

Benzocaine and menthol as anesthetics for rainbow trout males (*Oncorhynchus mykiss* Walbaum 1792).

Cortes - García A y Rodríguez - Gutiérrez M.

Universidad Autónoma Metropolitana Unidad Xochimilco. Departamento El Hombre y su Ambiente. Laboratorio de Reproducción, Genética y Sanidad Acuícola. Calzada del Hueso 1100. Colonia Villa Quietud, Delegación Coyoacán. C. P. 04960. Distrito Federal. México. Tel. 5483 74 94.

* Responsible email: rogm0211@correo.xoc.uam.mx

ABSTRACT

The efficiency of benzocaine and menthol in rainbow trout males (*Oncorhynchus mykiss*) of three years old, with an average weight of $1,590 \pm 0.2$ g and a pattern length of 6.60 ± 2.3 cm was compare. In evaluation, it was consider three times: sedation (ST), manipulation (MT) and recovery (RT). Anesthetics were prepared from stock solutions: benzocaine: 12 g in a litter of alcohol and it was use in doses of 3 mL L^{-1} of water. For menthol with 100 g L^{-1} of alcohol and 1 mL L^{-1} of water was use. Results with benzocaine were $ST 225.50 \pm 96$ sec; $MT 153 \pm 37$ sec and $RT 325 \pm 248$ sec while in menthol it was obtain $ST 244 \pm 80$ sec; $MT 152.6 \pm 37$ sec and $RT 399 \pm 163$ sec. Organisms were sedate and recover faster with benzocaine. ANDEVA just pointed out significate differences ($P < 0.05$) and in RT between treatments. Organisms sedation using both anesthetics allow manipulation during 152 sec, enough time for morphometric parameters determination or for other type of sampling like semen obtention, sanitary sampling, among others. With both treatments it was obtained an average of $ST = 234$ sec and in $RT 362$ sec. In any treatment, mortality was observe after 24 hours. Both anesthetics are available in markets and have low prices, so it was recommend in manipulation of this species with high commercial value.

Key words: aquaculture, anesthesia, sedation, recovery

RESUMEN

Se comparó la eficiencia de benzocaína y mentol en machos de trucha arco iris (*Oncorhynchus mykiss*) de tres años, con un peso promedio de $1,590 \pm 0.2$ g y una longitud patrón 6.60 ± 2.3 cm. En la evaluación se consideraron tres tiempos: sedación (TS), manipulación

(TM) y recuperación (TR). Los anestésicos se prepararon a partir de soluciones madre: benzocaína: 12 g en un litro de alcohol y se empleó en dosis de 3 mL L^{-1} de agua. Para mentol con 100 g L^{-1} de alcohol y se empleó 1 mL L^{-1} de agua. Los resultados con benzocaina fueron: $TS 225.50 \pm 96$ seg; $TM 153 \pm 37$ seg y $TR 325 \pm 248$ seg mientras que para mentol se obtuvo $TS 244 \pm 80$ seg; $TM 152.6 \pm 37$ seg y $TR 399 \pm 163$ seg. Los organismos se sedaron y se recuperaron más rápido con benzocaína. El ANDEVA solo señaló diferencias significativas ($P < 0.05$) en el TR entre los tratamientos. La sedación de los organismos utilizando ambos anestésicos permitió la manipulación durante 152 seg, tiempo suficiente para la determinación de los parámetros morfométricos o bien para muestreos de otro tipo como obtención de semen, muestreo sanitario, entre otros. Con ambos tratamientos se tuvieron en promedio en $TS = 234$ seg y en $TR 362$ seg. En ninguno de los tratamientos se observó mortalidad después de las 24 horas. Los dos anestésicos están disponibles en el mercado y tienen bajo costo, por lo que se recomiendan en la manipulación de ésta especie con alto valor comercial.

Palabras claves: acuicultura, anestesia, sedación, recuperación

INTRODUCTION

The use of anesthetics in aquatic organisms intends minimize stress especially during manipulation, because it negatively affects in its physiology (Ashley 2007) especially in males because of its hormonal nature they are more aggressive. It is recommend its use to facilitate handling in diverse procedures such as biopsies,

Anesthetics in rainbow trout
Cortes - García A y Rodríguez - Gutiérrez M.

transportation, reproduction induction, gametes extraction, taking measurements and weight (Rodríguez *et al.* 2013).

