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Anesthetic induction and recovery of *Hippocampus reidi* exposed to the essential oil of *Lippia alba*

Mauro Alves da Cunha[1], Bruno Ferreira da Silva[2], Frederico Augusto Cariello Delunardo[2], Simone Cristina Benovit[3], Levy de Carvalho Gomes[2], Berta Maria Heinzmann[3] and Bernardo Baldisserotto[1]

The aim of this study was to identify the times of anesthetic induction and recovery in slender seahorses (*Hippocampus reidi*) that were exposed to the essential oil of *Lippia alba* (EO), as well as the efficacy of EO as a stress-reducing agent in the transport of this species. Slender seahorses were placed in 1-L aquaria containing different concentrations of EO (0, 10, 20, 50, 150, 300 and 450 μL L^{-1}), and after induction, fish were transferred to aquaria that were free of anesthetic to evaluate their recovery time. In an additional experiment, slender seahorses were transported in plastic bags with 15 μL L^{-1} of EO for 4 or 24 h. The increased concentration of EO proportionally decreased the time required for the induction of anesthesia. EO treatment (15 μL L^{-1}) inhibited the increase in blood glucose levels that was provoked by transportation for 4 or 24 h. Transportation for 24 h also decreased the number of lymphocytes and increased the neutrophil count, and these effects were avoided with the addition of EO to the water. These results demonstrate that EO was effective as an anesthetic at concentrations of 10-20 μL L^{-1} for slight sedation and transport and at 150 μL L^{-1} for deep anesthesia in the slender seahorse.

O objetivo deste estudo foi identificar os tempos da indução e recuperação anestésica em cavalos marinhos (*Hippocampus reidi*) expostos ao óleo essencial de *Lippia alba* (OE), assim como a eficácia do OE como um agente redutor de estresse no transporte desta espécie. Os cavalos marinhos foram colocados em aquários contendo 1 litro de água e diferentes concentrações de OE (0, 10, 20, 50, 150, 300 e 450 μL L^{-1}), após a indução, os peixes foram transferidos à aquários livre de anestésico para avaliar o tempo de recuperação. Em um outro experimento os cavalos marinhos foram transportados em sacos plásticos contendo 15 μL L^{-1} do OE por 4 ou 24h. A concentração crescente do OE diminuiu proporcionalmente o tempo exigido para a indução da anestesia. O óleo essencial (15 μL L^{-1}) inibiu o aumento nos níveis de glicose sanguínea provocada pelo transporte por 4 ou 24 h. O transporte por 24 h igualmente diminuiu o número de linfócitos e aumentou o número de neutrófilos, estas alterações foram evitadas com o uso do OE na água. Estes resultados demonstram que no cavalo marinho o OE é eficaz para a sedação e transporte na faixa de 10-20 μL L^{-1} e para a anestesia profunda recomenda-se 150 μL L^{-1}.

Key words: Blood glucose, Leukocyte count, Seahorse, Stress.

Introduction

The slender seahorse, *Hippocampus reidi*, is one of the most exported Brazilian marine ornamental fish species (Monteiro-Neto *et al*., 2003). This species is also collected for folk medicine, souvenirs and religious purposes (Rosa *et al*., 2002, 2005). The seahorses produced in Brazil are usually exported to the United States. Mortality generally occurs either during transport or within weeks of arrival at the destination as a delayed physiological stress response to collection, handling and transport. Stress-related damage is most likely to be highest between collection and export, as the conditions for holding and transport are poorest at this level (Koldewey *et al.,* 2005). Severe or chronic stress is often associated with poor performance and has long been suspected to cause immunosuppression in cultured fish (Pickering, 1998). Stress may induce the release of epinephrine and norepinephrine by chromaffin tissue in response to stimulation of the sympathetic nervous system, which might increase plasma glucose levels (Gomes *et al.*, 2006), neoglucogenesis, the deposition of

[1]Universidade Federal de Santa Maria, Departamento de Fisiologia e Farmacologia, 97105-900 Santa Maria, RS, Brazil. bbaldisserotto@hotmail.com (BB)
[2]Universidade de Vila Velha, Laboratório de Ecotoxicologia Aquática, Vila Velha, ES, Brazil.
[3]Universidade Federal de Santa Maria, Departamento de Farmácia Industrial, 97105-900 Santa Maria, RS, Brazil.

glycogen in the liver, immunosuppression and leukocyte counts (Ross & Ross, 2008). Similarly, Ellsaesser & Clem (1987) showed that both the number and immunological competence of circulating lymphocytes in the blood of channel catfish (*Ictalurus punctatus*) were reduced following chronic stress.

