

Transportation of Blackmoor (*Carassius auratus*) Goldfish Variety with Low-Temperature Treatment at Different Durations

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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 analyze the induction time and recovery time of Blackmoor Goldfish (*Carrasius auratus*) with different low temperatures as a transportation method, to find out how to handle it with a proper temperature reduction in its transportation, and to find out the optimum time period in its transportation. This study was carried out from November 2020 to February 2021 in the Ciparanje wet laboratory, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. This research was conducted experimentally using the Factorial Randomized Block Design (FRBD) which consisted of two factors, namely four levels of treatment (12 °C, 16 °C, 20 °C, and 24°C (control)) and three levels of transportation duration (3, 5, and 7 hours) which were repeated three times. This research uses a closed transportation system. The research results showed that the low-temperature treatment of 16°C and a transportation duration of 3 hours was the best treatment with an induction time of 02:37, recovery time of 07:13, and having a post-transportation survival rate of 90% and post-maintenance for 7 days of 85 %. The DO parameter is ± 7.53 mg/L, pH is ± 7.38 and ammonia is ± 0.0016 mg/L.

Keywords: Anesthesia; closed transport system; low temperature; blackmoor goldfish; survival rate.

1. INTRODUCTION

In freshwater fisheries, ornamental fish commodities are a contributor to foreign exchange and their export value is extremely high and tends to increase from year to year [1]. According to Basrah [2] demand continues to increase, both for cultivation and consumption in several areas, for urban and abroad. This is a major driver to improve existing live fish systems and technologies, and may lead to the creation of new, more effective, and efficient transport technologies. If during transportation the fish are still alive with a high-density level, then the fish transportation process can be said to be successful [3].

Goldfish are one of the most popular ornamental fish. This fish has various body shapes and has a wide variety of colors, namely red, yellow, green, black, and silvery [4] in Sholichin, 2012). Goldfish (*Carassius auratus*) is one type of ornamental fish which is non-temporary, its market opportunity is always stable and even shows an increase. As an example, goldfish production in South Jakarta had increased from 2000 to 2004, from 512,365 to 798,428 (South Jakarta City Government, 2005). The eyes of these fish are what make their appearance stand out. This blackmoor goldfish is black in color and has protruding round telescope eyes that cause it to seem as though its body is formed around the eye. Goldfish in the domestic market has a stable market and even shows an increase, for example, goldfish production in 2016 in the Tulungagung area of East Java reached a total production of 4,416.166,660 fish [5].

Live fish transportation is a method of transporting live fish in certain packaging and methods [6].

Prakoso & Radona [7] stated that the transportation of fish seeds from hatcheries/ponds to nurseries or rearing areas is an important factor in the success of fish farming. Poor transportation handling techniques will cause fish conditions to deteriorate, stress and fish mortality rates can be very high [8, Chandrasoma & Pushpalatha, 2016).

Temperature is one of the physical parameters which affect the life processes of aquatic organisms, specifically fish, because fish are poikilothermic animals whose body temperature is influenced by their surrounding environment [9]. This low-temperature anesthesia process is

carried out in order for the fish's metabolism to be at a basal condition. The condition of the fish is in a basal state. Basal metabolism is defined as the minimal level of energy expenditure to maintain the structure and function of body tissues (to stay alive). Basal metabolism includes energy requirements for blood circulation, replacing damaged cells, respiration, and intestinal peristaltic movements [10]. The anesthesia process is divided into two, which are gradual temperature reduction and direct temperature reduction.

Based on these matters mentioned above, it is necessary to conduct further research on the use of nutmeg seed oil at different concentrations as a natural anesthetic in blackmoor goldfish during closed transportation with the aim to maintain the highest survival rate.

2. MATERIALS AND METHODS

2.1 Time and Place

This research was conducted from November 2020 to February 2021. The transportation activity of blackmoor goldfish (*Carassius auratus*) was performed in the Jatinangor area. Meanwhile, the pisciculture of the blackmoor goldfish was carried out in the Ciparanje wet laboratory, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran.

2.2 Tools and Materials

The tools which were used are 1 unit of concrete tub measuring with the size of 200 x 100 x 60 cm³, 12 units aquarium of 60 x 30 x 30 cm³ size, aerator set, bamboo fish net, thermometer, pH meter, DO meter, syringe, oxygen cylinder, stopwatch, polyethylene plastic with the size of 60 x 40 cm², zipper plastic, rubber bands, measuring cups, 6 units of Styrofoam boxes of 60 x 40 x 30 cm³ size, duct tape, stationery, pick-up car, Erlenmeyer flask, test tubes, spectrophotometer, cuvette, paper filters, plastic bottles, funnels, volume pipettes, test tube racks, a camera, and a logbook.

