# ESSENTIAL OILS OF *Ocimum basilicum* AND *Cymbopogon flexuosus* IN THE SEDATION, ANESTHESIA AND RECOVERY OF TAMBACU (*Piaractus mesopotamicus* MALE X *Colossoma macropomum* FEMALE)

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**ABSTRACT**

The objective of this study was to verify use of the essential oils of basil (*Ocimum basilicum*) (EOOB) and lemongrass (*Cymbopogon flexuosus*) (EOCF) for the sedation, anesthesia and recovery in tambacu juveniles. Eight fish were tested at concentrations of 0, 10, 25, 50, 100, 200 and 300 µL $L^{-1}$. The sedation time was less than 30 seconds at all concentrations, and 10 or 25 µL $L^{-1}$ are recommended for sedation for both essential oils to be the most economic doses. The fastest anesthesia and anesthetic recovery times of 113.90 and 152.12 seconds for EOOB and 194.88 and 116.25 seconds for EOCF, respectively, occurred at 300 µL $L^{-1}$. In conclusion, we recommend the use of EOOB and EOCF for sedation and anesthesia of tambacu at concentrations of 10-25 and 300 µL $L^{-1}$, respectively.

**Keyword**s: anesthetic, basil, lemongrass, anesthesia recovery.

# ÓLEOS ESSENCIAIS DE *Ocimum basilicum* E *Cymbopogon flexuosus* NA SEDAÇÃO, ANESTESIA E RECUPERAÇÃO DE TAMBACU (*Piaractus mesopotamicus* MACHO X *Colossoma macropomum* FÊMEA)

**RESUMO**

Objetivou-se verificar os óleos essenciais de manjericão (*Ocimum basilicum*) (OEOB) e capim limão (*Cymbopogon flexuosus*) (OECF) para a sedação, anestesia e recuperação de juvenis de tambacu. Foram utilizados oito exemplares para cada concentração: 0, 10, 25, 50, 100, 200 e 300 uL $L^{-1}$. Os tempos de sedação foram inferiores a 30 segundos em todas as concentrações, e 10 ou 25 uL $L^{-1}$ são recomendados para a sedação por ambos os óleos essenciais por serem as doses mais econômicas. Os melhores tempos de anestesia e recuperação anestésica ocorreram na concentração de 300 uL $L^{-1}$: 113,90 e 152,12 e 194,88 e 116,25 segundos para os OEOB e OECF, respectivamente. Dessa forma, é recomendado o uso de OEOB e OECF para sedação e anestesia de tambacu nas concentrações de 10-25 e 300 µL $L^{-1}$, respectivamente.

**Palavras-chave:** anestésico, manjericão, capim limão, recuperação anestésica.

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## INTRODUCTION

The tambacu is a hybrid resulting from reproduction between tambaqui (*Colossoma macropomum*) females and pacu (*Piaractus mesopotamicus*) males. The tambacu is omnivorous and integrates the hardiness and resistance to low temperatures of the pacu with the fecundity of the tambaqui (GONÇALVES *et al.*, 2010). The production of tambacu in Brazilian fish farming in 2010 was 21,621 tons, with a 85% increase in production compared to 2009. In 2011, production reached 49,818 tons, an increase of 125% compared to 2010, making the third species most cultivated in Brazil (BRASIL, 2011).

Activities in fish farming systems in general involve physical activity (i.e., capture, handling and transportation). According BARCELOS *et al.* (2000), response to stress that is excessive in either by intensity or duration can result in undesirable consequences, such as a growth rates reduction, weight loss, illness and even mortality. The use of anesthetic/sedative substances in of fish can prevent physical injury and reduce metabolism by reducing physical activity (COYLE *et al.*, 2004), reducing the effects of stress. The choice of appropriate anesthetic must account for its economic viability, practicality and effectiveness (CHO and HEATH, 2000).

The synthetic anesthetics (such as MS-222 and benzocaine hydrochloride) cause adverse effects such as irritation of the gills of fish and pollute the environment through their waste products (INOUE *et al.*, 2005). Additionally, they can contaminate the environment with toxic residues. Restrictions on the use of anesthetics in fishery operations relate not only to the ineffectiveness of the materials but also to the legality of their use on food fishes. And in the Brazil, there is no specific legislation for the use of anesthetics for fish farming. Other limiting factors were the high cost, safety, and slow recovery (MARKING and MEYER, 1985).

