



The Use of Natural Anesthetic Ingredients for Clove Oil in Transportation of Juvenile Swordtail (*Xiphophorus helleri*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research aims to determine the most effective concentration of clove oil and the best period in the transportation of swordtail (*Xiphophorus helleri*) with the highest survival rate. This research used the factorial randomized block design (FRBD) which consisting of four treatments of concentration (10×10^{-3} mL, 13×10^{-3} mL, 16×10^{-3} mL), three treatments of duration (3 hours, 5 hours, 7 hours), and repeated three times. The measured parameters are induction time, conscious recovery time, and survival rate after transportation. The results showed that the treatment of 10×10^{-3} mL with the duration of 5 hours was an effective treatment with an average induction time of 17 minutes 56 seconds, a conscious recovery time of 7 minutes 37 seconds, the survival rate of 100% at post-transportation and after 7 days of rearing is 83%. Water quality after transportation are temperature (24.6 °C), DO (14.72 mg / L), pH (6.42) and ammonia (0.0043 mg/L).

Keywords: Conscious recovery time; clove oil; induction time; survival rate; swordtail.

1. INTRODUCTION

The swordtails are known for their beautiful color variations on their bodies and fins, making them popular in the freshwater ornamental fish market. The swordtails can classify into various types based on variations in the color hues of the body and fins [1]. Transportation is a solution for distributing seeds or broodstock to a destination area with the principle of maintaining maximum possible survival until the consumers accepted the fish [2]. The transport of fish classified into two, with an open system and a closed system [3]. Some of the factors that cause fish mortality during transportation are much carbon dioxide because of respiration, the accumulation of ammonia is formed from fish metabolism, and consumes much oxygen [2]. One of the efforts to reduce mortality during the transportation is anesthesia.

Anesthetic was used to be calm the fish so that they are less active, reducing oxygen consumption, reduce the production of carbon dioxide that can biodegradable so that it does not harm fish [4]. According to Rusda (2004), The mechanism of the anesthetic agent works to block the transmission of nerve stimuli to the central nervous system and vice versa, where it works mainly on the cell membrane while the effect on the nerves is minimal. Anesthetic substances used for induction fish are in the form of chemicals such as MS-222 (tricaine methane sulphate), CO₂, and quinaldine while the natural ingredients such as rubber seed extract, nutmeg extract, clove extract.

One of the anesthetic ingredients is clove oil, the quality of clove oil is influenced by eugenol compounds. Eugenol compounds have a sharp and spicy aroma that can provide stimulant and anesthetic effects that can suppress the rate of oxygen consumption (O₂) and affect fish metabolism, but there is no accumulation of residues in fish bodies caused it reissued [5]. This research aims to determine the best clove oil concentration and duration for transporting swordfish (*X. helleri*) with a high survival rate.

2. RESEARCH AND METHODS

2.1 Time and Place

This research was conducted from November 28, 2020 to December 31, 2020. This research took place at the Ciparanje Wet Laboratory, Padjadjaran University's Faculty of Fisheries and Marine Sciences. This research carried out the

transportation is done at 09:00 pm until 07:00 pm.

2.2 Preparation of Tools and Materials

The tools used in this research were an aquarium with size (60 x 30 x 30) cm³ with 20 fish per aquarium, aerator, DO meter, pH meter, thermometer, fishing net, oxygen cylinder, plastic, polyethylene, stopwatch, styrofoam, rubber, and pick-up cars. The materials used 720 swordtail seeds, diluted clove oil, pearl seed feed, pure oxygen, water samples before and after transportation.

2.3 Treatment and Work Step

This research used the factorial randomized block design (FRBD) process, which included of four treatments of concentration (control, 10 x 10³ mL, 13 x 10⁻³ mL, 16 x 10⁻³ mL), three treatments of duration (3 hours, 5 hours, 7 hours), and repeated three times. The measured parameters are induction time, conscious recovery time, and survival rate after transportation and water quality. The induction time was the time calculated from the test fish put into a plastic containing clove oil with a concentration according to the treatment until it shows symptoms of the total fainting phase of the fish. The conscious recovery time calculated from stunned test fish and continued until the fish showed signs of regaining consciousness.. The work steps in this research consisted of the preparation of materials and tools, anesthetizing procedures for test fish, transportation procedures for fish, procedures for conscious recovery time, procedures for maintaining test fish after transportation, and procedures for measuring water quality.

2.4 Data Analysis

Data of induction time, time to recover to the consciousness recovery of fish, and water quality are analyzed descriptively and comparatively. Survival rate tested with Annova at the 95% confidence level. If there is a significant difference between treatments, then it is continued by Duncan's multiple distances (Gasperz 1991). Water quality measurements include DO, pH, temperature, and ammonia.

