hypoxia, make them to remain alive and conscious for long periods and therefore the time of death is delayed (Wall, 2001, Poli et al., 2005). Thus, it is not known whether this method makes fish truly insensible (Lines et al., 2003). Nevertheless, this method produces good quality fish, through reduction of enzymatic and bacterial spoilage activity, despite not being accepted by the Farm Animal Welfare Council because of the prolonged period of sensibility to the stimuli that trout has before death (Wall, 2001; Poli et al., 2005). For instances, eels stunned by this method roughly take 12 min to be unconscious and insensible (Lambooj et al., 2002) and gilthead sea bream take less than 20 min (Huidobro et al, 2001). Wall (2001) suggested doing more research to improve this method since it has noteworthy advantages, such as quick and easy to handle and appropriate flesh quality. One of the possibilities is to use carbon dioxide or an inert gas in ice slurry to hasten death (Wall, 2001).

4.5 Chemical anaesthesia

Anaesthesia stunning consists in sedating fish by adding an anaesthetic compound (e. g. iso-eugenol) to the water (Lines at al., 2003). Its addition does not result in visible aversive response by the fish and after ten to 30 min fish appear insensible (Robb, 2001; Lines at al., 2003). Then, fish are netted from the pen or the tank and killed using a percussive blow or spike (Robb, 2001). This technique is considered humane and good for flesh quality since it does not provoke too much stress to fish, which accomplish to the Farm Animal Welfare Council requirements.

This procedure is only allowed in countries like New Zealand and Australia. This method is not more widely used since there are concerns regarding addition of chemicals to fish just prior slaughter (Robb, 2001), e. g. in Europe and in the USA. This procedure does not give the fish a chance to get rid of this substance from the flesh (Robb, 2001). Furthermore, the cost required to overcome legislation barriers and possible public response to eating fish that could be perceived as having been poisoned (Lines et al., 2003) are other bottlenecks that make this technique difficult to put in practice.

5 - Slaugthering (inducing death)

5.1 Cerebral concussion

This method is also named as percussive stunning, knocking or blow in the head. Already described as stunning method in section 4.1, here it will be analysed as killing method, and in this perspective this is a very common method, of worldwide spread use. It is very simple, easy to perform (although some practice is needed to achive perfection) and it is common in all salmon farming, where several "killing machines" were developed based on this method. As previously mentioned, this is a very effective and welfare-supportive method, but only if the efficacy is verified, as sometimes fish show recover signs (so, later it becomes evident that fish was not killed).

5.2 Electrocussion

This is a method normally used as a complementary method in combination with others, like CO_2 narcosis and lowering the temperature. Results depend mainly on species, water salinity and other chemical and physical properties, characteristics of the electrical current applied and other indirect factors. The fish unconsciousness or killing obtained depends highly on the intensity and length of the current applied (Poli et al., 2005). As in the previous method, the final killing of the fish is frequently a result from asphyxia and not the low temperature and the consequent low metabolism, but the electricity seems to be a very effective way of shortening the processing times, thus increasing welfare.

5.3 CO_2 narcosis

It is used commonly as a complementary method to fish crowding and electricity. This method helps to reduce killing times of other methods, or it can be used alone. In this last case, respiration is difficult or impossible and fish dies, within a period of time that depends of species, water characteristics and other factors.

5.4 Thermal shock

Thermal shock in ice slurry or in a mixture of ice+water is used as stunning method and was described above, but the efficiency (both for stunning and for killing) is arguable as, depending on species and many other unclear factors, fish can take too long to die and welfare is then not guaranteed as it should be. But it must be emphasized that this is most common method used nowadays by the farming industry, although the final killing of the fish is frequently a result from asphyxia and not the low temperature and the consequent low metabolism.

5.5 Asphyxia

Also known as death in air, this is a very common killing method, specially when applied after a stunning method. It is very simple and easy to apply, cheap and convenient for the industry, as it only implies leaving the fish out of water. Fish will die by lack of oxygen in the tissues, showing signs of stuggle or being quiet depending on species and other factors. To increase efficiency or to avoid taking fish from the water, this can also be done by crowding the fish in a small space and by the inclusion of a high percentage of CO_2 in the same water.

5.6 Brain spiking

This method is also named pin-bolt, decerebration or brain destruction. This is a very specific method, as it is almost exclusively applied to some species like tuna and other fish of considerable dimensions. A metallic pin or special sharp-pointed hand-held instrument is used by experienced workers to achieve the brain tissues and, by lateral movements, to destroy the integrity of the organ, inducing immediate death. It is considered as an appropriate welfare method, because it is very quick (it takes onlya few seconds to induce death).

