



Fish welfare: the state of science by scientometrical analysis

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ABSTRACT. In 2014, Brazil produced 474.33 thousand tons of captive-bred fish. In addition, regulatory agencies of animal ethics and welfare have recently encouraged experiments to be done using simpler vertebrates, such as fish. The aim of this article was to perform a scientometric analysis of scientific production that deals with fish welfare, in an attempt to find trends and gaps in this line of research. Our analyses showed a growing concern about fish welfare, although several questions remained inadequately covered. The most studied species was the Atlantic salmon, with Norway having the most publications on this theme. There are controversies among scientists about fish capacity for suffering and enjoyment (sentience). As regards slaughter or euthanasia, some studies showed that some methods are more endorsed than others, because they effectively reduce suffering and improve the appearance of the meat. In respect of animals used for experimentation, the most recommended substances were benzocaine and MS222. Thus, despite the importance of this subject, few studies are decisive and there is still no consensus on how to improve fish welfare or even on how to reduce suffering at the moment of slaughter.

Keywords: suffering, pain, euthanasia, stress, humane slaughter.

Bem-estar em peixes: o status da ciência por meio de análise cienciométrica

RESUMO. Em 2014, o Brasil produziu 474,33 mil toneladas de peixes em cativeiro. Além disso, há um recente incentivo dos órgãos reguladores da ética e do bem-estar animal para que experimentos sejam feitos com vertebrados mais simples, tais como peixes. O objetivo do presente trabalho foi realizar uma análise cienciométrica da produção científica que trata do bem-estar de peixes e se preocupa com eles, em uma tentativa de encontrar tendências e lacunas nessa linha de pesquisa. Nossas análises mostraram uma crescente preocupação com o bem-estar em peixes, apesar de várias questões continuarem deficientes. A espécie mais utilizada nos estudos foi o salmão do Atlântico, e o país com mais publicações nessa temática foi a Noruega. Percebe-se que existem controvérsias entre cientistas quanto à capacidade de sofrimento desses animais, a sentiência. Quanto ao abate ou à eutanásia, estudos mostraram que alguns métodos são mais aconselháveis que outros, pois efetivamente irão reduzir o sofrimento e melhorar a apresentação da carne. Para animais de experimentação, as substâncias mais recomendadas foram a benzocaína e o MS222. Assim, apesar da importância do tema, poucos estudos são definitivos, inexistindo ainda consenso sobre os métodos de melhoria do bem-estar em peixes e sobre como reduzir o sofrimento no momento do abate.

Palavras-chave: sofrimento, dor, eutanásia, estresse, abate humanitário.

Introduction

A fish is any member of a group of paraphyletic organisms that include non-tetrapod, aquatic animals, which present a cranium and most of the time, breathe through gills and which do not have members with digits. The many fish groups make up more than half the vertebrate species on Earth. The total number of species can add up to or even surpass 32,500 (Pough, Jani, & Heiser, 2008). For this reason it is hard to identify only one fish species used in animal production. Instead of terrestrial animal farming, of which the total global production is based on a limited number of mammals and poultry, aquaculture includes more than 240 animal

and plant species used directly in human consumption (Crepaldi et al., 2006).

The importance of fish meat for human consumption has changed fish farming into an essential food source, in addition to relieving the capture pressure over the natural stocks of some species (Hilsdorf & Orfão, 2011). The production of fish and other water organisms in captivity is increasing in Brazil. Accordingly, studies that use fish species, with many goals, have increased as well. In 2013, Brazilian aquaculture was included for the first time in the Municipal Stockbreeding Production annual report (PPM), of the *Instituto Brasileiro de Geografia e Estatística* [IBGE] (2013). Also

in 2014, Brazil produced 474.33 thousand tons of captive-bred fish, 20.9% more than in the previous year. Since 2013, the numbers of fish farms pointed to a new reality in pisciculture, which migrated to the North of the country, especially with the contribution of Rondônia state (IBGE, 2014).

The 2013 and 2014 productions data show that despite the relevant ichthyodiversity in Brazil, the most commonly used species in the different farming systems is tilapia, which is not native to South America (41.9% of national production, with 198,664 tons produced). Carp, which in 2007 occupied the second position (17% of national production) (Crepaldi et al., 2006), appears with only 4.4% of the 2014 production, having lost space to native fish, namely tambaqui (29.3%) and tambacu/tambatinga (8.5%). Other more than 14 fish types, mostly native to Brazil, occupy the remaining 16%. The increase in fish farming has led to concerns about fish welfare, in all production stages (IBGE, 2014).

But, who cares about fish welfare? The fish can feel pain and demonstrate suffering? Animal welfare has been defined as the balance between positive and negative experiences or affective states (Milot et al., 2014). The concern for animal welfare has increased, and until some consumers demonstrate willingness to pay more for fish welfare (Solgaard & Yang, 2011). However, the animal welfare debate has tended to focus on terrestrial species. Second Håstein, Scarfe, and Lund (2005), this can be attributed to the diversity of fish species, as cited above, to our understanding of practices involved in aquatic animal production, and the relative scarcity of scientific information, as well as difficulties in navigating policies, guidance and regulations affecting aquatic animal welfare.

