An Acad Bras Cienc (2021) 93(Suppl. 4): e20210078 DOI 10.1590/0001-3765202120210078
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ANIMAL SCIENCE

Linalool induces relaxation of the mantle of golden apple snail (*Pomacea canaliculata*)

ADRIANE E. BIANCHINI, SHARINE N. DESCOVI, BERTA M. HEINZMANN & BERNARDO BALDISSEROTTO

Abstract: The objective of this study was to evaluate the possible relaxing effect of essential oils (EOs) (*Aloysia triphylla* and *Lippia alba*) and phytochemicals (citral and linalool) in the gastropod *Pomacea canaliculata*. Animals were exposed to compounds at the concentrations range of 25-750 µL L^{-1}. Magnesium chloride ($MgCl_2$, 10-50 g L^{-1}) and control group (ethanol 6.75 mL L^{-1}, highest concentration used for treatment dilution) were also tested. The EOs, citral and $MgCl_2$ had no relaxing effect at the concentrations range tested, and citral caused aversive behavior (closure of the operculum) from 90 µL L^{-1}. Exposure to linalool at 25, 50, 100, 200 and 400 µL L^{-1} relaxed 28, 76, 88, 96 and 100% of the animals, respectively. The concentrations of 25, 50 and 400 µL L^{-1} differed statistically from each other, while 100 and 200 µL L^{-1} were equal to 50 and 400 µL L^{-1}. All animals recovered up to 40 min, except at of 400 µL L^{-1}. Linalool is effective for relaxing *P. canaliculata* and can be useful in management techniques that require relaxation. However, further studies are needed to certify whether linalool is appropriate for maintaining animal welfare in invasive procedures that require total insensitivity.

Key words: anesthetics, essential oil, gastropods, mollusks, relaxation.

INTRODUCTION

Mollusks are important for various sectors of the economy, such as pearl production and food. The Food and Agriculture Organization (FAO) estimates that in 2016 mollusk production reached 17.1 million tons (metric) (FAO 2018). In addition, mollusks are extensively used for research purposes due to the anatomy of their nervous system (Winlow et al. 2018). In this context, anesthetics are used to perform many procedures (Garr et al. 2012), to ensure animal welfare, to assist in performing neurobiological research (Lewbart & Mosley 2012), by promoting animal relaxation and/or complete anesthesia. Among anesthetics/relaxants for mollusks, magnesium hydrochloride ($MgCl_2$) is widely used in gastropods (Garr et al. 2012, Zeidan et al. 2018), bivalves (Puchnick-legat et al. 2015), and cephalopods (Winlow et al. 2018). Other substances used are ethanol (Lewbart & Mosley 2012), 1-phenoxy-2-propanol (Wyeth et al. 2009), 2-phenoxyethanol, benzocaine (Mamangkey et al. 2009), and isoflurane (Polese et al. 2014).

Natural products constitute an important source of bioactive and biodegradable molecules (Bakkali et al. 2008). Among the different vegetable extractives, essential oils (EOs) extracted from aromatic plants and/or isolated compounds are successfully used as anesthetics in fish (Baldisserotto et al. 2018, Bianchini et al. 2017b, Hoseini et al. 2019) and crustaceans (Li et al. 2018). Eugenol is effective as an anesthetic/relaxant in marine *Haliotis tuberculata coccinea* (Bilbao et al. 2010) and freshwater *Pomacea poludosa* and *Pomacea* *canaliculata* gastropods (Bianchini et al. 2017a, Garr et al. 2012), but not in the bivalve *Pinctada maxima* (Mamangkey et al. 2009). Recently, the anesthetic/relaxant action of *Origanum majorana* and *Ocimum americanum* in *P. canaliculata* have been described (Bianchini et al. 2017a).