5.7 Evisceration

This is considered as a non-acceptable method in terms of welfare, but is still used in some parts of the world, namely in Asiatic countries. No references were found for the use of this method within Europe (as direct killing method), but unintentionally it still can exist, as in some evisceration rooms after killing, there is no complete confidence on fish being always dead and consequently some can be eviscerated still alive.

5.8 Gill excision

Like for the previous method, this is considered as a non-acceptable method in terms of welfare. Gills are taken of by precise cuts and fish dies as a consequence of the bleeding (loss of blood, low blood pressure, heart stop).

5.9 Stress of each method

As a summary of what was described as methods and having in mind that:

• ↓ time suffering and ↓ stress, ↑ welfare and ↑ fish quality
• industry is already using several methods, nowadays mainly ice slurry+asphixia
• it is difficult to change industrial activities in a short time
• there is a controversy about the ice slurry method
• trustable indicators of welfare or even stress have not yet been developed
• processes depend considerably on tradition, facilities, purposes, times and costs

In this manual and for the purposes to achieve with it, the welfare/stress induced by the methods can be seen mainly by measuring the time needed for complete death. If a method implies suffering for a short period of time (as the scientific ways of measuring precisely the level of welfare are still in study) than other, it can be considered as better.

5.10 Recommended methods

There is no definitive answer for this. As a consequence of the previous considerations, the recommended methods are the ones that kill more rapidly, considering the whole of the fish to process. Blow in the head and brains spiking seem to be the most recommendable methods. But considering that probably brain spiking induces unacceptable pain (due to intensity and not due to time) it is only acceptable if a previous stunning method is applied. All farmers using now immersion in an ice slurry as killing method must think about other possible killing and stunning methods and they must be prepared to change their procedures if, as it is expectable, scientific evidence will support the unacceptability of this method, at least used alone.

6 - Final quality

It was already mentioned that fortunately the recent fish welfare concern is supported by a better quality. This means the farmers are interested in better welfare methods at least because the quality of the product they will obtain is improved. So, it can also be said that a very effective (although indirect) way of measuring welfare is to look at the final quality of the fish. In the next section some of the main methods to evaluate fish quality, currently mentioned as freshness, although the definition of freshness is very complex (Bremner and Sakaguchi, 2000).

6.1 Quality measurements

All methods used to evaluate freshness can theoretically be used to infer indirectly conclusions about the welfare of the processing options, but the most directly measures are the methods based on ATP breakdown products and on *rigor mortis* measurements, as they are considered as *freshness indicators* that give information earlier in time (and not *spoilage indicators*, because they are "late indicators").

6.2 Measurement of ATP metabolites

ATP, a compound that is able to concentrate energy for later use, exists in fish cells at levels that depend on the struggle, stress and, after death, on peri-mortal circumstances, as defined by Bremner and Sakaguchi (2000). It is considered as one of the most promising methods for freshness correct evaluations, but it involves specialized work on chemistry laboratories as it is measured by HPLC (High Performance Liquid Chromatography). It is predicted that some simplifications will be developed, and after that maybe it wioll become the most common and precise method.

6.3 Measurement of *rigor mortis*

Rigor mortis or cadaveric rigidity occurs in all muscular tissues after death and it is the most visible physical effect within some hours or a few days after death. It can be measured using simple procedures and equipments, by persons with short or even no previous experience at all, by simple measurement of the angle that fish makes with vertical, when resting in an horizontal surface which supports the head-half of the fish body. The lower the peri-mortal stress, the later rigor starts and the longer it will last, which is definitely sign of a superior quality. The project Seacase published a set of simple instructions that farmers or other fish workers can follow step-by-step in order to measure correctly rigor on fusiform (cigar-shaped) fish, that can be obtained free by asking to the project task leader Paulo Vaz-Pires (vazpires@icbas.up.pt).

7 - Future perspectives and *Seacase* contribution

7.1 Predictable future slaughter problems

As it is predictable that the current situation, that was derived from many years of no concern about fish welfare, will change soon and that many new scientific and technical work will arise soon, Seacase participants considered useful that the project contributes to this new approach. Seacase is a project with a close and frequent contact with several farmers and farms within Europe and this contact is a valuable and positive basic circumstance that allows the collection and trial of several slaughter changes and methods, and consequently all opportunities to collect data, to perform studies and to spread results will be intensively used.

7.2 Most needed results and studies to achieve them

Seacase participants agree that it is preferable to obtain answers to simple and practical questions than to pose questions that are to big with answers that are unattainable. Examples of simple questions that the project is able to try to answer are:

- It is possible to improve the ice slurry welfare by combining it with other methods?
- Can we be sure that these improvements are feasible and effective?
- Can we develop a simple method to measure rigor mortis in field conditions?
- Can we use this method to analyse and compare several stunning or killing methods?
- Can we finally achieve one recommended method or, at least, can we choose the best?

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