



Farmed fish welfare: stress, post-mortem muscle metabolism, and stress-related meat quality changes

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Abstract Over the past few decades, a considerable scientific progress has been achieved in the area of fish welfare at all stages—farming, transportation, various pre-slaughter manipulations, stunning/killing procedures, etc. The increasing scientific interest is mainly due to the serious need of developing and implementing specific fish welfare requirements across the whole chain for both ethical (welfare) and commercial (meat quality) reasons. Since there has been substantial evidence that fish are able to experience pain and suffering, the provoked stress response could be a major cause of altered post-mortem metabolism and impaired meat quality. The present review was aimed to present the relationship between ante-mortem stress, course of post-mortem metabolic processes and the potential alterations in some quality traits of fish for human consumption.

Keywords Fish welfare · Sentience · Stress · Post-mortem metabolism · Meat quality

Introduction

The concept of fish welfare includes several main moments borrowed from the standards of warm-blooded animals welfare (FAWC 1996), or so-called “Five freedoms”. However, “measuring” fish welfare can be extremely difficult for two main reasons. The first reason is related to the lack of consensus on whether fish can feel pain and anxiety. In this regard, in the scientific literature, there has been a serious debate on the presence of awareness and well-developed sentience in these animals (Braithwaite and Ebbesson 2014).

Some authors (Rose 2002; Rose et al. 2014) believe that some essential parts of the brain are not developed in fish, making the claim of fear and pain unrealistic. The presence of consciousness in mammals (including humans) is due to the function of the neocortex, which does not exist in the brain of fish and has no adequate analogue, and therefore, there can be no awareness of pain and fear. In response to noxious stimuli, fish exhibit unconscious neuroendocrine and physiologic reactions mainly controlled by the brain stem, spinal cord and other subcortical brain regions.

In contrast, a number of authors have concluded that there is anatomical, physiological, and behavioral evidence that fish have well-developed nociception and adequately perceive and realize pain (Chandruo et al. 2004; Braithwaite and Boulcott 2007; Sneddon 2009). Dunlop and Laming (2005) observed neuronal responses in the spinal cord, cerebellum, tectum, and telencephalon of goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*) subjected to mechanoreceptive and nociceptive stimuli. These results

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indicated that mechanoreceptive and nociceptive responses in fish were not limited to the spinal cord and lower brain centers, but involved higher parts of the central nervous system, including telencephalon. Furthermore, other studies have proven that fish are capable of showing emotional fever (Rey et al. 2015), as well as learning to avoid frightening stimuli (Yue et al. 2004). It has been considered that medial pallium region in fish is functionally homologous to amygdala in mammals (Salas et al. 2006), which are part of the limbic system and are responsible for emotional memory (including fear). This was supported by the results of Portavella and Vargas (2005), showing that lesions of the medial pallium led to impaired acquisition of avoidance conditioning response.

The second reason is associated with the need of objective methodologies to assess the impact of farming, ante-mortem procedures, stunning, and killing methods on welfare and fish meat quality. As reviewed by Poli (2009): “a number of indicators can be used to assess fish welfare-suffering, both in a scientific and practical context, such as behavioral, haematic, cellular, tissue post-mortem fish stress and quality indicators, but none of them are optimal.”. In this context, the main purpose of the present work was to trace the relationship between the pre-mortem stress, the course of post-mortem metabolic processes and the potential changes in some quality indicators of fish meat.

Stress in fish

Stress, according to Selye (1973), is “the nonspecific response of the body to any demand made upon it”. A stressor can be any change in the environment that disturbs homeostasis. Neutral stress causes responses that are neither detrimental nor beneficial to the organism. However, distress results in reactions that may affect animal welfare or reproduction and cause pathological changes (Squires 2003).

