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Assessment of the effectiveness of clove oil, tricaine methanesulfonate (MS-222), and 2-phenoxyethanol for anesthetizing juvenile Pacific White Snook (*Centropomus viridis*)

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ABSTRACT. In marine fish farming, the use of anesthetics is necessary to reduce the stress associated with routine procedures, such as capturing, handling, and obtaining biometric measurements. The objective of this study was to determine the minimum effective concentration of 3 anesthetic agents: clove oil, tricaine methanesulfonate (MS-222), and 2-phenoxyethanol. Each anesthetic was evaluated in 9 juvenile Pacific White Snook (*Centropomus viridis*) at different concentrations. The efficacy criteria considered were the induction time to deep anesthesia (stage III), within a duration of less than 180 s, and the recovery time (RT), no greater than 300 s. In addition, the survival of juveniles was evaluated during the experimental period and for 72 h after exposure to the anesthetics. The minimum effective concentrations were determined to be between 50–75 mg·L^{-1} for clove oil, 125 mg·L^{-1} for MS-222, and 500 µg·L^{-1} for 2-phenoxyethanol, with no visible adverse effects. Inverse correlations were observed between the anesthetic concentrations and induction time (stage III); however, only the clove oil concentration showed a positive correlation with the RT. No mortality was observed during exposure to the anesthetic agents or during the 72 h following the experiment at any of the evaluated concentrations of each anesthetic.

Key words: Pacific White Snook, anesthesia, induction time, recovery time, survival.

INTRODUCTION

The use of anesthetics in marine fish farming is essential to facilitate handling and reduce stress associated with performing routine procedures, such as biometrics, blood collection, artificial reproduction, and transportation (Sneddon 2012, Martins et al. 2019, Tchobanov et al. 2024). Anesthesia is a reversible state induced by an external agent, which causes loss of sensation as a result of the depression of the central nervous system (Ross and Ross 2008, Martins et al. 2019). In fish, anesthetics are administered mainly by immersion; they are dissolved in seawater, and fish are subsequently immersed in it (Neiffer and Stamper 2009, Sneddon 2012). These substances are absorbed by the gills and transported by the blood to the central nervous system (Ross and Ross 2008). In fish, anesthesia levels range from light sedation, intended to reduce stress during handling and non-invasive procedures, to deep anesthesia, required for invasive interventions (Zahl et al. 2012). The determination of these levels is based primarily on the assessment of gill ventilation rates, equilibrium maintenance (normal body position), and response to external stimuli (Ross and Ross 2008, Sneddon 2012, Schroeder et al. 2021). The induction time to deep anesthesia should be brief, not exceeding 180 s, and should not cause hyperactivity or tension. When fish are transferred back to anesthesia-free waters, the recovery time should be 300–600 s (Ross and Ross 2008, Bronstad 2022). The efficacy of the anesthetics used in fish handling depends on various factors, such as species, size, environmental factors, the concentration of the anesthetic, the method of application, availability, and cost (Priborsky and Velisek 2018, Jia

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et al. 2022, Simões-Bueno et al. 2024). In addition, anesthetic agents should be easy to manipulate and non-toxic for fish, humans, and the environment (Ross and Ross 2008).

The most commonly used anesthetics in the handling of marine fish are clove oil, tricaine methanesulfonate (MS-222), 2-phenoxyethanol, and benzocaine (Munday and Wilson 1997, Souza et al. 2012, Zahl et al. 2012, Ghanawi et al. 2013, Barata et al. 2016, He et al. 2020, Soldatov 2021, Bronstad 2022, Sorensen et al. 2023, Karim et al. 2024). However, it is necessary to determine the appropriate concentration of each anesthetic for induction in each species, since the use of inadequate concentrations can cause adverse effects such as increased stress and substantial alterations in cardiovascular and respiratory parameters, and even mortality (Readman et al. 2017, Priborsky and Velisek 2018, Martins et al. 2019, Soldatov 2021).

The Pacific White Snook (*Centropomus viridis*) is a species with high commercial value due to the organoleptic characteristics of its flesh, distinguished by its white color, texture, and exquisite flavor (Álvarez-Lajonchère and Tsuzuki 2008). In addition to the quality of its flesh, this species has great potential for aquaculture because it is hardy, adapts easily to captivity conditions and artificial feed, reaches commercial size in 6 months, and, because it is an euryhaline species, tolerates different salinities in aquaculture (Álvarez-Lajonchère et al. 2013, Labastida-Che et al. 2013, Montoya-Ponce et al. 2024). At the *Centro de Investigación en Alimentación y Desarrollo* (Center for Research in Food and Development; CIAD, for its acronym in Spanish), Mazatlán branch, Mexico, biotechnology has been developed for the production of juveniles of this species (Ibarra-Castro et al. 2017), and the grow-out phase of juveniles produced in captivity has recently begun in different culture systems distributed along the Mexican Pacific coast (Baldini et al. 2022). However, to the best of our knowledge, there is no published information on specific protocols for anesthetizing juveniles of Pacific White Snook to facilitate their handling.

