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BENZOCAINE AND EUGENOL AS ANESTHETICS FOR PANGASIUS JUVENILES, *Pangasianodon hypophthalmus*

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Abstract

The aim of this study was to evaluate the effect and efficacy of different concentrations of eugenol and benzocaine for pangasius juveniles (*Pangasianodon hypophthalmus*) and determine the anesthetic and concentration most appropriate for carrying out routine fish farming practices for this species. One hundred juveniles with an average weight of 32.10 ± 4.9 g and an average total length of 15.32 ± 0.57 cm were used. Four concentrations of both anesthetics were evaluated: 0, 25, 50, 75 and 100 mg/L, with four induction time stages and three recovery stages. During the time that the fish remained anesthetized, biometric procedures were performed. Then, they were transferred to a 10-liter aquarium containing clean water, without adding the anesthetic to observe the recovery time. After recovery, the animals were kept in aquariums for 72 hours to check for mortality. Eugenol, at all concentrations evaluated, and benzocaine, at a concentration of 25 mg/L, were not effective in sedating juveniles of pangasius. Benzocaine concentrations of 75 and 100 mg/L were effective for anesthesia and recovery of fish within the time span of five and seven minutes. However, considering animal welfare, the use of a concentration of 100 mg/L is recommended, as it resulted in shorter latency and recovery times.

Keywords aquaculture management, fish sedation, drugs.

Resumo

O objetivo deste trabalho foi avaliar o efeito e a eficácia de diferentes concentrações de benzocaína e eugenol em juvenis de pangasius (*Pangasianodon hypophthalmus)* para determinar o anestésico mais eficiente e a concentração mais adequada para realização das práticas rotineiras em piscicultura para esta espécie. Foram utilizados 100 juvenis, com peso médio de 32,10 ± 4,9 g e comprimento total médio de 15,32 ± 0,57 cm. Foram avaliadas quatro concentrações para ambos os anestésicos: 0, 25, 50, 75 e 100 mg/L, sendo observados quatro estágios de tempo de indução e três estágios de recuperação. Durante o tempo em que os peixes permaneceram anestesiados, foram realizados os procedimentos de biometria. Em seguida, os animais foram transferidos para um aquário de 10 L contendo água limpa, sem adição do anestésico, para observar o tempo de recuperação. Após a recuperação, os animais foram mantidos nos aquários por 72 horas para verificação da mortalidade. O eugenol, em todas as concentrações avaliadas, e a benzocaína, na concentração de 25 mg/L, não foram eficazes na sedação de juvenis de pangasius. As concentrações de benzocaína de 75 e 100 mg/L foram eficientes para anestesia e recuperação dos peixes considerando a faixa de tempo pré-estabelecida de cinco e sete minutos, respectivamente. Entretanto, considerando o bem-estar animal, recomenda-se o uso da concentração de 100 mg/L, uma vez que resultou em menor tempo de latência e recuperação.

Palavras chaves manejo aquícola, insensibilização em peixe, fármacos.

INTRODUCTION

The Brazilian production of fish from aquaculture was 529,623 tons in 2019 (IBGE, 2020). One of the species that has stood out in the national aquaculture scene due to its production potential is pangasius (*Pangasianodon hypophthalmus*). The State of São Paulo was the pioneer to regulate the cultivation of this species, through the Decree n° 62243, of January 1, 2016 (SÃO PAULO, 2016). It is noteworthy that the permission is only for cultivation in excavated or masonry ponds; the pangasius cannot be cultured in cages in the São Paulo State. Within the scope of the Decree and of the Resolution SAA 73 (SÃO PAULO-SAA, 2016), the pangasius was included in a list of various exotic aquatic species, suitable for cultivation in the basins of São Paulo State (INSTITUTO DE PESCA, 2016; INSTITUTO DE PESCA, 2018).

Regarding its zootechnical attributes, the species has fast growth, reaching 1.4 kg in 8 months, with an apparent feed conversion of 1.4:1, as observed in preliminary studies carried out. In addition, another interesting feature is the possibility of rearing at high densities, as the species has facultative air respiration.

According to Ross and Ross (2008a), common fish farming activities subject fish to various management procedures, such as biometrics, sanitary treatments, vaccination, reproductive practices, and transport, which cause a high level of stress. These management activities can cause physiological changes, directly affecting the zootechnical performance of fish as well as their health and, consequently, the cost of production (ZAHL et al., 2012; RIBEIRO et al., 2015).