Stress-inducing factors (so-called stressors) are numerous and vary in their nature and impact. Barton (2002) summarized them in three basic groups: perceived stressors (e.g., presence of a predator), physical stressors (e.g., handling, transportation, etc.) and chemical stressors (e.g., low oxygen concentration, increased acidity, etc.). In addition, according to the author, the physiological responses that arise under different stressors can also be grouped. Primary stress response includes mainly endocrine changes manifested by elevated levels of catecholamines and corticosteroids. Secondary stress response affects metabolism, water-salt balance and functions of the cardiovascular, respiratory, and immune systems. The tertiary stress response is associated with changes in growth, disease resistance and fish behavior, and may occur as a result of the primary and secondary responses.

Hormone secretion plays an important role in regulation of homeostasis and development of stress response. The neuroendocrine response resulting from stress is conventionally divided into rapid and long term, the first being associated with activation of the adrenal medulla, while the latter is due to the function of hypothalamic–pituitary–adrenal axis (Squires 2003). The adrenergic (rapid) stress response is mediated by the sympathetic nervous system and leads to release of catecholamines (adrenaline and noradrenaline) from the chromaffin cells, located in the anterior kidney area in fish (Reid et al. 1998). The long-term neuroendocrine response begins with release of corticotropin-releasing factor (CRF) from the hypothalamic nucleus preopticus. CRF activates release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary, leading to production of glucocorticoids (mainly cortisol) from the adrenal cortex (the interrenal cells in the head kidney of fish) (Flik et al. 2006).

As reviewed by Tort et al. (2004), the described mechanism is generally valid for each stressful situation, but the extent of the physiological changes that occur is significantly influenced by the duration of the stress factor. Acute stress usually involves immediate and significant secretion of stress hormones, followed by a rapid recovery of baseline levels and lack of severe effects on the organism. On the other hand, chronic or repeated stressors lead to moderate but steadily elevated steroid secretion, which may result in much more serious changes in the body, depletion of a large amount of energy resources and a longer recovery period. Moreover, fish species differ in their neurobehavioural reactions meaning that the provoked neurophysiological and metabolic responses might be significantly different as well (Fanouraki et al. 2011; Daskalova et al. 2016a).

According to Perry and Bernier (1999), the adrenergic response usually develops under the influence of acute and strong stress factors, and probably does not play a significant role in mediating the physiological and



metabolic reactions resulting from mild and moderate stress. Although catecholamines are important for the development of some adaptive reactions such as stress-induced changes in carbohydrate metabolism (Nakano and Tomlinson 1967; Wright et al. 1989), their role in some other physiological responses is still debatable or limited (Chen et al. 2002; Perry et al. 2004).

Cortisol, on the other hand, is considered to be a reliable stress indicator even in some cases of short-acting stressors (Pickering et al. 1982; Fanouraki et al. 2011). Increased cortisol levels have been observed in different fish species under the impact of a number of acute stressors such as: crowding of rainbow trout for 30–50 min (Merkin et al. 2010); short-term (less than 1 h) transportation of common carp (*Cyprinus carpio* L.) (Varga et al. 2014; Daskalova and Pavlov 2014); 3 min air exposure of gilthead seabream (*Sparus aurata* L.) (Arends et al. 1999); short-lasting exposure of barramundi (*Lates calcarifer*) to a combination of air, crowding and rapid temperature change (Wilkinson et al. 2008); etc. However, the physiological and metabolic changes are usually dependent on the duration of cortisol exposure. Vijayan et al. (1997) observed enhanced carbohydrate catabolism demonstrated by elevated glucose and lactate levels after 2-h confinement of tilapia (*Oreochromis mossambicus*), whereas the prolonged stress (24 h) and prolonged exposure to cortisol resulted in increased activity of some hepatic enzymes as well as increased free amino acid concentration showing accelerated proteolysis. Although some procedures such as air exposure, transportation, different stunning and slaughter methods play a role mostly as acute stressors, crowding/high stocking density is often a major chronic stressor. In this context, Bolasina (2011) reported that flounder (*Paralichthys orbignyanus*) subjected to high stocking density for 15 days maintained significantly elevated blood cortisol levels. In addition to increased blood cortisol, Montero et al. (1999) observed changes in a number of other physiological and biochemical parameters in juvenile gilthead seabream held under high-density conditions for 15 weeks. The results of the study revealed elevated hematocrit, hemoglobin and red blood cell count, decreased hepatosomatic index, altered liver fatty acid composition, and impaired immunity.