Anesthetics are known to be effective at reducing or minimizing stress in fish. Certain anesthetics have been used in the transport of fish, such as benzocaine in *Menidia estor* (Ross *et al*., 2007) and amylobarbitone, barbital sodium, chloral hydrate, MS222, quinaldine, tertiary amyl alcohol and urethane in Indian carp (Das & Goswami, 2003). The essential oil of *Lippia alba* (Mill.) N. E. Brown (EO) is effective as an anesthetic for silver catfish (*Rhamdia quelen*) (Cunha *et al*., 2010a), but no studies on EO have been performed in other species or on its effects on fish during transport. Therefore, the aim of this study was to identify the anesthetic induction and recovery times in slender seahorses that were exposed to EO, as well as the efficacy of EO as a stress-reducing agent in the transport of this species. This is also the first study dealing with seahorse transport.

Materials and methods

Animals

Juvenile slender seahorses were acquired from a local producer in the city of Serra - ES, Brazil. They were transported to the laboratory and kept in continuously aerated 100-L aquaria with controlled temperature (22.3ºC), salinity 27.5 ± 0.2 and dissolved oxygen levels 5.5 ± 0.5 mg L^{-1}. A voucher specimen was registered in the ichthyologic collection at the Universidade Federal do Espírito Santo (CI-UFES 1027).

Plant material

Lippia alba (Mill.) N.E. Brown was cultivated at the Universidade Federal de Santa Maria (UFSM) campus, Santa Maria, RS, Brazil. Samples of the aerial portions of the plant were collected in October, 2008. The plant material was identified by Dr. Gilberto Dolejal Zanetti from the Departamento de Farmácia Industrial, UFSM. A voucher specimen (SMDB No. 10050) was deposited in the herbarium of the Departamento de Biologia, UFSM.

Essential oil extraction

The essential oil used was obtained from fresh leaves of the plant by hydro-distillation using a Clevenger type apparatus for 2 h (European Pharmacopoeia, 2007). Essential oil samples were stored at -20 ºC in amber glass bottles.

Anesthesia induction and recovery

Slender seahorse juveniles (2.5 ± 0.5 g and 10.0 ± 1.0 cm) that had been fasted for 24 hours were transferred to aquaria containing 1 L of water and EO at concentrations of 10, 20, 50, 150, 300 and 450 μL L^{-1} (equivalent to 8, 16, 40, 120, 240 and 360 mg L^{-1}, respectively, because the density of this EO is approximately 0.80), with the EO first being diluted in ethanol (1:10). Control experiments were performed using aquaria that contained only ethanol at a concentration equivalent to the dilution used for the 450 μL L^{-1} EO treatment. To evaluate the time required for anesthesia induction, 10 seahorses, each of which were placed in individual aquaria, were used for each concentration tested, and each juvenile was used only once, according to the method of Schoettger & Julin (1967). The maximum observation time was 45 min, except in the seahorses exposed to the lower concentrations of EO (10 and 20 μL L^{-1}), which were observed for a period of 6 h to determine the concentration to be used for transport. After induction, the juveniles were transferred to anesthetic-free aquaria to measure the anesthesia recovery time.