In the current study, the possible relaxing effects of EOs from *Lippia alba* and *Aloysia triphylla* were evaluated. Both have anesthetic effects in fish (Almeida et al. 2018, Batista et al. 2018, Gressler et al. 2014) and shrimp (*Litopenaeus vannamei*; Parodi et al. 2012). The effectiveness of linalool and citral were also evaluated. These compounds are major constituents of the EOs of *L. alba* and *A. triphylla*, respectively, collected in certain regions (Almeida et al. 2018). In view of the anesthetic activity proven in other species, we believe that these EOs, as well as their isolated phytochemicals, can also be used in mollusk species. For this reason, we used the gastropod *P. canaliculata* as the study organism.

MATERIALS AND METHODS

Animals

The animals were obtained from a fish culture in São João do Polêsine city (Rio Grande do Sul, Brazil) and then acclimated in 250-L tanks in a water re-circulation system with activated charcoal/stone filters (temperature 25 °C, pH 7.32, and dissolved oxygen levels 7.43 mg L^{-1}). Animals were fed "ad libitum" with fresh lettuce. The identification of animals was carried out by Dr. Carla Bender Kotzian (Department of Biology, Federal University of Santa Maria, UFSM). Invertebrate experiments do not require approval by the ethics committee.

Essential oil and phytochemicals

The EOs were extracted from the leaves of *A. triphylla* (EOA) and *L. alba* (EOL) cultivated at the campus of the Universidade Federal de Santa Maria in the city of Frederico Westphalen, southern Brazil. The plant material was subjected to extraction by hydro-distillation in a Clevenger type apparatus. Subsequent chemical characterization was done by gas chromatography using an Agilent 7890A gas equipment coupled to an Agilent 5975C mass selective detector (GC-MS). The majoritarian constituents identified were geranial or citral A (24.32%), limonene (21.69%) and cis-Carveol (18.53%) in the EOA, and *S*-(+)-Linalool (66.34%), eucalyptol (10.63%) and aromadendrene (3.48%) in the EOL. More details on chromatographic conditions and chemical characterization can be found in Almeida et al. (2018).

Linalool (purity: 98%, density: 0.86 g mL^{-1}, Sigma-Aldrich®, Brazil), citral (purity ³ 96% density: 0.89 g mL^{-1}, Sigma-Aldrich®, Brazil) (racemic mixtures), and magnesium chloride ($MgCl_2$, PA, Synth®, Brazil) were obtained commercially.

Experimental design: relaxation

Animals (shell: 3.43 ± 0.28 cm) were placed in continuously aerated 1-L aquaria (n = 5/aquarium, 5 replicates) for induction of relaxation and recovery. EOA, EOL, linalool, and citral, previously dissolved in 95% ethanol (1:10) were tested at concentrations range of 25-750 µL L^{-1}. Magnesium chloride ($MgCl_2$, diluted in water) was tested at 10, 20, 40 and 50 g L^{-1}. A control group was exposed to ethanol (vehicle used for the dilution of EOs and phytochemicals) at the highest concentration used for treatment dilution (6.75 mL L^{-1}). The protocol used to evaluate the relaxing activity of these animals was performed as described previously by Bianchini et al. (2017a) and adapted from Garr et al. (2012). Briefly, animals were considered relaxed when they did not show any resistance to the pulling of the operculum with the aid of a forceps. The animals were evaluated every 10 min for up to 40 min. The relaxed animals were transferred to anesthetic-free aquaria and the recovery time was evaluated at 10-min intervals. The animal was considered recovered when it presented resistance to pulling of the operculum. Mortality was evaluated 24 h after the experiment.

Aversive behavior assessment

The animals exposed to citral had aversive behavior characterized by immediate closure of the operculum (Figure 1). This behavior may have hindered the absorption of citral by the animal. Therefore, a second experiment with gradual exposure of the animals to citral was carried out. For this, a re-use system with Aquaria 1 and 2 (total 4 L) was set up. Into Aquarium 1, 5 μL L^{-1} citral was added every 5 min, which slowly came into equilibrium with Aquarium 2 in which the animals (n = 5, duplicate) were present. Citral concentration gradually increased until the animals (60%) closed the operculum. This concentration was considered as the minimum concentration of citral capable of causing aversive behavior. The objective of this second experiment was also to verify whether citral would have relaxing activity when its concentration increased gradually. Therefore, the verification of a possible relaxing effect was also performed according to the methodology used in the first experiment.