On other hand, pain evaluation in fish is particularly challenging due to their evolutionary distance from humans, their lack of audible vocalization, and apparently expressionless behavior (Sneddon, Braithwaite, & Gentle, 2003). Many interesting studies have been published discussing the possibility of fish have nociceptors (reflex to a noxious stimulus) (Sneddon et al., 2003), and not only perceive the pain (Sneddon, 2011), but whether fish have emotions, and if so how they experience them (Braithwaite, Huntingford, & Bos, 2013; Kittilsen, 2013).

In relation to use of animal in research, there are recent social pressure to minimize the use of animal testing and the ever-increasing concern on welfare of the laboratory animals. The objective is one day in future not more use test in animals (Garcia-Reyero, 2015). During highly standardized

experiments, confounding parameters must be minimized which might lead to animals behaving unnaturally. Some authors show that substrate is suggested as a means to improve the olfactory environment, promoting fish welfare and enhance their activity (Meuthen, Baldauf, Bakker, & Thünken, 2011). Colored substrates, specially blue, may also attenuate stress response in fish of laboratory (Batzina et al., 2014).

Thus, the aim of this article is to perform a scientometric analysis of scientific production that deals with fish welfare, in an attempt to find trends and gaps in this line of research.

Material and methods

Searches were carried out in indexed databases of peer-reviewed articles: 'ISI – Web of ScienceTM' (<http://apps.webofknowledge.com/>) and 'SciELO' (<http://www.scielo.org/php/index.php>). The keywords searched were 'fish' and 'welfare', and 'bem-estar peixes'. The search covered every year from 1945 until the current search date (06/29/2016). Articles were refined as regards adherence to theme. Repeated papers were excluded. The reference list of the articles found was also considered. The abstract, and if necessary, the entire article was accessed to compile the important information in a spreadsheet. The more relevant information was computed to compose the revision. Recent publications of IBGE and the *Conselho Nacional de Controle de Experimentação Animal* [CONCEA] were consulted. This multidisciplinary council is of a normative, consultative, deliberative and appellate character, which is responsible for the use of animals and their welfare in teaching or scientific research.

Results and discussion

First, in ISI – Web of Science, we found 1,276 articles. We then excluded non-relevant articles, by abstract analysis, and 875 articles about fish welfare remained. In SCIELO, 16 articles were found. After comparison and exclusion by repeated reference and non-relevance, 851 articles remained, and offered all the information necessary for scientometric analysis.

Based on scientometry results, we see that fish welfare concerns began really in the 2000s (Figure 1A). The first publication found was from 1979 (Meyer, 1979) and cared more about the economic value of the fish than the well-being of the fish itself. In the 1980s, no publication was found. In the 1990s, on average, 2 publications were reported annually. In relation to fish welfare, Norway, with 23.5% of the papers, is the leader in publications among a total of 55 recorded countries (Figure 1B).

England and the USA appear with 11.97% and 11.85%, respectively. In this ranking, Brazil recorded 53 publications, the same number as Italy. The leadership of Norway is probably associated with the results of a model specie. Most publications (almost 20%) included *Salmo salar*, the popular Norwegian or Atlantic salmon (Table 1). Norway is the leading nation in salmon farming. In 2011 Norway produced approximately 60% of the world's farmed salmon and it is exported to over 100 countries worldwide (Ellingsen et al., 2015).

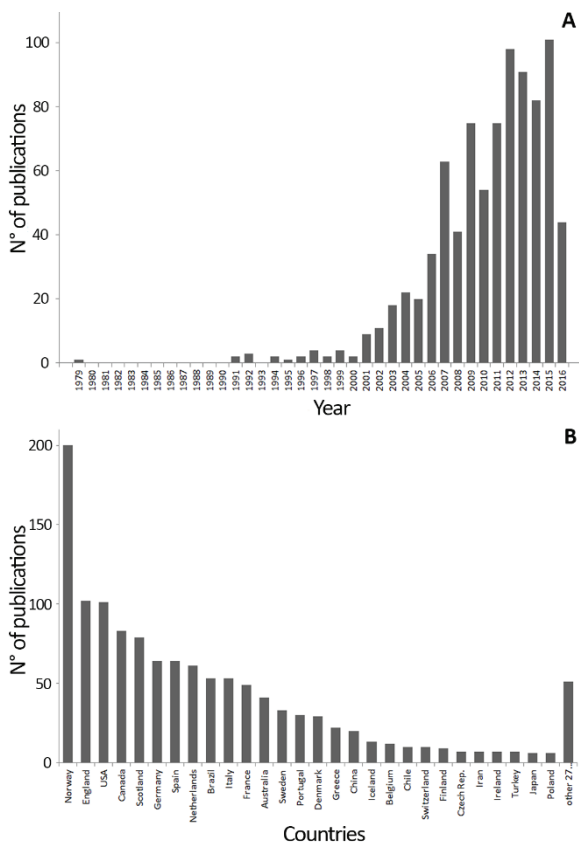


Figure 1. A. Distribution of publications by year. B. Distribution of publications by country.