In terms of post-mortem muscle metabolism, two types of stress-/cortisol-related changes might be observed. As discussed by Poli et al. (2005), acute stress and vigorous muscle activity prior to slaughter lead to increased anaerobic glycolysis demonstrated by elevated lactic acid production and lower muscle pH. If stress conditions are repeated or last for a longer period, the lactic acid produced will be cleared, whereas the energy reserves will be gradually exhausted. As a result, fish killed during such period of exhaustion will show high and unchanging in time muscle pH due to the lack of substrate for anaerobic glycolysis. The present work is focused mainly on the post-mortem changes that arise under acute short-lasting (minutes, hours) stress.

Post-mortem muscle metabolism

Immediately after death, oxygen flow to the tissues and organs ceases due to interrupted blood circulation. As a result, the aerobic ATP (adenosine triphosphate) production is blocked and anaerobic glycolysis remains the main pathway for generating ATP. This process involves muscle glycogen breakdown and formation of lactic acid through a series of phosphorylated sugar derivatives. Small amounts of ATP can also be generated from the available reserves of creatine phosphate and ADP (adenosine diphosphate). However, the amount of these energy supplies is insignificant and they are depleted in a few minutes. Soon after death, the generation of ATP under anaerobic conditions is also terminated, which is a result of two main factors: (1) depletion of the substrate, i.e., glycogen reserve and (2) inhibition of glycolytic enzymes as a result of the pH decline mainly due to lactic acid accumulation in the musculature (Hultin 1984; Gregory and Grandin 1998). Furthermore, the hydrolysis of ATP in the muscle tissue is associated with release of protons leading to additional pH decrease. However, it has been found that not more than 10% of the total post-mortem pH decline is a result of the protons released (Hamm 1977). It is also important to note that anaerobic glycolysis is significantly less efficient with respect to the amount of ATP produced by one molecule of glucose. In anaerobic conditions, two molecules of ATP are derived from one molecule of glucose, whereas the complete oxidation of glucose to CO₂ (glycolysis, Krebs cycle and oxidative phosphorylation) results in about 30 molecules of ATP (Berg et al. 2002).

ATP is essential for the processes that occur in muscles. It provides the energy required for muscle contraction, which begins with release of Ca²⁺ ions in the sarcoplasm after the muscle cell has received a nervous impulse to contract. Soon after death, the level of calcium ions in the sarcoplasm increases



significantly due to the impossibility to be retained in the mitochondria and sarcoplasmic reticulum under anaerobic conditions (Hultin 1984). In addition, ATP hydrolysis generates energy needed for normal functioning of the ion pumps, which regulate the concentration of Ca^{2+} ions released in the sarcoplasm. When the level of ionized Ca exceeds 10^{-6} M, muscle contraction is activated. Thus, after injecting buffers containing calcium and EGTA into intact muscle fibers from the crab *Maia squinado*, muscle contraction was obtained when the free Ca^{2+} ions reached concentrations between 0.5 and 1 μM (Portzehl et al. 1964). As a result of the post-mortem decrease of ATP, the energy required for normal functioning of the ion pumps is depleted, the concentration of Ca^{2+} ions released in the sarcoplasm cannot be reduced, and consequently, the muscle remains contracted. This condition is known as post-mortem stiffness or rigor mortis (Gregory and Grandin 1998). The dependence between post-mortem decline of ATP and onset of rigor mortis was confirmed by Bate-Smith and Bendall (1947). Furthermore, Bendall (1951) concluded that muscle contraction was more closely related to the fall in the ratio of rate of resynthesis to rate of breakdown of ATP than to the drop in the ATP level itself.

