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Original Article

Putting the Pacific fat sleeper *Dormitator latifrons* (Pisces: Eleotridae) to sleep: effects of clove oil and lidocaine anesthetic

Colocando o dorminhoco-gordo-do-pacífico *Dormitator latifrons* (Pisces: Eleotridae) para dormir: efeitos do óleo de cravo e do anestésico lidocaína

M. A. Aréchiga-Palomera[a,b], A. G. Figueroa-Salazar[b], K. N. Nieves-Rodríguez[a,b], H. Nolasco-Soria[c], S. R. Guerrero-Galván[b], D. Badillo-Zapata[b] and F. Vega-Villasante[b]*

[a]Universidad de Guadalajara, Centro Universitario de la Costa, Programa de Doctorado BEMARENA, Puerto Vallarta, Jalisco, México
[b]Universidad de Guadalajara, Centro Universitario de la Costa, Laboratorio de Calidad de Agua y Acuicultura Experimental, Puerto Vallarta, Jalisco, México
[c]Centro de Investigaciones Biológicas del Noroeste, Laboratorio de Fisiología Comparada y Genómica Funcional, S.C. La Paz, Baja California Sur, México

Abstract

Among the different handling techniques in aquaculture, the use of anesthetics has had a growing interest focused on guaranteeing animal welfare, and reducing possible stress situations during general handling. The aim of this study was to present the use of eugenol and lidocaine with non-invasive anesthesia procedures in *Dormitator latifrons*, in which the different stages of anesthesia (induction and recovery) were determined. One hundred and twenty healthy fish of average weight of 73.59 ± 13.53 g and standard length of 17 ± 1.36 cm were used. The experimental fish were subjected to fasting for 24 h prior to the tests. Five fish were subjected to eugenol (25, 50, 100, and 200 µL/L), and lidocaine (100, 200, 300, and 400 mg/L), in triplicate. The time to reach deep and recovery anesthesia were recorded and the data analyzed using ANOVA (α= 0.05). Organisms exposed to anesthetics evidenced early episodes of fast, short-distance swimming (initial hyperactivity) for short periods of time. Survival was 100% with both compounds and concentrations. Fish exposed to a eugenol concentration of 200 µL/L had longer anesthesia times and took longer time to recover ($P<0.05$). The most effective concentrations for eugenol and lidocaine were of 200 µL/L and 400 µL/L in juvenile fish, promoting rapid inductions, without compromising the conditions for the recovery of the fish. This work provides practical information for handling and transportation *D. latiforns* with the least possible stress and ensuring animal welfare.

Keywords: fish, eugenol, stress, handling, sedation.

Resumo

Dentre as diferentes técnicas de manejo na aquicultura, o uso de anestésicos tem despertado interesse crescente voltado para a garantia do bem-estar animal, reduzindo possíveis situações de estresse durante o manejo geral. O objetivo deste estudo foi apresentar o uso de eugenol e lidocaína com procedimentos anestésicos não invasivos em *Dormitator latifrons*, nos quais foram determinadas as diferentes etapas da anestesia (indução e recuperação). Foram utilizados 120 peixes saudáveis, com peso médio de 73,59 ± 13,53 g e 17 ± 1,36 cm de comprimento, em jejum de 24 horas antes dos testes. Cinco peixes foram submetidos a eugenol (25, 50, 100 e 200 µL/L) e lidocaína (100, 200, 300 e 400 mg/L), em triplicata. O tempo para atingir a anestesia profunda e de recuperação foi registrado, os dados foram analisados com ANOVA (α= 0,05). Organismos expostos a anestésicos evidenciaram episódios precoces de nado rápido de curta distância (hiperatividade inicial) por curtos períodos de tempo. A sobrevivência atinge 100% com ambos compostos e concentrações. Peixes expostos a uma concentração de eugenol de 200 µL/L tiveram tempos de anestesia mais longos e demoraram mais para se recuperar ($P<0,05$). As concentrações mais efetivas para eugenol e lidocaína foram de 200 µL/L e 400 µL/L em peixes juvenis, promovendo induções rápidas, sem comprometer as condições de recuperação dos peixes. Este trabalho fornece informações práticas para o manejo e transporte de *D. latifrons* com o mínimo de estresse possível e garantindo o bem-estar animal.

