

Humane Harvesting Initiative: The Influence of Humane Harvesting on Fish Quality, Nutritional Value and Safety

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Abstract

Recently, fish welfare particularly during pre-slaughtering and slaughtering procedures is become prominent in fishery and seafood industry. The response to stress starts in the brain and is followed by biochemical changes in the blood and subsequent behavioral changes. There are differences between fish welfare in aquaculture and fisheries. In aquaculture, the welfare could be controlled by managing the crowding, feeding, handling, disease, transportation, drug administration, water quality, temperature fluctuations, harvesting or slaughtering. However, in case of fisheries, the welfare is associated with pre-harvesting (pre-slaughtering) and slaughtering procedures. Stressful conditions result in biochemical changes in plasma followed by the texture and nutritional value changes in. Due to stress, the physical movement of muscles will be increased, the energy sources (mainly adenosine triphosphate (ATP)) are depleted, and lactic acid in muscles increased which can drop the post mortem pH levels in muscle. In addition, there is a relationship between fish welfare and food safety risk for consumers. Due to the stress, the intestinal microflora change, which the pathogenic bacteria can be colonized which the safety of the final products can be influenced. Understanding the biochemical and physical changes during the fish harvesting and slaughtering can provide this opportunity for industry to control the stressful conditions to provide high quality seafood products with higher nutritional value and safety are presented.

Keywords: Fish; Welfare; Biochemical Changes; Quality; Safety

Introduction

2012 estimates put the global production of fisheries and aquaculture at 158 mmt, with 91.3 mmt of that amount credited to capture and fisheries [1]. Recently, fish welfare has been gaining interest from both consumers and the fisheries industry (producers) concerning the production, processing, quality, shelf life and nutritional value [2]. Technically, it is difficult to define and measure the welfare of fish in fisheries. According to [3], welfare is defined as "the balance between positive and negative experiences or affective states. The state of this balance may range from positive (good welfare) to negative (bad welfare)." In the case of fisheries, good welfare means good production at the end of the fish's life, and is determined by the handling of two important processes: the pre-slaughter procedure and the final slaughter method employed. If these two steps are conducted in bad conditions, fish will experience stresss which can strongly impact their quality and nutritional value. However, there are instructions, rules and methods for terrestrial animal welfare and humane slaughtering, even if they haven't been effectively made available.

History of Animal Welfare in USA

The first attempts at humane slaughtering and improved animal welfare practices date back to the late 1800s when several new organizations were established in the United States, many linked to the Society for the Prevention of Cruelty to Children, which was founded by Mary Ellen Wilson in 1873. Four years later in 1877, representatives from 27 humane organizations in 10 states gathered for a meeting that ultimately resulted in the founding of the American Humane Association. Instantly, that association began to address inhumane treatment of farm animals. The American Humane Association, now a national non-profit organization dedicated to addressing the welfare of both children and animals, has continued the fight ever since. Its mission is to prevent cruelty, abuse, neglect, and the exploitation of children and animals, and to ensure that their interests and well-being are humanely guaranteed by an aware and caring society.

- On October 9, 1877, the first national humane organization, the American Humane Association, was established in Cleveland, Ohio by representatives from 10 states. The primary aim was to guarantee humane treatment for farm animals and livestock.
- In 1894, the link between violence against animals and violence against people was first identified.
- In 1898, under the pressure from the American Humane Association, Congress banned the practice of vivisection in schools.
- In 1915, the American Humane Association initiated a movement called Be Kind to Animals. It is still celebrated nationally during the first full week of May.