Given the growing interest in the cultivation of *C. viridis* and the lack of information on the use of anesthetics in this species, in the present work, we evaluated the efficacy of 3 anesthetics at different concentrations in juveniles in captivity; the induction time to deep anesthesia (stage III) and the recovery time (RT) were analyzed to determine the minimum effective concentration to improve their handling and reduce stress during activities for their cultivation.

Materials and methods

Origin of the *Centropomus viridis* juveniles

Approximately 250 juvenile *C. viridis* were obtained from the *Planta Piloto para la Producción de Juveniles de Peces Marinos* (Pilot Plant for the Production of Marine Fish Juveniles; PPPM, for its acronym in Spanish) at CIAD, Mazatlán branch, following the protocol described by Ibarra-Castro et al. (2017). The organisms had an average total length (TL) of 19.43 ± 2.23 cm and an average weight (W) of 53.42 ± 15.17 g. Juveniles were maintained at an ambient temperature of 25 °C in an open system, in a 5,000-L fiberglass tank with filtered seawater, with a water exchange rate of 83 $L \cdot min^{-1}$ and constant aeration. Feeding was suspended 24 h before the start of the experiment. The experiment was conducted in the PPPM pre-grow-out area, in 20-L plastic buckets equipped with aeration.

Anesthetics

We evaluated 3 anesthetics at different concentrations: clove oil (eugenol [90–95%], AE17, Storelab, Pymble, Australia) at 50, 75, 100, and 125 $mg \cdot L^{-1}$; MS-222 (A5040, Sigma Aldrich, Burlington, USA) at 100, 125, 150, and 175 $mg \cdot L^{-1}$; and 2-phenoxyethanol (P1126, Sigma Aldrich) at 125, 250, 500, 750, and 900 $\mu \cdot L^{-1}$. The concentrations selected for each anesthetic were based on previously reported values for other marine fish species (Ross and Ross 2008, Zahl et al. 2012, Priborsky and Velisek 2018). MS-222 and 2-phenoxyethanol were placed directly into the seawater of each experimental unit before starting each test; clove oil was pre-diluted in ethanol (1:10) and subsequently added to the seawater.

Experimental design

Each anesthetic concentration was evaluated in 9 fish. One fish at a time was randomly selected and placed in a bucket with 5 L of filtered seawater with the concentration of the anesthetic being tested. The induction time (s) was divided into 3 stages: deep sedation (stage I), light anesthesia (stage II), and deep anesthesia (stage III) (Table 1). The behavior of the fish was observed to determine the time elapsed to reach each stage using a digital stopwatch. Once stage III was reached, the fish was removed from the anesthesia; in less than 30 s, TL was measured using an ichthyometer, and the W was recorded with a digital scale with an accuracy of ± 0.05 g. Subsequently, the fish was placed in a recovery tank containing 10 L of filtered seawater without anesthetic and with aeration, and the total RT was recorded (Table 1). We ensured that the maximum exposure time to each anesthetic and concentration did not exceed 180 s (Ross and Ross 2008). If no anesthetic effect was observed for 3 min, the anesthetic concentration was considered insufficient and further evaluation was discontinued. At the end of the experiment, the fish were transferred to a 5,000-L circular fiberglass tank with a continuous flow of filtered seawater and constant aeration. The fish were fed after 24 h and observed for 72 h after the experiment to assess survival rate and behavior. The water temperature during the experiment was 25 ± 0.2 °C and the salinity was kept at 34 $g \cdot L^{-1}$.

Statistical analysis

Normality (Bartlett's test) and homoscedasticity (Levene's test) for the induction times to reach each stage (I, II, and III)

Table 1. Stages of anesthesia in fish.

Stage of anesthesia	Description	Physiological and behavioral changes
I	Deep sedation	Normal equilibrium, reduced movement, decreased ventilation, does not react to visual stimuli.
II	Light anesthesia	Partial loss of equilibrium and ventilation, and very reduced movements.
III	Deep anesthesia	Total loss of muscle tone, total loss of balance, almost absent ventilation.
R	Full recovery	Normal opercular frequency, reacts to external stimuli.