Transport

Slender seahorses (2.3 ± 0.8 g and 9.8 ± 1.1 cm) were placed in plastic bags (one seahorse per bag) containing 0.5 L of water with 15 μL L^{-1} of EO that had previously been diluted in ethanol (1:10) (n= 10 for treatment) and transported for 4 or 24h. This concentration was used because there was no significant difference observed between the 10 and 20 μL L^{-1} treatments with respect to the anesthetic stage reached. The control group was subjected to the same procedures but no anesthetic was added to the water. Bags were then inflated with oxygen, tied with rubber strings and packed in plastic boxes, as described by Gomes *et al*. (2006). Water samples were collected before the plastic bags were closed and after transport for determination of dissolved oxygen, temperature and salinity with an oxygen meter YSI (model Y5512 Yellow Springs, USA). Blood samples (n= 10 for each treatment) were collected at the end of the transport period and from a group that was not subjected to transportation. An aliquot of the blood was used for glucose determination with a digital Accu-Check™ apparatus, and another aliquot was smeared on clean slides (two per fish), which were dried at room temperature for 24 h, and then fixed in 100% methanol for 10 min. Subsequently, they were stained with 4% Giemsa solution for 10 min, air-dried, and then prepared for counting lymphocytes, eosinophils, neutrophils, thrombocytes, monocytes, and basophiles; one hundred random leukocytes cells had been counted for each individual with the aid of a Leica Galen III optical microscope, as described by Tavares-Dias *et al*. (2000).

Statistical analyses

To verify the homogeneity of variances, all data were submitted to Levene's test. As the data were homoscedastic, they were analyzed using two-way ANOVA and Tukey tests. STATISTICA (version 5.1) was used for these analyses, and significance was set at a level of 95% ($P < 0.05$).

Results

An increasing concentration of EO proportionally decreased the time required for anesthesia induction. Slender seahorses that were exposed to low concentrations (10 and 20 μL L^{-1}) of the EO for 6 h maintained a uniform state of

sedation, *i.e.*, they remained at stage 2 (Table 1). No mortality resulted from anesthesia induction within the range tested. The application of 4500 µL L^{-1} ethanol alone (concentration equivalent to the dilution used for the 450 µL L^{-1} EO treatment) did not produce an anesthetic effect. No significant difference was found in the recovery time at the different concentrations of the EO tested.

There was no significant difference in the dissolved oxygen levels (16.60±4.20 mg L^{-1}), salinity (27.6±0.2) or temperature (22.8±0.6 °C) after transportation, and no mortality was observed in any of the treatments. The lymphocyte counts of the control slender seahorses transported for 24 h presented significantly lower values than before transport. However, the neutrophil counts increased significantly after the transport compared to before transport. Slender seahorses transported in water with EO did not shown any significant change in these parameters at the end of transport. No significant change was observed in eosinophil, thrombocyte, monocyte and basophil counts (Table 2).

The blood glucose levels of control slender seahorses increased significantly after 4 and 24 h of transportation compared to before transport. Fish transported under conditions of 15 µL L^{-1} EO did not exhibit any significant change in their blood glucose levels compared to before transport (Fig. 1).

Discussion

Anesthesia induction and recovery

The maximum allowable time for the induction of deep anesthesia (stage 4) in fish is 10 min (Roubach *et al*., 2005; Ross & Ross, 2008). The lowest concentration of the EO used here that was capable of inducing deep anesthesia in the slender seahorse was 50 µL L^{-1}, but 150 - 300 µL L^{-1} was required to obtain rapid (approximately 3 - 4 min) deep anesthesia (Table 1). As slender seahorses anesthetized with these two concentrations did not show any significant difference in the recovery times, the lower concentration (150 µL L^{-1}) is suggested as the optimal concentration for deep anesthesia induction. In the silver catfish, the lowest concentration of EO of *L. alba* that is able to induce deep anesthesia is 100 µL L^{-1}, while rapid anesthesia is reached with concentrations of 300-500 µL L^{-1}, and recovery time is 350-450 s (Cunha *et al*., 2010a). Apparently, the slender seahorse is more easily anesthetized by EO of *L. alba* than the silver catfish, but the time of recovery was similar in both species. Recovery time is usually faster at lower concentrations of anesthetic, and it becomes more prolonged as the concentration increases (Ross & Ross, 2008). However, the different concentrations of EO tested in the slender seahorse did not affect recovery time.