Unlike mammalian and poultry meat, which shows strong post-mortem drop in pH reaching ultimate (peak) values of about 5.5–5.8 (Lundberg et al. 1987; Schilling et al. 2008), fish meat is poor in glycogen and the post-mortem pH decrease is significantly slighter. Although the ultimate pH in some fish, such as tuna (genus *Thunnus*) and halibut (*Hippoglossus hippoglossus*), may be as low as 5.4–5.6 (Huss 1995), most fish species do not exhibit ultimate pH values lower than 6.0–6.2 (Roth et al. 2007; Digre et al. 2011; Rahmanifarah et al. 2011, etc.).

The transition of rigor mortis to a stage of muscle relaxation is mostly related to the onset of proteolytic processes and meat tenderization, or so-called meat aging. It is important to note that these processes occur much faster in fish meat due to the lower muscle glycogen level. Meat tenderization is a process involving two main steps: (1) weakening and degradation of Z-disks and (2) degradation of desmin and titin, leading to destabilization of the myofibrils during meat storage. The essential enzymes involved in post-mortem proteolysis are mainly Ca-activating calpains, and also multicatalytic proteinase complex (MCP) and lysosomal cathepsins (Koochmarai 1994; Nowak 2011). Investigating the degradation of some muscle proteins (nebulin, dystrophin, metavinculin, vinculin, desmin, and troponin T), Geesink et al. (2006) concluded that μ -calpain was largely responsible for post-mortem proteolytic processes, whereas m-calpain was activated to some extent.

However, fish meat shows some substantial differences in the activity of proteolytic enzymes and in the extent and rate of degradation of some muscle proteins as compared to meat derived from mammals. In this regard, it has been found that desmin degradation could not be used as a suitable marker for assessment of post-mortem changes of stored fish, as the rate of its degradation significantly varies among fish species (Verrez-Bagnis et al. 1999). Furthermore, Chéret et al. (2007) reported that in comparison with beef, sea bass (*Dicentrarchus labrax*) white muscle showed higher activity of cathepsins B and L, lower cathepsin D activity, and higher calpastatin levels. In the musculature of sea bass, cathepsins play a major role in the degradation of the heavy chain of myosin and α -actinin. Tropomyosin and actin are only susceptible to cathepsin L, whereas troponin T and desmin are degraded by cathepsins B and L (Ladrat et al. 2003). The significant role of cathepsins B and L in post-mortem proteolysis was also evidenced by a study of Yang et al. (2015) in cod (*Gadus morhua*). Moreover, in some fish species, the proteolytic processes leading to meat softening could be associated with the activity of some other endogenous proteases, such as trypsin-like enzyme, collagenase-like enzyme, metalloproteases, etc. (Sriket 2014).

Stress-related changes in post-mortem muscle metabolism and some meat quality traits

Stress response in fish is usually characterized by increased muscle activity (swimming, wrestling, and escaping behavior). This inevitably leads to depletion of a large amount of energy reserves in the muscles and significant changes in the normal course of post-mortem muscle metabolism, usually manifested by accelerated occurrence of some of the described processes. Thus, Chinook salmon (*Oncorhynchus tshawytscha*) subjected to exhausted harvesting showed significantly lower (as compared to a group of fish harvested in a rested manner) levels of muscle glycogen, creatine phosphate, and ATP immediately after death, which was associated with increased concentrations of glucose and lactate and lower initial pH (Forgan et al. 2010). Low



