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HEALTH SCIENCES

Eugenol as an anesthetic for two African cichlid species (*Aulonocara nyassae* and *Aulonocara stuartgranti*): effects on induction time, recovery time and ventilatory frequency

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Abstract: This study evaluated the anesthetic efficacy of eugenol for two size classes of *Aulonocara nyassae* and *Aulonocara stuartgranti*. The experimental groups were classified as follows: *A. nyassae*, Juvenile I-Any (1.29 ± 0.35 g) and Juvenile II-Any (2.97 ± 0.67 g); and *A. stuartgranti*, Juvenile I-Ast (1.98 ± 0.60 g) and Juvenile II-Ast (3.69 ± 0.74 g). For each group, 80 specimens were randomly allocated to seven eugenol concentrations (25, 50, 75, 100, 125, 150 and 175 mg L^{-1}) and a control (ethanol) in a completely randomized design with 10 individuals per concentration. Anesthesia trials involved exposing one animal at a time to the different eugenol concentrations, and recording induction time, recovery time, but ventilatory frequency was also measured during induction and recovery. The efficacy of an anesthetic administered via immersion bath is related to rapid induction to deep anesthesia (<180 s) and short recovery (<300 s). Therefore, the study demonstrated that eugenol concentrations between 50 and 175 mg L^{-1} are recommended for Juvenile I-Any and between 25 and 175 mg L^{-1} for Juvenile II-Any. The ideal concentrations for *A. stuartgranti*, on the other hand, range from 25 to 175 mg L^{-1} for both evaluated size classes.

Key words: *Aulonocara* spp., biometric handling, clove essential oil, deep anesthesia, ornamental fish.

INTRODUCTION

Aquaculture, a rapidly expanding global sector, faces challenges arising from the intensification of production, particularly regarding animal welfare, climate change, and sustainability (Jones et al. 2022, Garlock et al. 2024, Chand & Dubey 2025). Notable among these challenges is the need to mitigate the impacts of physiological stress to fish, often triggered by specific but essential farming procedures such as biometry (Ferreira et al. 2021, Silva et al. 2026), transportation (Balamurugan et al. 2016), vaccination (Azizi et al. 2025), and reproduction (Corso et al. 2019). Effective stress management not only represents an ethical demand but also constitutes a decisive factor for fish productivity, health, and zootechnical performance (Luz & Favero 2024). In this sense, the choice of anesthetics for fish must follow criteria that consider their efficacy and toxicity not only for the animals, but also for handlers and the environment (Cunha et al. 2010, Aydın & Barbas 2020). Additionally, factors such as cost, availability, and impact on animal welfare also directly influence the choice of compounds (Ross & Ross 2008, Zahl et al. 2012, Sloman et al. 2019).

Although synthetic anesthetics, such as benzocaine, quinaldine, metomidate, propofol, 2-phenoxyethanol, and tricaine methanesulfonate (MS-222), have proven efficacy, their widespread use has raised growing regulatory and environmental concerns, in addition to the occurrence of adverse effects in fish exposed to these substances (Neiffer & Stamper 2009, Priborsky & Velisek 2018). Concerns about potential toxic residues, the requirement for long depuration periods before fish can be used for human consumption, and the environmental risks associated with the use of synthetic anesthetics have intensified the search for safer and more environmentally sustainable alternatives (see review by Purbosari et al. 2019). In this context, essential oils emerge as promising options as they are in line with the principles of environmentally responsible aquaculture (Aydın & Barbas 2020, Brandão et al. 2022).

Among natural alternatives, eugenol (4-allyl-2-methoxyphenol), the main component of clove (*Syzygium aromaticum*) essential oil (Keene et al. 1998), stands out for its anesthetic efficacy, low cost, and reduced environmental impact (Javahery et al. 2012, Priborsky & Velisek 2018, Vercellini et al. 2025). Although regulatory approval of eugenol for use with fish intended for human consumption is still a challenge (Ross & Ross 2008), its use is already a consolidated and preferred practice in ornamental aquaculture, being successfully applied to several ornamental species (Pattanasiri et al. 2017, Tarkhani et al. 2017, Fujimoto et al. 2018, Nuanmanee et al. 2024, Nascimento et al. 2025). However, despite its widespread use, the literature reports adverse effects in fish exposed to high eugenol concentrations, such as 250 mg L^{-1}, including severe gill damage (Abdel-Fattah et al. 2005). Furthermore, there are reports of potential genotoxic effects in fish (Nascimento et al. 2020) and more recent evidence of hepatotoxicity and neurotoxicity, such as prolonged cardiovascular recovery and spinal cord collapse (Rocha et al. 2024). These findings reinforce the need for investigations into anesthetic efficacy and species-specific sensitivity when using eugenol.

