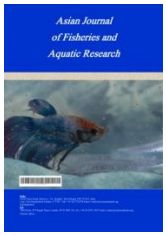

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The Used of Natural Anesthetic Materials Nutmeg Seed Oil in the Transportation of Sumatran Fish Fingerlings (*Puntius tetrazona*)

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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

The low survival rate in Sumatran fish fingerlings transportation is a problem related to metabolic disturbances that cause death. The addition of nutmeg seed oil in the transportation medium is expected to reduce the rate of respiration and metabolism, so that fish mortality can be minimized. The purpose of this study was to determine the effective concentration of nutmeg seed oil to maintain the highest survival rate of Sumatran fish fry transported during treatment and to analyze its effect on induction time and recovery time. This research was carried out from January to March 2021 in the Ciparanje wet laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. This research was conducted experimentally using a factorial randomized group design (FRGD) consisting of two factors, namely the concentration of four levels (0.03, 0.06, 0.09 ml/L and control) and duration of three levels (3, 5, and 7 hours) which was repeated three times. This research uses a closed transportation system. Parameters observed were induction time and conscious recovery time of test fish, post-transportation and post-maintenance survival for 7 days and water quality consisting of temperature, DO, pH, and ammonia. The results showed that the effective nutmeg seed oil for the anesthesia of Sumatran fish fry transported was 0.03 ml/L with a transportation duration of 3 hours because it had an induction time of 08:07, recovery time of 02:41

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and postoperative survival rate. transportation by 98.33% and post-maintenance for 7 days by 91.49%. Temperature parameters are 22.7^{o}C, DO is ±10.3 mg/L, pH is ± 6.83 and ammonia is ±0.0010 mg/L.

Keywords: Anesthesia; closed transportation system; nutmeg seed oil; Sumatran fish fingerlings; survival rate.

1. INTRODUCTION

Ornamental fish farming has good prospects because it has several advantages including not requiring large land, relatively short harvesting period so that capital turnover is fast, able to absorb labor and have promising market opportunities because it is in demand by various groups of people [1]. In the 2014-2017 period, it shows that the traffic of ornamental fish between provinces has increased significantly with an average growth of 27.51% per year. The total volume of ornamental fish trafficked between provinces in Indonesia in 2017 reached 23.32 million fish consisting of 20.61 million freshwater ornamental fish and 2.61 million seawater ornamental fish [2]. West Java Province is one of the largest suppliers of ornamental fish in Indonesia, which is 43.5% of the total national production. The main supporter of freshwater ornamental fish production in West Java is Bogor Regency, which is 70% of the total production [2].

The demand for the Sumatran ornamental fish market is increasing from year to year, efforts to meet this market demand must be balanced with the continuous supply of Sumatran ornamental fish seeds in large quantities and quality. To distribute Sumatran ornamental fish from farmers especially in the city of Bogor to various cities in West Java, such as the city of Bandung, it is necessary to transport fish. Fish transportation is an effort to move fish from one place to another in a limited environment accompanied by relatively sudden changes in the nature of the environment so that it can threaten the survival of fish [3].

Transport of live fish in water can be done in two systems, namely open systems and closed systems [4]. Fish transportation is influenced by several factors including the type of fish, density and water quality. Water quality is often the main factor causing fish death due to increased levels of ammonia in transport water media [5]. One way to overcome this is to anesthetize the fish using anesthetic agents.

Nutmeg is known as a spice plant that has high economic value because it has many benefits to be used as processed products. One of the products produced by the nutmeg plant is nutmeg seed oil. Nutmeg seed oil contains myristicin compounds which are hallucinogenic and toxic agents, are easily absorbed by other constituents in nutmeg oil and have a very intense odor so that if used in excessive doses it will cause poisoning. such as preventing the formation of tumors and can be used in the technique of stunning export fish so that the condition of the fish remains fresh during the trip. In addition, other benefits can prevent carbon tetrachloride liver poisoning in rats [6].

Various studies have been conducted regarding the oil content in nutmeg, one of which is research conducted by the Science and Technology Authority. Based on these studies, the skin and flesh of nutmeg contains a lot of essential oils and tanning substances. The mace or nutmeg also contains the same substance but there is another substance, namely starch, which is efficacious to encourage the release of gas in the intestine as well as to excite tense nerves because mace contains a sedative substance so that it can be used to stun fish.