In total, 141 fish species were reported (46 in more than one study), as can be seen in Table 1. The most studied fish were *Oncorhynchus mykiss* (rainbow trout), *Dicentrarchus labrax* (seabass), *Sparus aurata* (seabream) and *Danio rerio* (zebrafish). Four studies were carried out using hybrid fishes, and 37 dealt with more than one species simultaneously.

The results also show that more than 95% of the publications are in English. Other publication languages included German (19 papers), French (9), and Portuguese (7). Articles in Dutch, Spanish, Italian and Polish were also found. Of all the studies, at least 149 performed reviews, 88 took

another approach (without focusing on a single fish species), and at least 12 worked with models to maximize welfare (not directly with fish).

Table 1. Fish species cited in each publication. It was showed species that appears in two or more article. Other 95 species were cited in only one publication and are not presented in table.

Fish under study	N° of publications	%
<i>Salmo salar</i>	139	19.50
<i>Oncorhynchus mykiss</i>	72	10.10
<i>Dicentrarchus labrax</i>	46	6.45
<i>Sparus aurata</i>	32	4.49
<i>Danio rerio</i>	30	4.21
<i>Gadus morhua</i>	29	4.07
<i>Oreochromis niloticus</i>	23	3.23
<i>Cyprinus carpio</i>	20	2.81
<i>Clarias gariepinus</i>	13	1.82
<i>Solea senegalensis</i>	13	1.82
<i>Anguilla anguilla</i>	9	1.26
<i>Oreochromis mossambicus</i>	9	1.26
<i>Scophthalmus maximus</i>	9	1.26
<i>Salvelinus alpinus</i>	8	1.12
<i>Carassius auratus</i>	7	0.98
<i>Rhamdia quelen</i>	7	0.98
<i>Salmo trutta</i>	7	0.98
<i>Psetta maxima</i>	6	0.84
<i>Gasterosteus aculeatus</i>	5	0.70
<i>Hippoglossus hippoglossus</i>	4	0.56
<i>Micropterus salmoides</i>	4	0.56
<i>Seriola lalandi</i>	4	0.56
<i>Esox lucius</i>	3	0.42
<i>Hippocampus guttulatus</i>	3	0.42
<i>Oncorhynchus kisutch</i>	3	0.42
<i>Perca fluviatilis</i>	3	0.42
<i>Pimephales promelas</i>	3	0.42
<i>Acipenser brevirostrum</i>	2	0.28
<i>Arapaima gigas</i>	2	0.28
<i>Centropomus parallelus</i>	2	0.28
<i>Diplodus sargus</i>	2	0.28
<i>Huso huso</i>	2	0.28
<i>Iranocichla hormuzensis</i>	2	0.28
<i>Labeo rohita</i>	2	0.28
<i>Lepomis macrochirus</i>	2	0.28
<i>Micropterus dolomieu</i>	2	0.28
<i>Morone saxatilis</i>	2	0.28
<i>Oncorhynchus tshawytscha</i>	2	0.28
<i>Paracheirodon innesi</i>	2	0.28
<i>Pseudoplatystoma fasciatum</i> x <i>Leiarius marmoratus</i>	2	0.28
<i>Pterophyllum scalare</i>	2	0.28
<i>Silurus glanis</i>	2	0.28
<i>Solea solea</i>	2	0.28
<i>Tanichthys albonubes</i>	2	0.28
<i>Thunnus thynnus</i>	2	0.28
<i>Tilapia rendalli</i>	2	0.28

Recommendations have suggested that, in order to experience well-being, animals must be able to express their natural behavior. When it comes to terrestrial animals, a lot of knowledge has been accumulated over the centuries. Nonetheless, when it comes to fish, most of the time there is not enough information due to factors like the difficulty in observing animals in their natural environment, the number of fish species and the lack of knowledge about the biology of each species, considering that two species of the same gender can have totally different behaviors.

Despite the increasing concern, ethical matters involving fish are still deficient. There is controversy

among scientists about the capacity for suffering of these animals, i.e. if these animals are sentient. The study of Rose (2002) consider that fish probably cannot feel any pain, defending that these animals did not evolve biological features such as essential brain regions or any similar functional features that enable them to be sentient. On the other hand, other authors argue that fish feel pain and that animal welfare is a concept that can be rightfully applicable to fish (Gallardo & Oliveira, 2006; Schiermeier, 2003). Chandroo, Duncan, and Moccia (2004), broaching a fish's anatomical, psychological and behavioral aspects, suggest that they are sentient, and able to experience pain, fear and psychological stress.

When an injury occurs in a body of a vertebrate, specialized receptors, known as nociceptors, are responsible to detect the damage. A signal about the injury is then transmitted through specialized nerve fibres, named A-delta and C fibres, to the spinal cord where reflexive responses may be triggered, some of which involve physiological changes. From the spinal cord, information may then be transmitted to the brain where specific areas process it, leading to various behavioural and physiological responses (Braithwaite & Ebbesson, 2014). Studies with some fish species show that they have a nociceptive system very similar to the nociceptive systems of mammals and birds (Roques, Abbink, Geurds, Vis, & Flik, 2010; Sneddon et al., 2003; Sneddon, 2003).

