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Nutmeg Oil as an Anesthetic for Enhanced Gas Diffusion in High-Density Seabass Seedlings

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ABSTRACT

Asian seabass (*Lates calcarifer*) is a high-value aquaculture species cultivated to supply domestic and export markets. However, water quality deterioration during transport—marked by declining dissolved oxygen (DO) and rising carbon dioxide (CO_2) levels due to increased fish respiration and limited gas diffusion governed by Henry's Law—can induce stress and reduce seed survival. This study aimed to evaluate the effect of nutmeg oil (Myristica fragrans Houtt.) as an anesthetic on the survival and physiological response of Asian seabass seeds transported at different stocking densities, and to determine the optimal density for wet closed-system transportation. The experiment, conducted in February 2025 at the Faculty of Marine and Fisheries, Syiah Kuala University, employed a completely randomized design with five treatments and four replications. Treatments included a control (without anesthetic) and nutmeg oil administration (1.0 ppm) at densities of 25, 35, 45, and 55 fish. Transportation lasted for eight hours. Nutmeg oil significantly affected anesthesia induction and recovery time ($P<0.05$) but had no significant effect on survival ($P>0.05$). The highest survival rate (98.88%) was observed at 45 fish per container with 1.0 ppm nutmeg oil. The findings suggest nutmeg oil is an effective natural anesthetic for improving transport efficiency and fish welfare, supporting SDG 14 (Life Below Water) through sustainable aquaculture practices.

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1. INTRODUCTION

Asian seabass (*Lates calcarifer*) is a high-value aquaculture species (Putra *et al*., 2025), yet the transportation of fish seeds remains a significant bottleneck. During transport, rapid fluctuations in water quality specifically declining dissolved oxygen (DO) and rising carbon dioxide (CO_2) induce physiological stress and metabolic disruption, leading to high mortality rates (Monica et al., 2019).

In closed wet transportation systems, water quality deteriorates rapidly due to physical principles governing gas solubility and diffusion (Suratno *et al*., 2023). According to Henry's Law, the solubility of respiratory gases like O_2 and CO_2 is directly proportional to their partial pressures above the water surface; however, at high stocking densities, elevated fish respiration rates consume DO faster than it can diffuse across the air-water interface (governed by Fick's First Law of Diffusion, $J = -D * (\Delta C/\Delta x)$, where diffusion flux J decreases with limited surface area and gradient ΔC). This leads

to hypercapnia (CO_2 buildup) and hypoxia, exacerbating stress independent of biological factors (Putra et al., 2025).

Anesthetics like nutmeg oil (*Myristica fragrans* Houtt.) can mitigate these stressors by suppressing metabolic activity (Pellu *et al*., 2018) (Wirasakti et al., 2021; Zahl et al., 2012). While its bioactive components, such as eugenol, are known for their anesthetic properties (Nurdjannah, 2004), there is currently a lack of empirical data regarding the interaction between nutmeg oil anesthesia and the physical constraints of gas diffusion in high-density seabass transport. Specifically, the threshold at which nutmeg oil effectively offsets the physical limitations of O_2 diffusion at varying stocking densities remains unexplored.

Accordingly, this study evaluates the efficacy of nutmeg oil in optimizing the physiological response and survival of Asian seabass seeds during closed-system transportation. By analyzing the intersection of anesthetic sedation and gas exchange physics, this research seeks to identify the optimal stocking density for sustainable aquaculture management.

2. METHODS

2.1 Materials

2.1.1 Time and Location

This study was conducted in February 2025 at the Faculty of Marine and Fisheries, Syiah Kuala University, Darussalam, Banda Aceh City, Aceh Province, Indonesia.

2.1.2 Tools and Materials

The equipment used included a camera, drop pipette, dip net, polyethylene (PE) plastic bags, Styrofoam boxes, pH meter, thermometer, DO meter, measuring cylinder, rubber bands, pure oxygen cylinder, stopwatch, glass jars, aeration hose and stones, transport vehicle, concrete tanks, writing instruments, syringe, hemacytometer, Sahli hemometer, and hematocrit reader. The materials comprised Asian seabass (*Lates calcarifer*) seeds (5–7 cm in length), nutmeg oil (*Myristica fragrans* Houtt.), commercial feed, seawater, EDTA (Ethylenediaminetetraacetic acid), and fish blood samples.