The genus *Aulonocara*, comprising cichlids endemic to Lake Malawi (Hashem et al. 2022), represents a highly valuable and popular segment in the international ornamental fish market (Schwalbe et al. 2012), driven by their vibrant colorations and active behavior. These species, ecologically divided into smaller (African cichlids) and larger (associated with sandy substrates) lineages (Konings 1995, Hashem et al. 2022), include highly sought-after varieties such as the *Aulonocara nyassae* (Peacock blue cichlid) and the *Aulonocara stuartgranti* (Yellow regal peacock) (Konings 1995). Although *A. nyassae* is already bred in captivity (Silva et al. 2021, 2022, Bonifácio et al. 2022), there remains a knowledge gap regarding effective anesthetic protocols for this valuable genus. The only published study to date (Ferreira et al. 2020) revealed that juvenile *A. nyassae* exhibit distinct responses to benzocaine according to size class, emphasizing the influence of body size in determining the ideal anesthetic concentration.

Thus, this study aimed to evaluate the anesthetic efficacy of eugenol for these two species of African cichlids (*A. nyassae* and *A. stuartgranti*), considering two size classes (Juvenile I and II), through the analysis of anesthesia induction and recovery times, as well as ventilatory frequency during biometry.

MATERIALS AND METHODS

Ethics committee

The experiments were carried out at the Laboratório de Aquacultura (LAQUA), affiliated with the Escola de Veterinária of the Universidade

Federal de Minas Gerais (UFMG). All procedures involving the fish were previously submitted for review and approved by the Comitê de Ética no Uso de Animais at UFMG (CEUA/UFMG), under protocol number 326/2019.

Fish acclimation and environment

The specimens (with homogeneous weights, weighed during each test) were acclimated for two weeks in rectangular tanks with a useful volume of 42 L of water, maintained in a recirculating aquaculture system (RAS) with constant supplemental aeration. The fish were allocated to their respective experimental tanks, separated and classified according to African cichlid species (*A. nyassae* or *A. stuartgranti*) and size class (Juvenile I or Juvenile II), according to the composition of the experimental groups. The two size classes established for *A. nyassae* were Juvenile I-Any, with 80 specimens having an average weight of 1.29 ± 0.35 g and an average total length of 44.90 ± 4.90 mm, and Juvenile II-Any, with 80 fish weighing 2.97 ± 0.67 g and measuring 59.15 ± 4.08 mm. Similarly, the two size classes established for *A. stuartgranti* were Juvenile I-Ast, with 80 fish weighing 1.98 ± 0.60 g and measuring 50.93 ± 5.36 mm in length, and Juvenile II-Ast, with 80 juveniles weighing 3.69 ± 0.74 g and measuring 63.35 ± 4.21 mm. The animals were fed three times a day (at 9 h, 12 h and 15 h) until apparent satiety. Feeding was carried out with extruded commercial feed (Supra, AQUAline) measuring 1.7 mm in diameter and containing 460.0 g kg^{-1} of crude protein, 80.0 g kg^{-1} of ether extract, 140.0 g kg^{-1} of mineral matter, 15.0 g kg^{-1} of phosphorus, and 20.0 g kg^{-1} of calcium, according to information provided by the manufacturer. All juveniles were subjected to food deprivation for a period of 24 h prior to the start of the tests.

The physicochemical variables of the RAS water were measured daily, always before the first offering of food to the fish. The average values obtained were temperature 27.21 ± 0.09 °C, pH 7.08 ± 0.06 (measured with a Hanna® HI98130 multiparameter probe), dissolved oxygen 6.81 ± 0.58 mg L^{-1} (Water Quality Meter® AK87 oximeter) and total ammonia 0.53 ± 0.02 mg L^{-1} (Alfakit® Labcon Test colorimetric kit). A 50% renewal of the useful volume of the RAS water was performed weekly. A photoperiod of 12 hours of light and 12 hours of darkness was adopted.

Anesthetic and preparation of solutions

The eugenol (anesthetic) used in this study, with 99% purity, was purchased from Biodinâmica Química e Farmacêutica LTDA (Ibiporã, Paraná, Brazil). All eugenol concentrations (25, 50, 75, 100, 125, 150, 175 mg L^{-1}) were previously diluted in 5 mL ethanol, and the control group (0 mg L^{-1}) consisted of only ethanol (5 mL, 99%) without anesthetic (Ferreira et al. 2021).