No other studies besides Cunha *et al.* (2010a) have been reported on anaesthetizing fish using EO of *L. alba*, but menthol at concentrations of 100- 200 mg L^{-1} can provoke deep anesthesia in the tambaqui, *Colossoma macropomum*, after 1-2 min, and this results in a recovery time of 5-12 min (Façanha & Gomes, 2005), similar to what was found in the present study for slender seahorses. Deep anesthesia can be achieved in the tropical reef fishes Sergeant Major *Abudefduf saxatilis,* Cocoa damselfish *Stegastes variabilis,* Maria-nagô *Pareques acuminatus,* Doctorfish *Acanthurus chirurgus,* Budião-batata *Sparisoma axillare,* Schoolmaster snapper *Lutjanus apodus*, and Frillfin goby *Bathygobius soporator* with 20 mg L^{-1} clove oil, with associated induction and recovery times of less than 3 and 5 min, respectively (Cunha & Rosa, 2006). Silver catfish can reach deep anesthesia with 20 mg L^{-1} eugenol (the main compound of clove oil), but for rapid anesthesia is necessary to use 50 mg L^{-1} (Cunha *et al*., 2010b). The effect of clove oil varies according to the species, but usually 20-50 mg L^{-1} induced stage 4 of anesthesia within 120-360s (Cho & Heath 2000; Keene *et al*., 1998; Sladky *et al*., 2001; Iversen *et al*., 2003). Therefore a lower concentration of clove oil or eugenol than EO of *L. alba* is necessary to induce deep anesthesia in the teleosts species studied so far.

Transport

Sedation can be beneficial in fish transportation, especially in cases where long distances are to be covered, but there may also be advantages of sedating animals for short journeys. Stage two of anesthesia, or deep sedation, which was characterized by Schoettger and Julin (1967) as "partial loss of equilibrium, no reaction to external stimuli", is

Table 1. Time required for induction and recovery from anesthesia using the essential oil of *Lippia alba* in slender seahorse juveniles. Stages according to Schoettger & Julin (1967). Maximum observation time was 45 min. The time to reach each stage in seconds (s) is shown. N = 10 for each concentration tested. No relationship between concentrations of the essential oil of *L. alba* and time of recovery was found. y = time to reach the stage, and x = concentration of the essential oil of *L. alba* (µL L^{-1}).

Concentration (µL L^{-1})	Induction				Time of recovery
	Stage 2	Stage 3a	Stage 3b	Stage 4	
10	2280±290.9	-	-	-	-
20	1868±300.2	-	-	-	-
50	1011.4±250.3	1433.5±98.2	1564.4±305.3	1771.4±256.3	273.5±43.5
150	76.1±81.6	221.2±78.4	228.1±54.2	241.1±58.7	337.5±63.2
300	40.9±33.2	135.9±23.2	185.6±60.5	190.3±41.2	319.4±70.5
450	10.9±35.5	59.4±25.4	63.5±34.7	75.9±14.2	352.8±123.8
Equations	$Lny= 8.76- 0.30x^{0.5}$ $r^2= 0.974$	$Lny= -1.07+ 32.67/Lnx$ $r^2= 0.981$	$Lny= -0.77+ 31.77/Lnx$ $r^2= 0.948$	$Lny= -0.69+ 31.84/Lnx$ $r^2= 0.963$	

Table 2. Hematological parameters (%) of slender seahorses transported in plastic bags with the essential oil of *L. alba* (15 µL L^{-1}). N= 10 for each treatment tested. Different letters in the columns indicate a significant difference between treatments based on two-way ANOVA and Tukey's test ($P < 0.05$).

Treatment	Lymphocytes	Neutrophils	Monocytes	Thrombocytes	Eosinophils	Basophiles
Before transport	63.4±5.6[a]	3.33±1.4[a]	9.5±2.2[a]	4.08±1.3[a]	7.50±4.8[a]	12.17±4.3[a]
OE 4h	56.2±6.5[a]	4.33±2.4[a]	7.67±4.1[a]	6.17±5.8[a]	4.75±2.1[a]	20.83±11.9[a]
Control 4h	55.9±5.8[a]	6.87±3.8[a]	16.91±5.0[a]	4.33±3.2[a]	6.33±5.6[a]	11.58±5.6[a]
OE 24h	54.00±7.2[a]	4.75±1.9[a]	8.10±2.46[a]	4.50±3.3[a]	11.08±8.3[a]	15.08±7.4[a]
Control 24h	36.62±5.2[b]	11.70±5.2[b]	12.8±13.4[a]	4.08±1.9[a]	19.3±17.3[a]	15.42±14.9[a]

considered an ideal condition for transporting fish because fish sedated at this level exhibit reduced activity but are able to maintain partial equilibrium, swimming capacity, and avoid physical damage resulting from collision with plastic bags (Cooke *et al*., 2004).

