Global Scientific JOURNALS

GSJ: Volume 8, Issue 11, November 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

Concentration of Nutmeg Oil as an Anesthetic in Transportation of Neon Tetra Fish (*Paracheirodon innesi*) with Different Duration

Walim Lili[1], Eddy Afrianto[2], Azhra Fatimah Ramdhani[3] and Roffi Grandiosa[4]

[1]*Student at Faculty of Fisheries and Marine Science, Padjadjaran University, Bandung – Sumedang KM. 21 Jatinangor 45363, Indonesia*
[2]*Lecturer at Faculty of Fisheries and Marine Science, Padjadjaran University, Bandung – Sumedang KM. 21 Jatinangor 45363, Indonesia*
E- mail address: azhra17001@mail.unpad.ac.id

KeyWords
Closed system transportation, nutmeg oil concentration, induction time and conscious recovery, survival rate

ABSTRACT

Live fish transportation needs certain living conditions with specific methods to increase the survival rate. The suggested transportation of live fish needs to be carried out with an imotilization process by optimizing anesthetic agents which are know to reduce stress, metabolism and oxygen consumption. This research aims to analyze the time of induction, conscious recovery and the survival rate of neon tetra using nutmeg oil in closed system transportation. This research used a factorial randomized group design (FRGD) experimental method consisting of two factors, namely the four factors concentration factors (0.1 ml/L, 0.2 ml/L, 0.3 ml/L and control) and three stages of transport duration factors (3, 5 and 7 hours). The parameters observed during the research were induction time, conscious recovery, post transportation survival, 7 days post maintenance and observation of water quality in the form of temperature, DO, pH, and ammonia. The results showed that at a duration of 5 hours with a concentration of 0.1 ml/L the induction time was 1.26 minutes, the conscious recovery time was 3.10 minutes, the survival rate post transportation was 93.33% and after 7 days of maintenance was 85.00%. The average water temperature parameters were ± 25.1°C, DO was ± 8.4 mg/L, pH was ± 5.60 and ammonia was ± 0.0024 mg/L.

INTRODUCTION

Ornamental fish cultivation is a business that provides an alternative source of income to increase the income of ornamental fish cultivators or entrepreneurs. Ornamental fish farming has a good prospect to be developed, this is because in ornamental fish cultivation has advantages such as the simple technology to be applied [1].

The ornamental fish cultivation also is more attractive to the public because it ornamental fish hobbyists. Various ornamental fish can be cultured in Indonesia besides that, they are also important and promising to be developed because they have a wide market, both domestic and foreign. It was know that Indonesia was able to sell ornamental fish valued at US $ 24 million in 2014 [2].

Neon tetra fish is one of the ornamental fish with high economic potential which experienced a relatively rapid increase in export value in 2006 [3]. This fish is native to Rio Putumayo, East Peru [4], and is one of the most recognized domesticated fish among hobbyists [5].

Nutmeg oil has many uses in the pharmaceutical field. Nutmeg oil is used in the medicinal industry to treat, colic, diarrhea, bronchitis, and rheumatism. Nutmeg essential oil has other abilities, namely, it can kill insects (insecticidal) and also act as an antifungal (fungicidal), antibacterial while it also possess powerful antioxidants [6].

The myristicin in nutmeg oil is poisonous, easily absorbed by other constituents in nutmeg oil and has a very intense odor. Myristicin can be used as an anesthetic as a component in a mixture of certain drugs and applied pharmaceutical field. This substance is a hallucinogenic which agent that can cause poisoning in excessive doses. However, myristicin is very useful in preventing tumor formation and can be used in the technique of immotilization of fish so that the fish my have a high survival rate during transportation [7].

Fish anesthesia is a process that causes the fish's body to lose its ability to feel due to low respiratory and metabolic activities. Hence, the fish will undergo a physiological change from a conscious state to sedation [8].