Assessment of eugenol anesthetic efficacy

Anesthesia assessments were performed independently for each experimental group, categorized by species (*A. nyassae* and *A. stuartgranti*) and size class (Juveniles I and II) (see *Acclimation of specimens and environment*). For each group, 80 fish were randomly distributed among seven eugenol concentrations (25, 50, 75, 100, 125, 150, and 175 mg L^{-1}; 5 mL ethanol) and the control (0 mg L^{-1}; 5 mL ethanol), following Ferreira et al. (2021) with adaptations. The experimental design was completely randomized, with ten individuals per concentration (n = 10), and each fish was considered an experimental unit (Fig. 1).

The anesthetic induction tests were performed by transferring the specimens (one at a time) to a beaker containing 1 L of water with the pre-established concentration. Each fish was processed individually through induction, biometric handling, and recovery

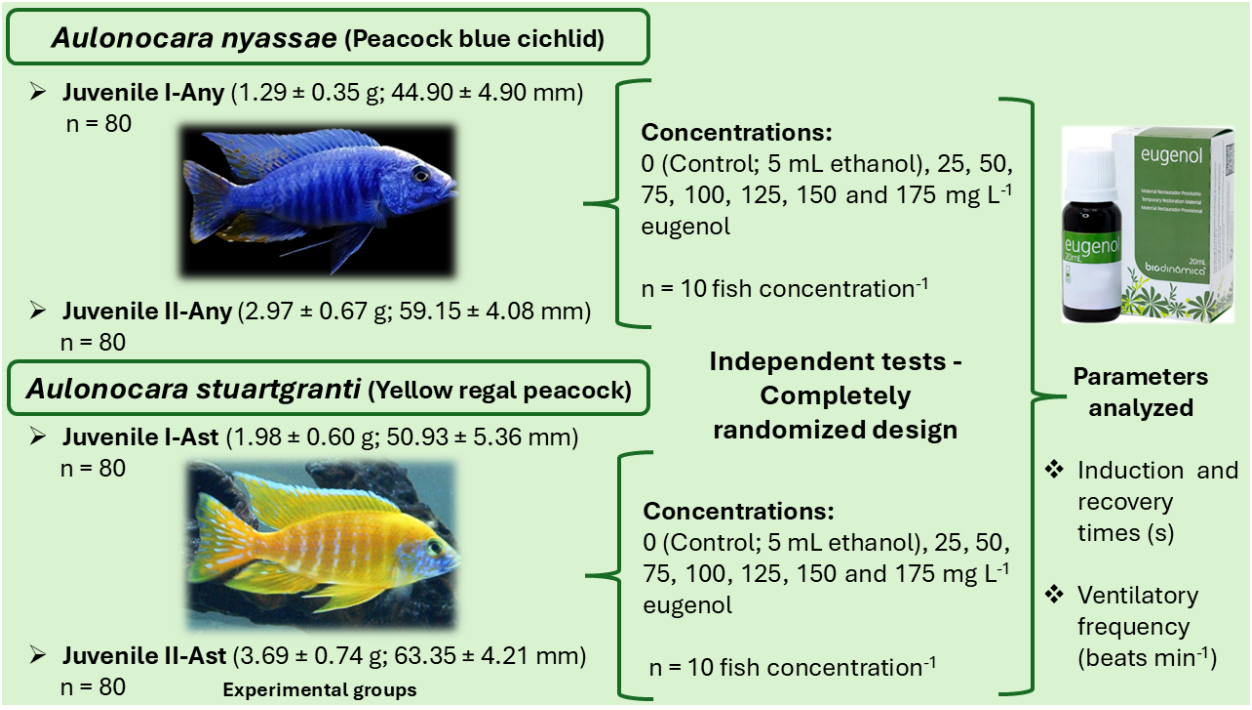

Figure 1. Schematic diagram of the materials and methods employed in the study.