Adapted from Ross and Ross (2008).

and the RT were verified for each of the concentrations evaluated. Because the data were normally distributed, they were analyzed using a one-way analysis of variance (ANOVA, $P < 0.05$), and significant differences between the concentrations of each anesthetic were determined using Tukey's multiple range test ($\alpha = 0.05$). Linear regression analyses were performed between the concentrations of each anesthetic and the induction time to reach stage III and RT. Statistical analyses were performed using Centurion XVI v. 16.204 software (Statgraphics Technologies, Inc., The Plains, USA).

Results

Clove oil

As shown in Table 2, the induction time to reach stage III with clove oil was significantly longer with the 50 mg·L^{-1} concentration than with the 100 and 125 mg·L^{-1} concentrations ($F_{(3, 32)} = 2.9$, $P = 0.0503$). The RT was significantly longer with the 125 mg·L^{-1} concentration than with the other concentrations ($F_{(3, 32)} = 11.24$, $P < 0.0001$). A moderately significant inverse correlation ($F_{(1, 34)} = 8.23$, $P = 0.0070$) was observed between clove oil concentrations and the induction time to reach stage III (lower clove oil concentrations were associated with longer induction times to stage III) (Fig. 1). A significant correlation ($F_{(1, 34)} = 31.25$, $P = 0.000$) was also observed between clove oil concentrations and RT (lower concentrations were associated with shorter recovery times) (Fig. 2). Upon contact with the anesthetic bath at concentrations of 100 and 125 mg·L^{-1}, the fish exhibited transient hyperactivity or erratic swimming, which ceased in all cases when stage I of anesthesia was reached. This behavior was not observed with the other 2 concentrations (50 and 75 mg·L^{-1}). No mortality was observed in juveniles during or after the experiment (72 h).

MS-222

The results obtained with the anesthetic MS-222 are shown in Table 3. Induction times to stage III were significantly shorter with higher concentrations ($F_{(3, 32)} = 25.65$, $P = 0.0000$). Regarding the RT, there were no significant differences between the evaluated MS-222 concentrations in juvenile *C. viridis* ($F_{(3, 32)} = 0.91$, $P = 0.4485$). A significant inverse correlation ($F_{(1, 34)} = 49.18$, $P = 0.0000$) was observed between induction time to stage III and MS-222 concentrations (Fig. 3); however, no significant correlation ($F_{(1, 34)} = 0.55$, $P = 0.4623$) was found between RT and the different concentrations. Unlike what occurred with juveniles exposed to high concentrations of clove oil, no hyperactivity was observed in juveniles exposed to different concentrations of MS-222. No mortality was observed in juveniles exposed to MS-222 during or after the 72 h of the experiment.

2-phenoxyethanol

Concentrations of 125 and 250 µg·L^{-1} were not effective in inducing stage III anesthesia in juveniles in less than 5 min and were therefore not included in the statistical analysis. The remaining concentrations were effective in inducing stage III anesthesia in 180 s or less (Table 4). The time to induction of stage III was significantly longer with the 500 µg·L^{-1} concentration ($F_{(2, 24)} = 3.63$, $P = 0.0420$) (Table 4). In contrast, no significant differences in RT were observed among the evaluated concentrations of 2-phenoxyethanol ($F_{(2, 24)} = 1.00$, $P = 0.3829$). A significant inverse correlation ($F_{(1, 25)} = 7.36$, $P = 0.0118$) was observed between the induction time to stage III and the 2-phenoxyethanol concentrations evaluated (Fig. 4). Regarding the RT, no significant correlation ($F_{(1, 25)} = 1.02$, $P = 0.3232$) was found between the different concentrations. No hyperactivity was observed in the juveniles upon contact with the anesthetic bath at the different concentrations evaluated; however, the 900 µg·L^{-1} concentration caused irritation of the caudal fin and mouth in some organisms. During and after the 72 h of the experiment, 100% survival was achieved.

Discussion

Optimizing anesthesia protocols for handling farmed fish is key to ensuring the well-being of the organisms and the

