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The Used of Clove Oil Anesthesia on Giant Gourami Fingerlings *(Osphronemus gourami)* Transported in The Morning With Different Durations

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KeyWords
Anesthesia, closed transportation system, clove oil, giant gourami fingerlings, survival rate.

ABSTRACT

The problem that often encountered in the transportation of the fish included low survival levels. The addition of clove oil in transportation media might suppress the metabolism rate of the fish; so that, the fish mortality during transportation could be minimized. This research aims to determine the concentration of clove oil that was effective for anesthesia of Giant gourami fingerling transported during the treatment time and to analyze the effects of induction time and recovery time and analyze the survival rate of Giant Gourami fingerling. This research was conducted in January-February 2020. The method of this research used an experimental method factorial randomized group design (FRGD) consists of two factors are the four-factor concentration factor (0,010, 0,015, 0,020 ml/L and control) and the three-stage transport duration factor (3, 5 and 7 hours). The transportation system in this study was a closed system. The parameters observed in the transportation period are the time of induction and recovery time of the test fish, survival rate post transportation, survival rate post maintenance 7 days and water quality including temperature, DO, pH, and ammonia. Based on the results of research, the effective concentration for transportation of giant gourami fingerling was 0,010 ml/L with a duration of transportation for 7 hours has an induction time during 14.71- 15.54 minutes, recovery time during 9.95-10.20 minutes and the survival rate post-transportation was 98.33% and post-maintenance 7 days was 77.97%. The parameters of water quality can be concluded that they were still within the tolerance limits of giant gourami had been specified.

INTRODUCTION

Gourami fish (Osphronemus gouramy) is one type of freshwater fish and is a quite popular consumption fish in Indonesia. Gourami fish is also one of the mainstay commodities of freshwater fisheries that has good prospects based on market demand and its price compared to other freshwater fish such as catfish and carp. The demand for gourami fish in the market is increasing from year to year in line with the increase in production with increasing fish consumption can be seen from the statistics of gourami fish consumption rate per capita which always increases every year. In 2013 the consumption per capita was 35.1 kg/ capita, in 2014 it increased to 38.14 kg/capita, in 2015 it increased to 41.1 kg/capita, in 2016 it increased to 43.88 kg/capita, in 2017 it increased to 47.12 kg/capita, (KKP, 2018).

The demand for gourami in the market is increasing from year to year, bearing in mind that the gourami cultivation business in a pond must also be balanced with the supply of continuous and sufficient quantity of quality fish seeds to ensure the availability of gourami throughout the year at maximum and sustainable production levels. Considering the high demand of consumers for live fish, transportation is important in distributing fish to various regions.

There are two transportation systems that are commonly used for fish transportation, namely open system transportation and closed system transportation [1]. Fish seeds transportation activities are generally carried out with high density to save costs. High density in transportation can cause fish seeds to become stressed and more prone to death. One way to reduce the death rate is by good transportation by anesthesia using anesthesia.

According to [2], anesthetic ingredients can be synthetic chemicals or natural ingredients. The use of chemicals as an anesthetic can leave residues that are harmful to fish, humans, and the environment. Therefore, it is recommended to use natural anesthetic ingredients such as clove oil for the transportation of fish.

Clove oil is rich in eugenol content of 70-90% in the form of eugenol which functions as a stimulant, local anesthetic, carminative, antiemetic, antiseptic, antimicrobial, and aromatherapy [2] so that it can be used in reducing stress [3]. Some of the advantages of clove oil from other anesthetics are the characteristics of a short induction time and a long time of fainting (sedation).

Based on this, it is necessary to further research the use of clove oil at different concentrations as an anesthetic material on a giant gourami fingerlings during closed transportation processes so as to reduce the mortality rate (mortality).

MATERIAL AND METHODS

Time and place

The research was conducted from January to February 2020. The research was conducted in 2 stages, namely the transportation phase of giant gourami fingerlings transported with a wet system starting from the Jatinangor area to Tasikmalaya and the fish-raising stage at Ciparanje wet laboratory, Faculty of fisheries and marine science, Padjadjaran University.