initial pH as a result of stress was also observed by Wilkinson et al. (2008) in barramundi. Muscle pH was significantly lower in the stressed as compared to the rested group until 18 h post-harvest. Furthermore, flesh pH of the stressed fish did not change significantly over time (from 1 h up until 24 h), while the anesthetized fish exhibited gradual decrease in pH reaching ultimate values at 18 h post-mortem. Similar findings with respect to post-mortem pH changes were reported by Ribas et al. (2007) in Senegal sole (*Solea senegalensis*) and Daskalova and Pavlov (2014) in common carp. Most of the aforementioned observations are probably due to high blood levels of stress hormones (mainly cortisol) and are accompanied by elevated plasma glucose concentrations as a result of accelerated glycogenolysis in the liver and muscles (Vijayan et al. 1997; Forgan et al. 2010). The depletion of glycogen reserves leads to lactic acid formation, which accumulates predominantly in muscles and less in liver and blood (Padmavathy and Ramanathan 2010). Moreover, the pre-mortem depletion of available glycogen explains the insignificant changes in muscle pH over time.

The rate and extent of decline of ATP and pH in muscle tissues are inextricably linked to the rate of rigor mortis development. The results obtained by Wilkinson et al. (2008) showed that the time to full rigor onset was significantly delayed (12 h) in rested fish, whereas the conventional harvest technique led to rapid occurrence of full rigor (3 h). Similarly, gilthead seabream subjected to net crowding for 20 min prior to slaughter exhibited extremely fast onset and resolution of rigor mortis—highest rigor score was observed 2 h after death and resolution of rigor mortis started 3 h after death. Contrariwise, onset of rigor mortis was delayed in anesthetized fish, reaching the highest score between 12 and 21 h after death, and starting resolution at 32 h (Matos et al. 2010).

The duration of pre-rigor period is essential in fish processing. When stress and muscle activity prior to slaughter are minimized, the processing of fish (filleting, packing, etc.) can be performed before the onset of rigor mortis, which will increase the yield and reduce the damage of fish flesh (Poli et al. 2005). A number of studies have shown that pre-rigor filleting leads to significantly reduced incidence and severity of gaping, improved visual color, and firmer texture of the fillets (Skjervold et al. 2001a, b; Einen et al. 2002; Roth et al. 2009). As discussed by Skjervold et al. (2001b), unlike meat for which consumers prefer tender texture, fish consumers prefer firm texture. This fact is not surprising, as fish meat softening is associated with breakdown of the muscle structure, and often results in low yield and decreased product quality (Godiksen et al. 2009). The effect of pre-rigor filleting on drip loss is still debatable. According to the results obtained by Einen et al. (2002), unfrozen pre-rigor fillets showed lower drip loss compared to unfrozen post-rigor fillets. Similar findings were reported by Roth et al. (2009) in smoked pre- and post-rigor fillets. Contrariwise, Skjervold et al. (2001a) observed higher content of dry matter in pre-rigor fillets, probably indicating higher drip loss, whereas other studies have not shown any significant differences in drip loss in relation to filleting time (Skjervold et al. 2001b). Furthermore, there are some data, showing that pre-rigor filleting might be associated with slower bacterial growth, resulting in prolonged shelf life of the fillets (Tobiassen et al. 2006).

To date, it has been found that the rate and extent of muscle pH decrease have a significant effect on muscle protein state and post-mortem proteolysis, and subsequently on some meat quality traits, such as color, texture, and water-holding capacity. The rapid pH decline after death leads to irreversible denaturation of myosin and it passes from a soluble to an insoluble state. This is a key factor for the increased liquid loss in the so-called pale, soft, exudative (PSE) meat in turkeys (Pietrzak et al. 1997) and pigs (Warner et al. 1997; Joo et al. 1999), as well as after electrical stimulation of beef (den Hertog-Meischke et al. 1997). Nevertheless, a study in broiler chickens has shown that the denaturation of sarcoplasmic and total proteins does not seem to be the essential cause of reduced water-holding capacity, but still, the lower pH remains the main determinant of paleness and increased drip loss (Van Laack et al. 2000). The rapid pH decline leads to accelerated activation of μ -calpain, followed by its early autolysis. Under such conditions, larger amounts of intact desmin and talin can be found in the musculature, which, in turn, affects the degree of muscle fiber shrinkage during rigor development and leads to greater liquid leakage due to increased amount of extracellular water (Bee et al. 2007). Furthermore, the rapid muscle pH decline and lower pH levels are associated with significantly paler color of pork and poultry meat. It has been proved that chicken breasts at low pH (about 5.91) have higher reflectance and paler color compared to high-pH (about 6.36) breasts which exhibit higher transmittance into their depth and considerably darker color (Swatland 2008). The results of Van Laack et al. (2000) in broiler breast meat showed a significant positive correlation between muscle pH levels and sarcoplasmic protein solubility, and negative correlation between these two parameters and L^* (lightness) value. A strong correlation between sarcoplasmic protein precipitation and pork color was also reported by Joo et al. (1999).