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- In 1916, the American Humane Association was invited by the U.S. Secretary of War to develop humane rules for Army animals similar to those the American Red Cross employs in its treatment of soldiers. Hence, the American Red Star Animal Relief was developed to rescue wounded animals in World War I.
- In 1920, the Red Star program initiated the rescue of animals caught in disasters, and started fundraising to save and provide for thousands of elk in Yellowstone National Park.
- In 1925, the American Humane Association started a committee to address animal cruelty during animal training.
- In 1935, the American Humane Association began a study aimed at banning the application of toxic chemicals to control predatory animals. Following an incident which caused 1400 lambs to freeze to death during transportation, the Association pressured both the Interstate Commerce Commission and Bureau of Industry to protect livestock during shipping.
- In 1937, the American Humane Association's Red Star rescued and fed animals from a flood near the Mississippi River.
- In 1940, after filming "Jesse James" in 1939, a film in which a horse was killed, the American Humane Association opened a new Western Regional Office in Hollywood, California. The office work to stop cruelty to animals in film and television specifically. President Franklin D. Roosevelt signed a law protecting the bald eagle.
- In 1941, following the US entry into World War II, the American Humane Association's Red Star trained more than 400 civilians in the use of animal aids while providing millions of copies of Air Raid Precaution and Wartime Diet for Pets.
- In 1946, due to the strike by railroad workers which caused many animals to be temporarily transported in inhumane conditions, the American Humane Association volunteered to provide food and water for a number of animals.
- In 1951, the first "stamp of approval" was developed by the American Humane Association's Western

Regional Office. It certified films as practicing humane methods with regard to its animal actors.

- In 1952, using animals as test subjects in a cigarette study was banned.
- In 1958, the Humane Slaughter Act was signed into law requiring animals to be stunned unconscious prior to slaughter.
- In 1959, The Royal SPCA in England and the American Humane Association in the US developed an international society for the animal protection.
- In 1966, the American Humane Association helped prevent pets from being stolen and used as research test subjects.
- In 1970, overcrowding of animals was banned.
- In 1976, more protections were added to animal transportation protocols, providing more humane conditions.
- In 1977, the American Humane Association celebrated its centennial.
- In 1984, the first National Horse Abuse Investigation School was established.
- In 1988, the first formalized guidelines for animal welfare in the film industry were issued.
- In 1990, the first National Cruelty Investigation School for animals was initiated.
- In 1991, guidelines were developed for the welfare of those pets belonging to military reservist sent to the Middle East during the Operation Desert Storm.
- In 1998, No Animals Were Harmed website was started.
- In 1999, guidelines were developed for advertising agencies that use animals in advertisements.
- In 2000, the farm animals program was initiated by the American Humane Association to establish standards for the treatment of animals in agriculture.

- In 2011, the Animal Welfare Research Institute was established to study the methods for improving the quality of life for animals.

The act passed by Congress in 1958 (P.L. 85-765; 7 U.S.C. 1901 et seq), details what qualifies as "humane slaughtering," saying:

"No method of slaughtering or handling in connection with slaughtering shall be deemed to comply with the public policy of the United States unless it is humane.

Either of the following two methods of slaughtering and handling are hereby found to be humane:

- (a) In the case of cattle, calves, horses, mules, sheep, swine, and other livestock, all animals are rendered insensible to pain by a single blow or gunshot or an electrical, chemical or other means that is rapid and effective, before being shackled, hoisted, thrown, cast, or cut; or
- (b) By slaughtering in accordance with the ritual requirements of the Jewish faith or any other religious faith that prescribes a method of slaughter whereby the animal suffers loss of consciousness by anemia of the brain caused by the simultaneous and instantaneous severance of the carotid arteries with a sharp instrument and handling in connection with such slaughtering."

Meanwhile, many states have their own rules and regulations. In Table 1, different states' rules are presented with date of enactment, methods, religious exemptions, animals covered and penalty.

Technically, animal welfare practices and slaughtering techniques are linked to the quality of the end product. Like terrestrial animals, fish should be stunned before and during slaughter to decrease fear, stress, and movement, as well as to increase the welfare and quality of the product [4].