Statistical analysis

Data were evaluated by Kruskal-Wallis test followed by Dunn post-hoc. The statistical tests were performed using GraphPad Prism software (San Diego, CA, EUA, version 6.0), and the minimum significance level for all analyses was set at $p < 0.05$. Data are reported as mean ± SEM.

RESULTS

Relaxing effect

EOL, EOA, $MgCl_2$ and ethanol control did not cause a relaxing effect up to the highest concentration tested (750 μL L^{-1} for Eos, 50 g L^{-1} for $MgCl_2$ and 6.75 mL L^{-1} for ethanol). In contrast, linalool caused relaxation in this species with maximum effect (100% relaxation) at 400 μL L^{-1} (Figures 1b and 2a). All animals recovered within the maximum observation period (40 min), except those exposed to the highest concentration (400 μL L^{-1}). The lowest concentration tested (25 μL L^{-1}) relaxed only 28 % (± 10.19%) of the animals tested. Regarding relaxation time, there was no

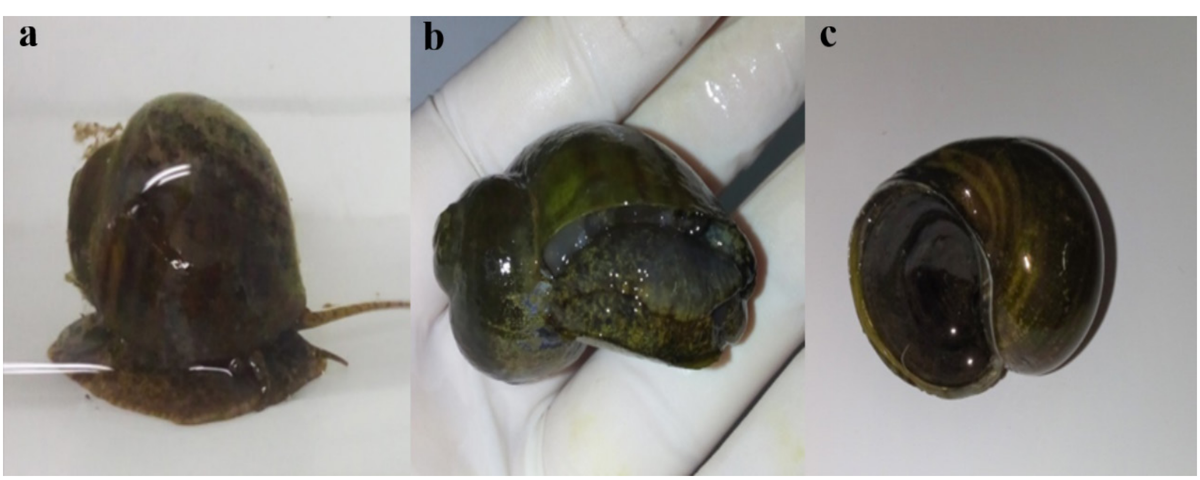

Figure 1. Different stages of *Pomacea canaliculata* exposed or not to substances with relaxing properties. (a) Normal stage or with essential oil without effect, (b) Relaxation stage, (c) Aversive-like effect observed for citral.

correlation between time and concentration. At the most effective concentrations (50, 100, 200 and 400 μL L^{-1}), the relaxation time did not differ significantly between them, with the exception of the 100 μL L^{-1} concentration which differed from 50 μL L^{-1}.

