UNIVERSIDADE FEDERAL DO PARANÁ

DANIEL SANTIAGO RUCINQUE GONZALEZ

PERCEPTION ON FISH SENTIENCE, WELFARE AND HUMANE SLAUGHTER BY LATIN AMERICAN CITIZENS AND ELECTRICAL STUNNING IN PACU AND SOUTH AMERICAN CATFISH

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Membro

I dedicate this work to all non-human animals

who suffer because of the human existence

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“Veterinarians can help change socially unacceptable agricultural and other animal use practices before society gets fed up with such practices and bans them or severely restricts them regardless of economic impact on producers”

Bernard Rollin

RESUMO

No Brasil, em 2011 foram produzidas 544.490 toneladas de peixes provenientes da aquicultura. A preocupação com o grau de bem-estar dos animais destinados à produção de alimentos é crescente no mundo ocidental. No entanto, os métodos tradicionais de abate de peixes causam um alto grau de sofrimento e estudos de métodos de abate humanitário em espécies nativas ainda são escassos. Além disso, as regulamentações de abate de animais de produção não incluem peixes. Desta forma, este trabalho teve como objetivo estudar parâmetros para eletronarcose de duas espécies, assim como conhecer a percepção de cidadãos da América Latina em relação à senciência, bem-estar e abate humanitário de peixes. Este estudo divide-se em cinco capítulos: (1) Apresentação; (2) Percepção sobre senciência, bem-estar e abate humanitário de peixes por cidadãos da América Latina; (3) Eletronarcose em pacu (*Piaractus mesopotamicus*); (4) Eletronarcose em jundiá (*Rhamdia quelen*) e (5) Considerações finais. O capítulo sobre a percepção dos cidadãos mostrou que os respondentes consideram os peixes como seres sencientes. A classificação da percepção de senciência entre os diferentes grupos taxonômicos parece estar em concordância com a proximidade filogenética, sugerindo uma maior sensibilidade com os mamíferos. Os cidadãos perceberam um alto grau de sofrimento em cenários de pesca esportiva, feira do peixe vivo e peixes usados em laboratórios. Além disso, os respondentes concordaram com a inclusão dos peixes nas normativas de abate humanitário. Os capítulos sobre electronarcose permitiram entender parâmetros de insensibilização elétrica com tempos de inconsciência maior que 1 min, sem alterações significativas no pH muscular e rigor mortis das carcaças. Ainda, os parâmetros foram seguros em termos de evitar fraturas, hemorragias ou petéquias nas carcaças. Os resultados obtidos apontam para a necessidade de desenvolvimento de pesquisa visando melhorar as atuais práticas de abate de peixes no Brasil, assim como a relevância de incluir os peixes na regulamentação de abate humanitário. Novos estudos são necessários para fornecer ferramentas aplicáveis na indústria, de modo de instaurar práticas de abate humanitário em peixes que diminuam o sofrimento de um grande número de indivíduos.

Palavras-chave: Abate humanitário. Bem-estar animal. Eletronarcose. Inconsciência. Percepção. Sofrimento. Termonarcose.

ABSTRACT

In Brazil, in 2011 it was produced 544,490 tons of fish from aquaculture. The concern with the level of welfare of animals for food production is increasing in the Western world. However, traditional methods of killing fish cause a high degree of suffering and studies of humane slaughter methods in native species are still scarce. Furthermore, the most regulations slaughter of farm animals include do not include fish. Thus, this work aimed to study parameters for electrical stunning in two species and to study the perception on fish sentience, welfare and slaughter by citizens from Bogotá, Colombia, and Curitiba, Brazil. This study is divided into five chapters: (1) Presentation; (2) Perception on fish sentience, welfare and humane slaughter by Latin American citizens; (3) Electrical stunning in pacu (*Piaractus mesopotamicus*); (4) Electrical stunning in South American catfish (*Rhamdia quelen*) and (5) Final considerations. The chapter on the perception of citizens showed that respondents consider the fish as sentient beings. The classification of sentience perception among taxonomic groups seems in accordance with the phylogenetic proximity to humans, suggesting greater sensitivity towards mammals. Citizens perceived a high degree of suffering in recreational angling, fair live fish and fish used in laboratories. In addition, respondents agreed with the inclusion of fish in humane slaughter regulations. The chapters on electrical stunning allowed identified parameters of electrical stunning to unconsciousness times greater than 1 min and no significant changes in muscle pH and rigor mortis of carcasses. Further, parameters used were safe in terms of avoiding fractures, bleeding or petechial in the carcasses. The results point to the need for research development to improve the current fish slaughtering practices in Brazil, as well as the importance of including fish in the regulation of humane slaughter. Further studies are needed to provide tools applicable in the industry in order to establish humane slaughter practices in fish reduce the suffering of a large number of individuals.

Keywords: Animal welfare. Electrical stunning. Humane slaughter. Hypothermia. Perception. Suffering. Unconsciousness.

LIST OF FIGURES

LIST OF TABLES

INDEX

1. PRESENTATION

Currently, the scientific community recognizes that fish, like other vertebrates, have the ability to feel pain and suffer, i.e. they are recognized as sentient beings (Braithwaite & Boulcott, 2007; Braithwaite et al., 2013; Braithwaite & Ebbesson, 2014; Broom, 2007, 2010; Brown, 2014; Bshary & Brown, 2014; Chandroo et al., 2004; Culum et al., 2011; Lund et al., 2007; Sneddon, 2002, 2003, 2009; Sneddon et al., 2003, 2014). Such scientific recognition has led to important practical changes. For example, the World Organisation for Animal Health - OIE, through the aquatic animal health code, recommends humane slaughter practices of fish destined for human consumption (OIE, 2015). Even though the slaughter legislation is not in accordance with the principles of animal welfare in several countries, there is a tendency for a greater interest in the issue by consumers. Studies in Europe, for example, show that citizens are concerned about the welfare of fish used for human consumption (Ellingsen et al., 2015). In Brazil, in a study performed in the metropolitan region of Curitiba, 88% of respondents recognized that fish feel pain and 89% believed that the traditional methods of slaughter may cause suffering (Pedrazzani et al., 2008).

Countries like Norway and Sweden maintain specific regulations of humane slaughter in fish for human consumption (Norway, 2009; Röcklinsberg, 2015). In Brazil and Colombia, the humane slaughter regulations do not include fish (Brasil, 2000, Colombia, 2007). However, the main animal protection law in Brazil (Brasil, 1998) includes fish, as Article 32 specifies that the law applies to wild, domestic, domesticated, native or exotic animals.

For a method of slaughter to be humane, fish must undergo stunning to induce unconsciousness and insensibility to pain stimuli; stunning must have sufficient duration to secure that an animal does not recover during exsanguination (Lambooij et al., 2008). In Brazil and other Latin American countries, the methods used in both pre-slaughter and slaughter of fish may not considered humanitarian. In Brazil, the hypothermia in ice for stunning is used in most fish slaughterhouses (Oliveira Filho et al., 2014). For example, it may take up to 20 minutes for fish that are placed in ice to lose sensitivity to pain (Van De Vis et al., 2003). During this time, the fish is completely conscious and

there might be high degrees of suffering. In a study conducted in Brazil, the authors showed that it takes about 13 minutes for tilapia submitted to hypothermia in ice to lose consciousness (Pedrazzani et al., 2009). In addition, bleeding without stunning as a method of slaughter in fish also causes a high degree of suffering (Robb et al., 2000).

Thus, this study aimed to deepen knowledge about the perception of citizens in Latin America in relation to sentience, welfare and slaughter of fish, as well as advance knowledge of electrical stunning parameters in two native species of economic interest in Brazil. In Chapter 2, we studied the perception on sentience, welfare and slaughter of fish by citizens in two cities in Latin America. We observed that the respondents recognized fish as sentient beings, and perceived suffering in specific scenarios. Participants agreed that fish should be included in the humane slaughter legislation. In chapter 3 and 4, the electrical parameters for electrical stunning in pacu (*Piaractus mesopotamicus*) and South American catfish (*Rhamdia quelen*) were studied, respectively. The results of these chapters allowed the identification of parameters that cause stunning and that can be used as a method for humane slaughter, reducing the high degree of suffering inflicted by the current fish killing methods in Brazil.

The results obtained in Chapter 2 were submitted for publication in the international journal PLOS ONE, with 3.2 impact factor and A1 Qualis in the field of veterinary medicine (Annex 1). Two simple data abstracts were presented as a poster at the *HSA International Symposium 2015: Recent Advances II*, in Zagreb, Croatia, from 16th to 17th of July 2015 (Appendix 1), and at the *III Congresso Medvep de Especialidades Veterinárias*, Curitiba, Paraná, from 22nd to 25th of July 2015 (Appendix 2). Additionally, two talks were given, one at the Live Fish Fair in the city of Araucária, Paraná, entitled "*Senciência e abate humanitário de peixes*", on the 30th and 31st March 2015 (Annex 2) and the other at the Federal Institute of Paraná, Paranaguá, Paraná, entitled "*Bem-estar e abate humanitário de peixes*" during the 2nd Academic Week and 4th week of Aquaculture, on September 3rd 2015 (Annex 3).

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2. PERCEPTION ON FISH SENTIENCE, WELFARE AND HUMANE SLAUGHTER BY CITIZENS OF BOGOTÁ, COLOMBIA AND CURITIBA, BRAZIL

RESUMO

Discussões sobre o bem-estar dos animais destinados à produção de alimentos são cada vez mais frequentes, principalmente em países desenvolvidos. O objetivo deste trabalho foi estudar a percepção sobre senciência, bem-estar e abate de peixes por cidadãos de Bogotá, Colômbia e Curitiba, Brasil. Um questionário *online* com 12 perguntas apresentadas em diferentes formatos abertas e fechadas, múltipla escolha e escala formato Likert foram avaliadas pelos respondentes. Respostas provenientes de 395 participantes de Bogotá e 387 de Curitiba foram avaliadas, e os resultados são apresentados na ordem de Bogotá seguidos por Curitiba. A proporção de respondentes que percebem os peixes como seres sencientes foi 79.7% e 71.8%. A classificação da percepção de senciência entre os diferentes grupos taxonômicos parece estar em concordância com a proximidade filogenética, sugerindo uma maior sensibilidade com os mamíferos. A ordem descendente relacionada à percepção de sofrimento em peixes nos diferentes cenários foi pesca com linha e anzol (75.6%, 70.6%); feira do peixe vivo (68.7% - somente em Curitiba); pesque-pague (59.7%, 54.4%); peixes como animais de laboratórios (58.0, 48.1%); peixes de produção (35.7, 36.8%); peixes em pet shops (35.5%, 26.1%); produção de peixes ornamentais (19.3%, 21.8%); peixes em aquários de exibição (18.8%,16.9%); e peixes mantidos como animais de estimação (12.4%,12.3%). Falta de conhecimentos sobre as condições de captura, manejo, transporte e venda de peixes ornamentais pode justificar a baixa percepção de sofrimento em estes últimos cenários. Em relação ao abate humanitário, 57.0% e 55.0% dos respondentes nunca tinham ouvido falar do assunto. Após a reflexão induzida pelo questionário, 76.0% e 72% dos participantes acham que os peixes devem ser incluídos na normativa de abate humanitário. Este trabalho apresenta dados inéditos que sugerem que os respondentes da Bogotá, Colômbia e Curitiba, Brasil consideram os peixes como seres sencientes. A percepção de sofrimento em cenários específicos deve levar a mudanças em atividades comuns. O reconhecimento de sofrimento pode auxiliar na modificação de regulações sobre abate humanitário, para reduzir dor em um grande número de peixes que são abatidos anualmente para consumo humano no Brasil e na Colômbia.

Palavras-chave: Abate. Bogotá. Curitiba. Opinião. Pesca esportiva. Sofrimento.

ABSTRACT

Discussions on farm animal welfare have become frequent, especially in developed countries. The aim of this research was to study the perception on fish sentience, welfare and slaughter by citizens from Bogotá, Colombia, and Curitiba, Brazil. An online survey with 12 questions presented as open-end, multiple choice and 5-point Likert-type scale formats was available to respondents. Answers from 395 participants in Bogotá and 387 in Curitiba were analyzed, and results are presented in the order Bogotá followed by Curitiba. The percentage of participants who perceived fish as sentient animals was 79.7% and 71.8%. The classification of sentience perception among taxonomic groups seems in accordance with the phylogenetic proximity to humans, suggesting greater sensitivity towards mammals. The descending order related to the highest perception on fish suffering in different scenarios was fishing with hook and line (75.6%, 70.6%); municipal live fish fair (68.7% - only in Curitiba); fish-and-pay ponds (59.7%, 54.4%); fish kept as laboratory animals (58.0, 48.1%); fish farming (35.7, 36.8%); fish in pet stores (35.5%, 26.1%); production of ornamental fish (19.3%, 21.8%); fish in aquarium exhibits (18.8%,16.9%); and fish kept as pets (12.4%,12.3%). Lack of knowledge about the conditions of capture, handling, transport and sale of ornamental fish may justify the perception of low level of suffering in the last scenarios. Regarding humane slaughter, 57.0% and 55.0% of respondents were unaware of the issue. After reflection induced by the questionnaire, 76.0% and 72% of participants believed that fish should be included in humane slaughter regulations. This study presents original data suggesting that respondents from Bogotá and Curitiba consider fish as sentient beings. The perception of suffering in specific scenarios challenges common activities. Recognition of suffering also endorses humane slaughter regulations to reduce pain in a large number of individuals of fish slaughtered annually for human consumption in Colombia and Brazil.

Keywords: Angling. Bogotá. Curitiba. Opinion. Slaughter. Suffering.

2.1 INTRODUCTION

Debates on farm animal welfare have become frequent, especially in developed countries. In European countries, citizens have put pressure for higher animal welfare standards for sentient animals (Spooner et al., 2014). Broom (2014) defined a sentient animal as one that has some ability to: i) evaluate the actions of others in relation to itself and third parties; (ii) remember some of its own actions and their consequences; (iii) assess risks and benefits; (iv) have some feelings; and (v) have some degree of awareness (Broom, 2014). In addition, Webster (2006) defined that sentient animals are those that experience emotions associated with pleasure and suffering and are motivated to promote their evolutionary behavior (Webster, 2006).

In relation to fish, researchs have revealed that teleost fish can feel pain due to their sensory system, and that they also have memory and consciousness (Smith & Boyd, 1991; Chandroo et al., 2004; Huntingford et al., 2006; Broom, 2007, 2010; Reilly et al., 2008; Braithwaite & Ebbesson, 2014). For example, fish are known to fall for optical illusions which suggests that they not only take in visual information but complex processing also occur at the level of perceptual organization (Agrillo et al., 2013). Unlike mammals, many fish are capable of detecting and creating electric currents in the water allowing them to find prey, navigate in their environment and communicate with their fellows (Brown, 2014). Learning association between sound and food occurred in 14 trials in rainbow fish (Bibost & Brown, 2014), while rats in took about 40 trials (Bouton & Peck, 1989). Fish have excellent long-term memories, develop complex traditions, show signs of Machiavellian intelligence, cooperate with and recognise one another and are even capable of tool use (Brown, 2014; Bshary et al., 2002; Culum et al., 2011). Fish have neuroanatomical, behavioural and physiological attributes that characterize conscious cognition or motivational affective states, then there may be compelling evidence to suggest that fish can suffer (Chandroo et al., 2004). Thus, on balance the evidence seems to suggest a degree of sentience in fish.

Concerns about fish sentience and capacity to suffer have contributed to improve animal handling and promoted practices that may increase animal welfare and avoid suffering states. Recently, the World Organization for Animal

Health, through the Aquatic Animal Code, set recommendations for the welfare of farmed fish, mainly in transport, stunning and killing for human consumption (OIE, 2015). This is particularly relevant since fish are the most consumed animal and are the second most used in scientific research worldwide (Brown, 2014; Huntingford & Kadri, 2014).

In Norwegian and Swedish humane slaughter regulations, stunning of farmed fish is mandatory (Norway, 2009; Röcklinsberg, 2015). Humane slaughter is characterized by stunning, which is applied to animals to induce unconsciousness and insensibility to pain stimuli for sufficient duration to secure that an animal does not recover during exsanguination (Lambooij et al., 2008). Killing methods such as asphyxia on ice, evisceration and exsanguination cause a high degree of suffering in conscious animals (Robb & Kestin, 2002). In Latin American countries, like Brazil and Colombia, these methods are commonly used without previous stunning, as fish are not included in the humane slaughter regulations (Brasil, 2000; Colombia, 2007).

The societal opinion is an important factor when discussing new regulations to improve fish welfare in different scenarios (Muir et al., 2013). However, it seems that people have low levels of knowledge about livestock production systems. For example, in Brazil, Bonamigo et al. (2012) observed that respondents did not know broiler chicken production systems, and Pedrazzani et al. (2008) observed that respondents had never heard about humane slaughter of animals.

Considering the increasing demand for products of animal origin, it is necessary to study the perception of society in relation to the quality of life of animals, because social concern dictates the need for animal welfare standards and regulations (Caporale et al., 2005). From such knowledge, changes in production systems and regulations may be proposed to improve fish welfare. Thus, the aim of this research was to study the perception on fish sentience, welfare and slaughter by citizens from Bogotá, Colombia, and Curitiba, Brazil.

2.2 MATERIAL AND METHODS

An online survey containing 12 questions was available through Survio® platform to the citizens of Bogotá (B), Capital District, Colombia and Curitiba (C), Paraná, Brazil, in the language of each country (TABLE 1). Questions were presented as open-end, multiple choice and 5-point Likert-type scale formats. Additionally, participants were asked demographics questions, such as gender, age and education. The minimum sampling size of 384 respondents in each city was calculated based on Schaeffer et al. (1990) considering a maximum error of 5.0% for a population of over one million people; a 95.0% confidence interval and a maximum uncertainty principle of 50.0%. The survey was distributed by e-mail and social networks. In addition, coordinators of higher education programs in both cities were contacted by e-mail to share the survey link amongst professors and students. A total of 20 universities in B and 5 in C were contacted. Responses were collected from August 2014 to April 2015. The study was approved by the Human Research Ethics Committee of the Federal University of Paraná (SCS/UFPR), protocol 814-835 2014 (ANNEX 4).

TABLE 1 - NON-DEMOGRAPHIC QUESTIONS (Q) INCLUDED IN THE ONLINE SURVEY ABOUT THE PERCEPTION ON FISH SENTIENCE, WELFARE AND SLAUGHTER IN BOGOTÁ, CAPITAL DISTRICT, COLOMBIA AND CURITIBA, PARANÁ, BRAZIL; AVAILABLE FROM AUGUST 2014 TO APRIL 2015

Questions	Content	Options of answers
Q1	Do you believe fish can feel emotions such as fear and joy, and also suffer? Why?	1 certainly not; 2 no; 3 undecided or I do not have an opinion on this; 4 yes; 5 certainly yes.
Q2	In a scale from 1 to 5, classify the ability of each animal to feel emotions: pigeon, butterfly, human baby, rat, dog, chicken, fish, sheep, cattle, cockroach and wolf.	1; 2 ; 3; 4; 5; I do not know 1 means do not feel emotions; 5 means certainly feel emotions; intermediate values are equivalent of a growing capacity to feel emotions.
Q3	Do you believe that removing fish from the water causes suffering to them?	1 certainly not; 2 no; 3 undecided or I do not have an opinion on this; 4 yes; 5 certainly yes.
Q4	Is catch-and-release angling acceptable? Why?	1 certainly not; 2 no; 3 undecided or I do not have an opinion on this; 4 yes; 5 certainly yes.
Q5	Have you ever practiced angling?	Yes, almost every day; yes, 1-3 times a week; yes, 1-3 times a month; yes, a few times a year; yes, only once; no, never.
Q6	In a scale from 1 to 5, in which 1=none and 5=maximum, how much pain do you believe fish can experience?	1; 2; 3; 4; 5.

Q7	What is the level of fish suffering in the following scenarios? Fish with hook and line; municipal live fish fair (*C); fish-and-pay ponds; fish kept as laboratory animals; fish farming; fish in pet stores; production of ornamental fish; fish in aquarium exhibits and fish kept as pets.	1 none; 2 a little; 3 moderate; 4 a lot; I do not know.
Q8	Which methods of fish slaughter have you ever seen?	Asphyxia; gutting; decapitation; percussion; death in dry plastic bags (*C); none; others:
Q9	Do you believe that the methods of slaughter you indicated in the previous question cause suffering to the fish?	1 certainly not; 2 no; 3 undecided or I do not have an opinion on this; 4 yes; 5 certainly yes.
Q10	Do you think suffering affects fish meat quality?	1 certainly not; 2 no; 3 undecided or I do not have an opinion on this; 4 yes; 5 certainly yes.
Q11	Have you ever heard of humane slaughter?	Yes; no
Q12	Do you believe that fish should be included in the humane slaughter regulation, as other animals used for food production? Why?	1 certainly not; 2 no; 3 undecided or I do not have an opinion on this; 4 yes; 5 certainly yes.

*C only presented to respondents in Curitiba

Questions 7 and 8 were not introduced to the respondents from B because in such country there is no similar scenario of a fair where fish are taken home by consumers without water supply.

From a total of 903 respondents, 782 answers were evaluated, being 395 from B and 387 from C. One hundred and twenty-one respondents were from cities other than B or C and their responses were not included in our analysis. Considering the demographic variables within the age group, the responses of categories "46-55 years-old" and "56 years-old or more" were combined due to the low number of participants in the latter. Thus, four age groups were compared (TABLE 2). Answers were not compared according to the education level of participants since 95.1% (744/782) of them declared themselves as having higher education (TABLE 2); this is related to our respondent-request strategy.

The demographic data showed that respondents were mostly female; 18 to 25 years old and with incomplete higher education.