3. RESULTS AND DISCUSSION

3.1 Induction Time

According to the result of the research, each treatment has a different induction time (Fig. 1).

According to Fig. 1, the treatment concentration of 10×10^{-3} mL with the induction time of 3 hours, 5 hours, and 7 hours yielded 17 minutes 12 seconds, 18 minutes 08 seconds, and 17 minute 42 seconds. The concentration of 13×10^{-3} mL with durations of 3, 5, and 7 hours was 15 minutes 17 seconds, 14 minutes 36 seconds and 14 minutes 15 second. Induction time of 16×10^{-3} mL with durations of 3, 5, and 7 hours was 12 minutes 40 second, 12 minutes 12 minutes 44 second, and 10 minutes 46 seconds. The effect of anesthetic treatment on test fish results in a balance disorder due to a decrease in the rate of oxygen consumption and metabolic rate, both of which are toxic and reduce the risk of injured fish (Kaya and Louhenapessy 2016). Increased

operculum movement or activity; after a few minutes, the fish's movement slows, becomes ataxic, swims unbalanced, and begins to turn sideways until it reaches the bottom of the pond, and respiration increases [6].

3.2 Consciousness Recovery Time

A stopwatch is used to observe and calculate the conscious recovery time. Observations began with the stunned test fish and continued until the fish showed signs of regaining consciousness. According to the research, each treatment takes a different amount of time to return to consciousness (Fig. 2).