2.1.3 Experimental Design

A Completely Randomized Design (CRD) with a non-factorial arrangement was applied, consisting of five treatments and four replications. The treatments were as follows:

- P1 = 25 fish (control, without nutmeg oil)
- P2 = 25 fish + 1.0 ppm nutmeg oil
- P3 = 35 fish + 1.0 ppm nutmeg oil
- P4 = 45 fish + 1.0 ppm nutmeg oil
- P5 = 55 fish + 1.0 ppm nutmeg oil

The 1.0 ppm nutmeg oil concentration was selected based on previous studies indicating its optimal anesthetic effect for tropical fish transport. The duration of transportation was standardized at eight hours for all treatments.

2.1.4 Experimental Procedures

1. Preparation of Test Fish

Test fish were acclimated in an aerated holding tank for 24 hours to eliminate metabolic waste and stabilize physiological conditions. Feeding was withheld during this period to reduce metabolic activity and clear intestinal contents. Only healthy, undamaged fish were selected for the experiment.

2. Blood Sampling

Blood samples were collected using syringes coated with EDTA as an anticoagulant. Erythrocyte and leukocyte counts were determined using a hemacytometer, hemoglobin concentration was measured with a Sahli hemometer, and hematocrit values were obtained using a hematocrit reader. These parameters were used to evaluate the fish blood profile.

3. Packing Process

Following the method of Suratno *et al*. (2023) each 60 cm × 40 cm PE plastic bag was filled with 2 L of water and treated with 1.0 ppm nutmeg oil, which was allowed to equilibrate for 15 minutes before fish introduction. The bags were filled with pure oxygen at a 1:3 ratio of water to oxygen volume in accordance with SNI 02-6487.2-2002. Initial measurements of pH, temperature, and dissolved oxygen (DO) were recorded prior to sealing.

4. Transportation

The sealed bags were placed into Styrofoam boxes and arranged in the rear compartment of a vehicle to simulate wet closed-system transport. The transport process was conducted for eight hours under ambient conditions.

5. Post-Transportation Handling

Upon arrival, post-transport water quality parameters (temperature, pH, and DO) were immediately measured. The fish were then transferred into aerated water without anesthetic to monitor their recovery time, defined as the period required for regaining equilibrium and normal swimming behavior.

6. Post-Transportation Maintenance

Post-transport maintenance was conducted for seven days to assess survival. Twenty glass jars (10 L each) were prepared with aerated seawater, and fish were fed commercial pellets three times daily (08:00, 12:00, and 17:00) to satiation. Partial water exchange was performed every two days, and daily mortality was recorded following (Syawalani et al., 2021).

Observed Parameters

1. Fish Behavior – Monitored descriptively by observing operculum movement, swimming activity, and response to external stimuli.
2. Induction Time – Time elapsed from fish exposure to anesthetic until complete loss of equilibrium (Khalil *et al*., 2018).
3. Recovery Time – Time required for fish to regain equilibrium and normal behavior after transport (Khalil *et al*., 2018).
4. Blood Profile – Measurements included erythrocyte, leukocyte, hemoglobin, and hematocrit levels.
5. Survival Rate (SR) – Calculated following Effendi (1979):
6. The survival rate was calculated using the formula by Effendi (1979); El Rahimi *et al* (2021):

$$SR(\%) = \frac{N_t}{N_o} \times 100\% \quad (1)$$

where Nt is the number of surviving fish at the end of the experiment, and N_0 is the initial number.

7. Water Quality Parameters – Temperature, pH, and DO were recorded before and after transportation.

2.1.5 Data Analysis

Quantitative data, including induction time, recovery time, and survival rate, were analyzed using Analysis of Variance (ANOVA) at a 95% confidence level with SPSS software. When significant differences were detected ($P < 0.05$), post-hoc tests were selected based on the Coefficient of Variation (CV): Duncan's Multiple Range Test (DMRT) for CV > 10%, Least Significant Difference (LSD) for CV > 5%, and Honestly Significant Difference (HSD) for CV < 5%. Behavioral observations, blood profiles, and water quality data were analyzed descriptively.