Based on this, it is necessary to conduct further research on the use of nutmeg seed oil at different concentrations as a natural anesthetic in Sumatran fish fingerlings during closed transportation so as to maintain the highest survival rate.

2. MATERIALS AND METHODS

2.1 Time and Place

This research was carried out from January to March 2021. Sumatran fish transportation activities were carried out in the Jatinangor area. Meanwhile, the maintenance of Sumatran fish fingerlings is carried out in the Ciparanje wet laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University.

2.2 Tools and Materials

The tools used are concrete tubs measuring 200 x 100 x 60 cm^3 as many as 1 piece, aquarium size 60 x 30 x 30 cm^3 as many as 12 pieces, aerator set, drain, thermometer, pH meter, DO meter, spuit, oxygen tube, stopwatch, plastic polyethylene 60 x 40 cm^2, zipper plastic, rubber bands, measuring cup, Styrofoam box measuring 60 x 40 x 30 cm^3, 6 pieces, duct tape, stationery pick-up car, Erlenmeyer flask, test tube, spectrophotometer, cuvette, paper filters, plastic bottles, funnels, volume pipettes, test tube racks, cameras and logbooks.

The materials used were 720 fish of 1-2 cm size Sumatran fish, happy green brand nutmeg seed oil, PF 800 feed, pure oxygen, water samples before and after transportation, signette's solution and Nessler's solution.

2.3 Methods

This research was conducted experimentally using a factorial Randomized Group Design (FRGD) which consisted of two factors, namely the concentration of four levels (control treatment 0.03, 0.06 and 0.09 ml/L) and duration of three levels (3, 5 and 7 hours). Each treatment consisted of 20 fish/2 liters of water. Transportation activities are carried out in the afternoon until the morning. Observation parameters include survival rate, induction time, recovery time and water quality parameters. Survival rate data were analyzed by ANOVA (F test) at a 95% confidence level, if there was a significant difference between treatments in the F test, it was continued with Duncan's multiple spacing (Gaspersz, 1991). While the data of induction time, recovery time and water quality parameters data were analyzed descriptively comparatively.

2.4 Observation Parameters

2.4.1 Induction time

The induction time was calculated from the test fish being put into each treatment until they showed symptoms of the fainting phase to total fainting.

2.4.2 Recovery time

Observation and calculation of recovery time started from the test fish being transferred to an aquarium that had been given high aeration until the fish showed normal symptoms again due to anesthesia. Symptoms return to normal due to anesthesia.

2.4.3 Survival rate

The survival of the fish was observed and calculated when opening the transport packaging and after being reared for 7 days. Survival of fish is calculated from the ratio of the number of fish that live at the end of the period with those that live at the beginning of the period [7].

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

Description:

SR = Survival 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

2.4.4 Water quality parameters

Observation of water quality is carried out by measuring water quality in transportation before and after transportation. Measurement of water quality in situ includes DO, pH, and temperature while ex situ includes ammonia at the Water Resources Management Laboratory of FPIK Unpad. Measurement of water quality parameters uses a thermometer to measure temperature, DO meter to measure dissolved oxygen, pH meter to measure pH and spectrophotometric methods to calculate ammonia.

Measurement of ammonia using the spectrophotometric method using the following formula:

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

Information:

Sample absorbance =Calculated absorbance of sample
Standard absorbance = Calculated absorbance from standard

3. RESULTS AND DISCUSSION

3.1 Induction Time

Based on the results of the research that has been carried out, the induction time for each

treatment was obtained based on the concentration of nutmeg seed oil and the duration of transportation in Fig. 1.

Based on the research that has been done, it shows that the use of nutmeg seed oil with different concentrations gives symptoms to the condition of the fish's body when it enters the fainting phase. Giving nutmeg seed oil with a concentration of 0.03 ml/L resulted in an induction time of 8-9 minutes. Entering the early minutes, the fish still look active to external stimuli so that the balance of muscle contraction remains in normal conditions. However, at the time of entering the 3-5th minute, the movement of the operculum gets faster, which is followed by the movement of fish that are spinning irregularly. At the 6-7th minute the movement of the fish decreases and begins to lose body balance. The fish entered the total induction phase at 8-9 minutes which was marked by the fish experiencing fainting so that the position of the fish was at the bottom of the plastic and the loss of response to external stimuli.

