

Fish welfare and quality as affected by pre-slaughter and slaughter management

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Abstract. A reliable assessment of animal welfare-suffering and of its impact on product quality requires a multidisciplinary approach that takes into account fish behaviour and the different biochemical and physiological processes involved. This might be done by the contemporary study of changes of indicators of brain function, endocrine responses, *post mortem* tissue biochemical processes and quality changes. This work reviewed some of the most used indices of stress at the time of slaughter, commercial slaughter methods and related stress effects on physical and biochemical parameters of fish quality. The set of the available data seemed to indicate that, although of some results appear contradictory, pre-slaughter and slaughter stressful practices could have an important effect on the flesh quality in fish. A clear effect emerged mostly on the physical properties of flesh, because severe stress at slaughter time exhausted muscular energies, produced more lactic acid, reduced muscular pH, increased the rate of *rigor mortis* onset. In this way they could have significant negative effects on technological traits, flesh quality and keeping quality of fish. Asphyxia and electrically stunned fish were more stressed than spiked, knocked and live chilled fish. Combining various methods together might be a more satisfactory strategy for both animal welfare and product quality.

Key words: Fish quality, Fish welfare, Pre-slaughter stress, Slaughter methods, Stress indices

Introduction

Concern about fish welfare, including the time of slaughter, is nowadays gaining interest both at consumer and at producer level, even if the choices made by aquaculture operators often respond to the need to maximise benefits, not always evaluating them in respect to animal welfare.

On the other hand, it has to be recognised that welfare is difficult to define and to measure. A useful definition can be that suggested

by Spruijt et al. (2001): “the balance between positive (reward, satisfaction) and negative (stress, aversion) experiences or affective states. The state of this balance may range from positive (good welfare) to negative (bad welfare)”. So, welfare of a farmed fish depends on to what extent a fish can adapt to the rearing conditions and find them rewarding (Kristiansen and Juell 2002). As clearly proved in many other terrestrial meat animals, good welfare means good production. The end point of animal life, i.e. the pre-slaughter procedures management and slaughter methods, if carried out without care to avoid severe stress conditions to the animal, can heavily influence the expression of quality and the subsequent changes during storage of the final product. This is an ethic aspect that also has clear reflexes on fish quality.

Animal welfare and product quality are linked aspects of the total quality of fish, therefore any conflict between the requirements of fish welfare and efficient aquaculture could be avoidable. To maintain the best original quality fish would be stunned until death and killed without any avoidable stress, through operations based on practical issues.

This work wishes to contribute to the limited number of reports on the relationship between management of pre-slaughter and slaughter procedures and quality of fish by summarising the most used indices of stress at the time of slaughter, the commercial slaughter methods currently used and their stress effects evaluated by behavioural, haematic, tissue and quality indices and by the changes in physical and biochemical parameters of fish quality.

The structure of this review considers first the physiological significance of the most used indicators of fish welfare/suffering, each of them able to contribute to achieving a clearer outline of the real fish stress condition and the relative consequence for the final product quality. The effects of pre-slaughter management practices, such as the most commonly used stunning/slaughter methods on the behaviour of those stress and quality indicators, are then considered. At the end, a direct comparison of combinations of different stunning/slaughter methods applied to the same lot of fish, aimed to provide a sort of grading of their stressful effects, is considered.

Fish welfare-suffering and quality indicators

The key issue in welfare evaluation at time of slaughter is animal suffering, which is also difficult to measure. Research has demonstrated that, even if less well developed than in other farmed animals, fish also

have sense organs able to detect painful stimuli, sensory pathways for processing such stimuli and brain mechanisms that process this information and generate behavioural responses (Kestin 1994; Wendelaar Bonga 1997; Huntingford 2002). A reliable assessment of animal welfare-suffering and of the impact on product quality, requires a multidisciplinary approach that, together with the study of fish behaviour in respect to stressful conditions, takes into account the different biochemical and physiological processes involved. This might be done by the contemporary study of the relative changes of significant indicator parameters of brain function, endocrine responses, *post mortem* tissue biochemical processes and quality changes.

Behavioural indicators

Behaviour can be a good noninvasive indicator of fish welfare as it often responds rapidly to environmental changes, even if it cannot be an exhaustive approach. At slaughter, fish behaviour gives macroscopic indications of the presence or absence of consciousness.

Behavioural studies consist of a series of observations carried out during or after the pre-slaughter and slaughter procedures. The most frequent observations are related to both the self-initiated behaviour, such as strength and persistence of *swimming motility*, *gill movement*, and the responses to stimuli such as the capability to maintain the *equilibrium* when the fish is turned upside down, the *movement of eye* following the changes in body postures in the longitudinal axis, the *reaction to the needle puncture* on head or tail (pricking), the *handling* along the lateral line and the *electricity application* of low voltage electricity (Marx et al. 1997; Tobiassen and Sørensen 1999; Van der Vis et al. 2001).

Brain function indicators

Electroencephalogram (EEG), Electrocardiogram (ECG), Visually evoked responses (VERs) from photic stimulation, i.e. flashes of light directed towards the eyes, and Somatosensory evoked responses (SERs) are objective ways to indicate the presence of fish sensibility and consciousness. EEG and VERs need the implant of four silver electrodes, two of them on the caudal and anterior margin of the optic lobe on the left side of the brain, and two ground electrodes on the left brain hemisphere (Kestin et al. 1991; Kestin 1994; Robb et al. 2000; Van der Vis et al. 2001, 2003; Lambooi et al. 2002; Robb and Roth 2003). Measurement of brain activity using EEG Technology provides

reliable information but the level of expertise required to perform such test makes its use very limited.

Haematic indicators

As in all the other vertebrates, fish react with a well-characterised neuroendocrine “stress response” of adaptive value (Wendelaar Bonga 1997) to whatever undesirable challenge is threatening the animal homeostasis, i.e. the complex physiological processes that contribute to maintain the specific ranges of temperature, pH and solute concentration, necessary for the maintenance of the normal physiological functions.