TABLE 2 - DEMOGRAPHIC DATA OF 395 RESPONDENTS IN BOGOTÁ AND 387 IN CURITIBA, FROM A SURVEY CONCERNING THE PERCEPTION ON FISH SENTIENCE, WELFARE AND SLAUGHTER; AUGUST 2014 TO APRIL 2015

Variable	Categories	Number of respondents (%)	
		B	C
Sex	Male	153 (38.7)	143 (36.9)

	Female	242 (61.3)	244 (63.5)
Age (years)	18-25	202 (51.1)	190 (49.1)
	26-35	110 (27.8)	106 (27.4)
	36-45	47 (11.9)	44 (11.4)
	46-55	27 (6.8)	34 (8.8)
	56 or more	9 (2.3)	13 (3.4)
Education	Primary (incomplete)	1 (0.3)	1 (0.3)
	Primary (complete)	1 (0.3)	1 (0.3)
	Secondary (complete)	26 (6.6)[1]	8 (2.1)[2]
	Higher (incomplete graduation*)	157 (39.7)	176 (45.5)
	Higher (complete graduation)	88 (22.3)	85 (22.0)
	Higher (post-graduation)	122 (30.9)	116 (30.0)

Different superscripts report statistical difference (P<0.05) between cities using Chi-square test.
* Graduation in progress or not concluded

Data were analyzed by city, including analysis according to gender and age groups. Descriptive statistics was applied and the Mann-Whitney test was used for comparison in pairs between B and C and between male and female respondents. The comparison within the four age groups was performed using the Kruskal-Wallis test, followed by Dunn's test when significant difference was found. Both tests were applied in Q1, Q2, Q3, Q4, Q6, Q7, Q9, Q10 and Q12. In Q5, Q8 and Q11 we used the Chi-square test. In addition, demographic data were compared between cities through Chi-square test. The significance level was set at $P<0.05$, through Minitab software, version 17.

2.3 RESULTS AND DISCUSSION

2.3.1 Demographic data

According to table 2, the percentage of male and female respondents differed from gender distribution within the population of each city, that is 47.8% male and 52.2% female in B (DANE, 2010) and 47.8% male and 52.3% female in C (IBGE, 2010). A higher percentage of female respondents may be related to higher sensitivity and positive attitudes of women to animal protection (Herzog, 2007). Therefore, women may have greater motivation to complete such type of survey.

Regarding education, there was a significant difference in the number of respondents with secondary education between B and C (Table 2). However, this may have low impact in survey results due to the low percentage of

respondents within this education level. A total of 92.9% of respondents from B and 97.5% from C were attending or attended higher education, differing from population average of 22.1% in B (DANE, 2010) and 43.6% in C (IBGE, 2010), which is most likely a consequence of the sampling method of our study that included most contacts from universities. Therefore, our respondents are a representative sample of citizens of Bogota and Curitiba with higher education.

2.3.2 Fish sentience

2.3.2.1 Sentience perception according to geographic origin

In relation to the perception on fish sentience, 79.7% of respondents from B and 71.8% from C, believed that fish are able to feel emotions (P=0.6195; FIGURE 1A). Some of the reasons cited by these respondents were: “fish have nervous system and sensory receptors as well as other vertebrates”, woman from B, 18-25 years old and secondary education; “fish have a complex nervous system that allows them to feel suffering, pain and fear”, man from B, 26-35 years old and higher education; “I believe fish feel fear, suffering and pain, but I am not sure about joy”, woman from C, 18-25 years old and secondary education; “As any other animal, fish are able to perceive what is happening around them and to react to stimuli”, woman from C, 18-25 years old and higher education. Most respondents (75.8%) considered fish as sentient beings, which may increase moral support of promoting changes in fish handling, aiming to prevent animal suffering.

Few respondents, 8.4% in B and 10.9% in C, believed fish are unable to feel emotions. Some reasons cited by them were: “animals act by instinct”, man from B, over 45 years old and higher education; “fish react to external stimuli, but they have no awareness to feel emotions”, man from B, 18-25 years old and higher education; “fish are irrational and emotion presupposes rationality not to be confused with instinct”, man from C, over 45 years old and higher education; “because fish are not as developed as mammals”, woman from C, 18-26 years old and higher education. These respondents would benefit from contact with the several studies that have reported behavioural complexity of fish, including data on cognition and consciousness (Braithwaite et al., 2013; Broom, 2007, 2010; Chandroo et al., 2004; Huntingford et al., 2006). Additionally, in a recent

study it is concluded that fish are able to retain memories of events with positive and negative valence which may be retrieved by environmental cues (Millot et al., 2014). Our results suggest the need to improve communication between science and society. This improvement may increase knowledge about fish sentience and behaviour, and thus promote the recognition of fish as sentient animals by all.

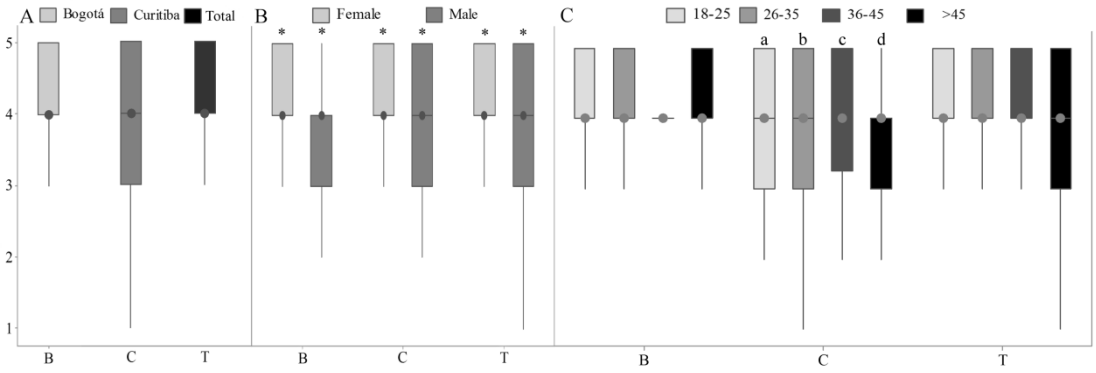

FIGURE 1 - PERCEPTION ON FISH SENTIENCE (Q1) BY 782 CITIZENS OF BOGOTÁ AND CURITIBA, 2014/2015; (A) DATA FOR CITIES, (B) DATA FOR GENDER, (C) DATA FOR AGE, BEING 1 = CERTAINLY NOT AND 5 = CERTAINLY YES; (T) REPRESENTS THE TOTAL DATA; THE ASTERISK INDICATES GENDER DIFFERENCE (P<0.05, MANN-WHITNEY); LETTERS INDICATE DIFFERENCES BETWEEN AGE GROUPS (P<0.05, KRUSKAL-WALLIS AND DUNN TEST)

2.3.2.2 Sentience perception according to gender and age of respondent

When data were compared by gender, most women gave significantly higher scores to fish sentience in both cities (P<0.01; FIGURE 1B). This is in accordance with other studies involving perception and attitudes towards animals, probably due to a higher sensitivity of women (Herzog, 2007; Kupsala et al., 2013; Taylor & Signal, 2005). Comparisons between age groups showed lower perception on fish sentience in respondents of over 45 years old in C (P=0.045; FIGURE 1C). This is in agreement with Kendall et al. (2006), who observed negative correlation between age and positive human attitudes towards the treatment of animals. Recent concerns about farm animal welfare in Brazil may also have influenced citizen opinion, especially the youth. However, reasons for the absence of significant differences among age groups in B are unknown; this result is in accordance with Signal & Taylor (2006), who did not observe correlation between age and positive attitudes to animals. The perceptions of responds from B presented less variance in comparison to

respondents from C (FIGURE 1C). Citizens from C may be going through a generation shift towards higher recognition of sentience by the younger age groups, represented by greater variance of scores. Results suggest that the influence of age in perception on fish sentience should be further studied in both cities, taking into considerations personal beliefs, values and familiarity with animal production, as proposed by Vanhonacker & Verbeke (2014).

2.3.2.3 Sentience perception according to animal species

The respondents rated lower levels of sentience to animals that are more phylogenetically distant from humans (FIGURE 2). The percentage of respondents who scored "I do not know" for each taxonomic group, presenting Bogotá results followed by Curitiba's, was: human baby (1.3 and 1.5%); dog (0.8 and 2.1%); wolf (2.8% and 3.9%); cattle (2.0 and 3.9%); sheep (3.3 and 4.7%); rat (2,0 and 5,7%); chicken (2.3 and 6.4%); pigeon (3.0 and 7.0%); fish (4.5 and 8.8%); butterfly (11.9 and 15.5%) and cockroach (13.7 and 15.2%). Results suggest that respondents had more difficulty to score sentience in animals that are more phylogenetically distant from humans.

Comparison among humans, mammals and birds showed no significant differences, which can be explained by high variance of the attributed scores. Comparing perception on fish sentience with other taxonomic groups, significant higher scores of sentience were assigned to human baby ($P<0.01$; FIGURE 2). Animals farther apart from humans, such as fish and invertebrates, were perceived with a lower degree of sentience, and this inverse relation between phylogenetic and animal sentience was also mentioned by other authors (Knight et al., 2009; Broom, 2010; Kupsala et al., 2013). Additionally, the difficulty of human beings to understand emotions in fish due to differences in body structure, facial expressions and living environment, may contribute to the attribution of low sentience levels in fish when compared to other animals (Röcklinsberg, 2015). For the first time here is reported the position of respondents from Bogotá, Colombia and Curitiba, Brazil, who declared their recognition of sentience in fish. This provides support for actions aiming the inclusion of these animals in welfare regulations.

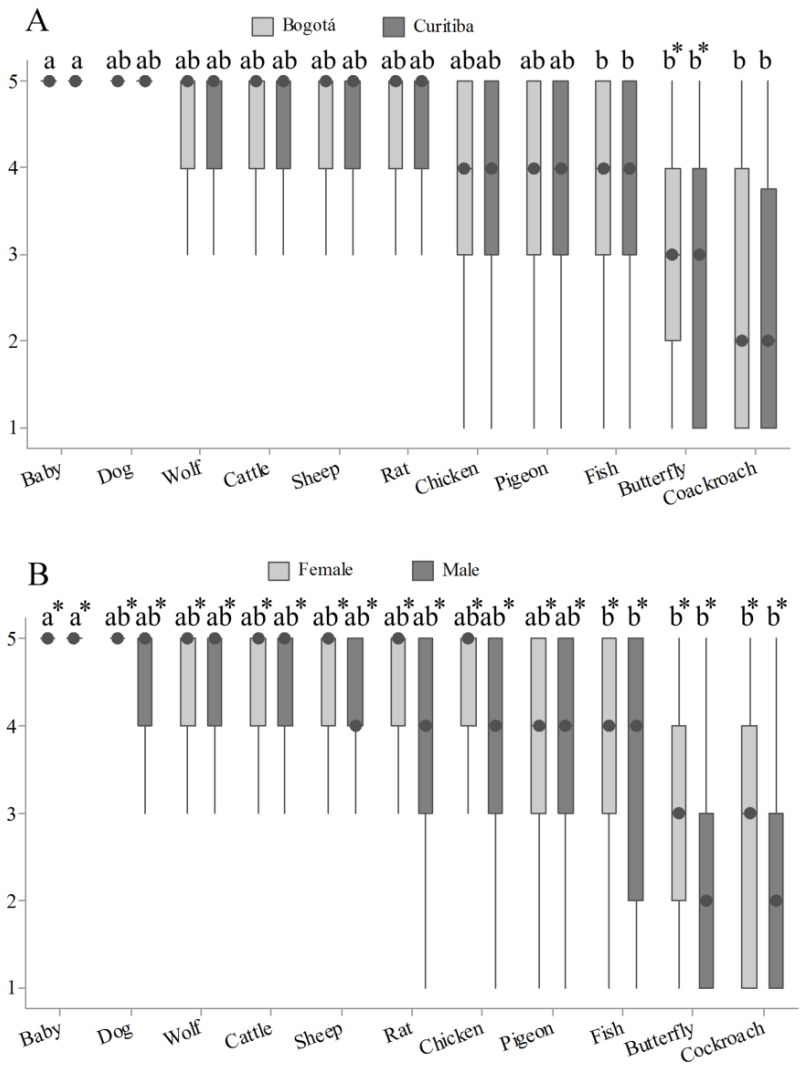

FIGURE 2 - PERCEPTION ON ANIMAL SENTIENCE (Q2) BY 782 CITIZENS OF BOGOTÁ AND CURITIBA, 2014/2015; (A) DATA FOR CITIES, (B) DATA FOR GENDER, BEING 1 = NO SENTIENCE AND 5 = MAXIMUM SENTIENCE; THE ASTERISK INDICATES BOTH CITY AND GENDER DIFFERENCE (P<0.05, MANN-WHITNEY); LETTERS INDICATE DIFFERENCES BETWEEN ANIMALS (P<0.05, KRUSKAL-WALLIS AND DUNN TEST)

Our intention was to study the perception on fish sentience in relation to others species. However, scores of perception on butterfly sentience were different between B and C (P=0.0124; FIGURE 2A). Further studies may help us understand the reason for this difference.

When the perception on sentience within species was compared by gender, women presented higher scores in all groups of animals (P<0.01, FIGURE 2A). This is in agreement with other studies (Taylor & Signal, 2005; Herzog, 2007; Kupsala et al., 2013). Classification and comparison of scores according to age is observed in FIGURE 3. Scores given to dog, wolf and rat were different within age groups (P<0.05). Despite the fact that wolf scores

presented the same median and variance, Dunn's test allowed classification of scores by average rank. Respondents of age group higher than 45 years old tended to assign lower scores to the cattle (P=0.06), and assigned lower scores for dog, wolf and rat. Higher sensitivity of young people is in agreement with the literature (Kendall et al., 2006).

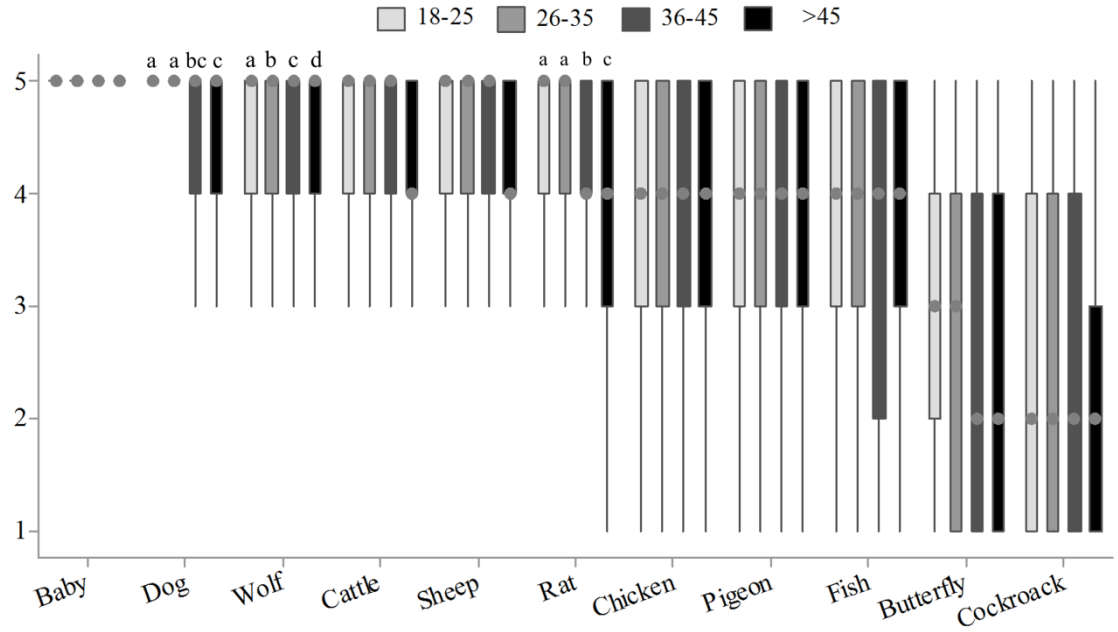

FIGURE 3 - PERCEPTION ON ANIMAL SENTIENCE (Q2) DATA FOR AGE, BY 782 CITIZENS OF BOGOTÁ AND CURITIBA, 2014/2015; BEING 1 = NO SENTIENCE AND 5 = MAXIMUM SENTIENCE; LETTERS INDICATE DIFFERENCES BETWEEN AGE GROUPS (P<0.05, KRUSKAL-WALLIS AND DUNN TEST)

2.3.3 Fish suffering

2.3.3.1 Perceptions about fishing

Most respondents in B (93.7%) and C (92.8%) declared that they recognize animal suffering when fish are removed from the water (P=0.005) and there was greater perception among citizens of C (FIGURE 4A). In addition, women have recognized such suffering more than men in C, resulting in difference between gender in general data (P<0.05; FIGURE 4B). When compared within data of age group, the group of over 45 years old showed lower perception on fish suffering out of the water (P=0.038) (FIGURE 4C). Few respondents, 19.5% from B and 19.9% from C, classified catch-and-release angling as an acceptable practice (P=0.6559; FIGURE 1C), and there were no differences of acceptability within the age groups (P>0.05; FIGURE 3C). Some reasons cited for accepting this practice were: "since it is a practice in which fish

does not die, I think that it may be an acceptable practice", man from B, 36-45 years old and higher education; “because fish will survive after being released” women from C, 18-25 years old and higher education.

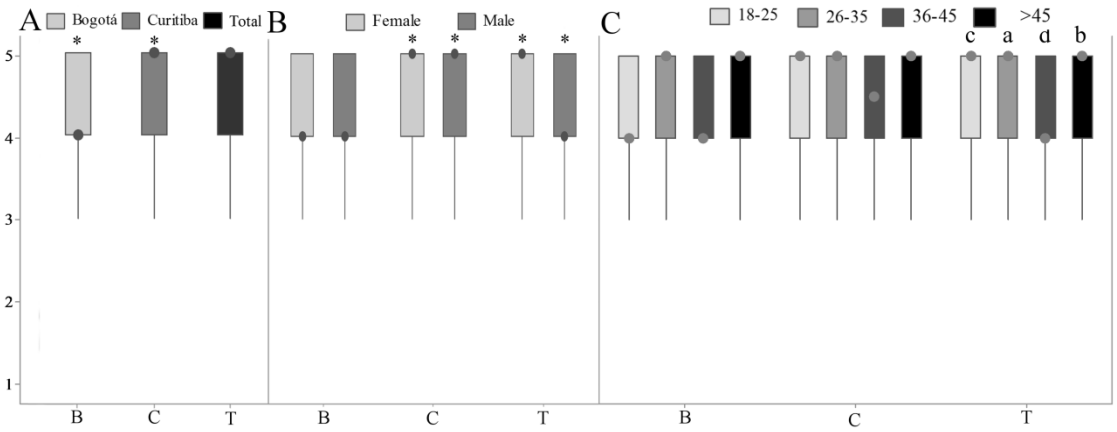

FIGURE 4 - PERCEPTION ON FISH SUFFERING OUT OF WATER (Q3) BY 782 CITIZENS OF BOGOTÁ AND CURITIBA, 2014/2015; (A) DATA FOR CITIES, (B) DATA FOR GENDER, (C) DATA FOR AGE, BEING 1 = CERTAINLY NOT AND 5 = CERTAINLY YES; (T) REPRESENTS THE TOTAL DATA; THE ASTERISK INDICATES CITY AND GENDER DIFFERENCE ($P<0.05$, MANN-WHITNEY); LETTERS INDICATE DIFFERENCES BETWEEN AGE GROUPS ($P<0.05$, KRUSKAL-WALLIS AND DUNN TEST)

Catch-and-release angling is questionable from an ethical point of view because fish are subjected to unnecessary suffering. This fact was recognized by some of the respondents of this study. Ferguson e Tufts (1992), observer that removing fish from water for one minute after exhaustive exercise increased fish mortality when compared to a control group. Cooke & Philipp (2004) also showed that the practice of capture and release increases predation risk for these animals. Furthermore, removing fish from water has already been showed to be a stressing condition, with increased blood cortisol levels (Pankhurst & Dedualj, 1994; Huntingford et al., 2006). The recreational angling, with or without releasing, affects directly the animal as it causes injury associated with pain (Muoneke & Childress, 1994; Huntingford et al., 2006). Additionally, the recreational angling with release is associated with a mortality rate of 30-68% which depends on the type of hook, fish size, location and number of perforations (Muoneke & Childress, 1994). In general, the majority of fish are hooked through the jaw region (Muoneke & Childress, 1994). This area is important for respiration, food acquisition and consumption, and in some cases for reproduction (e.g., mouth brooding, competition for mates) or social

interactions (e.g., yawning, displays) (Cooke and Sneddon, 2006). Additionally, Sneddon (2003) reported several types of sensory receptors in this area. As suffering is defined like an unpleasant state of mind that disrupts the quality of life, it is the mental state associated with unpleasant experiences such as pain, distress and injury. Thus, it seems that unnecessary suffering and poor welfare are consequences of catch-and-release angling in fish.

Most respondents from B, 67.3%, and C, 64.3%, did not accept catch-and-release angling as a recreational practice (FIGURE 5A), being women more opponent than men in both cities ($P<0.01$; FIGURE 5B). Common causes for rejecting this practice included the capacity of fish to suffer when removed from the water and the unnecessary injuries caused to animals for human entertainment. As example, one respondent declared “even if fish are slaughtered, there is no justification to catch them only for fun”, man from B, 25-35 years old and higher graduation; or “it causes unnecessary suffering of animal in my point of view”, woman from C, 36-45 years old and higher education. In the literature, level of support varied in different countries, being 35.0% in Germany, 65.0% in New Zealand, 71.0% in England (Arlinghaus et al., 2012; Muir et al., 2013). Respondents from B and C showed higher sensibility for fish suffering in catch-and-release angling, and the higher percentage of female respondents may have corroborated to this result.