Citral did not cause relaxation. However, it was observed that exposure to all citral concentrations caused an aversive behavior in the animals characterized by the immediate closure of the operculum, which remained in this way for all the time the animals were in contact with the compound (Figure 1c). In relation to other adverse effects, no mortality occurred with the compounds tested in the 24-h period after the experiments. However, in animals exposed to linalool 400 μL L^{-1} loss of mucus was observed.

Aversive behavior assessment

The minimum citral concentration that promoted an aversive effect in the animals was 90 μL L^{-1} (the same value in the two duplicates). Citral also did not promote a relaxing effect in this exposure protocol. Animals returned to normal (opening of the operculum) quickly after citral removal. No mortality occurred after 24 hours.

DISCUSSION

Anesthetic substances can produce muscle relaxation, analgesia, sedation and even general anesthesia that involves general CNS depression (Winlow et al. 2018). In this study, the effectiveness of the compounds was based on the ability to induce relaxation of the mantle, identified by the lack of resistance to the act of pulling the operculum (Garr et al. 2012). Therefore, we use the term relaxation to designate this effect, a standard term used in other studies with bivalves (Acosta-Salmón et al. 2005, Acosta-Salmón & Davis 2007, Butt et al. 2008, Mamangkey et al. 2009) and gastropods (Aquilina & Roberts 2000, Bianchini et al. 2017a, Garr et al. 2012) where a similar methodology was used. The assessment of general anesthetic effect in molluscs may require non-behavioral methodologies. In an *ex vivo* study, for example, it was found that 2-phenoxyethanol, in addition to decreasing the muscle contraction force in isolated deganglionized oral masses, also reduces the excitability in central neurons of *Hermissenda crassicornis* (Wyeth et al. 2009), which is indicative of a general anesthetic agent.

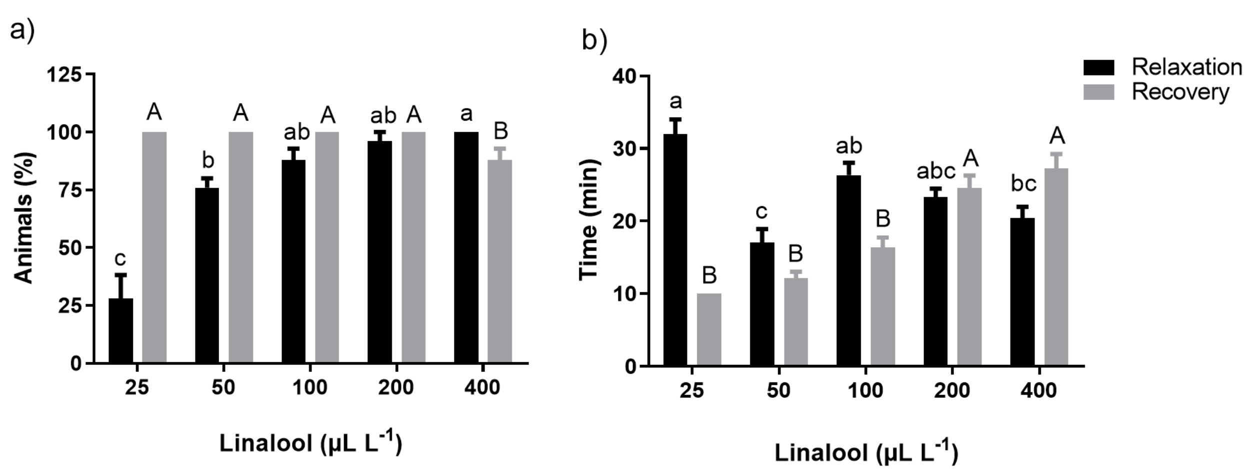

Figure 2. Percentage of relaxation (a) and recovery of *Pomacea canaliculata* exposed to linalool and their respective times (b). Lowercase letters indicate statistical difference between linalool concentrations in the relaxation stage, and uppercase letters indicate statistical difference between linalool concentrations in the recovery stage (Kruskal-Wallis test and Dunn post-hoc, $p < 0.05$, as mean ± SEM).