State	Citation	Date Enacted	Allowed Methods	Animals Covered	Penalty
AZ	AZ ST § 3- 2002 – 2017	1956	Single blow or gunshot or an electrical, chemical or other means that is rapid and effective.	Cattle, calves, horses, mules, sheep, swine, and other livestock.	No penalty is listed under the humane slaughter statute
СА	CA FOOD & AG § 19501 – 19503	1967	Captive bolt, gunshot, electrical or chemical means,	All cattle, calves, horses, mules, sheep, swine, goats, or fallow deer	Not listed in laws, but may be in rules
со	CO ST § 35- 33-203 – 407	1989 Note: this section is repealed July 1, 2009 pursuant to § 35- 33-407	Method whereby an animal is rendered insensible to pain by a mechanical, electrical, chemical, or other means that is rapid and effective. Cannot use a manually operated hammer, sledge, or poleax.	"Livestock" means cattle, calves, sheep, swine, horses, mules, goats	In addition to criminal penalties prescribed, violation incurs a civil penalty of not more than \$750 per violation for each day of violation.
FL	F. S. A. § 828.22	1961	A method whereby the animal is rapidly and effectively rendered insensitive to pain by electrical or chemical means or by a penetrating captive bolt or gunshot with appropriate caliber and placement.	"Livestock" means cattle, calves, sheep, swine, horses, mules, goats, ostriches, rheas, emus	Any person who violates the Act and any associated rule is subject to an administrative fine of up to \$10,000 for each violation.
IA	IA ST § 189A.18 - 189A.22	1965	Limited to slaughter by shooting, electrical shock, captive bolt, or use of carbon dioxide gas.	"Livestock" means alive or dead animal which is limited to cattle, sheep, swine, goats, farm deer	Any person who violates any provisions of this chapter for which no other criminal penalty is provided shall be guilty of a simple misdemeanor.
IL	IL ST CH 510 § 75/0.01 - 75/8	1967	A method whereby the animal is rendered insensible to pain by gunshot or by mechanical, electrical, chemical or other means that is rapid and effective.	Cattle, calves, sheep, swine, horses, mules, goats, and any other	Any violation of this Act or of the rules and regulations is a petty offense.
IN	IN ST 15- 2.1-24-1 - 15-2.1-24- 33	1996	State board establishes rules that require animals to be rendered insensible to pain prior to severance of the carotid artery.	Livestock and poultry (according to the Purpose of Chapter, Sec. 15-2.1-24-1(3)).	A person who recklessly violates this chapter commits a Class B misdemeanor.
KS	KS ST § 47- 1401 –	1961	A method whereby the animal is rendered	Cattle, calves, sheep, swine, horses, mules,	Misdemeanor (exact terms not specified).

	1405		insensible to pain by	goats, aquatic animals	
	1405		mechanical, electrical,	goats, aquatic ammais	
			chemical, or other means		
			that is rapid and effective.		A
MD	MD Code, Agriculture, § 4-101 – 4-131	2002	A method by which livestock are rendered insensible to pain, by a single blow or gunshot, or by an electrical, chemical, or other rapid and effective. Humane method" does not include the use of a manually operated hammer, sledge, or poleax during a slaughtering operation.	"Livestock" means cattle, calves, sheep, swine, horses, mules, goats, or other animals that may be used in the preparation of a meat product. "Livestock" does not include poultry or other fowl.	A person who violates this section is guilty of a misdemeanor and on conviction is subject to a fine not exceeding \$100 for each violation. The Secretary may refuse to provide or may suspend temporarily inspection services for an establishment that violates this section with respect to the slaughter of livestock.
ME	ME ST T.22 § 2521 - 2521-C	1999	Rendering animals insensible to pain by a single blow or gunshot or an electrical, chemical or other means that is rapid and effective.	Limited to "livestock" which is not defined in the section.	Not specified/no penalty given.
MI	MCL § 287.551	1962	A method whereby the animal is rendered insensible to pain by mechanical, electrical, chemical or other means that is rapid and effective. The use of a manually operated hammer, sledge or poleax is declared to be an inhumane method of slaughter within the meaning of this act.	"Livestock" means cattle, calves, sheep, swine, horses, mules, goats and any other animal which can or may be used in and for the preparation of meat or meat products.	Any person who violates any provision of this act shall be guilty of a misdemeanor.
MN	MN ST § 31.59 - 31.592	1961	Any method of slaughtering livestock which normally causes animals to be rendered insensible to pain by a single blow of a mechanical instrument or shot of a firearm or by chemical, or other means that are rapid and effective. The use of a manually operated hammer or sledge is declared an inhumane method of slaughter.	"Livestock" means cattle, horses, swine, sheep and goats.	A person violation the Act is guilty of a misdemeanor and shall be punished accordingly.
NH	NH ST § 427:33 –	1960	Any method of slaughtering livestock which normally	"Livestock" means cattle, horses, swine,	Unspecified Misdemeanor.