Stress can increase blood glucose levels (Biron & Benfey, 1994; Wendelaar-Bonga, 1997; Barcellos *et al.,* 2003) and affect leukocyte levels (Sopinska, 1984; Dick & Dixon, 1985, Ellsaesser & Clem, 1986; Pickering & Pottinger, 1987). The blood glucose level of the control slender seahorses increased after transport, which is in agreement with previous findings in Chinook salmon, *Oncorhynchus tshawytscha* (Barton *et al*., 1986), coho salmon, *Oncorhynchus kisutch* (Vijayan & Leatherland, 1989), brook trout, *Salvelinus fontinalis* (Biron & Benfey, 1994) and pirarucu, *Arapaima gigas* (Brandão *et al*., 2006), following transportation. Slender seahorses transported with EO did not exhibit this increase in blood glucose levels, indicating a lower stress level throughout transportation. Similarly, clove oil (5 mg L^{-1}) can mitigate the stress response in the matrinxã, *Brycon amazonicus*, subjected to transport, as it

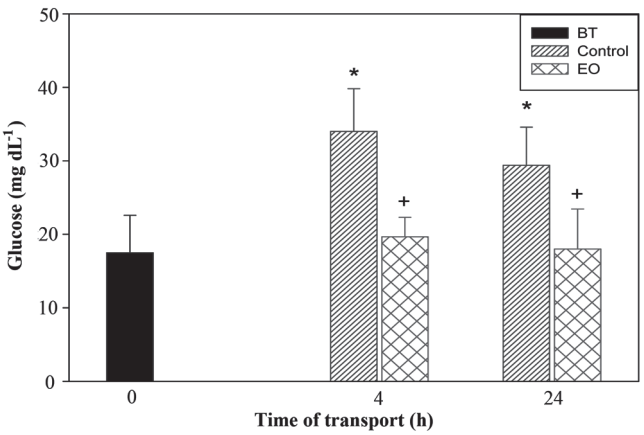

Fig. 1. Blood glucose levels of seahorses transported in plastic bags (one seahorse per bag) for 4 or 24 h. N= 10. BT= Before transport; Control= only water; EO = essential oil of *L. alba* previously diluted in ethanol (1:10) 15 µL L^{-1}. * significantly different from before transport using two-way ANOVA and Tukey's test ($P < 0.05$). + significantly different from control group at the same time of transport using two-way ANOVA and Tukey's test ($P < 0.05$).

prevents increases of plasma glucose, cortisol, lactate, ammonia, Cl^- and K^+ (Inoue *et al.,* 2005). Furthermore, benzocaine (0 or 20 mg L^{-1}) and clove oil (0, 2, or 5 mg L^{-1}) can be used as anesthetics for transport based on the observation that the survival of the Nile tilapia, *Oreochromis niloticus*, was satisfactory (general average of 97.26%) after 5 h of transport (Oliveira, 2009). Largemouth bass, *Micropterus salmoides*, transported using 5 -9 mg L^{-1} of clove oil presented a loss of reactivity and reduced cardiac output while maintaining equilibrium and recovering more rapidly than non-anesthetized controls (Cooke *et al*., 2004).