The CONCEA does not say much about fish sentience, but foreshadows two fascicles in the '*Guia brasileiro de produção, manutenção ou utilização de animais em atividades de ensino ou pesquisa científica*' (Brazilian guide of production, care and use of animals in teaching or scientific research activities) dedicated to them. The first fascicle has been published and broaches the most commonly used species for research or teaching purposes: lambari (*Astyanax fasciatus* and *A. altiparanae*), tilapia (*Tilapia rendalli* and *Oreochromis niloticus*) and zebrafish (*Danio rerio*). This guide tackles subjects such as species biology, adequacy of facilities, feeding, diseases, anesthesia, surgery and euthanasia, and serves as a good guide for those who work with these three groups of species (CONCEA, 2015).

In fishponds or in the laboratory, fish are kept until the proper moment for slaughter or euthanasia, specific for each species or purpose. Slaughter techniques in captive-bred fish have been the aim of many studies, with different goals and objectives, e.g. promoting quality, efficiency and safety control of procedures (e.g., Conte, 2004; Lines & Spence, 2014; Robb & Kestin, 2002; Vis et al., 2003). There

are diverse slaughter methods and species responses vary according to the different techniques (Ashley, 2007). Therefore, the proper slaughter (or euthanasia) method is an important step to ensure the quality of fish in commercial production (Santos, 2013). Some studies broach matters about the possibility of ethical considerations in industrial slaughter procedures (Lambooj, Vis, Kloosterboer, & Pieterse, 2002; Vis et al., 2003). In addition, it is important to obtain information about how usual management could affect the welfare and quality of captive-bred fish and to propose alternative procedures.

Euthanasia (from Greek) means 'death', and is a humane method of killing animals, without pain and with minimal discomfort. It is the practice to cause death in an animal in a controlled and assisted way to relieve pain and/or suffering (CONCEA, 2013a). According to Normative Resolution 13/2013, from CONCEA, the criteria usually adopted for the indication of euthanasia in an individualized way are: severely injured animals, without any possibility of treatment, animals with terminal diseases in intense suffering and elderly animals when there are no resources to attend their needs. However, other situations of death induction can occur, e.g. the humane slaughter of animals for food consumption and the production or keeping of animals for scientific and didactic purposes. Humane slaughter for food consumption is governed by specific legislation from the Agriculture, Livestock and Supply Office (*Ministério da Agricultura, Pecuária e Abastecimento* [MAPA]).

On the other hand, a great variety of slaughter methods is used in aquaculture and can induce different stress levels. Some of these methods are used for commercial purposes, e.g. air asphyxia and immersion in a salt water and ice solution, involving protracted periods of consciousness before death (Ashley, 2007). Slaughter methods like the use of carbon dioxide and electrical stunning have been used in sea fish, but both have been considered inhumane, and decrease the pH of the meat or speed up the beginning of *rigor mortis* (Poli, Parisi, Scappini, & Zampacavallo, 2005).

One of the most common slaughter methods is ice-water immersion (Ashley, 2007). This method consists of submerging fish into cold water (about 1°C) until their death. There is a question of welfare when this method is used; however, hypothermia anesthetizes animals, and is applied in studies that evaluate welfare, as well as its relationship to the quality of the final product (e.g. Bagni, Civitareale, Priori, & Ballerini, 2007). Moreover, cold-sensitive

nociceptors have not yet been found in fish (Braithwaite & Ebbesson, 2014).

The exsanguination slaughter method involves gill perforation, and, subsequently, submerging the fish into cold water (1°C) (Olsen, Sorensen, Larsen, Elvevoll, & Nilsen, 2008). To ensure animal welfare, exsanguination is done together with previous anesthetizing, using CO₂ (Roth, Johan, & Slinde, 2005), electrical stimulation and hypothermia (Lambooi, Kloosterboer, Gerritzen, & Vis, 2006). Asphyxia is considered one of the most stressful slaughter methods, compared to exsanguination, for example (Sigholt et al., 1997).

According to Conte (2004), death by asphyxia and thermal shock are not considered acceptable under the perspective of fish welfare, because they cause intense and protracted suffering, although they are still the most commonly used methods in fish processing industries, as they are easy to apply and offer positive quality results. Industrially applied methods aim to promote initial anesthetizing, followed by complementary methods that cause the death of the fish (e.g. percussive stun, usually used in large-sized fish coming from extractive fishing; brain spike by a compressed air gun or a perforating object). They are considered complementary methods and are only used when there is the intention of commercializing fish filets or cutlets, since the deformation of the head causes an unpleasant appearance (Santos, 2013).

