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The Effect of Stocking Densities on Mortality of Nile Tilapia (*Oreochromis niloticus*) Fingerlings (3-5 cm) in a Closed Wet Transportation System from Kediri to Sidoarjo

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ABSTRACT

Transportation of fish fingerlings is an important stage in aquaculture activities because it affects the success of fingerling distribution. One factor influencing transportation success is stocking density during transportation. This study aimed to determine the effects of different stocking densities on the mortality of Nile tilapia (*Oreochromis niloticus*) fingerlings (3-5 cm) in a closed wet-transport system and to identify the stocking density associated with the lowest mortality rate. This study used an experimental method with a Completely Randomised Design (CRD) comprising four treatments: A (100 fish/5 L), B (120 fish/5 L), C (150 fish/5 L), and D (200 fish/5 L), with six replications. Transportation lasted 4 hours with a water-to-oxygen ratio of 1:3. Parameters observed included mortality, survival rate, temperature, and pH. The results showed that stocking density affected the mortality of Nile tilapia fingerlings. The lowest mortality was observed in treatment A (1.83%), while the highest mortality was found in treatment D (7.83%). A density of 100 fish/5 L provided the best condition for minimising mortality during transportation.

Keywords: Nile Tilapia (*Oreochromis niloticus*), Density, Mortality, Closed Wet Transport, Survival Rate

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of the most important freshwater aquaculture commodities in Indonesia due to its rapid growth rate, ease of culture, and high adaptability to various aquatic environments (Andriani, 2018; Dailami et al., 2021). In addition, Nile tilapia has considerable economic value and stable market demand, making it a promising species for aquaculture development and income generation among fish farmers (Adi et al., 2024; Mistina & Maruanaya, 2025). The increasing expansion of Nile tilapia farming has consequently increased the demand for high-quality fingerlings to support sustainable production.

The availability and quality of fingerlings strongly influence the success of Nile tilapia aquaculture. High-quality fingerlings generally exhibit better growth performance and survival during the culture period (Dadiono et al., 2025). Therefore, an efficient distribution system is required to maintain fingerling quality from hatcheries to grow-out facilities. One of the most commonly used methods for live fish transportation is the closed wet transportation system, which utilises water and oxygen in sealed containers without water exchange during transportation. This system is considered practical and efficient for transporting fingerlings over relatively long distances (Imanto, 2008; Supriyanto & Dharmawantho, 2021).

Despite its advantages, closed wet transportation systems have several limitations. During transportation, fish continue to respire and excrete metabolic waste, which deteriorates water quality. This process may reduce dissolved oxygen concentration while increasing carbon dioxide and ammonia accumulation, which can negatively affect fish physiological conditions (Midani, 2022). Prolonged exposure to poor water quality may induce stress, reduce adaptive capacity, and increase mortality during transportation (Maulana et al., 2025).

One of the main factors influencing water quality deterioration during transportation is stocking density. Stocking density refers to the number of fish placed within a certain volume of water. High stocking densities increase oxygen consumption, metabolic activity, and waste

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accumulation, thereby accelerating water quality degradation (Iyeda et al., 2024). Furthermore, excessive density may intensify competition for space and oxygen, resulting in elevated stress levels and increased mortality (Syamsunarno et al., 2019). According to Andriani & Syauqibik (2025), stocking density significantly affects the physiological condition and survival of fish during transportation.

In commercial aquaculture practices, increasing stocking density is often used to improve transportation efficiency and reduce operational costs. However, excessive stocking density may compromise fish survival by accelerating deterioration of water quality and increasing physiological stress. Therefore, determining an appropriate stocking density is essential to balance transportation efficiency and fish survival.

The effect of stocking density is also influenced by fish size. Nile tilapia fingerlings measuring 3-5 cm are considered more vulnerable to environmental changes than larger fish because their physiological adaptation mechanisms are not yet fully developed (Ahmadi et al., 2024). Consequently, these fingerlings are more susceptible to stress and mortality during transportation, particularly under high-density conditions.