The use of anesthetics is a viable alternative to reduce the stressful effects of day-to-day management practices in fish farms (ROSS and ROSS, 2008a; ZAHL et al., 2012; RIBEIRO et al., 2015). Among numerous existing anesthetics, benzocaine (ethyl p-aminobenzoate) is widely used in research centers and by some fish farmers due to the low cost and ease of acquiring and handling this product (SOUZA et al., 2012; RODRIGUES et al., 2016; BRAZ et al., 2017). Another anesthetic widely used is eugenol (4-Allyl-2-methoxyphenol), which has aroused interest because it is a natural product, originating from clove oil (ROTILI et al., 2012; HOSEINI et al., 2015; MIRGHAED et al., 2016; BRAZ et al., 2017).

Given the scarcity of tests with anesthetics, as well as their dosages and effects for pangasius, studies aimed at consolidating good management practices and filling yet another gap in the physiology of this species, whose production has been growing throughout the country.

The aim of this study was to evaluate the effect and efficacy of different concentrations of eugenol and benzocaine for pangasius juveniles (*Pangasianodon hypophthalmus*) to determine the anesthetic and concentration most appropriate for carrying out routine fish farming practices for this species.

MATERIAL AND METHODS

The experiment was carried out at the Laboratory of Nutrition of Aquatic Organisms of the Center for Research on Continental Fish of the Instituto de Pesca (São José do Rio Preto, SP) and it was approved by the Experimental Ethics Committee of the Instituto de Pesca, nº 04/2019. One hundred specimens of pangasius (*P. hypophthalmus*) were used, with an average weight of 32.10 ± 4.9 g and total length of 15.32 ± 0.57 cm, acquired from a commercial fish farm (Global Peixes), located in Santa Fé do Sul (SP). The fish were acclimated for 14 days in four 500 L boxes, with aeration and constant water renewal. Feeding was carried out three times a day with commercial food (32% crude protein) in the amount of 2% of the live weight of the fish. Twenty-four hours before the anesthetic tests, the fish were kept fasting.

The experiment was carried out in 10 L aquariums. At the beginning of the tests, the water quality was verified, with the mean values (± standard deviation) of dissolved oxygen (6.25 ± 0.15 mg/L; YSI 55), temperature (26.0 ± 0.12°C YSI 55), pH (7.0; Alcon Labcon Kit Test) and ammonia (0.0 mg/L; Alcon Labcon Kit Test). The values observed are within the ideal range of cultivation for tropical fish according to Leonardo et al. (2018).

Tests with each anesthetic were performed individually, on different days. To obtain the evaluated concentrations, stock solutions of benzocaine (FARMOS Commercial e Industry LTDA, Brazil) and eugenol (Biodynamics; 99% minimum purity) were prepared from a 1:10 dilution of the chemical in ethylic alcohol (100 g/L of ethanol 92.8%, according to SOUZA et al., 2012). The tested concentrations (treatments) were: 0 (control), 25, 50, 75 and 100 mg/L for both anesthetics. Eight fish (n = 8) were used per treatment (repeats).

Groups of five animals were individually exposed to each treatment, from the lowest to the highest concentration, and at each concentration, the aquariums were properly washed (BITTENCOURT et al., 2012). The control received the same handling as the others, but without the anesthetic solution in the aquarium. The time for the appearance for the behavioral patterns related to anesthesia and recovery was monitored using a digital chronometer.

To assess the best concentrations of each anesthetic, the response time for anesthesia and fish recovery was used as a criterion, according to BRAZ et al. (2017), with change in the adopted time range (five minutes for anesthesia (sedation) and seven minutes for full recovery of the animals - Step 3).

The four stages of anesthesia were: Stage 1 - onset of loss of balance, characterized by swimming movement in the normal position, interspersed with lateral movements; Stage 2 - total loss of balance, corresponding to uncoordinated side movement; Stage 3 - decreased opercular movement; Step 4 - total loss of reaction to any stimulus or absence of tactile sensation (BITTENCOURT et al., 2012). The verification of stage 4 was carried out with the aid of a plastic stick, lightly touching the fish.

After the anesthesia induction procedure, each animal was submitted to biometric management (weight and length), simulating practical activities in fish farming.

Afterwards, each fish was transferred to a 10 L aquarium, with clean water, where its recovery was monitored, observing three steps: Step 1 - normalization of opercular movements; Step 2 - return from swimming; and Stage 3 - full recovery (BRAZ et al., 2017). After this period, all fish from each “anesthetic and concentration” treatment was transferred to 60 L boxes with oxygenation and constant water renewal, where survival for 72 h was observed.