Tools and materials

The tools used are concrete tub size 200 x 100 x 60 cm^3**,** aquarium size 60 x 30 x 30 cm^3 as much as 12 pieces, a set of aerators, drain, pH meter, thermometer, DO meter, oksigen tube, milimeter block, stopwatch, micropipette, polyethylene plastic bag size 40 x 60 cm^2 with a thickness of 2 mm, measuring cylinder, Styrofoam box size 60 x 40 x 30 cm^3 as much as 12 pieces, pick up car, test tubes, spectrophotometer, cuvette, filter paper, filtering bottles, funnel, pipette bulb and ice box.

The materials used are 720 giant gourami fingerlings 4-6 cm in length, clove oil, PF 100 fish fingerlings feed, pure oxygen, water samples before and after the transportation, seignette solution, and nessler solution.

Method

This research uses an experimental method with factorial randomized group design (FRGD), which consists of two factors, namely the four-level concentration and the three-level of duration that are repeated three times. This research carried out transportation of gourami fingerlings with a density of 20 fish/2 liters of water with each treated using different concentration at the beginning of the closed wet transportation system (control treatment , concentration of clove oil 0,010, 0,015 and 0,020 ml/L), and giant gourami fingerlings were transported for 3, 5 and 7 hours at morning. After transportation maintenance for 7 days is carried out to find out the post-transportation survival rate.

Observation Parameters

Induction Time

Induction time was calculated from the test fish inserted into each treatment from showing the symptoms of fainting to total fainting.

Recovery Time

Observation and calculation of recovery time using a stopwatch starts from the test fish transferred into an aquarium that has been given high aeration until the fish show normal condition

Survival Rate

The survival of giant gourami fingerlings was calculated after being transported and after 7 days of maintenance. The survival rate of fish is calculated from the comparison of the number of fish that live at the end of the period with those that live at the beginning of the period [10], using the following formula:

$$SR\ (\%) = \frac{Nt}{No}\ X\ 100\%$$

Description :
SR : Survival or Survival rate of fish during the experiment
Nt : Number of fish at the end of the experiment
No : Number of fish at the beginning of the experiment

Water Quality Parameters

Water quality parameters observed were temperature, DO, NH_3, and pH. *In situ* water quality measurement includes DO, pH, and temperature while *ex situ* includes ammonia. The measurement was done in the Faculty of Fisheries and Marine Science, Aquatic Resources Management Laboratory Universitas Padjadjaran . Measurement of each water quality parameter using a DO meter to measure DO, pH meter to measure pH, a thermometer to measure temperature, and a spectrophotometric method for measuring ammonia.

Ammonia measurement uses the spectrophotometric method to use the following formula:

$$\text{Ammonia Value} = \frac{1000}{25} \text{x} \frac{\text{Sample Absorbances}}{\text{Standard Absorbances}} \text{x 5 microgram}$$

Description :
Example absorbance : Calculated Absorbance from the sample
Sample absorbance : Calculated Absorbance from the standard

RESULT AND DISCUSSION

Induction Time

Induction time is a calculation of the from conscious fish until the fish finally fainting because of the anesthetic given. Based on the results of research that has been done, obtained induction time at each clove oil treatment and the duration of transportation in figure 1.

