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Effect of Color During Transport and Anesthetic Efficacy of Alcoholic Drink, 2-Phenoxyethanol, Clove Oil, MS-222, and Benzocaine in Silver Therapon, *Leiopotherapon plumbeus* (Kner 1864)

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Abstract

The study evaluated the survivability of silver therapon, *Leiopotherapon plumbeus* (Kner, 1864) in different color containers during transport and the efficacy of five anesthetic agents [alcoholic drink, 2-phenoxyethanol, clove oil, tricaine methanesulfonate (MS-222), and benzocaine] in the induction and recovery time of *L. plumbeus*. Different colored polyethylene bags (black, red, yellow, blue, and transparent) did not influence the survival rate of fish until the termination of the experiment (12-h transport time). The immersion experiment used three different concentrations in each anesthetic agent with three replicates (ten fish specimens per replicate). Different dosages significantly influenced the induction time, with decreased induction efficacies in high dosages. Moreover, the 200 ml L^{-1} and 300 ml L^{-1} alcoholic drinks anesthetized the fish specimens comparable to the induction efficacy of several dosages of 2-phenoxyethanol, MS-222, and benzocaine. Recovery time significantly varied among treatments, with a prolonged recovery period with increasing anesthetic concentrations. Regression analysis revealed a positive correlation between fish standard length and induction time ($P < 0.05$), albeit more pronounced in smaller dosages. Induction and recovery times were not correlated to fish size when exposed to higher dosages. The present finding demonstrated the anesthetic efficacy of four commercial anesthetic solutions, so as with alcoholic drinks with concentrations between or equal to 200 ml L^{-1} and 300 ml L^{-1}. Experimental trials for fish euthanization and field trials are open for further investigation.

Keywords: Ayungin, Bataan, induction time, anesthesia, recovery time

1. Introduction

Silver therapon (*Leiopotherapon plumbeus* Kner 1864), locally known as *ayungin* is an endemic commercial fish thriving in freshwaters (Paller *et al.* 2011) and brackishwaters of Luzon, Philippines (De Leon *et al.* 2017; Santos *et al.* 2020). It is regarded as an important fishery resource for subsistence fisheries which is priced from $ 4 to $ 8 per kilogram (Corpuz and Espaldon 2021). Wild populations of freshwater fishes, however, are imperiled by overfishing, habitat alterations (Corpuz *et al.* 2015), and the presence of alien invasive species causing their natural populations to dwindle (Corpuz *et al.* 2018). To save this terapontid, conservation management initiatives and repopulation through habitat restoration and hormonal-induced breeding have been implemented by the Philippine government and other state-funded universities (Aya *et al.* 2015; Consigna *et al.* 2019).

One of the vital factors to take into consideration for successful induced breeding is handling stress (Hseu *et al.* 1998; Weber *et al.* 2009). It was reported that physical stress may cause breeding inefficiency and at worst, mortality to the fish broodstock (Coyle *et al.* 2004). One method to minimize stress is to anesthetize the animals, which results in loss of sensitivity or insensitivity and induced sleep and muscle relaxation (general anesthesia). Moreover, the appropriate color background is used in aquaculture particularly in the hatchery to provide the best fish growth performance (Imanpoor and Abdollahi 2011; Brian 2015). In the same manner as transportation, a specific color background of transport bags may help the fish to reduce stress and eventual mass mortality (Manliclic *et al.* 2018).

In aquaculture, various anesthetic agents are being used to reduce the stress on the fish and injuries during transportation, breeding, and fish capture (Coyle *et al.* 2004). There are several types of anesthesia used to lessen the stress of fish in performing induced breeding including tricaine methanesulphonate (hereafter denoted as MS-222). The MS-222 is a water-soluble powdered substance that is typically buffered with sodium bicarbonate to lessen its acidity and is commonly delivered in a water bath (Popovic *et al.* 2012). Benzocaine (ethyl paraaminobenzoate) is known for its rapid induction and recovery times and good safety margin for several groups of fish (Ross and Ross 2008). Clove oil is derived from *Eugenia caryophyllate* tree, which contains methyleugenol, eugenol, and isoeugenol (Soto and Burhanuddin 1995), whereas clove oil has been widely used due to their efficacy and being inexpensive (Uçar and Atamanalap 2010). The 2–phenoxyethanol is a colorless oily liquid with a faint aromatic odor but moderately soluble in water. It is widely used for transporting live fish because it is cheap, reliable, and efficient and its active ingredients are ethylyn glycol monophenyl ether (Weber *et al.* 2009; Ucar and Atamanalap 2010). The alcoholic drink contains the recreational drug, ethanol which is produced through the fermentation of grains, fruits, or other sources of sugar. The practicality of using alcoholic drinks as anesthetic agents lies in the efficacy in reducing the mobility and reaction of fish to pressure, and the availability and ease of access in local markets. Attempts of using this substance for fish anesthesia previously showed a positive sedative effect in zebrafish (Hullinger 2014). However, the appropriate amounts or dosages to realize its anesthetic effect are yet determined for *L. plumbeus*.