Water-holding capacity and drip loss

Some of the aforementioned problems associated with deterioration of the quality traits of mammalian and poultry meat can also be observed in fish meat. Hultmann et al. (2012) reported significantly reduced muscle pH and slightly elevated muscle collagenase-like activity immediately post-slaughter in Atlantic cod subjected to a combination of stress factors. As a result, stressed fish showed significantly reduced water-holding capacity and hardness after 5 days of storage in ice. Considerably high percentage of drip loss (up to 4% at 7 days post-processing) was also found in channel catfish (*Ictalurus punctatus*) subjected to a simulated industry transport without prior anesthesia (Bosworth et al. 2007) and in acutely stressed Atlantic salmon (almost threefolds greater compared to the rested group) (Roth et al. 2006). Although the extent of pH decline, post-mortem proteolytic processes and muscle protein solubility are considered to be essential factors influencing water-holding capacity, Goes et al. (2015) reported for the first time the relationship between expression of ryanodine receptor gene encoders, ante-mortem stress, and water-holding capacity in fish. According to the results, pre-slaughter stress in Nile tilapia (*Oreochromis niloticus*) led to lower RyR1 (type 1 ryanodine receptor protein) gene expression and reduced meat water-holding capacity due to increased availability of cytosolic Ca^{2+} .

As it was discussed by Daskalova et al. (2016b), the impairment of water-holding capacity is of great importance, as it is substantial in commercial processing practice. Water-holding capacity is an essential fresh meat property, because it affects the yield and quality of the end product. The impaired ability of meat to retain water is almost always associated with unacceptable levels of moisture loss resulting in economic losses, such as reduction in salable product weight, production of poor quality products, and loss of water-soluble proteins and vitamins along with the lost moisture (Huff-Lonergan 2002). The recovery of water-holding capacity could be achieved by adding water-retaining agents, such as phosphates, used as food additives with the aim to retain natural moisture, i.e., reduce drip losses during processing and storage and inhibit fluid losses of fresh shipments (Campden 2012). It has been found that, although the addition of polyphosphates to fish fillets leads to improvements in yield after dry-salting and storage, all profits made by gain in weight may be lost due to poor quality rating. Moreover, after rehydration of such fillets, the weight gain could be totally lost (Thorarinsdottir et al. 2001). This suggests that the addition of water-retaining agents is not appropriate in some meat-processing technologies.

Meat color

Scientific data on the impact of stress on fish meat color are somewhat controversial. Erikson and Misimi (2008) reported that perimortem stress in Atlantic salmon produced darker color demonstrated by lower L^* values of the fillets. Furthermore, the initial values of redness, yellowness, and color saturation were also lower in the exhausted fish, but increased significantly during ice storage. Darker color of the fillets of stressed Atlantic salmon was also evidenced by Roth et al. (2006), but there were no differences in the other color parameters. Contrariwise, salmon subjected to CO_2 stunning showed higher redness and yellowness as compared to iso-eugenol-anesthetized fish, whereas no significant differences were observed in fillet lightness. Interestingly, the authors noticed a relationship between texture and color, as the fillets with high L^* values were softer than those with low L^* values (Kiessling et al. 2004). It is important to note that some of the stress-related color changes might be due to the rapid ATP depletion as well as due to the changes in meat pH. There have been some evidence that the acidic pH combined with lack of ATP is associated with rapid increase in the metmyoglobin ratio which, in turn, leads to faster meat discoloration demonstrated by brown color (Inohara et al. 2013).