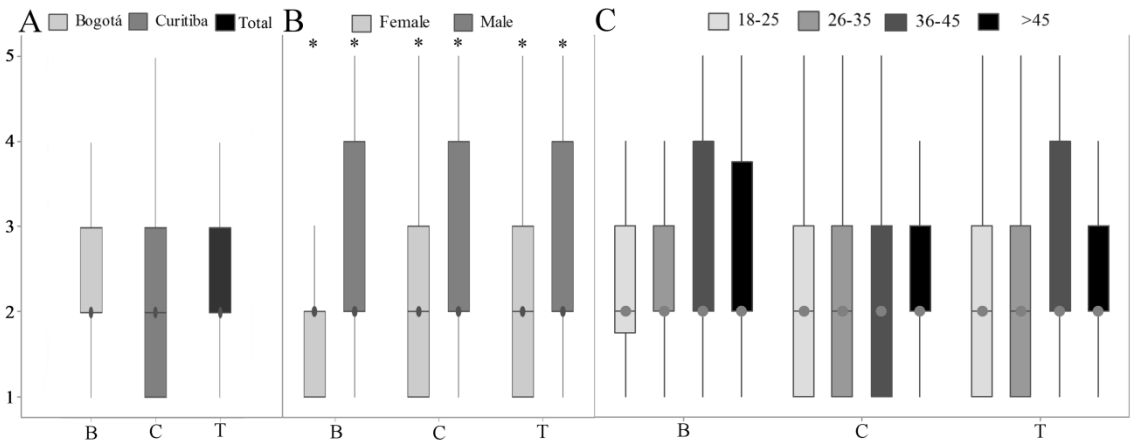

FIGURE 5 - ACCEPTABILITY OF CATCH-AND-RELEASE ANGLING (Q4) BY 782 CITIZENS OF BOGOTÁ AND CURITIBA, 2014/2015; (A) DATA FOR CITIES, (B) DATA FOR GENDER, (C) DATA FOR AGE, (T) REPRESENTS THE TOTAL DATA; BEING 1 = CERTAINLY NOT AND 5 = CERTAINLY YES; THE ASTERISK INDICATES GENDER DIFFERENCE (P<0.05, MANN-WHITNEY)

In Brazil, fishing was regulated by the Ministry of Fisheries and Aquaculture, which was incorporated in October 2015 by the Ministry of Agriculture. Amateur fishing license is issued by governmental body to a person or an entity for leisure and sports when there is no economic purposes (Brasil, 2009). This license is in accordance to the article 29 of the Environmental Crimes Act, law 9,605, which considers crime the act of killing, pursuing, hunting, catching and using wild, native or migratory animals without specific license or in disagreement with the license (Brasil, 1998). Nevertheless, this is incoherent with article 32 of the same law, which considers a crime to practice acts of abuse, mistreatment, to injury or to mutilate wild or domestic, native or exotic animals. In Colombia, sport fishing license is issued to any person (AUNAP, 2012). The same incoherence was observed in Colombian animal protection law, which considers cruelty to cause injury on animals (Colombia, 1989).

Some groups have suggested the development a code of standard practices to improve the welfare of fish on recreational fishing (Arlinghaus et al., 2007, 2012). In 2007, the European Inland Fisheries Advisory Commission of the Food and Agricultural Organization of the United Nations prepared a code of practice for recreational fisheries, to set minimum global requirements for this practice, including fish welfare (EIFAC, 2008). Codes of practice are non-mandatory, and the voluntary adoption by people involved with recreational

fishing may be strongly related to personal belief about fish sentience. Additionally, as most respondents of both B and C do not agree with catch-and-release angling, it seems important to develop specific regulation to avoid unnecessary suffering of fish that is commonly observed in this practice.

Fewer respondents from B have already practiced angling when compared to C (11.1% and 31.3%, P<0.01). Variation was also found in New Zealand (15.1%) (Muir et al., 2013) and Finland (40.0%) (Toivonen, 2008). The observed variation may be consequence of cultural characteristics of each country. For example, in the metropolitan region of Curitiba there is availability of fish-and-pay pond tanks where people practice recreational fishing. According to our results, angling is predominantly performed by men in B and C (P<0.01). About 63.2% of women declared they had never practiced angling in B, against 36.6% of men; and 30.3% of women in C, against 12.6% of men. As observed by Shrestha et al. (2002) 99.0% of sport fishing in Pantanal, Brazil, were practiced by men. These results may be an effect of higher concerns of women about fish welfare and sentience, leading to a reduced practice of angling. Total of respondents that had never practiced angling were 54.2% in B and 22.7% in C (P<0.01). It seems relevant to animal welfare to understand motivations of these respondents since they may not feel pleasure in an activity that is harmful to fish.

2.3.3.2 Perception on fish pain

Median score for fish capacity to feel pain was 4.0 (1.0-5.0) in both cities (P=0,1267; FIGURE 6). In general, few respondents believed fish do not feel pain, 2.3% in B and 5.4% in C, close to the low proportion of 1.2% found by Muir et al. (2013), in New Zealand (Muir et al., 2013). Gender and age also influenced this result, since women and young people gave higher scores to the capacity of fish to feel pain (P<0.01; FIGURES 6B and 6C). Scientific debates have pointed against (Rose, 2002; Rose et al., 2014) and in favor (Chandroo et al., 2004; Broom, 2007; Braithwaite, 2010; Sneddon, 2012; Sneddon et al., 2003, 2014) of fish capacity to feel pain. However, the precautionary principle is recommended in order to improve animal welfare, which means that one should protect a group of animals if there are doubts about their capacity to feel pain (Sneddon et al., 2014). In addition, the perception of great part of respondents

that fish can feel pain is important to encourage the adoption of procedures and the development of regulation to avoid fish suffering.

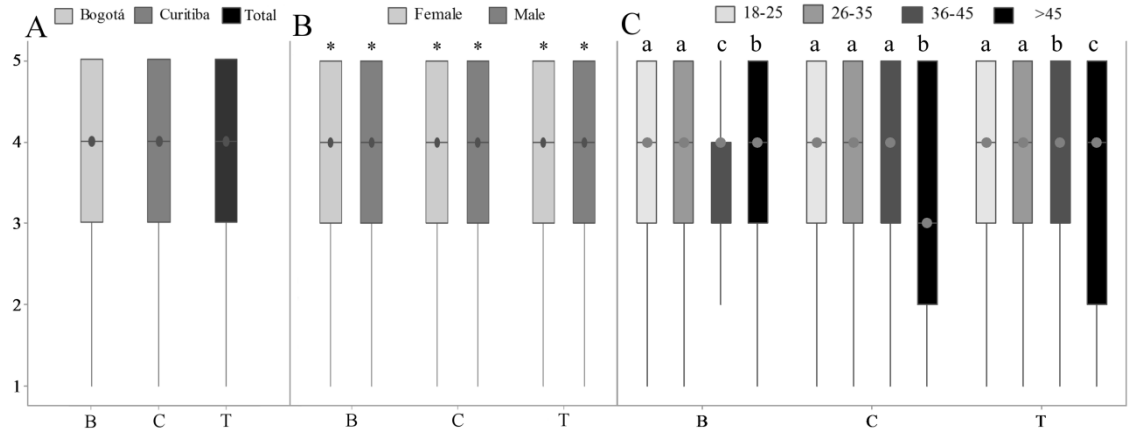

FIGURE 6 - PERCEPTION ON FISH PAIN (Q6) BY 782 CITIZENS OF BOGOTÁ AND CURITIBA, 2014/2015; (A) DATA FOR CITIES, (B) DATA FOR GENDER, (C) DATA FOR AGE, BEING 1 = NONE AND 5 = MAXIMUM; THE ASTERISK INDICATES GENDER DIFFERENCE (P<0.05, MANN-WHITNEY); LETTERS INDICATE AGE DIFFERENCE (P<0.05, KRUSKAL-WALLIS AND DUNN TEST)

2.3.3.3 Perception on fish suffering in different scenarios

Perception on fish suffering was different according to each scenario, as observed on FIGURE 7A. Descending order of maximum perception on fish suffering, presenting Bogotá results followed by Curitiba's, was fishing with hook and line (75.6 and 70.6%); municipal live fish fair (68.7% only C); fish-and-pay pond (59.7 and 54.4%); fish kept as laboratory animals (58.0 and 48.1%); fish farming (35.7 and 36.8%); fish in pet stores (35.5 and 26.1%); production of ornamental fish (19.3 and 21.8%); fish in aquarium exhibits (18.8 and 16.9%) and fish kept as pets (12.4 and 12.3%). Vanhonacker & Verbeke (2014) found that attitudes, perceptions, concerns and behaviour were much more strongly influenced by variables that relate to individual experiences and familiarity with the agricultural sector and to life-style, values and beliefs in relation to animals, animal welfare and animal production than by socio-demographic characteristics (Vanhonacker & Verbeke, 2014). Two scenarios differed between B and C, fish kept as laboratory animals and in pet stores (P<0.05). These scenarios presented great variance in C (FIGURE 7A), also observed in other questions, while respondents from B attributed significantly higher scores; which may be related to individual experience of respondents that may affect suffering perception.

Similar results were observed between women and men regarding municipal live fish fair. Women presented a tendency of higher perception on fish suffering in pet stores ($P=0.055$) and significant higher scores in all other scenarios ($P<0.05$; FIGURE 7B). Comparison between age groups also differed in four scenarios ($P<0.05$, FIGURE 7C), but without a logic conclusion since in some cases, e.g. fish in pet stores and kept as pet, young age group did not give the higher scores. It is desirable more research to understand the effect of age on perception on fish suffering in different scenarios.

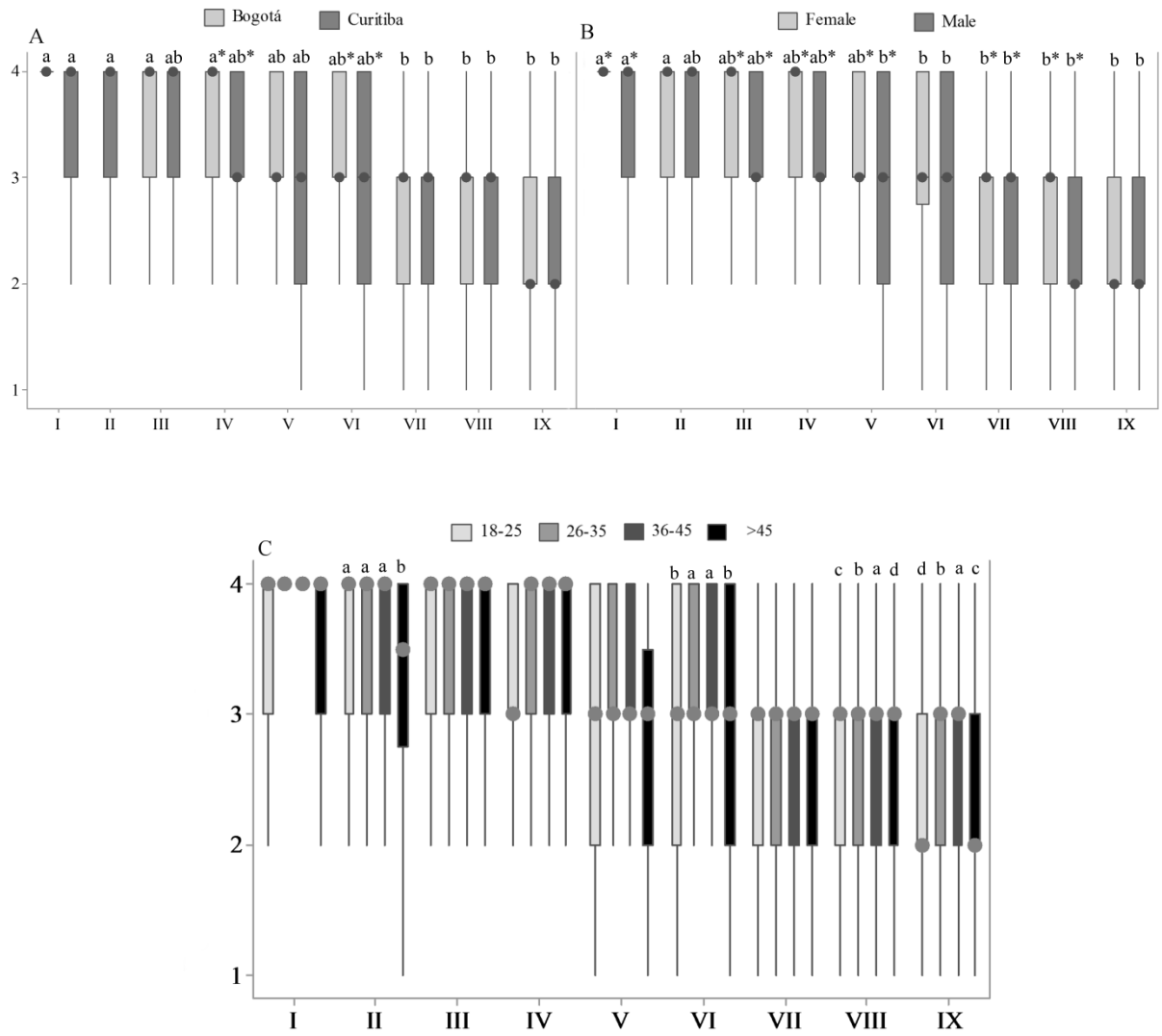

FIGURE 7 - PERCEIVED LEVEL OF SUFFERING IN DIFFERENT SCENARIOS (Q7) BY 782 CITIZENS OF BOGOTÁ AND CURITIBA, 2014/2015; (A) DATA FOR CITIES, (B) DATA FOR GENDER, (C) DATA FOR AGE, BEING 1 = NONE AND 5 = A LOT; (I) FISHING WITH HOOK AND LINE, (II) MUNICIPAL LIVE FISH FAIR, (III) FISH-AND PAY PONDS, (IV) FISH KEPT AS LABORATORY ANIMALS, (V) FISH FARMING, (VI) FISH IN PET STORES, (VII) PRODUCTION OF ORNAMENTAL FISH, (VIII) FISH IN AQUARIUM EXHIBITS, (IX) FISH KEPT AS PETS; THE ASTERISK INDICATES CITY AND GENDER DIFFERENCE (P<0.05, MANN-WHITNEY); LETTERS INDICATE DIFFERENCES BETWEEN SCENARIOS AND AGE GROUPS (P<0.05, KRUSKAL-WALLIS AND DUNN TEST)

The live fish fair, which is a scenario with high perception on animal suffering, has taken place for more than 25 years in metropolitan region of Curitiba during the Easter week. This may have affected suffering perception of older respondents, which presented lower scores in this scenario within the age groups (FIGURE 7C). It is also common in other States in Brazil, such as Santa Catarina, Rio Grande do Sul, Paraíba and Pernambuco (Carvalho & Callou, 2008; Pedrazzani et al., 2008; Cardoso et al., 2009; Mendes Filho et al., 2010; Perussantto et al., 2012). Live fish fair is an income complement for producers

because it is the opportunity to sell the product directly to final consumer, without intermediaries. In Araucária, for example, about 12,000 people visited the fair in two days (SMAG, 2010). High number of visitants seems to be related to a demand for fresh product. Most common species are tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), big head carp (*Aristichthys nobilis*), silver carp (*Hypoththalmichthys molitrix*) and native species like catfish (*Rhamdia quelen*) and pacu (*Piaractus mesopotamicus*) (Cardoso et al., 2009).

During the live fish fair in Araucária, metropolitan region of C, 20 tons of fish were sold in 2015 (Prefeitura de Araucária, 2015), and in Santa Maria, State of Rio Grande do Sul, amount sold reached 40 tons (Cardoso et al., 2009). Usually, there is a fast period of 24 to 48 hours prior the fair. Fish are kept in tanks with high densities on farm, during transport and at the fair. Transport of live fish is considered a limitation by producers since transport requires time and increase production cost (Perussantto et al., 2012). Fish are sold alive and consumers take them home in plastic bags without water supply; or fish are slaughetered and cleaned on place without previous stunning. Both situations are considered critical points for animal welfare (Robb & Kestin, 2002; Huntingford et al., 2006; Ashley, 2007; Pedrazzani et al., 2008), and similar slaughter situations with any other vertebrate farm animal would probably not be allowed by governamental bodies.

About 61.2% of respondents from C answered that fish suffer during live fair. Based on this, it seems relevant to assess fish welfare during the fair to implement measures that may improve animal welfare or may suggest the need of abolishing live fish fair. Establishing a fish producer association to centralize fish slaughter and processing, supported by local authorities, may be a viable alternative to replace those fairs (Perussantto et al., 2012). In this case, fresh fish would still be offered to consumers, avoiding unecessary suffering caused by poor welfare conditions on tanks during the fair and by asfixia in plastic bags.

Maximum perception on fish suffering on fish-and-pay pond scenario (59.7% B and 54.4% C) was lower than fishing with hook and line (75.6% B and 70.6% C). However, these two scenarios have similar characteristics. On fish-and-pay pond establishments, consumers have the option to take home the cleaned fish, which may lead to a reduced perception of suffering, probably due

to dissociation of the live animal from the final product that is consumed (Hoogland et al., 2005).

Fish kept as laboratory animals were also perceived by respondents as involved in animal suffering (58.9% B and 48.1% C; $P<0.05$). The amount of fish used for research purposes has increased since the 1990s (Borski & Hodson, 2003), and it tends to further increase. As an example, in the United Kingdom, the number of fish used in research increased by 15% from 2010 to 2011 (Home Office, 2011). Since fish represent the most diverse group of vertebrates, with aproximately 25,000 species, the development of guides for the care and use of these animals in research has been a challenge (Borski & Hodson, 2003). Other studies have observed opposition to the use of animal in research (Phillips & McCulloch, 2005; Knight et al., 2009; Metzger, 2014), which may indicate the need of further information about animal welfare in laboratory scenario. Welfare assessment of fish in laboratories is also desirable to identify critical points and provide actions to address them.

Fish farming ($P>0.05$; B 41.3%, C 31.5%), fish in pet stores ($P<0.01$; 41.6% B, 35.9% C), production of ornamental fish ($P>0.05$; B 42.3%, C 33.9%), and fish in aquarium exhibits ($P>0.05$; 37.9% B, 35.1% C) were perceived by respondents to cause moderate suffering. Results suggest that respondents may be aware of some items related to these scenarios to consider them as moderate suffering.

In Brazil, 61.8% (765,287 tons) of fish for human consumption are obtained by capture and 38.2% (473,429 tons) by inland aquaculture (FAO, 2013). In Colombia, these percentages are 40.0% (59,639 tons) and 60.0% (89,398 tons), respectively (FAO, 2013). Procedures of fish handling, removal from the water, transport, disease control, stocking density, feed withdrawal and slaughter are known as critical points that lead to poor welfare in a great number of fish (Ashley, 2007). Public perception on animal suffering should be taken into consideration by governmental bodies to invest in research aiming to provide support for the development of regulation for the protection of fish used for human consumption.

Considering the pet store scenario, animal abuse in pet stores located in metropolitan region of Curitiba have been reported (Hammerschmidt, 2012). Although complaints did not include fish, they suggest inability of some pet

stores to comply with minimum animal welfare requirements, demanding further investigation. According to Huntingford et al. (2006) and Volpato (2009) potential critical points of fish kept in pet stores include high stocking density, low water quality and abnormal behavior, which refer to poor welfare. In Brazil and Colombia, pet stores must contract a veterinarian, who is responsible for animal welfare (Bogotá, 2014; Conselho Regional de Medicina Veterinária do Paraná, 2014). Moderate perception on fish suffering in pet stores may indicate the need of more active participation of veterinarians on these establishments.

Perception on fish suffering in ornamental scenario may be influenced by previous knowledge of respondents about this production chain. According to FAO, more than 90% of freshwater ornamental fish are captive bred compared to only 0.3% in the case of marine fish (FAO, 2015). Fish exported from Brazil are almost all captured in both continental and marine waters, while internal market is mainly supplied by inland captive fish (IBAMA, 2008). Thus, perception on moderate suffering may be related to those fish obtained from aquaculture, which may be understood by respondents as having similar conditions of fish bred for human consumption. However, animal suffering may be even higher in captured wild fish, since it is estimated that only one in ten animals captured from nature survives (Renctas, 2001). Although the estimate does not include fish, high mortality is also expected to occur. Great losses of wild-caught fish occurs because fish are subjected to physical injury, extreme changes in water quality and temperature, and exposure to chemicals used as prophylactic treatment for disease control (Livengood & Chapman, 2007). Our results suggest the need of more information to consumer about the origin and keeping of ornamental fish, as well about welfare status of these animals.

Moderate perception on suffering in fish in aquarium exhibits may be related to conditions of aquariums, where animal welfare status is controversial (Bekoff, 2007; Huntingford et al., 2006; Volpato, 2009). Respondents perceived fish kept as pets as a low suffering scenario (37.9% B; 35.1% C), which may be motivated by a common sense that pet owners care about their pets. In the specific case of fish, water quality, inadequate nutrition and privation of social interaction are critical points to animal welfare (Huntingford et al., 2006).

Perception on fish suffering in different scenarios may motivate new studies about animal welfare assessment. From such knowledge,

establishments may implement protocols to ensure minimum standards of animal welfare. An effective communication on the level of fish welfare in different scenarios can value the work of responsible professionals and improve public perception.

2.3.4 Perceptions about fish slaughter

Results about respondent knowledge on slaughter methods are presented on FIGURE 8. Other methods mentioned by respondents from B were anesthesia (3.0%), exsanguination (1.3%) and electronarcosis (0.5%). Respondents from C also mentioned immersion in ice (4.5%) and electronarcosis (1.6%). Percussion and decapitation presented statistical difference between B and C ($P<0.05$; FIGURE 8A), which may be a consequence of previous contact of respondents with the slaughter methods. Besides that, answers about percussion differed according to gender and age (FIGURES 8B and 8C). Men mentioned percussion method more than women did. This may be explained because percussion stunning is likely the most common method used by anglers when fish is killed for harvest (Cooke & Sneddon, 2007) and, according to our results, angling is predominantly practiced by men in B and C.