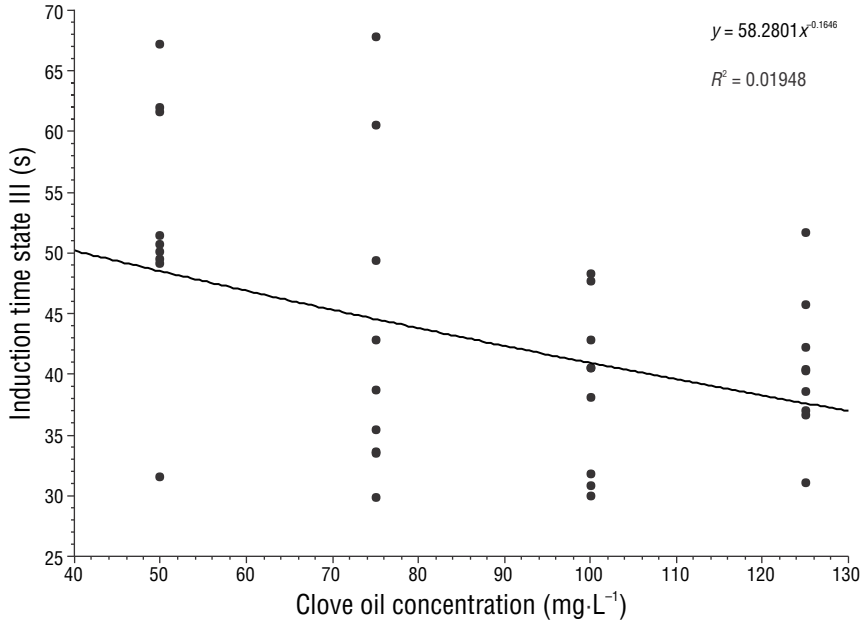

Figure 1. Relationship between clove oil concentrations and induction time to stage III of anesthesia in juvenile Pacific White Snook (*Centropomus viridis*).

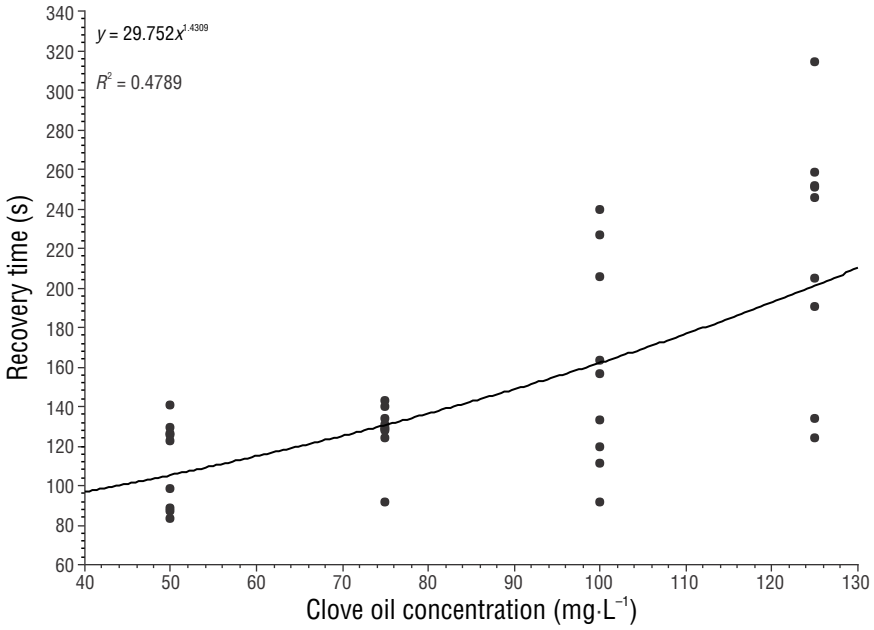

Figure 2. Relationship between clove concentrations and recovery time of juvenile Pacific White Snook (*Centropomus viridis*).

Table 2. Results of anesthesia induction and recovery times in juvenile *Centropomus viridis* exposed to various concentrations of clove oil.

		Induction time (s)			
Anesthetic	Concentración ($mg \cdot L^{-1}$)	Stage I: Deep sedation	Stage II: Light anesthesia	Stage III: Deep anesthesia	Recovery time (RT)
Clove oil	50	26.95 ± 7.29[b]	36.05 ± 3.33	52.59 ± 10.34[b]	111.46 ± 21.87[a]
	75	25.11 ± 6.24[b]	30.76 ± 5.3	43.54 ± 13.17[ab]	127.8 ± 14.73[a]
	100	18.22 ± 4.72[a]	30.10 ± 6.3	38.97 ± 6.89[a]	199.93 ± 53.72[a]
	125	27.07 ± 9.2[b]	34.28 ± 10.85	40.40 ± 9.39[a]	219.68 ± 61.85[b]

Values (mean ± SD, $n = 9$) with different letters in the same column are significantly different ($P < 0.05$).

economic and environmental viability of marine fish farming (Espinoza-Ramos et al. 2025).

Clove oil

Clove oil is obtained through the distillation of the leaves, flowers, and stems of the clove tree (*Eugenia aromaticum* or *Eugenia caryphyllata*). Its active ingredients are eugenol and isoeugenol, which constitute 90–95% of the weight of clove oil (Ross and Ross 2008). The oil is absorbed through the gills and skin of fish, entering the bloodstream due to its high lipophilicity, which facilitates its distribution to tissues such as the brain (Priborsky and Velisek 2018). One of the main advantages of clove oil is that it is inexpensive and easy to use, has few harmful effects on humans under normal conditions, and has a short period of side effects without posing a known environmental risk (Ross and Ross 2008, Soldatov 2021).