Palavras-chave: peixe, eugenol, estresse, manipulação, sedação.

1. Introduction

Dormitator latifrons (Richardson, 1844), also called Pacific fat sleeper, chococo, chalaco, chame, puyeque or popoyote, is a fish that has attracted biological interest for more than a hundred years (Vega-Villasante et al., 2021). Its distribution ranges from California (USA) to Peru, occupying rivers, streams and coastal lagoons and

*e-mail: fvillasante@cuc.udg.mx
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estuaries (Yáñez-Arancibia and Díaz-González, 1977). In Central America, it has great commercial potential, and its fishing represents an alternative source of employment (López-López et al., 2015). Although there are no official data on its fisheries and aquaculture production, this species has a commercial interest since it is part of the gastronomy in several coastal communities in Ecuador and southern Mexico mainly in the states of Guerrero and Oaxaca (Aréchiga-Palomera et al., 2022). In recent years, interest in its study in Mexico has increased, especially because it is considered a species with high potential to be cultivated as an alternative to exotic species such as tilapia (Basto-Rosales et al., 2019). The transport and handling of this fish, from the natural environment to the laboratory, is of essential importance since it is prone to suffer stress, which favors the appearance of diseases (Vega-Villasante et al., 2017). To date, there are no protocols to guarantee the adequate conditions for its correct handling.

In the aquaculture industry, capture, handling, confinements, and transport are some of the activities that cause stress and cause physiological alterations in organisms (Aréchiga-Palomera et al., 2016). Stress has a negative impact on animals with effects including reduced immunocompetence, increased susceptibility to disease, reduced egg quality, growth (Pickering, 1981), and loss of market value (Vander Salm et al., 2004). Recently, attention has been focused on minimizing stress, and the use of anesthetics is among the techniques toward this purpose during general handling that can range from transportation, clinical procedures, and obtaining tissue samples and body fluids to surgeries (Cho and Heath, 2000; Velasco-Santamaría et al., 2008; Scott et al., 2009).

Currently, anesthetics approved by the U.S. Food and Drug Administration (FDA) for use in aquaculture-derived feeds in the United States, are tricaine methanesulfonate (MS-222) and carbon dioxide (CO_2) (Schnick et al., 1986). However, it has been reported that the use of MS-222 can cause occupational hazards (retinopathy) to users and has a waiting period of 21 days before the product can be consumed (Smith et al., 1999).

Lidocaine is an amide-type local anesthetic that acts by blocking voltage-gated sodium channels in neuronal tissues, interrupting nerve transmission, and reducing the neurogenic response (Soto et al., 2018). The use of lidocaine as an anesthetic in fish has been documented and the doses used can vary depending on the species and the size of the fish (Avdesh et al., 2012; Vargas-Vargas, 2017).

On the other hand, there has been a growing interest in the use of natural compounds with an anesthetic effect in aquaculture, such as eugenol from clove oil (Anderson et al., 1997). Clove oil is a plant product, easy to acquire, cheap, environmentally friendly, and safe for operators and fish (Iversen et al., 2003). Some investigations have reported the effectiveness of clove oil as an anesthetic for fish species such as matrincha, *Brycon cephalus* (Inoue et al., 2003); tambaqui, *Colossoma macropomum* (Roubach et al., 2005); spotted, *Pseudoplatystoma corruscans* (Vidal et al., 2006); catfish, *Rhamdia quelen* (Cunha et al., 2006) and snook *Centropomus undecimalis* (Souza-Junior and Alves-Junior, 2006); Its ability to reduce pressure on transport and handling has also been demonstrated (Cunha et al., 2006). Some of these studies have reported sedation, moderate anesthesia, and deep anesthesia (Adel et al., 2016).