The materials used were 720 fish blackmoor goldfish (*Carassius auratus*.) of 4-5 cm size, nutmeg oil, PF 800 feed, pure oxygen, water samples of before and after transportation, Signette salt solution, and Nessler's reagent.

2.3 Methods

This research was conducted experimentally using a Factorial Randomized Block Design

(FRBD) consisting of two factors, namely the concentration of four levels (24°C (control), 20 °C, 16 °C, and 12 °C) and duration of three levels (3, 5 and 7 hours). Each treatment consisted of 20 fish/2 liters of water. The transportation activity was performed at night until the morning.

2.4 Observation Parameter

2.4.1 Induction time

The induction time was calculated starting from when the test fish were 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 when the test fish were transferred to an aquarium that had been given high aeration until the fish showed normal symptoms again due to anesthesia.

2.5 Survival Rate

Fish survival was observed and calculated from the unpacking of the transport packages and after being reared for 7 days. Fish survival was calculated from the ratio of the number of fish which lived at the end of the period with those that lived at the beginning of the period [11].

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

Description:

SR = Fish survival during the experiment

Nt = Number of fish at the end of the experiment

No = Number of fish at the beginning of the experiment

2.6 Water Quality Parameter

Observation of water quality is performed by measuring water quality in transportation before and after transportation. Measurement of water quality with in situ technique includes DO, pH, and temperature while with ex-situ technique includes ammonia at the Water Resources Management Laboratory of Faculty of Fisheries and Marine Sciences Universitas Padjadjaran. The 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.

The measurement of ammonia with the spectrophotometric method by using the following formula:

$$\text{Ammonia Value} = \frac{1000}{25} \times \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times 5 \text{ microgram}$$

Information:

Standard Absorbance : Absorbance calculated from sample

Sample Absorbance : Absorbance calculated from standard

3. RESULTS AND DISCUSSION

3.1 Induction Time

According to the research which has been conducted, the results show the induction time in each treatment based on the low-temperature treatment and duration of transport in Fig. 1.

Based on the research which had been conducted, it showed that the use of nutmeg seed oil with different treatments causes symptoms to the condition of the fish's body when they enter the fainting phase. The implementation of nutmeg seed oil with the 12°C treatment resulted in an induction time of 1:16-1:20 minutes. Entering the early minutes, the fish still seemed active to external stimuli so that the balance of muscle contraction remained in normal conditions. However, when entering the 1st minute, the fish had been seen swimming randomly, not in the same direction, which insinuated that the fish had entered the initial stage of anesthesia. At 1:16, the fish had entered the stage of a partial and total loss of balance, which was observed from the movement of fish affected by low-temperature conditions.

At a temperature of 16 °C, the induction time for transportation which was obtained was 2:21-2:50 minutes. In the second minute, the fish were seen swimming randomly, not in the same direction, which denoted that the fish had entered the initial stage of anesthesia. At 2:10, the fish had entered the stage of a partial and total loss of balance, which might be observed from the movement of fish affected by low temperatures. Fish that have entered the stage of partial loss of balance have the characteristics of starting to

relax their muscles, increase operculum movement, and react only to strong external stimuli [12]. At 2:50, the fish had entered the total induction stage which was indicated by the fish being in a dorsal recumbency position at the bottom of the plastic.

At a temperature of 20 °C, the induction time for transportation was 4:41-4:57 minutes. At the 3rd minute, the fish had entered the initial stage of anesthesia, marked by the fish starting to swim randomly, not in the same direction. In the fourth minute, the fish had started to calm down while swimming, which insinuated that the fish had entered the stage of partial loss of balance, which is characterized by the muscles beginning to relax and react when there is a strong stimulus from outside the container [12]. At 4:30, the fish had entered the stage of total induction where the fish had been completely anesthetized by the low-temperature treatment which was given. The characteristics of the fish that have fainted completely are that the fish is in a dorsal recumbency position at the bottom of the plastic, the movement of the operculum slows down and remains regular.