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	37		causes animals to be rendered insensible to pain by a single blow or shot of a mechanical instrument or by electrical, chemical or other means that is rapid and effective.	sheep, goats, as well as domesticated strains of buffalo, bison, llamas, alpacas, emus, ostriches, yaks, elk (cervus elephus canadensis), fallow deer (dama dama), red deer (cervus elephus), reindeer (Rangifer taradus), and other species of animals susceptible of use in the production of meat and meat products.	
NJ	NJ ST 4:22- 1 et seq.	1968	Not specified in the Act. Provides that the State Board of Agriculture shall develop and adopt (1) standards for the humane raising, keeping, care, treatment, marketing, and sale of domestic livestock; and (2) rules and regulations governing the enforcement of those standards.	For the purposes of this act, "domestic livestock" means cattle, horses, donkeys, swine, sheep, goats, rabbits, poultry, fowl, and any other domesticated animal deemed by the State Board of Agriculture and the Department of Agriculture.	No person may be cited or arrested for a first offense involving a minor or incidental violation, as defined by rules and regulations unless that person has first been issued a written warning.
он	OH ST § 945.01 - 945.99	1965	In the case of cattle, calves, horses, mules, sheep, swine, and other livestock, all animals are rendered insensible to pain by a single blow or gunshot or an electrical, chemical, or other means that is rapid and effective.	Cattle, calves, horses, mules, sheep, swine, and other livestock	Subject to fine not more than one hundred dollars (\$100).
OR	OR ST § 603.010 - 603.992	1973	Renders each such animal insensible to pain by a single blow or gunshot or by an electrical, chemical or other means that is rapid and effective.	Cattle, equines, sheep or swine.	Violation of ORS 603.065 (the humane slaughter law) is a Class B misdemeanor.
РА	PA ST 3 Pa.C.S.A. § 2361	1996	Humane methods are required, but undefined. However, the use of a manually operated hammer, sledge or poleax by slaughterers, packers or stockyard operators during slaughtering operations is	The term livestock is undefined.	Not listed in the law, but may be provided by departmental regulations.

			not a humane method of slaughter.		
RI	RI ST § 4- 17-1 - 4- 17-7	1961	A method through which the animal is rendered insensible to pain by mechanical, electrical, chemical or other means that is rapid and effective.	Cattle, cows, sheep, swine, horses, mules, goats and any other animal which can or may be used in and for the preparation of meat or meat products.	Subject to a fine of not more than five hundred (\$500) dollars, or by imprisonment for not more than one year.
VT	VT ST T. 6 § 3131 - 3134	1987	A method whereby the animal is rendered insensible to pain by mechanical, electrical, chemical or other means that is rapid and effective.	"Livestock" means cattle, calves, sheep, swine, horses, mules, goats, fallow deer, American bison and any other animal which can or may be used in and for the preparation of meat or meat products.	A person who violates this chapter shall be fined not more than \$100.00 nor less than \$50.00.
WA	West's RCWA 16.50.010 - 16.50.900	1967	A method whereby the animal is rendered insensible to pain by mechanical, electrical, chemical or other means that is rapid and effective. The use of a manually operated hammer, sledge or poleaxe is declared to be an inhumane method of slaughter.	"Livestock" means cattle, calves, sheep, swine, horses, mules and goats.	A misdemeanor and subject to a fine of not more than two hundred fifty dollars or confinement in the county jail for not more than ninety days.
wv	WV ST § 19-2E-1 - 19-2E-7	1982	Livestock, before being shackled, hoisted, thrown, cast or cut must be rendered insensible to pain by a single blow, gunshot or by electrical, chemical or other means that is safe, rapid and effective.	Cattle, swine, sheep or goats.	The section provides a graduating scheme of penalties for violation: - a first offense results in a misdemeanor punishable by a fine of \$100 - \$500. - a second offense results in a misdemeanor with a fine of \$500 - 1,000 <i>and</i> suspension of the license

Table 1: The States' Animal Slaughtering Rules (Adopted from Animal Legal & Historical Center, 2006).