Comparative studies have indicated that, compared to anesthetics such as MS-222 and 2-phenoxyethanol, clove oil allows most fish species to rapidly reach the resting state required for handling (Soldatov 2021). However, decreased ventilation and cardiovascular response have been reported as side effects, possibly associated with increased eugenol retention in the bloodstream (Priborsky and Velisek 2018). These effects depend on concentration, exposure time, and species (Sneddon 2012) and can be minimized by using optimal concentrations and appropriate operating conditions (Gonçalves-Gaveta 2020).

Reports have indicated that the effective concentration of clove oil to induce stage III and RT in less than 180 and 300 s, respectively, is 70 $mg \cdot L^{-1}$ in *Siganus rivulatus* (Ghanawi et al. 2013), 35 $mg \cdot L^{-1}$ in *Oplegnathus punctatus* (Jia et al. 2022), 40 $mg \cdot L^{-1}$ in *Rachycentron canadum* (Gullian and Villanueva 2009), 40–50 $mg \cdot L^{-1}$ in *Argyrosomus regius* (Cárdenas et al. 2016), and 175 $mg \cdot L^{-1}$ in *Eptatretus stoutii* (McCord et al. 2020). In the present study, all clove oil concentrations evaluated in juvenile *C. viridis* induced stage III anesthesia in less than 180 s and reached RT in less than 300 s.

These results are consistent with those reported for the previously mentioned species. Nevertheless, concentrations of 100 and 125 $mg \cdot L^{-1}$ caused hyperactivity or erratic swimming during the initial induction phase. This behavior has been observed in other species and is attributed to possible irritation caused by eugenol in the gill epithelium and other sensory tissues (Mylonas et al. 2005, Vidal et al. 2007, Viegas et al. 2020, Lopes-de Lima et al. 2021).

As in this work, a significant inverse correlation has been reported between clove oil concentrations and induction time to stage III for other marine fish species (shorter induction times with higher concentrations) (Barata et al. 2016, He et al. 2020). Conversely, the correlation was positive for the RT (longer recovery times with higher concentrations). This is because the more anesthetic absorbed during the induction period, the longer it takes for the fish to recover when placed in anesthesia-free water (Zahl et al. 2012). Overall, the results suggest that clove oil concentrations between 50 and 75 $mg \cdot L^{-1}$ are sufficient to induce stage III anesthesia in juvenile *C. viridis* in less than 180 s, with a RT of less than 300 s and no visible side effects.

MS-222

MS-222 is one of the most widely used anesthetics in aquaculture. It is a white, odorless, crystalline powder that is highly soluble in water (Ross and Ross 2008, Dheeran et al. 2023). Fish absorb it through their gills and skin, and it is distributed throughout the body via the bloodstream (Carter et al. 2011). MS-222 acts as a muscle relaxant by blocking sodium and potassium channels in both muscle and nerve membranes (Priborsky and Velisek 2018).

This anesthetic is non-toxic to humans and is the only one approved by the Food and Drug Administration (FDA) in the United States for use in fish intended for human consumption (Ross and Ross 2008, Carter et al. 2011). Fish excrete it in their urine within 24 hours, and tissue levels decrease to almost zero (Ross and Ross 2008). However, MS-222 can cause adverse effects in fish associated with cardiovascular,