Acute primary response to stress is an immediate massive release into the blood of adrenaline and noradrenaline from the chromaffin cells. This is followed by a gradual ACTH release that triggers off the release of corticosteroids, such as cortisol, from the interrenal tissue into the blood stream. These substances speed gluconeogenesis and lipid mobilisation which have repairing and adaptive significance. Prolonged stressful stimuli or frequent exposure to acute stressors (chronic stress) cause a redistribution of stored energy resources that can effect flesh quality more markedly than a single acute stress. *Catecholamines* (adrenaline and noradrenaline) are not commonly used as stress indicators because they are not easy to determine and quickly removed from blood (Wendelaar Bonga 1997). *Cortisol* is widely used both as long term and as short term stress condition index (Pickering et al. 1982; Pickering and Pottinger 1985), even if it may also vary with feeding, reproductive cycles, seasonal cycles and husbandry conditions. Multiple stress conditions amplify the cortisol response (Ortuño et al. 2002).

The consequences of these endocrine responses are an increase in heart-beat, higher oxygen uptake, energy source mobilisation and increase in *plasma glucose*. The last is easy to determine so it is frequently used as a stress indicator, although some authors have found a delay in its release (Barry et al. 1993). Higher energy mobilization and utilisation, following the increase of muscular activity, initiates the anaerobic glycolysis and a related increase in *plasma lactate*. Therefore, the increase of plasma lactate is used as stress index (Lowe et al. 1993; Erikson et al. 1999), even if most fish store lactate in muscle tissue. The increase in heart-beat and the need of higher oxygen uptake cause an increase in the number of moving erythrocytes and of the *haematocrit* value. This is also used as stress index because it is very simple to determine, even if standard values have to be validated

for each species before haematocrit is used as stress index (Reddy and Leatherland 1988).

The energy source mobilisation includes the fatty reserve, so also changes in *plasma free fatty acids* (FFA) could be a stress condition index, but this parameter generally did not show a clear response.

A rapid death does not allow a completion of secondary effects of stress, and pre-slaughter stress always has to be considered in the stress evaluation at slaughter by the haematic parameters.

Tissue indicators

Increased muscular activity, stress at slaughter and relative endocrine response can greatly influence fish *post mortem* biochemical processes, mostly the anaerobic glycolysis and ATP degradation rate. This in turn can markedly influence the onset and release of *rigor mortis*, which in turn largely determines the involution rate of fish freshness. In this way it leads to undesirable changes mostly in the marketable, physical and freshness quality parameters. The close interrelationships observed among endocrine acute stress responses and fish *post mortem* biochemical processes suggest to use not only haematic stress indicators like plasma cortisol, lactate and glucose, but also tissue stress indicators such as muscular pH, lactate and ATP and its catabolites.

Figure 1 presents an outline of the main *post mortem* biochemical processes with the most used stress indices used to measure the metabolic response to the pre-slaughter and slaughter management.

First of all, the muscle *phosphocreatine*, *ATP/ADP/AMP* and *glycogen* levels, that are the cellular energy charge indices, can be used as early stress indices. All of them can be influenced by the pre-slaughter and slaughter stress conditions and muscle activity markedly decreasing at death (Erikson 1997; Pottinger 2001; Tejada et al. 2001). The early onset of *rigor mortis*, as a consequence of the early lack of ATP, is the most macroscopic stress index.

Marked *muscle lactic acid* increase and *pH* decrease within the first day after death, linked to high anaerobic glycolysis activity also before death, are often good early stress and muscular activity indices (Oka et al. 1990; Lowe et al. 1993; Marx et al. 1997; Robb and Warriss 1997). Because ATP, ADP and AMP are degraded within the first day after death, the ATP catabolites *IMP*, *inosine* and *hypoxanthine* are used to follow the long term stress consequences. Some of them, such as IMP, have a good influence on fish taste while others, such as hypoxanthine, exert a marked negative effect.

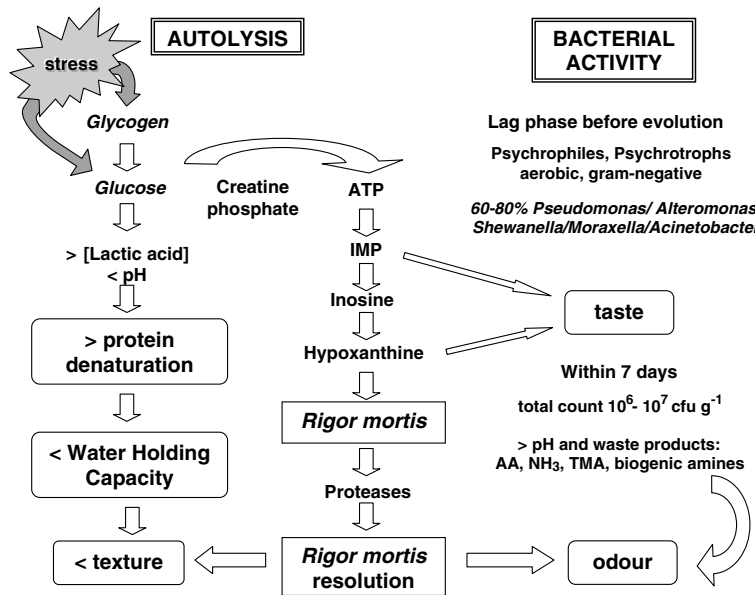


Figure 1. Tissue stress and quality indicators.

Fish quality indicators

Some quality traits are fixed at death, such as the fat content. This must be particular to the species and for the market requirements. Fat depots distribution in excess, can decrease the fish value. Many quality traits can change as affected by conditions at slaughter time (severity of the pre-slaughter and slaughter stresses) and during storage (handling and storage temperatures). The relative quality changes can be indicated by

1. fish and fillet appearance (*physical injuries, flesh gaping and colour*);
2. technological properties of the fish and fillet [*rigor evolution, texture (firmness, cohesiveness, elasticity), water holding capacity and fillet shrinkage*], *rigor mortis* onset and texture in particular are important for flesh processing;
3. freshness indicators, such as *dielectric properties* or *impedance*, *K value*, and spoilage indicators such as *biogenic amines*, lipid oxidation products such as *malonaldehyde*;
4. sensory qualities of raw fish (*appearance of skin, rigor status, eye, gills colour, smell, mucus, condition of flesh*), the *shelf life* and, even if less frequent, the differences in some sensory traits of cooked fillets as *texture, taste, flavour, odour*.