$MgCl_2$ is used as anesthetic in different species of mollusks and other invertebrates (Lewbart & Mosley 2012). Low concentrations usually cause relaxation in many species. In the gastropod *P. paludosa*, for example, 20 g L^{-1} of $MgCl_2$ was 80% effective (Garr et al. 2012). Contrary to expectation, for *P. canaliculata* it was not effective up to 50 g L^{-1}. It is likely that *P. canaliculata* has higher resistance to $MgCl_2$ compared to other species, due to some unknown factor. Adami et al. (2019) recently reported the difficulty in anesthetizing giant African snails (*Acathina fulica*) with alfaxalone and ketamine, well-known general anesthetics. It has also been demonstrated the existence of genetic variation for the phenotype of anesthesia efficacy in *Potamopyrgus antipodarum*, a New Zealand freshwater snail (Song et al. 2021).

Relaxation caused by linalool was expected, as this monoterpenoid has depressant activity in fish (*Rhamdia quelen* and *Cyprinus carpio*) and shrimp (*Litopenaeus vannamei*) species (Becker et al. 2015, Heldwein et al. 2014, Taheri Mirghaed et al. 2016). In addition, linalool is also known to act on the nervous system of mammals and rodents through mechanisms of action that include inhibition of glutamatergic receptors (Aprotosoaie et al. 2014), which can also be found in gastropods (Greer et al. 2019). Compared to other natural compounds previously tested in this species, linalool is as effective as eugenol (both promoted 100% relaxation) and more effective than the *O. americanum* EO (70%) and *Origanum majorana* EO (80%). Besides that, it was the most potent compound tested so far (Bianchini et al. 2017a).

Exposure to 50 µl L^{-1} linalool promoted relaxation in 76% or more of the animals, reaching 100% with 400 µl L^{-1}. However, at this concentration it did not occur total recovery of the animals up to 40 min, and besides there was loss of mucus. The increase in mucus production and release by gastropods is one of the first reactions to stressors, such as chemical irritation caused by molluscicidal agents. Its function is to create a protective barrier between the irritating agent and the animal's skin (Barker 2002). Therefore, due to the adverse effects observed with 400 µl L^{-1} linalool, concentrations higher than 200 µl L^{-1} are not recommended in this species. In addition, it was possible to achieve satisfactory efficacy, fast recovery time, and absence of the noticeable adverse effects at concentrations up to 200 µl $L^{-1.}$

While linalool promoted relaxation, EOL (around 66% *S*-(+)-linalool) had no relaxing effect on golden apple snails, even at the highest concentration tested, in contrast to the results obtained in fish (Almeida et al. 2018, Heldwein et al. 2014). The other constituents of EOL may have antagonized the relaxing effect of *S*-(+)-linalool in golden apple snails by some unknown mechanism (Bakkali et al. 2008). The EOL has the *S* enantiomer, and the linalool we tested is made up of a racemic mixture (*S*-(+) and *R*-(-)-linalool). According to this information, we should also consider the hypothesis that the R enantiomer may be more potent than the S in producing relaxation in this species, which would explain in part the lack of effect of the EOL. On the other hand, *S*-(+)-linalool is known for its anesthetic effect on *R. quelen* (Heldwein et al. 2014). Therefore, further studies are necessary to clarify this doubt. EOA was also not effective in *P. canaliculata* despite causing sedation/anesthesia in fish (Almeida et al. 2018, Gressler et al. 2014) and shrimp (Parodi et al. 2012).

