Effect of lemongrass oil (Cymbopogon sp.) as anesthesia material in the closed transportation process of white snapper seeds (Lates calcarifer)

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**ABSTRACT**

White snapper, *Lates calcarifer*, is a sea fish who was widely cultivated because the price reaches IDR 63,000.00/kg for market size (500 gram up). Demand of white snapper on the market tends to increase, but there are high mortality problems when transporting seeds from seed sources to the destination location where was relatively far away. Lemongrass oil had geraniol compounds, citronellol and aromatherapy odors could reduce anxiety (sedatives) and reduce the level of fish metabolism by making the fish faint. The purpose of this study was to determine the effect of lemongrass oil on survival rate, blood profile (total of erythrocytes, leukocytes, and glucose levels) post-transportation of white snapper seeds with length and weight average of 9.71 ± 0.62 cm and 11.33 ± 1.82 g, respectively. The method used was experimental and completely randomized design (CRD) with 4 treatments and 3 replications (0, 10, 20, 30 µl/L). The results showed that the allocation lemongrass oil with different concentrations gave a real effect on the survival rate but did not significantly affect the blood profile after transportation. The best concentration of lemongrass oil is 10 µl/L with survival rate of 100%.

**Introduction**

White snapper (*Lates calcarifer*) is a marine fish that is widely cultivated because it has high economic value. The price of white snapper can reach IDR 63,000.00/kg (MMFA, 2019). White snapper cultivation has become a commercial business to be developed, because of its relatively fast growth, easy maintenance and high tolerance for environmental changes. According to Girsang et al. (2014), that *L. calcarifer* or better known as white snapper/ baramundi is a fish that has economic value, both to meet domestic consumption and export needs. Retrieved from Bukalapak.com (2020), the domestic price is about IDR80,000/kg and the export price is about IDR 40,000/280 gram. The high market demand that demands continuity of production, but there are several obstacles in the field that affect the amount of production. Monica & Mardiana (2019) found that survival rate of white snapper transportation without anesthesia about 70%.

Constraints that are often faced by white snapper hatcheries are damage to packing packaging due to punctured dorsal fin spines, changes in water quality during transportation, such as decreased O₂ in water media, increased CO₂ which causes stress fish so that the survival rate of the seeds is low (Monica et al., 2019). The most influential factor in the transportation of live fish is water quality, namely physical and chemical parameters of water, such as dissolved oxygen, temperature, pH, carbon dioxide, and ammonia (NH₃) which causes fish to become stressed (Supriyono et al., 2010).

Prevention of fish death with the use of anesthetics initially used a lot of solid chemicals to leave residues in the fish's body. Therefore, alternative anesthetics are needed that are effective and do not cause negative effects for cultivators, consumers and the environment (Supriyono et al., 2010). Alternative anesthetics such as anesthetics made from natural ingredients such as lemongrass oil
(Cymbopogon sp.) which has many benefits, one of which contains essential oils which have a distinctive aromatherapy smell and can reduce anxiety. According to De Sousa et al. (2005). Lemongrass plant is calming (sedative) and helps the digestive process and smells good with a distinctive aroma. Citronella oil is an essential oil that contains a lot of geraniol and citronellol compounds which can reduce the metabolic rate of fish by making the fish faint or calming the fish. Geraniol and citronellol compounds play an important role in the anesthetic mechanism through the respiratory tissue (Pirhonen & Schreck, 2002; Hasan et al., 2016). Anesthetics are often used in aquaculture to minimize the stress response of fish and prevent negative impacts, in most cases their effectiveness depends on the procedure used, as too much anesthetic can itself trigger a stress response in fish (Ortuno et al., 2002; Girsang, 2013).

Citronella oil has the potential to be used as an anesthetic or anesthetic in the fish transportation process. The effectiveness of citronella oil as an anesthetic for grouper (Epinephelus fuscoguttatus) fry with an average length and weight of 7 cm and 4.02 grams has been investigated and informed by Supriyono et al. (2010). However, there is not much information about the use of citronella oil as an anesthetic in transporting white snapper seed, so it is necessary to conduct this research to determine the good concentration of citronella oil for anesthesia of white snapper seed.