assessment before the next individual was subjected to anesthesia. For each anesthetic concentration, a new solution was prepared and used for the anesthesia of five fish, after which it was discarded. The amount of time required for induction was recorded using a digital stopwatch (Taksun® Ts1809), starting at the moment the specimen was introduced into the solution and stopping when deep anesthesia was reached. Ventilatory frequency (VF) was monitored during the anesthetic induction process by counting the number of opercular beats per minute, according to the methodology adopted in previous studies (Alvarenga & Volpato 1995, Ferreira et al. 2020, Ananias et al. 2022). The phase of deep anesthesia, adopted in this study, was defined based on the same criteria observed by Keene et al. (1998) and Ferreira et al. (2020, 2021), considering the loss of balance, the complete absence of swimming and unconsciousness. Unconsciousness was defined as complete loss of equilibrium and absence of any response to tactile stimulation during manual handling. After reaching deep anesthesia, each juvenile underwent biometric management, being weighed (g) on an analytical balance (Marte® AD5002) and measured for total length (mm) with the aid of a digital caliper (Starrett® 799); biometric measurements lasted approximately 40 s. The specimens were then transferred to new beakers containing 1 L of clean water (from the RAS itself) to assess recovery. For recovery assessment, clean water was prepared and used for five fish per beaker. Recovery time was recorded and VF counted again. Both recovery time and VF were recorded individually for each fish. Full recovery was considered when the juveniles regained normal swimming balance and responded to tactile stimuli. Fish in the control group were observed for 10 min, without exposure to the anesthetic. Both criteria followed the methodologies described by Keene et al. (1998) and Ferreira et al. (2020, 2021).

At the end of the tests, the fish of each African cichlid species and size class were relocated to their original tanks (42 L), kept in the RAS with supplemental aeration, and distributed according to their respective experimental groups. Survival rate and return of appetite were monitored during the subsequent 24 hours, under the same conditions previously adopted in the acclimation period, according to the protocol previously established by Ferreira et al. (2021). Furthermore, return of appetite was assessed by visual observation during feeding, and was considered reestablished when all fish actively searched for and captured feed pellets.

Statistics

Data normality was verified using the Shapiro-Wilk test, while homogeneity of variance was assessed using Levene's test. Induction and recovery times, as well as VF, were subjected to analysis of variance (ANOVA), followed by regression analysis to identify the most appropriate model (significance level of 5%). Statistical analyses were performed using R software.

RESULTS

The control of 0 mg L^{-1} of eugenol (ethanol alone) did not induce anesthesia in either size class of either African cichlid species studied. Furthermore, survival rates were 100% for all concentrations tested (0, 25, 50, 75, 100, 125, 150 and 175 mg L^{-1}), both during the tests and during the subsequent 24 hours. Notably, feeding of all juveniles of both species was reestablished within the same interval.

Anesthetic effects on Aulonocara nyassae

Induction time for Juvenile I-Any showed a linear plateau response effect ($P < 0.05$) among the evaluated eugenol concentrations (Fig. 2a), with an inflection point at 70.80 mg L^{-1}, beyond which induction time remained constant. Recovery time also exhibited a linear plateau response effect ($P < 0.05$) (Fig. 2b), with an inflection point at 132.80 mg L^{-1}.

Induction time for Juvenile II-Any followed the same pattern, with a linear plateau response effect ($P < 0.05$) (Fig. 2c), but with an inflection point at 77.60 mg L^{-1}. On the other hand, recovery time showed a direct linear effect ($P < 0.05$) among the evaluated eugenol concentrations (Fig. 2d), ranging from 68 to 238 s.

Ventilatory frequency during anesthesia induction of Juvenile I-Any showed an inverse linear effect among the evaluated eugenol concentrations ($P < 0.05$), with values ranging from 101.82 to 77.82 beats min^{-1} (Table I). During recovery, VF also showed an inverse linear effect ($P < 0.05$) among the evaluated concentrations, ranging from 99.14 to 67.64 beats min^{-1}. In contrast, VF did not differ among the evaluated concentrations ($P > 0.05$) during induction of Juvenile II-Any. However, an inverse linear effect ($P < 0.05$) was observed during recovery, with VF values ranging from 87.71 to 74.21 beats min^{-1}.

Anesthetic effects on Aulonocara stuartgranti

Induction time for Juvenile I-Ast showed a linear plateau response effect ($P < 0.05$) among the evaluated eugenol concentrations (Fig. 3a), with an inflection point at 93.10 mg L^{-1}, beyond which induction time remained constant. Recovery time, in turn, showed a direct linear effect ($P < 0.05$), ranging from 63 to 245 s among the evaluated concentrations (Fig. 3b).