The average results of anesthetic induction time and total recovery of movements were submitted to Analysis of Variance (ANOVA) and, when significant differences were found, the Tukey test was applied. For all analyses, a significance level of 95% was used. It was used for the Sisvar 5.6 program (SISVAR, 2010).

RESULTS AND DISCUSSION

Mortality was not observed during anesthesia procedures, recovery and during the period of 72 hours after the manipulation of pangasius juveniles in any of the tests performed. During anesthetic induction with eugenol, fish showed a similar behavioral pattern when meeting different concentrations of anesthetics, with the three initial stages of anesthesia being verified (Stages 1 to 3). However, the fish did not reach Stage 4 (complete loss of reaction to any stimulus or absence of tactile sensation) during the observation period (limited to a maximum of 10 minutes) in any of the evaluated eugenol concentrations (Table 1). There were significant differences ($p \leq 0.05$) in anesthetic induction time between eugenol concentrations in Stages 2 and 3, with the shortest time verified at the concentration of 100 mg/L (Table 1).

Regarding the stages of recovery of fish submitted to eugenol anesthetic, all were reached within a period of less than seven minutes. Significant differences ($p<0.05$) were found between concentrations; recovery was achieved in a shorter time in fish subjected to the lowest concentrations (25 and 50 mg/L) (Table 1).

It is noteworthy that the fish did not reach the final stage of anesthesia (complete loss of reaction to external stimuli) after the observation period of 10 minutes in any of the concentrations, which may have influenced the animals' recovery time. As there was no total sedation, the management of biometrics may have resulted in greater stress for the animals; therefore, recovery was slower, especially at higher concentrations.

Table 1. Time (in minutes; mean ± standard deviation) to reach the stages of anesthesia and recovery of pangasius juveniles exposed to different levels of eugenol (mg/L).

Concentration (mg/L)	Stages - Anesthetic Induction (min)			
	1	2	3	4
25	1.03 ± 0.27	2.46 ± 0.57^{b}	7.14 ± 0.58^{b}	> 10
50	1.01 ± 0.28	2.48 ± 0.59^{b}	4.58 ± 0.78^{a}	> 10
75	1.02 ± 0.34	2.61 ± 0.63^{b}	4.57 ± 0.50^{a}	> 10
100	1.04 ± 0.68	1.06 ± 0.57^{a}	3.58 ± 0.49^{a}	> 10
Concentration (mg/L)	Stages - Anesthetic Recovery (min)			
	1	2	3	
25	0.66 ± 1.25^{a}	1.50 ± 0.71^{a}	3.04 ± 1.01^{a}	
50	0.77 ± 0.35^{a}	2.47 ± 0.41^{a}	3.87 ± 0.47^{ab}	
75	0.99 ± 0.64^{a}	3.91 ± 1.26^{b}	5.14 ± 1.58^{bc}	
100	2.46 ± 0.77^{b}	4.49 ± 1.47^{b}	5.89 ± 1.88^{c}	

* Values followed by different letters in the same column differ from each other by the Tuckey test, with 5% significance. Anesthesia Induction Phases: Phase 1 - onset of loss of balance, characterized by swimming movement in the normal position interspersed with lateral movements; Stage 2 - total loss of balance, corresponding to sideways movement, without coordination; Stage 3 - decreased opercular movement; Stage 4 - total loss of reaction to any stimulus or lack of tactile sensation. Stages of Anesthetic Recovery: Stage 1 - normalization of opercular movements; Step 2 - return to swimming; Step 3 - full recovery of movements.

Considering the results obtained, eugenol was not efficient as an anesthetic for pangasius with an average weight of 32 g at the concentrations evaluated, since complete anesthesia (Stage 4) was not achieved. Therefore, the evaluated doses cannot be considered safe for the purpose of sedating the species. On the contrary, Hoseini et al. (2015), evaluating different concentrations of eugenol in pangasius weighing from 2 to 20 g, concluded that the anesthetic was efficient. The concentration of 40 mg/L was indicated as adequate for the management of the species with 20 g of average weight. However, the model proposed by the authors indicated a minimum concentration of 81.5 mg/L of the drug to induce anesthesia in less than three minutes in animals with an average weight of 20 g, attesting to the relationship between animal size and concentration anesthetic (ie, the greater the weight of the fish, the greater the concentration of the compound).