Recently, the use of non-toxic anesthetics such as clove oil and the essential oils of *Aloysia triphylla* and *Lippia alba* has shown satisfactory results (CUNHA *et al.*, 2010, 2011; BECKER *et al.*, 2012; ZEPPENFELD *et al.*, 2014). The essential oils of basil (*Ocimum basilicum)* (EOOB) and lemongrass (*Cymbopogon flexuosus)* (EOCF) are natural products that have not been studied with respect to their potential as sedatives and anesthetics in fish. Their chemical constituents have anesthetic properties; in EOOB, the effective molecule is linalool (HUSSAIN *et al.*, 2008), and in EOCF, the effective molecule is citral, which is a mixture of the geranial and neural isomers (PARIKH and DESAI, 2011).

Studies on tambaqui and pacu have shown molecules such as eugenol and menthol to be efficient anesthetics (FAÇANHA and GOMES, 2005; GONÇALVES *et al.*, 2008); however, there are currently no studies on anesthetics in tambacu. Therefore, this study aimed to verify the best concentration of EOOB and EOCF for the sedation, anesthesia and recovery in tambacu juveniles.

## MATERIALS AND METHODS

The essential oils were extracted from dried and fresh leaves by drag steam distillation followed by filtration for two hours using a Clevenger-type apparatus performed according to SILVA and FISCHER (2010). The essential oil was stored at -4 °C in amber glass bottles until chemical analysis by CG-MS. Essential oil was obtained from the Pólo de Tecnologia da Universidade do Noroeste do Rio Grande do Sul (Unijui), Três Passos-RS. The chemical composition was determined in the Laboratório de Extrativos Vegetais, Universidade Federal de Santa Maria, Brazil.

Tambacu juveniles were purchased from Bahia Pesca, Camaçari, BA, Brazil. Specimens were transported to the Laboratory of Aquatic Organisms Maintenance at the *Universidade Federal da Bahia* (UFBA). The fish were housed for 14 days in continuously aerated 250 L tanks and fed twice per day with commercial feed containing 30.00% crude protein and 3500 kcal $kg^{-1}$ digestible energy. Individuals were fasted for a period of 24 h prior to the experiments. Tanks were maintained under continuous aeration and subjected to partial water changes twice a week. During the experiments, water quality parameters were maintained at 25 $^{o}$C, pH 7.5, 0.03 mg $L^{-1}$ un-ionized ammonia and a dissolved oxygen concentration above 7.0 mg $L^{-1}$.

Anesthesia induction and recovery times were studied using 128 juveniles weighing 17.45 ±

0.66 g and measuring 10.32 ± 0.19 cm. The experiments were conducted in accordance with the Ethical Committee of the Biology Institute-UFBA under registration number 17/2014.

The testing procedure involved transferring juveniles with a net to aquaria containing 2 L of water and EOOB or EOCF at concentrations of 0, 10, 25, 50, 100, 200 or 300 μL $L^{-1}$ from stock solutions that had been diluted 1:10 with 99.6% ethanol. The control group was transferred to aquaria that contained 2700 μL $L^{-1}$ ethanol (at a concentration of equivalent to that used for 300 μL $L^{-1}$ for essential oil). Eight juveniles were tested at each concentration to evaluate the time required for sedation, anesthesia induction and recovery, where each juvenile was used only once. Two fish were placed in each aquarium at the same time, and the induction time at different stages of anesthesia was evaluated for up to 30 min. Then, the juveniles were transferred to a 20 L anesthetic-free aquarium to measure the recovery from anesthesia time. We considered for sedation the partial loss of equilibrium and erratic swimming, for anesthesia the total loss of equilibrium and cessation of locomotion and for recovery, the normal swimming and reaction to external stimuli (adapted from SMALL, 2003).

All data are expressed as the mean ± SEM and were subjected to the Levene test to verify homogeneity of the variances. The evaluation of anesthetic activity was performed by ANOVA and the time of sedation, anesthesia and anesthetic recovery data were analyzed using the tukey tests. A regression analysis of concentration versus time and significance was set at a critical level of 95% ($p < 0.05$), with a confidence interval of 95%.

**RESULTS**

Linalool (53.35%) was the major component of EOOB and Geranial (50.13%) and Neral (40.32%) were the major chemical constituents of the EOCF.