Giving nutmeg seed oil with a concentration of 0.06 ml/L resulted in an induction time of 6-7 minutes. Each fish experienced symptoms of fainting at 3-4 minutes which was marked by the disruption of the nervous system in the fish so that its movements were not balanced and tended to spin irregularly. In the 5th minute, the fish's awareness slowly began to disappear, it could be seen from the increasing movement of the operculum and some fish swimming with their body upside down. At 6-7 minutes the fish have entered the total induction phase. In the total induction phase, the fish did not move but the gill cover was still moving slowly, some fish were on the plastic bottom and the response to external stimuli was lost.

Treatment with a concentration of 0.09 ml/L gave a very fast induction time, which was about 5-6 minutes. Symptoms towards the induction phase occurred at 2-4 minutes because the concentration of nutmeg seed oil given was higher. These symptoms are characterized by irregular fish movements and tend to circle around in the transport medium. At 5-6 minutes the fish have entered the total induction phase because the fish are no longer moving except for the gill cover which is still moving to take oxygen.

Based on the induction time diagram (Fig. 1), it can be seen that all concentrations of nutmeg oil given resulted in an induction time that tends to fluctuate in each duration. This happened because there were differences in the size of the fish used in each treatment duration. The fish used have different sizes, ranging from 1-3 cm. Fish that have a larger size will faint faster than small fish. According to [8] fish that have larger gill spaces can more quickly and efficiently absorb anesthetic substances.

The fastest induction time occurred at a concentration of 0.09 ml/L with a time of 05:58 while the longest induction time was at a concentration of 0.03 ml/L with a time of 09:43. A similar study using neon tetra fish conducted by Fatimah et al (2020) stated that the fastest induction time occurred at 0.3 ml/L treatment with a time of 1.09 minutes while the longest induction time was at a concentration of 0.1 ml/L with a time of 1.31 minutes.

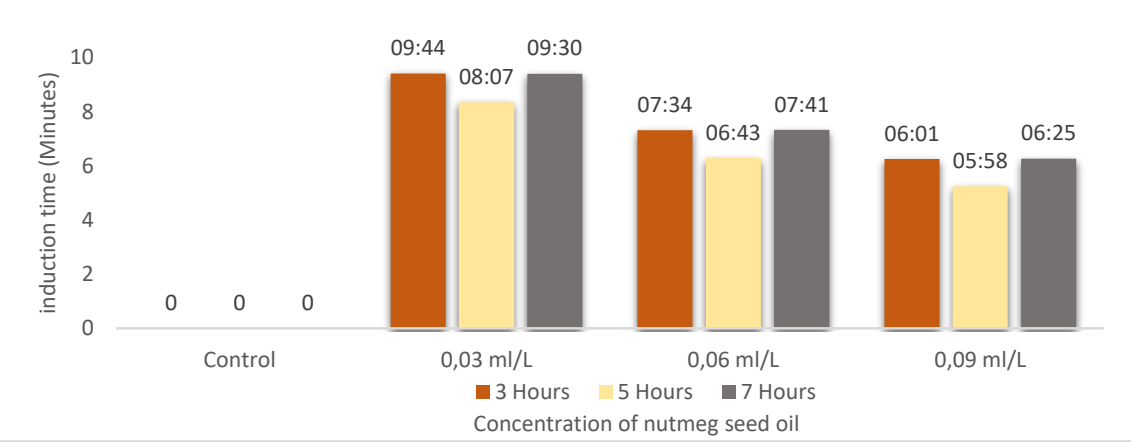

Fig. 1. Diagram of the effect of nutmeg seed oil concentration on sumatran fish fingerlings induction time at different transportation durations

Based on these results indicate a relationship between concentration and induction time. The higher the concentration of nutmeg seed oil given, the faster the induction time for Sumatran fish fingerlings. A good concentration to stun fish is a concentration that produces an induction time of under 15 minutes [9]. In this research, the induction time is under 10 minutes, that means the concentration is 0.03; 0.06 and 0.09 ml/L can be said to be good for stunning fish.