Materials and Methods

The tools used were concrete tubs, 50 x 30 x 30 cm aquarium as a maintenance container, plastic bags measuring 20 x 120 cm and Styrofoam as a seed container when packing, modified paralon to measure seed length, electric scales used to weigh seed weight, to measure water quality a DO meter as a DO meter was used, a pH meter to measure pH, a refractometer to measure salinity, a thermometer to measure water temperature, a measuring cup to measure the volume of a solution and a micropipette.

The test materials used in this study were citronella oil which was used as an anesthetic, ice cubes which functioned to lower the temperature during the transportation process and commercial feed for fish feed during rearing. Citronella oil obtained from citronella oil comercial and with content are citronellal and gerraniol. The test fish used in this study were white snapper (L. calcarifer) with a length, average weight of 9.71±0.62 cm, 11.33±1.82 g originating from BBPAP Jepara, totaling 168 fish which were each plastic packing amounted to 14 fish / IIµ (SNI : 02-6487.2-2002).

The white snapper used as the test fish is a healthy fish, not deformed or injured and with complete limbs. This research was conducted in January-March 2020 at the Center for Brackish Water Aquaculture Fisheries (BBPBAP) Jepara.

The graded white snapper was reared for 7 days. The reared fish were fasted for 36 hours (Ismi et al., 2016). During gratification, fish observations were still carried out. The containers used for maintenance during the study were concrete tanks and aquariums. The aquarium before use is cleaned and dried for 1 day, after drying the aquarium is filled with 14 liters of filtered seawater. Determination of concentration was carried out to obtain the desired concentration of citronella oil with 4 different levels including control. The concentration calculation uses the formula $V1×N1 = V2×N2$ to make a 1000 µL/L citronella oil solution from pure citronella oil.

This study consisted of 4 treatments and 3 replications, before packing, it was necessary to prepare sterile seawater by ultraviolet ray to prevented patogens as a test medium and then give lemongrass oil with different concentrations, namely 0 µL/L, 10 µL/L, 20 µL/L and 30 µL/L, then homogenized with aeration and checked for water quality, then white snapper fish were added with a density of 14 fish/L and given oxygen. The ratio of water and oxygen in plastic packing is 1:3 (SNI : 02-6487.2-2002), after that the plastic was tied with a rubber band, then put in a Styrofoam box (42x31x30 cm) and given ice cubes about 0.5-1 kg covered with newspaper. The Styrofoam box is tightly closed with duct tape. Transportation is carried out in a simulation in close pond or indoor pond namely by inserting styrofoam into the tub then water is flowed from the faucet and also moved with strong aeration so that waves can form. Fish observations were carried out every 3 hours for 12 hours by removing the plastic packing from the Styrofoam and checking the water temperature. The research data in the form of SR, the number of erythrocytes, leukocytes and glucose levels were analyzed using analysis of variance (ANOVA). Water quality data, fish behavior were analyzed descriptively by displaying a table.

Data analysis

Survival rate (SR)

The SR is calculated every 3 hours during transportation, post-transportation and 7 days of maintenance. according to Amalia et al. (2013). Survival rate of fish can be calculated using the formula:

$$SR(\%) = \frac{\text{Number of live fish at the end of treatment (Nt)}}{\text{Number of fish at the beginning of the treatment (No)}} \times 100\%$$
Results

The Survival rate of white snapper seeds post-transportation for 12 hours served on Table 1. The results showed the highest seed SR post-transportation in treatments B and C (100±0.00%), treatment D (95.24±4.12%) and treatment A (88.10±4.12%), after rearing 7 days in all treatments no death occurred.

The results of observing the behavior of white snapper seeds after 120 minutes of anesthesia with citronella oil can be seen in Table 2.

Observations for 120 minutes of packaged white snapper seeds showed different behavior in each treatment. Treatment A (0 µl/L): all fish were still conscious, high response to shock stimuli, active swimming. Treatment B (10 µl/L) the behavior of the fish began to change at 61st minutes: some fish were still conscious, the response began to decline to shock stimuli, lost balance and swam sideways, Treatment C (20 µl/L) at 61st minutes The response began to decline to shock stimuli, loss of balance and sideways swimming. Treatment D (30 µl/L): at the beginning the fish were given lemongrass oil, the fish movement was very active until they jumped to avoid the water, and at the 60th minute the fish showed a total loss of response to stimuli, however, they still responded to harsh stimuli.
Water quality is a supporting parameter in the transportation of white snapper fry. The water quality parameters measured were dissolved oxygen (DO), salinity, temperature, and acidity (pH). The results of water quality measurements are presented in Table 4.