The use of anesthetic agents in the form of anesthetic agents given to aquatic generally works through nerve impulses by inhibiting the delivery of sodium ions through selective sodium ion gates on the nerve membrane thereby reducing the metabolic rate [9].

Transportation of fish is an attempt to place the fish in a new environment that is different from the original environment. During this activity, the fish will experience a new environment when transporatation are relatively sudden hence it greatly threaten the life of the fish. The transportation of live fish is divided into two, namely the dry system transportation without using water and the wet system transportation using water [10].

MATERIALS AND METHODS

Time and Place of Research

The research was conducted for one month starting from March 1 to March 31, 2020. This research place was located at the Ciparanje Wet Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University.

Materials and Methods

The tools used in this research were 36 aquariums 60 x 30 x 30 cm^3, aerator, thermometer, DO meter, pH meter, oxygen cylinder, scale, millimeter block, stopwatch, plastic bag, rubber band, measuring cup, 60 x 40 x 30 cm styrofoam box as many as 6 pieces, duct tape, pickup truck, stationery, volume pipette, Erlenmeyer flask, test tube, spectrophotometer, cuvette, filter paper, filter bottle, funnel, syringe, test tube rack, camera. The materials used in this study were neon tetra fish from Tegalega Bandung, 1 cm neon tetra

fish, nutmeg oil, pearl pellets, pure oxygen, water samples, solution signette and nessler.

This research aims to analyze the time of induction, conscious recovery and the survival rate of neon tetra using nutmeg oil in closed system transportation. This research used a factorial randomized group design (FRGD) experimental method consisting of two factors, namely the four factors concentration factors (0.1 ml/L, 0.2 ml/L, 0.3 ml/L and control) and the three stage transport duration factors (3, 5 and 7 hours).

The parameters observed during the research were induction time, conscious recovery, post transportation survival, 7 days post maintenance, and water quality in the form of temperature, DO, pH, and ammonia.

The survival rate data were tested by ANOVA (F test) at the 95% confidence level. If there is a significant difference between treatments, it is followed by Duncan's multiple range test [11]. The length of time to faint, the time to recover to wake up and the water quality data were analyzed descriptively and comparatively.

RESULTS AND DISCUSSION

Induction Time

Based on the results of the research that has been done, it was found that the induction time in each treatment of nutmeg oil and transportation time was shown in **Figure 1**.

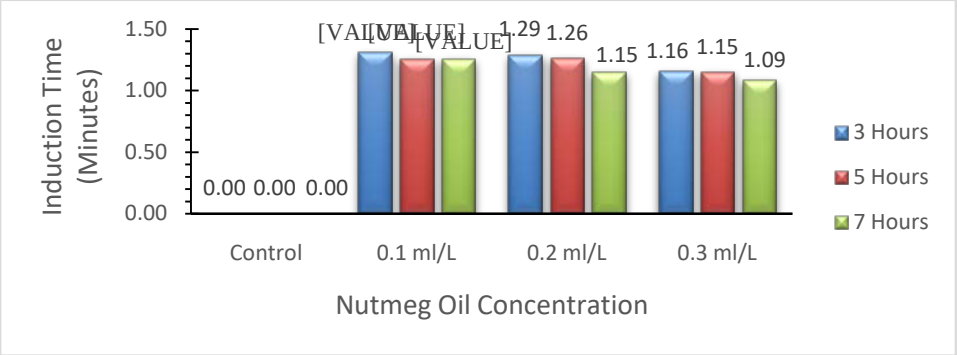

Figure 1. Diagram of the Effect of Nutmeg Oil Concentration on Induction Time of Test Fish at Different Transportation Duration

Based on the research that has been done, the fish showed symptoms in their body condition when they enter the fainting stage, when the nutmeg oil concentration is 0.1 ml/L, the fish show symptoms of fainting with fish swimming randomly when entering 0-1 minutes. This means that the fish has begun the initial stage of anesthesia. In the first minutes, some fish have entered the stage of total loss of balance. When entering 1.25 minutes the fish has entered the stage of losing reflex, which means that they have lost total consciousness, the operculum slows down.