3.2 Recovery Time

Based on the results of the research that has been carried out, the time to recover from each treatment of nutmeg oil and the duration of transportation is shown in Fig. 2.

Based on Fig. 2, the treatment with a concentration of 0.03 ml/L resulted in a recovery time of 2-3 minutes. During the awareness process, in the first minute the Sumatran fish fingerlings showed behavior towards the conscious phase which was indicated by the movement of the mouth, fins and operculum getting more normal but there was still the influence of the anesthetic agent. In the 1-2 minute the fish are more aware so that in a few moments the fish can swim normally and actively.

Concentration of 0.06 ml/L resulted in a recovery time of 4-5 minutes. The initial phase of the fish awareness process occurs at 0-2 minutes, this phase is longer than the initial phase of awareness, which is concentrated at 0.03 ml/L because the amount of concentration is higher. In this phase, it is characterized by the stable movement of the operculum and fins of the fish, but they are still not actively swimming because they are still under the influence of an anesthetic agent. Entering the 3-5th minute, the fish are able to move normally and are active again because there is no influence from nutmeg seed oil.

Treatment with a concentration of 0.09 ml/L resulted in a long recovery time of 6-7 minutes. The fish awareness process in each duration difference shows signs that are relatively the same. The same as the previous concentration in the early minutes, which is in the range of 0-4 minutes, the fish shows the mouth, fins and operculum starting to move as normal but the body is still unable to swim actively because it is still under the influence of the anesthetic agent. However, when entering 5-7 minutes the fish can move their body to swim actively because the influence of nutmeg seed oil begins to slowly disappear.

The fastest recovery time occurred at a concentration of 0.03 ml/L with a time of 02:41 while the longest recovery time was at a concentration of 0.09 ml/L with a time of 06:24. A similar study using neon tetra fish conducted by Fatimah et al (2020) stated that the fastest recovery time occurred in the 0.1 ml/L treatment with 2.53 minutes while the longest recovery time was at a concentration of 0.3 ml/L. with a time of 4.74 minutes.

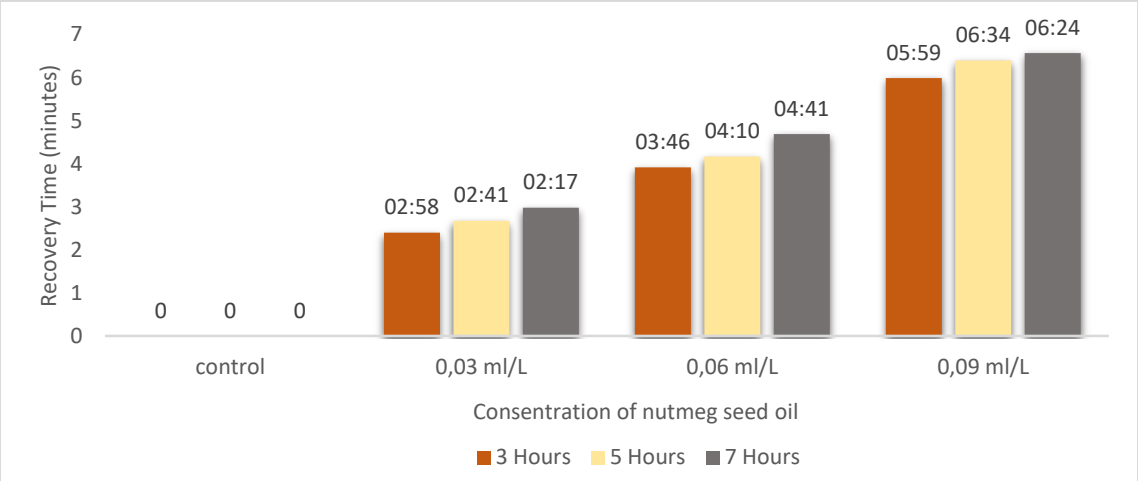

Fig. 2. Diagram of the effect of nutmeg seed oil concentration on recovery time of sumatran fish fingerlings at different transportation durations

Based on the diagram of the recovery time (Fig. 2), it shows that the higher the concentration of nutmeg seed oil and the longer the duration of transportation, the longer the time to recover will be required. According to [10] the use of anesthetics with different doses will affect the level of consciousness. The entry of anesthetic agents into the fish's body through blood cells causes the fish to become numb, so that the awareness process takes a long time and the length of awareness is also influenced by the duration of packaging [11] During the awareness raising process, the water used is aerated with the aim of accelerating the fish awareness process. Water containing sufficient dissolved oxygen can enter through the gills into the bloodstream and will clean the remnants of anesthetic substances in the fish's body and expel it through the [9]

3.3 Post Transportation Survival Rates

Based on the research that has been done, the survival rate of fish with different concentrations and duration of transportation is obtained in Fig. 3.