Based on the induction time diagram (Fig. 1), it can be observed that all the given low-temperature treatments resulted in an induction time that tends to fluctuate at each duration. This occurred because there were differences in the size of the fish used in each treatment duration. The fish which were used have different sizes, ranging from 4-4.5 cm. Fish that had a larger size would faint faster than the smaller fish. According

to Gunn [13], fish which have larger gill spaces are able to more quickly and efficiently accept environmental conditions with low temperatures.

These results indicate a relationship between treatment and induction time. The lower the low-temperature treatment given, the faster the induction time for blackmoor goldfish. The fastest induction time occurred at a temperature of 12°C with a time of 01:16 while the longest induction time was at a treatment of 20°C with a time of 04:57. A good treatment for fish anesthesia is a treatment that produces an induction time of under 15 minutes [14]. In this research, the induction time was under 10 minutes, which insinuates that the temperature treatment of 12°C, 16°C, and 20°C can be deemed to be good for fish anesthesia.

3.2 Recovery Time

According to the result of the research that has been carried out, the recovery time from each nutmeg seed oil treatment and the transportation duration is shown in Fig. 2.

The results in Fig. 2 shows that the average recovery time of goldfish fingerlings ranges between 2:10 - 07:42 minutes. The longest time of the recovery process is at 12°C for transportation treatment, in which the average recovery time is 05:06 minutes. In addition, the fastest time of the recovery process is at 20°C for transportation treatment, in which the average recovery time is 04:24 minutes.

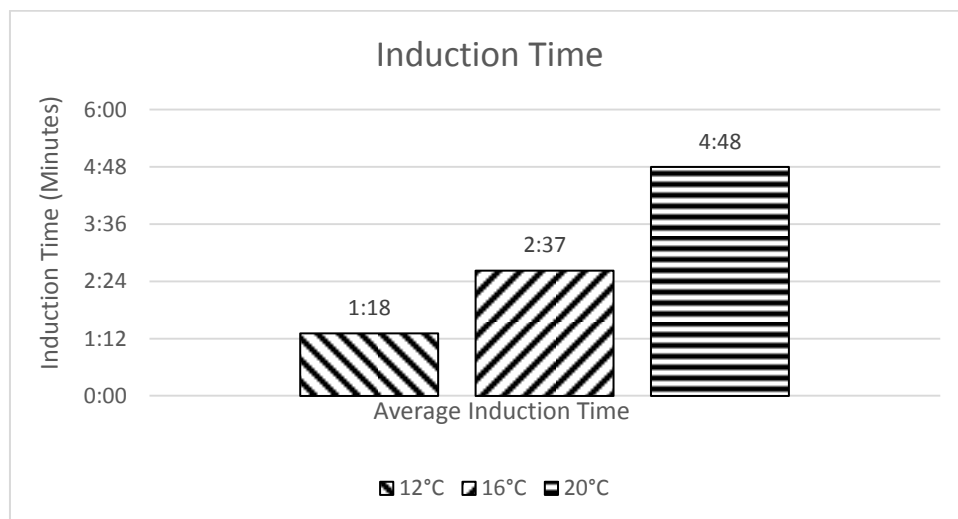


Fig. 1. Effect of Low-temperature Treatment on Induction Time of Blackmoor Goldfish at Different Transport Durations

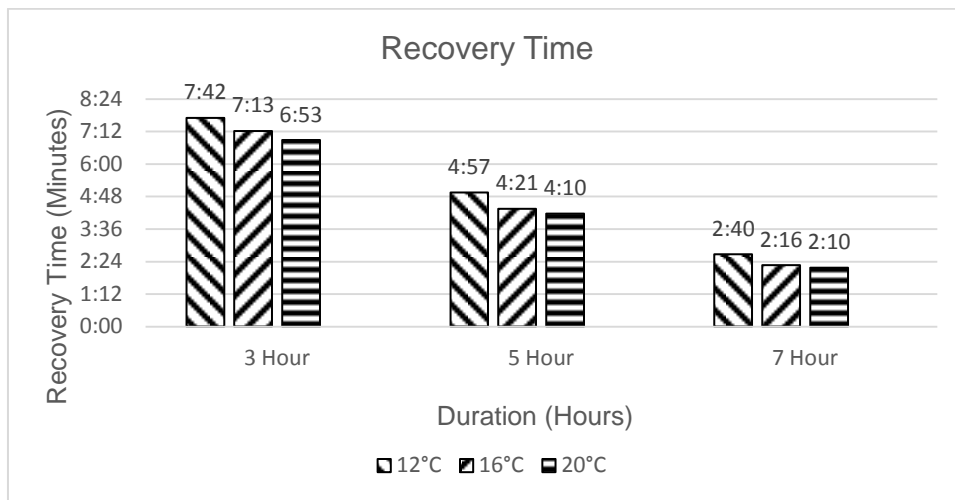


Fig. 2. Effect of Low-temperature Treatment on Recovery Time of Blackmoor Goldfish at Different Transportation Durations