Induction time for Juvenile II-Ast followed a similar pattern, with a linear plateau response effect ($P < 0.05$) (Fig. 3c) and an inflection point at 92.40 mg L^{-1}, beyond which induction time remained stable. Recovery time showed a direct linear effect ($P < 0.05$) among the evaluated

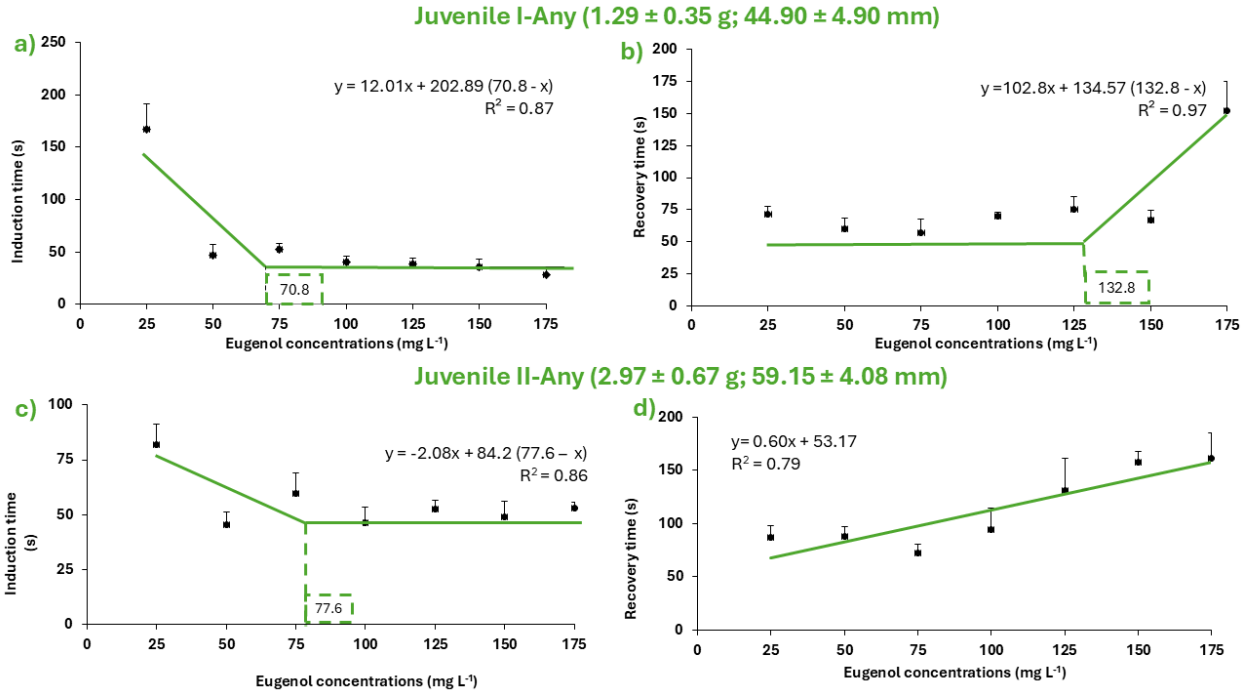

Figure 2. Anesthetic induction and recovery times (mean ± standard deviation, in seconds) for two size classes of *Aulonocara nyassae* exposed to eugenol.

concentrations (Fig. 3d), ranging from 119 to 176 s.

Ventilatory frequency during anesthesia induction of Juvenile I-Ast did not differ among the evaluated eugenol concentrations ($P > 0.05$) (Table II). However, an inverse linear effect was observed during recovery ($P < 0.05$), with VF ranging from 89.95 to 55.45 beats min^{-1}. Similarly, VF during induction of Juvenile II-Ast also did not differ among the evaluated concentrations ($P > 0.05$). Additionally, an inverse linear effect was observed during recovery ($P < 0.05$), with VF ranging from 85.46 to 64.46 beats min^{-1}.

DISCUSSION

The administration of eugenol resulted in distinct anesthetic responses among the juvenile size classes of the evaluated African cichlid species. The variation observed in induction and recovery times, as well as in VF, demonstrated that both species and body size significantly influence anesthetic efficacy.

Eugenol, due to its low aqueous solubility, requires a step of dilution in ethanol to ensure the homogeneity of anesthetic solutions (Neiffer & Stamper 2009). The absence of anesthetic effects and mortality in fish, even in the presence of reduced concentrations of ethanol, has been reported by other studies (Ribeiro et al. 2015, Oliveira et al. 2019, Silva et al. 2023, 2026) and was observed in the present findings. Similar results were reported by Fujimoto et al. (2018) for the *Paracheirodon axelrodi* (Cardinal tetra), *Heros severus* (Banded cichlid), and *Pterophyllum scalare* (Angelfish), also with no records of adverse effects after exposure to clove essential oil. Furthermore, all specimens of the present study resumed feeding within 24 h after anesthetic exposure, suggesting that the anesthetic (eugenol) and biometric handling did not compromise feeding behavior, as also

Table I. Ventilatory frequency (mean ± standard deviation, in opercular beats per minute) measured during anesthesia with eugenol in two size classes of *Aulonocara nyassae*.