A relaxing effect was also absent for citral, and aversive-like behavior was observed in the animals shortly after exposure. The closure of the operculum characterizes a defensive behavior against substances identified by the animal as potentially noxious. Similar behavior was observed in gastropods or bivalves exposed to sudden changes of environmental conditions (e.g. temperature) (McAlister & Fisher 1968). This same behavior was also observed during the attempt to anesthetize the giant African snails with ketamine and alfaxalone by different routes (Adami et al. 2019). The shell of mollusks, mainly bivalves and gastropods, has a protective function. Thus, the retraction into the shell and closing the operculum is one of the defense mechanisms and reflects the animal's perception of an unknown/dangerous situation, or chemical or physical stimulus (Goodchild et al. 2020). Considering that citral is an irritating substance for skin / mucous membranes in certain concentrations (OECD 2001), it is possible that citral caused irritation or discomfort in the mantle mucosa, triggering this aversive behavior. The absorption of citral was probably hindered by the closure of the operculum, and even when the animals came into contact with gradual concentrations, no relaxing effect was observed, possibly due to low concentrations (<90 µL L^{-1}). Studies with other mollusks, particularly those devoid of shell, would be of great interest to better elucidate the effects of citral in these animals.

In conclusion, linalool may be useful as relaxant in gastropods. Nevertheless, other studies needed to be performed in order to test the effectiveness of linalool on other mollusks species, as well as the ideal concentrations. Not less important, the effectiveness of linalool in procedures that require a state of deep anesthesia must be confirmed in order to maintain animal welfare.

Acknowledgments

A.E. Bianchini received a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) scholarship. B. Baldisserotto and B.M. Heinzmann received Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) research fellowships, and S. Descovi FAPERGS (Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul) fellowship.

REFERENCES

ACOSTA-SALMÓN H & DAVIS M. 2007. Inducing relaxation in the queen conch *Strombus gigas* (L.) for cultured pearl production. Aquaculture 262: 73-77.

ACOSTA-SALMÓN H, MARTÍNEZ-FERNÁNDEZ E & SOUTHGATE PC. 2005. Use of relaxants to obtain saibo tissue from the blacklip pearl oyster (*Pinctada margaritifera*) and the Akoya pearl oyster (*Pinctada fucata*). Aquaculture 246: 167-172.

ADAMI C, MONTICELLI P & D'OVIDIO D. 2019. Challenges encountered while attempting anaesthesia of giant African snails (*Acathina fulica*). Vet Anaesth Analg 46: 713-715.

ALMEIDA APG, HEINZMANN BM, VAL AL & BALDISSEROTTO B. 2018. Essential oils and eugenol as anesthetics for *Serrasalmus rhombeus*. Bol Inst Pesca 44: 44-50.

AQUILINA B & ROBERTS R. 2000. A method for inducing muscle relaxation in the abalone, *Haliotis iris*. Aquaculture 190: 403-408.

APROTOSOAIE AC, HĂNCIANU M, COSTACHE II & MIRON A. 2014. Linalool: A review on a key odorant molecule with valuable biological properties. Flavour Fragr J 29: 193-219.

BAKKALI F, AVERBECK S, AVERBECK D & IDAOMAR M. 2008. Biological effects of essential oils - A review. Food Chem Toxicol 46: 446-475.

BALDISSEROTTO B, BARATA LES, SILVA AS, LOBATO FF, SILVA LL, TONI C & SILVA LVF. 2018. Anesthesia of tambaqui *Colossoma macropomum* (Characiformes: Serrasalmidae) with the essential oils of *Aniba rosaeodora* and *Aniba parviflora* and their major compound, linalool. Neotrop Ichthyol 16(1): e170128.

BARKER GM. 2002. Molluscs as Crop Pests. New Zealand: ABI publishing, 400 p.

BATISTA ES, BRANDÃO FR, MAJOLO C, INOUE LAKA, MACIEL PO, DE OLIVEIRA MR, CHAVES FCM & CHAGAS EC. 2018. *Lippia alba* essential oil as anesthetic for tambaqui. Aquaculture 495: 545-549.

BECKER A, JENSEN L, GARCIA LO, SILVA LL, MONSERRAT JM, WASIELESKY W, HEINZMANN BM & BALDISSEROTTO B. 2015. The efficacy of linalool on transportation and anesthesia of white shrimp *Litopenaeus vannamei* (Crustacea, Penaeidae). FENACAN 54-60.