The concentration of 0.2 ml/L obtained the induction time for transportation for 3, 5 and 7 hours, respectively 1.29, 1.26 and 1.15 minutes. During minutes 0-1, the fish are seen swimming randomly out of the same direction, which means that the fish have entered the initial stage of anesthesia. In the 1.10 minutes, the fish have entered the partial and total loss stage. In 1.13 minutes, the fish have entered the total induction stage, which is indicated that the fish are in position *dorsal recumbency* in the plastic base and the response to external stimuli is completely absent.

The concentration of 0.3 ml/L obtained induction time for transportation for 3, 5 and 7 hours, respectively 1.16, 1.15 and 1.09 minutes. Minutes 0-1, the fish have entered the initial stage of anesthesia, marked by the fish starting to swim randomly out of

the same direction. Minutes to 1.07 the fish had started to calm down when swimming, which is a fish that has entered a phase of partial loss of balance, the characteristics of which the muscles have started to relax and react when there is a strong stimulation from outside the container. In 1.14 minutes the fish had entered the total induction stage where the fish was completely anesthetized by the anesthetic substance given.

The induction time diagram (**Figure 2**) showed a relationship between concentration and time of fainting. The higher the concentration of nutmeg oil (an anesthetic substance) was given, the faster the induction time, however when given at higher concentration it a affected the survival rate of fish [12].

Treatment with high nutmeg oil concentration tends to have a fast induction time. At the time of induction, the value sought is the treatment that gives the lowest value, the concentration of 0.3 ml/L is the concentration that has the fastest induction time of only 1.09 minutes compared to other concentrations. While the longest induction time was at a concentration of 0.1 ml/L with 1.31 minutes. Under anesthesia, the induction time was expected to be relatively faster, thereby reducing stress on fish. The characteristics of a good anesthetic agent were that it had an induction time of less than three minutes and could have a concious less than 15 minutes [13]. In this research, the induction time was less than 5 minutes, which means that the concentrations of 0.1, 0.2 and 0.3 ml/L were considered good in stun fish.

Conscious Recovery Time

Based on the results of the research that has been done, it is found that the conscious recovery time for each treatment of nutmeg oil and the duration of transportation is shown in **Figure 2**.

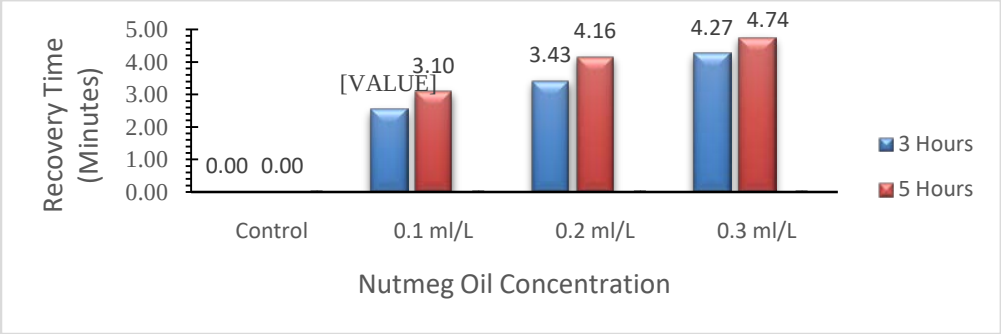

Figure 2. Diagram of the Effect of Nutmeg Oil Concentration on Conscious Recovery Time of Test Fish at Different Transportation Duration

Based on the results of research that has been carried out at a concentration of 0.1 ml/L, the behavior of the fish during the awakening process at 0-1 minutes was characterized by slow movement of the fish's mouth, fin and operculum from slow to normal. At 2-3 minutes some fish are already there moving slowly but still under the influence of nutmeg oil. After 3-4 minutes the fish were actively swimming.