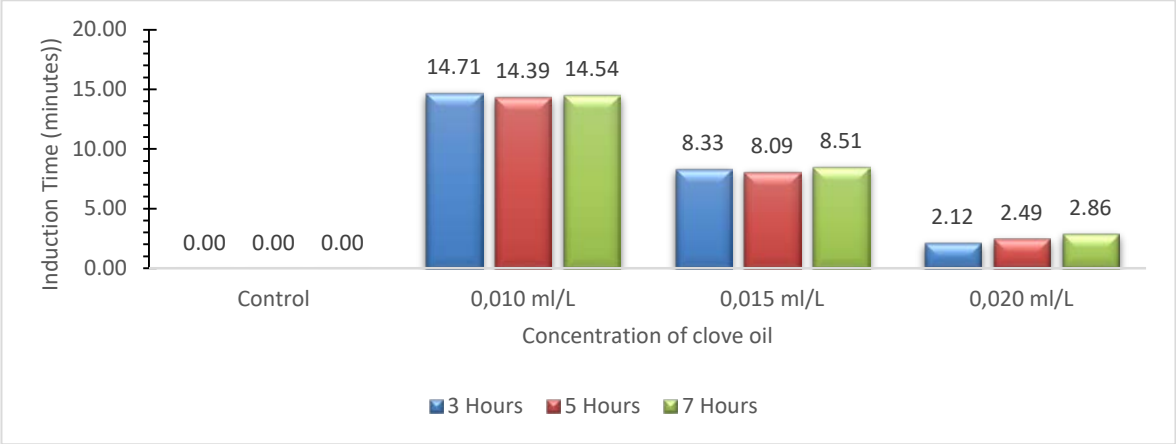


Figure 1. Diagram Of The Effect Concentration Of Clove Oil On Induction Time Of Giant Gourami Fingerling On Different Transportation Durations

Based on the research that has been done, basically fish show symptoms in the condition of the body when entering the fainting stage. Giving 0.010 ml / L clove oil concentration, the giant gourami fingerlings showed symptoms of fainting is the fish had been seen swimming randomly not unidirectionally when entering minutes 6-8 some showed tilted swimming movements. This means that fish that have begun to start the anesthesia stage show signs of panic. The occurrence of induction time or faint time at the 10th minute some fish has entered the stage of a partial and total loss of balance which shows the response to stimulation from outside is the weaker slow movement. Upon entering the minute 13-15 minutes the fish had entered the stage of losing reflexes which means it has been total fainting, marked by a slow operculum, the position of the fish is weak and collapsed.

Treatment of 0.015 ml / L concentration of clove oil, fish experienced induction time by showing symptoms of fainting in minutes 3-5 - Minutes 6-7 minutes the fish had entered the stage of a partial and total loss of balance seen from the movement of fish affected by clove oil. Fish are characterized by characteristics of increased operculum movements if there is strong stimulation, some swimming in reverse motion. If the phase of totals loss of balance has the characteristics of having lost consciousness, some are silent, swimming very slowly moving the operculum slowly decreases and stabilizes again. The fish had entered the total induction stage at 8-9 minutes which indicated that the fish was in a faint or dorsal recumbency position at the bottom of the plastic and the response to external stimuli was completely gone.

Treatment concentration of 0.020 ml / L, resulting in fish experiencing a fast induction time by showing symptoms of induction at 0-2 minutes. This is because fish react to their environment which has been given a concentration of 0.020 ml / L clove oil concentration characterized by symptoms that fish appear to be panicking, with a rather rapid operculum movement, the fish balanced begins to disappear partially, fish often swim sideways and upside down, even someone jumped to the surface. That is because the respiration rate is low enough to cause the test fish seed to be disturbed by its balance so that it cannot support its own body and fall in an oblique body position. Then in the less in 3 minutes the motion of the operculum is very slow, the fish often jump to the surface, the balance of the fish is to lost and some of the fish start to look collapsed. This shows the fish had fainted [5].

The induction time graph (Figure 1) shows that there is a relationship between concentration and faint time. The higher the concentration of clove oil (anesthetic substance) is given, the faster the induction time and even cause death in test fish. This is in line with the statement of Ratnasari (2000) stating that the higher the concentration of clove oil, the more eugenol is absorbed by the blood faster which then spreads to all parts of the body of the test fish seed so that ionic disruption in the brain causes fish to faint quickly due to nerves Mak functioning and causing gills to not function normally. The osmoregulation process of oxygen dissolved in water into blood cells and gills is disrupted. As a result, the process of respiration and metabolism has decreased. This is what caused the gourami fingerling was fainting.