The number of leukocytes present in an organism changes in situations of stress, depending on the studied species. Transportation of slender seahorses for 24 h decreased their lymphocyte and increased their neutrophil counts. No significant change occurred in slender seahorses transported with EO of *L. alba*, indicating that this EO is beneficial to reduce stress. A reduction in the concentration of lymphocytes and an increase in the number of neutrophils was also observed in the common carp, *Cyprinus carpio*, and the channel catfish, *Ictalurus punctatus*, after being subjected to the stress of capture or transport (Sopinska, 1984; Ellsaesser & Clem, 1986), as well as in Nile tilapia following the stress of capture (Martins *et al*., 2004), the European eel, *Anguilla anguilla*, under the stress of handling (Johansson-Sjöbeck *et al.,* 1978) and the common dab, *Limanda limanda*, after being subjected to acute stress (Pulsford *et al*., 1994). An increase in the number of leukocytes was found in tambaqui (Tavares-Dias *et al*., 2001) and in the hybrid tambacu *C. macropomum* x *Piaractus mesopotamicus* (Martins *et al*., 2002) after being subjected to handling. The number of thrombocytes decreased in pacu, *P. mesopotamicus* (Martins *et al*., 2000), and did not change in tambacu (Martins *et al*., 2002) subjected to handling.

Stress is associated with cortisol release in the blood following the activation of the hypothalamic–pituitary–inter-renal (HPI) axis. This hormone binds to receptors in leukocytes, leading to immunosuppression in most situations. One of the well-known effects of cortisol is the regulation of leukocyte migration in tissues. Stress increases the number of neutrophils (leukocytes involved in the inflammatory response) and reduces the counts of lymphocytes (leukocytes involved in the immune response) (Bauer *et al*., 2001). It is noteworthy that these changes are due to cortisol and norepinephrine, which induce leukocyte migration from blood to tissues and vice-versa (Pulsford *et al.,* 1994).

In conclusion, EO is effective in inducing slight sedation in the slender seahorse at concentrations of 10-20 μL L^{-1} and deep anesthesia at concentrations of 50–450 μL L^{-1}, and for rapid deep anesthesia, a concentration of 150 μL L^{-1} is recommended. Furthermore, adding 15 μL L^{-1} of EO to the water in which seahorses are transported inhibits the elevation of blood glucose and neutrophils and decrease in lymphocytes that occur without this anesthetic in the slender seahorse, and therefore, its use in the transport of this species is suggested because apparently reduces the stress of transport.

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Literature cited

Barcellos, L. J. G., L. C. Kreutz, R. M. Quevedo, I. Fioreza, L. B. Rodrigues, A. B. Soso, F. Ritter, J. Conrad, L. Cericato, M. Fagundes & S. R. Terra. 2003. Haematological and biochemical characteristics of male jundia (*Rhamdia quelen* Quoy & Gaimard Pimelodidae): changes after acute stress. Aquaculture Research, 34: 1465-1469.

Barton, B. A., C. B. Schreck & L. A. Sigismondi. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Transactions of the American Fisheries Society, 115: 245-251.

Bauer, M. E., P. Perks, S. L. Lightman & N. Shanks**.** 2001. Restraint stress is associated with changes in glucocorticoid immunoregulation. Physiology & Behavior, 73: 525-532.

Biron, M. & T. J. Benfey. 1994. Cortisol, glucose and hematocrit changes during acute stress, cohort sampling, and the diel cycle in diploid and triploid brook trout (*Salvelinus fontinalis* Mitchill). Fish physiology and Biochemistry, 13: 153-160.

Brandão, F. R., L. C. Gomes & E. C. Chagas. 2006. Respostas de estresse em pirarucu (*Arapaima gigas*) durante práticas de rotina em piscicultura. Acta Amazonica, 36: 349-356.

Cho, G.K. & D. D. Heayh. 2000. Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile Chinook salmon *Oncorhynchus tshawytscha* (Walbaum). Aquaculture Research, 31: 537-546.

Cooke, S. J., C. D. Suski, K. G. Ostrand, B. L. Tuft & D. H. Wahl. 2004. Behavioral and physiological assessment of low concentrations of clove oil anaesthetic for handling and transporting largemouth bass (*Micropterus salmoides*). Aquaculture, 239: 509-529.

Cunha, F. E. A. & I. L. Rosa. 2006. Anaesthetic effects of clove oil on seven species of tropical reef teleosts. Journal of Fish Biology, 69: 1504-1512.

Cunha, M. A., F. M. C. Barros, L. O. Garcia, A. P. L. Veeck, B. M. Heinzmannh, V. L. Loro, T. Emanuelli & B. Baldisserotto. 2010. Essential oil of *Lippia alba*: A new anesthetic for silver catfish, *Rhamdia quelen*. Aquaculture, 306: 403-406.