For anesthetizing or stunning, carbon dioxide has been used as an auxiliary method during fish capture. The use of stunning by CO₂ can cause a more precocious beginning of *rigor mortis* and the softening of muscular texture (Roth et al., 2005). The search for other more efficient gases and mixtures for stunning is suggested. According to CONCEA (2013b), although CO₂ is listed as a euthanasia method, recent data question it because there is a high risk of affecting animal welfare, in addition to the fact that the administration method of CO₂ can change negatively or exacerbate the risk of suffering. The possible risk to animal welfare with the use of CO₂ is based on evidence in aversion tests, behavioral observation and physiological responses (Gräns et al., 2016). Thus, the use of CO₂ as a euthanasia agent, as well as its administration method, is still under discussion. CONCEA states that CO₂ must not be employed on fish and amphibians, due to its acidity and the protracted maintenance of brain activity (CONCEA, 2013a).

The application of an electrical shock immediately after picking is seen as a method indicated to improve the welfare of fish during slaughter because it induces immediate

unconsciousness - the immobilization was close to instant, and they remains enough time until the slaughter (Gräns et al., 2016). This technique is already widely diffused for terrestrial production animal slaughter. Stunning by electrical shock is considered a viable alternative, since it can be applied to tanks or nets, reaching many fish at a time (Santos, 2013).

Electrical shock and gas addition to an ice and water mixture are methods applied by European industries in salmon and trout slaughter. The aim is a more efficient slaughter that can increase the commercial shelf life of the final product; nevertheless, they are considered expensive if applied to species of low commercial value (Scherer et al., 2005). Another possible option is the gas gun, common in livestock and pork slaughter, among others. This method has the advantage of causing fewer blood stains on meat and less bleeding (Gregory, 2005). According to CONCEA, the stunning caused by head hitting (concussion) or the use of a non-spiking gun are acceptable for fish, with some restrictions (when no other method is available or there is the complete impossibility for the use of other methods), and must always be followed by another method that ensures death (e.g. decapitation, brain spike or exsanguination).

Regarding research animals, immersion in liquid nitrogen may be acceptable for small-sized fish and some ornamental species, not exceeding 200 mg (0.2 g). It may be used for medium to large-sized species, embryo, larvae and post-larvae phases up to 200 mg (0.2 g). From this weight on, brain concussion or decapitation is recommended.

In experiment fish, when possible, euthanasia should be used in two stages: 1) anesthesia until equilibrium loss; 2) followed by a physical or chemical method which can cause brain failure. As regards anesthesia, the dilution of buffered anesthetic agents like tricaine ethanesulfonate or MS222 and benzocaine hydrochloride directly into the water is generally recommended (CONCEA, 2013a). Tricaine methanesulfonate or MS222 can be administered in many different ways in order to cause death. For fish and amphibians, it can be put into the water. Large-sized fish can be removed from the water and a concentrate solution of this substance can be flushed under the gill. Due to the acidity of this drug, when it is used in a concentration higher than 500 mg/L, the solution must be buffered with a saturated sodium bicarbonate solution, which results in a solution pH between 7.0 and 7.5 to be injected into the lymph spaces and pleuroperitoneal cavities. MS222 is an effective and low-cost method (CONCEA, 2013a).

and can be considered safe in terms of genotoxicity, because it does not induce primary DNA damage in fish (Barreto et al., 2007).

Benzocaine is the most commonly used anesthetic in Brazil (Gomes, Chipari-Gomnes, Lopes, Roubach, & Araujo-Lima, 2001). The only anesthetic approved by the Food and Drug Administration (FDA) (USA) is MS222 and it is recommended that meat should only be consumed 21 days after exposure. Benzocaine hydrochloride, similar to tricaine, may be used in an immersion and recirculation system for fish and amphibians in a buffered solution up to pH 7. The isolated form of benzocaine is not water soluble and must be prepared in a 100 g L⁻¹ concentration, in acetone or alcohol. On the other hand, benzocaine hydrochloride is water soluble and can be used directly for anesthesia or euthanasia in a concentration higher than 250 mg L⁻¹. Fish must be kept immersed in the solution for at least 10 minutes after ceasing opercular movements (CONCEA, 2013b).

Despite the widespread use of benzocaine, Blessing, Marshall, and Balcombe (2010) provide evidence favoring the use of ice-slurry over benzocaine in the humane killing of small-sized fish. According to the authors, most individuals in the benzocaine treatment exhibited what appeared to be a distressed behavior pattern of prolonged rapid swimming along the tank floor with a head-down attitude and rapid operculum movement. The ice-slurry method produced more rapid loss of equilibrium, more rapid death and generated less stressful behavior. Furthermore, the long duration of stress-related behavior in benzocaine-treated individuals suggests greater stress for fish in the benzocaine treatment. Another study suggests fish can perceive as aversive the most commonly recommended and used anesthetics: MS222 and benzocaine (Readman, Owen, Murrell, & Knowles, 2013). Two agents were found not to induce aversive behavioral responses: etomidate and 2,2,2-tribromoethanol. The authors suggest that compounds that are aversive, even at low concentration, should no longer be used routinely for anesthesia.

Methods using clove oil (which contains eugenol) and 2-phenoxyethanol are accepted in fish, with some restrictions, since tricaine and benzocaine are proven to interfere in research results. Eugenol causes a competitive neuromuscular-blocking and it is not clear if the chemical contention occurs because of this mechanism or because of an anesthetic effect. Therefore, there is still no information available for considering clove oil or

eugenol as a proper euthanasia method for fish (CONCEA, 2013b).