Welfare measurement

As discussed above, the definition of welfare is not clear or straightforward [2]. There are two major issues when it comes to defining and measuring animal welfare [5-9]. Generating appropriate guidelines for animal welfare depends on resolving these issues. There are differences between terrestrial animals and fish, but the five freedoms developed in the UK regarding terrestrial animals could easily be applied to fish. The five freedoms include freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury, and disease, freedom to express normal behavior and freedom from fear and distress [9]. All five could easily be applied to fish from aquaculture. However, for those harvesting from natural resources like fisheries, it's more complicated. The five freedoms are applied as a scientifically accurate index for measuring animal welfare [9]. For example, by periodically checking the environment of animals and their physical status within it, their welfare could be determined. However, good animal welfare can go beyond the physical aspects and includes mental changes, which is more complicated when it comes to fish [9]. The concept of animal welfare has been applied to those animals we believe can feel and experience pain, fear, stress and suffering. There is scientific evidence that fish also experience and feel pain, stress and suffering, and that they respond to the stressors in physical, behavioral and physiological scales [10-12]. Therefore, when fish respond to the stressors, it is hypothetically possible to measure their state of welfare.

Response to Stress

The response to stress starts in the brain and is closely followed by biochemical changes in the blood and subsequent behavioral changes. In aquaculture, the stress could be related to fish crowding, feeding, handling, disease, transportation, drug administration, water quality, temperature fluctuations, harvesting or slaughtering. However, when it comes to fisheries, the stressful conditions are related to pre-harvesting (preslaughtering) and slaughtering procedures. The response to stress starts with the release of catecholamines and activation of the hypothalamic-pituitary-interrenal (HPI) axis. Then, corticotropin released from the hypothalamus stimulates the pituitary gland to synthesize and release corticotropic hormones, which then stimulates the synthesis and mobilization of lipid and glucocorticoid hormones (cortisol in teleosts) from the interrenal cells [13,14]. The energy source mobilization includes the lipid, so changes in plasma-free fatty acids (FFA) could be a stress condition index if not for the fact that the

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parameter generally does not show a clear response [2]. Secondary and tertiary stress responses, as well as many adverse changes in the quality of the fish, will be initiated by both catecholamines and cortisol [15].

HPI activation results in energy source mobilization, depletion of glycogen stores, and an increase in plasma levels of glucose, along with high muscle activity which can cause anaerobic glycolysis and, as a result, an increase in plasma lactate and a big drop in muscle pH. Therefore, the levels of blood cortisol, glucose and lactate are often used to assess stress levels [16-18]. It has been shown that through diet modulation, and the use of supplements like vitamin C and vitamin E, the fish's resistance to stressors will be increased [19-21]. It cannot, however, be applied for the fisheries industry.

Catecholamines (adrenaline and noradrenaline) are not commonly used as stress indicators because they are not easy to determine and are quickly removed from the blood [14]. Cortisol is widely used as both a long-term and short-term stress condition index [22,23]. While under stress, heart rate will increase and the need for greater oxygen intake will cause a boost in the number of moving erythrocytes and of the hematocrit value, which can also being used as an indicator for stress. A rapid death does not allow a completion of the secondary effects of stress, and pre-slaughter stress always has to be considered in the stress evaluation at slaughter by the hematic parameters.