Cunha, M. A., C. C. Zeppenfeld, L. O. Garcia, V. L. Loro, M. B. Fonseca, T. Emanuelli A. P. L. Veeck, C. E. Copatti & B. Baldisserotto. 2010. Anesthesia of silver catfish with eugenol: time of induction, cortisol response and sensory analysis of fillet. Ciência Rural, 40: 2107-2114.

Das, S. K. & U. C. Goswami. 2003. Evaluation of a few locally available anaesthetics for potential use in fish seed transportation, Fishery Technology. Society of Fisheries Technologists, 40: 101-104.

Dick, P. T. & D. G. Dixon. 1985. Changes in circulating blood cell levels of rainbow trout, *Salmo gairdneri* Richardson, following acute and chronic exposure to copper. Journal of Fish Biology, 26: 475-481.

Ellsaesser, C. F. & L. W. Clem. 1986. Haematological and immunological changes in chanel catfish stressed by handling and transport. Journal of Fish Biology, 28: 511-521.

Ellsaesser, C.F. & L.W Clem. 1987. Cortisol-induced hematologic and immunologic changes in channel catfish (*Ictalurus punctatus*). Comparative Biochemistry and Physiology 87: 405-408.

European Pharmacopoeia. 2007. Strassbourg, European Directorate for the Quality of Medicines 6th ed.

Façanha, M. F. & L. C. Gomes. 2005. A eficácia do mentol como anestésico para tambaqui (*Colossoma macropomum,* Characiformes: Characidae). Acta Amazonica, 35: 71-75.

Gomes, L. C., B. Baldisserotto, E. C. Chagas, R. Roubach, R. P. Brinn & C. E Coppati. 2006. Use of salt during transportation of air breathing pirarucu juveniles (*Arapaima gigas*) in plastic bags. Aquaculture, 256: 521-528.

Inoue, L. A. K. A., L. O. B. Afonso, G. K. Iwama & M. G. Gilberto. 2005. Effects of clove oil on the stress response of matrinxã (*Brycon cephalus*) subjected to transport. Acta Amazonica, 35: 289-295.

Iversen, M., B. Finstad, R. S. McKinley & R. A. Eliassen. 2003. The efficacy of metomidate, clove oil, Aqui-S and Benzoak as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. Aquaculture, 221: .549-566.

Johansson-Sjobeck, M., G. Dave, A. Larsson, K. Lewander & U. Lidman. 1978. Hematological effects of cortisol in the European eel, *Anguilla Anguilla* L. Comparative Physiology and Biochemistry, 60: 165-168.

Keene, J. L., D. L. G. Noakes, R. D. Moccia & C. G. Soto. 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Onchorhynchus mykiss* (Walbaum). Aquaculture Research, 29: 89-101.

Koldewey, H., C. Delbeek, A. Marshall & C. E Keen. 2005. Collection and Transportation of Seahorses, In: Syngnathid Husbandry in Public Aquariums. Manual, 137.

Martins, M. L., F. R. Moraes, J. R. E. Moraes & E. B. Malheiros. 2000. Falha na resposta do cortisol estresse por captura por carragenina em *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae). Acta Scientiarum, 22(2): 545-552.

Martins, M. L., F. R. Moraes, R. Y. Fujimoto, D. T. Nomura & J. Fenerick. 2002. Respostas do híbrido tambacu (*Piaractus mesopotamicus* Holmberg, 1887 macho x *Colossoma macropomum* Cuvier, 1818 fêmea) a estímulos simples ou consecutivos de captura. Boletim do Instituto de Pesca, 28(2):195-204.

Martins, M.L., F. Pilarsky, E. M. Onaka, D. T. Nomura, J. Fenerick Jr., K. Ribeiro, D. M. Y. Myiazaki, M. P. Castro & E. B. Malheiros. 2004. Hematologia e resposta inflamatória aguda

em *Oreochromis niloticus* (Osteichthyes: Cichlidae) submetida aos estímulos único e consecutivo de estresse de captura. Boletim do Instituto de Pesca, 30: 71-80.