### Table 3. Results of water quality measurement on transportation media.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water quality</th>
<th>DO (mg/L)</th>
<th>Salinity (ppt)</th>
<th>Temperature (°C)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before applying oil</td>
<td></td>
<td>4.91</td>
<td>30</td>
<td>29.1</td>
<td>7.86</td>
</tr>
<tr>
<td>After applying oil</td>
<td></td>
<td>4.80</td>
<td>30</td>
<td>29.1</td>
<td>7.67</td>
</tr>
<tr>
<td>Post-transport A</td>
<td></td>
<td>3.10</td>
<td>30</td>
<td>26.9</td>
<td>6.18</td>
</tr>
<tr>
<td>Post-transport B</td>
<td></td>
<td>3.60</td>
<td>30</td>
<td>26.8</td>
<td>6.48</td>
</tr>
<tr>
<td>Post-transport C</td>
<td></td>
<td>3.73</td>
<td>30</td>
<td>26.8</td>
<td>6.59</td>
</tr>
<tr>
<td>Appropriateness D</td>
<td></td>
<td>3.52</td>
<td>30</td>
<td>26.5</td>
<td>6.40</td>
</tr>
</tbody>
</table>

Remarks: * SNI 6145.4:2014; ** Jaya et al. (2013)

### Discussion

#### Survival rate

The application of citronella oil to the transportation medium as an anesthetic agent had a significant effect on the survival rate (SR) of seeds during closed transportation. SR on fish transportation is very important to determine whether the application of citronella oil to the media has an effect or not. This research shows that treatment B (10 µL/L) and treatment C (20 µL/L) are the same result of survival rate (100%) According to Hasan et al. (2016), that the lower the concentration of citronella oil used 1 mL/L, the higher the survival of the test animals and the higher the concentration of citronella oil used (2 and 3 mL/L) the lower the survival of the test fish.

Low survival rate on treatment A (0 µL/L) caused transportation without citronella oil, so the metabolism not inhibit. Normal metabolism on seed transportation can cause reduce of dissolved oxygen and result higher mortality at the destination. In treatment D (30 µL/L) survival rate also reduce because in concentration citronella oil on the blood is high and can cause disturb of circulatory system. So, this cases can cause higher mortality.

#### Sedative time

According to Yanto (2009) the difference between the treatments given citronella oil was only at the time of induction to fainting, the treatment with high concentrations of citronella oil tended to have a fast induction time. according to Syawal et al., (2008), that stress in fish is an effort made by the physiological system to defend itself or adapt to changing environmental conditions, and movement fish that are not normal, if there are disturbing factors, either in the form of parasites, chemicals or physical stimuli that trigger stress on the fish, in addition, the presence of fish movements that jump up and down uncontrollably during immersion in the treatment indicates that the fish feel uncomfortable with the fish environment, thus trying to escape. As a result of the heat, the fish may be shocked, the body condition is weakened and it can even cause death.

Each treatments showed different sedative-time. Hasan et al. (2016) reported that each anesthetic dose has a different level of awakening time. The characteristics of fish that have been aware are that the fish begin to swim normally and receive a response to stimuli from the outside with a body condition that looks not weak.

#### Blood profile analysis erythrocyte count

The number of erythrocytes of white snapper fish after transportation for 12 hours and 7 days of maintenance showed no significant effect on each treatment. Data on the average number of erythrocytes are presented in Figure 1.

### Table 4. Awareness time and behavior of white snapper during acclimatization after 12 hours of transport.

<table>
<thead>
<tr>
<th>Observation Time (Minutes)</th>
<th>Treatment (Concentration)</th>
<th>Fish Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.41</td>
<td>A (10 µL/L)</td>
<td>The fish are all aware, swimming normally, responding to food not yet available (not close to feed), fish start eating at the 10th hour</td>
</tr>
<tr>
<td>12.36</td>
<td>B (0 µL/L)</td>
<td>The fish are all aware, swimming normally, responding to food not yet available (not close to feed), fish start eating at the 12th hour</td>
</tr>
<tr>
<td>14.55</td>
<td>C (20 µL/L)</td>
<td>The fish are all aware, swimming normally, responding to food not yet available (not close to feed), fish start eating at the 12th hour</td>
</tr>
<tr>
<td>19.13</td>
<td>D (30 µL/L)</td>
<td>The fish are all aware, swimming normally, responding to food not yet available (not close to feed), fish start eating at 13th hour</td>
</tr>
</tbody>
</table>
Figure 1. The number of erythrocytes of white snapper fish after transportation and after 7 days of rearing.