Pre-slaughter management practices

The application of stress factors *infra vitam* and/or in the *ante mortem* period may interact and influence both the animal's physiological reactions – in this way worsening the animal performance – and the *post mortem* biochemical processes that turn muscle into meat, in this way affecting the quality and durability of the final product (Azam et al. 1989; Izquierdo-Pulido et al. 1992; Lowe et al. 1993; Marx et al. 1997; Sigholt et al. 1997; Thomas et al. 1999; Parisi et al. 2001). Often there are unavoidable situations where multiple stressors interact and affect the final condition.

The capture process is a very traumatic time for the fish, because of the duration, struggling and crowding that occur during most capture protocols. The vigorous swimming during crowding implies an intense use of the white muscle. Anaerobic glycolysis will therefore increase as will lactic acid production and lowering of muscle pH.

If the fish is allowed to recover from the intense activity, the lactic acid produced will be cleared from the blood and muscle (Milligan 1996). This occurs during fish rearing, when a stressful condition may occur as a result of the stocking density, the quality and quantity of feed distributed, the quality of water and, above all, the oxygen level. A low concentration of dissolved oxygen (DO) in the water is responsible for many and serious effects on fish health, such as anorexia, respiratory stress, tissue hypoxia. It also negatively influences the growth and survival of fish (Thetmeyer et al. 1999). However, at the end of the production cycle the fish are slaughtered in a short time, so the recovery is not possible and muscle pH will remain low. On the contrary, if the traumatic pre-slaughter situation lasts for a longer time (i.e. repeated catching), the lactic acid produced will be gradually cleared from the blood and muscle, but the energy sources will become gradually exhausted. This is even more accentuated if fish are starved for a long time before slaughter. When a fish is killed, the pH will remain higher, due to the early end of *post mortem* anaerobic glycolysis caused by the energy source shortage. Starvation before slaughter, correctly carried out (i.e. for 1–3 days according to water temperature), is necessary to ensure complete gut emptying, reducing spoilage organisms charge and allowing higher oxygen levels during the pre-slaughter crowding period.

Increased stocking density, the related decrease of oxygen level due to crowding prior to slaughter, handling and disturbance prior to stunning and slaughter suffering are unavoidably associated with a degree of stress, particularly if it provokes scaling, external abrasion and protracted agony in the fish (Pankhurst and Sharples 1992; Tobiassen and

Sørensen 1999). In this regard a higher oxygen level in crowding condition prior to slaughter produces a less evident stress condition. Prior to slaughter, as summarised also by Wall (2001), fish should be rested, fasted only long enough to ensure gut emptying and have no external injuries or muscle bruising.

Parisi et al. (2001) reported the effects of pre-slaughter procedures such as crowding prior to slaughter and repeated catching on cortisol and haematocrit levels. They both increased in stressed fish. The same stressing conditions were studied through muscle contraction force after death.

High density and repeated catching showed different stressful effects and differences in *rigor mortis* phases. To summarise, high density produced a stressful response, shown by the haematological parameters. It caused a decrease in *pre-rigor*, a faster decrease in contraction force and a decrease in freshness. The stress by repeated catching produced a lower contraction force magnitude, due to the loss of a complete recovery from stress during handling and catching practices suffered in the preceding day. In addition, there was a pejorative effect on some quality parameters, such as the higher muscle pH and the 24 h shorter shelf life in comparison to the fish caught the preceding day. Sigholt et al. (1997) studied flesh quality in Atlantic salmon and reported that handling stress not only shortened the *pre-rigor* time but also resulted in a stronger *rigor*. Stress and exercise are linked to a reduction of fish quality and this is associated mainly with pre-slaughter stress (Pottinger 2001).

Stunning and slaughter methods

As already underlined, careful consideration should be paid in food animal slaughtering, and not only for an ethic reason. In fact, even if at the end point of fish life many quality traits are already fixed, many others will develop during the *post mortem* muscle turning into meat. These can present deleterious changes in fish submitted to severe stress and suffering conditions. Fish stunning/killing represents a highly stressful moment and can cause strength impact on flesh quality, this effect is more evident as more fish struggle or is stressed (Erikson, 1997). For this reason it is also important that the slaughterers well trained in the method in use and restare before and during the operations. There are fast (spiking and knocking) and slow methods. The first one is thought to be less aversive both for the animals and for meat quality, but cannot always be used for lots of small-sized

fish. The current stunning and slaughtering methods used in European farms for some of the most important cultured species are reported in Figure 2.

Stressful effects associated with some frequently used commercial and experimental methods are described as follows.

Death in air or asphyxia

Asphyxia, traditionally used for captured fish, consists in leaving fish to die out of the water. This is the oldest fish slaughter method and is characterised by a prolonged suffering period before death. The time it takes fish to die depends on the hypoxia resistance. For example, carp and eel are particularly resistant to hypoxia. The period necessary to reach stunning is variable (rainbow trout 15 min, sea bream 25 min, sea bass 60 min) (Kestin et al. 1991; Sebastio et al. 1996; Robb 2001; Bagni et al. 2002; Poli et al. 2002). Generally higher ambient temperatures result in faster fish death. The method is not humane and can cause deleterious change in fish shelf life and flesh quality. For example, when sea bass are left out the water they exhibit a quick violent reaction, followed by decreasing muscular activity and spasms, with death coming after protracted agony. Sea bass show high haematocrit and plasma cortisol, glucose and lactate values and very low muscle adenylate energy charge at 0 h and pH at 0 h and 24 h (Parisi et al. 2002; Poli et al. 2002).