Based on the conscious recovery time diagram (**Figure 2**), the higher the concentration given by the anesthetic agent, the longer the conscious recovery time was needed. The use of anesthetic with different concentrations will affect the level of consciousness.

Based on the research that has been carried out, the neon tetra awareness process after being transported with various concentrations and the duration of the transportation obtained an average conscious recovery time of 2-3 minutes. This was characterized by a decrease in the activity of the operculum movement during exposure and awakening. Fish recovery to normal movement and

swimming took 10 minutes or less and no mortality was found for 15 minutes after unloading if the fish were anesthetized at the effective concentration [14].

Gills play an important role in the fish awareness process. When the fish is transferred to a container filled with clean water, the gills will diffuse the substance (anesthetic) in the blood which will eventually be excreted in the fish's body [15].

Post Transportation Survival Rates

Based on the research that has been carried out, the survival rates of fish with different concentrations and durations of transportation are obtained in **Figure 3.**

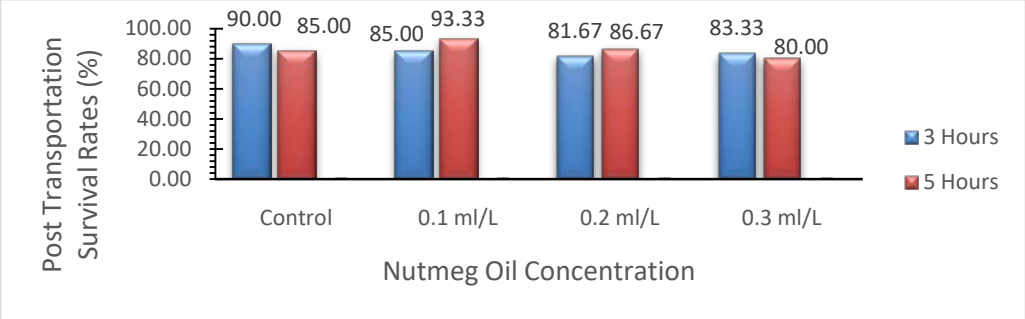

Figure 3. Diagram of the Effect of Nutmeg Oil Concentration on the Survival of Post-Transportation Test Fish at Different Transportation Duration

The survival rate of post-transport neon tetra (**Figure 3**) ranged from 80.00-93.33%. The highest post-transportation survival rate was at a duration of 5 hours with a concentration of 0.1 at 93.33% and the lowest at a concentration of 0.3 ml/L at 80.00%. At a duration of 7 hours, the fish experienced a mortality of 100%. Fish experience death due to environmental changes.

The cause of death during transportation was the stress test fish during the anesthetic process. It was also suspected that the fish could not tolerate anesthetic substances so that many died. The test fish experienced stress when the fish were transferred from the sink to the transportation container.

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 tissues which causes an increase in opening and closing [16]. Environmental changes can cause fish stress for example temperature and transportation. Temperature increases fish metabolism and transport causes stress on the immune system leading to disease and death [17]. The stress of transport can also lead to death [18]. The high survival rate at a concentration of 0.1 ml/L compared to other concentrations because when the fish were treated with this concentration the fish had fainted faster than other concentrations.

Fish fitness is important in transportation activities. Many fish become stressed and fish become floundering with a lot of physical activity so that the test fish spend a lot of energy adapting to the anesthetic environment [19].

The length of time the fish is anesthetized also affects the stress level of the fish. The high survival rate of fish in the control treatment and the concentration of 0.1 ml/L compared to other concentrations was because when the fish were treated with fish concentrations they could still tolerate the content of myristine compounds in the nutmeg oil.

7 Day Post Maintenance Survival Rate

Based on the research that has been conducted, the results obtained are the survival rate of fish after 7 days of being transported with different concentrations and duration of transportation.

