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Effects of Transport Stress (Duration and Density) on the Physiological Conditions of Marbled Rockfish (*Sebastiscus marmoratus, Cuvier 1829*) Juveniles and Water Quality

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Abstract: Live transportation is a critical component of fish farming and hatchery release. To optimize hatchery-release techniques and improve the survival rate of marbled rockfish (*Sebastiscus marmoratus*, Cuvier 1829) juveniles, the effects of varying transport durations (2, 4, 6, and 8 h) and densities (60, 90, 120, and 150 kg m^{-3}) on the physiological indicators of the fish and water quality were investigated under controlled laboratory conditions. We found that as transport duration and density increased, water quality significantly deteriorated, with ammonia nitrogen levels rising and dissolved oxygen content and pH levels decreasing. Physiological indicators including levels of lactate, cortisol, and malondialdehyde and activities of superoxide dismutase, alkaline phosphatase, and glutamate oxaloacetate transaminase notably increased, indicating that the fish experienced heightened stress during transport. Additionally, the mortality rate of juveniles increased significantly with increasing density and transport duration. The high mortality rate might be associated with sustained elevated cortisol levels and liver damage. Our results are helpful for determining the optimal transport conditions for *S. marmoratus* juveniles and also provide valuable insights for improving transport techniques for other aquatic animal species.

Keywords: *Sebastiscus marmoratus*; live transportation; time duration; oscillation; mortality rate

Key Contribution: The most significant contribution of this study is an improved understanding of the transport-induced stress responses in *Sebastiscus marmoratus* juveniles under varying densities and transport durations. Our results provide crucial insights for optimizing transport conditions for this species. They establish a scientific basis that can enhance survival rates in hatchery-release programs and potentially improve live transportation methods for other species.

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1. Introduction

Live transportation is a critical stage in the hatchery-release process. During transport, fish are exposed to various stressors, such as capture, changes in density, agitation, hypoxia, and water quality fluctuations, which contribute to transport stress [1,2]. Transport duration and density are the important human-controlled factors that influence transport stress. Excessive transport duration and high density can impair fish physiological functions, reduce resistance to pathogens, and even result in mortality [3,4]. Moreover, fish are transported in a confined aquatic environment in which their metabolic waste directly degrades water quality, which further disrupts normal metabolic functions and reduces survival rates [5]. Although previous studies have attempted to mitigate transportation stress through the use of sedatives or water treatment methods [6,7], optimizing transportation duration and density to reduce stress and improve survival rates remains a critical area of focus. This is particularly critical for juvenile fish, which are primary-release targets that have lower immune resistance than adults. Juveniles are more susceptible to environmental changes, leading to heightened stress and reduced survival during transport.

Multiple transport stressors, as well as their interactions, are likely to provoke various physiological responses in fish, such as accelerated respiration, increased metabolic rate, and heightened energy consumption. These changes disrupt the normal physiological and biochemical processes of the fish, leading to the production of metabolic byproducts such as lactate and malondialdehyde (MDA) [8,9], which further exacerbate the physiological burden. During closed-water transport, an accelerated metabolism of the fish increases oxygen consumption and leads to the accumulation of metabolic waste, causing a rise in ammonia nitrogen (NH_4^+-N) levels [10], a decrease in dissolved oxygen content, and fluctuations in pH [11,12], which in turn results in water quality deterioration. Typically, fish respond to stress by adjusting hormone secretion and enzyme activity [13]. For instance, increased cortisol secretion helps to maintain water–salt balance and enhances environmental adaptability [14], while elevated superoxide dismutase (SOD) activity helps preserve the redox balance within cells [15]. Therefore, when examining the effects of transport stress on fish physiological responses, water quality parameters and fish-related physiological and biochemical indicators can be used to assess the stress levels in fish.