It is extremely important to study and search for anesthetics that help reduce stress and mortality and ensure animal welfare during the handling of aquatic organisms, including *D. latifrons*, which is a species of inland waters, and which at the date, represents an important alternative for sustainable aquaculture of native fish on the Pacific coast (Aréchiga Palomera et al., 2022; Vega-Villasante et al., 2021). This work intends to provide information on the standardization of anesthesia protocols in *D. latifrons* regarding dose, anesthesia stage level, time, and body weight, comparing the effectiveness of a conventional anesthetic versus a natural compound.

2. Materials and Methods

The studies were carried out in the Laboratorio de Calidad de Agua y Acuicultura Experimental (LACUIC) of the Department of Biological Sciences of the Centro Universitario de la Costa, Puerto Vallarta, Jalisco.

One hundred twenty relatively healthy organisms (*D. latifrons*) selected from stock of mixed-sex juvenile organisms collected in the Estero El Quelele located in the State of Nayarit, Mexico (between 105° 17' W and 20° 43' N) and subjected to 60-day quarantine, with an average weight and standard length of 73.59 ± 13.53 g and 17 ± 1.36 cm respectively were selected from stock. The fish were kept in a cistern, and starved for 24 hours prior to anesthesia tests. The anesthesia procedures were non-invasive, being the case for this study by immersion and approved by the Animal Welfare Committee of the Centro Universitario de la Costa (approval code: CUCPV/SA/CBA/2/2022). For the study of both anesthetics, the organisms were subjected to different concentrations in tubs with a capacity of 50 L with constant aeration with a filter. The temperature and oxygen conditions were the same for all treatments.

The final time when the organisms reached the degree of deep anesthesia (phase 5) was recorded according to the criteria of Cooke et al. (2004) (Table 1). Once the maximum state of anesthesia was reached, the organisms were removed from induction and transferred to individual reservoirs free of the anesthetic with constant aeration to record and evaluate the recovery time (phase 0). The individual evaluation facilitated the observation of the different planes of anesthesia described in Cooke et al., (2004). The time was measured using a chronometer. Finally, survival at the following hours (1, 2, 24, 48, and 72 h) was recorded.

3. Anesthetics

Two anesthetic compounds used were clove oil eugenol (Viarden®) and lidocaine (Pisa®). All the anesthetics were used in water immersion directly on the experimental aquariums. These were handled with adequate protective equipment: gloves, face mask, gown, and safety glasses; and ensuring proper animal welfare practices.

D. latifrons juveniles were selected to evaluate the effect of eugenol (25, 50, 100 and 200 µL/L) and lidocaine (100, 200, 300, and 400 mg/mL). Five juvenile fish were exposed to anesthetics, placed in different aquariums with dechlorinated water and constant aeration, in triplicate.

3.1. Statistic analysis

With Sigma 11® software, the data were subjected to prior normality and homoscedasticity tests (α= 0.05), After which a Kruskall-Wallis test was applied. Significant differences between treatments were determined from each other by multiple comparisons by Tukey test (α= 0.05).

4. Results

The results show a high survival in the organisms induced with anesthesia eugenol and lidocaine, even organisms exposed to the highest concentrations. With both compounds, survival was 100% for all concentrations. At all concentrations prior to phase 2 induction of anesthesia, organisms exhibited short-distance, fast swimming episodes (initial hyperactivity) for short periods. This hyperactive swimming behavior did not appear in the recovery phases (except for some individuals when they were handled and transferred to their respective aquariums).

At the lowest concentrations for eugenol (clove) that is, 25 µL/L, organisms exhibited phase 3 stages of anesthesia with a tendency to phase 4 (Table 1) without any being fully defined, and the trial had to be discontinued after 30 minutes of exposure to the anesthetic. In the stage of induction and recovery time, significant differences were observed between treatments ($P<0.05$) (Table 1 and Table 2).