No mortality was observed during the experiments. The application of ethanol in the control group did not induce sedation or anesthesia. EOOB and EOCF were effective as sedatives in all tested concentrations, and fish exposed at 100, 200 and 300 μL $L^{-1}$ reached anesthesia. The sedation times were significantly higher in 10-50 μL $L^{-1}$ for EOOB and 10 μL $L^{-1}$ for EOCF, compared to the other treatments ($p < 0.05$) (Tables 1 and 2). Fish that only reached sedation recovered immediately after they were removed from the tanks.

Anesthesia occurred at concentrations above 100 μL $L^{-1}$ of EOOB and EOCF, and the time to anesthesia was reduced with increasing concentrations, where 300 μL $L^{-1}$ presented time to anesthesia significantly lower than 100 and 200 μL $L^{-1}$ for both essential oil ($p < 0.05$) (Tables 1 and 2). Fish exposed at a concentration of 300 μL $L^{-1}$ presented the fastest times to anesthesia: 113.875 and 152.12 seconds for EOOB and EOCF, respectively. The best times to recovery were found at the 100 (144.88 s) and 300 (116.25 s) μL $L^{-1}$ for EOOB and EOCF, respectively. The anesthetic recovery time increased for EOOB (Table 1) and decreased for EOCF (Table 2) with increasing concentrations according to the regression equation, although no significant differences existed between treatments ($p < 0.05$).

**Table 1**. Time (s) required for induction and recovery from anesthesia using the essential oil of *Ocimum basilicum* in tambacu juveniles. Stages are according to SMALL (2003). N=8 for each concentration tested.

| Concentration (µL L$^{-1}$) | induction (s) | | Recovery (s) |
|---|---|---|---|
| | Sedation | Anesthesia | |
| 10 | 17.75 ± 1.31$^{a}$ | - | - |
| 25 | 17.75 ± 1.63$^{a}$ | - | - |
| 50 | 14.12 ± 1.32$^{a}$ | - | - |
| 100 | 10.72 ± 1.42$^{b}$ | 744.50 ± 48.96$^{a}$ | 144.88 ± 9.35$^{a}$ |
| 200 | 11.00 ± 0.73$^{b}$ | 161.00 ± 29.26$^{b}$ | 161.25 ± 22.22$^{a}$ |
| 300 | 10.25 ± 1.43$^{b}$ | 113.88 ± 14.29$^{c}$ | 194.88 ± 12.19$^{a}$ |
| Equations | y = 16.425 – 0.025x<br>$R^2$=0.66 | y = 969.75 – 3.151x<br>$R^2$=0.81 | y = 117.00 – 0.250x<br>$R^2$= 0.96 |

Different letters in the column indicate significant difference between concentrations by Tukey. Where y=time to reach the stage and x=concentration of the essential oil of *Ocimum basilicum* (µL L$^{-1}$).

**Table 2**. Time (s) required for induction and recovery from anesthesia using the essential oil of *Cymbopogon flexuosus* in tambacu juveniles. Stages are according to SMALL (2003). N=8 for each concentration tested.

| Concentration (µL L$^{-1}$) | induction (s) | | Recovery (s) |
|---|---|---|---|
| | Sedation | Anesthesia | |
| 10 | 29.14 ± 3.84$^{a}$ | - | - |
| 25 | 15.75 ± 3.20$^{b}$ | - | - |
| 50 | 16.00 ± 2.69$^{b}$ | - | - |
| 100 | 12.86 ± 2.60$^{b}$ | 740.20 ± 62.30$^{a}$ | 189.83 ± 66.95$^{a}$ |
| 200 | 13.57 ± 2.54$^{b}$ | 216.75 ± 18.90$^{b}$ | 170.17 ± 45.34$^{a}$ |
| 300 | 13.50 ± 2.38$^{b}$ | 152.12 ± 16.39$^{c}$ | 116.25 ± 22.98$^{a}$ |
| Equations | * | y = 957.767 – 2.940x<br>$R^2$=0.83 | y = 232.333 – 0.368x<br>$R^2$=0.93 |

Different letters in the column indicate significant difference between concentrations by Tukey. Where y=time to reach the stage and x=concentration of the essential oil of *Cymbopogon flexuosus* ((µL L$^{-1}$). * No regression.