Monteiro-Neto, C., F. E. A. Cunha, M. C. Nottingham, M. E. Araujo, I. L. Rosa & G. M. L. Barros. 2003. Analysis of the marine ornamental fish trade at Ceará State, Northeast Brazil. Biodiversity Conservation, 12: 1287-1295.

Oliveira, J. R., J. L. Carmo, K. K. C. Oliveira & M. C. F. Soares. 2009. Cloreto de sódio, benzocaína e óleo de cravo-da-índia na água de transporte de tilápia-do-nilo. Revista Brasileira de Zootecnia, 38: 1163-1169.

Pickering, A.D. 1998. Stress responses of farmed fish. In: Black, K. D., Pickering, A.D. (Eds.), Biology of Farmed Fish. Sheffield Academic Press, Sheffield, Pp. 222–243.

Pickering, A. D. & T. G. Pottinger 1987. Crowding causes prolonged leucopenia in salmonid fish, despite interrenal acclimation. Journal of Fish Biology, 30: 701-712.

Pulsford, A. L., S. Lemaire-Gony, M. Tomlinson, N. Collingwood & P. J. Glynn. 1994. Effects of acute stress on the immune system of the dab, *Limanda limanda*. Comparative Biochemistry and Physiology**,** 109: 129-139.

Ross, L. G., J. S. Blanco, C. Martínez-Palacios, I. S. Racotta & M. T. Cuevas. 2007. Anaesthesia, sedation and transportation of juvenile *Menidia estor* (Jordan) using benzocaine and hypothermia, Aquaculture Research, 38: 909-917.

Ross, L. G. & B. Ross. 2008. Anaesthetic and sedative techniques for aquatic animals. 3. ed. Oxford: Blackwell Science, 240 p.

Rosa, I. L., T. L. Dias & J. K. Baum. 2002. Threatened fishes of the world: *Hippocampus reidi* Ginsburg, 1933 (Syngnathidae). Environmental Biology of Fishes, 64: 378.

Rosa I. M. L., R. R. N. Alves, K. M. Bonifácio, J. S. Mourão, F. M Osório, T. P. R. Oliveira & M. C. Nottingham. 2005. Fishers' knowledge and seahorse conservation in Brazil. Journal of Ethnobiology and Ethnomedicine, 1(12).

Roubach, R., L. C. Gomes, F. A. L. Fonseca & A. L. Val. 2005. Eugenol as an efficacious anaesthetic for tambaqui, *Colossoma macropomum* (Cuvier). Aquaculture Research, 36: 1056 -1061.

Schoettger, R. A. & M. Julin. 1967. Efficacy of MS-222 as an anesthetic on four salmonids. Investigations in Fish Control, U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 13: 1-15.

Sladky, K. K., C. R. Swanson, M. K. Stoskopf, M. R. Loomis,G. A. Lewbart. 2001. Comparative efficacy of tricaine methanosulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachipomus*). American Journal of Veterinary Research**,** 62: 337-342.

Sopinska, A. 1984. Effect of physiological factors, stress, and disease on hematological parameters of carp, with a particular reference to leukocyte pattern. II. Hematological results of stress in carp. Acta Ichthyologica et Piscatoria, 14: 121-139.

Tavares-Dias, M., S. H. C. Schalch, E. D. Silva, M. L. Martins & F. R. Moraes. 2000. Características hematológicas de *Oreochromis niloticus* (Osteichthyes: Cichlidae) cultivada intensivamente em pesque-pague do município de Franca, SP, Brasil. Arquivos de Veterinária, 16: 76-82.

Tavares-Dias, M., E. F. S. Sandrim, F. R. Moraes & P. C. F. Carneiro. 2001. Physiological responses of "tambaqui" *Colossoma macropomum* (Characidae) to acute stress. Boletim do Instituto de Pesca, 27: 43-48.

Vijayan, M. M. & J. F. Leatherland. 1989. Cortisol-induced changes in plasma glucose, protein, and thyroid hormone levels, and liver glycogen content of coho salmon (*Oncorhynchus kisutch* Walbaum). Canadian Journal of Zoology, 7: 2746-2750.

Wendelaar Bonga, S. E. 1997. The stress response in fish. Physiological Reviews 77: 591-625.

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