In general, organisms have less handling resistance when they are under effect of some anesthetic, which reduce traumas incidence during manipulation, due to its nervousness and alteration that are conducive to falls that might cause severe injuries such as internal damage in ovaries or testicles or mucus and squama remotion, favoring entrance of opportunistic pathogenic bacteria (Soto and Burhanuddin 1995).

Anesthetics in aquatic organisms, in general are applied in water, so they must being soluble, have brief induction and recovery time. Recovery have to be spontaneous, as well as a large safety factor, being nontoxic for organisms and man too, no leaving residues and allow, if required, intensification of anesthesia (Simões and Gomes 2009).

Anesthetics more used in aquaculture are tricaine methanesulfonate or MS 222; propanidid (Jeney *et al.* 1986); xylocaine (Rodríguez-Gutiérrez and Esquivel-Herrera 1995); benzocaine (Gilderhus 1990, Gomes 2001) and 2-phenoxyethanol (Quinn *et al.* 1988). First three require importation which increase its cost, because of that, options are being investigated that includes clove oil and menthol, that for being “natural”, are a viable alternative due to its easy obtention in market and affordable costs.

Clove oil solution was use as anesthetic in different species like *Carassius carassius* and *Cyprinus carpio* (Endo *et al.* 1972); *Centropomus striata* (King *et al.* 2005); *Ictalurus punctatus* (Waterstrat 1999); *Liza parsia* (Sonawane and Kulkarni 2001); *Oncorhynchus tshawytscha* (Cho and Heath 2000); *Phoxinus erythrogaster* (Detar and Mattingly 2005); *Xiphophorus helleri*, *Trichogaster leeri* and *Micropterus dolomieu* (Peake 1998); *Seriola dimerii* (García-Gómez *et al.* 2002); *Centropomus parallelus* (Tsuzuki and Berestinas 2008); and rainbow trout *Oncorhynchus mykiss* (Keene *et al.* 1998).

Menthol has been widely used to anesthetic in marine invertebrates (Ruppert and Barnes 1996) and has been little used in fish as *Colossoma macropomum* (Gomes *et al.* 2001), *Centropomus*

parallelus (Souza *et al.* 2012), *Oreochromis niloticus* (Simões y Gomes 2009). Nevertheless, there is a lack of studies about elimination time and it has been use as alternative to MS 222 for being less expensive (Gomes *et al.* 2000; González and González 2007).

On other hand, anesthetic concentration in muscle according to Noga (1996) and Stoskopf (1993) must be superior to 0.1 mg kg⁻¹ to be detectable. Studies about elimination of anesthetics in fish are diverse: Allen (1988) in trout and largemouth bass he did not detected benzocaine in muscle after 24 hours, while in red salmon MS 222 was detectable after 21 days (Wood *et al.* 2002) and clove oil in chinook salmon, it was metabolized in less than 24 h (Cho and Heath 2000).

The foregoing indicates that each anesthetic has its particularities besides that at contact; fish has resistance reflexes that might be severe or mild and altered levels of coagulation, glucose, cortisol and cholesterol (Wedemeyer 1970).

Because of importance that has to minimize stress during manipulation of aquatic organisms, this study has as purpose to compare the anesthetic effect of benzocaine and menthol in male rainbow trout's with an average age of three years.

MATERIAL AND METHODS

Organism's obtention

Thirty breeding organisms were obtain at installations of Centro Acuícola El Zarco, Mpo. of Ocoyuacac, Edo. of México (Fig.1). Organisms were adult stage three years old, with mean pattern length of 46.6 ±2.3 cm and weight of 1.590 ±0.2 g.

Experimental design

Variables using in this study were sedation time (ST), manipulation time (MT) and recovery time (RT). Each one was register with a chronometer and in anesthesia and control formats.