The results in Fig. 2 can be seen that the longer transportation time is (3, 5, and 7 hours) and the lower temperature treatment is (12 °C, 16 °C, 20 °C, and 24 °C), will have an effect on the length of the goldfish fingerlings recovery process. The lower temperature is given, the recovery process will be longer because the fish is in weak condition and lose more energy during the transportation so that the goldfish fingerlings need a longer time to be conscious. Moreover, the longer the duration of transportation, the faster the recovery process because the fish need time to adjust to the conditions of their habitat when they are rehabilitated.

The fish awareness process was carried out in water media that has been aerated with a temperature that has been adjusted to the fish habitat. According to Sufianto [1], the awareness process is carried out by putting the fish that have been in an unconscious state into the water with a normal temperature (± 27 °C). In order that the normal temperature of 28°C is sufficient to support the success of the test carried out. The response of conscious fish is generally shown by the increased operculum movement and the fish position has returned to stability and the movement of the fish's fins has returned to normal.

3.2.1 Post-transportation survival rate

Based on the research that has been carried out, the survival rate of fish which is treated with different transportation durations is shown in Fig. 3.

Fig. 3 shows the average post-transportation survival rate of blackmoor goldfish ranging between 53,3 - 100%. The highest post-transportation survival rate of the fish was found at the duration of 3 hours with a low-temperature treatment of 16°C at 100%, and the lowest rate is at a duration of 7 hours with a temperature of 24°C by 53.33%. These values indicate that the higher the temperature and the longest transportation duration are, the lower the blackmoor goldfish survival rate is.

During 5 hours and 7 hours of transportation treatments (W_2 dan W_3), the blackmoor goldfish fingerlings have experienced death and mortality quite significantly. These deaths and mortalities were caused by the duration of transportation and oxygen solubility in the water, According to Junianto [15], in closed system fish transportation, factors that need to be considered include oxygen solubility in water, density, and transportation time. This long transportation time causes the temperature to rise due to environmental influences, this rising temperature causes a decrease in dissolved oxygen and an increase in carbon dioxide (CO_2), causing increased activity and high levels of stress so that fish suffocate during transportation. According to Junianto [15], hyperactive fish will consume a lot of O_2 and emit a lot of CO_2 , so that the dissolved O_2 in the water of the transport media will decrease quickly and CO_2 gas will increase too quickly. Fish transported at relatively high temperatures will require more oxygen. For every 10°C increase in water temperature, oxygen consumption by fish will

increase 3-5 times, fish acclimatized at 20°C have a dead point at 31-34°C [15].

Based on the F test at 95% level (Table 1), administration of low temperatures of 12°C and 16°C with a duration of 3 hours was the treatment that resulted in the highest survival rate of 100%. However, for the duration of 5 and 7 hours, the treatment showed a very significant decrease so that it cannot be said to be the best treatment. Low-temperature treatment should use a temperature of 16°C and has the highest viability. Therefore, the temperature of 16°C can be used for transportation activities in 3 hours, which is the right treatment because the survival of fish is 100.0%.

3.2.2 7-day survival rate after maintenance

Here's the following diagram of the average 7-day survival rate after maintenance.

Based on the data in Fig. 4, the post-transportation survival rate of blackmoor goldfish using low-temperature treatments with different transportation durations resulted in various test fish survival rates. The results of the F test analysis with a level of 5%, the transportation temperature treatment showed significantly different results, but the duration of transportation treatment and the interaction between the

transportation duration and the treatment showed results that were not significantly different. Because the F test analysis of the duration of transportation treatment had significantly different results, it was continued with Duncan's test. After Duncan's test, the duration of treatment showed significantly different results.