When analyzing the total anesthesia induction time in eugenol (Table 2), the 200 µL treatment presented the lowest times with 2.77 minutes ($P<0.05$), and the 25 µL treatment, the highest to reach induction (22.97 min). The 25 µL, 50 µL, and 100 µL treatments were not sigficantly different among themselves, but they did with respect to 200 µL ($P<0.05$). In the induction time with lidocaine (Table 2), the concentration of 300 µL/L was a rapid induction ($P<0.05$, 1.7 min), in relation to 100 µL/L ($P<0.05$, 10.2 min), however, the concentrations of 200, 300 and 400 µL/L were not significant differences among themselves ($P>0.05$), but they were with respect to 100 µL/L ($P<0.05$), which displayed the highest induction times with six minutes ($P<0.05$).

For the recovery times with eugenol (Table 3), the 200 µL treatment had the highest time (11.48 min), followed by 100 µL (10.20 min), 50 µL (6.51 min), and 25 µL (6.4 min), although there were no significant differences between them ($P>0.05$).

Table 1. Criteria for the evaluation of states of induction of anesthesia. Criteria described in Cooke et al. (2004).

State of anesthesia	Descriptor	Characteristics
0	Normal	**Reactive to external stimuli**; opercular rate and muscle tone normal
1	Light sedation	**Slight loss of reactivity to stimuli**; slight decrease in opercular rate; **equilibrium normal**
2	Deep sedation	**Total loss of reactivity to all but strong stimuli**; slight decrease in opecular rate; **equilibrium normal**
3	Partial loss of equilibrium	Partial loss of muscle tone**; swimming erratic**; increased opercular rate; **reactive only to strong tactile or vibrational stimuli**
4	Total loss of equilibrium	**Total loss of** muscle tone and **equilibrium**; slow opercular rate; loss of spinal reflexes
5	Loss of reflexes reactivity*	**Total loss of response to stimuli**, slow opercular rhythm**, total loss of all reflexes**
6	Medullary collapse	Opercular movements cease; cardiac arrest follows

The letters in bold indicate that they were the characteristics evaluated in the present study. *Deep anesthesia.

Table 2. Evaluation of anesthesia with eugenol and lidocaine for *D. latifrons* juveniles in four different concentrations.

Eugenol induction				
Dose	25 µI/L	50 µI/L	100 µI/L	200 µI/L
Time (min)	22.97±12.57^{a}	8.95±6.33^{ab}	5±4.94^{bc}	2.77±0.83^{c}
Survival	100%	100%	100%	100%
Lidocaine induction				
Dose	100 µI/L	200 µI/L	300 µI/L	400 µI/L
Time (min)	6.91±4.60^{a}	2.69±1.16^{b}	1.80±0.44^{b}	2.09±0.63^{b}
Survival	100%	100%	100%	100%

Different superscripts show statistically significant differences between treatments ($P<0.05$).

Table 3. Evaluation with eugenol and lidocaine for *D. latifrons* juveniles in four different concentrations.

Eugenol recovery				
Dose	25 µl/L	50 µl/L	100 µl/L	200 µl/L
Time (min)	6.44±2.55	6.51±2.03	10.20±5.51	11.48±6.12
Survival	100%	100%	100%	100%
Lidocaine recovery				
Dose	100 µl/L	200 µl/L	300 µl/L	400 µl/L
Time (min)	3.94±2.84^{c}	8.90±3.32^{b}	9.81±3.49^{b}	16.34±5.06^{a}
Survival	100%	100%	100%	100%

Different superscripts show statistically significant differences between treatments (P<0.05).

Anesthesia recovery times for lidocaine treatments were higher for 400 µL/L (16.34 min) compared to all treatments (P<0.05). The 200 µL (8.9 min) and 300 µL/L (9.81 min) treatments were similar to each other (P>0.05), while the 100 µL/L treatment recorded the fastest recovery time of all treatments (P<0.05).

5. Discussion

The use of anesthetics in activities related to aquaculture have been of great importance in many countries, especially due to the requirements of good practices in which animal welfare is guaranteed, as a requirement for the commercialization of the products (Jerez-Cepa et al., 2019). Proof of this are the various methods that have been developed for its application. A fairly common method is by immersion in anesthetic solution, since a large number of fish can be applied simultaneously, since the gills in fish are the main route of entry and excretion of anesthetic agents (Sneddon, 2012).