![Figure 1](image1.png)

Figure 2. White snapper seed leukocyte count after transportation and after 7 days of rearing.

![Figure 2](image2.png)

Figure 3. Blood glucose levels of white snapper after transportation and after 7 days of rearing.

![Figure 3](image3.png)

Giving citronella oil with different concentrations did not have a significant effect on the number of erythrocytes. Erythrocytes increased in treatment B, C and D, treatment A decreased. The highest average number of erythrocytes was in treatment B (4.13±1.7×10⁹ cells/mL) followed by treatment D (3.67±2.67×10⁹ cells/mL), treatment C (2.43±1.33×10⁹ cells/mL) and the lowest was in treatment A (1.90±1.04 ×10⁹ cells/mL) which was not significantly different in each treatment. So the range of the number of erythrocytes in all treatments was 1.90-4.13×10⁹ cells/mL, while the normal white snapper seeds were 1.80-2.90×10⁹ cells/mL. Observation of 7 days of rearing all treatments, the
number of erythrocytes decreased in treatment B, C and D and increased in treatment A. According to Kathirkaman et al. (2018), the number of white snapper erythrocytes ranged from $1.45 \times 10^9$ cells/mL. The increase in the number of erythrocytes, leukocytes and post-transport glucose in all treatments compared to normal snapper. The increase in the number of erythrocytes occurred in the B>D>C>A treatment. It is suspected that the white snapper was stressed because of the treatment. According to Yanto (2012), if the time required for the anesthetic agent is too long, it will stress the fish. The faster the anesthetist’s ability to anesthetize, the less stress it causes. The higher the dose, the faster the time needed for the anesthetic agent to anesthetize the fish.

These results indicate that there is no significant effect of giving lemongrass oil on the blood profile. Load profile of normal white snapper seed with length, average weight $9.71 \pm 0.62$ cm, $11.33 \pm 1.82$ g is erythrocyte count $1.80 \times 10^9$-$2.90 \times 10^9$ cells/mL. According to Gbore et al. (2006) the number of erythrocytes in fish is generally in normal conditions ranging from $1.05$ to $3.50 \times 10^6$ cells/mL.

**Leukocyte count**

The number of leukocytes of white snapper fish after transportation for 12 hours and 7 days of rearing showed no significant effect on each treatment. Data on the average number of leukocytes are presented in Figure 2. The results of the calculation of the leukocyte count of post-transported snapper, the highest value was found in treatment B ($1.94 \pm 1.8 \times 10^9$ cells/mL) followed by treatment C ($1.70 \pm 0.66 \times 10^9$ cells/mL), treatment D ($1.46 \pm 0.73 \times 10^9$ cells/mL) and treatment A ($1.26 \pm 1.21 \times 10^9$ cells/mL) which were not significantly different in each treatment. So the range of leukocyte counts in all treatments was $1.26 \times 10^9$-$1.90 \times 10^9$ cells/mL, while the normal white snapper seeds were $1.10 \times 10^9$-$2.10 \times 10^9$ cells/mL and accordingly to Kathirkaman et al. (2018), the number of white snapper leukocytes ranged from $1.81 \times 10^7$ cells/mL. Observation of 7 days of maintenance of leukocyte count decreased in all treatments. The lowest results were in treatment A ($0.32 \pm 0.03 \times 10^9$ cells/mL), B ($0.42 \pm 0.16 \times 10^9$ cells/mL), D ($0.68 \pm 0.27 \times 10^9$ cells/mL) and C ($0.87 \pm 0.34 \times 10^9$ cells/mL) and the results were below normal values. according to Arlanda et al. (2018). The increase in leukocytes in tilapia is caused by the entry of foreign compounds into the body which causes stress in fish, so that the total leukocytes in the fish’s body increases. This is thought to be related to the administration of tobacco extract which increases the body’s defense response which is characterized by an increase in leukocytes. Maintenance of 7 days the number of leukocytes is decreasing, according to Susanto & Taqwa (2014). The number of leukocytes can be influenced by physiological factors and environmental conditions.