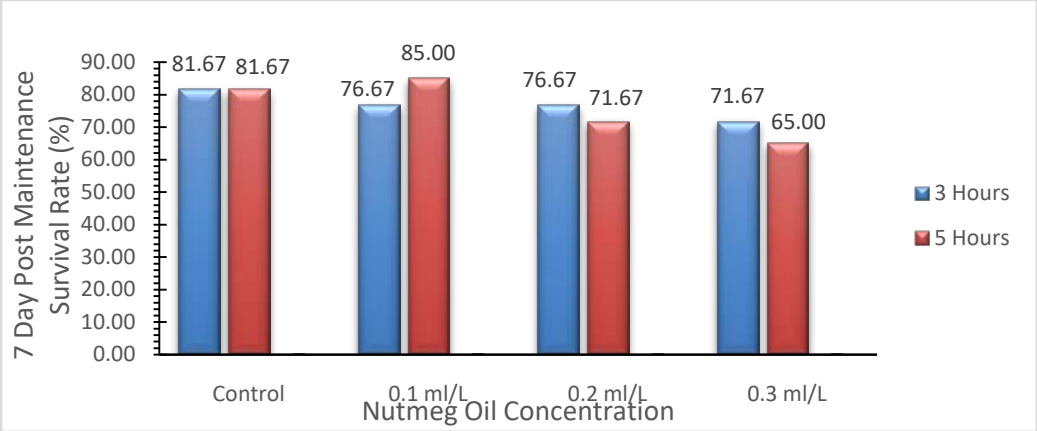

Figure 4. Diagram of the Effect of Nutmeg Oil Concentration on the Survivalof the Test Fish after 7 Days of Maintenance at Different Transportation Duration

Based on the research results presented in (**Figure 4**), it shows that there was a tendency of the survival rate of neon tetra fish to decrease after 7 days of maintenance, the average value obtained is around 65.00-85.00%. The lowest value was at a concentration of 0.3 with a transportation duration of 5 hours of 65.00%, while the highest value in addition to the control treatment was 81.67%, it was also obtained in the concentration treatment 0.1 with a transportation duration of 5 hours of 85.00% . The initial 3 days of rearing is a critical period for test fish where the greatest mortality rate occurs at the beginning of rearing.

The following days, after 3 days of rearing the test fish, they still experienced death but not as much as at the beginning of rearing. It is possible to predict the existence of temperature fluctuations that occur at night so that the fish are stressed and die. Usually during the day at the Hatchery (where the test fish rearing takes place) the temperature ranges from 28-30℃, while at night the temperature ranges from 22-24℃. It can see the possibility of extreme temperature fluctuations that cause fish in the following days after 3 days of rearing still die. The routine water change process every 2 days is an effort so that water quality does not decline with the aim of reducing the mortality rate before it occurs.

Water Quality During Transport of Neon Tetra Fish

The quality parameter of the transportation media is an important factor in the anesthesia activity in closed media. Water quality parameters that are in the good range for aquatic ecosystems guarantee that neon tetra fish are fainted due to treatment not due to environmental conditions which are internal stressors. Temperature, dissolved oxygen (DO), pH and ammonia were some of the parameters of water quality that can be observed [20]. Water quality during transportation can be seen in **Table 1**.

Table 1. Water Quality During Transport of Neon Tetra Fish

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	25	25.5	4.8	8.7	6.91	6.08	0.018	0.0126
	0.1		25.4		8.5		5.76		0.0028
	0.2		25.3		8.1		5.84		0.0031
	0.3		25.5		8.3		5.67		0.0041
5 hours	Control	25	25.2	5.5	8.3	6.79	5.40	0.005	0.0036
	0.1		25.1		8.4		5.60		0.0024
	0.2		25.2		8.4		5.45		0.0028
	0.3		25.1		8.3		5.64		0.0031
Feasibility According to Literature: SNI (2013)		25-27 °C		>4 mg/L		6-7		<0.02 mg/L	

Based on research that has been done, the temperature before transportation ranges from 25 °C and after transportation ranges from 25.1-25.5 °C. The increase in post-transport temperature will increase the respiration process in fish, the increase in temperature can lead to an increase in the metabolic rate [21].