The present study evaluated the anesthetic efficacy of alcohol drinks, 2-phenoxyethanol, clove oil, MS-222, and benzocaine as anesthetic agents in *L. plumbeus*. Specifically, this study determined the appropriate dosages for anesthesia, compared the induction and recovery times across different concentrations and anesthetic solutions, and analyzed the correlation of induction and recovery times with fish size. Moreover, we evaluated the survival of *L. plumbeus* stocked in various colored transport containers for 12-h transport time.

2. Method

2.1 Fish Specimens and Acclimation

The *L. plumbeus* (n = 312) were obtained from the fishponds in Orani, Bataan (14˚48.50” N, 120˚32.60” E) using cast nets and net traps set at the outlet of each pond. The specimens were transported at the hatchery facility of Bataan Peninsula State University and acclimated in three 1,200 L fiberglass tanks with a continuous supply of running freshwater and artificial aeration. Fish specimens were maintained for two weeks under ambient conditions (1:1 light-dark). During acclimation, daily monitoring of dissolved oxygen (5.30 ± 0.05 mg L^{-1}), temperature (26.63 ± 0.24 °C), and pH (7.81 ± 0.01) was done. Total ammonia-nitrogen concentration was recorded every other day and was constantly below 0.01 mg L^{-1}. Fish were fed *ad libitum* twice per day with commercial extruded feed (crude protein = 32%). Feeding was terminated 24 h before the experiment. No mortality was observed during the acclimation period. Fish specimens were size-sorted five days before the initiation of the experiment. Two hundred (200) unsexed fish individuals were used in the study.

2.2 Experimental Trials

Five anesthetics were used in this study — alcoholic drink (40% alcohol by volume, 80-proof, Ginebra San Miguel Inc., Philippines), 2-phenoxyethanol (ethylene glycol monophenyl ether, Sigma Aldrich Co., USA), MS-222 (Sigma Aldrich Co, USA), clove oil (90–95% eugenol, Sigma Aldrich Co., USA) and benzocaine (ethyl 4-aminobenzoate 99%, Sigma Aldrich Co, USA).

The different dosages of each anesthetic agent were prepared 30 min before the actual induction experiment. The experiment used 3-L plastic containers filled with 1 L of water with a specific dosage of anesthetic solution. Artificial aeration was provided throughout the experiment. The alcoholic drink, 2-phenoxyethanol, and MS-222 (see Table 1 for each concentration) were diluted directly into the anesthetic bath, whereas benzocaine and clove oil were initially dissolved in ethanol (92.8%) in a ratio of 1:9 (anesthetic to ethanol) since the two anesthetics are slightly soluble in water. The aliquot of the stock solutions was then used to attain the specific dosages in each treatment. Treatments and control groups (no anesthetic) were buffered with sodium bicarbonate to attain a pH level of 7.5.

The fish individual (ten fish per treatment of each anesthetic agent) was randomly placed in an experimental container with a specific concentration of the anesthetic solution. Treatments for all the anesthetic agents were investigated. The concentrations of alcoholic drinks were 100, 200, and 300 ml L^{-1}, while the four commercial anesthetic agents had 0.25, 0.50, and 1.00 ml L^{-1} (Coyle *et al.* 2004). The induction time was recorded for each the fish when fish displayed loss of balance, cessation of swimming, decrease in opercular rate, and no reaction to external stimuli (Pawar *et al.* 2011). Anesthetized individuals were weighed (g) and the standard length (SL) was measured. The SL (cm) of fish specimens was statistically homogeneous (9.07 ± 0.65 cm), ranging from 8.61 to

9.39 mm (Table 1), whereas weight was 12.27 ± 2.62 g, varying from 7.38 to 22.76 g. Consequently, the fish was placed into freshwater of similar temperatures to the experimental container. Water in the recovery container was constantly renewed. Recovery time was noted when the fish exhibited normal swimming and reaction to stimuli (Silva *et al.* 2012). The different stages of induction and recovery times followed the criteria proposed by Gullian and Villanueva (2009). Recovered specimens were brought back to the hatchery; whilst dead specimens (no vital signs after 30 min) were preserved in a 10% formaldehyde solution for fish collection.