Using the anesthetic benzocaine, the fish showed the same behavior observed with eugenol when meeting with the solution (they reached the three initial stages of induction of anesthesia). However, with benzocaine, fish also reached Stage 4 (complete loss of reaction to stimulus or absence of tactile sensation) except at the lowest concentration (25 mg/L).

The 50 mg/L concentration was less efficient ($p \leq 0.05$) in providing stages 3 and 4 sedations when compared to the 75 mg/L and 100 mg/L doses (Table 2).

Table 2. Time (in minutes; mean ± standard deviation) to reach stages of anesthesia and recovery in juveniles of pangasius exposed to different benzocaine concentrations in mg/L.

Concentration (mg/L)	Stages - Anesthetic Induction (min)			
	1	2	3	4
25	0.14 ± 0.05[a]	0.36 ± 0.31	0.89 ± 0.62[a]	> 10
50	0.56 ± 0.43[b]	0.71 ± 0.43	4.88 ± 1.02[c]	6.75 ± 1.50[c]
75	0.59 ± 0.30[b]	0.70 ± 0.30	2.56 ± 0.87[b]	3.84 ± 1.88[b]
100	0.35 ± 0.07[ab]	0.37 ± 0.17	1.27 ± 0.58[a]	1.39 ± 0.15[a]
Concentration (mg/L)	Stages - Anesthetic Recovery (min)			
	1	2	3	
25	0.99 ± 0.43[b]	1.54 ± 0.72[b]	3.12 ± 2.54[a]	
50	0.17 ± 0.09[a]	0.45 ± 0.32[a]	1.50 ± 1.57[a]	
75	0.77 ± 0.64[b]	3.91 ± 1.26[c]	5.14 ± 1.58[b]	
100	1.55 ± 0.37[c]	2.05 ± 0.57[b]	2.58 ± 0.57[a]	

* Values followed by different letters in the same column differ from each other by the Tuckey test, with 5% significance. Anesthesia Induction Phases: Phase 1 - onset of loss of balance, characterized by swimming movement in the normal position interspersed with lateral movements; Stage 2 - total loss of balance, corresponding to sideways movement, without coordination; Stage 3 - decreased opercular movement; Stage 4 - total loss of reaction to any stimulus or lack of tactile sensation. Stages of Anesthetic Recovery: Stage 1 - normalization of opercular movements; Step 2 - return to swimming; Step 3 - full recovery of movements.

The highest concentrations of benzocaine (75 to 100 mg/L) were effective to anesthetize and recover pangasius juveniles considering the established time of five and seven minutes, respectively, with a significant difference ($p \leq 0.05$) between these concentrations observed for sedation and recovery in Stages 3 and 4. Although the fish recovery time at the concentration of 50 mg/L of benzocaine was significantly lower than the highest concentrations, total sedation was verified after five minutes, considered as limiting to attest to the drug's efficiency. Therefore, benzocaine concentrations below 75 mg/L are not efficient for pangasius in this evaluated weight class.

The longer recovery time observed for the concentration of 25 mg/L may be the result of the absence of total sedation in animals exposed to this dose of anesthetic for 10 min. As the fish were not completely anesthetized during the biometrics management, they probably suffered greater stress, influencing the recovery time.

Sedation and recovery times observed in the present study were similar to those found in the literature for tainha juveniles (*Mugil liza)* (BRAZ et al., 2017), quinguio (*Carassius auratus)* (BITTENCOURT et al., 2012) and patinga (*Piaractus mesopotamicus* x *P. brachypomus*) (RODRIGUES et al., 2016). These authors describe times for sedation ranging from 2 to 11 minutes and recovery from 3 to 14 minutes. Souza et al. (2012), working with sea bass (*Centropomus parallelus*), report that the time for sedation occurred between 1 minute and recovery was observed between 1 to 5 minutes. In the present work, only the concentration of 100 mg/L of benzocaine provided a sedation time close to the results of the super-cited author, however, the recovery times at the concentrations in which the fish reached stage 4 are within the described time.

According to Ross and Ross (2008b) and Serra et al. (2016), the choice of anesthetic should take into account some points such as: efficiency (anesthesia time and recovery time), toxicity (taking into account fish, man and the environment), cost (value per kg of product) and management (fish species to be used and life stage). The efficacy of the anesthetic it is in the short latency time (average time of 3 minutes) and rapid recovery (average time of 5 minutes) of animals exposed to the product (ROSS and ROSS, 2008b).