Based on the above statement, it can be concluded that the temperature in transportation in this research is still feasible for maintenance and during transportation because the results obtained in this research can still be tolerated because temperature fluctuations are not too extreme and not much different from the feasibility level.

Based on (**Table 1**) the solubility value of oxygen (DO) was measured using a DO meter. In determining the DO value before and after transportation. The solubility value of oxygen before transportation is 4.8-5.5 mg/L. The average value of oxygen solubility after being transported is 8.1-8.7 mg/L. DO values obtained before transportation are still in the recommended range. Optimal dissolved oxygen for neon tetra fish is >4 mg/L [22].

The optimal value for the degree of acidity (pH) for the survival of neon tetra fish is 6-7. Based on these statements, the pH value obtained during research before transportation and after transportation is still at an optimal level to support fish survival. The average pH value before transportation is 6.79-6.91 and after transportation is 5.40-6.08.

Ammonia value is obtained by taking water samples before and after transportation and then tested at the Laboratory of Fisheries Resource Management, Faculty of Fisheries, Marine Sciences, Padjadjaran University using the spectrophotometric method. The average value of ammonia before transportation ranges from 0.005-0.018, while after transportation is 0.0024-0.0126. The feasibility level of ammonia for the survival of neon tetra in rearing media should be less than 0.2 mg/L. The ammonia value obtained in this research is still categorized as safe because the value obtained is still far from the value of the ammonia feasibility level.

CONCLUSION

Based on the research that has been done, it can be concluded that:

1. The best concentration of nutmeg oil for the transportation of neon tetra fish at a duration of 5 hours with a concentration of 0.1 ml/L resulted in an induction time of 1.26 minutes, a conscious recovery time of 3.10 minutes, a post transport survival rate of 93.33% and after 7 days maintenance by 85.00%.
2. The water quality parameters after transportation show a temperature of 25.5 °C, DO of 8.7 mg/L, a pH of 6.08 and ammonia of 0.0126 mg/L.

REFERENCES

[1] Prayudha., Hendrik., Hamid. 2016. Pemasaran Ikan Hias pada Usaha Kelompok Diamond Fish Club di Kelurahan Tampan Kecamatan Payung Sekaki Kota Pekanbaru Provinsi Riau. Fakultas Perikanan dan Kelautan Universitas Riau. Riu.

[2] KKP. 2014. Perikanan Budidaya Indonesia. Direktorat Jenderal Perikanan Budidaya. Kementerian Kelautan dan Perikanan. Diakses dari http://djpb.kkp.go.id pada tanggal 17 Oktober 2019.

[3] Budiardi, T., Gemawaty, N. Wahjuningrum, D. 2008. Produksi Ikan Neon Tetra *Paracheirodon innesi* Ukuran L pada Padat Tebar 20, 40, dan 60 Ekor/Liter dalam Sistem Resirkulasi. Jurnal Akuakultur Indonesia, 6: 211–215.

[4] Alderton, D. 2005. *Encyclopedia of Aquarium and Pond Fish.* New York, USA: DK Publication Inc.

[5] Saxby A, Adams L, Snellgrove D, Wilson RW, Sloman KA. 2010. The Effect of Group Size on the Behaviour and Welfare of Four Fish Species Commonly Kept in Home Aquaria. Applied Animal Behaviour Science 125: 195-205.

[6] Nurdjanah N. Teknologi Pengolahan Pala. Bogor: Badan Penelitian dan Pengembangan Pertanian. 2007.

[7] Wallis, T.E. 1960. Text Book of Pharmacognosy. J.& A Churchill Ltd, London.

[8] Abid, M.S., Endang D.M., dan Prayogo. 2014. Potensi Senyawa Metabolit Sekunder Infusum Daun Durian (*Durio zibethinus*) Terhadap Kelangsungan Hidup Ikan Nila (*Oreochromis niloticu*) pada Transportasi Ikan Hidup Sistem Kering. J. Ilmiah Perikanan dan Kelautan. 6(1): 93-99.