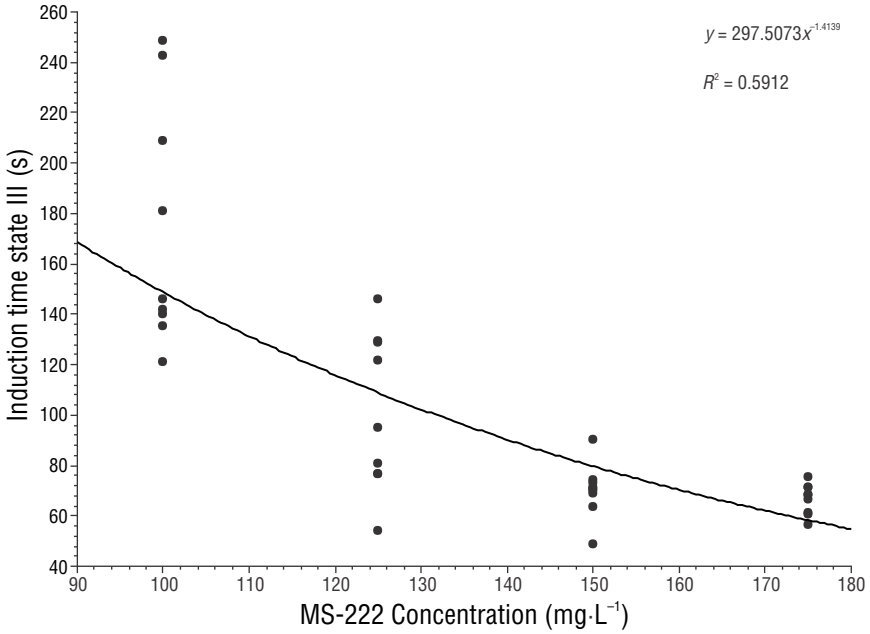

Figure 3. Relationship between MS-222 concentrations and induction time to stage III of anesthesia in juvenile Pacific White Snook (*Centropomus viridis*).

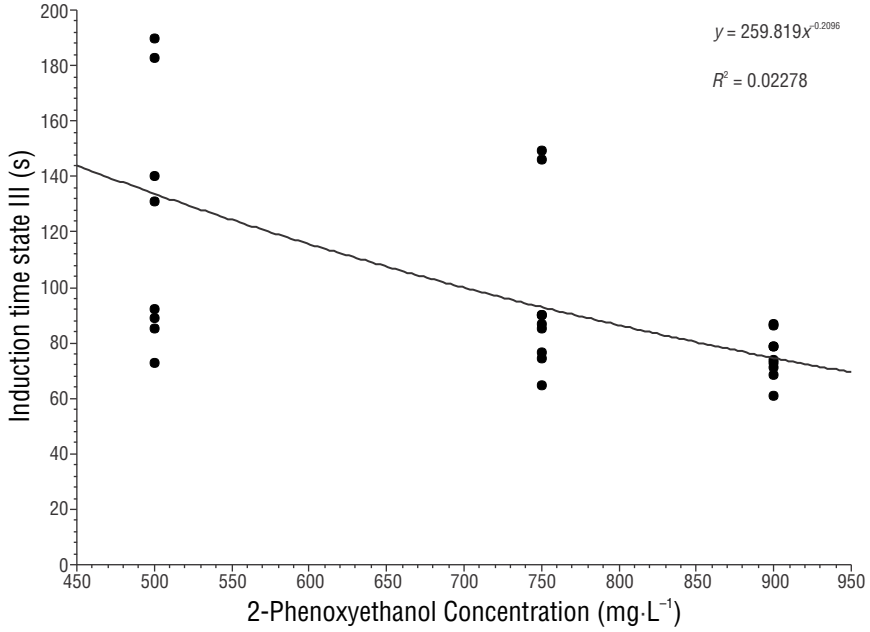

Figure 4. Relationship between 2-phenoxyethanol concentrations and induction time to stage III of anesthesia in juvenile Pacific White Snook (*Centropomus viridis*).

Table 3. Results of induction times to the different stages of anesthesia and recovery of juvenile Pacific White Snook (*Centropomus viridis*) exposed to various concentrations of MS-222.

		Induction time (s)			
Anesthetic	Concentración ($mg \cdot L^{-1}$)	Stage I: Deep sedation	Stage II: Light anesthesia	Stage III: Deep anesthesia	Recovery time (RT)
MS-222	100	36.02 ± 13.63^{b}	59.47 ± 24.18^{c}	174.2 ± 48.49^{c}	55.24 ± 8.35
	125	29.49 ± 10.12^{ab}	56.95 ± 7.27^{bc}	101.22 ± 31.39^{b}	57.31 ± 4.21
	150	22.96 ± 6.65^{a}	45.75 ± 9.64^{ab}	70.24 ± 10.83^{a}	62.23 ± 10.58
	175	22.53 ± 4.74^{a}	42.38 ± 6.89^{a}	66.70 ± 6.08^{a}	57.11 ± 12.88

Values (mean ± SD, $n = 9$) with different letters in the same column are significantly different ($P < 0.05$).

endocrine, and osmoregulatory disturbances (Carter et al. 2011, Martins et al. 2019), which makes it necessary to determine the minimum effective concentration (MEC) for each species (Dheeran et al. 2023). The MEC is defined as the concentration of anesthetic in the blood required to achieve stage III anesthesia (Hsu et al. 2023).