We can state that benzocaine at concentrations 75 and 100 mg/L is able to induce anesthesia and recovery of fish in less than 5 and 7 minutes, respectively, and may be indicated as a sedative in the management of pangasius.

After the tests, the fish remained under observation for 72 hours and none of the fish showed irritation in the gills and corneal lesions, neither died nor showed the clinical signs described by Inoue et al. (2003) when chemical are use in high doses.

According to Bittencourt et al. (2012), each fish species has a specific time for anesthetic induction according to its physiology. However, other factors are also correlated by the author, such as the stage of life (fingerlings, juveniles, adult fish) and the management that will be carried out (biometry, surgeries, transport).

The use of anesthetics in common fish farming practices is of paramount importance to minimize management stress (weighing, transport, capture for reproduction, change of facilities). When good management practices are not adopted, fish become stressed and can reduce food intake, resulting in less growth. Furthermore, stress can reduce the immunity of animals, favoring the onset of diseases and/or causing mortality. Thus, providing the fish with maximum well-being will result in higher rates of rearing areas, greater growth and production of healthy animals.

CONCLUSIONS

Eugenol, at the four concentrations tested, was not effective to totally desensitize juveniles of pangasius. Benzocaine was effective in anesthetic induction and recovery at concentrations above 50 mg/L; however, considering animal welfare, we recommend the use of a concentration of 100 mg/L, as it has a low latency time and fast recovery.

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REFERENCES

BITTENCOURT. F.; SOUZA, B.E.; BOSCOLO, W.R.; RORATO, R.R.; FEIDEN, A.; NEU, D.H. Benzocaína e eugenol como anestésicos para o quinguio (*Carassius auratus*) **Arquivos Brasileiros Medicina Veterinária e Zootecnia**, v.64, p.1597-1602, 2012. https://doi.org/10.1590/S0102-09352012000600028

BRAZ, R.S.; SILVA, I.O.; TESSER, M.B.; SAMPAIO, L.A.; RODRIGUES, R.V. Benzocaína, MS-222, Eugenol e Mentol como anestésico para juvenis de Tainha Mugil Liza. Boletim do Instituto de Pesca, v.43, p.605-613, 2017. https://doi.org/10.20950/1678-2305.2017v43n4p605

HOSEINI, S.M.; RAJABIESTERABADI, H.; TARKHANI, R. Anesthetic efficacy of eugenol on iridescent shark, Pangasius hypophthalmus (Sauvage, 1978) in different size classes. Aquaculture Research, v.46, p.405-412, 2015. https://doi.org/10.1111/are.12188

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. 2020. Pesquisa da Pecuária Municipal 2017, 2018 e 2019. Disponível em: https://sidra.ibge.gov.br/tabela/3940#resultado. Acesso em: 12 maio 2021.

INOUE, L.AK.A.; SANTOS-NETO, C.; MORAES, G. Clove oil anaesthetic for juveniles of matrinxã *Brycon cephaleus* (Gunther, 1869). **Ciência Rural,** v.33, n.5, p.943-947, 2003. https://doi.org/10.1590/S0103-84782003000500023

INSTITUTO DE PESCA. Portaria do Diretor, de 30 de novembro de 2016. Dispõe sobre a lista de espécies aquícolas alóctones, exóticas e híbridos cultiváveis no Estado de São Paulo. **Diário Oficial do Estado de São Paulo** - Poder Executivo, Seção I, São Paulo, SP, 01 de dez. 2016, v. 126, n.224, p.34-35.

INSTITUTO DE PESCA. Portaria do Diretor, de 30 de novembro de 2018. Dispõe sobre a lista de espécies aquícolas alóctones, exóticas e híbridos cultiváveis no Estado de São Paulo. **Diário Oficial do Estado de São Paulo** - Poder Executivo, Seção I, São Paulo, SP, 05 de dez. 2018, v. 128, n.225, p.70-71. Disponível em: <https://www.cdrs.sp.gov.br/portal/themes/unify/arquivos/produtos-e-servicos/legislacao-dcaa/PortariaPesca2018-ListaExoticas.pdf>. Acesso em: 18 dez. 2021.

MIRGHAED, A.T.; GHELICHPOUR, M.; HOSEINI, S.M. Myrcene and linalool as new anesthetic and sedative agents in common carp, *Cyprinus carpio – Comparison with eugenol. Aquaculture, v.464, p.165-170, 2016. https://doi.org/10.1016/j.aquaculture.2016.06.028*

RIBEIRO, P.A.P.; MIRANDA-FILHO; K.C.; MELO, D.C.; LUZ, R.K. Efficiency of eugenol as anesthetic for the early life stages of Nile tilapia (*Oreochromis niloticus*). **Anais da Academia Brasileira de Ciências,** v.87, p.529-535, 2015. http://dx.doi.org/10.1590/0001-3765201520140024.