Eugenol concentrations (mg L^{-1})	Juvenile I-Any (1.29 ± 0.23 g; 44.90 ± 4.90 mm)	
	Induction during ventilatory frequency (beats min^{-1})	Recovery during ventilatory frequency (beats min^{-1})*
25	95.10 ± 2.47	104.85 ± 13.90
50	98.08 ± 6.90	91.22 ± 4.13
75	89.69 ± 6.54	82.73 ± 5.10
100	94.92 ± 3.28	74.33 ± 9.93
125	91.88 ± 6.50	94.93 ± 5.09
150	80.01 ± 6.79	74.12 ± 1.77
175	72.32 ± 10.37	63.99 ± 5.81
Equation	y = - 0.16x + 105.82	y = - 0.21x + 104.39
R^2	0.80	0.63
Eugenol concentrations (mg L^{-1})	**Juvenile II-Any (2.97 ± 0.67 g; 59.15 ± 4.08 mm)**	
	Induction during ventilatory frequency (beats min^{-1})	**Recovery during ventilatory frequency (beats min^{-1})***
25	77.88 ± 8.89	86.42 ± 13.92
50	91.03 ± 7.96	89.31 ± 9.84
75	64.78 ± 10.50	79.29 ± 7.13
100	85.92 ± 6.61	75.99 ± 9.65
125	82.13 ± 13.38	84.82 ± 9.95
150	92.34 ± 9.52	77.81 ± 5.61
175	84.21 ± 24.37	70.33 ± 5.91
Equation	no significant	y = - 0.09x + 89.96
R^2	-	0.59

***Significance denoted as ($P < 0.05$).**

observed for other species (Pirhonen & Schreck 2003, Cupp et al. 2014, Ferreira et al. 2021).

According to the criteria established by Keene et al. (1998) and Ross & Ross (2008), the effectiveness of an anesthetic administered to fish via immersion bath should ensure anesthetic induction in less than 180 s and recovery that does not exceed 300 s. Based on these criteria, the present study demonstrated that, concentrations between 50 and 175 mg L^{-1} are recommended for Juvenile I-Any and between 25 and 175 mg L^{-1} for the larger Juvenile II-Any. In contrast, eugenol concentrations between 25 and 175 mg L^{-1} are recommended for both of the evaluated size classes (Juvenile I-Ast and Juvenile II-Ast). It is noteworthy that the literature already documents wide variation in ideal eugenol concentrations among different ornamental fish species. For example, a concentration of 15 mg L^{-1} was reported for the *Betta splendens* (Siamese fighting fish) (Pattanasiri et al. 2017); 20 mg L^{-1} for *Baryancistrus xanthellus* (Golden