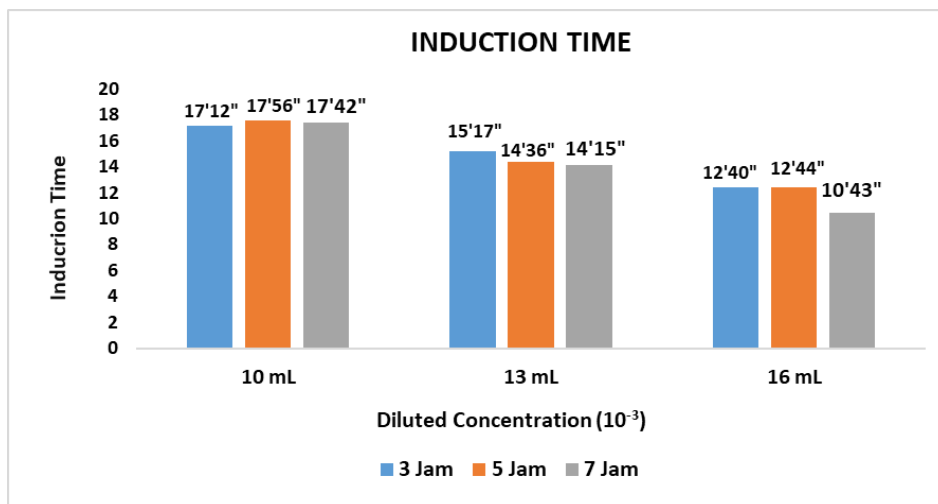


Fig. 1. Average induction time

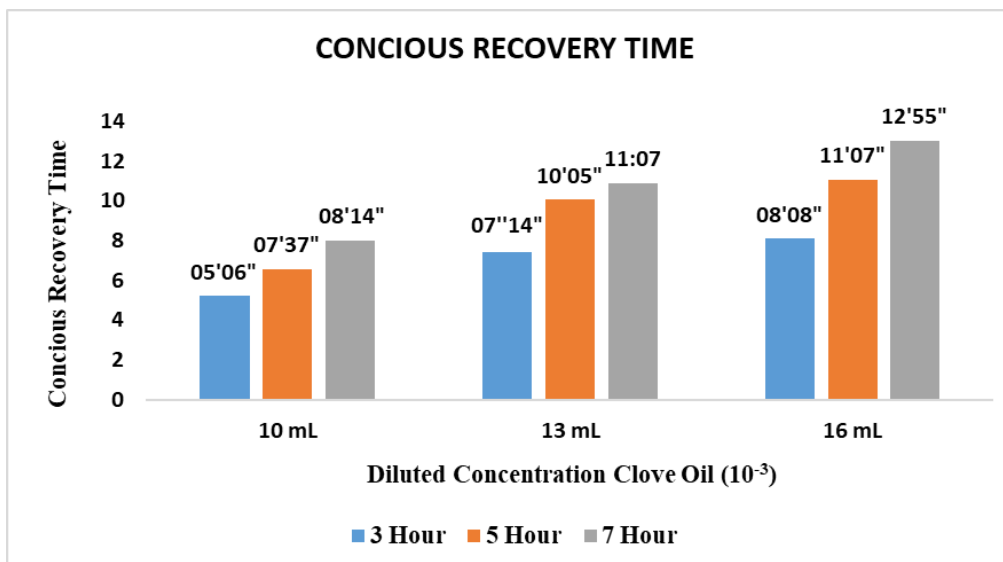


Fig. 2. Average conscious recovery time

According to Fig. 2, the treatment with a concentration of 10×10^{-3} mL, the length of time to recover to the consciousness of swordtail at 3 hours, 5 hours, and 7 hours was 05 minutes 06 seconds, 07 minutes 37 seconds, and 08 minutes, and 14 seconds. minutes. The concentration of 13×10^{-3} mL, conscious recovery time in 3, 5, and 7 hours is 07 minutes 14 seconds, 10 minutes 05 seconds, and 11 minutes 07 seconds. The concentration of 16×10^{-3} mL of time to regain consciousness in 3, 5, and 7 hours was 08 minutes 08 seconds, 11 minutes 07 seconds, and 12 minutes 55 seconds. The process of recovering from the consciousness of the test fish was caused by the weak condition of the fish and the effect of the concentration of clove oil so that the test fish needed a longer time to recover consciousness.

The consciousness process for transported fish that has done is put in an aquarium then given continuous aeration. Aeration used to add air to the water so that the dissolved oxygen content in the water becomes sufficient [7]. During the consciousness process, water with sufficient dissolved oxygen will enter through the gills and then enter the blood, cleaning the anesthetic remains in the fish's body before being discharged through the drain [8].

3.3 Survival Rate of Swordtail Post Transportation

Survival rate post transportation is the percentage of all fish that pre transported by the fish that are alive post transportation. Below is interaction between concentration treatment and duration of the swordtail survival rate (Table 1).

Based on Table 1, the survival rate after transportation with several treatments of concentration and duration, 10×10^{-3} mL 5 hours treatment is an effective treatment with the highest survival rate of 100%. Control treatment 7 hours is the lowest treatment with a survival rate of 88%. Based on the table Anova of treatment interactions, there was no significant difference between the treatment concentration and duration caused the survival of the fish treated with clove oil anesthetic, and control was not much different at the duration of 3, 5, 7 hours. Before being transported, fish gave additional pure oxygen so that fulfilled oxygen, and transportation activities are done out in the early morning and in the rain where dissolved oxygen levels are high due to a decrease in temperature.

Table 1. The interaction between treatment of concentration and duration on swordtail survival rate

Duration (Hour)	Concentration (mL)	Average (%)
3	Control	93
	10×10^{-3}	100
	13×10^{-3}	98
	16×10^{-3}	97
5	Control	93
	10×10^{-3}	100
	13×10^{-3}	98
	16×10^{-3}	93
7	Control	88
	10×10^{-3}	97
	13×10^{-3}	95
	16×10^{-3}	92

Table 2. Effect of treatment concentration on average survival rate of fish post transportation

Concentration (mL)	Average (%)
Control	91,67 ^a
16×10^{-3}	93,89 ^{ab}
13×10^{-3}	97,22 ^{bc}
10×10^{-3}	98,89 ^c

Based on Table 2, the concentration of 10×10^{-3} mL (c) is the best treatment with an average survival of 98.7% in all treatment duration (3 hours, 5 hours, 7 hours) after transportation. Clove oil contains a large amount of euganol (70-80%) so it is very effective for anesthetic agents even in low concentrations [9]. The Using of higher concentrations can inhibit excitability in the ganglion due to prolonged depolarization, and cause impaired peripheral nerve blood flow [10].

Based on Table 3, treatment duration of 7 hours (a) was not significantly different from the duration of 5 hours (ab) but significantly different from 5 hours. The 5 hours (ab) treatment was not significantly different from the 7 hours (b). There is a clear difference between the treatment duration of 3 hours and 7 hours because, in the control treatment, the fish is in an active state so that the increase in metabolism and oxygen consumption can reduce the length of time, thus stressing the fish and eventually dying.

3.4 Survival Rate of Swordtail Post Rearing for Seven Days

The cultivation of swordtail shows to determine the effect of using clove oil anesthetic with different concentrations and the duration of transportation. The following is a table of the

results of the survival rate research after seven days of maintenance.