The effect of Stress on Fish Quality and Nutritional Value

Along with the biochemical changes in plasma due to stress, the texture and nutritional value of the final product will also be adversely influenced by stress and inhumane harvesting practices. Due to stress, the physical movement of muscles will be increased, depleting energy sources, mainly adenosine triphosphate (ATP), and, as discussed above, increasing the lactic acid in muscles and dropping their post mortem pH levels. Hence, those fish which face stressful conditions during harvesting go into rigor mortis rapidly, which negatively effects the quality of the fillets with drip loss and a softening of the muscle texture [24-29]. Harvesting time is critical for fish in terms of stress, and the amount of stress experienced by a fish depends heavily on the duration, struggle and crowding of the harvesting process. Labored swimming during harvesting causes an intense use of white muscles and results in anaerobic glycolysis followed by increase in lactic acid and less muscle pH (Figure 1).

In contrast, in humanely slaughtered fish, the reduction of stress means that only some muscles will enter rigor mortis while others do not. This difference in timing results in a higher quality fillet in terms of texture. Another physical attribute that can change with harvesting techniques is the color of the fillet. It has been shown that pre-harvesting and harvesting stress significantly affects salmonids fillet color [30,31] which may be due to the insolubilization of muscle proteins as a result of low pH and drip loss that occurs in the development of prerigor and rigor mortis [32].

In addition, harvesting and post harvesting stress exposes the flesh of fish to the oxidation of polyunsaturated fatty acids (PUFAs), which can result in the production of reactive oxygen metabolites (ROMs). The production of ROMs is proven to induce severe damage in nucleic acid, proteins and lipids, and act as a carcinogen leading to inflammation, glomerular disease and aging for consumers [29,33-36]. In fish with a low level of activity, only some muscles have been used. These are the first muscles to enter rigor mortis, while the others do so later. Because of this difference in timing, not all muscles enter rigor mortis at the same time, so the fish as a whole is less stiff [37]. What's more, stress conditions in harvesting and post harvesting processes can induce peroxidase and aldehyde production in the fish fillet during short storage, which can decrease the nutritional quality of the fish fillet. As a result, the nutrient quality will be decreased, as well as both the texture and flavor of the product due to the degradation and loss of fatty acids and protein. Hence, at the end of the procedure, both physical and nutritional quality will be altered by stress which can influence the marketing to health-conscious consumers. Bagni et al. (2007) [29] found that the ROM was higher in sea bass subjected to stress compared with those kept in normal conditions.

One of the major reasons for seafood consumption, particularly marine harvested fish from fisheries, is obtaining the high quality protein and PUFAs. Fish PUFAs have several benefits for humans. They include the ability to decrease blood triglyceride counts, cure rheumatoid arthritis, boostthe effects of antidepressants and combat the depressive symptoms of bipolar disorder. They also help develop the visual and neurological faculties in infants, improve the cognition in children with Attention Deficit Hyperactivity Disorder (ADHD), protect against Alzheimer's disease and reduce the risk of ischemic stroke, particularly in older people.



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The Relationship between Stress and Food Safety Risk

The relationship between stress and food safety risk has been studied by researchers in animal science. They have found that there is a link between stress and pathogenic bacteria which can increase the food safety risk for humans. The gastrointestinal tract is the main habitat of different bacteria, including both useful and pathogenic bacteria. Technically, the nervous system is controlling the digestive tracts microcirculation, motility and secretions-this is called the brain-gut axis. Since, the first response to stress starts in the brain, it can influence functional and inflammatory disorders as well as infection. Catecholamine hormones particularly norepinephrine are normally presented in the gastrointestinal tract. However, during the stress period, the nervous system of the digestive tract releases catecholamines, causing significant local increases. This increase can strongly influence the intestinal microbial populations. The pathogen can colonize through different methods following stress, including suppression of the immune system, and promoting physiological alterations in the gastrointestinal tract, increasing their virulence and multiplication rate in the gut. Consequently, exposure of farm animals to stressors will lead to increased levels of foodborne pathogens in the gastrointestinal tract and increased risk of contamination of their carcasses [38]. This is important for farmed animals and aquaculture sectors, but it's worthwhile to predict the stressful conditions in pre-harvesting procedure which might include crowding, duration, and fish movement.