Slaughter techniques in aquaculture are diverse and fish species have varying responses to the different methods. The main effect of severe stress in slaughter and pre-slaughter is observed in the physical properties of the meat, with depletion of muscle energy, producing more lactic acid, reducing muscle pH and increasing the entrance speed of *rigor mortis*, in addition to causing a non-desirable muscle softening (Santos, 2013).

As regards fish welfare, attention on and concern with stress in pisciculture have increased considerably in recent years (Ashley, 2007), mainly regarding the negative effects on production and meat quality features (Lambooj et al., 2002). The level at which meat quality features are affected by stress depends directly on the severity, length and speed of the stressor. Stress can occur due to production management, stocking rate, transport and slaughter (Millán-Cubillo, Martos-Sitcha, Ruiz-Jarabo, Cárdenas, & Mancera, 2016; Shabani, Erikson, Beli, & Rexhepi, 2016). Problems caused by intensive production, together with consumer demand for better quality products, are changing producer interest for sustainable production, because fish quality also includes ethical aspects during their production (Lambooj et al., 2006).

Along with other cultivation systems, pisciculture also aims at greater production at the lowest possible cost. However, increasingly, there is a search for optimization in the welfare of the animal that is being produced. There is even the example of Papoutsoglou et al. (2013), which tested the performance of rainbow trout reared under the stimuli of two music compositions (Mozart and Romanza) and compared to those raised using a white noise treatment or control. The results indicate that the musical stimuli benefited fish growth.

Rodrigues, Junior, Balista, and Freitas (2015) aimed to highlight through questionnaires, what the members of a fishermen's association understood about fish welfare, and whether it is considered during the production process. This suggests that, even without scientific proof of sentience in fish, fish farmers treat them humanely. The answers appeared to be influenced by potential concerns over welfare (targeting the commercial side of the activity) and the fact that consumers desired the fish to be treated humanely.

A limiting factor in fish welfare in Brazil is the lack of information about humane slaughter. Even ordinance no. 3, from January, 17th 2000, from the *Ministério da Agricultura, Pecuária e Abastecimento*

(Brasil, 2000), which approves the technical regulation of anesthetizing methods for humane slaughter, includes only mammals, poultry and captive wild animals, without any mention of fish.

More recently, ordinance no. 524, from 2011, aimed to institute the *Comissão Técnica Permanente de Bem-Estar Animal* [CTBEA], to coordinate actions for the welfare of production animals or animals of economic interest in the many common bonds of the livestock production chain. Nonetheless, little or no attention has been directed to fish welfare.

Conclusion

In conclusion, neither researchers nor producers of fish have shown great concern about the welfare of these animals. This can be seen in recent articles and resolutions published by regulatory institutions. However, many studies about humane slaughter and which are the best stunning and/or anesthesia methods for fish are still necessary, aiming at the production of new information to create laws that could defend fish welfare.