Concentration treatments that have high concentrations of clove oil tend to have fast induction times. At the time of induction, the value sought is the treatment that gives the lowest value, then the concentration of 0.020 ml / L is the concentration that has the fastest induction time in only 2.12 minutes compared to other concentrations. Riesma et al (2016) stated that in anesthesia it is expected that the induction time is relatively faster to reduce stress in fish, the characteristics of a good anesthetic material are having an induction time of lesson than 15 minutes and better less than 3 minutes. In general, it can be stated that the concentration of clove oil in this study has been classified as good because it is less than 15 minutes, and even the concentration of 0.020 ml / L is less than 3 minutes.

Recovery time

Conscious recovery time is a calculation of the time in the process of raising fish affected by the anesthetic effect that is given and is calculated since the test fish are in a container of clean water awareness and the calculation is completed when the fish has regained consciousness or the fish are swimming normally again. Based on the results of research that has been done, it was found that the recovery time was conscious of each clove oil treatment and the duration of transportation in Figure 2.

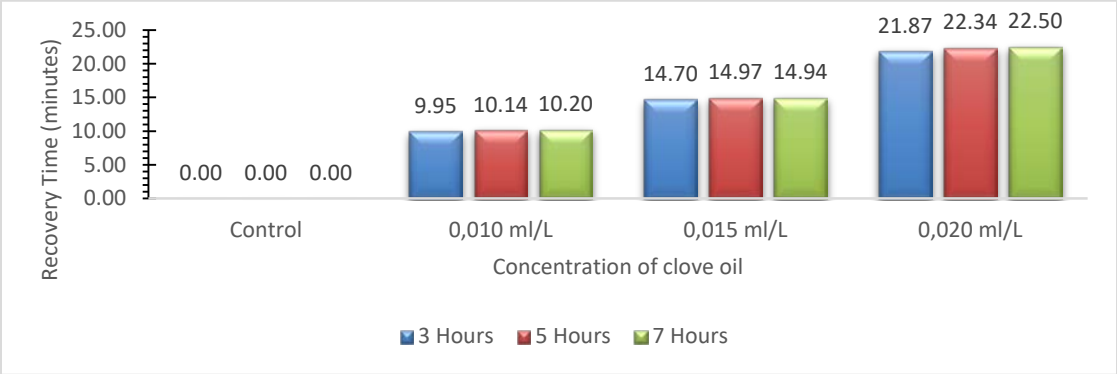


Figure 2. Diagram Of The Effect Concentration Of Clove Oil On Recovery Time Of Giant Gourami Fingerling On Different Transportation Durations

Based on Figure 2, the concentration treatment of 0.010 ml / L for the long recovery time is conscious around 9 to 10 minutes. During the process of awareness-raising, giant gourami fingerlings showed the same behaviour in each hour treatment. Recovering conscious fish ranges from 0 - 4 minutes. Fish exhibit behaviour, namely the movement of the mouth, fins and operculum of the fish from slow to normal. Then in minutes 5-7, some of the fish have moved slowly but are still under the influence of clove oil. In the range of 9-10 minutes, the fish are actively swimming normally.

Treatment concentration of 0.015 ml / L, giving conscious recovery time ranged from 14-15 minutes and showed the same behaviour at each hour treatment. The behaviour of fish when awakening is the same as the previous concentration in the range of minutes 0-5. Mouth, fins and operculum movements begin to move slowly from slow to normal. In minutes 6-8 fish begin to show behaviour began to move very slowly. At 9-11 minutes the fish have moved but are still not stable due to the influence of clove oil. At minute 12-15 the fish was already active and was swimming again because the influence of clove oil slowly began to disappear.

The concentration of 0.020 ml / L, giving a conscious recovery time range of 21-23 minutes. In the process of raising the giant gourami fingerlings, each hour treatment shows the same characteristics. The behaviour of the fish is seen starting in the range of minutes 0–11 by showing the mouth, fins, and operculum begin to move towards normal. At 12-17 minutes the fish have started to move with very slow motion and some fish try to swim but because it is still under the influence of clove oil. Then suddenly the fish fall back to the bottom of the container of awareness, not yet responding to stimuli from the outside. In the range of 18-23 minutes, the fish had been active swimming again.