The influences of the stress on nutritional value and filet quality were discussed earlier, however, the preharvesting and slaughtering methods can influence the microbial growth in fillet too. In 2009, the European Food Safety Authority discussed an opinion related to the influence of farmed fish slaughtering and food safety risk relationship (EFSA 2009). After slaughtering, postmortem chemical changes begin to take place, including glycolysis, enzymatic activity, nucleotide catabolism. pH drop and an increase in concentration of free non-protein nitrogen (NPN) compounds. The direct relationship between post-mortem changes and fillet quality has been proven, however, these biochemical changes also influence the food safety levels. The level of glycolysis inside the fish at the time of slaughter has a direct effect on the level of NPN, with high levels supporting bacterial growth.

Pre-Harvesting and Harvesting

The term "harvesting" is related to the slaughtering of fish, while "pre-harvesting" includes all activities the fish experiences between catch and slaughter. The term "slaughter," or "slaughtering," is used in reference to the termination of an animal life for human consumption. "Euthanasia" is the term used in reference to the humane killing of an animal which is not intended for human consumption. These days, animal slaughtering is an interesting topic for both consumers and the industry, not only in terms of ethics, animal welfare and humane harvesting, but also in terms of the quality, nutritional value and consumer health. Inherently, slaughter is a highly stressful moment for fish [39]. An important part of the slaughter process is decreasing the time between harvesting and slaughtering with fast stunning. Technically, there are two slaughtering methods, fast and slow. Fast methods are being used for big fish, however, it's not always possible to apply this method for small fish. Some of the slow methods include asphixiation in air, asphixiation in ice, exsanguination, carbon dioxide narcosis, evisceration, decapitation, anesthetics, salt or ammonia bath, anoxic water bath, electro-immobilisation and electro-stimulation as well as electro-fishing [40]. These methods are slow, inhumane and do not promote improved animal welfare. The slaughtering procedure influences on fish welfare could be shown as a model (Figure 2) [41].



In this model, section A shows that fish is killed in an un-humane condition in which fish is conscious, while it should be stunned before that; section B is an ideal condition, in which fish has been stunned and is killed or dies after it is unconscious; in C fish has been stunned but it can recover its consciousness and shows un-humane slaughtering; in section D a fish is killed in un-welfare way such as asphyxia while it is conscious but has also suffered from the aversive nature of the stunning method; in section E fish that has been stunned and is killed or dies after it is unconscious but has also suffered from the unhumane stunning method.

Asphixiation or Death in Air

This traditional method for slaughtering fish is based on leaving fish in the air to die. It is the most stressful slaughtering method, with fish experiencing prolonged suffering before death. The death time depends on the hypoxia resistance which is based on on species and temperature. This method is inhumane and can adversely influence the fish quality and shelf life of the products. Some fish, such as eel and carp are resistant to hypoxia, however most other fish are sensitive to hypoxia conditions. Due to the higher metabolism and faster consumption of oxygen, at higher temperatures, death will occur faster once hypoxia sets in. Lower muscle pH, faster rigor mortis and higher muscle lactate were all observed in those fish slaughtered via this method [40,42].

Asphixiation in Ice

Another method for slaughtering fish is to immediately place them in ice after harvesting. Consequently, the body temperature, metabolic rate, oxygen requirement and movement will all be decreased. The death time is prolonged, however. This method is not humane. Since the death time is prolonged, fish can still feel pain, stress and fear. Hence, it is not recommended [2,40].

Electrical stunning and electrocution

This method is used for fresh water fish due to the high conductivity of freshwater for stunning with electricity [2,43]. This method is fast, however, it can cause a violent reaction, including an open mouth and opercula as well as blood spots and other color changes along the backbone muscles. In addition, applying electricity could be harmful for workers.