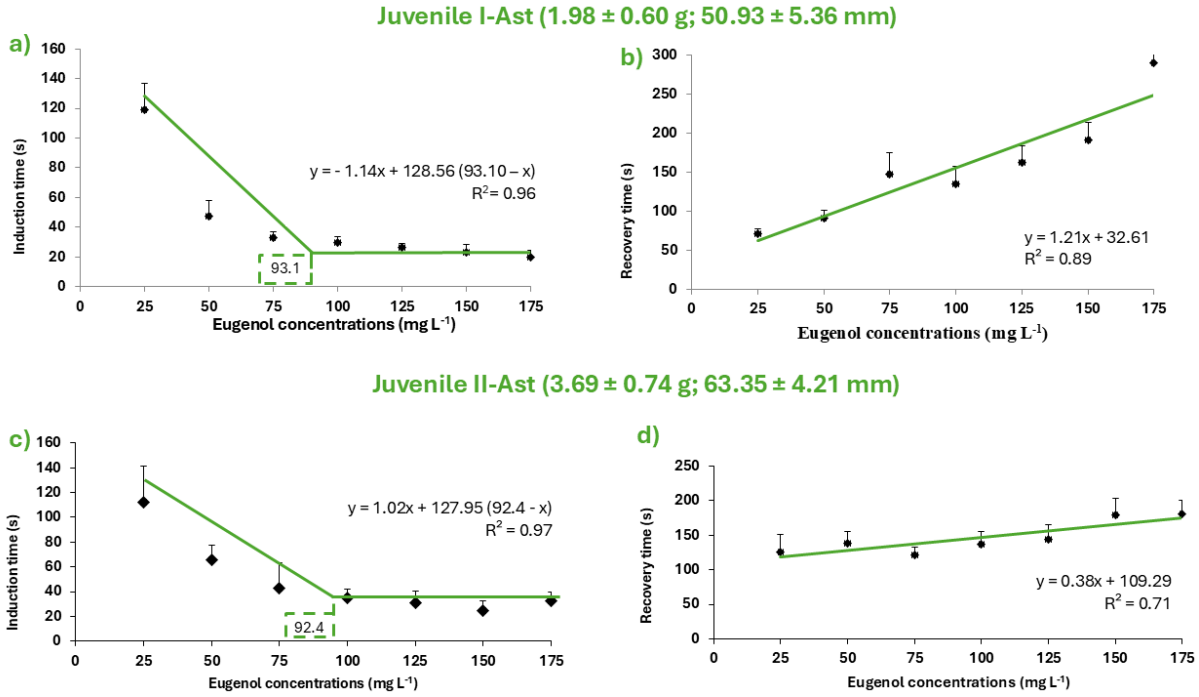

Figure 3. Anesthetic induction and recovery times (mean ± standard deviation, in seconds) for two size classes of *Aulonocara stuartgranti* exposed to eugenol.

nugget pleco) (Nascimento et al. 2025); 50 mg L^{-1} for *Amphilophus labiatus* × *Amphilophus trimaculatus* (Flowerhorn) (Tarkhani et al. 2017); 53 mg L^{-1} for *P. scalare* (Oliveira et al. 2019); 50 mg L^{-1} for *Cnesterodon decemmaculatus* (Ten spotted live-bearer) (Vercellini et al. 2025); 85 mg L^{-1} for *Danio rerio* (Zebrafish) (Ayala-Soldado et al. 2024); 70 mg L^{-1} for *Poecilia reticulata* (Guppy) (Nuanmanee et al. 2024); and 100 and 200 mg L^{-1} for *Poecilia vivipara* (Guppy) (Bolasina et al. 2017). In general, the eugenol concentrations tested in the present study (25, 50, 75, 100, 125, 150 and 175 mg L^{-1}) for African cichlids are within the recommended range for other ornamental species, demonstrating both its efficacy and its feasibility of application.

The time to anesthesia induction using eugenol showed a linear-plateau effect for most groups of the studied African cichlids. This pattern indicates that, above a certain eugenol concentration, further increases do not significantly accelerate induction. This response may be attributed to receptor saturation or the maximum rate of anesthetic absorption, either of which would limit induction speed regardless of higher concentrations, as previously reported (Ferreira et al. 2021, Ayala-Soldado et al. 2024, Zeng et al. 2024). Distinct patterns were observed for recovery time among the size classes of the studied African cichlids. Juvenile I of *A. nyassae* exhibited a linear-plateau effect, suggesting a stabilization of recovery time. However, Juvenile II of *A. nyassae* and both size classes (Juvenile I and II) of *A. stuartgranti* showed a direct linear response. This latter behavior, with prolonged recovery with increasing eugenol concentration, is frequently reported and indicates greater bioaccumulation of the compound or greater time for its elimination (Ayala-Soldado et al. 2024). For practical and animal welfare purposes, this prolonged recovery highlights the importance of optimizing anesthetic concentrations to avoid excessively long recovery times (Zahl et al. 2012, Ayala-Soldado et al. 2024).

Table II. Ventilatory frequency (mean ± standard deviation, in opercular beats per minute) measured during anesthesia with eugenol in two size classes of *Aulonocara stuartgranti.*

Eugenol concentrations (mg L^{-1})	Juvenile I-Ast (1.98 ± 0.60 g; 50.93 ± 5.36 mm)	
	Induction during ventilatory frequency (beats min^{-1})	Recovery during ventilatory frequency (beats min^{-1})*
25	76.94 ± 6.63	95.89 ± 3.20
50	85.79 ± 12.98	79.88 ± 7.17
75	70.39 ± 8.56	77.97 ± 3.70
100	69.24 ± 9.84	67.77 ± 2.44
125	104.13 ± 13.32	71.16 ± 2.86
150	78.14 ± 5.21	61.30 ± 3.98
175	76.95 ± 2.62	57.87 ± 3.46
Equation	no significant	y = - 0.23x + 95.70
R^2	-	0.81
Eugenol concentrations (mg L^{-1})	**Juvenile II-Ast (3.69 ± 0.74 g; 63.35 ± 4.21 mm)**	
	Induction during ventilatory frequency (beats min^{-1})	**Recovery during ventilatory frequency (beats min^{-1})***
25	71.44 + 11.29	81.02 + 8.74
50	45.79 + 6.62	82.41 + 6.39
75	52.16 + 8.13	87.19 + 8.01
100	78.19 + 14.92	75.95 + 5.76
125	75.79 + 12.13	64.00 + 8.59
150	88.80 + 12.03	75.12 + 3.38
175	88.50 + 9.51	61.89 + 6.07
Equation	no significant	y = - 0.14x + 88.96
R^2	-	0.61