Table 1. Mean ± standard error in standard length (mm) of experimental *Leiopotherapon plumbeus* (n = 200)

Anesthetic Solutions	**Standard Length (mm)**				***F* value**	***p***
	Control	**T1**	**T2**	**T3**		
Alcoholic drink	9.16 ± 0.21	8.61 ± 0.24	9.04 ± 0.19	9.25 ± 0.25	2.00	0.154
2-Phenoxyethanol	9.05 ± 0.15	8.89 ± 0.30	9.11 ± 0.13	9.12 ± 0.16	0.38	0.687
Clove oil	8.85 ± 0.26	8.82 ± 0.28	9.39 ± 0.22	9.24 ± 0.19	1.56	0.229
MS-222	9.24 ± 0.22	9.35 ± 0.27	9.16 ± 0.14	9.01 ± 0.21	0.61	0.548
Benzocaine	8.98 ± 0.18	9.02 ± 0.20	9.04 ± 0.12	9.07 ± 0.29	0.01	0.986

Treatments for alcoholic drink: T1 = 100 ml L^{-1}, T2 = 200 ml L^{-1}, T3 = 300 ml L^{-1}

Treatments for commercial anesthetic agents: T1 = 0.25 ml L^{-1}, T2 = 0.5 ml L^{-1}, T3 = 1.0 ml L^{-1}

2.3 Influence of Color Containers

In separate experiment, juvenile unsexed *L. plumbeus* from other conditioning tanks were randomly placed in various colored polyethylene bags (black, red, yellow, blue, and transparent) as colored containers (25.4 x 50.8 x 0.008 cm). The procedure was adopted from the study of Manliclic et al. (2018). Each bag contained 5 L of freshwater with a stocking density of one fish per liter (five fish x three replicates x five color containers = 75 fish individuals). The water used was obtained from the conditioning tanks. Bags were sealed after the addition of medical oxygen. The amount of oxygen was standardized for all treatments by ensuring that all plastic bags were air-filled of the same height. An improvised carrier with wheels was utilized to facilitate the simulated transport. The carrier was manually agitated by periodic push-and-pull actions and was operated under ambient conditions for 12 hours. Fish mortalities were monitored at 0-h, 6-h, and 12-h transport times.

2.4 Data Analyses

The homoscedasticity (Levene's test) and normality assumptions (Shapiro-Wilk test) for parametric terse were met. With that, analysis of variance (ANOVA) was employed for the mean comparison of induction and recovery time, followed by a posthoc test using Tukey's test ($p < 0.05$). Regression analyses were used to determine the relationship between SL and induction time, and between SL and recovery time ($p < 0.05$). In the color container experiment, the data on survival rates, expressed as percentages were arc sine-transformed prior to analyses. Treatment means (mean ± SD) in every transport time were compared using ANOVA ($P < 0.05$). Data were presented as mean ± standard deviation (SD). All statistical analyses were performed with the SPSS v 17 and Paleontological Statistic v 3.0.

3. Results

3.1 Influence of Color Containers

A summary of the survival rate of experimental fish is detailed in Table 2. Fish mortality occurred in the yellow and blue containers. The first mortality was observed in the yellow container, commenced at 6-h transport period, whereas the second mortality was recorded in the blue container at 12-h transport period. Nevertheless, no mortality happened in transparent, red, and black containers. Throughout, only two mortalities were recorded in the present study. Statistically, no significant difference among treatments was observed ($P > 0.05$).

Table 2. Data on the survival (%) of juvenile *Leiopotherapon plumbeus* packed in containers of different colors during the 12-h transport period

Color of Containers	Survival (%)					
	2 h	4 h	6 h	8 h	10 h	12 h
Transparent (Control)	100.00	100.00	100.00	100.00	100.00	100.00
Red	100.00	100.00	100.00	100.00	100.00	100.00
Yellow	100.00	100.00	93.33	93.33	93.33	93.33
Black	100.00	100.00	100.00	100.00	100.00	100.00
Blue	100.00	100.00	100.00	100.00	100.00	93.33

3.2 Induction Time

Different concentrations significantly affected the induction time, with a significant decrease in the induction period recorded (no more than 2 min) in T3 in each anesthetic solution (Table 3). The T1 of alcoholic drink had the longest mean induction time (9.73 ± 1.16 min). Nevertheless, the T3 of alcoholic drinks was able to anesthetize the fish individuals at 0.77 ± 0.14 min, considerably comparable to T3 of 2-phenoxyethanol (0.63 ± 0.12 min; $Q = 1.11$; $p = 0.44$), and T1 of MS-222 (0.74 ± 0.13 min; $Q = 0.19$; $p = 0.89$). Moreover, the mean induction time in T3 of benzocaine (1.96 ± 0.14 min) was not significantly different from T2 of the alcoholic drink (1.98 ± 0.30 min) ($Q = 0.12$; $p = 0.93$). Among all the treatments, the T3 of MS-222 solution had the most reduced mean induction time (0.21 ± 0.02 min).