Several studies have investigated fingerling transportation; however, most have focused on different fish sizes, additional treatments, or experimental conditions that do not fully represent field transportation practices. Information regarding the effect of stocking density on the mortality of 3-5 cm Nile tilapia fingerlings transported in a closed wet transportation system under conditions similar to commercial distribution routes, particularly the Kediri-Sidoarjo route, remains limited. Therefore, this study aimed to evaluate the effect of different stocking densities on the mortality of 3-5 cm Nile tilapia fingerlings (*Oreochromis niloticus*) during closed wet transportation and to determine the stocking density that produces the lowest mortality rate.

MATERIAL AND METHOD

Research Location and Time

This study was conducted at UD. Sumbermas Minajaya, Kediri, East Java, Indonesia, from April 13 to May 12, 2026. Transportation trials were conducted on the Kediri-Sidoarjo route, covering approximately 120 km and taking ±4 h. The route was selected to represent actual Nile tilapia fingerling distribution practices in East Java.

Equipment and Materials

The equipment used in this study included polyethylene (PE) plastic bags (60 × 100 cm, double layer), oxygen cylinders equipped with regulators, a digital pH meter, a digital water thermometer, plastic buckets, a digital balance, a hand net, rubber bands, styrofoam boxes (60 × 40 × 40 cm), and a stopwatch.

The test animals were healthy Nile tilapia (*Oreochromis niloticus*) fingerlings measuring 3-5 cm in length and weighing approximately 0.5-1 g per fish. Freshwater and pure oxygen were used as transportation media.

Research Methods

This study employed an experimental method using a Completely Randomised Design (CRD). According to Kusriningrum (2010), the minimum number of replications was determined using the formula $(t-1)(n-1) \geq 15$, resulting in six replications for each treatment. Therefore, four treatments with six replications were applied, resulting in a total of 24 experimental units.

Stocking density treatments were determined based on previous studies on Nile tilapia transportation (Afriansyah et al., 2016; Restu et al., 2024), consisting of: (a) Treatment A: 100 fish/5 L; (b) Treatment B: 120 fish/5 L; (c) Treatment C: 150 fish/5 L; (d) Treatment D: 200 fish/5 L.

Research Procedures

Prior to transportation, all plastic bags were prepared using a double-layer system to minimise leakage. Each bag was filled with 5 L of freshwater. Nile tilapia fingerlings were selected based on health condition, uniform size, active swimming behaviour, and absence of visible abnormalities. The fish were fasted for approximately 12 h before transportation to reduce metabolic activity and waste production during transport.

Fingerlings were stocked into transportation bags according to each treatment density. Pure oxygen was added at a water-to-oxygen ratio of 1:3, and the bags were tightly sealed using rubber bands. All bags were placed vertically inside styrofoam boxes to maintain temperature stability and reduce physical disturbance during transportation. Ice packs were positioned outside the bags inside the styrofoam boxes to maintain water temperature at approximately 25-30°C.

Transportation was conducted for 4 h using a closed wet transportation system without water exchange. At the end of transportation, mortality, survival rate, temperature, and pH were recorded for each experimental unit.

Data Analysis

The observed parameters included mortality, survival rate (SR), water temperature, and pH. Survival rate was calculated according to Effendie (2002) as the percentage of fish remaining alive at the end of transportation relative to the initial number of fish transported, using the formula $SR = (N_t/N_0) \times 100\%$, where N_0 is the number of fish at the beginning of transportation and N_t is the number of fish at the end of transportation. Mortality was considered the primary response variable and was calculated as the percentage of dead fish relative to the initial number of fish transported using the following formula, which represents the inverse expression of the survival rate formula:

$$M = ((N_0 - N_t) / N_0) \times 100\%$$

Where:
M = Mortality (%)
N_0 = number of fish at the beginning of transportation (fish)
N_t = number of fish at the end of transportation (fish).

Survival rate and water quality parameters were recorded as supporting indicators of transportation performance.

Prior to statistical analysis, mortality data were tested for normality using the Shapiro–Wilk test (Shapiro et al., 1965) and for homogeneity of variance using Levene's test (Levene, 1960). Data that met the assumptions of parametric analysis were subjected to one-way Analysis of Variance (ANOVA) at a 95% confidence level ($\alpha = 0.05$) to determine the effect of stocking density on mortality. When significant differences were detected, Tukey's Honestly Significant Difference (HSD) test and Duncan's Multiple Range Test (DMRT) were applied for multiple comparisons among treatments (Gomez & Gomez, 1984). Statistical analyses were performed using SPSS version 25.00.