Marbled rockfish (*Sebastiscus marmoratus*, Cuvier 1829) is a typical reef-dwelling fish species that inhabits warm temperate nearshore benthic waters. Its activity is mainly concentrated in seabed caves, coral reef crevices, and seaweed beds. It is one of the economically important fish species along the Zhejiang coast [16]. However, due to overfishing and habitat loss, its population has been severely decreased, with its resources in an overfished situation. To restore the resources, China has actively promoted the hatchery release of *S. marmoratus*, making it one of the primary species released along the Zhejiang offshore area [17]. Although the hatchery release of *S. marmoratus* has significant ecological and economic potential, the effectiveness of such releases is greatly influenced by the physiological condition and pre-release survival rate of individual fish. Given the potential transport stress that *S. marmoratus* face during hatchery release, it is essential to conduct research focused on optimizing release conditions to improve survival rates and enhance overall release efficiency.

The goal of this study was to investigate the physiological stress levels of *S. marmoratus* and water quality changes under different transport durations and densities in a controlled laboratory setting. We aimed to determine suitable transport conditions for *S. marmoratus* juveniles that would support their physiological responses and survival. To explore the stress response levels of the species under varying degrees of transport stress, changes in water quality and physiological indicators of individual fish were compared under different transport durations and densities in a controlled laboratory setting. The results of this study can be applied to transport method development for this species, and they provide technical support for live transport of other fish species.

2. Materials and Methods

2.1. Sample Collection and Source

The samples of juvenile *S. marmoratus* were artificially bred at the Xi Xuan Fisheries Science and Technology Island Hatchery in Zhoushan, Zhejiang Province. The average body length of the fish used in the experiment was 116.91 ± 4.43 mm, and the average body weight was 48.96 ± 5.34 g. Before the experiment, the fish were transferred to a 500 L recirculating water tank for acclimation over the course of one week. The fish were fed commercial feed ("Yubao") (Sichuan Yubao Ltd., Mianyang, Sichuan, China) at 09:00 and 16:00 daily, with a daily feed amount equivalent to 3% of their body weight. To minimize the impact of feed digestion on fish physiology, feeding was stopped 24 h before the experiment began. The experimental water was sand-filtered seawater with a temperature of 27.4 ± 0.3 °C and a pH of 7.7 ± 0.2. One-third of the tank's water volume was exchanged daily.

2.2. Experimental Design and Indicator Measurement

At the start of the experiment, 5 L of seawater was added to 64 food-grade plastic buckets (25 cm × 25 cm). Following the method of Hu et al. [18], a 4 × 4 two-factor experimental design was used. Four transport density groups were established (60 kg/m^3, 90 kg/m^3, 120 kg/m^3, and 150 kg/m^3). Based on the average body weight of the experimental fish, 6, 9, 12, and 15 fish were placed in the buckets for each density group. The fish were then subjected to oscillation for 2, 4, 6, and 8 h, with four parallel groups for each condition. Based on the findings of Zhou et al. [19] and Zhu et al. [20], as well as actual transport conditions, variable-speed multifunctional shakers (HY-4A, Lichen Technology, Changsha, China) were used for oscillation at a frequency of 80 r/min, chosen to simulate the typical dynamic conditions commonly observed in land transport (e.g., low-speed movement on uneven roads and medium-to-high-speed movement on smooth roads) and water transport (e.g., mild waves). During oscillation, an air stone was used to continuously aerate the water.

A water quality meter (Bante900P, Bante Instruments, Shanghai, China) was used to measure NH_4^+-N content, pH, and dissolved oxygen (DO) content in the seawater before and after oscillation. Fish from the culture tanks before placement in the buckets served as the control group. After oscillation, one fish from each parallel group was randomly selected, along with 10 individuals from the control group, and they were anesthetized with eugenol (40 mg/L). Dorsal muscle and liver tissues were collected and stored at –80 °C for further analysis. All samples were sent to Shanghai Enzyme-linked Biotechnology Co., Ltd. (Shanghai, China), for testing. Lactate content was measured using a microplate method with a lactate assay kit (Catalog No. MLsh0151, Shanghai Enzyme-linked Biotechnology Co., Ltd.) following the manufacturer's protocol. Malondialdehyde (MDA) content was determined using an MDA assay kit (Catalog No. YJ402139), and cortisol level was analyzed using a cortisol ELISA kit (Catalog No. YJ003467). The activities of superoxide dismutase (SOD), alkaline phosphatase (ALP), and glutamate oxaloacetate transaminase (GOT) were measured using their respective assay kits (Catalog Nos. YJ103520, YJ440639, and YJ449051). All assay kits were provided by Shanghai Enzyme-linked Biotechnology Co., Ltd. Absorbance for all ELISA assays was measured at 450 nm using a microplate reader (RT-6100, Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China). Each measurement was performed in triplicate to ensure reliability and accuracy. All tests strictly followed the protocols provided by the kit manufacturer to ensure consistency and reproducibility.