3.3 Recovery Time

The recovery period was significantly varied among treatments in each anesthetic solution, with induced recovery time with increasing dosages (Table 4). The clove oil was found to cause longer mean recovery time relative to other anesthetic solutions (particularly for T1 and T3), whilst specimens exposed to 2-phenoxyethanol exhibited the fastest mean recovery time (1.02 ± 0.09 min). Fish specimens in all treatments of 2-phenoxyethanol and benzocaine had the fastest recovery time (no more than 3 min). However, two specimens died in T3 of the latter. Additionally, the mean recovery time for T1 of alcoholic drink (2.51 ± 0.50 min) was statistically homogeneous to T1 of MS-222 (2.68 ± 0.10 min), T2 (2.14 ± 0.28 min) and T3 (2.42 ± 0.32 min) of 2-phenoxyethanol, and T3 of benzocaine (2.85 ± 0.27 min). Mean recovery time in T2 of alcoholic drinks (6.85 ± 0.97 min) showed considerable similar response to T2 of clove oil (6.03 ± 0.33 min; $Q = 1.13$; $p = 0.43$), and T3 of MS-222 (7.14 ± 1.36 min; $Q = 0.24$; $p = 0.86$).

Table 3. Induction rate (min) of different anesthetic solutions of varying concentrations for *Leiopotherapon plumbeus* ($n = 200$).

Anesthetic Solutions	Induction Time (min)				*F* value	*p*
	Control	T1	T2	T3		
alcoholic drinks	na	9.73 ± 1.16^{a}	1.98 ± 0.30^{b}	0.77 ± 0.14^{b}	32.74	< 0.001
2-phenoxyethanol	na	5.65 ± 1.21^{a}	2.96 ± 0.64^{ab}	0.63 ± 0.12^{b}	13.97	< 0.001
clove oil	na	1.26 ± 0.13^{a}	1.11 ± 0.21^{a}	0.37 ± 0.03^{b}	28.19	< 0.001
MS-222	na	0.74 ± 0.13^{a}	0.57 ± 0.12^{ab}	0.21 ± 0.02^{b}	12.08	< 0.001
benzocaine	na	4.50 ± 0.29^{a}	2.27 ± 0.27^{b}	1.96 ± 0.14^{b}	32.94	< 0.001

Treatments for alcoholic drink: T1 = 100 ml L^{-1}, T2 = 200 ml L^{-1}, T3 = 300 ml L^{-1}
Treatments for commercial anesthetic agents: T1 = 0.25 ml L^{-1}, T2 = 0.5 ml L^{-1}, T3 = 1.0 ml L^{-1}
For each anesthetic solution, means with the same superscript letter are not significantly different (Tukey post-hoc tests); na = not applicable

Table 4. Recovery time (min) of Leiopotherapon plumbeus exposed to different anesthetic solutions of varying concentrations (n = 200)

Anesthetic Solutions	Recovery Time (min)				*F* value	*p*
	Control	T1	T2	T3		
alcoholic drinks	na	2.51 ± 0.50^{a}	6.85 ± 0.97^{b}	8.98 ± 0.84^{b}	24.08	< 0.001
2-phenoxyethanol	na	1.02 ± 0.09^{a}	2.14 ± 0.28^{b}	2.43 ± 0.32^{b}	14.18	< 0.001
clove oil	na	5.34 ± 0.27^{a}	6.03 ± 0.33^{a}	13.43 ± 0.91^{b}	34.73	< 0.001
MS-222	na	2.68 ± 0.10^{a}	5.87 ± 0.91^{ab}	7.14 ± 1.36^{b}	10.72	< 0.001
benzocaine	na	1.97 ± 0.07^{a}	1.92 ± 0.09^{a}	2.85 ± 0.27^{b}	5.27	< 0.001

Treatments for alcoholic drink: T1 = 100 ml L^{-1}, T2 = 200 ml L^{-1}, T3 = 300 ml L^{-1}
Treatments for commercial anesthetics: T1 = 0.25 ml L^{-1}, T2 = 0.5 ml L^{-1}, T3 = 1.0 ml L^{-1}
For each anesthetic solution, means with the same superscript letter are not significantly different (Tukey post-hoc tests); na = not applicable

3.4 Correlation of Fish Length with Induction and Recovery Time

Correlation analysis indicated that the induction and recovery time is size-influenced, with smaller specimens anesthetized faster than the larger ones (Table 5). This observation was significantly evident in T1 of all anesthetic solutions, as well as in T2 of alcoholic drinks. The size of the fish did not affect the induction time when the concentration of commercial anesthetics was 0.5 ml L^{-1} and above. No significant correlation between size and recovery time was observed in T1 of alcoholic drinks and MS-222, including T2 and T3 of all anesthetic solutions (Table 5).