RESULT AND DISCUSSION

Result

Based on the study on the effect of different stocking densities on the mortality of Nile tilapia (*Oreochromis niloticus*) fingerlings measuring 3-5 cm during transportation in a closed wet transportation system, differences in mortality were observed among treatments. The mortality data are presented in Table 1.

Table 1. Range, Average, and Standard Deviation of Mortality of Nile Tilapia (*Oreochromis niloticus*) Fingerlings

Treatment	Mortality Range (%)	Average (%)	Standard Deviation (SD)
A (100 fish/5 L)	0.00-4.00	1.83	1.47
B (120 fish/5 L)	1.67-6.67	4.00	1.79
C (150 fish/5 L)	2.67-8.00	4.83	1.94
D (200 fish/5 L)	5.00-10.50	7.83	2.32

Based on Table 1, treatment A (100 fish/5 L) produced the lowest average mortality of Nile tilapia fingerlings, while treatment D (200 fish/5 L) resulted in the highest mortality. Mortality increased progressively with increasing stocking density during transportation.

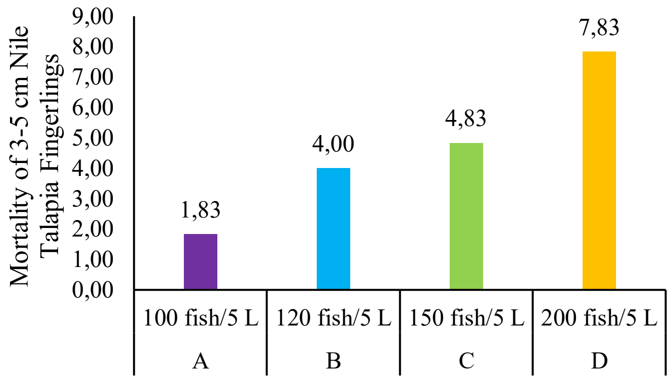

Figure 1. Average Mortality of Nile Tilapia (*Oreochromis niloticus*) Fingerlings under Different Stocking Densities

The trend shown in Figure 1 indicates that mortality increased with increasing stocking density. Treatment A resulted in an average mortality of 1.83%, whereas treatment D produced the highest mortality of 7.83%. Intermediate mortality values were observed in treatments B and C, with averages of 4.00% and 4.83%, respectively.

To determine whether the observed differences among treatments were statistically significant, a one-way ANOVA was performed. The results are presented in Table 2.

Table 2. One-way ANOVA of Mortality of Nile Tilapia (*Oreochromis niloticus*) Fingerlings

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.011	3	0.004	10.218	0.000
Within Groups	0.007	20	0.000		
Total	0.018	23			

Based on Table 2, stocking density significantly affected the mortality of Nile tilapia fingerlings during transportation ($P < 0.05$). The significance value of 0.000 indicates that differences in mortality among treatments were not caused by random variation but were associated with differences in stocking density.

To identify differences among treatments, a multiple comparison test was conducted using Duncan's Multiple Range Test (DMRT). The results are presented in Table 3.

Table 3. Mean Mortality and DMRT Grouping of Nile Tilapia (*Oreochromis niloticus*) Fingerlings

Treatment	Mean mortality (%)	Notation
A (100 fish/5 L)	1.83	a
B (120 fish/5 L)	4.00	ab
C (150 fish/5 L)	4.83	b
D (200 fish/5 L)	7.83	c

Means followed by different letters indicate significant differences at $\alpha = 0.05$ according to Duncan's Multiple Range Test (DMRT).

Based on Table 3, treatment A produced the lowest mortality and differed from treatment D. Treatments B and C were grouped within overlapping subsets, indicating that mortality between these treatments was not significantly different. Treatment D exhibited the highest mortality and formed a separate group, indicating a higher mortality response than the lower stocking density treatments.

In addition to mortality observations, survival rate and water quality parameters were also recorded as supporting indicators of transportation performance. The average survival rate ranged from 92.83% to 98.17%, with the highest value observed in treatment A and the lowest value observed in treatment D. Water temperature ranged from 26.20 to 27.58°C. In contrast, pH ranged from 6.75 to 7.46 throughout the transportation period.