3. RESULT AND DISCUSSION

3.1 Behavior of Asian Seabass (*Lates calcarifer*) Fry

The behavior of Asian seabass (*Lates calcarifer*) fry before and after transportation is presented in Table 1 and Table 2.

Table 1. Behavior of Fish Fry Prior to Loss of Consciousness

Behavioral Aspect	P1	P2	P3	P4	P5
Swimming Activity	Swimming normally and actively.	Swimming actively, then slightly panicked, followed by erratic movements and collisions with the plastic wall.	Actively swimming, then gradually losing balance.	Appears motionless and settles at the bottom.	Appears panicked and swims erratically, then stops responding to external stimuli.
Gill Condition	Fin movements remain normal, and operculum opening is also normal.	Fin movements begin to weaken.	Fin movements begin to weaken.	Fin movements weaken, and operculum opening slows down.	Fin movements weaken, and operculum opening also slows down.
Fish Response	Still responds to external physical stimuli.	Still responds to external physical stimuli.	Still responds to external physical stimuli.	Does not respond to external physical stimuli.	Does not respond to external physical stimuli.

Based on the observations presented in Table 1, it can be seen that the physiological conditions and behavioral responses of Asian seabass (*Lates calcarifer*) fry anesthetized with nutmeg oil as an alternative anesthetic agent were generally similar across all treated groups.

Table 2. Behavior of Asian Seabass (*Lates calcarifer*) Fry Prior to Recovery

Behavioral Aspect	P1	P2	P3	P4	P5
Swimming Activity	Swimming normally and actively.	Fish fry begin to move slowly.	Fish fry begin to move slowly.	Fish fry begin to move slowly.	Fish fry begin to move slowly.
Gill Condition	Gill movements remain normal.	Gill movements begin to appear.	Gill movements begin to appear.	Gill movements begin to appear.	Gill movements begin to appear.
Fish Response	Fish fry start to respond to external stimuli.	Fish fry start to respond to external stimuli.	Fish fry start to respond to external stimuli.	Fish fry start to respond to external stimuli.	Fish fry start to respond to external stimuli.

Based on the results of the study on the anesthesia of Asian seabass (*Lates calcarifer*) fry using nutmeg oil at different stocking densities during an eight-hour transportation process, the fish were subsequently revived using aeration and clean water (Table 2).

3.2 Induction and Recovery Time of Asian Seabass Fry

The induction and recovery times observed in Asian seabass (Lates calcarifer) fry are detailed in Table 3, which summarizes the duration of each phase across the different treatments applied in the study.

Table 3. Induction and Recovery Time of Fish Fry

Treatment	Induction Time (minutes)	Recovery Time (minutes)
P1	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
P2	23.96 ± 1.89^{d}	15.20 ± 0.11^{d}
P3	31.79 ± 2.64^{c}	16.22 ± 0.21^{c}
P4	40.17 ± 0.78^{b}	18.18 ± 0.06^{b}
P5	49.02 ± 1.05^{a}	19.37 ± 0.10^{a}

Note: Mean values within a column followed by different superscripts indicate significant differences ($P<0.05$), while identical superscripts indicate no significant difference. Values are presented as mean ± standard deviation (SD)

Based on the results of the ANOVA test, the application of nutmeg oil as an anesthetic agent for Asian seabass (*Lates calcarifer*) fry had a significant effect ($P<0.05$) on the induction time. The post-hoc test results indicated that treatments P1, P2, P3, P4, and P5 were significantly different ($P<0.05$) from one another. As shown in Table 3, the longest induction time was observed in treatment P5 (density of 55 fish), with an average duration of 49.02 minutes, while the shortest induction time occurred in treatment P2 (density of 25 fish), with an average duration of 23.96 minutes.