The results of the ANOVA test at the 95% confidence level showed that the administration of nutmeg oil for anesthesia in Sumatran fish fry has a significant effect on the survival rate of post-transportation. Fig. 3 shows the average survival rate of Sumatran fish fry after transportation ranging from 81.67 - 100%. The highest post-transportation survival rate was found at a duration of 3 hours with a concentration of 0.09 ml/L at 100% and the lowest value at a duration of 7 hours with a concentration of 0.09 ml/L at 81.67%. This value indicates that the higher the concentration of nutmeg seed oil and the longer the duration of transportation, the lower the survival rate of Sumatran fish fingerlings.

Post-transportation mortality was caused by external factors that caused the test fish to become stressed. These factors include the amount of concentration of nutmeg seed oil that cannot be tolerated by the test fish and the process of transferring the fish from the trough to the transportation container. Another factor that can cause fish to become stressed is environmental changes such as changes in temperature and pressure. High temperatures can increase the metabolic rate of fish while the pressure caused by the transportation process can put pressure on the immune system, causing disease and death [12]. According to [13] stressed fish can cause respiration and metabolism to increase. When metabolism increases, hypoxia occurs in fish, where hypoxia is a condition of fish that lacks oxygen in body tissues which causes increased operculum movement.

Based on the F test at the 95% level (Table 5), a concentration of 0.03 ml/L was the best treatment because it had a cd notation with a fish survival rate of 98.33%.

3.4 7-day Survival Rate after Maintenance

The following is a diagram of the average survival rate of fish after 7 days of rearing.

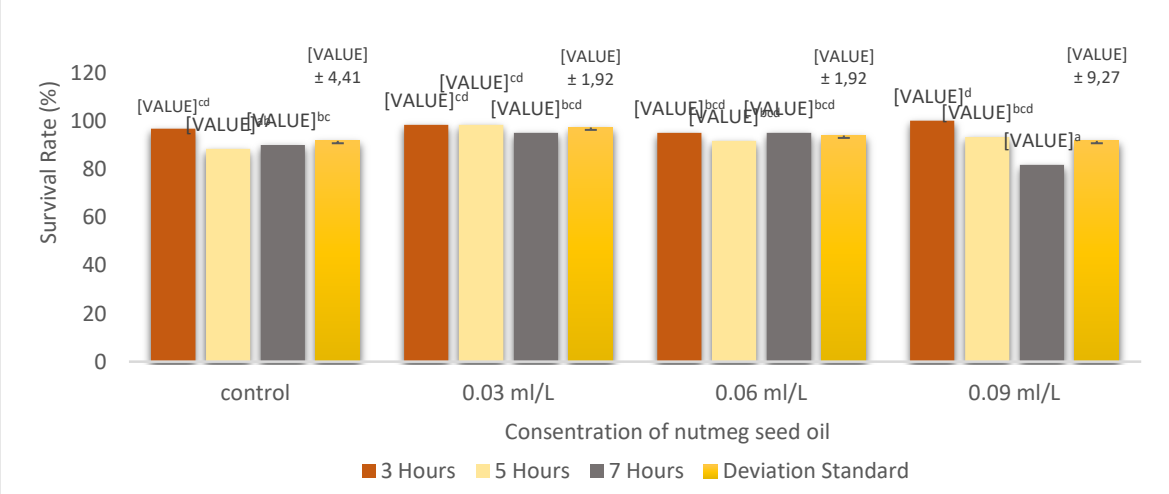

Fig. 3. Diagram of the effect of nutmeg seed oil concentration on the survival rate of fish test post-transportation