Table 5. Relationship of standard length (mm) with induction and recovery times in each treatment. Significant relationships are set in bold.

Anesthetic Solutions		T1		T2		T3	
		R^2	*p*	R^2	*p*	R^2	*p*
Alcoholic Drink	SL vs Induction	**0.78**	0.001	**0.85**	0.002	0.34	0.329
	SL vs Recovery	0.48	0.157	0.28	0.583	0.46	0.181
2-Phenoxyethanol	SL vs Induction	**0.71**	0.019	0.59	0.073	0.52	0.126
	SL vs Recovery	**0.76**	0.010	0.61	0.060	0.54	0.110
Clove Oil	SL vs Induction	**0.69**	0.026	0.59	0.072	0.55	0.102
	SL vs Recovery	**0.66**	0.038	0.18	0.622	0.19	0.597
MS-222	SL vs Induction	**0.71**	0.020	0.55	0.098	0.61	0.062
	SL vs Recovery	0.62	0.057	0.20	0.573	0.41	0.267
Benzocaine	SL vs Induction	**0.73**	0.018	0.34	0.337	0.62	0.056
	SL vs Recovery	**0.72**	0.020	0.30	0.392	0.45	0.268

Treatments for alcoholic drink: T1 = 100 ml L^{-1}, T2 = 200 ml L^{-1}, T3 = 300 ml L^{-1}
Treatments for commercial anesthetic agents: T1 = 0.25 ml L^{-1}, T2 = 0.5 ml L^{-1}, T3 = 1.0 ml L^{-1}

4. Discussion

The present study demonstrated that the color background did not affect the survival of *L. plumbeus* juveniles during the 6-h and 12-h transport times. Previous studies demonstrated that background color affects the performance of various aquatic species (Luchiari and Freire 2004; Ninwichian *et al.* 2018; Manliclic *et al.* 2019). In the works of Manliclic and his colleagues (2018), survival of Nile tilapia juveniles improved when exposed to the blue color container in 24-h conditioning time before transport. In the present study, no significant difference was observed in the mortality rate of *L. plumbeus* juveniles. It is comparable to the result of Manliclic et al. (2018), where there are no significant differences in four different color backgrounds during 6-h and 12-h transport time.

The induction times decreased significantly as concentrations increased in 2-phenoxyethanol, clove oil, MS-222, and benzocaine. The results are similar to the previous studies suggesting an inversed relationship between induction time and concentration of anesthetic agents in teleost fishes (Pawar *et al.* 2011; Yildiz *et al.* 2013; Varkey and Sajeevan 2014; Kucuk and Coba 2016; Ogretmen *et al.* 2016; Bolasina *et al.* 2017; Park 2019). It is also apparent that the alcoholic drinks have comparable effect in several dosages of commercial anesthetic agents

The study provides evidence that alcoholic beverages containing 40% ethanol can be used as an effective anesthetizing agent for *L. plumbeus*. The presence of an active component, ethanol is known to inhibit or depress the central nervous system activity of many mammalian species (Banerjee 2014). In most fish farms in the Philippines, alcoholic drinks are usually used as an anesthetic agent for aquaculture fish (e.g., *Clarias gariepinus*). In the absence of commercial anesthetics, alcoholic drinks can be practical options to facilitate the smooth handling of fish during induced spawning. The present finding thus opens further studies as to the anesthetic effect of alcoholic drinks in *L. plumbeus* during actual breeding and transport.

Recovery times increased gradually with increasing concentrations of all the anesthetic agents. Prolonged recovery with increased anesthetic dosage had been reported in seahorse (Pawar *et al.* 2011), rainbow trout (Yildiz *et al.* 2013), redline torpedo fish (Varkey and Sajeevan 2014), goldfish (Kucuk and Coba 2016), shabbout fish (Ogretmen *et al.* 2016), guppy (Bolasina *et al.* 2017), catfish (Park 2019). However, several studies have documented a decreasing recovery time with an increase in the concentration of clove oil and 2-phenoxyethanol for European sea bass (*Dicentrachus labrax*) and gilthead seabream (*Sparus aurata*) (Mylonas *et al.* 2005).