<hr/> Salmon (<i>Salmo salar</i>) <ul style="list-style-type: none"> • Gill cut • CO₂narcosis + gill cut • Percussion + gill cut • Cooling + gill cut 	<hr/> Sea bream (<i>Sparus aurata</i>), sea bass (<i>Dicentrarchus labrax</i>) and other marine fish <ul style="list-style-type: none"> • Asphyxia • Ice plus water or ice only
Rainbow trout (<i>Oncorhynchus mykiss</i>) <ul style="list-style-type: none"> • Asphyxia • CO₂narcosis + gill cut • Ice plus water or ice only • Gill cut • Electrical stunning + evisceration 	Eel (<i>Anguilla anguilla</i>) <ul style="list-style-type: none"> • Salt bath + evisceration (Netherlands) • Electrical stunning (50 V for 5 minutes) + evisceration (Germany) • Ice plus water + evisceration
Other experimental methods: Spiking and anaesthesia	

Figure 2. Stunning and slaughtering methods used in European farms.

Death in ice slurry

After harvesting fish are directly transferred to water/ice slurry tubs. Variable water/ice ratios are used to give liquid ice. This easy and quick procedure is used in Mediterranean Countries for Mediterranean small-sized species and in the UK for rainbow trout. Fish body temperature decreases rapidly as does their metabolic rate and movements (live chilling). Fish oxygen requirements also decrease markedly and the time to death can be prolonged. The fish die of anoxia. The length of the stunning period also can be long: trout 28–198 min, salmon 60 min, turbot 20 min, sea bream 20–40 min, sea bass 20 min, eel 12 min (Kestin et al. 1991; Huidobro et al. 2001; Wall 2001; Lambooij et al. 2002; Poli et al. 2002). Some authors have debated the “humane” aspect of this method (Kestin et al. 1991; Wedermeyer 1997). The Farm Animal Welfare Council (1996) were very critical of the prolonged period that trout remain sensible to stimuli. They therefore recommend that the method should be prohibited. However, in warm Mediterranean species, such as sea bream (Bagni et al. 2002) and sea bass (Zampacavallo et al. 2003) comparative experimental trials showed that the method does not seem to be particularly stressful, particularly if compared to asphyxia. For example, live chilled sea bass did not show any violent reaction. They also had lower haematocrit, plasma glucose and lactate, lactate in muscle (linked to low muscular activity), reduced metabolic rate, later *rigor mortis* onset, higher adenylate energy charge and lower hypoxanthine at death and at 24 h after death. They also showed improved flesh quality and shelf life as result of the rapid reduction of core body temperature (Parisi et al. 2002; Poli et al. 2002; Zampacavallo et al. 2003). The lowering of *pre-rigor* phase and of rigor release, together with longer shelf life and decrease of gaping were reported by Skjervold et al. (2001). Wall (2001) suggested researching the method and modifying with the aim of speeding the onset of death, for example using CO₂ or inert gas. In sea bass Zampacavallo et al. (2003) obtained a faster effect (10 vs 20 min) with an ice/water mixture 1:2 saturated by 40% N₂ and 60% CO₂.

Electrical stunning and electrocution

This method is used for trout and salmon, the fish being stunned and slaughter in a film of freshwater (good conductor of electricity). It is very efficient in that fish are stunned immediately with 1 sec of electricity applied across the head (Kestin et al. 1995). The duration of unconsciousness depends on the intensity and length of current

application until death. However electrical stunning can cause a quick and violent reaction, with mouth and opercula wide open, muscle blood spots and vertebral fractures, according to the duration of the current application. Moreover, the immobility of fish does not assure that fish are unconscious. On the other hand, when well applied, with the optimisation of the electrical parameters for each species (500 mA trout, 600 mA eel, 200 mA sea bream), electrical stunning and electrocution could really give sudden immobilisation and loss of consciousness and in this way be humane and very practical. The method can be promising (Van der Vis et al. 2003).

Carbon dioxide narcosis

This method is used mostly with salmon and trout. Fish are placed in a water bath saturated by CO_2 that dissolves in water giving H_2CO_3 in equilibrium with HCO_3^- and H^+ . This exerts a lowering effect on blood pH and, in this way, a toxic effect on the brain. Death is obtained by gill cutting. CO_2 narcosis is aversive to the fish, as clearly indicated by a quick and violent reaction, such as repeated swimming around, attempts to escape from the tub and abnormal activity before stunning. Immobility is reached within 2–4 min but it was demonstrated that fish still remain conscious until stunning time, varying with the fish species (2 min salmon; 3 min trout; 9 min carp; 109 min eel; 7–10 min sea bass) (Kestin et al. 1995; Marx et al. 1997; Robb et al. 2000; Poli et al. 2002). Stunning and killing time in rainbow trout was 4.5 min (Kestin et al. 1995). Our previous investigation (Poli et al. 2002) showed that sea bass stunned by CO_2 narcosis had a high energy charge in the muscle and low plasma cortisol. The method is efficient and quick in narcotising a large number of fish and is valid for several species and for all sizes, but it is aversive for the fish in the first minutes after death. Wall (2001) suggested the use of a controlled CO_2 delivery system or the use of inert gases to promote anoxia in a more humane way.

Knocking or percussive stunning

This method is frequently used for salmon, trout and other large fish, when it is possible to kill a single fish. The fish are taken from the water, restrained manually in an apposite place and stunned by one or two blows on the brain using a wood or plastic club, also termed priest. If the energy of the blow is sufficient there is a massive brain disruption and an immediate insensibility in fish. A high energy blow can kill the animal immediately. The bleeding that follows assures death of the fish

and improves flesh quality. This method is not easily applied because the blow must be precise and the slaughterers well-trained and rested. For salmon there are automatic tools such as a pneumatic club (Kestin et al. 1995; Marx et al. 1997; Tobiassen and Sørensen 1999; Robb et al. 2000; Van der Vis et al. 2001; Wall 2001). A confirmation that the method is among the least stressful ones in sea bass is evidenced by the lowest haematocrit, plasma glucose and lactate and the highest muscle energy charge (Poli et al. 2002). The method is one of the fastest and least stressful, therefore can be considered humane for fish but is too time consuming for batches of small-sized species.