***Significance denoted as ($P < 0.05$).**

Ventilatory frequency has been widely used as a physiological indicator during fish anesthesia as it allows real-time monitoring of respiratory changes (Alvarenga & Volpato 1995, Becker et al. 2018, Ananias et al. 2022). In the present study, during anesthesia induction, VF showed variable behavior between species and size classes. While Juvenile I-Any showed a tendency for VF to decrease with increasing anesthetic concentration, Juvenile II-Any and both size classes of *A. stuartgranti* did not exhibit a clear linear relationship, suggesting the absence of a concentration-response pattern. This heterogeneity may be related to physiological differences among the experimental groups, such as ontogenetic stage, metabolic rate, and individual sensitivity to the drug (Meka & McCormick 2005, Ross & Ross 2008, Neiffer & Stamper 2009, Ribeiro et al. 2015). Similar results were obtained by Ferreira et al. (2021) for two

size classes of juvenile *Piaractus brachypomus* (Pirapitinga), which also did not show significant changes in VF during induction when exposed to eugenol. During recovery, on the other hand, VF showed a linear reduction with increasing eugenol concentration for both species and size classes. This pattern is consistent with the depressant action of eugenol, which leads to a decrease in neural activity and, consequently, in metabolic oxygen demand (Lehotzky et al. 2023). However, the consistent results observed with eugenol contrast with the findings of Ferreira et al. (2020), who evaluated the anesthetic effects of menthol and benzocaine on *A. nyassae* (one of the species evaluated in the present study) and did not identify a clear relationship between the concentration of the agents and VF. This finding reinforces that the physiological response can vary among different anesthetics, even when applied to the same species. Additionally, the VF response to eugenol appears to be modulated not only by anesthetic concentration, but also by the biological characteristics of the species, such as body size, developmental stage, and metabolism (Ross & Ross 2008, Neiffer & Stamper 2009, Ribeiro et al. 2015, Ferreira et al. 2021). This variability should be carefully considered when defining anesthetic protocols for ornamental fish, especially in situations involving repeated or prolonged exposure to eugenol (Ayala-Soldado et al. 2024).

Overall, the distinct response patterns observed in this study, as well as the discrepancies in the literature, indicate that eugenol sensitivity is not uniform across species nor ontogenetic groups. Factors such as metabolism, respiratory physiology, and the anesthetic affinity for lipid tissues appear to play a determining role in this variability (Meka & McCormick 2005, Ross & Ross 2008, Ribeiro et al. 2015, Zeng et al. 2024). Despite its widely recognized benefits for fish welfare and safety during handling procedures (Javahery et al. 2012, Zahl et al. 2012), eugenol has limitations that deserve attention. For example, Ayala-Soldado et al. (2024) compared eugenol with MS-222 for *D. rerio* and reported prolonged recovery times after multiple anesthesias with eugenol, attributing this effect to its lipophilicity and bioaccumulation potential. The authors recommend caution when using eugenol repeatedly in short intervals. In the present study, however, eugenol proved effective for anesthetizing African cichlids for procedures such as biometry and other short-term applications.

CONCLUSIONS

Eugenol was shown to be an effective anesthetic for both evaluated African cichlid species. Concentrations between 50 and 175 mg L^{-1} are recommended for Juvenile I-Any of *A. nyassae* and between 25 and 175 mg L^{-1} for Juvenile II-Any. The ideal concentrations for *A. stuartgranti* range from 25 to 175 mg L^{-1} for both of the evaluated size classes. The observed variation in VF between species and size classes reinforces the need to consider specific physiological characteristics when defining anesthetic protocols. Future studies should investigate the effects of eugenol on hematological and biochemical parameters and oxidative stress, as well as its efficacy in repeated exposures, to contribute to improving anesthetic management for ornamental African cichlids.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.