BIANCHINI AE, CUNHA JA, BRUSQUE ICM, PINHEIRO CG, SCHINDLER B, HEINZMANN BM & BALDISSEROTTO B. 2017a. Relaxing effect of eugenol and essential oils in *Pomacea canaliculata*. Ciênc Rural 47(10): e20170210.

BIANCHINI AE, GARLET QI, DA CUNHA JA, BANDEIRA JUNIOR G, BRUSQUE ICM, SALBEGO J, HEINZMANN BM & BALDISSEROTTO B. 2017b. Monoterpenoids (thymol, carvacrol and S-(+)-linalool) with anesthetic activity in silver catfish (*Rhamdia quelen*): evaluation of acetylcholinesterase and GABAergic activity. Braz J Med Biol Res 50(12): e6346.

BILBAO A, DE VICOSE GC, PINO VIERA MD, SOSA B, FERNÁNDEZ-PALACIOS H & HERNÁNDEZ MDC. 2010. Efficiency of Clove Oil as Anesthetic for Abalone (*Haliotis Tuberculata Coccinea*). J Shellfish Res 29: 679-682.

BUTT D, O'CONNOR SJ, KUCHEL R, O'CONNOR WA & RAFTOS DA. 2008. Effects of the muscle relaxant, magnesium chloride, on the Sydney rock oyster (Saccostrea glomerata). Aquaculture 275: 342-346.

FAO - FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. 2018. The State of World Fisheries and Aquaculture: Meeting the sustainable development goals. http://www.fao.org/3/i9540en/I9540EN.pdf.

GARR AL, POSCH H, MCQUILLAN M & DAVIS M. 2012. Development of a captive breeding program for the Florida apple snail, *Pomacea paludosa*: Relaxation and sex ratio recommendations. Aquaculture 370-371: 166-171.

GOODCHILD CG, SCHMIDT LM & DURANT SE. 2020. Evidence for the 'behavioural character' hypothesis: does boldness programme disparate antipredator strategies? Anim Behav 164: 123-132.

GREER JB, MAGER EM & FIEBER LA. 2019. Altered expression of ionotropic L-Glutamate receptors in aged sensory neurons of *Aplysia californica*. PLoS ONE 14(5): e0217300.

GRESSLER LT ET AL. 2014. Silver catfish *Rhamdia quelen* immersion anaesthesia with essential oil of *Aloysia triphylla* (L'Hérit) Britton or tricaine methanesulfonate: effect on stress response and antioxidant status. Aquac Res 45: 1061-1072.

HELDWEIN CG, SILVA LL, GAI EZ, ROMAN C, PARODI TV, BÜRGER ME, BALDISSEROTTO B, FLORES MDM & HEINZMANN BM. 2014. S-(+)-Linalool from *Lippia alba*: Sedative and anesthetic for silver catfish (*Rhamdia quelen*). Vet Anaesth Analg 41: 621-629.

HOSEINI SM, MIRGHAED AT & YOUSEFI M. 2019. Application of herbal anaesthetics in aquaculture. Rev Aquac (3): 550-564.

LEWBART GA & MOSLEY C. 2012. Clinical anesthesia and analgesia in invertebrates. J Exot Pet Med 21: 59-70.

LI Y, SHE Q, HAN Z, SUN N, LIU X & LI X. 2018. Anaesthetic effects of eugenol on Grass Shrimp (*Palaemonetes sinensis*) of different sizes at different concentrations and temperatures. Sci Rep 8: 1-9.

MAMANGKEY NGF, ACOSTA-SALMON H & SOUTHGATE PC. 2009. Use of anaesthetics with the silver-lip pearl oyster, *Pinctada maxima* (Jameson). Aquaculture 288: 280-284.