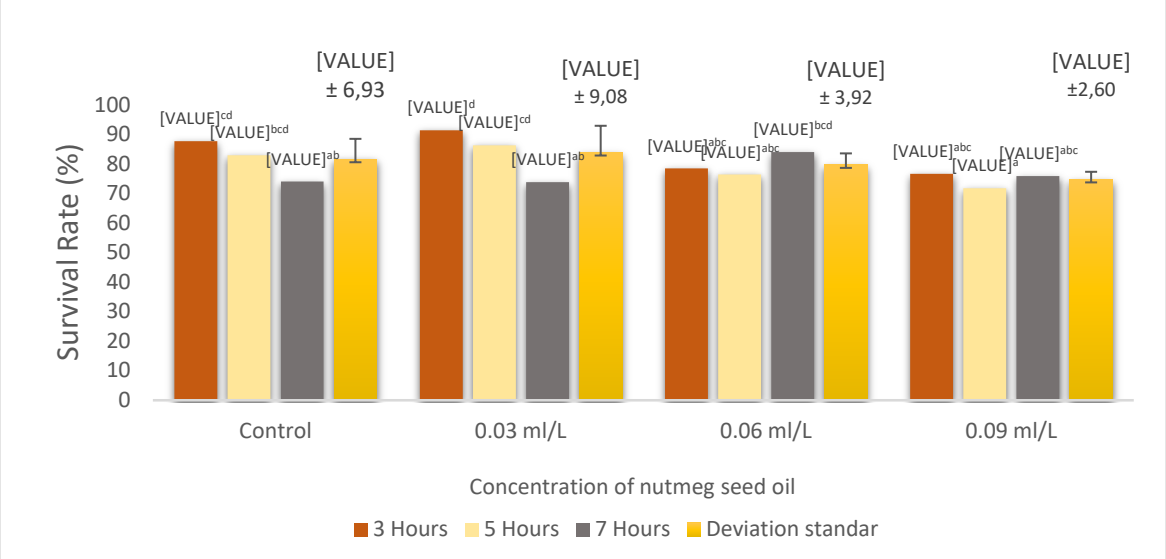

Fig. 4. Diagram of the effect of nutmeg seed oil concentration on the survival rate of fish test after 7 days of maintenance on different transportation duration

Table 1. Water quality parameters

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	24	22,6	6,94	10,2	7,9	6,89	0,00	0,0013
	0,03		22,7		10,3		6,83		0,0010
	0,06		22,7		10,9		6,75		0,0005
	0,09		22,7		10,8		6,73		0,0008
5 Hours	Control	24	22,9	6,74	9,8	7,7	6,33	0,00	0,0014
	0,03		22,2		10,1		6,24		0,0008
	0,06		22,2		9,9		5,78		0,0008
	0,09		22,3		9,9		5,80		0,0012
7 Hours	Control	23	21,8	7,63	9,2	7,2	6,51	0,00	0,0028
	0,03		21,9		9,6		6,55		0,0028
	0,06		21,9		9,8		6,55		0,0038
	0,09		21,8		9,7		6,76		0,0008

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

The results of the ANOVA test at the 95% confidence level showed that the administration of nutmeg oil for anesthesia in Sumatran fish fry had a significant effect on post-maintenance survival for 7 days. The diagram of the 7-day post-maintenance survival rate based on the difference in duration showed a decreasing trendline in the control treatment and 0.03 ml/L while the treatment with concentrations of 0.06 and 0.09 ml/L showed a fluctuating trendline. The highest value was found at a concentration of 0.03 ml/L with a transportation duration of 3 hours at 91.49%. While the lowest value was found at a concentration of 0.09 ml/L with a transportation duration of 5 hours of 71.83%.

The critical period for test fish during post-transportation rearing occurred in the first 3 days because the highest average fish mortality occurred at the beginning of rearing. The death was strongly suspected because there was still nutmeg seed oil left in the fish's body that had not been removed at the time of awakening and had to adapt to the new environment from the transportation container into the aquarium so that the fitness of the test fish decreased and then became stressed and died. According to [14], fish mortality in rearing aquariums mostly occurs at the beginning of the rearing due to the adaptation process to the new environment which causes high mortality rates.

The next day, the test fish continued to die but the number was less than the initial rearing. The test fish have been able to adapt to the new environment so that many fish can survive. The siphoning process which is carried out every day is also an effort to maintain water quality so that it does not decrease with the aim of reducing the mortality rate. There are still deaths suspected to be due to significant temperature fluctuations. During the day at the maintenance site the temperature is 28-30^{o}C while the temperature at night is around 22-24^{o}C.