References

- Ashley, P. J. (2007). Fish welfare: current issues in aquaculture. *Applied Animal Behaviour Science*, 104(3-4), 199-235.
- Bagni, M., Civitareale, C., Priori, A., & Ballerini, A. (2007). Pre-slaughter crowding stress and killing procedures affecting quality and welfare in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). *Aquaculture*, 263(1-4), 52-60.
- Barreto, R. E., Gontijo, A. M. M. C., Alves-de-Lima, R. O., Raymundi, V. C., Pinhal, D., Reyes, V. A. V., ... Salvadori, D. M. F. (2007). MS222 does not induce primary DNA damage in fish. *Aquaculture International*, 15(2), 163-168.
- Batzina, A., Kalogiannis, D., Dalla, C., Papadopoulou-Daifoti, Z., Chadio, S., & Karakatsouli, N. (2014). Blue substrate modifies the time course of stress response in gilthead seabream *Sparus aurata*. *Aquaculture*, 420-421, 247-253.
- Blessing, J. J., Marshall, J. C., & Balcombe, S. R. (2010). Humane killing of fishes for scientific research: A comparison of two methods. *Journal of Fish Biology*, 76(10), 2571-2577.
- Braithwaite, V. A., & Ebbesson, L. O. E. (2014). Pain and stress responses in farmed fish. *Revue Scientifique et Technique (International Office of Epizootics)*, 33(1), 245-253.
- Braithwaite, V. A., Huntingford, F., & Bos, R. van den. (2013). Variation in emotion and cognition among fishes. *Journal of Agricultural and Environmental Ethics*, 26(1), 7-23.
- Brasil. Ministério da Agricultura. (2000). Instrução normativa nº 3, de 17 de janeiro de 2000. Retrieved from: <http://www.agricultura.gov.br/>
- Chandroo, K. P., Duncan, I. J. H., & Moccia, R. D. (2004). Can fish suffer?: perspectives on sentience, pain, fear and stress. *Applied Animal Behaviour Science*, 86(3-4), 225-250.
- Conselho Nacional de Controle de Experimentação Animal [CONCEA]. (2013a). *Diretrizes da prática de eutanásia do Conce* (Vol. 1). Brasília, DF: Ministério da Ciência e Tecnologia.
- Conselho Nacional de Controle de Experimentação Animal [CONCEA]. (2013b). *Resolução normativa nº 13, de 20 de setembro de 2013*. Brasília, DF: Ministério da Ciência e Tecnologia.
- Conselho Nacional de Controle de Experimentação Animal [CONCEA]. (2015). 5A. Peixes mantidos em instalações de instituições de ensino ou pesquisa científica – I. In *Guia brasileiro de produção, manutenção ou utilização de animais em atividades de ensino ou pesquisa científica* (1st ed., Vol. 5, p. 43). Brasília, DF: Ministério da Ciência, Tecnologia e Inovação.
- Conte, F. S. (2004). Stress and the welfare of cultured fish. *Applied Animal Behaviour Science*, 86(3-4), 205-223.
- Crepaldi, D. V., Faria, P. M. C., Teixeira, E. D. A., Ribeiro, L. P., Costa, Â. A. P., Melo, D. C. de, ... Moraes, V. E. de. (2006). A situação da aquicultura e da pesca no Brasil e no mundo. *Revista Brasileira de Reprodução Animal*, 30(3-4), 81-85.
- Ellingsen, K., Grimsrud, K., Nielse, H. M., Mejdell, C., Olesen, I., Honkanen, P., ... Sandøe, P. (2015). Who cares about fish welfare?: a Norwegian study. *British Food Journal*, 117(1), 257-273.
- Galhardo, L., & Oliveira, R. (2006). Bem-estar animal: um conceito legítimo para peixes? *Revista de Etologia*, 8(1), 51-61.
- Garcia-Reyero, N. (2015). Are adverse outcome pathways here to stay? *Environmental Science and Technology*, 49(1), 3-9.
- Gomes, L. C., Chipari-Gomnes, A. R., Lopes, N. P., Roubach, R., & Araujo-Lima, C. A. R. M. (2001). Efficacy of benzocaine as an anesthetic in juvenile Tambaqui *Colossoma macropomum*. *Journal of the World Aquaculture Society*, 32(4), 426-431.
- Gräns, A., Niklasson, L., Sandblom, E., Sundell, K., Algers, B., Berg, C., ... Kiessling, A. (2016). Stunning fish with CO₂ or electricity: contradictory results on behavioural and physiological stress responses. *Animal*, 10(2), 294-301.
- Gregory, N. G. (2005). Recent concerns about stunning and slaughter. *Meat Science*, 70(3), 481-491.
- Håstein, T., Scarfe, A. D., & Lund, V. L. (2005). Science-based assessment of welfare: aquatic animals. *Revue Scientifique et Technique (International Office of Epizootics)*, 24(2), 529-547.
- Hilsdorf, A. W. S., & Orfão, L. H. (2011). Aspectos gerais do melhoramento genético em peixes no Brasil. *Revista Brasileira de Zootecnia*, 40(supl.), 317-324.