In the present study, concentrations of 125, 150, and 175 $mg \cdot L^{-1}$ induced stage III anesthesia in *C. viridis* juveniles in less than 180 s, with RTs of less than 300 s. These results are similar to those reported for other marine fish species such as *Thunnus albacares* (100–200 $mg \cdot L^{-1}$; Cano et al. 2014), *S. rivulatus* (100 to 125 $mg \cdot L^{-1}$; Ghanawi et al. 2013), *Alosa pseudoharengus*, (112 $mg \cdot L^{-1}$; Berlinsky et al. 2016), and *Lates calcarifer* (150 $mg \cdot L^{-1}$; Hsu et al. 2023). However, in species such as *Centropristis striata*, *O. punctatus*, and *Anisotremus scapularis*, lower MEC values (70–80 $mg \cdot L^{-1}$) have been reported (King et al. 2005, Jia et al. 2022, Espinoza-Ramos et al. 2025), which confirms that MS-222 MEC values differ markedly between species (King et al. 2005, Ross and Ross 2008, Popovic et al. 2012).

As observed in this study, the induction time to stage III in other species has been reported to decrease when MS-222 concentration increases, which is attributed to the easy absorption of the anesthetic through the gills (Weber et al. 2009, Matsche 2011, Espinoza-Ramos et al. 2025). However, in the present study, no correlation was observed between MS-222 concentrations and the RT, which has been observed in *Solea senegalensis* (Weber et al. 2009). One possible explanation for the lack of correlation between the RT and concentrations is that the fish does not remain in contact with the anesthetic for very long at higher levels, which reduces its absorption and promotes faster recovery (Weber et al. 2009).

In the present study, the survival rate of *C. viridis* juveniles during and at 72 h post-exposure to MS-222 was 100% at all concentrations evaluated, which is similar to that observed in *A. pseudoharengus* (Berlinsky et al. 2016). On the other hand, in some species such as *R. canadum* and *Acipenser oxyrinchus oxyrinchus*, moderate to vigorous hyperactivity has been reported at concentrations of 100 to 150 $mg \cdot L^{-1}$; however, the duration and intensity of this response decreased when the concentration of MS-222 increased to 200–250 $mg \cdot L^{-1}$ (Gullian and Villanueva 2009, Matsche 2011).

This behavior can be attributed to the fact that, at low concentrations, slow induction to stage III anesthesia allows the fish to detect the anesthetic agent based on its chemical properties, either through taste and smell or as a skin irritant, whereas, at high concentrations, rapid induction can limit this perception (Popovic et al. 2012). Although concentrations of 100 to 175 $mg \cdot L^{-1}$ of MS-222 were evaluated in this study, no hyperactivity was observed when the juveniles were placed in the container with the anesthetic, possibly because hypersensitivity to the agent can also vary among species. Based on the results of this study, a MEC of 125 $mg \cdot L^{-1}$ of MS-222 is suggested to induce stage III anesthesia in juvenile *C. viridis* without visible adverse effects.

2-phenoxyethanol

The anesthetic 2-phenoxyethanol is a clear, colorless or straw-colored, oily liquid with a faint aromatic odor and moderate solubility in water. It exhibits antimicrobial and fungicidal activity and is one of the most widely used anesthetics in aquaculture due to its efficiency, ease of preparation, and low cost (Ross and Ross 2008, Barata et al. 2016). Fish absorb it through the gills and skin, and it is transported by arterial blood to the central nervous system (Priborsky and Velisek 2018). Although the exact mechanism of its anesthetic action has not yet been described, it has been proposed that it involves the expansion of neuronal cell membranes and the suppression of neuronal activity in higher regions of the nervous system (Zahl et al. 2012).

Among the adverse effects reported in fish, 2-phenoxyethanol can cause a temporary reduction in olfactory capacity (McCord et al. 2020), and a decrease in ventilation, heart rate, blood pressure, and blood pH (Zahl et al. 2012). However, these effects can be minimized by carefully

Table 4. Relationship between 2-phenoxyethanol concentrations and induction time to stage III of anesthesia in juvenile Pacific White Snook (*Centropomus viridis*).

Anesthetic	Concentración ($mg \cdot L^{-1}$)	Induction time (s)			
		Stage I: Deep sedation	Stage II: Light anesthesia	Stage III: Deep anesthesia	Recovery time (RT)
2-phenoxyethanol	500	31.38 ± 6.35	69.64 ± 45.25	157.51 ± 112.23[b]	78.17 ± 25.88
	750	35.14 ± 10.58	54.19 ± 8.40	96.0 ± 30.55[ab]	76.71 ± 11.07
	900	34.64 ± 9.21	47.93 ± 11.69	75.33 ± 8.31[a]	88.86 ± 19.91

Values (mean ± SD, $n = 9$) with different letters in the same column are significantly different ($P < 0.05$).

selecting species-specific concentrations (Priborsky and Velisek 2018).