Bleeding or exsanguination

Many large fish such as tuna and Atlantic salmon are subjected to bleeding by the cutting of gills or veins and left to die in the water. The most common method is bleeding after stunning, however, in many regions, producers use bleeding methods without any prestunning method [40]. Bleeding alone is a slow slaughtering method which can induce stress, pain and fear. However, the quality of the final product is high. This method, employed alone, is inhumane. According to the research, there is no scientific evidence which shows that exsanguination without stunning produces a better result than exsanguination after stunning [40]. Studies about the quality of red and white muscles showed that the bleeding of an animal without a heartbeat is similar to the bleeding with a heartbeat [40,44,45]. Hence, it is strongly recommended to use bleeding after stunning the fish [40]. There are other slaughtering methods which are fast and more humane, including percussive stunning, hydraulic shock, spiking, shooting and electrical stunning [40].

Percussive stunning

This is one of the most common slaughter methods used in the salmon industry, in cod and halibut fisheries, and by anglers [40,46]. After removing the fish from the water, a club will be immediately used, either by workers or an automatic machine, to strike the fish's brain, instantly stunning it. It has been shown that the quality of the fish fillet was higher after applying percussive stunning [40,47].

Hydraulic stunning

This method uses a hydraulic device to stun the fish. However, due to the damage in muscles, the backbone, gut and gas bladder, fish quality will be negatively influenced [40]. Robb, et al. [40] compared different slaughtering methods in terms of humane practice and resulting quality (Table 2). Some of these methods have both low negative impacts on welfare and quality, however, due to some limitations their application is not appropriate. For example, using medicine for fish anesthetics is humane and has a minimal impact on the fish quality, but, due to the regulations, is not recommended. In addition, some other methods such as spiking, shooting and electrical stunning have a low negative effect on welfare and quality, but due to the difficulties in practicing, they are not appropriate methods at an industrial scale. It has been recommended that using two humane methods such as percussive stunning and bleeding not only provides the humane slaughtering aspect, but can also increase the quality of the final products due to the better texture, color and nutritional value. Each of these methods has its own advantages and limitations.

In many cases, the method can decrease the stress and increase the fish welfare, but due to the technical demands, cost, low efficiency, and extensive labor required, its application on an industrial scale is not

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Method ¹	Negative Impact on Welfare	Negative Impact on Quality	Negative impact on Food Safety
Asphyxiation	High	High	Low
Asphyxiation in ice	High	Low	Low
Exsanguination	Very High	High	High
Carbon dioxide narcosis	High	High	Low
Evisceration	Very High	High	High
Decapitation	Very High	-	High
Anesthetics	Very Low	Very Low	Very Low
Salt or ammonia bath	Very High	High	Low
Desliming in eel	High	High	Low
Electro-immobilisation	Very High	Very High	Low
Percussive stunning	Low	Low	Very Low
Hydraulic shock	Very Low	Very High	Low
Spiking, coring, ikejime	Low	Low	High
Shooting	Low	Low	High
Electrical stunning	Very Low	Low	Low
Combined method Percussive-Bleeding	Very Low	Very Low	Low

possible. By combining two or three methods, industry can overcome these limitations.

Table 2: Comparison of different methods of slaughtering on fish welfare and quality.

Conclusion

Seafood is one of the most important natural vectors for high nutritional value protein and omega-3s for human. Since fish can feel pain and stress, these factors can easily influence their quality, nutritional value, shelf life and consumption safety. On the other side, the fisheries' efforts to harvest fish in ways that maintain the quality result in not only high value products for consumers, but also a decreased carbon footprint for the industry. A sustainable fisheries and seafood production chain will be established. Once the quality begins to deteriorate, it is almost impossible to reclaim. On the other hand, deterioration which includes lipid oxidation and protein denaturation is an irreversible procedure which should be controlled from the very beginning of harvesting. Some pioneering companies, like Blue North Fisheries, have developed a novel approach called "Moon Pool" as part of pre-slaughtering procedure to decrease the fish's stress before bringing the fish aboard. It seems there is a big space for humane fisheries in the seafood production supply chain to strongly improve the quality, nutritional value and safety of the final products. Sustainable fisheries practices could also provide a vantage point from which to consider animal welfare.

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