[9] Stoskopf, MK 1993. Fish Medicine. WB Saunder s Company. Mexico. Thing. 79-112.

[10] Nani, M., Abidin Z., Setyono B.D.H. 2015. Efektivitas Sistem Pengangkutan Ikan Nila (*Oreochromis* sp.) Ukuran Konsumsi Menggunakan Sistem Basah, Semi Basah dan Kering. *Jurnal Akuakultur Rawa Indonesia*, Vol. 3(2). 84-90 hlm.

[11] Gasperz, V. 1991. *Metode Rancangan Percobaan*. CV. Armico. Bandung. Hlm 442.

[12] Ratnasari, D. 2002. Pengaruh Penggunaan Minyak Cengkeh Terhadap Ikan Klon (*Amphirion percula*) sebagai Alternatif Pengganti Potasium Sianida. *Skripsi*. Fakultas Perikanan dan Ilmu Kelautan. IPB. Bogor. 72 Hal.

[13] Riesma, B. A., Hasan, H., Raharjo, E. I. 2016. *Pengaruh Konsentrasi Minyak Cengkeh (Eugenia aromatica) Terhadap Kelangsungan Hidup Benih Ikan Patin Siam (Pangasianodon hypophthalmus) Dalam Transportasi Sistem Tertutup*. http://repository.unmuhpnk.ac.id/42/. Diunduh Pada Tanggal 25 Januari 2020.

[14] Pramono, V. 2002. Penggunaan Ekstrak (*Caulerpa racemosa*) Sebagai Bahan Pembiusan pada Transportasi Ikan Nila (*Oreocrhomis niloticus*) Hidup. Institut Pertanian Bogor. Bogor.

[15] Gunawan. 2015. Pengaruh Konsentrasi Minyak Cengkeh (*Eugenia Aromatica*) Terhadap Kelulusan Hidup Benih Ikan Nila (*Oreochromis Niloticus*) dalam Transportasi. *Thesis*. Fakultas Pertanian. Universitas Bengkulu.

[16] Sumartini *et al*. 2009. Respon Daya Cerna dan Respirasi Benih ikan mas *(Cyprinus carpio)* Pasca Transportasi dengan Menggunakan Daun Bandotan *(Ageratum conyzoides)* sebagai Bahan Anti Metabolik. Univeristas Airlangga.

[17] Hariyanto, S.E. Pranata.F.S. Aida.Y. 2008. Pemanfaatan Daun Kecubung *(Datura Metel L.)* sebagai Pembius Ikan Mas Koi *(Cyprinus carpio L.)* pada Saat Pengang-

kutan. Universitas Atma Jaya Yogyakarta.

[18] Zonneveld, N., Huisman E. A, dan Boon, J. H. 1991. Prinsip-Prinsip Budidaya Ikan. Gramedia Pustaka Utama, Jakarta, 318 hlm.

[19] Khalil, M. Yuskarina. Hartami.P. 2013. Efektifitas Konsentrasi Minyak Pala untuk Pemingsanan Ikan Nila *(Oreochromis niloticus)* Selama Transportasi. Jurnal Agrium, Vol. 10 No. 2 Hal 61-68.

[20] Imam, T. 2010. Uji Multi Lokasi pada Budidaya Ikan Nila dengan Sistem Akuaponik. Laporan Hasil Penelitian. Badan Riset Kelautan dan Perikanan (BRKP). 30 hal.

[21] Putra, A. N. 2015. Metabolisme Basal pada Ikan. Jurnal Perikanan dan Kelautan vol. 5 no.2:57-65.

[22] Standar Nasional Indonesia (SNI). 2013. Ikan Neon Tetra *(Paracheirodon inesi)*. Kelas Benih Sebar. SNI: 7951:2013.