Based on Table 4, the survival of the fish after transportation with several treatment concentrations and duration, treatment 10×10^{-3} mL, 5 hours (d) with significant difference to each treatment with the highest survival rate of 83%, treatment 13×10^{-3} mL, 3 hours (a) was the lowest treatment with survival rate 60%. This is in accordance with the results of the Din 2012 research, the concentration of clove oil has a significantly different effect on the survival rate of Pangas catfish.

The test results showed that the treatment (10 mL) was the best concentration with 92.86% post-transport survival and 46.74% post-maintenance survival rate. Based on the monitoring during seven days of breeding, a critical period for the test fish is the day after transport after 3rd day, where many test fish are weak and die from significant temperature changes. affect the physiological processes of fish so that fitness body can reduce and fish are kept strained [11]. Fish deaths can be reduced in the following days by maintaining water quality, like regularly changing water, monitoring the status, and regular feeding of fish two times a day.

Table 3. Effect of treatment duration on average survival rate of fish post transportation

Duration (Hour)	Average (%)	Notation
7	92,90	a
5	96,25	ab
3	97,10	b

Table 4. Survival rate post rearing for seven days

Duration (Hour)	Consentration (mL)	Average (%)
3	Control	75 ^c
	10×10^{-3}	78 ^{cd}
	13×10^{-3}	60 ^a
	16×10^{-3}	82 ^d
5	Control	82 ^d
	10×10^{-3}	83 ^d
	13×10^{-3}	82 ^d
	16×10^{-3}	77 ^c
7	Control	78 ^{cd}
	10×10^{-3}	72 ^{bc}
	13×10^{-3}	68 ^b
	16×10^{-3}	77 ^c

3.5 Water Quality

Water quality in the aquarium refers to the content of materials present in water concerning supporting survival. Fish deaths can be reduced in the following days by maintaining water quality, like regularly changing water, monitoring the status, and regular feeding of fish two times a day [12]. Temperature, dissolved oxygen, pH and ammonia is a limiting factor in fish culture. The results of water quality observations before and after transportation can be seen at Table 5.

Based on the research that has done, it is known that the temperature pre transportation range 24.6 – 25.6°C and post transportation 22.9 – 25.2°C. The water temperature must control every day during the 7 day maintenance of tested fish after transport, as the temperature directly affects the aquatic organisms' life processes, because fish are poikilotherm [13]. Temperature change of 5° C above normal can cause stress to fish, even tissue damage and death. Safe temperatures for keeping swordtails are in the range between 10 – 30°C [14].

Based on Table 5, that the DO value pre transportation ranges from 8.6 - 8.8 mg / L, and the DO value post transportation ranges from 9.1-14.84 mg / L. The increase in DO value caused before the plastic is bound to rubber then

filled with pure oxygen so that the fish transported don't run out of oxygen. Changes to the concentration of dissolved oxygen may have a direct effect on aquatic organisms' deaths, the indirect effect is to increase the toxicity of pollutants that in turn affect the organism itself [15].

Based on the research that has done, it is known that the pH pre transportation range 6.9– 7.2°C. The decrease in pH value caused high activity before anesthesia and the addition of clove oil which changes the pH of the water. Swordtail can live ranges from pH 6-8 [16].

Post transportation, ammonia values were measured and tested in the MSP FPIK Unpad laboratory with an average of 0.0029 to 0.021 mg/L. During maintenance seven days, the level of ammonia in the water must be controlled by the way the aquarium siphon three times for a week, and change the water every day and give enough fish feed so that not a lot of leftover feed settles bottom. The effect of high ammonia levels on fish is a narrowing of the gill, which causes the gas exchange process in the gills to decrease. Besides, it causes a decrease in the number of cells in the blood, reduced body resistance, and tissue damage to various organs [17]. Kadar Free ammonia levels are not more than 0.02 in aquatic water. 0,02 mg/ L [18].

Table 5. Average result the average results of water quality observations before and after transportation

Treatment		Water Quality						
Duration (Hour)	Concentration (mL)	Temperature (°C)		DO (mg/L)		pH		Ammonia (mg/L)
		pre	pasca	pre	pasca	pre	pasca	
3	Control	24,6	22,9	8,8	12,9	7,2	6,72	0,0029
	10 x 10 ⁻³	24,6	23,0	8,8	14,72	7,2	6,42	0,0043
	13 x 10 ⁻³	24,6	23,0	8,8	14,69	7,2	6,6	0,0067
	16 x 10 ⁻³	24,6	23,0	8,8	14,84	7,2	6,55	0,01
5	Control	24,7	24,3	8,5	10,04	6,9	6,72	0,014
	10 x 10 ⁻³	24,7	24,3	8,5	12,27	6,9	6,5	0,021
	13 x 10 ⁻³	24,7	24,4	8,5	12,92	6,9	6,62	0,014
	16 x 10 ⁻³	24,7	24,3	8,5	13,08	6,9	6,57	0,006
7	Control	25,6	25,0	8,6	9,10	7,1	6,81	0,0053
	10 x 10 ⁻³	25,6	25,0	8,6	11,23	7,1	6,82	0,0095
	13 x 10 ⁻³	25,6	25,2	8,6	11,33	7,1	6,47	0,013
	16 x 10 ⁻³	25,6	25,0	8,6	12,37	7,1	6,61	0,011