Based on the observation results, the survival value showed that all treatments received various survival values between 71.46% - 96.16% (Fig. 4). Effects after transportation that occur affect the physiological processes of fish which can reduce the health condition and body resistance of fish, so handling using low temperatures does not have an effect on the life of blackmoor goldfish fingerlings after transportation. According to Junianto [15], handling at low temperatures is declared safe because it does not contain chemicals that can harm fish. This shows that handling using low temperatures in the transportation of goldfish fingerlings has no impact on the life of goldfish fingerlings during maintenance after transportation. The maintenance of goldfish fingerlings is intended to determine the effect of low temperatures usage on the transportation of goldfish fingerlings. Observations were made on the survival of goldfish fingerlings since the transportation. This maintenance is carried out for 7 days.

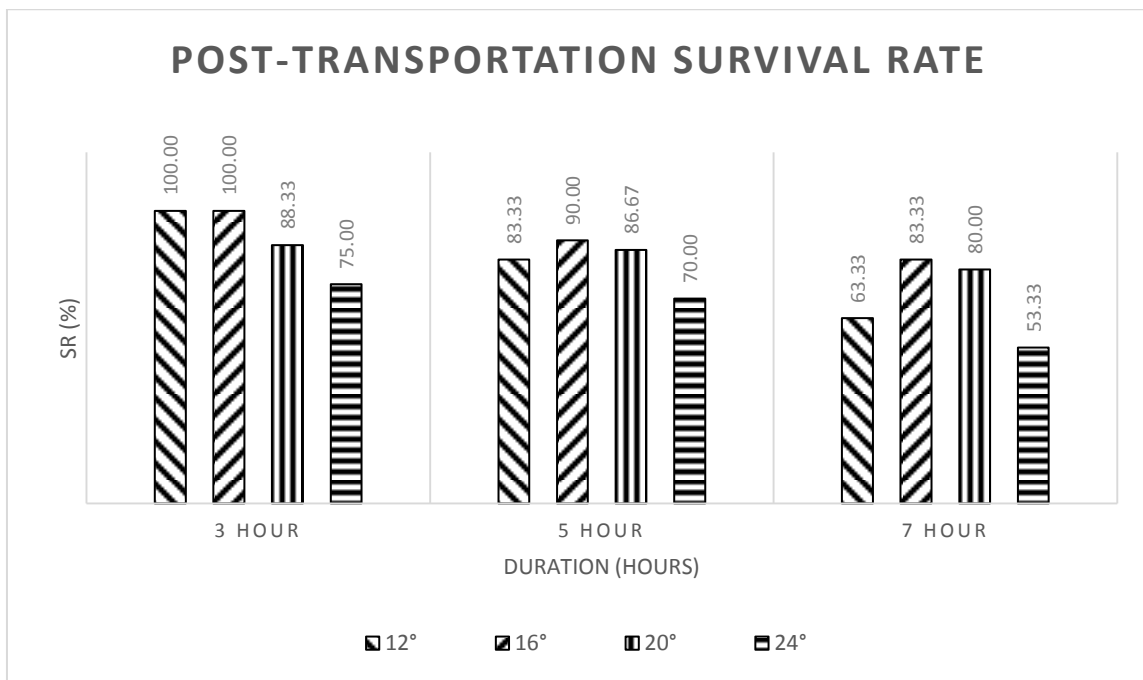


Fig. 3. Effect of Nutmeg Seed Oil Treatment on Post-transportation Survival Rates of Goldfish fingerlings

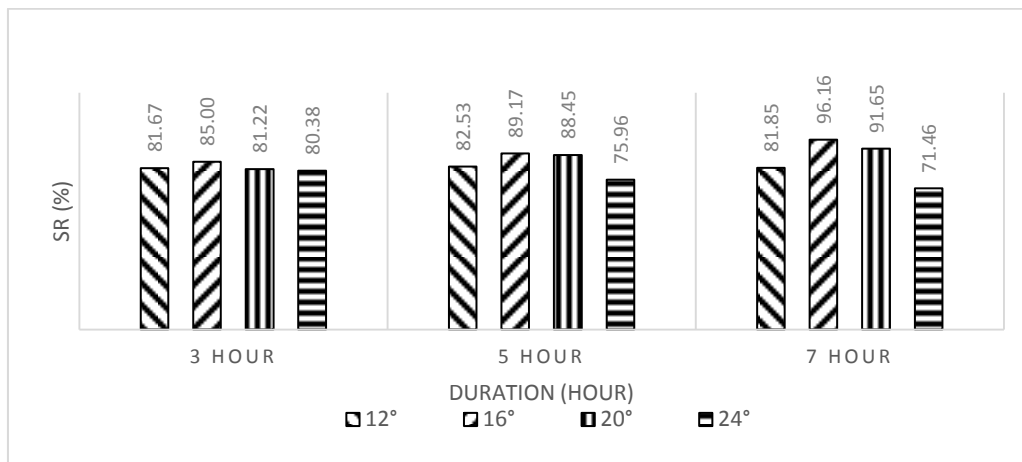


Fig. 4. Effect of nutmeg seed oil treatment on 7-day survival rate after maintenance at different transportation durations