During sedation, fishes were place in 40 L beakers one by one, changing it every five organisms. Used doses was 3 mL per liter of benzocaine stock solution and 1 mL of menthol stock solution per liter

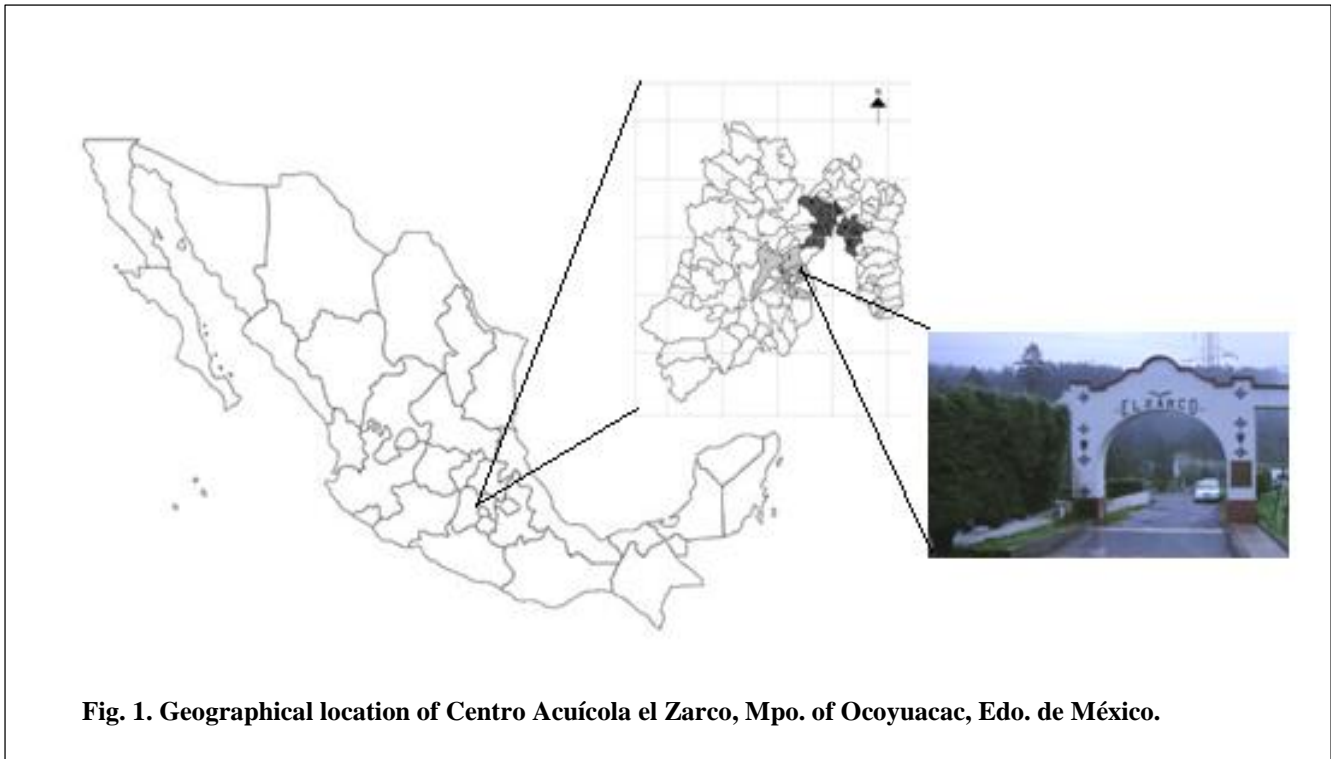


Fig. 1. Geographical location of Centro Acuicola el Zarco, Mpo. of Ocoyuacac, Edo. de México.

of water. Study was realized under the next conditions: water temperature 12°C with pH of 8.

Anesthetics preparation

The stock solution was elaborate with 12 g of Benzocaine and 100 g of menthol diluted in 1 L of alcohol.

Sedation time (ST)

Sedation time was obtained during organisms were introduce into the anesthetic solution until sedation, and then they were extracted. An organisms was considered sedated when it was going sideways and lost balance, went to the bottom, reduced opercula movement and did not move away when touched (Stoskopf 1993).