Based on Figure 2, the higher the concentration of clove oil used, the longer the conscious recovery time needed. According to Robertson et al (1987) in [6], the use of drugs with different doses will affect the level of awareness. The entry of fainting material in the blood causes the fish to numb, so the process of raising fish takes a longer time, and the duration of awareness is also influenced by the length of packaging [7]. When the awareness process takes place, water containing enough dissolved oxygen enters through the gills into the bloodstream and will clean up the remnants of anesthetic material in the body of the fish and release it through the drain [6].

Based on research results of conscious recovery time of giant gourami fingerling after transporting with various concentrations and treatments every hour showed the conscious time in the range of 9 to 23 minutes, while the average conscious recovery time ranged from 9.95 to 22.50 minutes.

According to [8], gills play an important role in the process of raising fish. When the fish are transferred into a container filled with clean water, the gills will diffuse the substance (anesthetic material) that is in the blood which will eventually be excreted in the body of the fish [9]. In other words, when a fish faints is placed in a water medium that does not contain anesthetic ingredients, the gills will work efficiently to clean the anesthetic material. Fish are starting to wake up, their metabolic processes are increasing and the need for oxygen ready to use for respiration will also increase. This is because the higher the concentration of clove oil, the longer the fish will wake up because the sedative content of the clove oil, eugenol, which is absorbed by the fish is different, so the higher the concentration that will be used to eat, the longer the fish will be conscious.

Post Transportation Survival Rates

Based on research that has been done, it is found that the survival rate of fish after transportation can be presented in Figure 3.

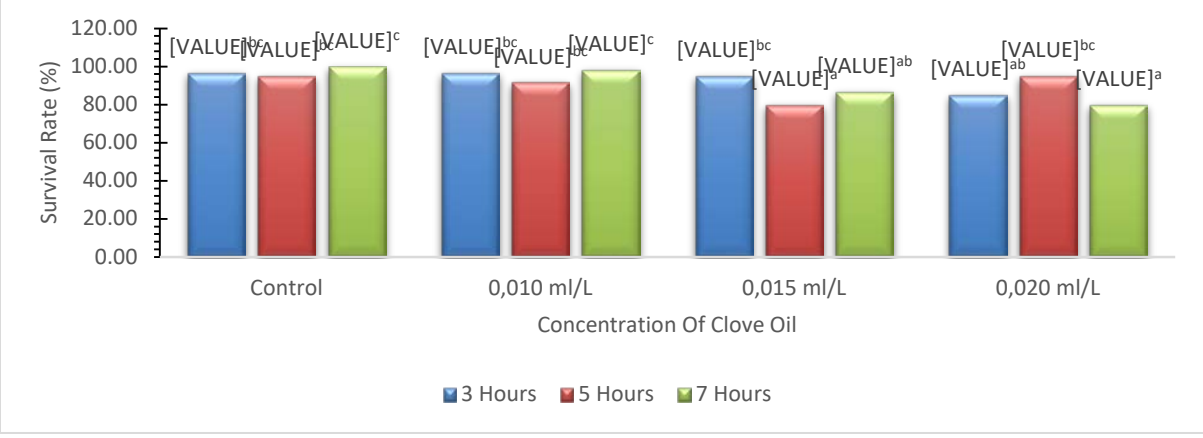


Figure 3. Diagram Of The Effect Of Concentration Of Clove Oil On The Survival Rate Of Post-Transportation Fish Test On Different Transportation Durations

Based on the data presented in Figure 3, that at each time treatment, the best concentration to use is a concentration of 0.010 ml / L because it gets the greatest average value after control and the lowest at a concentration of 0.020 ml / L. It can be seen that the higher concentrations given can affect the survival rate of test fish which tends to decrease. That is because fish cannot tolerate the concentration of clove oil if the concentration given is too high so the gourami fingerlings are already above the tolerance threshold [10].