**DISCUSSION**

Linalool has been reported as the most abundant component in the OEOB by BLANK et al. (2003) (78.1%) and OLIVEIRA et al. (2013) (29.50-32.26%). Geranial and neural have been reported as the most abundant components in the OECF for by BRUNETON (1991), PINO and ROSADO (2000) and KASALI *et al.* (2001) in proportions between 47 to 85%. Although the proportion found in this study was different from the previous studies, EOOB and EOCF were demonstrated effective.

Sedation is an early state of anesthesia in which sensory perception is reduced, but there is no loss of equilibrium. In contrast, there is a widespread loss of sensory perception and loss of equilibrium in anesthetized animals (ROSS and ROSS 2008). Sedation is the recommended condition for transport because reducing the fish's metabolism and rate of swimming (CUNHA *et al.*, 2010; 2011) can decrease damage caused by the abrasion between the fish and the transport plastic packaging. Although all concentrations between 10 and 300 μL $L^{-1}$ of EOOB and EOCF are effective as sedatives, the use of higher doses may be uneconomic (ROUBACH *et al.*, 2005); therefore, we recommend 10 or 25 μL $L^{-1}$ for tambacu sedation.

Although it is clear for transport is required sedation stage, on the other hand, for handling procedures, the fish must be anesthetized (MARKING and MEYER, 1985). In our study, anesthesia occurred at concentrations of 100 μL $L^{-1}$ or higher for both anesthetics. The results of this study are consistent with those of MARKING and MEYER (1985); for concentrations of 200 and 300 μL $L^{-1}$, anesthesia induction and recovery times of less than 180 and 300 seconds were observed for both essential oils, but the fastest times for anesthesia occurred in 300 μL $L^{-1}$.

Previous studies have reported the times to anesthesia and recovery for pacu and tambaqui with other anesthetics. The recommended concentrations of menthol and eugenol for tambaqui are 100 μL $L^{-1}$ and 65 μL $L^{-1}$, with anesthesia and recovery times of 152 and 105 seconds and 305 and 442 seconds, respectively (FAÇANHA and GOMES, 2005; ROUBACH *et al.*, 2005). For pacu, the recommended concentrations are 100 μL $L^{-1}$ of menthol and 50 μL $L^{-1}$ of eugenol, with anesthesia and recovery times of 102 and 92 seconds and 113 and 145 seconds, respectively (GONÇALVES, 2008). In addition, exposing tambaqui to concentrations of 50 and 100 μL $L^{-1}$ of benzocaine induced a total loss of equilibrium (representing anesthesia) in less than 120 seconds, with recovery times between 181 and 299 seconds (GOMES *et al.*, 2001). It is possible that tambacu be more resistant than the parental species because it is more hardiness, but this response will only be fully understood the extent that further studies with anesthetic in tambacu be realized.

Similarly to this study, studies of the essential oil of *Lippia alba* in silver catfish (*Rhamdia quelen*) and seahorse (*Hippocampus reidi*) (CUNHA *et al.*, 2010; 2011) and eugenol in Nile tilapia (*Oreochromis niloticus*) (VIDAL *et al.*, 2008) and pacamã (*Lophiosilurus alexandri*) (RIBEIRO *et al.*, 2013) and benzocaine, menthol and eugenol in fat snook (*Centropomus parallelus*) (SOUZA *et al.*, 2012) have shown that increased concentrations of anesthetic reduce the time needed to reach anesthesia.

Similar to the observations for tambacu juveniles exposed with EOOB in this study, other researchers found that the highest concentration of anesthetic required a greater recovery time, as verified for eugenol and clove oil (*Syzygium aromaticum*) in the Nile tilapia (VIDAL *et al.*, 2008; SIMÕES *et al.*, 2010) and essential oil of spearmint (*Mentha spicata*) and methyl salicylate in common carp (*Cyprinus carpio*) (ROOHI and IMAMPOOR, 2015). In general, the recovery time is usually faster at lower concentrations of anesthesia, and becomes more prolonged as the concentration increases ROSS and ROSS (2008); this is in agreement with our observations for EOOB in tambacu.

However, similar to the tambacu exposed with EOCF in this study, the anesthetic recovery time in pacu juveniles decreased with increasing concentrations of menthol (GONÇALVES *et al.*, 2008). It is possible that this decreased recovery time occurs due to the shorter exposure at the highest anesthetic concentrations. The results of this study, as well as those of previous studies, show that the anesthetic recovery time varies according to the anesthetic and species of fish used.