Manipulation time (MT)

This period consider the time to make a sanitary

procedure, gonadal or meristic sampling and starts now when organisms is extracted from anesthetic solution, and ends when it is introduced to water for its recovery. In this case, MT was standardize to obtain biometrics: total length, pattern and cephalic, as well as height and thickness, with a 60 cm modified measuring board. In addition, it was took the weight using a balance brand Adam with maximum capacity of 32 kg ± 1 g.

Recovery time (RT)

RT was count from the moment that the organism was introduce into water pond flow and ended organisms recovered balance and began to move in a normal way.

Results analysis

Results were analyze using a statistical package Statistica 9. One-way ANDEVA was use and if there were differences, Tukey test was apply for

comparison of multiple measures. In all analyzes a significance level of 95% was used (Zar 1996).

RESULTS

In fish, it was observe a minor resistance to get in contact with benzocaine and regarding to average sedation time with benzocaine (STB) was of 225.50 ± 96 sec and with menthol (STM) 244 ± 80 sec, which means that it took an average of 234 sec to sedate.

MT with benzocaine (MTB) was of 153 ± 37 sec and with menthol (MTM) 152.6 ± 37 sec. Time when organisms were kept water outside and manipulated from over 152 sec to obtain length and weight parameters. Variance analysis did not determined significate differences for ST ($P=0.535$) and MT ($P=0.906$) and deviations were similar (Fig. 2). Regarding to average RT with benzocaine (RTB) was of 325 ± 248 sec and with menthol (RTM) 399 ± 163 sec. Variance analysis determined significant differences ($P<0.05$) (Fig. 2), benzocaine having the lowest RT (Fig.2).

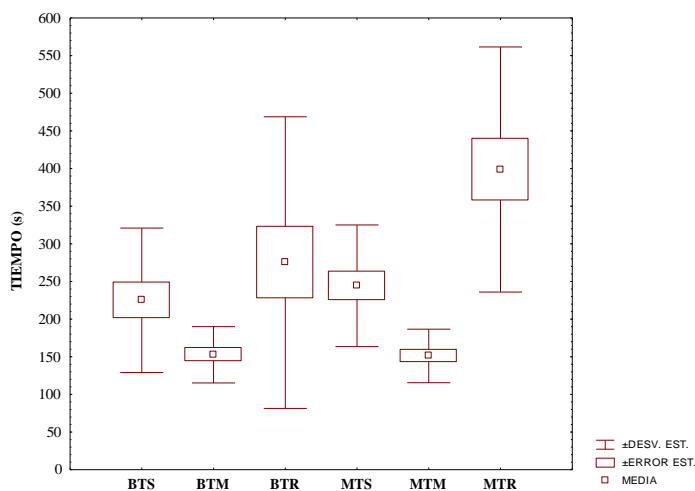


Fig. 2. Measures comparison of sedation (ST), manipulation (MT) and recovery (RT) times with benzocaine (B) and menthol (M) anesthetics.

DISCUSSION

A good anesthetic is one that induces sedation in less than 180 sec and recovery in less than 300 sec according Marcado and Meyer (1985). Considering this, anesthetics in this study have a ST and RT values slightly superior to recommended, but on the other side, it allowed adequate manipulation of organisms during measurement, both are readily available on market and low cost, in addition that no mortality was registered during test and 24 hours later.

Registered average sedation time with both anesthetics was of 234 sec, above value obtained by Gilderhus (1990) in juvenile Pacific salmon or chinook salmon (*Oncorhynchus tshawytscha*) using MS222 at 30 mg L^{-1} concentration in addition, clove oil at 25 mg L^{-1} obtaining an average of 180 and 125 sec respectively, with water temperature of 10 - 13 °C. Even though ST was lower, it is likely to be due to fish size and anesthetics concentration; nevertheless, it refers that trout's presented deficiency in respiratory activity.

Souza *et al.* (2012) reported anesthetized juvenile of little snook (*Centropomus parallelus*) with menthol at 150 mg L^{-1} concentration and registered average times of ST 120 sec and RT of 142 sec being lower than ones are determined in this study. The difference was attribute to concentration of each anesthetic because it have different specific behavior and it have to be consider organisms weight and size.