2.3. Statistical Analysis

Experimental data were processed using EXCEL 2021 software and expressed as mean ± standard error (SE). Transport duration and density were treated as independent variables. One-way analysis of variance and Student–Newman–Keuls tests were conducted using SPSS 17.0 (IBM Corp., Armonk, NY, USA), with statistical significance set at $p < 0.05$. Spearman correlation analysis was performed to assess the relationship between the fish sample mortality rates and physiological indicators. Additionally, the maximal information-based nonparametric exploration (MINE) algorithm was applied to analyze correlations between various indicators and the mortality rate, with the maximal information coefficient (MIC) used to measure the strength of these associations. The MINE algorithm was executed through the minerva package in R software version 4.0.0.

3. Results

3.1. Effects of Transport Duration and Density on Water Quality

NH_4^+-N concentration increased across all transport groups with increasing transport duration and density and was significantly higher in the treatment groups than in the control group (no transport) ($p < 0.01$) (Figure 1a). When the transport duration reached 4 h, a significant difference in NH_4^+-N concentration was observed between the 60 kg/m^3 and 150 kg/m^3 density groups ($p = 0.033$), and, at 6 h, the concentrations in the 60 and 90 kg/m^3 groups differed significantly compared to those in the 120 and 150 kg/m^3 groups ($p < 0.05$).

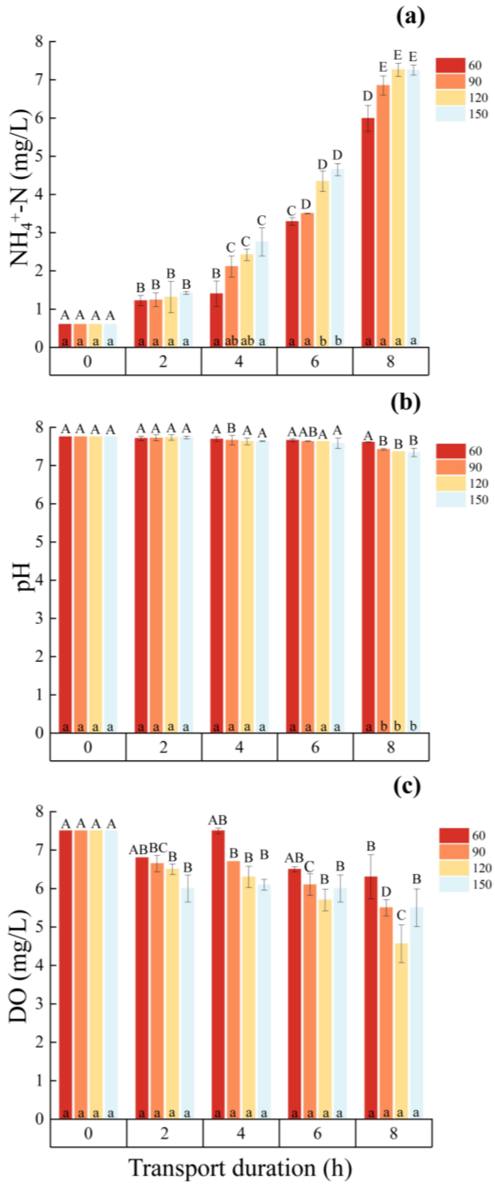

Figure 1. Water quality parameters including (**a**) NH_4^+-N (unit: mg L^{-1}), (**b**) pH, and (**c**) DO (mg L^{-1}) for different density groups (60, 90, 120, and 150 kg m^{-3} represented by the red, reddish brown, light yellow, and light blue bars, respectively) after different transport durations (0, 2, 4, 6, and 8 h). Different uppercase letters indicate significant differences between different transport durations within the same density group ($p < 0.05$). Different lowercase letters indicate significant differences between different density groups at the same transport duration ($p < 0.05$). The same is true for subsequent figures.