Spiking

This method is used for tuna and salmon and consists of the physical destruction of the brain by a sharp spike inserted through the skull and moving it into the brain to destroy it. The stunning is immediate, but the method is fast and efficient only if well applied. It is possible to miss the small target point and then fish suffer during the procedure (Tobiassen and Sørensen 1999; Robb et al. 2000; Van der Vis et al. 2001; Lambooi et al. 2002). As knocking, this is one of the fastest and least stressful methods, therefore it can be considered humane for fish but unsuitable for batches of small-sized species.

Comparison of the effects of different slaughter methods on the same lots of fish

Overall, the comparison of the effects of different slaughter methods using the same lots of fish (on animal behaviour and stress haematic, tissue and quality indicators) generally confirms the information above with the individual methods, even if some of them gave contradictory results.

Azam et al. (1989) compared three methods of stunning fish on trout (electrocution, exposure to elevated CO₂ concentration and a blow on the head), subsequently bled. Slaughter by electrocution and by carbon dioxide narcosis led to a greater initial lactic acid production and slightly lower pH than a blow to the head, but no differences emerged in other quality index of the ungutted fish covered by ice during 15 days of *post mortem* storage. The lowest pH observed was 6.42 (pH of fresh fish range around 6.5) and no softening of the flesh was observed. The sensory panel that evaluated toughness, elasticity, firmness and succulence was unable to identify differences between samples on the first day

and on the fifteenth day. After concluding that any advantage or disadvantage seemed associated with the slaughter methods examined, the authors reported that differences have also been found with regard to the onset and duration of *rigor mortis* and that further work in this area would be useful. In contrast, Sebastio et al. (1996) slaughtered rainbow trout by percussive stunning, asphyxiation, CO₂ narcosis and electrocution. By evaluating several biochemical parameters and muscle tissue quality, they found that percussive stunning preserved the highest glycogen reserve and glycolytic potential, together with the lowest pH during shelf life and the longest duration of *rigor mortis*. The CO₂ narcosis showed slight differences in comparison to the percussive stunning with a slight acidification that limited microbial proliferation with better preservability but had lower biochemical parameters. Electrocution showed biochemical parameters comparable to or better than those of CO₂ narcosis and lower acidification of muscle. Asphyxia did not allow good preservability of fish that showed low glycogen content, high post mortem pH values and the short duration of *rigor mortis*. Marx et al. (1997) compared the blow on the head, electricity and CO₂ narcosis with trout, carp and eel. It was observed that CO₂ narcosis gave rise to the lowest pH, water holding capacity and panellist evaluation. Also *rigor mortis* in carp and eel advanced most with CO₂ narcosis. *Rigor* indices were lowest after anaesthetising the fish manually. After 24 h the eel anaesthetised manually showed significantly higher pH values than the others but after 8 days of storage on ice the pH values of fish from the different stunning methods were very close to each other. As reported by Robb (2001) in rainbow trout, a very rapid drop in muscle pH due to stress can affect colour parameters (higher L*, angle of hue, chroma and lower Roche colour card score) making fish flesh appearance lighter and more opaque. Sigholt et al. (1997) compared meat quality in Atlantic salmon slaughtered by CO₂ narcosis and gill bleeding with or without a pre-slaughter period of confinement stress. They found that stressed fish had lower pH, phosphocreatine, ATP level, a more rapid onset of *rigor* and softer fillets. Sørensen and Carlehoeg (1999) compared quality in Atlantic salmon slaughtered by CO₂ narcosis, Eugenol as anaesthetic and gill cut, concussion or pin-bolt (concussion + decerebration) as quick killing methods and found only a few and small differences. Changes in pH, *K* value and onset and development of *rigor mortis* have been reported but they did not find that such differences were critical when sensory quality of product was determined. Only Eugenol resulted in a difference with regard to “*own smell and flavour*”.

Van der Vis et al. (2003) reported carbon dioxide stunning followed by gill cutting or gill cutting alone as not “humane” methods for salmon slaughter, even if automated percussive stunning is still not well resolved. They suggest that electrical stunning can be humane if applied properly, after the optimisation of electrical parameters in order to avoid occasional bloodspots. For gilt-head seabream the same authors reported that quality of fish slaughtered by percussive stunning was similar to that obtained by immersion in an ice slurry, but neither asphyxia in air nor transfer of the fish to an ice slurry were considered to be humane. For eel the authors suggest electricity in combination with nitrogen gas 0.64 A dm^{-2} for 1 sec, followed by 0.17 A dm^{-2} combined with nitrogen flushing for 5 min.

Several subsequent experiments with the aim of finding a practical low stress slaughtering method and the best quality of small warm species like European sea bass were carried out by Poli et al. (2002, 2003) on 300–600 g sea bass, stunned and slaughtered by: 1:2 ice in water (WI), ice–water mixture saturated with N_2 (WI- N_2) or 40% N_2 and 60% CO_2 (WI- N_2 CO_2), asphyxia (AS), CO_2 narcosis (CO_2), electrical stunning ($24 \text{ V} \times 2 \text{ min}$ –EL), spiking (SP) and knocking (KN). All stunning methods were followed by 30 min in ice covering, and fish behaviour, plasmatic and tissue stress indices, *rigor* onset and quality involution at 1°C with ice covering by EU scheme were studied. Asphyxia always produced the most prolonged agony, a remarkable physical activity, a relevant mobilisation of energy reserves with earlier *rigor* onset and shorter shelf life. This was especially prominent in comparison to spiking, knocking and water and ice, as there was often no difference among them (Figures 3 and 4). The use of gases during fish live chilling shortened death time without big differences in stress and quality indicators and produced a day longer shelf life in Water and Ice- N_2 CO_2 fish in respect to Water and Ice alone. *Rigor* was reached at 3 h (asphyxia), 6 h (electrical stunning) or about 9 h (spiking, knocking, water and ice, CO_2) after death. Live chilling was not particularly stressful, in comparison both to spiking and knocking, the fastest and generally less aversive slaughtering methods, and to CO_2 narcosis, which in turn was generally less stressful than asphyxia and electrical stunning. The most constant results in the experiments were found with asphyxia and water and ice slaughtering methods; the most variable ones with CO_2 narcosis and electrical stunning.