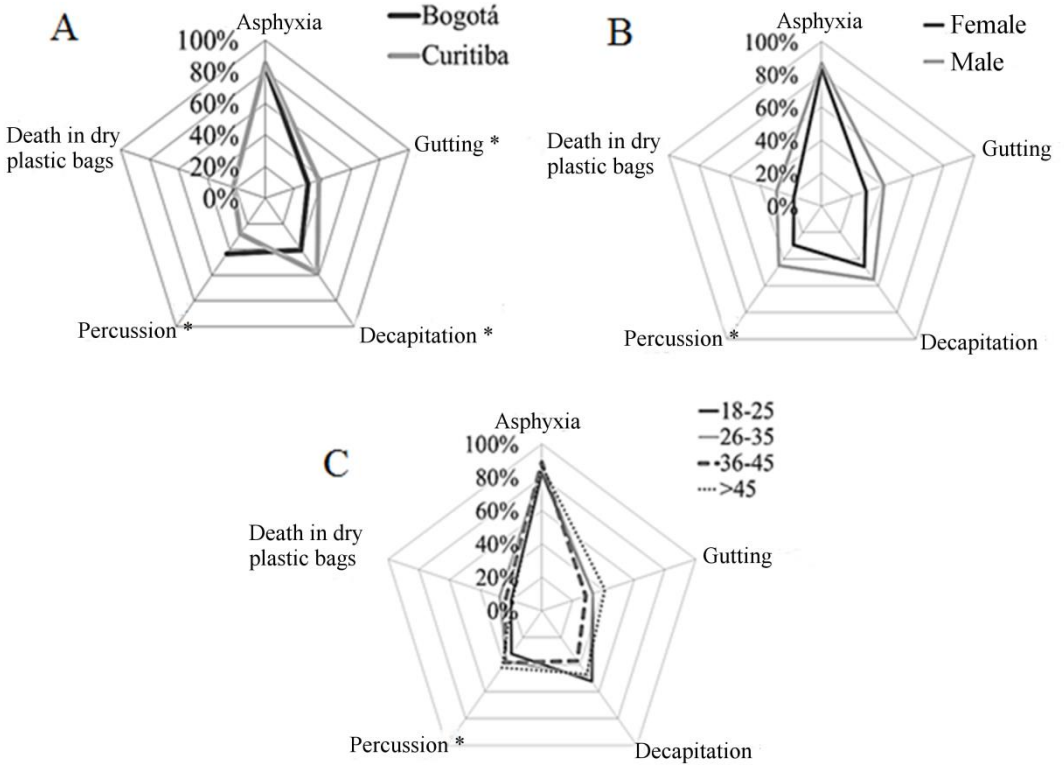

FIGURE 8 - PERCENTAGE OF RESPONSES REGARDING THE SLAUGHTER METHODS (Q8) MOST OBSERVED BY 782 CITIZENS OF BOGOTÁ AND CURITIBA, 2014/2015; (A) DATA FOR CITIES, (B) DATA FOR GENDER, (C) DATA FOR AGE, THE ASTERISK INDICATES DIFFERENCE BETWEEN GROUPS (P<0.05; CHI-SQUARE TEST)

Most respondents (84.2% B; 90.4% C; P=0.0027) believed that slaughter methods indicated on question Q8 (Table 1) may cause fish suffering (FIGURE 9), which is in agreement with Pedrazzani et al. (2008) who observed percentages of 85.0% and 89.0%. Higher scores for fish suffering were given by women and young groups (FIGURES 9B and 9C, P<0.05). Slaughter methods mentioned on question Q8 are known to cause animal suffering, and are not recommended (EFSA, 2009). The development of new methods of fish slaughter is in agreement with advances of society's ethical concerns and with new demands of fish welfare of the Word Organization for Animal Health – OIE (OIE, 2015).

A total of 64.6% of respondents in B and 63.9% in C agreed that fish suffering may affect meat quality (P<0.05; FIGURE 9). Pedrazzani et al. (2008) observed lower percentages of 52.9% and 44.1% from respondents interviewed during the live fish fair and at a bus station in Curitiba. High education level of our respondents may have contributed to higher knowledge about the relation between animal suffering and meat quality. Procedures such as pre-slaughter, catching and transport may cause fish stress (EFSA, 2009), and there is a

negative correlation between suffering level and meat quality (Poli et al., 2005). Higher scores were observed on women and young groups in C (P<0.05; FIGURES 9B and 9C). Future studies may better describe differences found on age and gender groups. Since expected quality is one of the most important factors in consumer intention to purchase food (Font-i-Furnols & Guerrero, 2014), it seems important to inform consumers about the relationship between animal suffering and meat quality in order to increase consumer demand for higher welfare on fish production for human consumption.

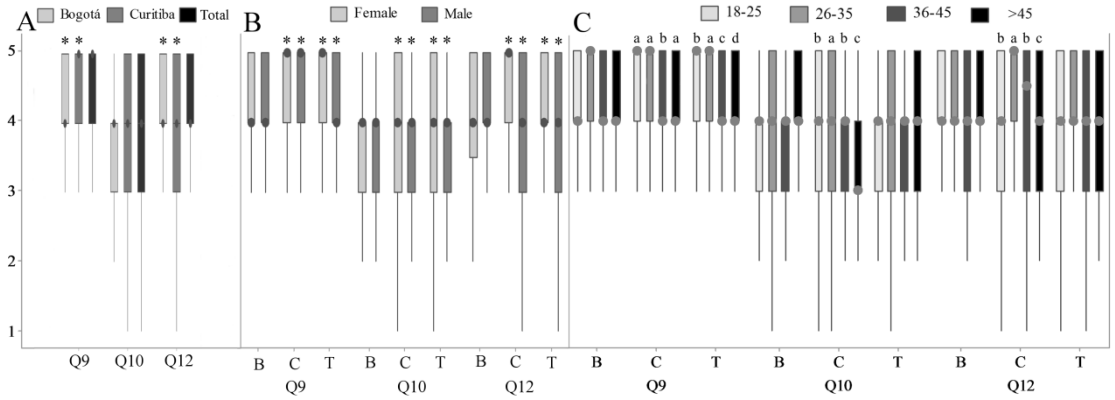

FIGURE 9 - PERCEPTION ON SUFFERING IN SLAUGHTER (Q9), MEAT QUALITY (Q10) AND REGULATION (Q12) BY 782 CITIZENS OF BOGOTÁ AND CURITIBA, 2014/2015; (A) DATA FOR CITIES, (B) DATA FOR GENDER, (C) DATA FOR AGE, BEING 1 = CERTAINLY NOT AND 5 = CERTAINLY YES; THE ASTERISK INDICATES GENDER DIFFERENCE (P<0.05, MANN-WHITNEY); LETTERS INDICATE DIFFERENCES BETWEEN AGE GROUPS (P<0.05, KRUSKAL-WALLIS AND DUNN TEST)

Considering humane slaughter, 57.0% of respondents from B and 55.0% from C had never heard about it (P>0.05), differing from Pedrazzani et al. (2008) who observed higher percentage of 91.1%. Difference between these studies may be consequence of higher educational level of respondents in our research. Therefore, on general population of B and C, where educational level is lower (DANE, 2010; IBGE, 2010), percentage of people who does not know humane slaughter is expected to be even higher. Many people enjoy eating meat but few enjoy harming or killing other sentient creatures. These inconsistent beliefs create a ‘‘meat paradox’’; people simultaneously dislike hurting animals and like eating meat (Loughnan et al., 2010). To solve this problem, people become vegetarian or fail to recognize that animals are killed to produce meat. Although few people live in true ignorance, some meat-eaters may live in a state of tacit denial, failing to equate beef with cow or pork with pig

(Loughnan et al., 2010). Perhaps for this reason, respondents do not know the meaning of humane slaughter. In C, women were more familiar to the term humane slaughter (P=0.009; 50.0%) when compared to men (36.4%). Additionally, only in C, the same was observed on young respondents of 18-25 years old (P=0.005; 44.7%) when compared to age group older than 45 years old (23.4%). Society awareness about the existence of procedures of fish slaughter that prevent animals from feeling pain is an important tool to increase demand for stunning methods on fish production. This is particularly important because in Colombia (Colombia, 2007) and Brazil (Brasil, 2000), regulations about humane slaughter of farmed animals do not include fish.

Most of respondents, 76.0% in B and 72.0% in C, agreed that fish should be included on humane slaughter regulation, with higher variation on C (P<0.05; FIGURE 9). This demonstrates a positive attitude of participants towards the inclusion of fish on the existing regulation in both countries, with higher scores in B. Women and respondents under 45 years old presented higher scores than older respondents in C (FIGURES 9B and 9C). Besides the fact that half of respondents did not know the term “humane slaughter”, results suggest that they understood its meaning and expressed their opinion. Reasons given by respondents that agreed with the inclusion of fish on humane slaughter regulation were based on recognition of fish sentience, concerns about meat quality and equality among farm animals. For example, “As any other animal, fish are sensitive and feel pain. If fish are intended for human consumption, we should be careful to cause the least possible suffering”, woman from B, 26-35 and higher education; “to improve meat quality and increase added value, avoiding excessive suffering”, woman from B, 18-25 years old and higher education; “because, similarly to other animals, fish feel stress and pain”, woman from C, 18-25 years old and higher education; “less suffering and higher meat quality, following what happens on slaughter of broiler chickens, pigs and cattle in some slaughterhouses”, man, over 45 years old and higher education.

This study presents original data from two cities in Latin America about perception on fish sentience, welfare and slaughter. Our results suggest that well educated citizens from Bogotá and Curitiba may accept the inclusion of fish in the circle of moral consideration. It is necessary important to study public

attitude in other regions of Latin America. Different cultures, even in the same country, may be related to different perceptions.

2.4 CONCLUSIONS

This study presents original data suggesting that well educated citizens from Bogotá and Curitiba consider fish as sentient beings. There was less variability in responses from citizens in B as compared to those in C; the latter showing slightly lower scores of sentience and suffering perception, apparently due to responses from higher age-groups. The perception on suffering in specific scenarios challenges activities such as recreational fishing, live fish fair and laboratory fish use. There seems to be a need to better inform citizens about procedures applied to fish in other scenarios, where suffering was not considered high by respondents, such as ornamental fish, aquarium exhibits and fish kept as pets. Recognition of suffering also endorses humane slaughter regulations to reduce pain in a large number of fish slaughtered annually for human consumption in Colombia and Brazil.

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3. ELECTRICAL STUNNING IN PACU (*Piaractus mesopotamicus*)

RESUMO

No Brasil, a imersão no gelo é usada no pré-abate na maioria dos frigoríficos de peixes. O objetivo deste trabalho foi estudar parâmetros elétricos para insensibilização em pacu (*Piaractus mesopotamicus*) para reduzir o sofrimento durante o abate e seus subsequentes efeitos sobre o pH e rigor mortis. Foram avaliados 70 peixes, divididos em dois grupos. No grupo G205, os peixes foram insensibilizados por meio dos parâmetros: 205 Volts (V), 50 Hz, ciclo de trabalho de 70% e 1.3 Amp (A) aplicados durante 45 s. No grupo G400, os peixes foram insensibilizados com 400 V, 30 Hz, ciclo de trabalho de 30% e 0.9 A, durante 30 s. A insensibilização elétrica foi aplicada com um equipamento de pesca elétrica portátil (Smith Root LR-24) em uma caixa plástica com 80 L de água. O tempo de inconsciência foi determinado por meio de avaliação comportamental. Imediatamente após determinar o retorno à consciência, todos os peixes foram colocados em uma caixa plástica com 35 L de água e 200 mg L^{-1} de benzocaína antes da sangria. Os 10 primeiros peixes de cada grupo foram identificados e colocados sob refrigeração em caixas plásticas com gelo, para medir o pH no músculo epaxial, assim como o índice de rigor mortis (RMI) às 0, 3, 6, 24, 72, 120 e 168 horas após a sangria. Todos os peixes foram efetivamente insensibilizados. Ao aplicar a corrente elétrica, os peixes perderam todos os reflexos e respostas comportamentais: os animais cessaram o nado, perderam o eixo de nado, não responderam a estímulos dolorosos, cessaram movimentos operculares e não mostraram reflexo vestíbulo-ocular. O tempo de inconsciência foi maior no tratamento G205, igual a 61.7 ± 13.4 s ($P<0.01$), em relação ao G400 50.1 ± 9.6 s, possivelmente relacionado a um maior tempo de exposição à corrente elétrica e a um maior ciclo de trabalho. Os valores de pH muscular (mediana, min-max) imediatamente após o abate foram 6.80 (6.70-6.90) no G205 e 6.75 (6.60-6.80) no G400 ($P<0.05$). Todos os peixes atingiram o rigor mortis máximo (RMI=100%) às 6 horas pós-abate. Os peixes não apresentaram nenhum dano externo, fratura vertebral, hemorragias ou petéquias no filé. A insensibilização elétrica em pacu parece viável a partir dos parâmetros utilizados no G205, devido ao tempo de inconsciência maior que 1 min, o que pode permitir um tempo mínimo de sangria sem sofrimento. Novos estudos são necessários para fornecer melhores estratégias para a indústria, de modo que regulamentações sobre abate humanitário de peixes possam ser implantadas.

Palavras-chave: Abate humanitário. Bem-estar animal. Inconsciência. Qualidade da carne.

ABSTRACT

In Brazil, pre-slaughter immersion in ice is used in most fish slaughterhouses. The aim of this paper was to study parameters for electrical stunning in pacu (*Piaractus mesopotamicus*) as a way to reduce animal suffering during slaughter and its subsequent effects on pH and rigor mortis. We evaluated 70 fish, divided into two groups. In one group (G205), fish were stunned following the parameters: 205 Volts (V), 50 Hz, duty cycle of 70% and 1.3 Amp (A) applied for 45 s; in the other group (G400), fish were stunned with 400 V, 30 Hz, duty cycle of 30% and 0.9 A, for 30 s. Electrical stunning was applied using a backpack electrofisher (Smith Root LR-24) in a plastic box with 80 L of fresh water. We determined the unconsciousness time through behavioral evaluation. Immediately after checking the return of consciousness, all fish were placed in a plastic box with 35 L of fresh water and 200 mg L^{-1} of benzocaine before bleeding. The first 10 fish from each group were identified and placed in refrigerated plastic boxes with ice for measurements of pH in the epaxial muscle as well as the rigor mortis index (RMI) at 0, 3, 6, 24, 72, 120 and 168 h after bleeding. All fish were effectively stunned. When the current was applied, we observed that all fish lost behavioral responses and reflexes instantly: fish stopped swimming, lost equilibrium, did not respond to painful stimuli, ceased opercular movements and did not show any vestibulo-ocular reflex. The unconsciousness time was significantly higher in the G205 treatment, equal to 61.7±13.4 s ($P<0.01$), in relation to G400 50.1 ± 9.6 s, possibly related to longer exposure to the electric current and the longer duty cycle. The pH values (median, min-max) immediately after slaughter were: 6.80 (6.70-6.90) for G205 and 6.75 (6.60-6.80) for G400 ($P>0.05$). All fish reached maximum rigor mortis (RMI=100%) within 6 h post mortem. The animals did not show any external damage, vertebral fracture, hemorrhages or blood spotting in the meat. Electrical stunning in pacu seems to be feasible through the G205 parameters, due to the unconsciousness period longer than 1 min, which may allow minimum time period to proceed bleeding without suffering. More research is needed to provide best strategies to the fish industry, so that fish regulations regarding humane slaughter may be implemented.

Keywords: Fish welfare. Humane slaughter. Meat quality. Unconsciousness.

3.1 INTRODUCTION

Brazil presents important potential for aquaculture because of its extensive coast and plentiful continental waters. In 2011, 1.43 million tons of fish were produced, being 544.490 tons (38.0%) from inland aquaculture (Brasil, 2011). The main species produced are tilapia and carp, and both represent 53.6% the production on farms; native species such as cachama (*Colossoma macropomum*), tambacu (*Colossoma macropomum* X *Piaractus mesopotamicus*), pacu (*Piaractus mesopotamicus*) and jundia (*Rhamdia quelen*) represent 33.9% of production (Brasil, 2011).

Piaractus mesopotamicus (Ostariophysi: Characiformes: Characidae), commonly known as pacu, is one of the most promising species for Brazilian fish farming, because of its fast growth rate, easy adaptation to artificial feeding and high consumer acceptance (Jomori et al., 2005; Ostrensky et al., 2007). It is the third most cultivated fish species in Brazil, being the production in 2011 equal to 21.689 tons (Brasil, 2011). The production of pacu is of interest for South American countries along the Parana River Basin: Paraguay, Uruguay, Argentina and Brazil (Jomori et al., 2005).

Recently, the World Organization for Animal Health - OIE, through the Aquatic Animal Code, set recommendations for the welfare of farmed fish, mainly in transport, stunning and killing for human consumption (OIE, 2015). In Brazil, the hypothermia in ice for stunning is used in most fish slaughterhouses (Oliveira Filho et al., 2014). Such method is recognized as causing prolonged suffering before loss of consciousness in many fish species (Lambooij et al., 2006; Lines et al., 2003; Pedrazzani et al., 2009; Robb & Kestin, 2002; Van de Vis et al., 2003), and there is ethical concern to avoid pain and suffering in animals destined for human consumption (Vanhonacker et al., 2008). In a study with participants of Latin America, 75.3% (589/782) of respondents considered that fish should be included in humane slaughter regulations (Rucinque & Molento, 2015).

Thus, stunning is typically applied to animals to induce unconsciousness and insensibility to pain stimuli for sufficient duration to secure that an animal does not recover during exsanguination (Lambooij et al., 2008).

Electricity is commonly used for stunning poultry and mammals that are killed for food. Effective electrical stunning is associated with the induction of a generalized epileptiform insult, if the electrical current has sufficient strength (Cook et al., 1995). In this state, the fish are unconscious and therefore unable to sense pain (Robb & Roth, 2003). Unconsciousness may be assessed through electroencephalografy EEG (Robb, D. H. et al., 2000) or behavioral assessment (Kestin et al., 2002). Some fish species used for food production, which were studied regarding electrical stunning, are salmon *Salmo salar* (Robb & Roth, 2003); African catfish *Clarias gariepinus* (Lambooij et al., 2004); trout *Oncorhynchus mykiss* (Lines et al., 2003); European turbot *Psetta maxima* (EFSA, 2009), carp *Cyprinus carpio* (Lambooij et al., 2007), tilapia *O. niloticus* (Lambooij et al., 2008) and eels *Anguilla anguilla* (Lambooij et al., 2002).

Various stunning methods and electrical parameters have been reported to have different effects on postmortem rigor development and subsequent meat quality (Lambooij, 2014). Electrical stunning can improve the quality of fish by a delay of rigor mortis and firmer texture as compared to fish stunned with CO_2 (Roth et al., 2002). However, research that relate electrical stunning to meat quality in Brazilian native species are scarce (Vargas et al., 2013; Vargas, 2015; Oliveira Filho et al., 2016). Thus, the aim of this study was to study parameters for electrical stunning in pacu (*Piaractus mesopotamicus*) as a way to reduce animal suffering during slaughter and its subsequent effects on pH and rigor mortis.

3.2 MATERIAL AND METHODS

This study was approved by the Animal Use Ethics Committee of the Agricultural Sciences Sector, Federal University of Paraná (UFPR), Curitiba, Paraná, Brazil, under protocol number 058/2014 (ANNEX 5).

3.2.1 Animals and localization

The animals used in this study (*Piaractus mesopotamicus)* belonged to “Estação de Pesquisa em Piscicultura e Ecologia de Espécies Nativas” – Research Station in Fish Farming and Ecology of Native Species of ITAIPU Binacional hydropower plant, located in the city of Foz do Iguaçu, Southwest of Parana, Brazil, where they were produced in cages located in a water reservoir

during ten months. Twenty-four hours before starting the experiment, fish were transported to an aquaculture laboratory (S 25° 25’ 46.678” O 54° 34’ 51.873”), placed in concrete tanks with no food. The experiment was conducted in September 2015.

We used six fish in a pilot study for testing the efficacy of currents to cause unconsciousness (TABLE 3). Then 70 fish of 0.84 ± 0.18 kg and total length of 33.02 ± 2.06 cm were divided into two groups; the minimum sampling size of 35 fish in each group was calculated based on Rosner´s formula for comparing two means (Rosner, 2010), considering a 95.0% confidence interval and a power of 90.0%. The values for mean and standard deviation of unconsciousness time for two different types of current (51 ± 26) and (37 ± 10) for stunning in tilapia were obtained from a previous study (Lambooij et al., 2008).

In the first group (G205), fish were stunned using the input parameters: pulsed direct current waveform, 205 Volts (V), 50 Hz and duty cycle equal to 70% applied for 45 s. In the second group (G400), fish were stunned using pulsed direct current waveform, 400 V, 30 Hz and duty cycle equal to 30% applied for 30 s. It is noteworthy mentioning that the output parameters were calculated and displayed by the equipment when the current was applied. Group parameters were established according to the pilot study as well as the equipment capacity (Kolz, 2008), with the aim to compare two different values of voltage in terms of longer periods of unconsciousness (TABLE 3).