The survival rate of goramy seeds after transportation (Figure 3) ranges from 80-100%. The highest survival rate of fish after transportation was also obtained at a concentration of 0.010 at 96.67% and the lowest at a concentration of 0.020 ml / L at 85.00%. The cause of death during the transportation is the cause of stress test fish during the anesthesia process. It is also suspected that the fish cannot tolerate the anesthetizing agent so that many experience death. The test fish experiences stress originating when it transfers fish from the feed tub to the transportation container.

Sumartini (2009) states that stress in fish causes respiration and metabolism to increase. Increased metabolism causes hypoxia in fish, where hypoxia is a condition where there is a lack of oxygen in the body's tissues which causes an increase in opening and closing the operculum. Also states that changes in the environment can cause fish stress such as temperature and transportation. The temperature will increase fish metabolism and transportation causes pressure on the immune system which causes illness and death.

At a concentration of 0.020 ml / L, goramy seeds experienced a mortality rate of 20% which was the lowest mortality in 7-hour transportation with a value of 80.00%. According to [12] fish fitness becomes an important thing in transportation activities. Many fish become stressed and fish flounder with lots of physical activity so that many test fish spend energy to adapt to the anesthetized environment.

Treatment with C notation is the best treatment that is controlled with a survival value of 100%. However, this research aims to determine how much optimal concentration that can be used for fish anesthesia, therefore a concentration of 0.010 can be used for transportation activities for 7 hours where this concentration is a more effective treatment than other treatments because of the survival of fish seeds by 98, 33%.

7-day Survival Rate After Maintenance.

A diagram of the average survival rate of fish after 7-day maintenance that can be presented in Figure 4

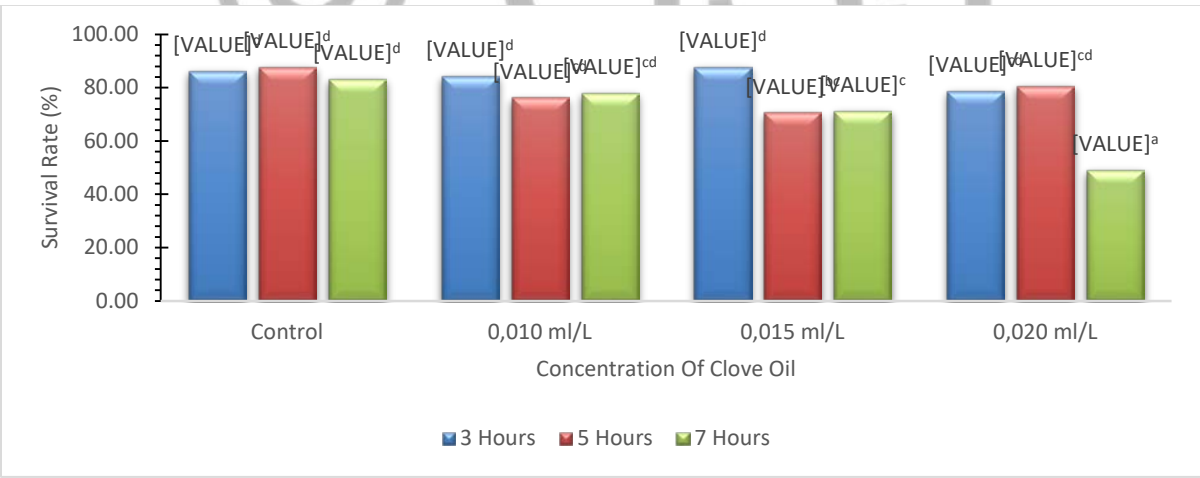
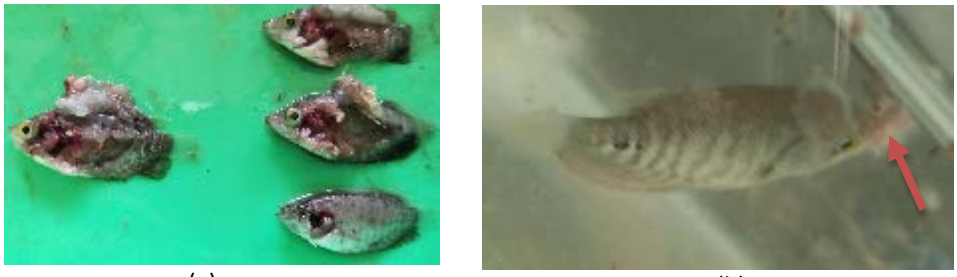