All groups showed a downward trend in pH value with increasing density and duration (Figure 1b). Significant differences in pH levels compared to the control group were observed in the 90 kg/m^3 group at 4 h and in the 90, 120, and 150 kg/m^3 groups at 8 h ($p < 0.05$). Additionally, only the 8 h group showed significant differences in pH among density groups at the same duration ($p < 0.05$).

The DO levels in all groups decreased gradually with increasing duration and density (Figure 1c). Except for the 60 kg/m^3 group, the DO levels in all other transport groups were significantly lower than that in the control group ($p < 0.05$). At the same duration, no significant differences were observed among the groups. The lowest DO level was recorded in the 120 kg/m^3 group after 8 h of transport.

3.2. Effects of Transport Duration and Density on Physiological Indicators of S. marmoratus

Figure 2 shows changes in the physiological indicators of the fish samples under different transport durations and densities. As transport duration increased, MDA and cortisol levels in all four density groups were significantly higher than those in the control group ($p < 0.05$) (Figure 2b). Other physiological indicators also showed statistically significant differences under certain conditions ($p < 0.05$) (Figure 2c–f). We found that SOD activity in the 120 kg m^{-3} group was significantly higher than that of the control group across all transport durations ($p < 0.05$) (Figure 2d). ALP activity in the 150 kg m^{-3} group after 8 h of transport was significantly higher than that in the control group ($p = 0.04$) (Figure 2e). Relative to the control group, GOT activity significantly increased in the 120 and 150 kg/m^3 groups after 2 h of transport and in the 90 kg/m^3 group after 6 h of transport ($p < 0.05$) (Figure 2f). However, no significant differences in lactate levels were observed between any transport group and the control group.