Pre-slaughter cooling of Atlantic salmon caused a longer time lapse before *rigor* onset and resolution (Skjervold et al. 2001) leaving a potential for filleting *pre-rigor*. This would not be a slaughter method itself but would have the added benefit of improving flesh quality.

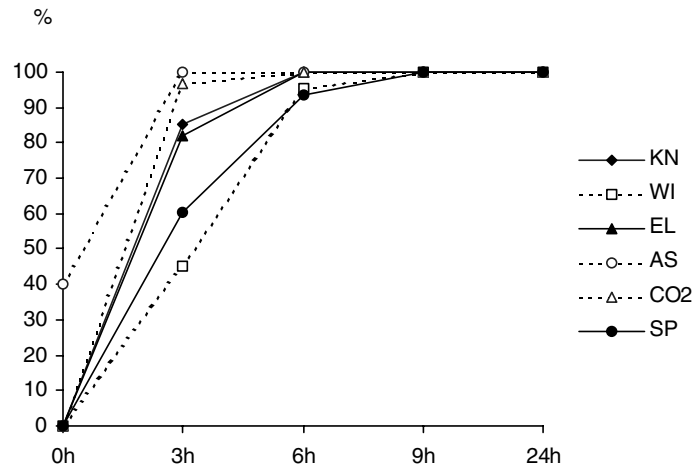


Figure 3. Rigor index changes after different stunning/slaughtering methods: knocking (KN), water + ice (WI), electricity (EL), asphyxia (AS), CO₂ narcosis (CO₂) and spiking (SP).

The comparison of different fish stunning and killing methods generally confirmed the results of the single methods, whilst providing a grading of stressful effects on the quality of fish. Asphyxia and electrical stunning fish were more stressed than spiked and live chilled fish. Regarding the relationship between killing method and fish quality the following results are found:

1. stress and exercise are linked to a reduction of fish quality and this is associated mainly with pre-slaughter stress;
2. stunning/killing methods induce more or less muscular activity and energy source consumption;
3. intensity and length of stress factors exerted at the time of death may give critical results.

Conclusion

The complexity of the available data seems to indicate that, although some results appear in some respects contradictory, pre-slaughter and slaughter stressful practices can have an important effect on the flesh quality in fish, in a similar fashion to mammals and poultry. Some general trends can be listed:

		1	2	3	4	5	6	7	8	9	10	11
	days	24	48	72	96	120	144	168	192	216	240	264
	hours	24	48	72	96	120	144	168	192	216	240	264
	BW	285.1	40.7	E ¹		A ²		B ³		UNFIT		
	Øsd	285.1	40.7	E ¹		A ²		B ³		UNFIT		
Knocking		285.1	40.7	E ¹		A ²		B ³		UNFIT		
Water+Ice		477.6	51.3	E	A		B		UNFIT			
Electricity		358.1	53.6	E	A		B		UNFIT			
CO₂		320.0	19.9	E	A		B		UNFIT			
Asphyxia		435.4	83.7	E	A		B		UNFIT			
Spiking		429.8	43.1	E	A		B		UNFIT			

¹ E class: Extra fish

² A class: Fresh fish

³ B class: Stale fish

Figure 4. Sensorial analysis (EU scheme) on raw fish after different stunning/slaughtering methods: knocking (KN), water + ice (WI), electricity (EL), asphyxia (AS), CO₂ narcosis (CO₂) and spiking (SP).

1. less stressful pre-slaughter practices and killing methods produce less intense physical exercise in fish and minimise the stress response, in this way minimising changes in the normal *post mortem* processes and the quality traits development and involution;
2. slaughter methods have an effect on the physical properties of flesh;
3. stressful pre-slaughter and killing methods exhaust muscular energies, produce lactic acid, reduce muscular pH and increase the rate of *rigor mortis* onset. In this way they can have significant negative effects on flesh quality, and in particular on keeping quality of fish;
4. with reduced pre-slaughter stress and activity, fish will take longer to go into *rigor*, giving the possibility of handling the fish while processing and packing before they enter into *rigor*, increasing fillets yield and reducing damage of flesh;
5. severe stress due to the pre-slaughter practices can be so aversive to the fish that it masks the benefits of good slaughter practices;
6. differences in sensory evaluation on cooked fish are less frequently found than physiological measures.

Therefore, the first aim should be to minimise and keep under control the pre-slaughter stress that interferes with slaughter stress. In parallel, further research is required for more humane and less stressful slaughter practices. In particular, it is important to study methods that will be useful when the achievement of instantaneous induction of insensibility is not possible, with the objective that the animal should be rendered unconscious and insensitive without avoidable stress, pain or suffering. The improvement of pre-slaughter and slaughter management has to be achieved not only from an ethical point of view, but also because there is a close relationship between fish welfare even at death and quality of the final product.

An optimal slaughter method should render fish unconscious until death without avoidable excitement, pain or suffering prior to killing. Therefore, combining various killing methods together (e.g. ice slurry plus CO₂ and/or N₂ or electrical stunning set up for each species plus N₂) may be a more satisfactory strategy for both animal welfare and product quality. A longer time both before *rigor* onset and resolution from pre-slaughter cooling gives a potential for filleting *pre-rigor* and has added benefit of improving flesh quality. Aquaculture industry can then provide high quality fish to processors and consumers by using improved pre-slaughter handling and slaughter methods.