Figure 4. Diagram Of The Effect Of Concentration Of Clove Oil On The Survival Rate Of Seven Days Post Maintenance On Different Transportation Durations

Based on the research results presented in Figure 4, it shows that there is a tendency to decrease the survival rate of gourami fingerlings after 7-day rearing. The average value obtained is around 86.23 - 49.15%. The lowest value at a concentration of 0.020 with a duration of transportation 9 hours has a percentage of 49.15% while the highest value other than the control treatment that is at a duration of 5 hours by 87.70% is also obtained at a concentration of 0.015 treatment with a duration of transportation for 3 hours which has the same percentage by 87.70%. The first three days of rearing is a critical period for test fish where the highest mortality rate occurs at the beginning of rearing. The decrease in survival during the maintenance of the test fish is thought to be caused by the remaining clove oil that cannot be neutralized and has not been removed and cleaned by the body of the test fish at the time of raising so that the test fish fats decline than the test fish becomes weak, stressed, and dies. In addition to the proficiency level that affects the decline in survival rates is an adaptation to the new environment. Fish mortality in aquarium maintenance mostly occurs at the beginning of maintenance due to adaptation to the new environment which causes high mortality rates [13].

In the following days, after 3 days of maintenance, the test fish continued to experience death but not as much as at the beginning of maintenance. Possible predictions of temperature fluctuations that occur at night so that the fish test stress and die. In addition to temperature fluctuations, another factor causing fish mortality was the test fish contracting Saprolegnia sp. most of these Saprolegnia sp attack on the mouth of the fish which makes the fish lose its appetite, unable to eat food so that it dies.

This is the documentation of test fish when dissected and fish affected by Saprolegnia sp. on going maintenance.



(a) (b)

FIgure 5. a) Fish Sampel Test ; (b) Test Fish Infected *Saprolegnia* sp.

Water Quality Parameters

Quality parameters in the transport media are one of the important factors in anesthetic activity in closed media. Water quality can indicate whether or not a source of water within a certain time by comparing the water quality standards set [14]. Water quality during transportation can be seen in Table.

Table 1. Water Quality Of Giant Gourami Fingerlings Transportation

Duration Factor	Concentration (ml/L)	Temperature (°C)		DO (mg/L)		pH		Ammonia (mg/L)	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
3 Hours	Control	22	24,8	7,5	9,4	7,78	6,79	0,025	0,009
	0,010		23,6		9,5		7,11		0,006
	0,015		24,2		9,7		7,29		0,007
	0,020		24,4		9,8		6,83		0,005
5 Hours	Control	21,8	24,6	8	9,9	7,41	7,09	0,013	0,007
	0,010		24,3		10,1		6,90		0,004
	0,015		24,4		11,0		7,01		0,004
	0,020		24,8		11,1		7,05		0,004
7 Hours	Control	22,9	25,2	7,7	9,4	7,51	7,01	0,018	0,010
	0,010		24,6		9,6		6,78		0,004
	0,015		24,8		10,7		6,79		0,004
	0,020		24,8		10,9		6,79		0,004

Ket : Pre (Before Transportation) Post (After Transportation)

Based on research that has been done, the temperature is measured before transportation and after transportation. The temperature is measured using a mercury thermometer. The temperature before transportation ranges from 21.8 – 22.9 ° C and after transportation ranges from 23.6 – 25.2 ° C.