Other studies have indicated that the rapid and intense pH decline (which is often associated with stress) results in paler meat color. Gatica et al. (2010) observed significantly lighter flesh color in crowded Atlantic salmon on day 0 and day 1 post-mortem by measuring the color on a scale ranging from 21 (light red) to 34 (dark red). Lighter color (higher L^* value) of the fillets was also reported in stressed channel catfish at 1, 4 and 7 days post-processing (Bosworth et al. 2007). Robb et al. (2000) recreated a model of increased muscle activity prior to death by electro-stimulation of rainbow trout immediately after slaughter. Results showed lower pH and higher L^* values (i.e., paler color) of the fillets obtained from electro-stimulated fish throughout the whole study (up to 75 h). The lighter color, as discussed by the authors, was due to denaturation of muscle



proteins and loss of fluids, which resulted in changes in the reflection of light from the meat surface. This statement was supported by a study of Daskalova and Pavlov (2015) which revealed that the lack of significant differences in ultimate pH and levels of liquid loss was further associated with similar L^* values.

Meat texture

The changes in fish meat texture (incl. higher gaping scores) which could be related to stress and increased muscle activity prior to death are also a considerable quality problem. Unfortunately, this issue is still not fully understood. Roth et al. (2006) reported that Atlantic salmon subjected to a combination of stress factors showed significantly higher gaping score and softer texture as measured by shear force (5.6N vs. 12.5N in rested fish) after 4 days of storage on ice. Interestingly, post-mortem electro-stimulation of rested salmon led to better quality parameters despite the earliest onset of rigor mortis. Based on this observation, authors concluded that post-mortem energy metabolism and rigor mortis might not be the main factors for activating muscle proteases, and the meat softening observed in stressed salmon could be due to physical stress of the myofibrils and connective tissues as a result of vigorous muscle activity prior to slaughter. As it was discussed above, some lysosomal cathepsins play a leading role in post-mortem proteolysis in fish. Lerfall et al. (2015), suggested that stress-related flesh softening might be a result of increased cathepsin L activity due to enhanced release of cathepsin L over time induced by some stress-related factors, such as low initial muscle pH and high blood lactate concentrations. Moreover, Bahuaud et al. (2010) reported a significant inverse correlation between muscle pH values and cathepsin B + L activity, incidence of myofibre–myofibre detachments, as well as myofibre–myocommata detachments, and a significant positive correlation between pH and fillet firmness. It was also found that the activity of some other enzymes, such as collagenase-like enzymes, could be slightly influenced by handling stress and it was significantly elevated at pH 7 than at pH 6. Cathepsin B- and B/L-like enzymes, on the other hand, showed higher activity at pH 6 than at pH 7, but might not be affected by pre-slaughter stress (Hultmann et al. 2012). Interestingly, according to the results from the same study, flesh hardness was significantly reduced in stressed fish compared to control group despite the insignificant differences in enzyme activity. The exact reasons for this discrepancy could be an object of further research. However, it can be suggested that stress-related fish meat softening is mainly associated with increased cathepsin B and L activity, as stress in fish is usually manifested by lower initial (and sometimes ultimate) pH values which are often closer to the optimal pH ranges for these enzymes.