In pompano fish (*Trachinotus marginatus*) in juvenile stage Okamoto *et al.* (2009) compared efficiency of benzocaine at different concentrations, in 25 mg L^{-1} TS was of 660 sec and RT of 180 sec, while with 150 mg L^{-1} ST was of 120 sec and RT of 840 sec, founding that at higher concentrations, ST and RT are inversely proportional. Compared with obtained in this study, values of ST was higher and RT was lower, even though RT can be excessive for fish in both studies. There was no skin irritation signs or other reaction as reported by Ross *et al.* (2007), which founded those signs in topsmelt *Menidia estor* with benzocaine at 40 mg L^{-1} ; the anesthesia was fast and profound but it resulted with some deaths after

an hour of exposition. It was recommend not use higher doses than 30 mg L⁻¹ for this specie.

Façanha and Gomes (2005) in pacu juvenile (*Colossoma macropomum*) using menthol at 100 mg L⁻¹, obtained an average for ST of 120 and 300 sec and for RT prove that at higher concentration (250 mg L⁻¹), lower ST but higher RT were present. They also mention that exposition to menthol did not cause any alteration in glucose and blood plasma Na⁺ concentration, two stress indicators. While obtained results in this investigation with breeding trout's, ST and RT were higher.

In contrast Simões and Gomes (2009) in mojarra (*Oreochromis niloticus*) using menthol at 100 mg L⁻¹ obtained between 300-330 sec in ST, a value above obtained in this study, in addition to this, their results showed that higher concentration than 50 mg L⁻¹ produced a rise of glucose in blood and therefore stress.

Gholipour (2013) who studied with 100 g juvenile rainbow trout (*Oncorhynchus mykiss*) with MS-222 at 50 mg L⁻¹ and clove oil at 25 mg L⁻¹ as anesthetics found that ST 114 ±11 sec, RT 150 ±20 sec values for first one, while for second were ST 125 ±23 sec and in RT 180 ±12 sec mean values. Comparing these results with this experiment it is observed that times were lower, but although, it is same species. The difference was attribute to development stage and organisms weight.

However, according to Wedemeyer (1970) one of the main inconvenience of MS 222 is that even when fish are anesthetized and rapidly recovered, it raises plasma cortisol levels. On the other hand, Coyle *et al.* (2004) indicated that water pH decrease, which cause olfactory epithelium irritation; and obtained results by Quinn *et al.* (1998) mentions that olfactory epithelium damage, occasioned problems when breeding, because it causes changes in behavior that affects spawning.

According to Façanha and Gomes (2005) and Gonçalves *et al.* (2008), menthol is an efficient anesthetic. Nevertheless, it must take into account the concentration to avoid producing stress in fish, even though it is obtained from a medicinal plant, there are not studies about possible permanency of residues in

muscle that produce a flavor change, as well as impact on the immune system.

Finally, as it was observe in Fig. 2, ST and MT values was similar with both anesthetics. Induction was relatively fast in 234 sec and MT with 152 sec was enough to take the meristic parameters or any other type of sampling as sanitary or manipulation during spawning induction between others. So determined RT was attribute to intrinsic characteristics of each anesthetic, obtaining a lower time of 325 sec with benzocaine and 399 sec with menthol.

Anesthetics performance depends in specie and development stage (Cassara *et al.* 1999; Small 2003 and Rodríguez *et al.* 2013), water physicochemical characteristics (Carrasco *et al.* 1984) and concentrations (Souza *et al.* 2012).

CONCLUSIONS

Benzocaine and menthol used to sedate adult male rainbow trout are adequate because with both of them, ST was in average 234 sec, while average RT was of 325 sec with benzocaine and 399 sec with menthol.

Mean values of MT was 152 sec, enough time to made manipulation of organisms in different procedures.

Both anesthetics were recommend for adult stage trout, because they allow manipulation and there are not any mortality even 24 h later, and are accessible and affordable.

ACKNOWLEDGMENTS

Authors thank to fishing sub delegation of Mexico State, for provided facilities, in Aquaculture Center "El Zarco" and to Biology career students of fifth trimester of UAM-X.