Temperature optimal maintenance of giant gourami fingerlings is 25-30 ° C [14]. The temperature in transportation tends to be low between before and after transportation because the time used for transportation is early morning. Temperature indirectly affects fish metabolism, at high temperatures or above 30 ° C can cause oxygen consumption can increase because the metabolic rate of fish will increase so that it can cause death. While at low temperatures or below 25 ° C fish metabolism slows and oxygen consumption also decreases (Kurniawan 2012). Based on the statement above, it can be concluded that the temperature in transportation in this research is still suitable for maintenance and when the transportation takes place because the results obtained in this research can still be tolerated.

One important factor that influences the survival of fish is oxygen. The oxygen needed by fish has dissolved oxygen in the water. DO is needed for the respiratory process and is a major component for fish metabolism. Based on Table 1, the oxygen solubility value before transportation is 7.5 - 8 mg / L. DO values obtained before transportation are still in the recommended range. The optimal dissolved oxygen range for carp is 3-8 mg. L-1. This shows that the dissolved oxygen content is quite good for the environmental conditions of giant gourami fingerlings [15].

In general, dissolved oxygen decreases after transportation compared to before transportation. The decrease in dissolved oxygen is as a result of its use by fish during transportation. The statement was inversely proportional to the results of the study, DO after transportation tends to increase. The average value of oxygen solubility after transport is 9.4 - 11.1 mg / L. The increased DO value can be said to be very high due to the injection or addition of pure oxygen to each plastic bag. As long as a fish passes out one of its basic needs for oxygen persists. However, with the anesthesia of fish during transportation can cause oxygen demand to be reduced [16] which causes a decrease in respiration and activity.

The feasibility of the optimal degree of acidity (pH) for the survival of giant gourami fingerlings according to [14] is 6.5-8.5. Based on this statement, the pH value obtained during the study before transportation and after transportation is still at an optimal level to support the graduation of fish. The average pH before transportation is 7.41 - 7.78 and after transportation is 6.78 - 7.29. The degree of acidity tends to decrease after transportation compared to before transportation. The decrease in pH is related to the addition of clove oil anesthetic to the transportation media. There is a tendency that the higher the anesthetic given the lower the pH of the water.

One of the problems in transporting fish using anesthetic agents is the decrease in pH when adding them to water which is thought to potentially give acidity when dissolved in water [17]. The low pH after transportation is also thought to be due to high fish activity during the anesthesia process. Before the fish fainted due to the clove oil, the movement of the fish operculum tended to be fast because of the ongoing adaptation to the new environment. With rapid respiration makes the amount of CO2 content produced and in this reaction free H + into Walter and make the pH drop.

Ammonia values were obtained by taking water samples before and after transportation and then tested in the Laboratory of Aquatic Resources Management at the Faculty of Fisheries and Science of Padjadjaran University using the spectrophotometric method. The average value of ammonia before transportation ranges between 0.013 - 0.025, while after transportation is 0.004 - 0.010. According to [18] the level of eligibility of ammonia for giant gourami fingerlings survival on maintenance media must be smaller than 0.2 mg.L. Ammonia value obtained in this research is still categorized as safe because the value obtained is still far from the value of the feasibility level of ammonia according to Effendi [18].

Based on the research results obtained, the concentration of ammonia has decreased. Ammonia reduction after transportation occurs due to the presence of injected oxygen which has an impact on improving water quality. If there is a lot of oxygen, ammonia will be converted to nitrate. Ammonia decreases due to oxidation. In addictive it is thought to occur during the anesthesia process which causes reduced fish activity so that the fish is in a fainting condition.

Conclusion

Based on the research that has been carried out, it can be concluded that the optimal treatment in the administration of clove oil as an anesthetic for transportation was a concentration of 0.010 ml / L with a duration of transportation for 7 hours due to a survival rate of 98.33% in post-transportation and 77.97% in post maintenance 7 days.

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