4. CONCLUSION AND SUGGESTION

4.1 Conclusion

Based on the result of the research held. That treatment of 10×10^{-3} mL clove oil with with a duration of 5 hours is an effective treatment with an average induction time of 17 minutes 56 seconds and a conscious recovery time of 7 minutes 37 seconds post transportation, the survival rate post transportation is 100%, and 83% after seven days of maintenance. Water quality parameters in transportation are temperature (24,6°C), DO (14,72 mg/L), pH (6,42), and ammonia (0,0043 mg/L) still safe for swordtail.

4.2 Suggestion

The best treatment recommended when transporting swordtail using clove oil as an anesthetic is 10×10^{-3} mL with a duration of 5 hours. It is necessary to carry out further research on the transportation of swordfish, preferably using other natural anesthetic ingredients and the time of transportation from morning to afternoon.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lesmana, Darti Satyani. Water quality for freshwater ornamental fish. Penebar Swadaya. Jakarta. 2001;88.
2. Junianto. Fish handling techniques. Penebar Swadaya. Jakarta; 2003.
3. Ismi S. Effect of oxygen replacement on grouper seed transport with a closed system. Journal of Marine and Tropical Science. 2017;9(1):385–391.
4. Tahe S. The use of phenoxy ethanol, cold temperature, and combination of cold temperature with phenoxy in anesthetizing milkfish. Media Akuakultu. 2008;3(2):133–136.
5. Effendie MI. Fisheries biology. Yayasan Pustaka Nusatama. Yogyakarta; 1997:163.
6. Weber EPS, Weisse C, Schwarz T, Innis C, Klide AM. Anesthesia, diagnostic maging, and surgery of fish. Compendium (Yardley, PA). 2009;31(2):1–9.
7. Boyd CE. Water quality in pound aquaculture. Birmigham Publishing co, Alabama; 1990.
8. Abid M, Endang DM, dan Putri. The secondary metabolites potential of infusum durian's (*Durio zibethinus*) leaves effect to survival rate of nila (*Oreochromis niloticus*) on the livefish dry transport system. Journal of Fisheries and Maritime. 2014;6(1):93–99.
9. Nurjanah N. Diversification of the use of cloves. Balai besar penelitian dan pengembangan pasca panen pertanian, Bogor; 2004.
10. Gunawan. The effect of clove oil (*Eugenia aromatica*) concentration on the survival rate of tilapia (*Oreochromis niloticus*) seeds in transportation. Thesis. Faculty of Agriculture. Bengkulu University; 2015.
11. Anggraini D, Taqwa FH, Yulisman Y. The mortality of koi fish (*Cyprinus carpio*) seed at base height of sugarcane dregs cork and different transportation time. Journal of Fisheries and Maritime Affairs. 2014;19(1):78–89.
12. Nasution SH. Or namental fish water rainbow. Penebar Swadaya, Jakarta; 2000.
13. Putra AN. Basal metabolism in fish. Journal Fisheries and Maritime. 2015; 5(2):57-65.
14. Englund RE. The loss of native biodiversity and continuing nonindigenous species introduction in fresh water estuarine and wetland communities of pearl harbour, Oahu, Hawaiian Island. Estuaries. 2008;25(3):418–430.
15. Simanjuntak M. Seawater quality reviewed from nutrient, dissolved oxygen and ph aspects in banggai waters, central sulawesi sea water quality observed from nutrient aspects, dissolved oxygen and pH in the banggai waters, Central Sulawesi. Journal of Tropical Marine Science and Technology. 2012;4(2):290-303.
16. SNI. Production of ornamental swordtail (*Xiphophorus* sp, Heckel 1848) SNI 8112:2015. Badan Standarisasi Nasional, Jakarta; 2015.
17. Sutomo S. Effect of ammonia on fish in closed system culture. Oseana.1989;14(1):19–26.
18. Hasanah U, Haeruddin H, Widyorini N. Effect of enzymes with different

concentrations in tilapia (*Oreochromis niloticus*) feed on ammonia, nitrite, and sulfide concentrations in maintenance.

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