The mechanisms of fish in recovery times during anesthetizing seem to be complex. The observed delayed recovery may be attributed to its persistence on the gill surface (Sladky *et al.* 2001; King *et al.* 2005). Moreover, the difference in the respective recovery times is highly affected by species, size, physiological status, and environmental conditions (Ross and Ross 1999). Different factors, i.e., biological and/or environmental factors affect the efficacy of anesthetics in fish. According to Coyle *et al.* (2004), the efficacy of anesthetic drugs depends on the gill area to body weight ratio, metabolic rate, and temperature related. Moreso, larger fish has a slower absorption rate of anesthetic drugs compared to smaller fish because of the smaller gill area surface relative to body mass for drug diffusion (Popovic *et al.* 2012). Our observation is in agreement with the work of Park (2019), i.e., small-sized fish were more easily anesthetized and recovered more rapidly from anesthesia than large-sized fish. Despite that, there are other reports that found that larger individuals had quicker recovery times than smaller ones (Woody *et al.* 2002; Fernandes *et al.* 2017). It is noteworthy to mention that all recovered and survived fish individuals in the two experiments were returned to hatchery facility for future research initiatives.

5. Conclusion and Recommendations

This preliminary study demonstrated that the color of containers had no profound effect on the survival of juvenile *L. plumbeus*, although this may be attributed to low stocking density and short transport period. For further investigation, it is suggested to increase the number of experimental fish per container and increase the transport time under the ambient setting.

The present study demonstrated the anesthetic efficacy of alcoholic drinks and four commercial anesthetic agents in *L. plumbeus*. Higher dosages, *viz* 200 ml L^{-1} to 300 ml L^{-1} of alcoholic drinks were found to be effective, and are comparable to the induction response of other commercial anesthetics. The results imply cost-effective use of anesthetic during induced spawning and transportation of this terapontid. The results of the study provide a practical method for minimizing stress in fish handling, which is vital in aquaculture and conservation endeavors for this terapontid, and possibly in other diminutive native fish species.

The study can be replicated in induced spawning and transportation under ambient set-up. Fish euthanization using experimental anesthetic agents is also open for further investigation. Likewise, investigation of the dose-response in biological status (reproductive state, sizes, and sexes), hematological profile, and cortisol levels will contribute more to the total efficacy of anesthetics in experimental *L. plumbeus*.

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References

Aya, F. A., Nillasca, V. S. N., Corpuz, M. N. C., & Garcia, L. M. B. (2015). Larval rearing of silver therapon (*Leiopotherapon plumbeus*) in outdoor tanks. In Romana-Eguia MRR, Parado-Estepa FD, Salayo ND, & Lebata-Ramos MJH (Eds.), *Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia: Challenges in Responsible Production of Aquatic Species: Proceedings of the International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia 2014* (p. 331). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Banerjee, N. (2014). Neurotransmitters in alcoholism: A review of neurobiological and genetic studies. *Indian Journal of Human Genetics, 20*(1), 20–31. https://doi.org/10.4103/0971-6866.132750

Bolasina, S. N., de Azevedo, A., & Petry, A. C. (2017). Comparative efficacy of benzocaine, tricaine methanesulfonate and eugenol as anesthetic agents in the guppy *Poecilia vivipara. Aquaculture Reports, 6*, 56–60. https://doi.org/10.1016/j.aqrep.2017.04.002

Brian, O. (2015). Effect of tank background colour on the hatchability of *O. niloticus* eggs and survival of fry. *International Journal of Fisheries and Aquatic Research*, *2*(6), 81–86.

Consigna, M. J. S., Corpuz, M. N. C., Rabadon, M. L. L., & Manliclic, A. D. M. (2019). Hormone-induced spawning and gonadosomatic index of brackishwater silver therapon (*Leiootherapon plumbeus* Kner 1864). *Asia Life Sciences, 28*(2), 387–397.

Corpuz M. N. C., & Espaldon, M. V. O., (2021). Socio-ecological system assessment for conservation planning in riverine and mangrove fishery areas in Bataan, Philippines. In National Conference on Food, Environment, Engineering and Technology. Rubiano M. F. O., Roldan L., & Paguia H. M. (Eds), *Balanga City, Bataan, Philippines (*p. 87).

Corpuz, M. N. C. (2018). Diet variation and prey composition of exotic clown featherback, *Chitala ornata* (Gray 1831) Osteoglossiformes: Notopteridae) in Laguna de Bay, Luzon Island, Philippines. *Asian Fisheries Science, 31*(4), 252–264. https://doi.org/10.33997/j.afs.2018.31.04.001

Corpuz, M. N. C., Paller, V. G. V., & Ocampo, P. P. (2015). Ichthyofaunal survey in selected freshwater habitats in Camarines Sur, Philippines. *Asian Journal of Biodiversity, 6,* 80–99.