Discussion

Treatment A (100 fish/5 L) produced the lowest average mortality of Nile tilapia (*Oreochromis niloticus*) fingerlings during transportation, whereas treatment D (200 fish/5 L) resulted in the highest mortality. The lower mortality observed in treatment A indicates that the stocking density was still within the tolerance range of the fingerlings and provided sufficient space, oxygen availability, and environmental stability during transportation. Under these conditions, fish maintained normal physiological functions and adapted more effectively to transportation stress. Pane et al. (2023) reported that fish responses to environmental changes are closely related to physiological adaptation mechanisms, while Hertika et al. (2022) stated that environmental conditions play an important role in maintaining the physiological balance of cultured aquatic organisms.

Compared with treatment A, mortality increased progressively in treatments B, C, and D as stocking density increased. Higher stocking densities increased oxygen consumption and accelerated the accumulation of metabolic wastes, such as carbon dioxide and ammonia, in the transport medium. Consequently, fish experienced greater physiological stress due to limited space and intensified competition for available oxygen. Pratama et al. (2023) explained that increased stocking density can trigger physiological stress responses in aquatic organisms, while Djauhari et al. (2019) reported that high stocking densities may alter physiological conditions and increase stress levels in fish.

The highest mortality observed in treatment D (200 fish/5 L) suggests that the stocking density exceeded the optimal carrying capacity of the transportation medium. Under crowded conditions, fish movements become restricted and physical interactions among individuals increase, resulting in greater environmental pressure during transportation. Similar findings were reported by Lake & Tjendanawangi (2019), who found that increasing stocking density in wet transportation systems reduced the survival of transported organisms. Restu et al. (2024) also reported that increasing the density of Nile tilapia fingerlings during transportation reduced their survival. Furthermore, Sova et al. (2026) stated that high stocking density in closed transportation systems tends to reduce fish survival due to increased environmental stress during transport.

The survival rate showed an inverse relationship with mortality. Treatment A, which produced the lowest mortality, had the highest survival rate (98.17%), whereas treatment D, which produced the highest mortality, had the lowest survival rate (92.83%). These findings indicate that increasing stocking density reduces fish's ability to maintain normal physiological conditions during transportation. Similar results were reported by Bakrie & Olgani (2020), Rohman et al. (2023), and Kurniawan (2022), who demonstrated that transportation conditions and stocking density significantly influence the survival of Nile tilapia fingerlings.

Water quality measurements during transportation remained within acceptable ranges for Nile tilapia fingerlings. Water temperature ranged from 26-28°C, while pH values ranged from 6.7-7.5. These conditions were still within the optimal ranges reported for Nile tilapia culture. Cahyanti & Awalina (2022) stated that the optimum temperature range for Nile tilapia growth is 25-30°C, while Pramleonita et al. (2018) reported that Nile tilapia can tolerate pH conditions ranging from neutral to slightly alkaline. Water quality plays an important role in supporting fish life, growth, development, and survival (Dari et al., 2023). Since water quality remained relatively stable among treatments, the differences in mortality observed in this study were more likely associated with the effects of stocking density and transportation stress rather than changes in temperature or pH. Similar conclusions were reported by Dewi et al. (2022), who found that fish mortality is influenced not only by water quality but also by environmental stress experienced during culture and transportation.

Overall, this study found that increasing stocking density in a closed wet-transport system increases the risk of mortality in Nile tilapia fingerlings. Therefore, a stocking density of 100 fish/5 L is considered the most suitable density for transporting 3-5 cm Nile tilapia fingerlings for approximately four hours under the conditions applied in this study.

CONCLUSION

Different stocking densities significantly affected the mortality of Nile tilapia (*Oreochromis niloticus*) fingerlings measuring 3-5 cm during transportation using a closed wet transportation system. The lowest mortality rate (1.83%) and the highest survival rate (98.17%) were obtained at a stocking density of 100 fish/5 L, indicating that this density provided the most suitable transportation conditions among the treatments tested. Increasing stocking density tended to increase mortality and reduce the survival rate during transportation. Water temperature (26-28°C) and pH (6.7-7.5) remained within acceptable ranges for Nile tilapia fingerlings throughout the study and therefore did not appear to be the primary factors affecting mortality during transportation.

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