This is the first study on the use of eugenol and lidocaine by immersion in *D. latifrons*, in which the different stages of anesthesia (induction and recovery), behavior and apparent post-anesthesia health status were determined.

An ideal anesthetic should be easily administered, keep the animal in the desired stage, and act quickly with low doses; it must be rapidly cleared to achieve recovery once the anesthetic is removed from the animal (Coyle et al., 2004); sedation should be achieved in a period not exceeding 10 minutes (preferably less than 5 min), and recovery from normal swimming in a period of less than 15 minutes, not more than 10 minutes (Oliveira Vidal et al., 2006).

The results obtained suggest that eugenol is an alternative for anesthesia given its low cost, low toxicity and low bioaccumulation in tissues (Cooke et al., 2004), and it is easily metabolized and is rapidly excreted (Wagner et al., 2002). Its use reduces hypermobility or stress caused by animal handling, reducing the risk of damage to fish (Inoue et al., 2003). While, for lidocaine, its use as an anesthetic is widely used in humans and animals due to its low cost and easy access (Muir et al., 2008), and its metabolism takes place in the liver before being excreted (Lauretti, 2008).

The effectiveness of clove oil as an anesthetic has been reported for other fish species such as matrincha, *Brycon cephalus* (Inoue et al., 2003); tambaqui, *Colossoma macropomum* (Roubach et al., 2005); spotted, *Pseudoplatystoma corruscans* (Vidal et al., 2006); catfish, *Rhamdia quelen* (Cunha et al., 2006). In cyprinids, its effectiveness as an anesthetic with sedation, moderate anesthesia and deep anesthesia has been reported, with concentrations from 25-100 mg/L, in golden perch 15-50 mg/L or in catfish 100-150 mg/L, to mention a few (Neiffer and Stamper, 2009). On the other hand, the effectiveness of lidocaine in fish has been documented, where the doses used can vary depending on the size of the fish; for zebrafish <2mg/kg (Avdesh et al., 2012), for trout between 100-350 mg/L (Vargas-Vargas, 2017), and for crappie 300 mg/L (Urzúa Pizarro et al., 2022). The concentrations of clove oil (eugenol) used for *Piaractus mesopotamicus* in concentrations of 120 and 150 mg/L, determine even faster inductions, without damaging the recovery of the animals (Solís-Murgas et al., 2010). Similar case for this study with *D. latifrons*, in which concentrations of 100-200 µl/L induced deep anesthesia (2-5 minutes), and recovery was within the ideal range (10 minutes) with successful survival. With *Cheirodon interruptus*, Urzúa et al. (2022), report that a concentration of 300 mg/L of lidocaine, produces deep anesthesia in a few minutes (1-3 minutes), with recovery time of 8 minutes, which agrees with what was recorded for *D. latifrons*, in induction with lidocaine at concentrations of 300-400 µL/L, but with a prolonged recovery time (8-20 minutes).

It has been reported that low concentrations of clove oil can induce light anesthesia, as in the case of *Pterophyllum scalare*, which at concentrations of 20 mg/L allow procedures such as morphometry, weighing, marking, transportation, and classification (Millán-Ocampo et al., 2012). However, in *D. latifrons* the lowest concentrations are not effective in terms of induction, due to the long time it takes to take effect, which is detrimental to animal welfare.

The concentrations used in the results shown for *D. latifrons* suggest the use of 200 µl/L clove oil (eugenol) to induce deep anesthesia in less than two minutes, whereas for lidocaine, deep anesthesia is achieved in one to three minutes with a concentration of 400 µl/L. Both eugenol (clove oil) and lidocaine turned out to be anesthetic compounds with possibilities of application in the handling and transport of *D. latifrons*.

In the case of clove oil, it is also a compound of natural origin that is easy to excrete and has good performance at the time of dosing. It is easy to obtain and low cost. Therefore, it is presented as the best choice for use with the species of interest in this study.

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