Coyle, S. D., Durborow, R. M., & Tidwell, J. H. (2004). Anesthetics in Aquaculture. SRAC Publication No. 3900. 6 pp. Retrieved from https://fisheries.tamu.edu/files/2013/09/SRAC-Publication-No.-3900-Anesthetics-in-Aquaculture.pdf

De Leon, K. J. A., Manliclic, A. D. C., & Corpuz, M. N. C. (2017). Spatial and sexual variation on morphometrics, length and weight, and condition factor dynamics of endemic silver therapon (*Leiopotherapon plumbeus* Kner). *International Journal of Agricultural Technology, 13*(7), 1567–1577.

Fernandes, I. M., Bastos, Y. F., Barreto, D. S., Lourenco, L. S., & Penha, J. M. (2017). The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes. *Brazilian Journal of Biology, 77*(3), 444–450. https://doi.org/10.1590/1519-6984.15015

Gullian, M., & Villanueva, J. (2009). Efficacy of tricaine methanesulphonate and clove oil as anesthetics for juvenile cobia *Rachycentron canadum*. *Aquaculture Research*, *40*, 852–860. https://doi.org/10.1111/j.1365-2109.2009.02180.x

Hseu, J. R., Yeh, S. L., Chu, Y. C., & Ting, Y. T. (1998). Comparison of efficacy of five anesthetics in gold-lined sea bream. *Sparus sabra*. *Acta Zoologica Taiwanica, 9*(1), 00–00.

Hullinger, J. (2014). *Here's what happens when fish get drunk.* Retrieved 13 August, 2020, from http://mentalfloss.com/article/57027/heres-what-happens-when-fish-get-drunk.

Imanpoor, M. R., & Abdollahi, M. (2011). Effects of tank color on growth, stress response and skin color of juvenile Caspian Kutum *Rtilus frisii Kutum. Global Veterinaria*, *6*(2), 118–125.

King, W. V., Hooper, B., Hillsgrove, S., Benton, C., & Berlinsky, D. (2005). The use of clove oil, metomidate, tricaine methanesulphonate and PE for inducing anaesthesia and their effect on the cortisol stress response in black sea bass (*Centropristis striata* L.). *Aquaculture Research, 36*, 1442–1449. https://doi.org/10.1111/j.1365-2109.2005.01365.x

Kucuk, S., & Coban, D. (2016). Effects of tricaine as an anaesthetics on Goldfish, *Carassius auratus* (Linnaeus 1758) at different salinities and concentrations. *Turkish Journal of Fisheries and Aquatic Sciences, 16*, 611–616. https://doi.org/10.4194/1303-2712-v16_3_13

Luchiari, A. C., & Freire, F. A. M. (2009). Effects of environmental colour on growth of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), maintained individually or in groups. *Journal of Applied Ichthyology*, *25*(2), 162–167.

Manliclic, A. D. C., Consigna, M. J. S., Rabadon, M. L. L., & Corpuz, M. N. C. (2019). Black background improves the population growth of euryhaline rotifer, *Brachionus rotundiformis* reared in two photoperiod regimes. *AACL Bioflux, 12*(5), 1762–1770.

Manliclic, A. D. C., Corpuz, M. N. C., & Vera Cruz, E. M., (2018). Optimum conditioning period before packing, salt-treated water, and blue background color improves the survival of Nile tilapia *Oreochromis niloticus* (Linn.) fingerlings during transport. *The Philippine Agricultural Scientists*, *101*(4), 379–390.

Mylonas, C. C., Cardinaletti, G., Sigelaki, I., & Polzonetti-Magni, A. (2005). Comparative efficacy of clove oil and 2-phenoxyethanol as anaesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures. *Aquaculture, 246*, 467–481. https://doi.org/10.1016/j.aquaculture.2005.02.046

Ninwichian, P., Phuwan, N., Jakpim, K., & Sae-Lim, P. (2018). Effects of tank color on the growth, stress responses, and skin color of snakeskin gourami (*Trichogaster pectoralis*). *Aquaculture International*, *26*(2), 659–672.

Ogretmen, F., Golbasi, S., & Kutluyer, F. (2016). Efficacy of clove oil, benzocaine, eugenol, 2-phenoxyethanol as anaesthetics on shabbout fish (*Barbus grypus* Heckel, 1843). *Iranian Journal of Fisheries Sciences, 15*(1), 470–478.