- Instituto Brasileiro de Geografia e Estatística [IBGE]. (2013). *Produção da Pecuária Municipal*, 41. Retrieved from: http://biblioteca.ibge.gov.br/visualizacao/periodicos/84/ppm_2013_v41_br.pdf
- Instituto Brasileiro de Geografia e Estatística [IBGE]. (2014). *Produção da Pecuária Municipal*, 42. Retrieved from: http://biblioteca.ibge.gov.br/visualizacao/periodicos/84/ppm_2014_v42_br.pdf
- Kittilsen, S. (2013). Functional aspects of emotions in fish. *Behavioural Processes*, 100(376), 153-159.
- Lambooi, E., Kloosterboer, R. J., Gerritzen, M. A., & Vis, J. W. van de. (2006). Assessment of electrical stunning in fresh water of African Catfish (*Clarias gariepinus*) and chilling in ice water for loss of consciousness and sensibility. *Aquaculture*, 254(1-4), 388-395.
- Lambooi, E., Vis, J. W. van de, Kloosterboer, R. J., & Pieterse, C. (2002). Welfare aspects of live chilling and freezing of farmed eel (*Anguilla anguilla* L.): neurological and behavioural assessment. *Aquaculture*, 210(1-4), 159-169.
- Lines, J. A., & Spence, J. (2014). Humane harvesting and slaughter of farmed fish. *Revue Scientifique et Technique (International Office of Epizootics)*, 33(1), 255-264.
- Meuthen, D., Baldauf, S. A., Bakker, T. C. M., & Thünken, T. (2011). Substrate-treated water: A method to enhance fish activity in laboratory experiments. *Aquatic Biology*, 13(1), 35-40.
- Meyer, P. A. (1979). Publicly vested values for fish and wildlife: criteria in economic welfare and interface with the law. *Land Economics*, 55(2), 223-235.
- Millán-Cubillo, A. F., Martos-Sitcha, J. A., Ruiz-Jarabo, I., Cárdenas, S., & Mancera, J. M. (2016). Low stocking density negatively affects growth, metabolism and stress pathways in juvenile specimens of meagre (*Argyrosomus regius*, Asso 1801). *Aquaculture*, 451, 87-92. doi:10.1016/j.aquaculture.2015.08.034
- Millot, S., Cerqueira, M., Castanheira, M. F., Øverli, Ø., Martins, C. I. M., & Oliveira, R. F. (2014). Use of conditioned place preference/avoidance tests to assess affective states in fish. *Applied Animal Behaviour Science*, 154, 104-111. doi:10.1016/j.applanim.2014.02.004
- Olsen, S. H., Sorensen, N. K., Larsen, R., Elvevoll, E. O., & Nilsen, H. (2008). Impact of pre-slaughter stress on residual blood in fillet portions of farmed Atlantic cod (*Gadus morhua*) - Measured chemically and by Visible and Near-infrared spectroscopy. *Aquaculture*, 284(1-4), 90-97.
- Papoutsoglou, S. E., Karakatsouli, N., Skouradakis, C., Papoutsoglou, E. S., Batzina, A., Leondaritis, G., & Sakellaris, N. (2013). Effect of musical stimuli and white noise on rainbow trout (*Oncorhynchus mykiss*) growth and physiology in recirculating water conditions. *Aquacultural Engineering*, 55, 16-22. doi:10.1016/j.aquaeng.2013.01.003
- Poli, B. M., Parisi, G., Scappini, F., & Zampacavallo, G. (2005). Fish welfare and quality as affected by pre-slaughter and slaughter management. *Aquaculture International*, 13(1-2), 29-49.
- Pough, F. H., Jani, C. M., & Heiser, J. B. (2008). *A vida dos vertebrados* (4th ed.). São Paulo, SP: Ateneu.
- Readman, G. D., Owen, S. F., Murrell, J. C., & Knowles, T. G. (2013). Do fish perceive anaesthetics as aversive? *PLoS ONE*, 8(9), e73773. doi: 10.1371/journal.pone.0073773
- Robb, D. H. F., & Kestin, S. C. (2002). Methods used to kill fish: Field observations and literature reviewed. *Animal Welfare*, 11(3), 269-282.
- Rodrigues, D. A., Junior, A. G. C., Balista, W. C., & Freitas, R. R. de. (2015). Animal welfare concerns at a fish farming operation in southeastern Brazil. *Journal of Integrated Coastal Zone Management*, 15(3), 417-424.
- Roques, J. A. C., Abbink, W., Geurds, F., Vis, H. van de, & Flik, G. (2010). Tailfin clipping, a painful procedure: Studies on Nile tilapia and common carp. *Physiology and Behavior*, 101(4), 533-540.
- Rose, J. D. (2002). The neurobehavioral nature of fishes and the question of awareness and pain. *Reviews in Fisheries Science*, 1(800), 1-38.
- Roth, B., Johan, O., & Slinde, E. (2005). The effect of slaughtering procedures on blood spotting in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture*, 250(3-4), 796-803.
- Santos, E. C. B. (2013). *Método de abate e qualidade da Tilápia do Nilo*. Jaboticabal, SP: Universidade Estadual Paulista.
- Scherer, R., Augusti, P. R., Steffens, C., Bochi, V. C., Hecktheuer, L. H., Lazzari, R., ... Emanuelli, T. (2005). Effect of slaughter method on postmortem changes of grass carp (*Ctenopharyngodon idella*) stored in ice. *Food and Chemical Toxicology*, 70(5), c348-c353.
- Schiermeier, Q. (2003). New evidence that fish feel pain. *Nature*, (April), 1-2. doi:10.1038/news030428-9
- Shabani, F., Erikson, U., Beli, E., & Rexhepi, A. (2016). Live transport of rainbow trout (*Oncorhynchus mykiss*) and subsequent live storage in market: water quality, stress and welfare considerations. *Aquaculture*, 453, 110-115. doi:10.1016/j.aquaculture.2015.11.040
- Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T. S., & Seland, A. (1997). Handling stress and storage temperature affect meat quality of farmed-raised Atlantic Salmon (*Salmo salar*). *Journal of Food Science: Food Chemistry*, 62(4), 898-905.
- Sneddon, L. U. (2003). Trigeminal somatosensory innervation of the head of a teleost fish with particular reference to nociception. *Brain Research*, 972(1-2), 44-52.
- Sneddon, L. U. (2011). Pain perception in fish evidence and implications for the use of fish. *Journal of Consciousness Studies*, 18(9-10), 209-229.
- Sneddon, L. U., Braithwaite, V. A., & Gentle, M. J. (2003). Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proceedings. Biological Sciences; The Royal Society*, 270(1520), 1115-1121.

- Solgaard, H. S., & Yang, Y. (2011). Consumers' perception of farmed fish and willingness to pay for fish welfare. *British Food Journal*, 113(8), 997-1010.
- Vis, H. van de, Kestin, S., Robb, D., Oehlenschläge, J., Lambooy, B., Münkner, W., ... Nesvadba, P. (2003). Is humane slaughter of fish possible for industry? *Aquaculture Research*, 34(3), 211-220.

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