References

- Azam, K., Mackie, M. and Smith, J. 1989. The effect of slaughter method on the quality of rainbow trout (*Salmo gairdneri*) during storage on ice. *International Journal of Food Science and Technology* 24: 69–79.
- Bagni, M., Priori, A., Finoia, M.G., Bossù, T. and Marino, G. 2002. Evaluation of pre-slaughter and killing procedures in sea bream (*Sparus aurata*). In: Proceedings of the “Aquaculture Europe 2002: Sea Farming Today and Tomorrow”. Special Publication n. 32, pp. 135–136.
- Barry, T.P., Lapp, A.F., Kayes, T.B. and Malison, J.A. 1993. Validation of a microtitre plate ELISA for measuring cortisol in fish and comparison of stress responses of rainbow trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*). *Aquaculture* 117: 351–363.
- Erikson, U. 1997. Muscle quality of Atlantic salmon (*Salmo salar*) as affected by handling stress. Doctoral Thesis. Norwegian University of Science and Technology, Trondheim, Norway, p. 68.
- Erikson, U., Sigholt, T., Rustad, T., Einarsdottir, I.E. and Jorgensen, L. 1999. Contribution of bleeding to total handling stress during slaughter of Atlantic salmon. *Aquaculture International* 7: 101–115.
- Farm Animal Welfare Council 1996. Report on the welfare of fish. FAWC, Block D, Government Buildings, Hook Rise South, Tolworth, Surbiton, Surrey KT6 7NF, pp. 1–52. <http://www.fawc.org.uk>.
- Huidobro, A., Mendes, R. and Nunes, M.L. 2001. Slaughtering of gilthead seabream (*Sparus aurata*) in liquid ice: influence on fish quality. *European Food Research and Technology* 213: 267–272.
- Huntingford, F. 2002. Welfare and Aquaculture. In: Proceedings of the “Aquaculture Europe 2002: Sea Farming Today and Tomorrow”. Special Publication n. 32, pp. 52–54.
- Izquierdo-Pulido, M.-L., Hatae, K. and Haard, N.F. 1992. Nucleotide catabolism and changes in texture indices during ice storage of cultured sturgeon, *Acipenser trasantanus*. *Journal of Food Biochemistry* 16: 173–192.
- Kestin, S.C. 1994. Pain and stress in fish. Report of the Royal Society for the Prevention of Cruelty to Animals. Causeway, Horsham, West Sussex, UK, p. 36.
- Kestin, S.C., Wootton, S.B. and Adams, S. 1995. The effect of CO₂, concussion or electrical stunning of rainbow trout (*Oncorhynchus mykiss*) on fish welfare. In: Proceedings of the “Quality in aquaculture”, Special Publication n. 23, pp. 380–381.
- Kestin, S.C., Wootton, S.B. and Gregory, N.G. 1991. Effect of slaughter by removal from water on visual evoked activity in the brain and reflex movement of rainbow trout (*Oncorhynchus mykiss*). *The Veterinary Record* 128: 443–446.
- Kristiansen, T.S. and Juell, J.E. 2002. How can we document the welfare of farmed fish? In: Proceedings of the “Aquaculture Europe 2002: Sea Farming Today and Tomorrow”. Special Publication n. 32, pp. 285–286.
- Lambooj, E., Van Der Vis, J.W., Kloosterboer, R.J. and Pieterse, C. 2002. Welfare aspects of live chilling and freezing of farmed eel (*Anguilla anguilla* L.): neurological and behavioural assessment. *Aquaculture* 210: 159–169.
- Lowe, T., Ryder, J.M., Carrager, J.F. and Wells, R.M.G. 1993. Flesh quality in snapper, *Pagrus auratus*, affected by capture stress. *Journal of Food Science* 58: 770–773, 796.

- Marx, H., Brunner, B., Weinzierl, W., Hoffman, R. and Stolle, A. 1997. Methods of stunning freshwater fish: impact on meat quality and aspects of animal welfare. *Zeitschrift für Lebensmittel Untersuchung und Forschung* 204: 282–286.
- Milligan, C.L. 1996. Metabolic recovery from exhaustive exercise in rainbow trout. *Comparative Biochemistry and Physiology* 113A: 51–60.
- Oka, H., Ohno, K. and Ninomiya, J. 1990. Changes in texture during cold storage of cultured yellowtail meat prepared by different killing methods. *Nippon Suisan Gakkaishi* 56: 1673–1678.
- Ortuño, J., Angeles Esteban, M. and Meseguer, J. 2002. Lack of effect of combining different stressors on innate immune responses of seabream (*Sparus aurata* L.). *Veterinary Immunology and Immunopathology* 84: 17–27.
- Pankhurst, N.W. and Sharples, D.F. 1992. Effects of capture and confinement on plasma cortisol concentrations in the snapper, *Pagrus auratus*. *Australian Journal of Marine and Freshwater Research* 43: 345–356.
- Parisi, G., Mecatti, M., Lupi, P., Scappini, F. and Poli, B.M. 2002. Comparison of five slaughter methods for European sea bass. Changes of isometric contraction force and pH during the first 24 hours *post mortem*. In: Proceedings of the “Aquaculture Europe 2002: Sea Farming Today and Tomorrow”. Special Publication n. 32, pp. 417–418.
- Parisi, G., Mecatti, M., Lupi, P., Zampacavallo, G. and Poli, B.M. 2001. Fish welfare and quality: experimental results on rearing and harvesting practices. In: Proceedings of the Third Congress of the European Society for Agricultural and Food Ethics (EURSAFE 2001) “Food Safety, Food Quality and Food Ethics”, 3–5 October 2001, Florence, Italy, pp. 337–340.
- Pickering, A.D. and Pottinger, T.G. 1985. Factors influencing blood cortisol levels of brown trout under intensive culture conditions. In: Lofts, B. and Holms, W.N. (eds.), *Current Trends in Endocrinology*. Hong Kong University, pp. 1239–1242.
- Pickering, A.D., Pottinger, T.G. and Christie, P. 1982. Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. *Journal of Fish Biology* 20: 229–244.
- Poli, B.M., Parisi, G., Zampacavallo, G., Scappini, P., de Francesco, M. 2003. The effect of slaughter methods on European sea bass (*Dicentrarchus labrax*) behaviour, rigor onset, plasmatic and tissue stress indexes and quality. In: Proceedings of the “First Joint Trans-Atlantic Fisheries Technology Conference (TAFT)”, 33rd and 48th Atlantic Fisheries Technology Conference, 11–14 June 2003, Reykjavik, Iceland.
- Poli, B.M., Zampacavallo, G., Iurzan, F., de Francesco, M., Parisi, G. and Mosconi, G. 2002. Biochemical stress indicators changes in sea bass as influenced by the slaughter method. In: Proceeding of the “Aquaculture Europe 2002: Sea Farming Today and Tomorrow”. Special Publication n. 32, pp. 429–430.
- Pottinger, T.G. 2001. Effects of husbandry stress on flesh quality indicators in fish. In: Kestin, S.C. and Warriss, P.D. (eds.), *Farmed Fish Quality Fishing News Books*, Oxford, pp. 145–160.
- Reddy, P.K. and Leatherland, J.F. 1988. Stress Physiology. In: Leatherland, J.F. and Woo, P.T.K. (eds.), *Fish Diseases, Volume III: Non-infectious disorders*, C.A.B. International, pp. 279–302.
- Robb, D.H.F. 2001. The relationship between killing methods and quality. In: Kestin, S.C. and Warriss, P.D. (eds.), *Farmed Fish Quality*. Fishing News Books, Oxford, pp. 220–233.