Paller, V. G. V., Ocampo, P. P., & Corpuz, M. N. C. (2011). *Fish Ark Philippines: direction for the conservation of native and endemic Philippine Freshwater Fishes Project 1: Survey of diminutive freshwater fishes indigenous to isolated crater lakes, mountain crater lakes, mountain streams and cataracts in Southern Luzon, Philippines.* Retrieved 15 July 2020 from http://agris.fao.org/agris-search/search.do?recordID=PH2012000274.

Park, I. S. (2019). The anesthetic effects of clove oil and MS-222 on far eastern catfish. *Silurus asotus. Development & Reproduction*, *23*(2), 183–191. https://doi.org/10.12717/DR.2019.23.2.183

Pawar, H. B., Sanaye, S. V., Sreepada, R. A., Harish, V., Suryavanshi, U., & Tanu Ansari, Z. A. (2011). Comparative efficacy of four anaesthetic agents in the yellow seahorse. *Hippocampus kuda* (Bleeker, 1852). *Aquaculture, 311*, 155–161. https://doi.org/10.1016/j.aquaculture.2010.12.007

Popovic, N., Strunjak-Perovic, I., Coz-Rakovac, R., Barisic, J., Jadan, M., Persin Berakovic, A., & Sauerborn Klobucar, R. (2012). Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. *Journal of Applied Ichthyology*, *28*, 553–564. https://doi.org/10.1111/j.1439-0426.2012.01950.x

Ross, L. G., & Ross, B. (2008). Anaesthetic and sedative techniques for aquatic animals (3rd ed.). Oxford: Blackwell Publishing. p. 240. https://doi.org/10.1002/9781444302264.ch7

Santos, D. A., Manliclic, A. D. M., & Corpuz, M. N. C. (2020). Length-weight relationship and condition factor of silver therapon, *Leiopotherapon plumbeus* (Terapontidae) from two brackishwater habitats. *AACL Bioflux*, *13*(3), 1495–1503.

Silva, L. de L., Parodi, T. V., Reckziegel, P., Garcia, V. de O., Bürger, M. E., Baldisserotto, B., Malmann, C. A., Pereira, A. M. S., & Heinzmann, B. M. (2012). Essential oil of *Ocimum gratissimum* L.: anesthetic effects, mechanism of action and tolerance in silver catfish, *Rhamdia quelen*. *Aquaculture, 350-353*, 91–97. https://doi.org/10.1016/j.aquaculture.2012.04.012

Sladky, K. K., Swanson, C. R., Stoskopf, M. K., Loomis, M. R., & Lewbart, G. A. (2001). Comparative efficacy of tricaine methanesulfonate and clove oil for use as anaesthetics in red pacu (*Piaractus brachypomus*). *American Journal of Veterinary Research, 62*, 337–342. https://doi.org/10.2460/ajvr.2001.62.337

Soto, C. G., & Burhanuddin, G. (1995). Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish (*Siganus lineatus*). *Aquaculture, 136*(1), 149–152. https://doi.org/10.1016/0044-8486(95)01051-3

Uçar, A., & Atamanalp, M. (2010). The effects of natural (clove oil) and synthetical (2-phenoxyethanol) anesthesia substances on hematology parameters of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta fario*). *Journal of Animal and Veterinary Advances, 9*(14), 1925–1933. https://doi.org/10.3923/javaa.2010.1925.1933

Varkey, A. M. T., & Sajeevan, S. (2014). Efficacy of 2-phenoxyethanol as an anaesthetic for adult redline torpedo fish, *Sahyadria denisonii* (Day 1865). *International Journal of Zoology*. https://doi.org/10.1155/2014/315029

Weber, R. A., Peleteiro, J. B., Garcia Martin, L. O., & Aldegunde, M. (2009). The efficacy of 2-phenoxyethanol, metomidate, clove oil and MS-222 as anaesthetic agents in the Senegalese sole (*Solea senegalensis* Kaup). *Aquaculture*, *288*, 147–150. https://doi.org/10.1016/j.aquaculture.2008.11.024

Woody, C. A., Nelson, J., & Ramstad, K. (2002). Clove oil as an anaesthetic for adult sockeye salmon: field trials. *Journal of Fish Biology, 60*(2), 340–347. https://doi.org/10.1006/jfbi.2001.1842

Yildiz, M., Kayim, M., & Akin, S. (2013). The anaesthetic effects of clove oil and 2-phenoxyethanol on rainbow trout (*Oncorhynchus mykiss*) at different concentrations and temperatures. *Iranian Journal of Fisheries Sciences, 12*(4), 947–961.