TABLE 3 - ELECTRICAL PARAMETERS USED IN THE PILOT STUDY TO TEST STUNNING IN SIX PACU FISH (*Piaractus mesopotamicus*), IN THE STATE OF PARANA, SOUTH BRAZIL, SEPTEMBER 2015

Waveform	Input parameters			Output parameters - Average (peak)			Application time (s)	Results	
	Tension (V)	Frequency (Hz)	Duty cycle (%)	Power Watts (W)	Tension (V)	Current (A)		Effective stunning	Duration of unconsciousness (s)
Smooth direct current	205	-	-	295 (296)	193 (193)	1.3 (1.3)	30	Not	0
Pulsed direct current	280	30	43	246 (266)	156 (165)	1.4 (1.6)	30	Yes	47
Pulsed direct current	205	50	70	266 (440)	204 (204)	1.3 (2.2)	15	Yes	30
Pulsed direct current	400	30	25	308 (440)	204 (204)	1.5 (2.2)	30	Yes	50
Pulsed direct current	205	50	70	266 (440)	204 (204)	1.3 (2.1)	45	Yes	48
Pulsed direct current	400	30	30	305 (1075)	326 (321)	0.9 (3.4)	30	Yes	58

3.2.2 Electrical stunning

Electrical stunning was executed using a backpack electrofisher (Smith Root LR-24) within a plastic box as stunning tank, containing 80 L of fresh water. The distance between the ring-shaped and the wire-shaped electrodes was 40 cm. The general characteristics of water were controlled according to the following parameters: 20 °C, dissolved oxygen greater than 6 mg L^{-1}, pH greater than 6.5 and conductivity of 0.700 mS. Small quantities of salt (NaCl) were added to increase the conductivity for the required level. Water measurements were directly in the water through the Horiba U-50 Series (Multiparameter Water Quality Meters).

Fish were removed from the concrete tanks using a net and placed in the stunning tank. The current was applied to the first five fish individually in both groups, to observe the consistency of stunning. The other 30 fish were stunned by pairs. We followed the reports from Kestin et al. (2002) to determine the unconsciousness time through behavioral evaluations. The same evaluator observed all fish. The processes of electrical stunning were recorded with a digital camera (Sony SteadyShot DSC-W320). Immediately after checking the

return of consciousness, all fish were placed in a plastic box with 35 L of fresh water with 200 mg L^{-1} of benzocaine. When fish stopped opercular movements, which corresponds to stage 6 of anesthesia (Woolsey et al., 2004), they were bled through gill-cutting in ice during 3 minutes. Subsequently the carcasses were measured and weighed. The recordings were analyzed to confirm the unconsciousness time of each fish and the first observed indicator was registered.

3.2.3 Rigor mortis and pH

Carcasses of the first 10 fish from each group were identified and placed in refrigerated plastic boxes with ice for measurements of pH in the muscle as well as the rigor mortis index (RMI) at 0, 3, 6, 24, 72, 120 and 168 hours after slaughter. The proportion of ice per fish was 2:1. For pH determination, we performed a 1 cm-cut in the skin to access the epaxial muscle in order to insert the electrode linked to the pH meter (MS TECNOPON, mPA 2010). A new cut was done in each measurement, alternating the left and right musculature. The electrode was calibrated every 10 measurements.
Rigor mortis index was calculated according to Bito et al. (1983), using the following formula:

$$RMI\ (\%) = [(L_0 - L_T)/L_0] \times 100$$

Where L_0 (cm) is the vertical distance between the base of the caudal fin and the table surface measured immediately after slaughter and L_T (cm) is the vertical distance between the base of the caudal fin and the table surface at selective time intervals (Concollato et al., 2014). For the other 25 fish of each group, we analyzed the carcasses and fillets in order to observe hemorrhage and spinal fractures.

3.2.4 Statistical analyses

Prior to data comparison between groups, a normality test was performed using Shapiro-Wilk test. For comparisons between treatments, we used one-way analysis of variance (ANOVA) for parametric data and Mann-Whitney test for non-parametric data.

3.3 RESULTS AND DISCUSSION

All 70 fish in the main experiment were effectively stunned. When the current was applied, the animals instantly lost behavioral responses and reflexes: fish stopped swimming, lost equilibrium, did not respond to painful stimuli, ceased opercular movements and did not show vestibulo-ocular reflex. Such results are in agreement with studies in tilapia (Lambooij et al., 2008), salmon (Robb & Roth, 2003), trout (Robb et al., 2002) and pacu (Oliveira Filho et al., 2016). Electrical stunning appears to be better in terms of animal welfare, because it reduces stress and suffering caused by the traditional methods of stunning fish in Brazil (Mendes et al., 2015; Oliveira Filho et al., 2014; Pedrazzani et al., 2009). According to our observations, stunned pacu showed patterns of behavior compatible with apparent unconsciousness. Further studies with EEG are important to validate unconsciousness and insensitivity results obtained in this work.

A total of 71.4% (50/70) fish showed breathing as first behavioral response during consciousness recovery after electrical stunning. Breathing seems to be a promising indicator, considering the correlation between the incidence of recovery of Visual Evoked Responses through EEG and initiation of opercular movements in Atlantic salmon (Robb & Roth, 2003). Other behavioural responses in the present study were eye roll (17/70) and swimming (2/70). Thus, opercular movements seem to constitute the choice behavioral indicator of consciousness recovery after electrical stunning in pacu, with high practical viability.

The mean duration of unconsciousness was 61.7 ± 13.4 s for G205 and 50.1 ± 9.6 s for G400 ($P<0.01$, FIGURE 10). The confidence interval for the mean of unconsciousness time in G205 was 57.1 – 66.3 s. The unconsciousness time was significantly higher in the G205 possibly because of the longer exposure to the electric current and the higher duty cycle. Oliveira Filho et al. (2016) showed that pacu stunned in water with alternating current, 200 V and 50 Hz applied for 180 s induced apparent unconsciousness of up to 4 min. Such period of unconsciousness may be related to the exposition to the current during 180 s. Lines et al. (2003) demonstrated the proportional relationship between exposure time and time unconscious in trout submitted to

an electrical field using a sinusoidal waveform, 230 V and 50 Hz (Lines et al., 2003). In accordance with the present results, Robb and Roth observed that Atlantic salmon stunned with high voltage, using electric field strength equal to 200 V for 1 s, resulted in earlier recovery as per EEG observation (154 ± 30 s) when compared with the use of 50 V for 3 s (288 ± 50 s) and 25 V for 12 s (212 ± 50 s) (Robb & Roth, 2003). Lambooij, (2014) reported that 40 s may be a suitable period of unconsciousness to facilitate exsanguination and onset of death in unconscious animals. In broiler chickens, unconsciousness and insensibility for 60 s is considered the minimum duration required for effective stunning, assuming that a neck cut is performed within 30 s after stunning (Hindle et al., 2010). Nevertheless, fish should be bled immediately following stunning to secure that the animal does not recover. Stunning of pacu for humane slaughter may be possible considering such parameters: standard pulse, 205 V, 50 Hz, duty cycle 70%, 1.3 A and application for 45 s, since they induced unconsciousness during 61.7 ± 13.4 s.

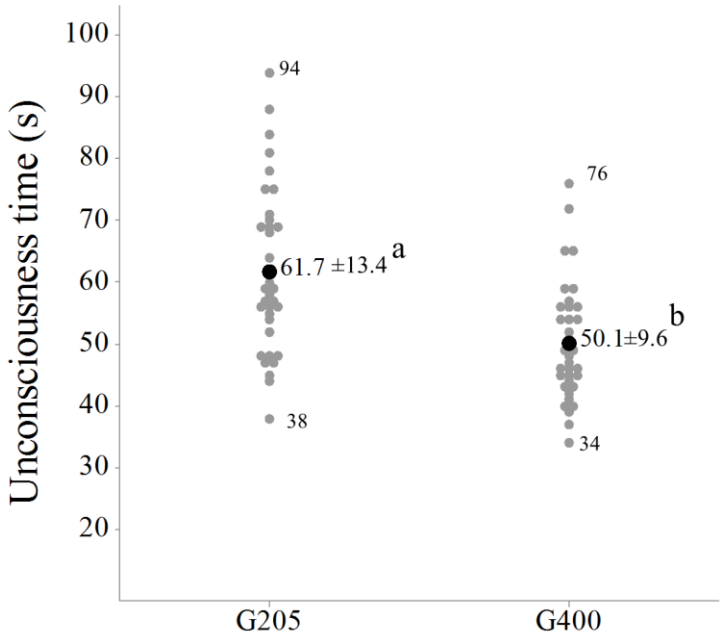


FIGURE 10 - UNCONSCIOUSNESS TIME AFTER ELECTRICAL STUNNING (MEAN±SD), 35 PACU (*Piaractus mesopotamicus*) PER GROUP, STATE OF PARANA, SOUTH OF BRAZIL, SEPTEMBER 2015; DIFFERENT LETTERS INDICATE SIGNIFICANT DIFFERENCES (P<0.01, ANOVA)

The hypothesis that the main factor that determines unconsciousness time in pacu is the time of exposure to the current is worth testing in an experiment designed with this goal. Probably, the current has an important role

because the current average was 1.3 A in G205, coinciding with the longer unconsciousness (FIGURE 11). Similar result was reported in trout submitted to an electrical field using a sinusoidal waveform, 230 V and 50 Hz during 5 s, and showed the proportional relationship between the current magnitude and unconsciousness time (Lines et al., 2003). Chicken must be stunned using a minimum current of 100 mA, applying a sinusoidal waveform to ensure effective stunning when currents of less than 200 Hz are used (European Union, 2009). Lambooij et al. (2008) reported that Nile tilapia is effectively stunned during 27 ± 10 s, with an average current of 0.4 ± 0.1 A, 365 V and 50 Hz of alternating current. For an instantaneous stunning in individual carp, 113 V is applied across the electrode plates at 16 cm distance for 1 second. These conditions resulted in an overall current density of 0.14 A (EFSA, 2009). Besides, studies report that the alternating current is more efficient in terms of time of unconsciousness in comparison with direct current (Nordgreen et al., 2008; Oliveira Filho et al., 2016). Our results can motivate Brazilian industry to develop equipment that enable effective stunning in fish.

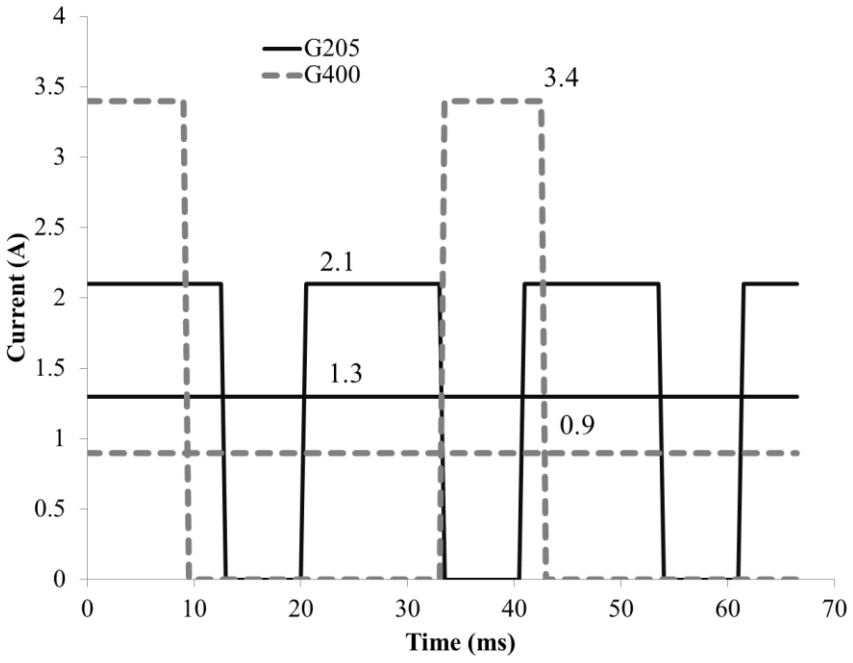
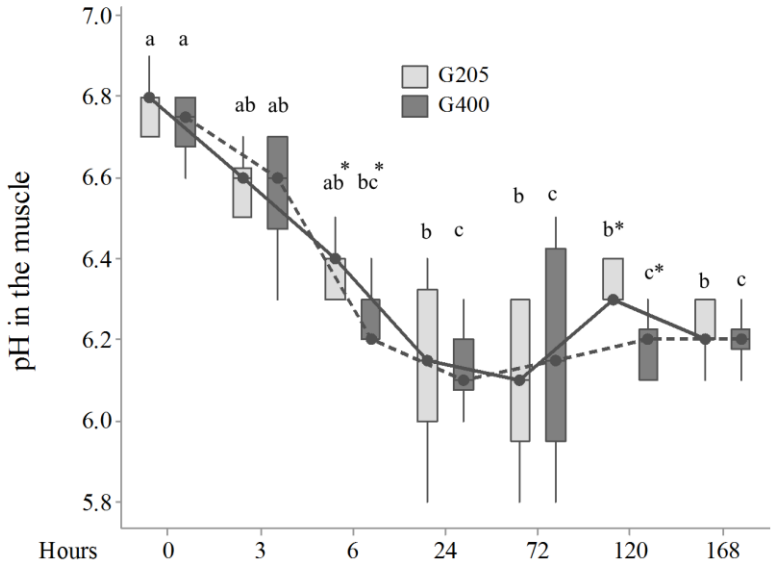


FIGURE 11 – CURRENT DIAGRAM (AVERAGE AND PEAK) OF PARAMETERS USED IN 35 PACU (*Piaractus mesopotamicus*) PER GROUP, STATE OF PARANA, SOUTH OF BRAZIL, SEPTEMBER 2015

The pH values (median, min-max) immediately after slaughter were: 6.80 (6.70-6.90) for G205 and 6.75 (6.60-6.80) for G400 (FIGURE 12). Similar

values were reported in cachama killed by hypothermia in ice, with values of 6.5 and 6.7 (Bello & Rivas, 1992). In agreement with our results, Almeida et al., (2006) report values of 6.08, immediately after slaughter, in cachama killed by hypothermia in ice. Higher pH values were reported in tilapia slaughtered using CO_2 or iced water immersion; 7.0 ± 0.09 was the mean pH value immediately after death (Oliveira Filho et al., 2014). The mean pH value in the muscle immediately postmortem in Atlantic cod (*Gadus morhua* L.) killed by percussion was 7.9 (Kristoffersen et al., 2006). Future studies may evaluate other humane



slaughter methods in pacu, such as percussion, and compare the effects of each method on muscle pH. Our results show that electrical stunning does not significantly affect pH values in pacu, compared to traditional methods of killing fish.

FIGURE 12 - VALUES OF PH IN EPAXIAL MUSCLE, 35 PACU (*Piaractus mesopotamicus*) PER GROUP, STATE OF PARANA, SOUTH OF BRAZIL, SEPTEMBER 2015; LETTERS INDICATE DIFFERENCE BETWEEN INTERVALS OF MEASUREMENT (P<0.01, KRUSKAL-WALLIS TEST); THE ASTERISK INDICATES DIFFERENCE BETWEEN GROUPS (P<0.01, MANN-WHITNEY TEST)

The values of pH in the muscle were significantly higher in G205 at 6 and 120 hours of mensuration. Robb et al, (2000) found that electrical stimulation of fish muscle accelerated the anaerobic metabolism and the drop in muscle pH (Robb et al., 2000). Possibly, the parameters used in G400 causes a decrease in muscle pH in pacu. Post-mortem glycolysis results in accumulation

of lactic acid which in turn lowers the pH of the muscle (Huss, 1995; Poli et al., 2005). Such pH reduction is important for meat quality, since it prevents bacterial growth. The bacterial metabolic activity decomposes nitrogen compounds to form basic compounds such as ammonia and trimethylamine (Huss, 1995). The slight increase in pH value observed after 72 hours may be due to the production of volatile nitrogen bases. Muscle pH values observed in this study are in accordance with Brazilian regulations of fish, i.e. less than 6.8 in external musculature (Brasil, 1952). The values of the last measurement at 168 hours were not significantly different. Our results show that electrical stunning does not significantly affect pH values muscle in pacu, because they are in the range allowed by the Brazilian legislation.

All fish reached maximum rigor mortis (RMI=100%) within 6 h post mortem (FIGURE 13). Increased muscular activity, stress at slaughter and endocrine response influence fish post-mortem biochemical processes, mostly the anaerobic glycolysis and ATP degradation rate (Huss, 1995). Such biochemical processes can influence the onset and release of rigor mortis, which in turn largely determines the involution rate of fish freshness (Huss, 1995). In cachama (*Colossoma macropomum*), a species of the family Characidae, the same as pacu, slaughtered by asphyxiation in ice, total muscle contraction occurred after 30 minutes (RMI=99.4%) (Almeida et al., 2005). Additionally, in hybrid of cachama (*Colossoma macropomum* X *Piaractus brachypomus*) killed by bleeding, reached the maximum state of rigor mortis at 2 hours after slaughter (Kodaira et al., 2001). Therefore, the results of our study show a possible delay in the installation of maximum rigor mortis. In Atlantic salmon stunned with CO_2, percussion or electrical stunning, the authors observed that fish stunned with CO_2 had an earlier onset and resolution of rigor mortis than the other stunning methods (Roth et al., 2002). Perhaps, the electrical stunning in pacu delay the onset of rigor mortis, compared to traditional methods of pre-slaughter and slaughter in fish. Therefore, the results of rigor mortis stunned with electrical stunning are not a barrier for the introduction of methods of humane slaughter in pacu.

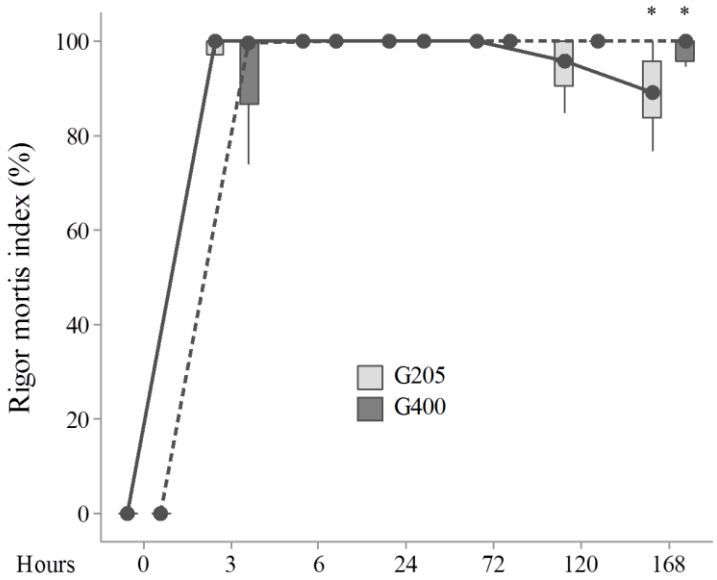


FIGURE 13 - VALUES OF RIGOR MORTIS INDEX (%), 35 PACU (*Piaractus mesopotamicus*) PER GROUP, STATE OF PARANA, SOUTH OF BRAZIL, SEPTEMBER 2015, ASTERISK INDICATES DIFFERENCE BETWEEN GROUPS (P<0.01, MANN-WHITNEY TEST)

The RMI at 168 h was significantly different between groups (P=0.0038). Therefore, G205 initiated earlier rigor mortis resolution. This difference may be due to the parameters used in the electrical stunning. The G205 treatment started resolution of rigor mortis at 120 h and G400 at 168 h after death. This result can be explained by the higher pH in G205 at 120 hours, which could promote the growth of bacteria and thus possibly, lead to the resolution of rigor mortis. A longer time both before rigor onset and resolution from pre-slaughter cooling gives a potential for filleting pre-rigor and has added benefit of improving flesh quality (Poli et al., 2005). An earlier resolution of rigor mortis results in softer texture and loss of shelf life (Nakayama et al., 1996). The results of resolution of rigor mortis in pacu are in concordance with them reported in a study with cachama (*Colossoma macropomum*), killed by asphyxiation in ice, where the resolution of rigor mortis began at 144 h post-mortem (Almeida et al., 2005). Therefore, the electrical stunning seems to not affect the resolution of rigor mortis in pacu significantly.

The problem of electrical stunning, especially when fish are immersed in water during stunning, is that carcass damage might occur, such as muscle hemorrhages or a broken vertebral column (Van de Vis et al., 2014). No problems were observed in the carcass quality. Fish did not show any external damage, vertebral column fracture, macrohemorrhages or blood spotting in the

fillet. The used electric parameters seem safe in terms of avoiding bleeding or fractures in the carcass.

3.4 CONCLUSION

We report parameters for electrical stunning in pacu. Such information provides support for new studies seeking to reduce animal suffering during slaughter. Electrical stunning in pacu is possible with pulsed direct current waveform, 205 V, 50 Hz, duty cycle 70% and 1.3 A applied for 45 s, as such parameters induce unconsciousness during 61.7 ± 13.4 s. This duration of unconsciousness may allow for a proper industry flow that couples stunning to bleeding in a way that avoids consciousness recovery before death. Additionally, this stunning did not cause significant alterations on muscle pH values and rigor mortis. The electrical parameters employed seem safe in terms of avoiding muscle bleeding or fractures in the carcass. Our results contribute to the development of new humane slaughter methods in fish that maintain or improve the quality of meat, and which are necessary to improve animal welfare in aquaculture. We recommend new studies assessing unconsciousness by EEG as well as searching for methods with longer unconsciousness duration.