- Robb, D.H.F. and Roth, B. 2003. Brain activity of Atlantic salmon (*Salmo salar*) following electrical stunning using various field strengths and pulse duration. *Aquaculture* 216: 363–369.
- Robb, D.H.F. and Warriss, P.D. 1997. How killing methods affect salmonid quality. *Fish Farmer*, Nov/Dec: 48–49.
- Robb, D.H.F., Wotton, S.B., McKinstry, J., Sørensen, N.-K. and Kestin, S.C. 2000. Commercial slaughter methods used on Atlantic salmon: determination of the onset of brain failure by electroencephalography. *Veterinary Record* 147: 298–303.
- Sebastio, P., Ambroggi, F. and Baldrati, G. 1996. Influenza del sistema di sacrificio su trote iridee di allevamento. I Considerazioni biochimiche. *Industria Conserve* 71: 37–49.
- Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T.S. and Seland, A. 1997. Handling stress and storage temperature affect meat quality of farm-raised Atlantic salmon (*Salmo salar*). *Journal of Food Science* 62: 898–905.
- Skjervold, P.O., Fjæra, S.O., Østby, P.B. and Einen, O. 2001. Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). *Aquaculture* 192: 265–280.
- Sørensen, N.K. and Carlehoeg, M. 1999. Effect of killing methods for farmed Atlantic salmon (*Salmo salar*) on quality and storage life, iced in boxes. In: Proceedings of the “Aquaculture Europe 1999: Towards predictable quality”, Trondheim, Norway, August 1999. Special Publication n. 27, pp. 271–272.
- Spruijt, B.M., Van den Bos, R. and Pijlman, F.T.A. 2001. A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. *Applied Animal Behaviour Science* 72: 145–171.
- Tejada, M., Huidobro, A. and Pastor, A. 2001. Slaughter methods affecting adenosine triphosphate and derivatives in chilled stored gilthead seabream (*Sparus aurata*). In: Kestin, S.C. and Warriss, P.D. (eds.), *Farmed Fish Quality*. Fishing News Books, Oxford, p. 410.
- Thetmeyer, H., Waller, U., Black, K.D., Inselmann, S. and Rosenthal, H. 1999. Growth of European sea bass (*Dicentrarchus labrax*) under hypoxic and oscillating oxygen conditions. *Aquaculture* 174: 355–367.
- Thomas, P.M., Pankhurst, N.W. and Bremner, H.A. 1999. The effect of stress and exercise on *post mortem* biochemistry of Atlantic salmon and rainbow trout. *Journal of Fish Biology* 54: 1177–1196.
- Tobiassen, T. and Sørensen, N.K. 1999. Influence of killing methods on time of death of Atlantic salmon (*Salmo salar*) and rainbow trout (*Salmo gairdneri*) as measured by behavioural indices of sensibility and reflexes. In: Proceedings of the “Aquaculture Europe 1999”, EAS Special Publication n. 27, p. 244.
- Van der Vis, H., Kestin, S., Robb, D., Oehlenschläger, J., Lambooi, B., Munkner, W., Kuhlmann, H., Kloosterboer, K., Tejada, M., Huidobro, A., Ottera, H., Roth, B., Sørensen, N.K., Akse, L., Byrne, H. and Nesvadba, P. 2003. Is humane slaughter of fish possible for industry? *Aquaculture Research* 34: 211–220.
- Van der Vis, H., Oehlenschläger, J., Kuhlmann, H., Munkner, W., Robb, D.H.F. and Schelvis-Smit, A.A.M. 2001. Effect of the commercial and experimental slaughter of eels (*Anguilla anguilla* L.) on Quality and Welfare. In: Kestin, S.C. and Warriss, P.D. (eds.), *Farmed Fish Quality*. Fishing News Books, Oxford, pp. 234–248.
- Wall, A.J. 2001. Ethical considerations in the handling and slaughter of farmed fish. In: Kestin, S.C. and Warriss, P.D. (eds.), *Farmed Fish Quality*. Fishing News Books, Oxford, pp. 108–115.

- Wedemeyer, G.A. 1997. Effects of rearing conditions on the health and physiological quality of fish in intensive culture. In: Iwama, G.K., Pickering, A.D., Sumpton, J.P. and Schreck, C.B. (eds.), *Fish Stress and Health in Aquaculture*, Soc. Exp. Biol. Semin., Ser. Vol. 62. Cambridge University Press, pp. 35–71.
- Wendelaar Bonga, S.E. 1997. The stress response in fish. *Physiological Reviews* 77: 591–625.
- Zampacavallo, G., Scappini, F., Mecatti, M., Iurzan, F., Mosconi, G. and Poli, B.M. 2003. Study on methods to decrease the stress at slaughter in farmed sea bass (*Dicentrarchus labrax*). *Italian Journal of Animal Science*, 2 (Suppl. 1): 616–618.