The results of this study showed a clear tendency that the higher the stocking density, the longer it took for the Asian seabass (*Lates calcarifer*) fry to lose consciousness, and vice versa. This phenomenon is presumed to occur because the effectiveness of nutmeg oil at a concentration of 1.0 ppm influences the induction time in fish subjected to different densities. This finding is consistent with (Syawalani et al., 2021), who stated that increasing the stocking density of fish within a packaging medium prolongs the induction period. Fish maintained at higher densities tend to experience delayed anesthesia.

Megi *et al*. (2024) further explained that in denser environments, fish may exhibit delayed anesthesia due to the more complex distribution of the anesthetic agent, as well as changes in metabolic rates resulting from higher density conditions. As an environmentally friendly natural substance, nutmeg oil is effective in inducing anesthesia and reducing stress during transportation, thereby decreasing mortality among fish fry. This finding aligns with Khalil *et al*. (2018), who reported that nutmeg oil contains essential oil compounds such as myristicin and eugenol-like alcohols, which can affect the nervous system—causing fish to become lethargic, move slowly, and eventually lose consciousness.

Based on the ANOVA results presented in Table 3, the application of nutmeg oil as an anesthetic agent had a significant effect ($P<0.05$) on the recovery time of Asian seabass (*Lates calcarifer*) fry at different stocking densities. Post-hoc tests revealed that treatments P1, P2, P3, P4, and P5 differed significantly ($P<0.05$) from one another. The results indicated that treatment P5 (density of 55 fish) showed the longest recovery time at 19.37 minutes, whereas treatment P2 (density of 25 fish) demonstrated the shortest recovery time at 15.20 minutes.

The results of this study showed that as the stocking density increased, the time required for fish to regain consciousness also tended to increase, and vice versa. This phenomenon is presumed to occur because a higher number of fish within the same volume reduces their ability to recover quickly from anesthesia. The recovery time increased with stocking density, consistent with previous findings on density effects during fish anesthesia and transport (Hasan *et al*., 2017; Syawalani *et al*., 2021). The effect of the active compounds in nutmeg oil may differ according to the stocking density used, influencing the duration of recovery.

Hasan *et al*. (2017) also stated that the use of anesthetics under different density conditions can affect the recovery duration of fish. In this study, the recovery time of fish fry was observed when anesthetized fish were transferred into another container equipped with aeration and free of nutmeg oil. The recovery period refers to the duration required for the fish to regain full consciousness from an anesthetized state, characterized by the resumption of normal behavior, restored equilibrium, and responsiveness to external stimuli (Ross & Ross, 2008).

3.3 Blood Profile Test

The blood profile test was conducted on two treatments: the control group (without nutmeg oil) with the lowest stocking density (25 fish) and the treatment group with nutmeg oil at the highest density (55 fish). The results of the laboratory blood profile analysis of Asian seabass (*Lates calcarifer*) fry are presented in Table 4.

Table 4. Blood Profile of Asian Seabass (*Lates calcarifer*) Fry

Sample	Hemoglobin (g/dL)	Hematocrit (%)	Leukocytes (WBC) (cells/mm^3)	Erythrocytes (RBC) (cells/mm^3)
Control	6.0 g/dL	9%	94,350 cells/mm^3	5,460,000 cells/mm^3
Nutmeg oil	6.2 g/dL	28%	52,575 cells/mm^3	10,040,000 cells/mm^3

Based on the results presented in Table 4, the hemoglobin concentration of Asian seabass (*Lates calcarifer*) fry in the control treatment was 6 g/dL, while that in the nutmeg oil treatment was 6.2 g/dL. These values appear lower than the normal hemoglobin range of 8.5–10.2 g/dL as reported by Raghuveer and David (2015). According to Dellman and Brown (1989), a hemoglobin level below the normal range may indicate poor water quality or the presence of infection. Hemoglobin concentration is closely related to the erythrocyte count, where higher hemoglobin levels correspond to higher erythrocyte values. The increase in hemoglobin levels is influenced by the rise in erythrocyte count within the fish's body (Putranto et al., 2019).

The normal hematocrit level in Asian seabass fry ranges around 18.26% (Tan *et al*., 2023). The present study showed that the hematocrit value in the control group was 9%, while that in the nutmeg oil treatment reached 28%. The increase in hematocrit beyond the optimal range may represent a physiological response to stress caused by unfavorable environmental conditions, such as high stocking density (Dellman & Brown, 1989; Refaey et al., 2018; Thophon et al., 2003)(Permana *et al*., 2019).