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4. ELECTRICAL STUNNING IN SOUTH AMERICAN CATFISH (*Rhamdia quelen*)

RESUMO

No Brasil, a imersão no gelo é usada como método pré-abate na maioria dos frigoríficos de peixes. O objetivo deste trabalho foi estudar parâmetros para eletronarcose em jundiá (*Rhamdia quelen),* para reduzir o sofrimento animal durante o abate e seus subsequentes efeitos sobre pH e rigor mortis. Foram avaliados 70 peixes divididos em dois grupos. No grupo G125, os peixes foram insensibilizados por meio dos parâmetros: 125 Volts (V), 30 Hz, ciclo de trabalho de 90% e 1.2 Amp (A), aplicados durante 30 s. No grupo G400, os peixes foram insensibilizados com: 400 V, 30 Hz, ciclo de trabalho de 27% e 0.9 A durante 30 s. A insensibilização elétrica foi aplicada usando um equipamento portátil de pesca elétrica (Smith Root LR-24) em uma caixa plástica com 80 L de água. A determinação do tempo de inconsciência foi por meio de avaliação comportamental. Imediatamente após determinar o retorno à consciência, todos os peixes foram colocados em uma caixa plástica com 35 L de água e 300 mg L^{-1} de benzocaína no momento prévio à sangria. Os 10 primeiros peixes de cada grupo foram identificados e colocados sob refrigeração em caixas plásticas com gelo, para medir o pH na musculatura epaxial, assim como o índice de rigor mortis (RMI) às 0, 3, 6, 24, 72 e 120 h após a sangria. No G125, 14.4% (5/35) dos peixes não foram efetivamente insensibilizados, em contraste com G400, em que 100% (35/35) dos peixes foram efetivamente insensibilizados ($P<0.05$). Ao aplicar a corrente elétrica, os peixes insensibilizados perderam instantaneamente os reflexos e respostas comportamentais: os animais cessaram o nado, perderam a posição de nado, não responderam a estímulos dolorosos, cessaram os movimentos operculares e não apresentaram reflexo vestíbulo-ocular. Quando a corrente foi desligada, a maior parte dos peixes (73.3% no G125; 80.0% no G400) apresentou boca e opérculos abertos ($P>0.05$). O tempo de inconsciência foi significativamente maior no tratamento G400, igual a 87.7 ± 16.1 s, em relação a 66.6 ± 16.1 s para o G125 ($P<0.01$), possivelmente relacionada à maior voltagem utilizada. As medianas (min-max) de pH imediatamente após o abate foram 6.65 (6.20-6.90) no G125 e 6.70 (6.40-7.10) no G400 ($P>0.05$). Noventa por cento dos peixes atingiram o máximo rigor mortis (RMI=100%) às 6 h pós-abate. As carcaças não apresentaram dano externo, fraturas vertebrais, hemorragias ou petéquias. A eletronarcose no jundiá parece viável a partir dos parâmetros utilizados no G400, devido ao tempo de inconsciência maior que 1 min, o que pode permitir um tempo mínimo de sangria sem sofrimento. Novos estudos são necessários para fornecer melhores estratégias para a indústria, de modo que regulamentações sobre abate humanitário de peixes possam ser implantadas.

Palavras-chave: Abate humanitário. Bem-estar animal. Inconsciência. Qualidade da carne.

ABSTRACT

In Brazil, pre-slaughter immersion in ice is used in most fish slaughterhouses. The aim of this paper was to determine parameters for electrical stunning in South American catfish (*Rhamdia quelen*) as way to reduce animal suffering during slaughter and its subsequent effects on pH and rigor mortis. We evaluated 70 fish, divided into two groups. In one group (G125), fish were stunned using as parameters 125 Volts (V), 30 Hz, duty cycle of 90% and 1.3 Amp (A) applied for 30 s; in the other group (G400), fish were stunned with 400 V, 30 Hz, duty cycle of 27%, 0.9 A, for 30 s. Electrical stunning was applied using a backpack electrofisher (Smith Root LR-24) in a plastic box with 80 L of fresh water. We determined the unconsciousness time through behavioral assessment. Immediately after checking the return of consciousness, all fish were placed in a plastic box with 35 L of fresh water and 300 mg L^{-1} of benzocaine before bleeding. The first 10 fish from each group were identified and placed in refrigerated plastic boxes with ice for measurements of pH in the epaxial muscle as well as the rigor mortis index (RMI) at 0, 3, 6, 24, 72 and 120 h after bleeding. In G125, 14.4% (5/35) of fish were not effectively stunned, in contrast with G400 in which 100% of fish (35/35) were effectively stunned ($P<0.05$). When the current was applied, stunned fish lost behavioral responses and reflexes instantly: fish stopped swimming, lost equilibrium, did not respond to painful stimuli, ceased opercular movements and did not show any vestibulo-ocular reflex. When the electrical current was turned off, most of fish (73.3% in G125; 80.0% in G400) had open mouth and operculum ($P>0.05$). The unconsciousness time was significantly higher in the G400 treatment, equal to 87.7±16.1 s ($P<0.01$), in relation with 66.6 ± 16.1 s in G125, possibly due to a higher voltage used. The median (min-max) pH values immediately after slaughter were 6.65 (6.20-6.90) for G125 and 6.70 (6.40-7.10) for G400 ($P>0.05$). Ninety percent of fish reached maximum rigor mortis (RMI=100%) within 6 h post-mortem. The carcasses did not show any external damage, vertebral fracture, hemorrhages or blood spotting. Electrical stunning in South American catfish seems to be feasible through the G400 parameters, due to the unconsciousness period longer than 1 min, which may allow a minimum time period to proceed bleeding without suffering. More research is needed to provide best strategies to the fish industry, so that fish regulations regarding humane slaughter may be published and implemented.

Keywords: Animal welfare. Humane slaughter. Meat quality. Unconsciousness.

4.1 INTRODUCTION

Global fish production has grown steadily in the last five decades, with food fish supply increasing at an average annual rate of 3.2 percent, outpacing world population growth which is at 1.6 percent (FAO, 2014). In 2011, 1.43 million tons of fish were produced in Brazil, being 544.490 tons (38%) from inland aquaculture (Brasil, 2011).

The South American catfish *Rhamdia quelen* (Osteichthyes, Teleostei, Siluriformes, Pimelodidae, Rhamdia) locally known as jundiá, is one of the most promising species for Brazilian fish farming. *Rhamdia quelen* has neotropical distribution, from Southwest Mexico to central Argentina (Gomes et al., 2000). South American catfish production is 1.747 tons in Brazil (Brasil, 2011) and they are commonly used in polyculture with other species, such as tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), big head carp (*Aristichthys nobilis*), silver carp (*Hypoththalmichthys molitrix*), African catfish (*Clarias gariepinus*), American catfish (*Ictalurus punctatus*) and pacu (*Piaractus mesopotamicus*) (Ostrensky et al., 2007). *R. quelen* lives naturally in lakes and deep wells of rivers, preferring calmer waters environments with sand and mud bottom, along the banks and vegetation. They hide among rocks and rotting logs, from which they come out at night looking for food (Gomes et al., 2000). In experiments on larvae and fingerlings of South American catfish marked aversion to light and search for dark places was observed (Piaia et al., 1999).

Recently, with the intensification in animal production, concerns about animal welfare have grown in developed countries. The animal welfare debate has tended to focus on terrestrial species. However, in a study on Norwegian perception about animal welfare, Ellingsen et al. (2015) noted that fish welfare was considered important by the respondents. Additionally, Latin Americans consider fish as sentient animals and believe that fish should be included in the regulations of humane slaughter (Rucinque & Molento, 2015). The slaughter methods in fish are a critical point that can cause high degree of suffering in a large number of fish slaughtered annually for human consumption. Thus, in Norwegian and Swedish humane slaughter regulations, stunning of farmed fish

is mandatory (Norway, 2009; Röcklinsberg, 2015). However, norms for humane slaughter in Brazil do not include fish.

Killing methods such as hypothermia in ice followed by evisceration and exsanguination cause high degree of suffering in conscious animals (Robb & Kestin, 2002). In Brazil, the hypothermia in ice for stunning is used in most fish slaughterhouses (Oliveira Filho et al., 2014). Humane slaughter is characterized by stunning, which is applied to animals to induce unconsciousness and insensibility to pain stimuli for sufficient duration to secure that an animal does not recover during exsanguination (Lambooij et al., 2008). It is evident that fish slaughter in Brazil is not humane.

Electricity is widely used for stunning poultry and red meat animals that are killed for food. Effective electrical stunning is associated with the induction of a generalized epileptiform insult, if the current has sufficient strength (Cook et al., 1995). In such state, fish are unconscious and therefore unable to sense pain (Robb & Roth, 2003). The determination of unconsciousness can be achieved through electroencephalografy (EEG) (Robb et al., 2000) or behavioral assessment (Kestin et al., 2002). The aim of this paper was to study parameters for electrical stunning in South American catfish *Rhamdia quelen* to reduce animal suffering during slaughter and its subsequent effects on pH and rigor mortis.

4.2 MATERIAL AND METHODS

This experiment was approved by the Ethics Committee for Animal Use of the Agricultural Sciences Campus of the Universidade Federal do Paraná (Federal University of the State of Paraná, Brazil), during session on October 10, 2014, and it is registered under protocol number 058/2014 (ANNEX 6).

4.2.1 Animals and localization

Fish evaluated in this study (*Rhamdia quelen*) belonged to the “Estação de Pesquisa em Piscicultura e Ecologia de Espécies Nativas (EPPEEN) – Research Station in Fish Farming and Ecology of Native Species” of ITAIPU Binacional hydropower plant, in the city of Foz do Iguaçu, Southwest of Parana, Brazil, where they were produced in cages located in the water reservoir during twelve months. Twenty-four hours before starting the experiment, fish were

transported to an aquaculture laboratory (S 25° 25’ 46.678” O 54° 34’ 51.873”), placed in concrete tanks with no food. The experiments were conducted in September 2015.

We used six fish in a pilot study for testing the efficacy of currents to cause unconsciousness (TABLE 4). Then 70 fish of 0.12 ± 0.04 kg and a total length of 22.73 ± 2.00 cm were divided into two groups. The minimum sampling size of 35 fish in each group was calculated based on Rosner´s formula for comparing two means (Rosner, 2010), considering a 95.0% confidence interval and a power of 90.0%. The values for mean and standard deviation of unconsciousness time for two different types of current (51 ± 26) and (37 ± 10) for stunning in tilapia were obtained from a previous study (Lambooij et al., 2008).

In the first group (G125), fish were stunned using the input parameters: pulsed direct current waveform, 125 V, 30 Hz and duty cycle equal to 90% applied for 30 s. In the second group (G400), fish were stunned with pulsed direct current waveform, 400 V, 30 Hz and duty cycle equal to 27% applied for 30 s. It is noteworthy mentioning that the output parameters were calculated and displayed by the equipment when the current was applied. Group parameters were established according to pilot studies and the equipment capacity (Kolz, 2008), with the goal of comparing two parameters in terms of longer times of unconsciousness (TABLE 4).

TABLE 4 - ELECTRICAL PARAMETERS USED IN THE PILOT STUDY TO TEST STUNNING IN SIX SOUTH AMERICAN CATFISH (*Rhamdia quelen*), IN THE STATE OF PARANA, SOUTH BRAZIL, SEPTEMBER 2015

Waveform	Input parameters			Output parameters – Average (peak)			Application time (s)	Results	
	Tension (V)	Frequency (Hz)	Duty cycle (%)	Power Watts (W)	Tension (V)	Current (A)		Effective stunning	Duration of unconsciousness (s)
Smoot direct current	205	-	-	295 (296)	193 (193)	1.3 (1.3)	30	Not	0
Pulse direct current	125	30	30	127 (160)	123 (123)	1.0 (1.3)	30	Not	0
Pulse direct current	205	50	70	266 (440)	204 (204)	1.3 (2.2)	15	Not	0
Pulse direct current	400	30	25	308 (440)	204 (204)	1.5 (2.2)	30	Yes	46
Pulse direct current	125	30	90	142 (157)	123 (127)	1.2 (1.3)	30	Yes	60
Pulse direct current	400	30	27	288 (1075)	319 (320)	0.9 (3.4)	30	Yes	50

4.2.2 Electrical stunning

Electrical stunning was executed using a backpack electrofisher (Smith Root LR-24) within a plastic box functioning as stunning tank with 80 L of fresh water. The distance between the ring-shaped and the wire-shaped electrodes was 40 cm. The general characteristics of water were controlled according to the following parameters: 20 °C, dissolved oxygen greater than 6 mg L^{-1}, pH greater than 6.5 and conductivity of 0.700 mS. Small quantities of salt (NaCl) were added to increase the conductivity for the required level. Water measurements were performed directly in the water through the Horiba U-50 Series (Multiparameter Water Quality Meters).

Fish were removed from the concrete tanks using a net and placed in the stunning tank. The current was applied to the first five fish individually in both groups, to observe the consistency of stunning. The other 30 fish were stunned by pairs. We followed the protocol to determine the unconsciousness time through behavioral evaluations (Kestin et al., 2002). The same observer evaluated all fish. The processes of electrical stunning were recorded with a digital camera (Sony SteadyShot DSC-W320). The recordings were analyzed to

confirm the unconsciousness time of each fish and the first observed indicator was registered. Immediately after checking the return of consciousness, all fish were placed in a plastic aquarium with 35 L of fresh water with 300 mg L^{-1} of benzocaine. When fish stopped opercular movements, which corresponds to stage 6 of anesthesia (Lambooij et al., 2008), they were bled through gill-cutting in ice during 3 minutes. The carcasses were subsequently measured and weighed.

4.2.3 Rigor mortis and pH

Carcasses of the first 10 fish from each group were identified and placed in refrigerated plastic boxes with ice for measurements of pH in the epaxial muscle as well as the rigor mortis index (RMI) at 0, 3, 6, 24, 72, 120 and 144 hours after slaughter. The proportion of ice per fish was 2:1. For pH determination, we performed a 1 cm-cut in the skin to access the epaxial muscle in order to insert the electrode linked to the pH meter (MS TECNOPON, mPA 2010). A new cut was made in each measurement, alternating the left and right muscles. The electrode was calibrated every 10 measurements.

Rigor mortis index was calculated according to Bito et al. (1983) using the following formula:

$$RMI\ (\%) = [(L_0 - L_T)/L_0] \times 100$$

Where L_0 (cm) is the vertical distance between the base of the caudal fin and the table surface measured immediately after slaughter and L_T (cm) is the vertical distance between the base of the caudal fin and the table surface at selective time intervals (Concollato et al., 2014). For the other 25 fish of each group, we analyzed the carcasses and fillets in order to observe hemorrhage and spinal fractures.

4.2.4 Statistical analyses

Prior to data comparison between groups, a normality test was performed using Shapiro-Wilk test. For comparisons between treatments, we used one-way analysis of variance (ANOVA) for parametric data and Mann-Whitney test for non-parametric data. The Binomial test was used to compare proportion of animals performing specific behaviours between treatments.

4.3 RESULTS AND DISCUSSION

In G125 a total of 14.4% (5/35) fish were not effectively stunned, in contrast with G400, in which 100% of fish (35/35) were effectively stunned ($P<0.05$). The five fish from G125 were excluded from subsequent behavioral assessments. Probably, a lower voltage used in the G125 may have influenced the final current. In African catfish (*Clarias gariepinus*), voltages of 223, 225, 232 and 264 V at 50 Hz applied for 1 s did not cause unconsciousness (Lambooij. et al., 2006). Particularly, four of five fish (4/5) were not effectively stunned (G125); they were located in the lower left corner as shown in FIGURE 14. Probably, in this position, the current does not effectively pass from one electrode to the other.

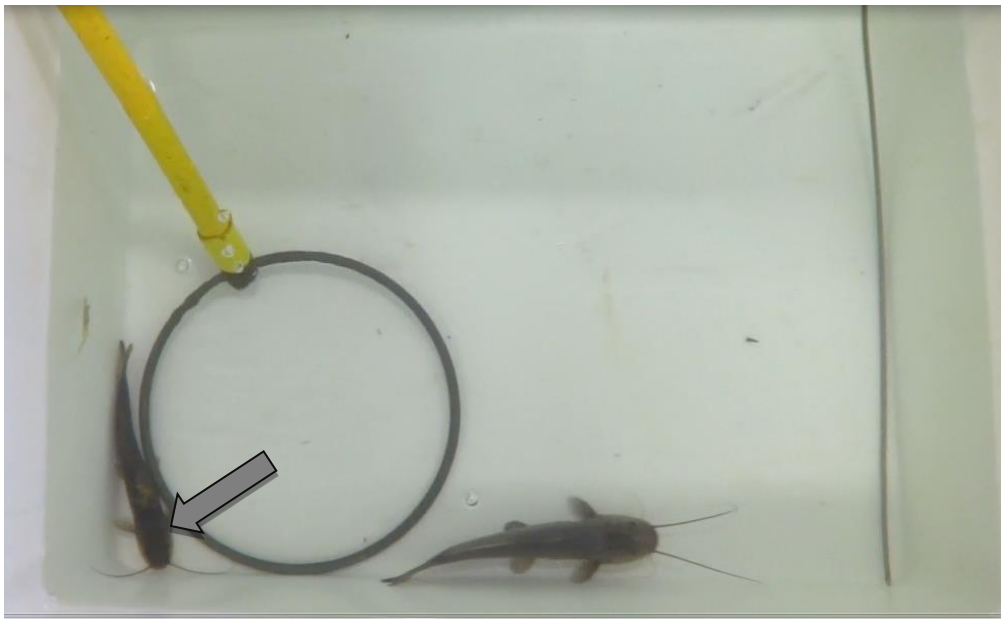

FIGURE 14 - THE ARROW INDICATES THE POSITION OF 4/5 SOUTH AMERICAN CATFISH (*Rhamdia quelen*) THAT WERE NOT EFFECTIVELY STUNNED; STATE OF PARANA, SOUTH OF BRAZIL, SEPTEMBER 2015

When the current was applied, the animals instantly lost behavioral responses and reflexes: fish stopped swimming, lost equilibrium, did not respond to painful stimuli, ceased opercular movements and did not show vestibulo-ocular reflex. Such results are in agreement with studies in tilapia (Lambooij et al., 2008), salmon (Robb & Roth, 2003) and trout (Robb et al., 2002). When the electrical current was turned off, most of fish (73.3% in G125; 80.0% in G400) had open mouth and operculum, with no difference between groups ($P>0.05$). Subsequently, fish showed spasms along the length of the

body. The proportion of fish that had spams was similar between groups, showing tendency to significant difference (P=0.06), with a total of 30.0% in G125 and 48.6% in G400. The median (min-max) number of spasms was similar between groups (P>0.05), with 6.0 (2.0–17) spasms for G125, and 6.0 (1.0–28) for G400. The latency until the first spasm was different among groups (P<0.05) with mean equal to 24.0 ± 7.6 s for G125 and 47.9 ± 16.5 s for G400. Spasms ceased when the fish performed breathing movements. Data of the assessed behavior post-stunning can be found on FIGURE 15.

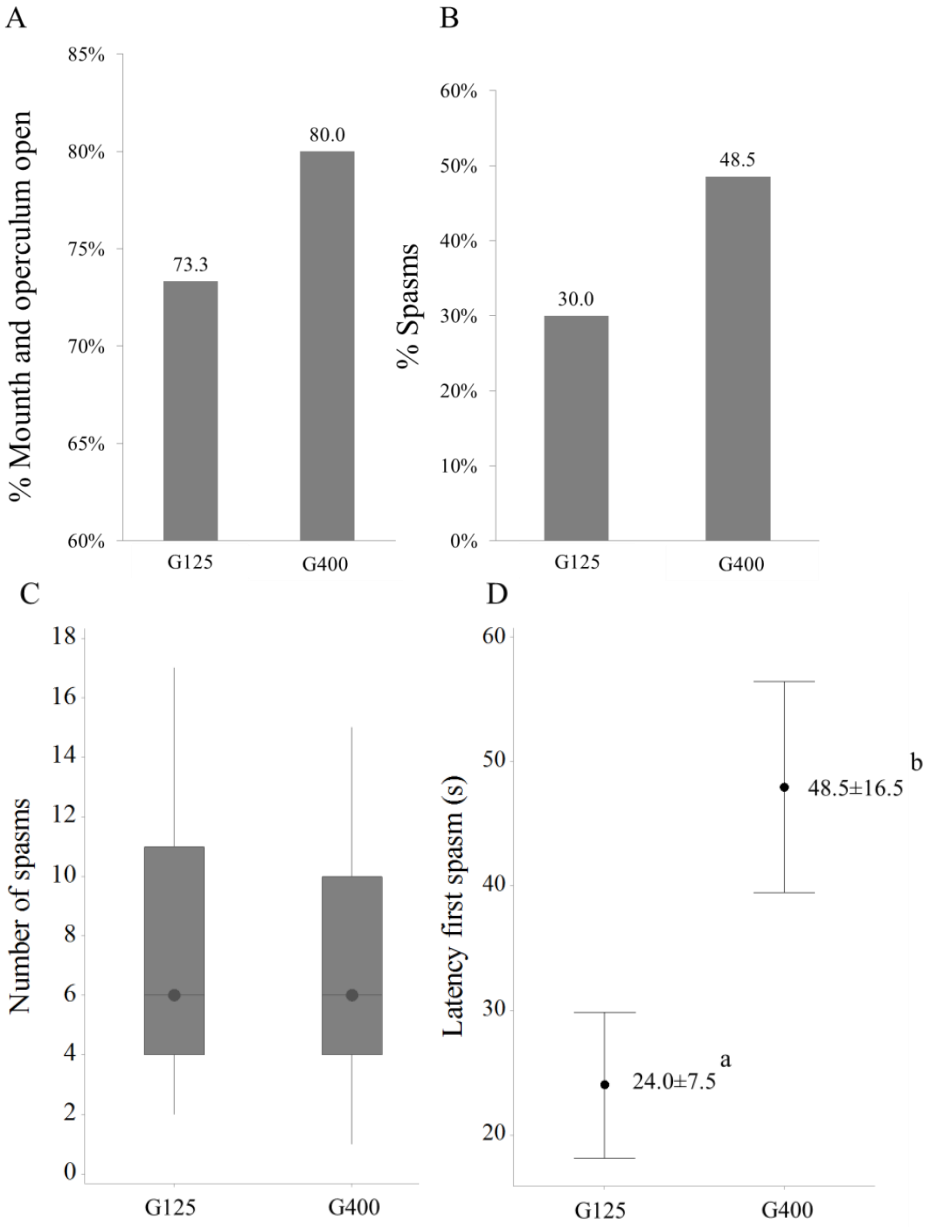

FIGURE 15 - PROPORTION OF SOUTH AMERICAN CATFISH (*Rhamdia quelen*) WITH OPEN MOUTH AND OPERCULUM (A), SPASMS (B), MEDIAN NUMBER OF SPASMS (C), AND MEDIAN LATENCY FOR THE FIRST SPASM (D); DIFFERENT LETTERS INDICATE DIFFERENCES BETWEEN GROUPS (P<0.05)

The behaviors observed in South American catfish were also reported in rainbow trout (*Oncorhynchus mykiss*) stunned by electrical stimulus (Robb et al., 2002). Effective stunning leads to tonic/clonic seizures which are considered a sign for unconsciousness (EFSA, 2004). Electrical stunning can provoke immediate loss of consciousness in fish; it is based on the induction of a general epileptiform insult, called grand mal or seizure-like state, caused by an electrical current going through the brain (Lambooij et al., 2010). This epileptic process is characterized by rapid and extreme depolarization of the membrane potential (Lambooij, 2014). The brain is unable to respond to stimuli, and there is a consensus that a vertebrate animal is unconscious and thus cannot

experience pain during a generalized epileptiform insult (Lambooij, 2014). When measured on the EEG, such insult consists of relatively small waves increasing in amplitude in the tonic phase and decreasing in frequency in the clonic phase to result ultimately in a period of strong depression of electrical activity as found in pigs, sheep and calves. The general epileptiform insult on the EEG of broiler chickens, ostriches and eels is characterized by a tonic/clonic phase and an exhaustion phase (Lambooij et al., 2004). Probably, the behaviors observed in our study for South American catfish (*Rhamdia quelen)* indicate a tonic/clonic phase and, thus unconsciousness. Such finding is a step forward for animal welfare, due to a possible alternative to the prolonged suffering caused by the traditional methods of stunning fish in Brazil (Mendes et al., 2015; Oliveira Filho et al., 2014; Pedrazzani et al., 2009).