Table 1. Water quality after the transportation of blackmoor goldfish

Duration	Temperature (°C)		DO (mg/L)			pH		Ammonia (mg/L)	
	t ₀	t ₂	t ₀	t ₁	t ₂	t ₀	t ₂	t ₀	t ₂
3 Hours	12	14,3	6,80	8,9	8,6	7,00	7,14	0	0,00
	16	18,6	6,73	8,4	7,53	7,08	7,38	0	0,00
	20	22,9	6,83	8,2	7,07	7,00	7,46	0	0,00
	24	26,1	6,93	9,6	5,07	6,77	7,01	0	0,01
5 Hours	12	14,0	6,80	9,2	8,77	6,97	6,26	0	0,00
	16	18,2	6,73	8,1	7,37	6,99	6,67	0	0,01
	20	22,21	6,87	9,8	7,4	6,94	6,57	0	0,01
	24	25,8	6,77	8,4	5,83	7,01	6,99	0	0,00
7 Hours	12	15,8	6,37	8,6	7,27	7,06	7,37	0	0,00
	16	18,5	6,93	9,7	8,8	6,85	7,42	0	0,02
	20	23,4	5,83	9,3	8,1	8,53	7,31	0	0,01
	24	26,5	6,80	8,6	4,27	6,99	7,36	0	0,02

3.3 Water Quality Parameters

Temperature measurement in this research was carried out before and after the transportation. Temperature parameters are measured by a thermometer. The temperature before transportation is in the range of 12-24°C and after transportation is in the range of 14,0 – 26,5°C. The temperature in the transportation media has increased due to the time used for the fish transportation activities carried out at night until morning. Temperature below 25°C can reduce the metabolic rate of fish so that the rate of oxygen consumption is reduced. Meanwhile, high temperatures or above 30°C can increase the metabolic rate of fish, causing the rate of oxygen consumption to increase [16].

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 sinking tank, 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 5.83 – 6.93 mg/L. but after being given pure oxygen the value of pure oxygen content increased to 8.1 – 9.7 mg/L. Meanwhile, the value of dissolved oxygen after transportation decreased to 4.27 – 8.77 mg/L.

Dissolved oxygen value increased by 8.1-9.7 mg/L, so it can be said it was quite high because of the addition of pure oxygen which was carried out before the plastic bag was tied with a rubber band. The addition of pure oxygen into the plastic bag aims to maintain the supply of oxygen stock so it does not lack during transportation.

However, due to 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.

The measurement of the value 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 ranges from 6.85 to 8.53 and after transportation ranging from 6.26 to 7.36. According to Lesmana [18], the optimal and appropriate acidity (pH) value for the survival of blackmoor goldfish ranges from 6.5 to 7.0. The decrease in the value of the degree of acidity (pH) was caused by fish activity during transportation and the addition of an anesthetic agent into the transportation medium.

The pH in this research has decreased but is not harmful to the survival of fish because it is still within the tolerable limit. The decrease in pH was caused by the increased movement of fish during the anesthetic process which increased 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 operculum was very fast due to the effect of giving the nutmeg seed oil anesthetic which cause the fish to adapt to the new environment. The decrease in pH is caused by a large amount of CO₂ produced by an aquatic organism through the respiration process which tends to release H⁺ ions, in which the pH of the water will decrease [19].

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 0.00047 – 0.0183. According to Tamaru [20], the range of ammonia for fish survival is 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 results of this research, it can be concluded that the treatment with a low temperature of 16°C and a transportation duration of 3 hours is the best treatment with an induction time of 02:37, recovery time of 07:13, and has a post-transportation survival rate of 100% and post-maintenance for 7 days by 85%.

The DO parameter is ± 7.53 mg/L, pH is ± 7.38 and ammonia is ± 0.0016 mg/L.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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