Based on the F test at a 95% confidence level, the best treatment was the treatment with a concentration of 0.03 ml/L at a transportation duration of 3 hours because it had a notation d with the highest survival rate of 91.49%.

Temperature measurements in this research were carried out before transportation and after transportation. Temperature parameters were measured using a thermometer. The temperature before transportation is in the range of 23-24^{o}C and after transportation is in the range of 21.8 - 22.9^{o}C. The temperature in the transportation media has decreased due to the time used for fish transportation activities carried out at night to the morning. The decrease in temperature will not harm the fish because the decrease is not too drastic and can even increase the survival rate of the fish. At temperatures below 25^{o}C can reduce the metabolic rate of fish so that the rate of oxygen consumption is reduced. While at high temperatures or above 30^{o}C can increase the metabolic rate of fish, causing the rate of oxygen consumption to increase [15]. According to [16] Sumatran fish fingerlings thrive in the optimal temperature range of 22-25^{o}C. Based on this statement, it can be said that the temperature in the transportation medium is still safe and feasible to use for maintenance and ongoing transportation because temperature fluctuations are not too drastic.

The measurement of dissolved oxygen in the transportation medium was carried out using a DO meter on water samples before and after transportation. Dissolved oxygen measurement before transportation is done by measuring directly from the water sample in the sink while the dissolved oxygen measurement after transportation is done by measuring the water sample from each plastic bag according to the treatment. Based on the table, the oxygen solubility value before transportation is 6.74 – 7.63 mg/L. While the value of dissolved oxygen after transportation is 9.2 – 10.8 mg/L.

Dissolved oxygen value after transportation has increased by 2.46 – 3.17 mg/L so it can be said to be quite high because of the addition of pure oxygen which is done before the plastic bag is tied with a rubber band. The addition of pure oxygen into the plastic bag aims to maintain the supply of oxygen stock so that it does not lack during transportation. However, as a result of anesthesia during transportation, oxygen demand is reduced due to decreased respiration rate [17]. The dissolved oxygen value measured during this research showed a good and optimal value for the test fish. Sumatran fish fingerlings can grow optimally and reproduce in waters that have dissolved oxygen values >2.0 mg/L.

The measurement of the degree of acidity (pH) in this research was carried out using a pH meter on water media before and after transportation. The average value of the degree of acidity before transportation ranged from 7.2 to 7.9 and after transportation ranged from 5.78 to 6.89. According to [18] the optimal and appropriate acidity (pH) value for the survival of Sumatran fish ranges from 6.5 to 7.0. The decrease in the value of the degree of acidity (pH) was caused by the activity of fish during transportation and the addition of an anesthetic agent into the transportation medium.

The pH in this study decreased but did not endanger the survival of fish because it was still within the tolerable limit. The decrease in pH is caused by the increased movement of fish during the anesthetic process so that it can increase the value of ammonia. The movement is very active but only a few moments before the fish faints. Before the fish fainted, the movement of the body and the overculum was very fast because of the effect of giving the nutmeg seed oil anesthetic so that the fish adapted to the new environment. The decrease in pH is caused by the number of CO2 compounds produced by an aquatic organism through the respiration process which tends to release H+ ions so that the pH of the water will decrease [19].

The ammonia value was obtained by taking water samples before and after transportation and then tested in the MSP FPIK UNPAD laboratory using the spectrophotometric method. The average value of ammonia after transportation is 0.0005 – 0.0038. According to [20] the range of ammonia for the survival of

Sumatran fish fingerlings is below 0.1 mg/L. Based on this statement, the ammonia value obtained in this research can be said to be feasible and safe.

4. CONCLUSION

Based on the research that has been done, it can be concluded that the anesthetic treatment of nutmeg seed oil at a concentration of 0.03 ml/L with a transportation duration of 3 hours can maintain the highest survival rate of 98.33% after transportation and 91.49% in post-maintenance for 7 days, and produces water quality parameters that are quite good including temperature of 22.7^{o}C, DO of 10.3 mg/L, pH of 6.83 and ammonia of 0.0010 mg/L.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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