BIBLIOGRAPHY

- Allen JL. 1988. Residues of benzocaine in rainbow trout, largemouth bass, and fish meal. *Progressive Fish-Culturist*, Bethesda 50(1):59-60.
- Ashley PJ. 2007. Fish welfare: Current issues in aquaculture. *Applied Animal Behaviour Science* 104: 199-235.
- Carrasco SH, H Sumano, R Navarro-Fierro. 1984. The Use of Lidocaine-Sodium Bicarbonate as Anaesthetic in Fish. *Aquaculture* 41: 395-398.
- Cassará C, SE Gómez, A Giusto, RA Ferriz y V. Asikian. 1999. Resistance to different toxics and anaesthetics in *Poecilia reticulata* Peters, 1958. *Bioikos* 13(1/2):29-39.
- Cho GK, y DD Heath. 2000. Comparison of tricaine methanesulphonate (MS 222) and clove oil anesthesia effects on the physiology of juvenile chinook salmon *Oncorhynchus tshawytscha* Walbaum). *Aquaculture Research* (31): 537-546.
- Cotter P y K Rodnick. 2006. Differential Effects of Anesthetics on Electrical Properties of the Rainbow Trout (*Oncorhynchus mykiss*) Heart. *Comparative Biochemistry and Physiology Part A*. 145:158-165.
- Coyle SD, RM Durborow y JH Tidwell. 2004. Anesthetics in Aquaculture. Southern Regional Aquaculture Center. Publication No 3900. Kentucky State University Aquaculture Research Center, Texas, USA.
- Detar JE y HT Mattingly. 2005. Response of the Southern Redbelly dace to clove oil and MS-222: effects of anaesthetic concentration and water temperature. *In: Proceedings of the annual conference of the South-eastern Association of Fish and Wildlife Agencies* 58:219-227.
- Endo T, K Ogihima, H Tanaka y S Oshima. 1972. Studies on the anaesthetic effect of eugenol in some fresh water fishes. *Bulletin of the Japanese Society of Scientific Fisheries*, 38:761-767.
- Façanha MF y LC Gomes. 2005. A eficácia do mentol como anestésico para tambaqui (*Colossoma macropomum*, Characiformes: Characidae). *Acta Amazónica* 35(1):71-75.
- García-Gomez A, F Gandara y T Raja. 2002. Use of clove, *Syzygium aromaticum* L. (Merr. & Perry), oil as a cost-effective anaesthetic for routine tasks in handling cultured marine fish. *Boletín del Instituto Español de Oceanografía* 18: 21-23.
- Gilderhus A. 1990. Benzocaine as a Fish Anesthetic: Efficacy and Safety for Spawning-Phase Salmon. *The Progressive Fish Culturist* 52: 189-191.
- Gomes LC, AR Chippari-Gomes, NP Lopes, R Roubach, CAR Araujo-Lima. 2001. Efficacy of benzocaine as an anesthetic in juvenile tambaqui *Colossoma macropomum*. *Journal of the World Aquaculture Society* 32:426-431.
- Gonçalves AFN, EC Santos, JBK Fernandes, LS Takahashi. 2008. Mentol e eugenol como substitutos da benzocaína na indução anestésica de juvenis de pacu. *Acta Scientiarum Animal Science* 30(3): 339-344.
- González CA y JF González (2007). Comparación del efecto anestésico entre mentol y metansulfonato de tricaina en tilapia roja (*Oreochromis* sp.) y yamu (*Brycon amazonicus*). *Revista Médica Veterinaria y Zootécnica*. 54(2): 220 – 221.
- Green, CJ. 1982. *Laboratory animal handbook 8. Animal anesthesia*. London: Laboratory Animals Ltd.
- Jeney Z, G Jeney, J Oláh, A Siwicki y I Danko. 1986. Propanidid a New Anaesthetic for Use in Fish Propagation. *Aquaculture* 54: 149-156.
- Keene J, D Noakes, R Moccia y C Soto. 1998. The Efficacy of Clove Oil as an Anaesthetic for Rainbow Trout, *Oncorhynchus mykiss* (Walbaum, 1792). *Aquaculture Research* 29: 89-101.
- King W, B Hooper, S Hillsgrove, C Benton y DL Berlinsky. 2005. The use of clove oil, metomidate, tricainemetha-nesulphonate and 2-phenoxyethanol for inducing anaesthesia and their effect on the cortisol stress response in black sea bass (*Centropristis striata* L.). *Aquatic Research* 36:1442-1449
- Lorenzo D, D Paz, E Dellesaca, P Davies, R Vila y S Cañigual. 2002. Essential Oils of *Mentha pulegium* and *Mentha rotundifolia* from Uruguay. *Brazilian Archives of Biology and Technology* 4 (45): 519-524.
- Noga EJ. 1996. *Fish Disease: Diagnosis & Treatment*. St. Louis (USA): Mosby. Chapter 22. *General Concepts in Therapy*. pp. 253 – 270.
- Peake S. 1998. Sodium bicarbonate and clove oil as potential anesthetics for nonsalmonid fishes. *North American Journal of Fisheries Management* 18:919-924.
- Quinn T, A Olson y J Konecki. 1988. Effects of Anaesthesia on the Chemosensory Behaviour of Pacific Salmon. *Journal Fish Biology* 33: 637-641.