The normal leukocyte (WBC) count in Asian seabass fry ranges between 5.3–69.9×10^9 cells/L or 5.3–69.9 million cells/mm^3 (Tan *et al*., 2023). The study results showed that the leukocyte count in the control group was 94,350 cells/mm^3, whereas in the nutmeg oil treatment it was 52,575 cells/mm^3. This indicates that fish without anesthesia experienced a significant increase in leukocyte levels compared to anesthetized fish. Leukocytes play a key role in enhancing immunity like defending the body against pathogenic invasion through phagocytosis, identifying, and destroying foreign cells (Ramadhan & Sari, 2019).

The normal erythrocyte (RBC) count in Asian seabass ranges between 4.1–11.2×10^9 cells/L or 4.1–11.2 million cells/mm^3. In this study, the erythrocyte count in the control treatment was 5,460,000 cells/mm^3, while in the nutmeg oil treatment it increased to 10,040,000 cells/mm^3. This increase may be a physiological response to a higher oxygen demand during transportation (Yanto *et al*., 2009). An increase in erythrocyte count reflects the adaptive mechanism of fish to challenging environmental conditions, such as stress induced by transportation (El Rahimi et al., 2021; Putranto et al., 2019; Syawalani et al., 2021).

3.4 Survival Rate of Asian Seabass Fry

The survival rate of Asian seabass (*Lates calcarifer*) fry is presented in Table 5.

Table 5. Survival Rate of Asian Seabass (*Lates calcarifer*) Fry

Treatment	Survival Rate (%)
P1	97 ± 3.82^a
P2	96 ± 5.65^a
P3	97.14 ± 3.29^a
P4	98.88 ± 2.22^a
P5	95.90 ± 3.10^a

Note: Mean values within a column followed by different superscripts indicate significant differences (P<0.05), while identical superscripts indicate no significant difference. Values are expressed as mean ± standard deviation (SD).

Based on the ANOVA results, the application of nutmeg oil at the same concentration (1.0 ppm) showed no statistically significant effect (P>0.05) on the survival rate of Asian seabass (*Lates calcarifer*) fry at different stocking densities. The Duncan's post-hoc test also indicated that treatments with nutmeg oil did not differ significantly (P>0.05) from one another. As shown in Table 5, the highest survival rate was observed in treatment P4 (density of 45 fish) with a value of 98.88%, while the lowest was recorded in treatment P5 (density of 55 fish) with a value of 95.90%.

The results suggest that the use of nutmeg oil at a constant concentration of 1.0 ppm effectively maintained a stable survival rate across different stocking densities. The high survival rate observed in all treatments demonstrates the effectiveness of nutmeg oil as a natural anesthetic in reducing stress during transportation. This finding is supported by Zahl *et al*. (2012), who stated that the use of anesthetics during fish transportation can reduce metabolic activity and oxygen consumption, thereby minimizing stress that may lead to mortality. Nutmeg oil contains eugenol and myristicin, compounds known for their sedative and anesthetic properties, which help calm the fish during transport and reduce physical trauma caused by collisions within the transport container.

Based on the observations, different stocking densities in this study indicated that nutmeg oil at a concentration of 1.0 ppm remained effective at P4 (density of 45 fish). However, at P5 (density of 55 fish), although still within the tolerance limit, a slight decrease in survival rate was observed. This finding is consistent with (El Rahimi et al., 2021), who stated that high stocking densities can increase oxygen competition and the accumulation of toxic metabolites such as ammonia, although the use of anesthetics can mitigate these negative effects. The decline in survival rate at higher densities may also be attributed to increased physical interactions among individuals, which can cause injuries and stress, as reported by (Refaey et al., 2018) in tilapia. According to (Gil et al., 2016), a survival rate above 50% is considered good, a rate between 30–50% is moderate, and a rate below 30% is classified as poor.

3.5 Water Quality Parameters

The water quality parameters measured during the study included temperature, pH, and dissolved oxygen (DO). Measurements were taken before and after transportation, as shown in Table 6.