The open operculum and mouth in the absence of spasms are suggestive of tonic phase and the spasms reflect the onset of clonic phase. We could not identify an exhaustion phase because when fish ceased spasms, they initiated breathing movements. In African catfish (*Clarias gariepinus*) stunned with 291 ± 5 V and 1.60 ± 0.11 A during 1 s in fresh water, the characteristics of a general epileptiform insult on the EEG were observed. The tonic, the clonic phase and the exhaustion phase were 7 ± 3, 12 ± 7 and 9 ± 6 s on the EEG, respectively (Lambooij, B. et al., 2006). The latency to first spasms in South American catfish was longer in G400, possibly related to the higher voltage used compared to G125. In trout submitted to electrical field using a sinusoidal waveform and 50 Hz, Robb et al. (2002) demonstrated the proportional relationship between current magnitude (V) on survival and the time to recovery of opercular movements. The post-stunning behaviors, observed by us: open mouth and operculum, spams and latency until the first spasm suggest an effective unconsciousness in South American catfish (*Rhamdia quelen*). However, we suggest future studies with evaluation of unconsciousness through EEG.

A total of 71% (58/70) of fish showed breathing as first response after electrical stunning. Breathing seems to be a promising indicator, considering the correlation between the incidence of recovery of Visual Evoked Responses through EEG and initiation of opercular movements in Atlantic salmon (Robb & Roth, 2003). Other first behavioural responses in the present study were

swimming (4/70) and eye roll (2/70). Thus, opercular movements seem to constitute an important behavioral indicator of consciousness recovery after electrical stunning in South American catfish, with high practical viability.

The mean duration of unconsciousness was 66.6 ± 16.1 s for G125 and 87.7 ± 21.6 s for G400 (P<0.01) (FIGURE 16). The unconsciousness time was significantly higher in the G400 treatment, possibly due to higher voltage (400 V) in contrast with G125 (125 V) and latency to the first spasm. Lambooij et al. (2006) reported 28 ± 8 s of unconsciousness in African catfish stunned through a voltage of 291 ± 5 V, 1.60 ± 0.11 A during 1 s.. Lambooij (2014) reported that 40 s may be a suitable period of unconsciousness to facilitate exsanguination and onset of death in unconscious animals. In broiler chickens, unconsciousness and insensibility for 60 s is considered the minimum duration required for effective stunning, assuming that a neck cut is performed within 30 s after stunning (Hindle et al., 2010). Nevertheless, fish should be bled immediately following stunning to secure that the animal does not recover. Thus, stunning of South American catfish for humane slaughter may be possible considering such parameters: pulsed direct current waveform, 400 V, 30 Hz, duty cycle of 27% and 0.8 A applied for 30 s, once they induce unconsciousness during 87.7 ± 21.6 s. Further studies can evaluate those parameters followed by bleeding for humane slaughter in fish.

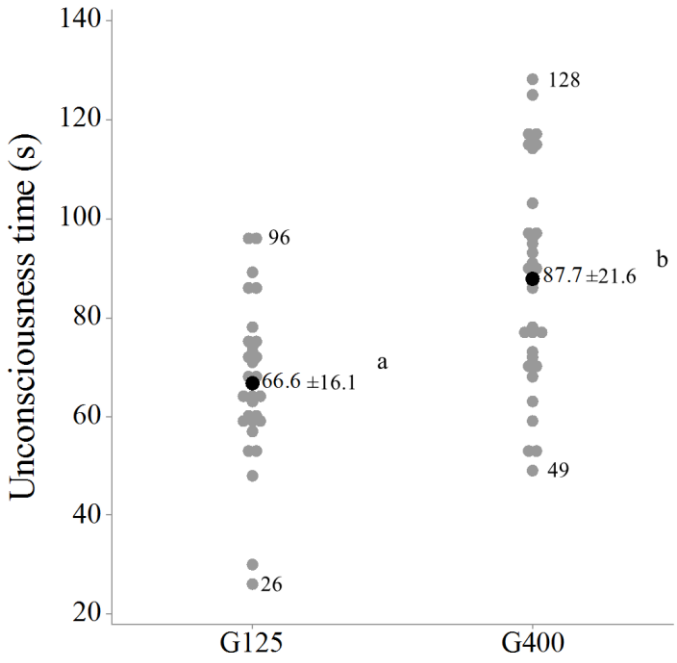

FIGURE 16 - UNCONSCIOUSNESS TIME AFTER ELECTRICAL STUNNING (MEAN±SD), 35 SOUTH AMERICAN CATFISH (*Rhamdia quelen*) PER GROUP, STATE OF PARANA, SOUTH OF BRAZIL, SEPTEMBER 2015; DIFFERENT LETTERS INDICATE SIGNIFICANT DIFFERENCES (P<0.01, ONE-WAY ANOVA)

The hypothesis that the main factor that determines the time of unconsciousness in pacu is the voltage is worth testing in an experiment designed with this goal. Probably, the current peak has an important role because it was equal to 3.3 A in G400, coinciding with longer unconsciousness (FIGURE 17). Recently, Daskalova et al. 2015 studied the effectiveness of tail-first dry electrical stunning for turbot (*Scophthalmus maximus*) and common sole (*Solea solea*). The authors used a current peak of 2.39 ± 0.91 A for turbot and 1.22 ± 0.68 A for sole applied by 1 s to induce unconsciousness during 15.1 ± 9.4 s and 13.6 ± 12.2 s for turbot and sole, respectively (Daskalova et al., 2015). Probably, ours results on unconsciousness time in G400 are related to the current peak.

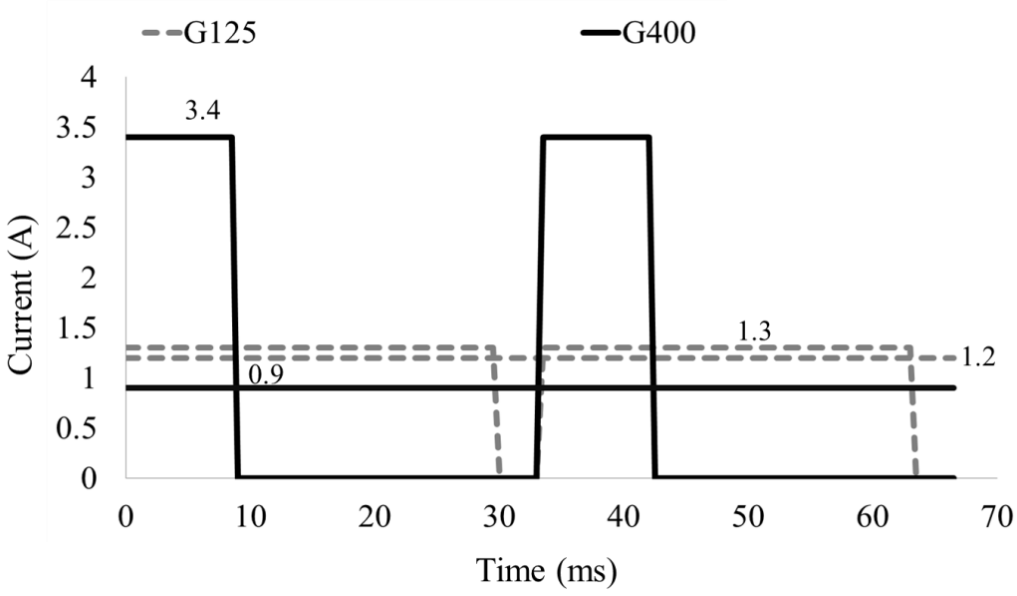

FIGURE 17 - CURRENT DIAGRAM (AVERAGE AND PEAK) OF PARAMETERS USED IN 35 SOUTH AMERICAN CATFISH (*Rhamdia quelen*) PER GROUP, STATE OF PARANA, SOUTH OF BRAZIL, SEPTEMBER 2015

The pH values (median, min-max) immediately after slaughter were 6.65 (6.20-6.90) for G125 and 6.70 (6.40-7.10) for G400 (FIGURE 18). Higher values were observed in African catfish slaughtered by cranial spiking, the initial pH of the muscle immediately after death was 7.10. It decreased to 6.00 within 4 days, and then gradually increased to 7.25 at the lapse of 18 days storage period, in which the fish were organoleptically unacceptable (Adoga et al.,

2010). Nevertheless, similar results were reported in European catfish (*Silurus glanis*) stunned with a blow on the head, eviscerated and bled with values of pH equal to 6.2 ± 0.2 and 6.1 ± 0.5 at 24 hours and 10 days after slaughter, respectively (Manthey et al., 1988). Therefore, electrical stunning in catfish probably does not alter the values of muscle pH significantly. Other methods of humane slaughter, as percussion, improve the quality of meat (Robb & Kestin, 2002). In Atlantic cod (*Gadus morhua* L.) stunned by CO2, lower pH values after slaughter were found, in comparison with fish stunned by percussion (Kristoffersen et al., 2006). The values of pH for South American catfish were similar in G125 and G400 in all the measurement times. The initial reduction in pH could be attributed to post-mortem glycolysis which led to accumulation of lactic acid, which in turn lowered the pH of the muscle (Huss, 1995). Values of pH in South American catfish were in accordance with Brazilian regulations, which require a pH of less than 6.8 in external musculature (Brasil, 1952). Our results show that electrical stunning does not affect the muscle pH values significantly in South American catfish, because they are in the range allowed by the Brazilian legislation.

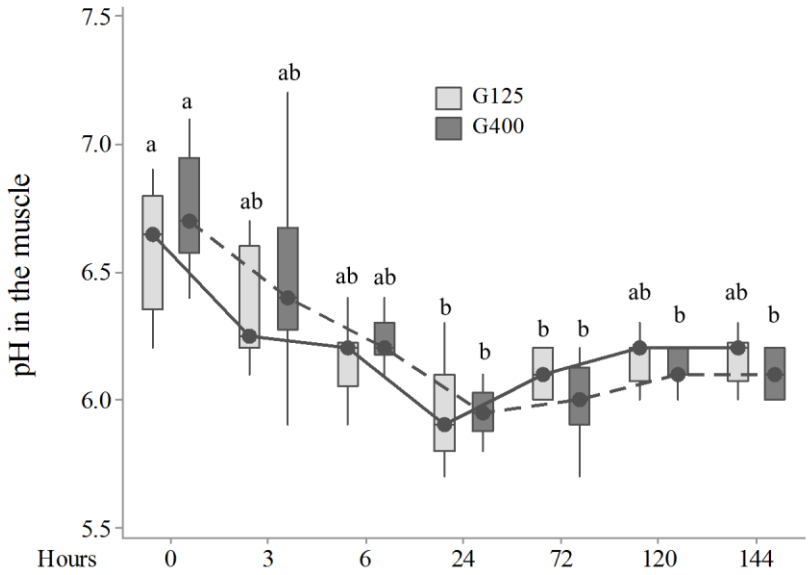

FIGURE 18 - MUSCLE PH VALUES, IN 10 SOUTH AMERICAN CATFISH (*Rhamdia quelen*) PER GROUP, STATE OF PARANA, SOUTH OF BRAZIL, SEPTEMBER 2015; DIFFERENT LETTERS INDICATE DIFFERENCE BETWEEN INTERVALS OF MEASUREMENT (P<0.01, KRUSKAL-WALLIS TEST)

The majority of fish (90%) reached maximum rigor mortis index (RMI = 100%) within 6 h post-mortem (FIGURE 19). Increased muscular activity, stress

at slaughter and endocrine responses may highly influence fish post-mortem biochemical processes, mostly the anaerobic glycolysis and ATP degradation rate (Huss, 1995). The maintenance of fish quality depends largely on the length of the pre-rigor period. During pre-rigor phase, fish flesh remains in a state of freshness. Deterioration sets in when relaxation starts, in post-rigor period. In general, the slower the rate of progress of rigor-mortis, the longer is the shelf-life of the fish (Poli et al., 2005). A prolongation of pre-rigor period can be achieved by delaying the onset of rigor, decreasing the rate of progress of rigor or by both, which are obviously of great commercial importance. Delay the onset of rigor is particularly important as fish meat in pre-rigor state is considered good for the market (Islami et al., 2014). A total of 90.0% of fish in G125 and 60.0% in G400 onset resolution of rigor mortis at 72 h, without significant differences ($P>0.05$). In African catfish slaughtered by cranial spiking, the authors found that complete relaxation (35%) occurred within 12 h after death, with no sign of spoilage (Adoga et al., 2010). Fish deterioration begins only after the resolution of rigor mortis (Poli et al., 2005). Nevertheless, Adoga et al. (2010) observed African catfish in acceptable condition sensorially up to 12 days in ice storage, despite onset the resolution of rigor 12 hours after death. Humane slaughter methods can delay the onset and resolution of rigor mortis in comparison to stunning with CO2 (Poli et al., 2005; Roth et al., 2002). Probably, electrical stunning in South American catfish accelerates the resolution of rigor mortis. It is important that future studies determine the quality of the meat for human consumption.

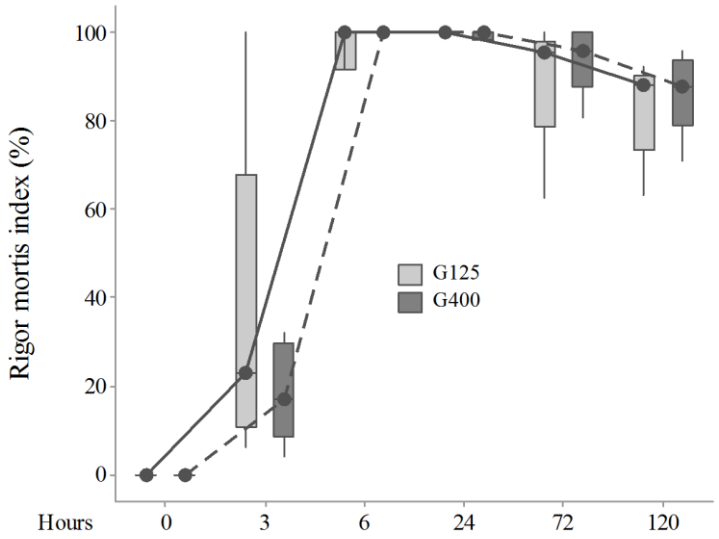

FIGURE 19 - VALUES OF RIGOR MORTIS INDEX (%) IN 10 SOUTH AMERICAN CATFISH (*Rhamdia quelen*) PER GROUP, STATE OF PARANA, SOUTH OF BRAZIL, SEPTEMBER 2015

The problem of electrical stunning, especially when fish are immersed in water during stunning, is that carcass damage might occur, such as muscle hemorrhages or a broken vertebral column (Van de Vis et al., 2014). No problems were observed in the carcass quality in our study. Fish did not show any external damage, vertebral fracture, hemorrhages or blood spotting in the meat. The used electric parameters are probably safe in terms of avoiding bleeding or fractures in the carcass.

4.4 CONCLUSION

For the first time, we report parameters for electrical stunning in South American catfish. Such information provides support for new studies seeking to reduce animal suffering during slaughter. Electrical stunning in South American catfish (*Rhamdia quelen)* is possible through pulse direct current, 400 Volts, 30 Hz, duty cycle of 27% and 0.9 A applied for 30 s, as unconsciousness is maintained during 87.7 ± 21.6 s in 100% of fish. Additionally, this stunning method did not cause significant alterations on muscle pH values. The electrical parameters employed seem safe in terms of avoiding muscle bleeding or fractures in the carcass. Our results contribute to the development of new

humane slaughter methods in fish that maintain or improve the quality of meat, which are necessary to improve animal welfare in aquaculture. We recommend new studies assessing unconsciousness by EEG as well as searching for methods with longer unconsciousness duration.

4.5 ACKNOWLEDGEMENTS

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5. FINAL CONSIDERATIONS

The results showed that the citizens from the two cities in Latin America recognized fish as sentient beings. Moreover, such recognition is linked to the perception of a high degree of suffering in the different scenarios of fish use, as well as the current slaughter methods. Respondents agreed with the inclusion of fish in humane slaughter regulations. It seems relevant to take into account the opinion of the population during the preparation and updating of standards on humane slaughter of animals for food production; thus, the inclusion of fish seems essential. Minimum standards for animal welfare should be established in different scenarios where fish are used also to avoid infractions to animal protection laws.

The studies of electrical stunning produced original knowledge regarding parameters providing stunning duration higher than one minute in two species of economic interest in Brazil. Even though behavioral indicators suggested unconsciousness, further studies with evaluation by EEG are needed. Additionally, we observed that the used electrical parameters are safe in terms of preventing bleeding, muscle petechiae or fractures in the carcass. These results allow for the advancement in fish welfare, since they offer tools for the development of humane slaughter methods which may be further adapted to regular slaughterhouse flow.

The results of this study provide support to relevant practical changes, such as the inclusion of fish in the humane slaughter legislation and the production of technology for the stunning of fish species that are relevant to Brazil. As a general final message, the inclusion of animal welfare issues in aquaculture should be conducted proactively, with research aimed at developing a product that is associated to high quality and ethical acceptance by the consumer.

APPENDIX 1 – POSTER PRESENTED IN HSA INTERNATIONAL SYMPOSIUM 2015: RECENT ADVANCES II

PERCEPTION ON FISH WELFARE ISSUES BY LATIN AMERICAN CITIZENS

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*carlamolento@ufpr.br

Introduction

Recent studies on cognition, emotional states and nociception have suggested that fish are sentient beings (1). However, surveys about public perceptions on fish sentience and welfare are scarce. Additionally, humane slaughter regulations in most countries do not include fish, as is the case in Brazil and Colombia. This study aimed to assess the perception of Brazilian and Colombian citizens in relation to sentience, welfare and humane slaughter in fish.

Material and Methods

An online survey was distributed to the population of Bogotá, Bogotá D.C., Colombia, and Curitiba, Paraná, Southern Brazil. We received responses of 782 respondents, 395 from Bogotá and 387 from Curitiba. The survey consisted of questions on demographics and people perception regarding sentience, slaughter methods and different practices involving fish. Comparisons were made using Mann-Whitney, Kruskall-Wallis, Dunn and chi-square tests; the calculated error was 5%.

Results and Discussion

The participants were asked about the level of sentience in eleven different species, on a 5 point-scale. The descending order in perception can be observed in the Figure 1. Results seem related to the level of human affinity to each species and phylogenetic proximity. In general, women's perception on sentience was higher for all species ($p<0.01$). The perception of fish suffering in different scenarios is presented in the Figure 2. Amongst all participants, (18.4%; 144/782) found catch-and-release recreational angling acceptable; being (29.4%; 87/296) amongst men and (13.8%; 67/486) amongst women ($p<0.01$); in New Zealand, (65%; 284/435) of the participants accepted such practice (2). Slaughter methods cited as most commonly observed were asphyxia (84.6%; 662/782), decapitation (49.2%; 385/782), percussion (35.3%; 276/782), gutting (33.6%; 263/782) and transportation of live fish in dry plastic bags, characterizing slow death by asphyxiation (11.0%; 86/782). Most respondents (92.3%; 722/782) pointed out that these methods of slaughter cause suffering in fish. Forty-three percent (344/782) of respondents have heard the term humane slaughter and (75.3%; 589/782) considered that fish should be included in humane slaughter regulations.

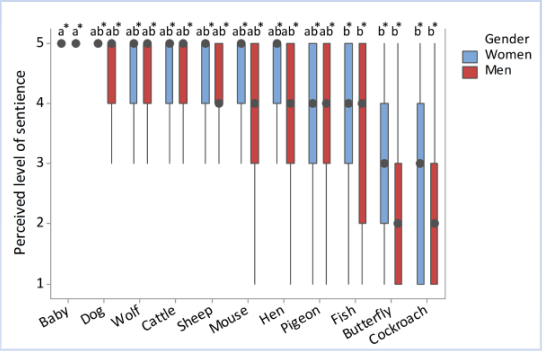

Figure 1. Median notes for sentience, being 1 = no sentience and 5 = maximum sentience; letters indicate differences between groups ($p<0.05$, Kruskal-Wallis and Dunn Test); the asterisk indicates gender difference ($p<0.05$, Mann-Whitney); considering 782 citizens of Bogotá and Curitiba, 2014/2015.