Meat freshness

A number of studies have shown that stress in fish might also be associated with a faster loss of meat freshness. This fact is not surprising, since it is mainly a result of the acceleration in post-mortem metabolism, i.e., accelerated breakdown of ATP, enhanced proteolysis, etc. In this relation, levels of ATP, ATP-related compounds and K value have been commonly used as indicators of freshness. Digre et al. (2011) reported that Atlantic cod subjected to chasing and reduced water quantity had significantly lower levels of ATP and higher levels of IMP (inosine monophosphate) at the day of slaughter. After 7 days of ice storage, the stressed cod showed faster loss of freshness demonstrated by significantly lower IMP contents and higher K value as compared with the control or anesthetized group. Similarly, studies in silver carp (*Hypophthalmichthys molitrix*) and sea bass showed that slaughter procedures provoking aversive behavior, such as asphyxia and gill cut without prior stunning, were associated with faster degradation of ATP, enhanced activity of adenosine monophosphate deaminase and acid phosphatase, and rapid accumulation of IMP and end products—HxR (inosine) and Hx (hypoxanthine) (Zampacavallo et al. 2003; Zhang et al. 2017). As pointed by Zhang et al. (2017), IMP is the main umami substance of fish products, whereas HxR and Hx are the main substances causing off-flavor. Thus, the increased activity of acid phosphatase leading to faster hydrolysis of IMP to HxR should be inextricably linked to a rapid loss of desired flavor.

According to a study of Özogul and Özogul (2004) stressful slaughter procedures such as immersion in ice slurry may lead not only to a rapid ATP depletion and higher K value, but also to higher levels of some biogenic amines in the muscle tissue. This finding was probably associated with greater activity prior to death leading to favorable conditions for growth of microbial flora involved in decarboxylation of the available amino acids. Furthermore, eels (*Anguilla anguilla* L.) slaughtered by the commercial method called “salt-



bath” showed accelerated lipid oxidation demonstrated by strong increase in TBARS (thiobarbituric acid reactive substances) levels. Although the observed muscle pH was far lower than the optimal for the enzyme lipoxygenase, it was suggested that the physical damage to the muscle increasing the cell ruptures and accessibility of catalytic enzymes was a possible cause of the enhanced lipid oxidation (Morzel and Van de Vis 2003). Giuffrida et al. (2007) found that the greater ATP depletion was associated with more intense lipid oxidation of smoked trout and fresh gilthead seabream. The authors concluded that the rapid consumption of ATP induced accelerated conversion of xanthine dehydrogenase to xanthine oxidase which, in turn, could produce hydroxyl radicals in the presence of oxygen and redox iron.

Conclusion

The present work provides insights into stress-related mechanisms and pathways leading to alterations in post-mortem metabolic processes and some of the important meat quality parameters. The capacity of fish to be aware of pain, fear and suffering is still debatable. However, they are probably sentient beings, as it has been proved by a number of neurobehavioral studies. Moreover, neuroendocrine stress responses in fish are very similar to those in mammals and poultry leading to similar changes in the course of post-mortem metabolism and some meat quality traits. The most probable mechanisms and alterations arising under stress conditions can be briefly summarized, as follows:

1. Pre-mortem depletion of a large amount of energy reserves resulting in accelerated post-mortem metabolism;
2. Lower initial and ultimate muscle pH, followed by a rapid increase;
3. Rapid rigor mortis onset, followed by its early resolution > impossibility to process the fish during the pre-rigor period, leading to reduced yield, increased incidence of gaping and impaired water-holding capacity;
4. Accelerated activity (and sometimes rapid autolysis) of some proteolytic enzymes, lower RyR1 gene expression and reduced meat water-holding capacity;
5. Denaturation of muscle proteins and liquid loss due to low pH levels > changes in the reflection of light from the meat surface resulting in paleness;
6. Physical stress of the myofibrils and connective tissues as a result of vigorous muscle activity prior to slaughter > activation of muscle proteases > meat softening. It can be hypothesized that the increased cathepsin B and L activity is the main reason for occurrence of stress-related fish meat softening;
7. Rapid ATP depletion and physical damage to the muscles > faster accumulation of HxR, Hx, biogenic amines, TBARS > loss of meat freshness.

Further elucidations of the described processes and mechanisms would be helpful to understand in detail the exact relations between stress and meat quality alterations, as well as to find the proper solution for the commercial practice.

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