In this context, the results of the present study showed that 2-phenoxyethanol concentrations of 125 and 250 $\mu g \cdot L^{-1}$ were not effective in inducing stage III anesthesia in juvenile *C. viridis*, which is consistent with reports for other species such as *Huso huso* (Shaluei et al. 2012), *A. regius* (Barata et al. 2016), *A. pseudoharengus* (Berlinsky et al. 2016), and *Acipenser gueldenstaedtii* (Kübra 2022). Conversely, these concentrations have been effective in inducing stage III anesthesia in less than 3 min in juvenile *C. striata* (King et al. 2005), and in *Diplodus sargus* and *Diplodus puntazzo* (Tsantilas et al. 2006). Concentrations of 500, 750, and 900 $\mu g \cdot L^{-1}$ of 2-phenoxyethanol effectively induced stage III anesthesia in juvenile *C. viridis* in less than 180 s, with RTs below 300 s, which is considered optimal when evaluating anesthetic agents (Ross and Ross 2008). These results are similar to those reported for *S. senegalensis* (Weber et al. 2009), *T. albacares* (Cano et al. 2014), *A. pseudoharengus* (Berlinsky et al. 2016), and *R. canadum* (Sorensen et al. 2023).

Differences in MEC can be attributed to factors such as species, size, age, and environmental conditions (Jia et al. 2022, Simões-Bueno et al. 2024). In the present study, an inverse relationship was observed between the evaluated 2-phenoxyethanol concentrations and the induction time to stage III, consistent with reports for other species, such as *D. sargus* and *D. puntazzo* (Tsantilas et al. 2006), and *H. huso* (Shaluei et al. 2012). Although some authors have observed positive exponential correlations between the RT and anesthetic concentration (Tsantilas et al. 2006, Barata et al. 2016, Akgul and Can 2020), this correlation was not significant in the present study; the RT of juvenile *C. viridis* exposed to different concentrations of 2-phenoxyethanol was independent of the concentration, coinciding with what has been reported for *S. rivulatus* (Ghanawi et al. 2013), *A. regius* (Serezli et al. 2012), and *E. stoutii* (McCord et al. 2020).

The lack of correlation could be explained by the fact that the fish were exposed to high concentrations of the anesthetic for shorter periods, which implied less absorption of the anesthetic and, consequently, a faster recovery. Nevertheless, the species-specific physiological responses to different anesthetic agents must also be considered (Mylonas et al. 2005, Weber et al. 2009, Ghanawi et al. 2013). For example, Kübra (2022) reported that 2-phenoxyethanol is not recommended for anesthetizing adult *A. gueldenstaedtii* due to the long induction time and the high concentration required, and noted that high concentrations can affect fish gill tissue.

In the present study, the highest concentration (900 $\mu g \cdot L^{-1}$) caused irritation of the mouth and caudal fin in some juveniles, probably because 2-phenoxyethanol is an irritant (Kübra 2022). On the other hand, no mortality was observed in juvenile *C. viridis* exposed to the different concentrations of 2-phenoxyethanol, either during the experimental period or 72 h later. Similar results have been reported in other marine fish species anesthetized with 2-phenoxyethanol (Maršić-Lučić et al. 2005, Serezli et al. 2012, Barata et al. 2016, Akgul and Can 2020).

Conclusions

This study demonstrated that the 3 anesthetics evaluated were effective in inducing stage III anesthesia in juvenile *C. viridis*. The MECs without visible adverse effects were 50–75 $mg \cdot L^{-1}$ for clove oil, 125 $mg \cdot L^{-1}$ for MS-222, and 500 $\mu g \cdot L^{-1}$ for 2-phenoxyethanol, under the experimental conditions of this work.

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Declarations

Supplementary Material

This work includes no supplementary material.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Conceptualization: JMMB, MIAP; Data curation: MIAP, LERI; Formal analysis: MIAP, LERI; Funding acquisition: JMMB, MIAP, GVB; Research: MIAP; Methodology: MIAP, LERI, GVB; Supervision: MIAP; Validation: JMMB, MIAP; Visualization: MIAP, LERI, Writing—original draft: MIAP, Writing—review and editing: JMMB, LERI, GBV, MIAP.

Data Availability

The data for this study are available from the corresponding author upon reasonable request.

Ethical approvals and permits for studies involving animals

For the execution of the present study, the general animal welfare procedure based on Mexican legislation on animal welfare (NOM-062-ZOO-1999, Technical specifications for the production, care, and use of laboratory animals), which does not include aquatic organisms, was followed; however, we followed the ethical standards described in the ARRIVE Guidelines: Animal Research Reporting In Vivo Experiments.

Use of AI Tools

The authors did not employ any AI tools in this work.

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