RODRIGUES. W.A.; MELO, I.W.A.; ROCHA, J.D.M.; SILVA, C.T.; BRIDI, V.R.C.; SIGNOR, A.; BITTENCOURT, F.; BOSCOLO, W.R. Tempo de indução e recuperação à anestesia da benzocaína para patinga (*Piaractus mesopotamicus* X *Piaractus brachypomus*). **Revista Brasileira de Higiene e Sanidade Animal,** v.10, p. 364-373, 2016.

ROSS, L.G.; ROSS, B. Introduction. In: ROSS, L.G.; ROSS, B. **Anesthetic and sedative techniques for aquatic animals**. 3ª ed. Oxford, UK.: Blackwell Publishing, 2008a. p.1-5. https://doi.org/10.1002/9781444302264.ch1

ROSS, L.G.; ROSS, B. The Features of Anesthetic Agents. In: ROSS, L.G.; ROSS, B. **Anesthetic and sedative techniques for aquatic animals**. 3ª ed. Oxford, UK.: Blackwell Publishing, 2008b. p.53-56. https://doi.org/10.1002/9781444302264.ch5

ROTILI, D.A.; DEVENS, M.A.; DIEMER, O.; LORENZ, E.K.; LAZZARI, R.; BOSCOLO, W.R. Uso de eugenol como anestésico em pacu. **Pesquisa Agropecuária Tropical,** v.42, p.288-294, 2012. https://doi.org/10.1590/S1983-40632012000300013

SÃO PAULO. Decreto n°. 62.243, de 1 de novembro de 2016. Dispõe sobre as regras e procedimentos para o licenciamento ambiental da aquicultura, no Estado de São Paulo, e dá providências correlatas. **Diário Oficial do Estado de São Paulo** – Poder Executivo, Seção I, São Paulo, SP, 2 de novembro de 2016, v.126, n.206, p.1-4. Disponível em: <https://governo-sp.jusbrasil.com.br/legislacao/401315535/decreto-62243-16-sao-paulo-sp> Acesso em: 18 dez. 2021.

SÃO PAULO. Secretaria da Agricultura e Abastecimento – SAA. Resolução SAA - 73, de 24 de novembro de 2016. Dispõe sobre critérios e procedimentos a serem seguidos pelo Instituto de Pesca para a edição e revisão da lista de espécies alóctones, exóticas e híbridas, cujo cultivo está permitido, e os locais autorizados para o cultivo de cada espécie. **Diário Oficial do Estado de São Paulo** - Poder Executivo, Seção I, São Paulo, SP, 25 de nov. de 2016. Disponível em: <https://www.cdrs.sp.gov.br/portal/themes/unify/arquivos/produtos-e-servicos/legislacao-dcaa/RESOLU%C3%87%C3%83O%20SAA%2073%2024-11-2016%20editada%20do%20DOESP.doc>. Acesso em: 18 dez. 2021.

SERRA, M.; WOLKERS, C.P.; ZANUZZO, F.; GIMBO, R.; URBINATI, E. O estresse na criação de peixes. In: CAMARGO, A.C.S.; NOGUEIRA, W.C.L.; TORRES, A.F.B.; ALMEIDA, A.C.; STEFANELLO, C.M. (eds) **Piscicultura**: Aspectos Relevantes, 1ª ed., UNIPAMPA, 2016, 271-316.

SISVAR - **Sistema de análise de variância**. Versão 5.7. Lavras-MG: UFLA, 2010. https://des.ufla.br/~danielff/programas/sisvar.html

SOUZA, R.A.R.; CARVALHO, C.V.A.; NUNES, F.F.; SCOPEL, B.R.; GUARIZI, J.D.; TSUZUKI, M.Y. Comparative effect of benzocaine, menthol and eugenol as anesthetics for juvenile fat snook. **Boletim do Instituto de Pesca**, v.38, p.247-255, 2012.

ZAHL, I.H.; SAMUELSEN, O.; KIESSLING, A. Anesthesia of farmed fish: implications for welfare. Fish Physiology and Biochemistry, v.38, p.201-218, 2012. https://doi.org/10.1007/s10695-011-9565-1