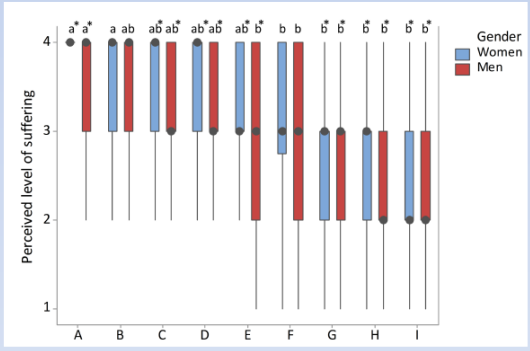

Figure 2. Median notes for suffering, being 1 = no suffering and 4 = much suffering; (A) Fishing with hook and line, (B) Municipal live fish fair, (C) Fish-and-pay ponds, (D) Fish kept as laboratory animals, (E) Fish farming, (F) Fish in pet stores, (G) Production of ornamental fish, (H) Fish in aquarium exhibits, (I) Fish kept as pets; letters indicate differences between scenarios ($p<0.05$, Kruskal-Wallis and Dunn Test); the asterisk indicates gender difference ($p<0.05$, Mann-Whitney); considering 782 citizens of Bogotá and Curitiba, 2014/2015.

Conclusion

The perception of fish sentience and welfare issues in citizens from two Latin American cities supports changes in the way these animals are treated, including proactive steps to improve legislation.

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APPENDIX 2 – POSTER PRESENTED IN "CONGRESSO MEDVEP DE ESPECIALIDADES VETERINÁRIAS"

PERCEPÇÃO DE CIDADÃOS DA COLÔMBIA E DO BRASIL EM RELAÇÃO À SENCIÊNCIA EM DIFERENTES GRUPOS ANIMAIS

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Introdução

O reconhecimento da senciência animal é fundamental para modificar normas que evitem atos de maus-tratos. Webster (2006) definiu um animal senciente como aquele que experimenta emoções associadas a sentimentos de prazer e sofrimento (1). Neste sentido, os animais cientificamente considerados sencientes pertencem ao grupo dos vertebrados e cefalópodes (2). Na América Latina, o conhecimento sobre a percepção da sobre senciência animal é ainda escasso. O objetivo do presente estudo foi comparar a percepção de cidadãos de Bogotá D.C., Colômbia e Curitiba, PR, Brasil sobre senciência em diferentes grupos de animais.

Material e Métodos

Um questionário online foi disponibilizado às populações de Bogotá (B) e Curitiba (C), em seus respectivos idiomas, de agosto de 2014 a março de 2015. Os respondentes foram convidados a atribuir uma nota em relação à capacidade de sentir emoções a 11 grupos de animais. No total foram avaliadas 395 respostas de Bogotá e de 415 de Curitiba. A fim de comparar as cidades e o gênero, utilizou-se teste de Mann-Whitney ao nível de significância de 5%. O teste Kruskal-Wallis seguido do teste de Dunn foi utilizado para comparar as notas atribuídas os grupos de animais

Resultados e Discussão

A distribuição das notas atribuídas em cada grupo de animais nas duas cidades encontram-se na Figura 1. Animais mais distantes como aves, peixes e invertebrados são percebidos com um menor grau de senciência, questão também mencionada por Broom (2007) (3). Das notas atribuídas aos 11 grupos de animais, 10 apresentaram diferença ($p<0,05$) evidenciando uma atribuição maior de senciência em cidadãos de C. As diferenças podem estar relacionadas às características de avanço na legislação de proteção animal de cada país. Na Colômbia, a legislação encontra-se desatualizada desde 1989, e ainda são permitidas rinhas de galo, corridas de touros, entre outras práticas consideradas como maus-tratos. No Brasil rinhas de galo e corridas de touros, e outras práticas, são proibidas desde 1994 (4). As mulheres apresentaram maior percepção de senciência em 10 dos 11 grupos de animais ($p<0,05$) Figura 2. Não foi observada diferença nas notas atribuídas ao lobo, possivelmente homens e mulheres têm contato escasso com tal animal silvestre.

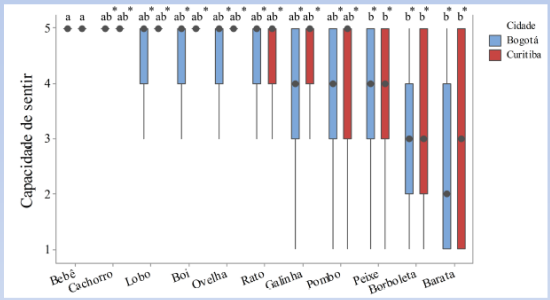

Figura 1. Medianas das notas atribuídas à capacidade de sentir, sendo que: 1 = o animal não sente e 5 = o animal certamente sente, por 395 cidadãos de Bogotá e 415 de Curitiba, de agosto 2014 a março de 2015; letras diferentes indicam diferença entre grupos de animais ($p<0,05$, Kruskal-Wallis e Teste Dunn); * indica diferença entre as cidades ($p<0,05$, Mann-Whitney).

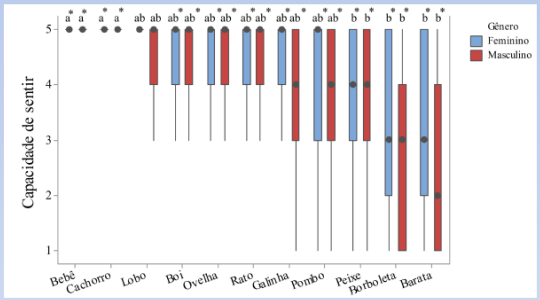

Figura 2. Medianas das notas atribuídas à capacidade de sentir, sendo que: 1 = o animal não sente e 5 = o animal certamente sente, por 537 mulheres e 282 homens cidadãos de Bogotá e Curitiba, de agosto 2014 a março de 2015; letras diferentes indicam diferença entre grupos de animais ($p<0,05$, Kruskal-Wallis e Teste Dunn); * indica diferença entre os gêneros ($p<0,05$, Mann-Whitney).

Conclusão

A percepção de senciência é maior nos animais pertencentes à classe dos mamíferos. Em geral, os cidadãos de Curitiba apresentaram uma maior percepção de senciência quando comparados aos cidadãos de Bogotá. As mulheres mostraram uma maior sensibilidade em comparação aos homens. Os resultados sugerem a necessidade de fomentar uma melhoria na percepção da população em relação à capacidade de sofrer de animais menos próximos do ser humano como aves e peixes.

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ANNEX 1 – SUBMISSION OF CHAPTER 2 IN THE JOURNAL PLOS ONE

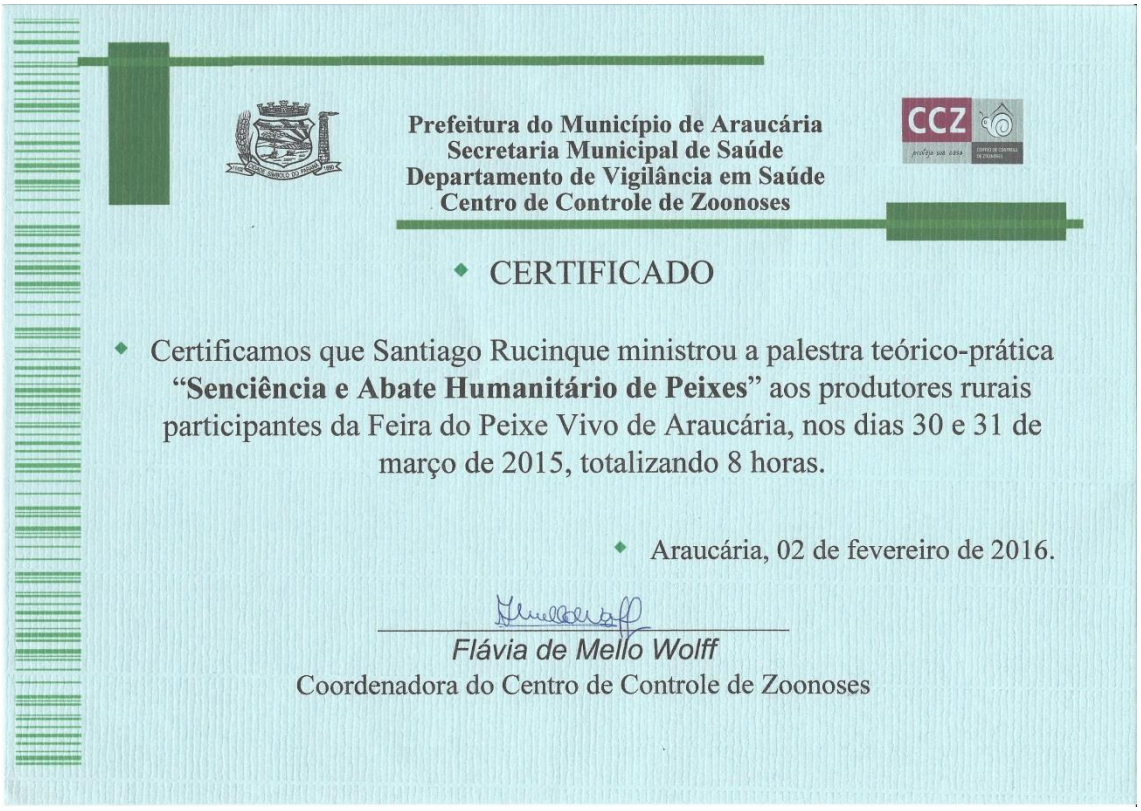
PLOS ONE

Perception on Fish Sentience, Welfare and Humane Slaughter by Latin American Citizens

--Manuscript Draft--

Manuscript Number:	PONE-D-15-49984
Article Type:	Research Article
Full Title:	Perception on Fish Sentience, Welfare and Humane Slaughter by Latin American Citizens
Short Title:	Latin American Perception on Fish Sentience
Corresponding Author:	Carla Forte Maiolino Molento, MSc, PhD Universidade Federal do Paraná Curitiba, Paraná BRAZIL
Keywords:	angling; Bogota; Curitiba; opinion; slaughter; suffering
Abstract:	Discussions on farm animal welfare have become frequent, especially in developed countries. The aim of this research was to study the perception on fish sentience, welfare and slaughter by citizens from Bogotá, Colombia, and Curitiba, Brazil. An online survey with 12 questions presented as open-end, multiple choice and 5-point Likert-type scale formats was available to respondents. Answers from 395 participants in Bogotá and 387 in Curitiba were analyzed, and results are presented in the order Bogotá followed by Curitiba. The percentage of participants who perceived fish as sentient animals was 79.7% and 71.8%. The classification of sentience perception among taxonomic groups seems in accordance with the phylogenetic proximity to humans, suggesting greater sensitivity towards mammals. The descending order related to the highest perception on fish suffering in different scenarios was fishing with hook and line (75.6%, 70.6%); municipal live fish fair (68.7% - only in Curitiba); fish-and-pay ponds (59.7%, 54.4%); fish kept as laboratory animals (58.0, 48.1%); fish farming (35.7, 36.8%); fish in pet stores (35.5%, 26.1%); production of ornamental fish (19.3%, 21.8%); fish in aquarium exhibits (18.8%,16.9%); and fish kept as pets (12.4%,12.3%). Lack of knowledge about the conditions of capture, handling, transport and sale of ornamental fish may justify the perception of low level of suffering in the last scenarios. Regarding humane slaughter, 57.0% and 55.0% of respondents were unaware of the issue. After reflection induced by the questionnaire, 76.0% and 72% of participants believed that fish should be included in humane slaughter regulations. This study presents original data suggesting that Latin Americans consider fish as sentient beings. The perception of suffering in specific scenarios challenges common activities. Recognition of suffering also endorses humane slaughter regulations to reduce pain in in a large number of individuals of fish slaughtered annually for human consumption in Latin America.
Order of Authors:	Daniel Santiago Rucinque, DMV, MSc
	Ana Paula Oliveira Souza
	Carla Forte Maiolino Molento

ANNEX 2 – TALK IN ARAUCÁRIA-PR



Prefeitura do Município de Araucária
Secretaria Municipal de Saúde
Departamento de Vigilância em Saúde
Centro de Controle de Zoonoses

CCZ

CERTIFICADO

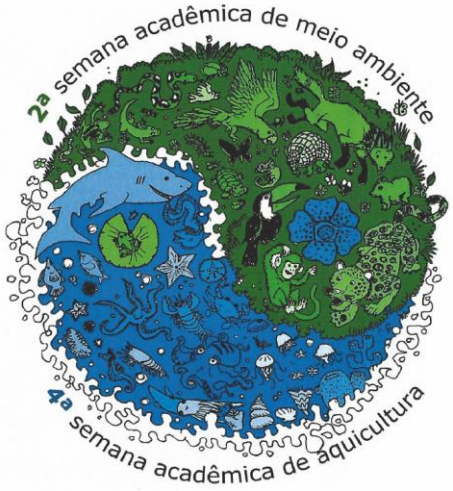
Certificamos que Santiago Rucinque ministrou a palestra teórico-prática "**Senciência e Abate Humanitário de Peixes**" aos produtores rurais participantes da Feira do Peixe Vivo de Araucária, nos dias 30 e 31 de março de 2015, totalizando 8 horas.

Araucária, 02 de fevereiro de 2016.

Flávia de Mello Wolff
Coordenadora do Centro de Controle de Zoonoses

ANNEX 3 – TALK IN PARANAGUÁ-PR

CERTIFICADO



Certificamos que **Daniel Santiago Rucinque Gobnzalez** ministrou a palestra "Bem-estar e abate humanitário de peixes" na 2ª. Semana Acadêmica de Meio Ambiente e 4ª. Semana Acadêmica de Aquicultura do Instituto Federal do Paraná – Campus Paranaguá. A palestra foi realizada no dia 3 de setembro de 2015, tendo duração de 02 horas.

Paranaguá, 3 de setembro de 2015

Izabel Carolina Raittz Cavallet
Coordenadora do evento

Mateus das Neves Gomes
Coordenador de Pesquisa e Extensão
IFPR Paranaguá

ANNEX 4 - APPROVAL BY THE HUMAN RESEARCH ETHICS COMMITTEE OF THE FEDERAL UNIVERSITY OF PARANÁ

UNIVERSIDADE FEDERAL DO PARANÁ - SETOR DE CIÊNCIAS DA SAÚDE/ SCS -

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: PERCEPÇÃO E ATITUDES HUMANAS SOBRE A SENCIÊNCIA ANIMAL E QUESTÕES RELACIONADAS AO BEM-ESTAR ANIMAL

Pesquisador: Carla Forte Maiolino Molento

Área Temática:

Versão: 3

CAAE: 34820114.0.0000.0102

Instituição Proponente: Programa de Pós-graduação em Ciências Veterinárias

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 814.835

Data da Relatoria: 26/09/2014

Apresentação do Projeto:

PERCEPÇÃO E ATITUDES HUMANAS SOBRE A SENCIÊNCIA ANIMAL E QUESTÕES RELACIONADAS AO BEM-ESTAR ANIMAL

Carla Forte Maiolino Molento

Trata-se de projeto de pesquisa amplo, linha de pesquisa da Prof. Dra. Carla Forte Maiolino, que visa avaliar a percepção e atitudes humanas sobre a senciência animal e questões relacionadas ao bem estar animal.

Objetivo da Pesquisa:

Geral: Estudar a percepção humana a respeito da capacidade de sentir (senciência) em diferentes espécies de animais e questões de bem-estar.

Específicos:

- Estudar a percepção de biólogos, médicos veterinários, zootecnistas, consumidores e produtores de ovinos no estado do Paraná em relação à presença de senciência e estados afetivos nos animais desta espécie,

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aplicando-se um questionário e comparando à população europeia;- Estudar a percepção de produtores em relação a diferentes práticas de rotina realizadas em animais de
produção;
- Avaliar atitudes dos produtores para a realização de diferentes práticas de manejo e/ou rotina realizadas em animais de produção;
- Conhecer as percepções da população em questões relacionadas ao bem-estar e abate de peixes no Brasil e na Colômbia;
- Avaliar as atitudes relacionadas ao sofrimento e maus tratos em diferentes espécies.
- Realizar pesquisa com produtores de ovinos associados à Associação Paranaense de Criadores de Ovinos (OVINOPAR) e/ou à Castrolanda para determinar a motivação para realização da caudectomia;

Avaliação dos Riscos e Benefícios:
De acordo com os pesquisadores a aplicação de questionários para avaliar a percepção dos respondentes pode ser considerada como de baixo risco. Os tipos de risco mais prováveis são a possibilidade de exposição de informações demográficas (idade, sexo, renda, grau de escolaridade), as quais serão confidenciais, anônimas, e constrangimento pela possibilidade de provocar experiências ou recordações de situações vividas que causaram algum tipo de sofrimento psíquico. Além disso, o respondente pode acreditar que está correndo risco de ser julgado, entretanto será disponibilizado espaço apropriado para que sejam realizados comentários referentes à pesquisa, com o objetivo de minimizar tal efeito.

Comentários e Considerações sobre a Pesquisa:
A maior força motriz para a melhoria do bem-estar animal é a opinião pública. Há grande demanda do público por melhorias no bem-estar animal dos animais. Essa demanda se reflete nas atividades de várias organizações sem ânimo de lucro e através dos meios de comunicação social e, finalmente, a nível político, através de uma melhor legislação. Consequentemente a melhoria de vida dos
animais pode acarretar em bem-estar humano a partir do consumo de produtos provenientes de

animais com qualidade de vida adequada e condições de manutenção eticamente aceitáveis.

Considerações sobre os Termos de apresentação obrigatória:
Todos os termos obrigatórios foram anexados.

Recomendações:
Solicitamos que sejam apresentados a este CEP, relatórios semestrais e final, sobre o andamento da pesquisa, bem como informações relativas às modificações do protocolo, cancelamento, encerramento e destino dos conhecimentos obtidos, através da Plataforma Brasil - no modo: NOTIFICAÇÃO. Demais alterações e prorrogação de prazo devem ser enviadas no modo EMENDA. Lembrando que o cronograma de execução da pesquisa deve ser atualizado no sistema Plataforma Brasil antes de enviar solicitação de prorrogação de prazo.

Conclusões ou Pendências e Lista de Inadequações:
As pendências foram atendidas.
É obrigatório retirar na secretaria do CEP/SD uma cópia do Termo de Consentimento Livre e Esclarecido com carimbo onde constará data de aprovação por este CEP/SD, sendo este modelo reproduzido para aplicar junto ao participante da pesquisa.

O TCLE deverá conter duas vias, uma ficará com o pesquisador e uma cópia ficará com o participante da pesquisa (Carta Circular nº. 003/2011CONEP/CNS)

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Considerações Finais a critério do CEP:

UNIVERSIDADE FEDERAL DO PARANÁ - SETOR DE CIÊNCIAS DA SAÚDE/ SCS -

Continuação do Parecer: 814.635

CURITIBA, 01 de Outubro de 2014

Assinado por:
IDA CRISTINA GUBERT
(Coordenador)

Endereço: Rua Padre Camargo, 280
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ANNEX 5 - APPROVAL BY THE ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES SECTOR, FEDERAL UNIVERSITY OF PARANÁ

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 058/2014, referente ao projeto "Parâmetros para eletronarcose de tilápia (*Oreochrmis niloticus*) e pacu (*Piaractus mesopotamicus*) e sua demanda social", sob a responsabilidade de Daniel Santiago Rucinque Gonzalez, na forma em que foi apresentado (utilização de 152 peixes e como grau E de invasividade), foi aprovado pela Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias, em reunião realizada dia 10 de Outubro de 2014.

CERTIFICATE

We certify that the protocol number 058/2014, regarding the project "Parameters for electronarcosis *tilapia* (*Oreochrmis niloticus*) and *pacu* (*Piaractus mesopotamicus*) and its social demand", under Daniel Santiago Rucinque Gonzalez's supervision, in the terms it was presented (use of 152 fish and was classified as grade E of invasiveness), was approved by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Universidade Federal do Paraná (Federal University of Paraná, Brazil) during session on October 10th, 2014.

Curitiba, 10 de Outubro de 2014.

Ricardo Guilherme D'Otaviano de Castro Vilani
Presidente CEUA-SCA

Ananda Portella Félix
Vice-Presidente CEUA-SCA

ANNEX 6 - APPROVAL BY THE ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES SECTOR, FEDERAL UNIVERSITY OF PARANÁ

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO ADICIONAL

Certificamos que o protocolo número 058/2014, referente ao projeto **"Parâmetros para eletronarcose de tilápia (*Oreochromis niloticus*) e pacu (*Piracatus mesopotamicus*)"**, aprovado pela CEUA em 10/10/2015, sob a responsabilidade de **Daniel Santiago Rucinque Gonzalez** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro, de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada à substituição de 76 tilápias por 76 jundiás pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, em reunião de 27/08/2015.

ADDITIONAL CERTIFICATE

We certify that the protocol number 058/2014, regarding the project "**Parameters for electronarcosis of tilapia (*Oreochromis niloticus*) and pacu (*Piracatus mesopotamicus*)",** approved by CEUA in 10/10/2014, under **Daniel Santiago Rucinque Gonzalez** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October, 2008, of Decree nº 6.899, of 15 July, 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved the substitution of 76 tilapia to 76 jundia by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), in session of 08/27/2015.

Curitiba, 27 de Agosto de 2015.

Ananda Portella Félix
Presidente CEUA-SCA

Simone Tostes de Oliveira Stedile
Vice-Presidente CEUA-SCA