While previous work addresses the use of anesthetics in the paternal species pacu and tam-

baqui, appropriate dosages for tambacu cannot be based upon these studies. In addition, the suggested dosages for anesthesia in the parental species may not have the desired effect on tambacu.

## CONCLUSION

Based on the induction and recovery times we recommend the use of EOOB and EOCF for sedation and anesthesia of tambacu at concentrations of 10-25 and 300 μL $L^{-1}$, respectively.

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## REFERENCES

BARCELLOS, L.J.G.; SOUZA, S.M.G.; WOEHL, V.M. 2000 Estresse em peixes: fisiologia da resposta ao estresse, causas e conseqüências (revisão). *Boletim do Instituto de Pesca, 26*: 99-111.

BECKER, A.G.; PARODI, T.V.; HELDWEIN, C.G.; ZEPPENFELD, C.; HEINZMANN, B.M.; BALDISSEROTTO, B. 2012 Transportation of silver catfish, *Rhamdia quelen*, in water with eugenol and the essential oil of *Lippia alba*. *Fish Physiology and Biochemistry, 38*: 789-796.

BLANK, A.F.; SOUZA, E.M.S.; ARRIGONI-BLANK, M.F.; PAULA, J.W.A.; ALVES, P.B. 2007. Maria Bonita: cultivar de manjericão tipo linalol. *Pesquisa Agropecuária Brasileira, 42*: 1811-1813.

BRASIL, 2011 BOLETIM ESTATÍSTICO DA PESCA E AQUICULTURA. Ministério de Pesca e Aquicultura. Capítulo 8, Aquicultura. *Ministério da Pesca e Aquicultura*, Brasília. [Online] URL: <www.mpa.gov.br/files/docs/Boletim_MPA_2011_pub.pdf>. Acessado em: 01 set. 2015.

BRUNETON, J. 1991 Elementos de fitoquímica y de farmacognosia. Zaragoza: Acribia.

CHO, G.K. and HEATH, D.D. 2000 Comparison of tricaine methanesulphonate (MS 222) and oil clove anaesthesia effects on the physiology of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) (Walbaum). *Aquaculture Research, 31*: 537-546.

COYLE, S.D., DURBOROW, R.M., TIDWELL, J.H. 2004 Anesthetics in aquaculture. Texas: SRAC (SRAC Publications, 3900).

CUNHA, M.A.; de BARROS, F.M.C.; GARCIA, L.O.; VEECK, A.P.L.; HEINZMANN, B.M.; LORO, V.L.; EMANUELLI, T.; BALDISSEROTTO, B. 2010 Essential oil of *Lippia alba*: A new anesthetic for silver catfish, *Rhamdia quelen*. *Aquaculture, 306*: 403-406.

CUNHA, M.A.; DA-SILVA, B.F.; DELUNARDO, F.A.C.; BENOVIT, S.C.; GOMES, L.C.; HEINZMANN, B.M.; BALDISSEROTTO, B. 2011 Anesthetic induction and recovery of *Hippocampus reidi* exposed to the essential oil of *Lippia alba*. *Neotropical Ichthyology, 9*: 683-688.

FAÇANHA, M.F. and GOMES, L.C. 2005 A eficácia do mentol como anestésico para o tambaqui (*Colossoma macropomum*). *Acta Amazônica, 35*: 71-75.

GOMES, L.C.; CHIPPARI-GOMES, A.C.; LOPES, N.P.; ROUBACH, R.; ARAUJO-LIMA, C.A.R.M. 2001 Efficacy of benzocaine as an anesthetic in juvenile tambaqui *Colossoma macropomum*. *Journal of the World Aquaculture Society*, *32*: 426-431.

GONÇALVES, A.C.S.; MURGAS, L.D.S.; ROSA, P.V.; NAVARRO, R.D.; COSTA, D.V.; TEIXEIRA, E.A. 2010 Desempenho produtivo de tambacus alimentados com dietas suplementadas com vitamina E. *Pesquisa Agropecuária Brasileira, 45*: 1001-1011.

GONÇALVES, A.F.N.; SANTOS, E.C.C.; FERNANDES, J.B.K.; TAKAHASHI, L.S. 2008 Mentol e eugenol como substitutos da benzocaína na indução anestésica de juvenis de pacu anestésica de juvenis de pacu. *Acta Scientiarum, Animal Science, 30*: 339-344.

HUSSAIN, A.J.; ANWAR, F.; SHERAZI, S.T.H.; PRZYBYLSKI, R. 2008 Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry, 108*: 986-995.