MCALISTER RO & FISHER FM. 1968. Responses of the false limpet, Siphonaria pectinata Linnaeus (Gastropoda, Pulmonata) to osmotic stress. Biol Bull 134(1): 96-117.

OECD. 2001. SIDS Initial Assessment Report for 13th SIAM. Citral. CAS N°:5392-40-5. Japan.

PARODI TV ET AL. 2012. The anesthetic efficacy of eugenol and the essential oils of *Lippia alba* and *Aloysia triphylla* in post-larvae and sub-adults of *Litopenaeus vannamei* (Crustacea, Penaeidae). Comp Biochem Physiol C Toxicol Pharmacol 155: 462-468.

POLESE G, WINLOW W & DI COSMO A 2014. Dose-Dependent Effects of the Clinical Anesthetic Isoflurane on *Octopus vulgaris* : A Contribution to Cephalopod Welfare. J Aquat Anim Health 26: 285-294.

PUCHNICK-LEGAT A, LEGAT JFA, GOMES CHAM, SUHNEL S & MELO CMR. 2015. Anesthesia in oysters of the genus *Crassostrea* cultured in Brazil. Bol Inst Pesca 41: 785-793.

SONG Q, MAGNUSON R, JALINSKY J, ROSEMAN M, & NEIMAN M. 2021. Intraspecific genetic variation for anesthesia success in a New Zealand freshwater snail. Genetica 149: 47-54.

TAHERI MIRGHAED A, GHELICHPOUR M & HOSEINI SM. 2016. Myrcene and linalool as new anesthetic and sedative agents in common carp, *Cyprinus carpio* - Comparison with eugenol. Aquaculture 464: 165-170.

WINLOW W, POLESE G, MOGHADAM HF, AHMED IA & DI COSMO A. 2018. Sense and insensibility - An appraisal of the effects of clinical anesthetics on gastropod and cephalopod molluscs as a step to improved welfare of cephalopods. Front Physiol 9: 1147.

WYETH RC, CROLL RP, WILLOWS AOD & SPENCER AN. 2009. 1-Phenoxy-2-propanol is a useful anaesthetic for gastropods used in neurophysiology. J Neurosci Methods 176(2): 121-128.

ZEIDAN GC, BARROSO CM & BOEHS G. 2018. Efficiency of anesthetics on female gastropod *Littoraria angulifera*. Bol Inst Pesca 44(3): e304.

How to cite
BIANCHINI AE, DESCOVI SN, HEINZMANN BM & BALDISSEROTTO B. 2021. Linalool induces relaxation of the mantle of golden apple snail (*Pomacea canaliculata*). An acad Bras Cienc 93: e20210078. DOI 10.1590/0001-3765202120210078.

Manuscript received on January 19, 2021;
accepted for publication on July 4, 2021

ADRIANE E. BIANCHINI[1]
https://orcid.org/0000-0002-9141-0085

SHARINE N. DESCOVI[2]
https://orcid.org/0000-0001-9496-2195

BERTA M. HEINZMANN[1,3]
https://orcid.org/0000-0002-6509-949X

BERNARDO BALDISSEROTTO[1,2,4]
https://orcid.org/0000-0002-8770-0100

[1]Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria, Camobi, Av. Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

[2]Programa de Pós-Graduação em Zootecnia, Universidade Federal de Santa Maria, Camobi, Av. Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

[3]Universidade Federal de Santa Maria, Departamento de Farmácia Industrial, Camobi, Av. Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

[4]Universidade Federal de Santa Maria, Departamento de Fisiologia e Farmacologia, Camobi, Av. Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

Correspondence to: **Bernardo Baldisserotto**
E-mail: bbaldisserotto@hotmail.com

Author contributions

AEB: Study design, data interpretation, writing - review and editing of manuscript. SND: Study design and preparation of manuscript. BMH: Supervision, funding acquisition, writing – review and editing of manuscript. BB: Study design, writing - review and editing, supervision, project administration, funding acquisition.