- Rodríguez GM, GA Cortés y GD Contreras. 2013. Comparación del Aceite de clavo y la benzocaína como anestésicos en el manejo de la trucha arco iris (*Oncorhynchus mykiss*). Avances de las ciencias en las humanidades y todas las disciplinas (2):29-33.
- Rodríguez-Gutiérrez M y A Esquivel-Herrera. 1995. Evaluation of the Repeated Use of Xylocaine as Anesthetic for the Handling of Breeding Carp (*Cyprinus carpio*). Aquaculture 129: 431-436.
- Ross L, G Ross, JB Sánchez, C Martínez-Palacios, SI Racotta y MC Toledo. 2007. Anesthesia, sedation and transportation of juvenile *Menidia estor* (Jordan) using benzocaine and hypothermia. Aquaculture Research 38:909-917.
- Simões L y L Gomes. 2009. Eficácia do Mentol como Anestésico para Juvenis de Tilápia-do-Nilo (*Oreochromis niloticus*). Arquivo Brasileiro de Medicina Veterinária e Zootecnia 61 (3): 613-620.
- Small BC. 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.
- Sonawane UD y GN Kulkarni. 2001. Anaesthetic effects of clove oil and sodium bicarbonate on the fry of *Liza parsia*, Iranian Journal Fish Science 3:49-62
- Soto GC y S. Burhanuddin. 1995. Clove Oil as a Fish Anaesthetic for Measuring Length and Weigh of Rabbitfish (*Siganus lineatus*). Aquaculture 136: 149-152.
- Souza R A, CV Carvalho, FF Nunes, BR Scopel, JD Guarizi y MY Tsuzuki. 2012. Efeito comparativo da benzocaína, mentol e eugenol como anestésicos para juvenis de robalo peva. Bol Inst Pesca, 38: 247-255.
- Stoskopf M. 1993. Anaesthesia. In: Brown L. (Eds). Aquaculture for Veterinarians: fish husbandry and medicine. London, UK: Pergamon Veterinary Handbook Series. 161- 168.
- Tsuzuki MY y AC Berestinas. 2008. Desempenho de juvenis de robalo-peva *Centropomus parallelus* com diferentes dietas comerciais e frequências alimentares. Boletim do Instituto de Pesca, São Paulo. 34(4): 535-541.
- Waterstrat P. 1999. Induction and recovery from anesthesia in channel catfish *Ictalurus punctatus* fingerlings exposed to clove oil. Journal of World Aquaculture Society, Baton Rouge, 30(2): 250-255.
- Wedemeyer G. 1970. Stress of Anesthesia with MS-222 and Benzocaine in Rainbow Trout (*Salmo gairdneri*). Journal of the Fisheries Research Board of Canadá 27: 909-914.
- Wood CA, J Nelson y K Ramstad. 2002. Clove oil as an anaesthetic for adult sockeye salmon: field trials. Journal of Fish Biology (60): 340-347.
- Zar JH. 1996. Biostatistical Analysis. Third edition. Prentice-Hall, New Jersey. 718 p.