INOUE, L.A.K.A.; AFONSO, L.O.B.; IWAMA, G.K.; MORAES, G. 2005 Efeito do óleo de cravo na resposta de estresse do matrinxã (*Brycon cephalus*) submetido ao transporte. *Acta Amazonica 35*: 289-295.

KASALI, A. A.; OYEDEJI, A. O.; ASHILOKUN, A. O. 2001 Volatile leaf oil constituents of *Cymbopogon*

*citratus* (DC.) Stapf. *Flavour and Fragrance Journal, 16*: 377-8.

MARKING, L.L. and MEYER, F.P. 1985 Are better anesthetics needed in fisheries. *Fisheries 10*: 2-5.

OLIVEIRA, R.A.; MOREIRA, I.S.; OLIVEIRA, F.F. 2013. Linalool and Methyl Chavicol present basil (*Ocimum* sp) cultivated in Brazil. *Revista Brasileira de Plantas Medicinais, 15*: 309-311.

PINO, J.A.; ROSADO, A. 2000 Chemical composition of the essential oil of *Cymbopogon citratus* (DC.) Stapf, from Cuba. *Journal of Essential Oil Research, 12*: 301-2.

PARIKH, J.K. and DEAI, M.A. 2011 Hydrodistillation of Essential Oil from *Cymbopogon flexuosus*. *International Journal of Food Engineering, 7*: 1-9.

RIBEIRO, P.A.P.; MIRANDA-FILHO, K.C.; MELILLO-FILHO, R.; SANTOS, A.E.H.; SILVA, W.S.; RODRIGUES, L.A.; LUZ, R.K. 2013 Efeito anestésico do eugenol em juvenis de pacamã. *Pesquisa Agropecuária Brasileira, 48*: 1136-1139.

ROOHI, Z.; IMANPOOR, M.R. 2015 The efficacy of the oils of spearmint and methyl salicylate as new anesthetics and their effect on glucose levels in common carp (*Cyprinus carpio* L., 1758) juveniles. *Aquaculture, 437*: 327-332.

ROSS, L.G. and ROSS, B. 2008 Anaesthetic and sedative techniques for aquatic animals. 3nd ed. London: Blackwell.

ROUBACH, R.; GOMES, L.C.; FONSECA, F.A.L.; VAL, A.L. 2005 Eugenol as an efficacious anaesthetic for tambaqui, *Colossoma macropomum* (Cuvier). *Aquaculture Research, 36*: 1056-1061.

SILVA, P.S.C.; FISCHER, G.N. 2010 Plantas aromáticas como alternativa para a agricultura familiar de Bom Progresso: Estudo de caso. *Revista Varia Scientia Agrárias, 1*: 55-66.

SIMÕES, L.N.; PAIVA, G.; GOMES, L.C. 2010 Óleo de cravo como anestésico em adultos de tilapia-donilo. *Pesquisa Agropecuária Brasileira, 45*: 1472-1477.

SMALL, B.C. 2003 Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish *Ictalurus punctatus*. *Aquaculture, 218*: 177-185.

SOUZA, R.A.R.; CARVALHO, C.V.A.; NUNES, F.F.; SCOPEL, B.R.; GUARIZI, J.D.; TSUZUKI, M.Y. 2012 Eefeito comparativo da benzocaína, mentol e eugenol como anestésicos para juvenis de robalo peva. *Boletim do Instituto de Pesca, 38*: 247-255.

VIDAL, L.V.O.; ALBINATI, R.C.B.; ALBINATI, A.C.L.; LIRA, A.D.; ALMEIDA, T.R.; SANTOS, G.B. 2008 Eugenol como anestésico para a tilápia-do-nilo. *Pesquisa Agropecuária Brasileira, 43*: 1069-1074.

ZEPPENFELD, C.C.; TONI, C.; BECKER, A.G.; MIRON, D.S.; PARODI, T.V.; HEINZMANN, B.M.; BARCELLOS, L.J.G.; KOAKOSKI, G.; DA- ROSA, J.G.S.; LORO, V.L.; DA-CUNHA, M.A.; BALDISSEROTTO B. 2014 Physiological and biochemical responses of silver catfish, *Rhamdia quelen*, after transport in water with essential oil of *Aloysia triphylla* (L'Herit) Britton. *Aquaculture, 418-419*: 101-107.