These results indicated that there is no significant effect of giving lemongrass oil on the blood profile. Blood profile of normal white snapper seed with length, average weight $9.71 \pm 0.62$ cm, $11.33 \pm 1.82$ g leukocyte counts $1.10 \times 10^9$-$2.10 \times 10^9$ cells/mL. Compared to the number of leukocytes in normal fish, which was $2.72 \times 10^4$ cells/mm$^3$, all post-transport leukocyte counts in the treatment were above normal conditions. The high number of leukocytes in each treatment was caused by the level of stress on the fish as well as infection or damage to the gills due to deteriorating water quality and environmental influences on the transportation media which were getting worse Supriyono et al. (2010).

**Glucose level**

Glucose levels of white snapper after transportation for 12 hours and rearing 7 days showed no significant effect on each treatment presented in. The average blood glucose data is presented in Figure 3.

Blood glucose levels after 7 days rearing analyzed by normality, homogeneity and additivity. From Anova showed that results of measuring the glucose levels of white snapper after transportation showed no significant difference. The highest average glucose level was in treatment B ($379.00 \pm 187.95$ mg/dl) then treatment C ($362.67 \pm 50.03$ mg/dl), treatment D ($179.33 \pm 79.12$ mg/dl ) and the lowest was in treatment A ($147.33 \pm 40.93$ mg/dl), which was not significantly different in each treatment. So the range of glucose levels in all treatments was $362.67$-$147.33$ mg/dl while the normal white snapper seeds were $120-166$ mg/dl. According to Abdelwahab et al. (2012), white snapper glucose levels ranged from $187-192$ mg/dl. After maintenance for 7 days glucose levels all decreased. When experimental fish are exposed to stressors, the blood glucose value immediately increases. Seabream fish glucose levels increased significantly to $100$ mg/dl immediately after exposure to foreign bodies (anesthesia), but not in untreated or normal fish, where glucose levels remained constant. After being maintained for 4 days glucose levels started to normal (Ortuno et al., 2002). According to Hastuti & Supriyono (2003), glucose levels in fish that were stressed with sudden changes in cold temperatures experienced an increase in
glucose and were the highest stress level but were not lethal to fish.

The research results of Supriyono et al. (2010) revealed that the measurement of glucose levels after transportation and after 7 days of maintenance showed that the highest increase in glucose levels occurred in the 30 mg/L lemongrass oil treatment (79.73). The results of statistical tests showed that treatment N (untransported normal fish) was not different from treatment K (without lemongrass oil), A (10 mL/L lemongrass oil), and B (20 mL/L lemongrass oil) but treatment N was different with treatment C (30 mL/L citronella oil). After 7 days of rearing the results of statistical tests on tiger grouper glucose levels showed that treatment N was not different from treatment A, but treatment N was different from treatments B and C. From these results, it was shown that the treatment that was close to normal fish glucose values was treatment A. Fish with Treatments of 20 and 30 mL/L lemongrass oil (B and C) had lower glucose levels than normal fish, because the fish had a low appetite and resulted in a lack of nutrients and energy reserves (protein and fat) in the fish’s body.

Water quality

Measurement of water quality in transportation media. Salinity (30 ppt) and temperature (29.1 °C) were relatively stable but on measurement of DO and pH decreased, before being given citronella oil DO was 4.91 mg/L after added citronella oil to 4.80 mg/L and initial pH was 7, 89 to 7.67. The pH of the water after adding lemongrass oil decreased because the pH of the lemongrass oil used was 4.5 which is acidic so it can lower the pH of the water. Measurements of post-transportation water quality have all decreased. It is suspected that the increase in CO2 levels in the water is caused by the respiratory activity of fish. according to Irianto (2005), that the higher the CO2 produced from a respiration will tend to liberate H+ so that the pH of the water will decrease. The normal pH for snapper is 7-8.5 (SNI 6145.4:2014)

Conclusion

Based on the results of this study, the concentration of citronella oil 10 µL/L gave the best results as an anesthetic in the closed transport process of white snapper fish seeds. The administration of citronella oil as an anesthetic during transportation of white snapper fry had a significant effect on the survival rate of post-transportation seeds with an SR of 100%. The behavior of white snapper seeds in treatment A did not change, while treatments B, C and D after being exposed to citronella oil experienced changes, namely the operculum movement slowed down, the response to stimuli decreased, lost balance and jumped to avoid water. As an anesthetic, the administration of citronella oil when transporting white snapper fry had no significant effect on the blood profile (the number of erythrocytes, leukocytes and glucose levels.

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