Table 6. Water Quality Parameters

Treatment	Temperature (°C)		pH		DO (mg/L)	
	Initial	Final	Initial	Final	Initial	Final
P1	28	29	7.5	7.3	6.8	6.7
P2	28	29.8	7.5	7.0	6.3	6.0
P3	29	30	7.3	6.84	5.9	5.5
P4	29	30.6	7.2	6.77	5.6	5.3
P5	29	30.6	7.0	6.69	5.4	5.1

The temperature of the transportation medium at the beginning of the experiment ranged between 28°C and 29°C. This temperature range falls within the optimal level for Asian seabass (*Lates calcarifer*) fry, as specified by the Indonesian National Standard (SNI 6145.4:2014), which is 26–32°C. According to (Syamsunarno et al., 2019), the increase in temperature may be influenced by the use of air-conditioned vehicles that function as heat retainers during transport. From a physics perspective, temperature rise also stems from reduced convective heat loss and limited thermal diffusion across the closed plastic packaging, where Fourier's Law ($q = -k * \Delta T/\Delta x$) governs slower heat transfer at higher densities due to diminished water circulation. Temperature fluctuations can also occur due to the limited dissolved oxygen within the plastic packaging and the reduced diffusion of air across the water surface (Afriani et al., 2023)

According to SNI 6145.4:2014, the optimal pH range for Asian seabass (*Lates calcarifer*) fry is 7.0–8.5. The results of this study showed that the pH values ranged from 7.0 to 7.5 before anesthesia and decreased to 7.3–6.9 after anesthesia. The decline in pH levels is attributable to CO_2 accumulation from fish respiration, which physically dissolves into water per Henry's Law ($C = k_H * P_CO2$), forming carbonic acid and lowering pH, with diffusion-limited off-gassing in closed systems exacerbating the effect at higher densities (Hassan *et al*., 2022). Nevertheless, the pH values recorded in this study remain within the acceptable range set by the SNI standard.

According to the Indonesian National Standard (SNI 6145.4:2014), the optimal dissolved oxygen (DO) concentration for Asian seabass (*Lates calcarifer*) fry is ≥ 5 mg/L. Ramadhan and Sari (2018) stated that an adequate DO concentration for fish survival should be above 2 mg/L. In this study, the DO concentration at the beginning of the experiment ranged between 5.4–6.8 mg/L, while at the end of the experiment it ranged between 6.7–5.1 mg/L. The results showed that DO levels decreased across all treatments by the end of the experiment. The decline in DO concentration corresponded with an increase in stocking density due to physics-driven imbalances: fish respiration depletes DO faster than replenishment via Fick's First Law of Diffusion ($J = -D * \Delta C/\Delta x$), where limited air-water surface area and shallow concentration gradients (ΔC) at high densities restrict O_2 influx. This finding is consistent with Wirasakti *et al*. (2021), who reported that higher fish densities in rearing or transport containers tend to reduce DO levels, and vice versa. Nevertheless, the DO range observed in this study remained within the acceptable limits established by the SNI standard.

4. CONCLUSION

This study demonstrates that nutmeg oil (*Myristica fragrans* Houtt.) at a concentration of 1.0 ppm acts as a viable natural sedative for the wet closed-system transportation of Asian seabass (*Lates calcarifer*) fry. The application of nutmeg oil significantly influenced both induction and recovery times ($P < 0.05$), with durations increasing in correlation with higher stocking densities. While survival rates remained high across all treatments ranging from 95.90% to 98.88%—statistical analysis confirmed that stocking density had no significant effect on survival ($P > 0.05$). This suggests that the anesthetic maintains high survival regardless of density within the tested range, likely by balancing metabolic respiration against diffusion-limited oxygen replenishment. Furthermore, the improved hematological profiles in anesthetized fish relative to the control indicate a reduction in physiological stress responses. In conclusion, nutmeg oil at 1.0 ppm is a technically effective anesthetic for inducing sedation and managing stress, supporting sustainable aquaculture practices and fish welfare during transport without compromising survival rates at higher densities.

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