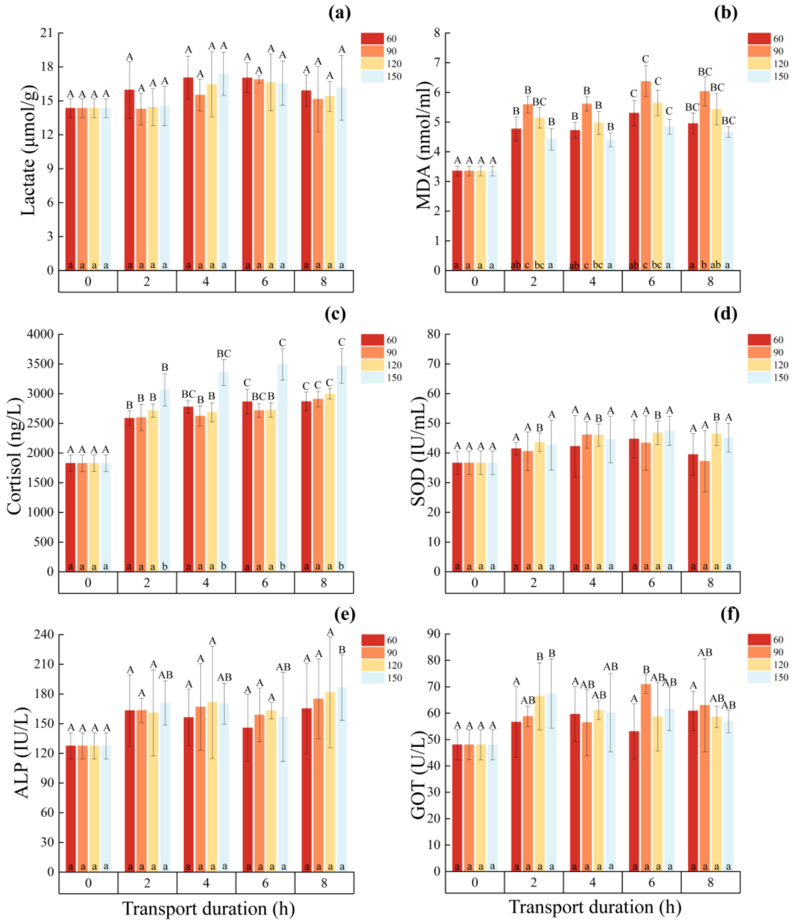

Figure 2. Physiological indicators including (**a**) lactate (μmol g^{-1}), (**b**) MDA (nmol mL^{-1}), (**c**) cortisol (ng L^{-1}), (**d**) SOD (IU mL^{-1}), (**e**) ALP (IU L^{-1}), and (**f**) GOT (U L^{-1}) in each density groups (60, 90, 120, and 150 kg m^{-3} represented by the red, reddish brown, light yellow, and light blue bar) after different transport duration (0, 2, 4, 6, and 8 h).

As transport density increased, significant differences in MDA levels were observed between the density groups ($p < 0.05$) (Figure 2b). Cortisol levels in the 150 kg/m^3 group were significantly higher than those in the other groups ($p < 0.05$) (Figure 2c). However, other physiological indicators did not show significant differences among the density groups, suggesting that density had a relatively smaller impact on these indicators.

3.3. Mortality Rate of *S. marmoratus* and Correlation Analysis with Physiological Indicators

In the 60 kg/m^3 group, the fish sample exhibited a 100% survival rate across all transport durations. In the 90, 120, and 150 kg/m^3 groups, mortality began to occur after 6 h of transport, with mortality rates of 5.60%, 8.30%, and 13.30%, respectively; after 8 h of transport, the mortality rates increased to 38.90%, 70.80%, and 83.30%, respectively (Figure 3).

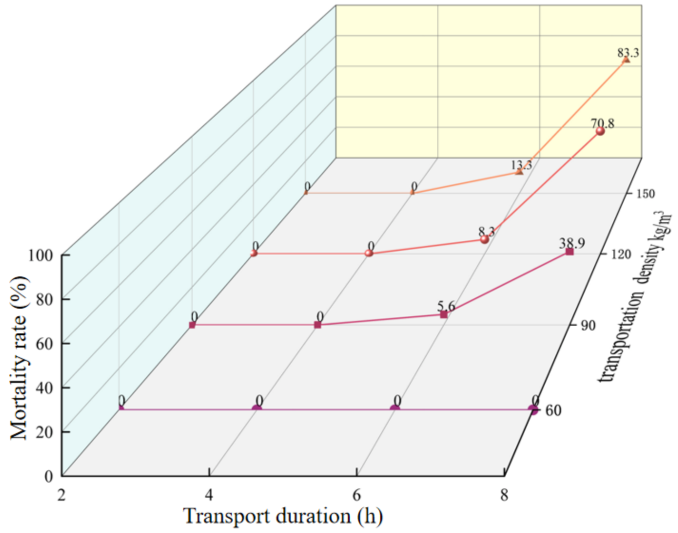

Figure 3. Mortality rates (%) of the samples under different transport durations (2, 4, 6, and 8 h) and densities (0, 60, 90, 120, and 150 kg m^{-3}).

Under different transport durations and densities, the mortality rate of the fish samples showed a significant positive correlation with cortisol levels and a significant negative correlation with MDA level and GOT activity (Figure 4). We used the MINE algorithm to show the correlation (represented by strength of MIC) between the mortality rate of the fish samples versus various physiological indicators and obtained the following results: cortisol (0.709), MDA (0.376), ALP (0.366), GOT (0.344), SOD (0.325), and lactate (0.328). Cortisol exhibited the highest correlation, which identified it as a key factor influencing the mortality rate. Although the MIC values of other physiological indicators were lower than that of cortisol, they still indicated a certain degree of correlation with mortality.

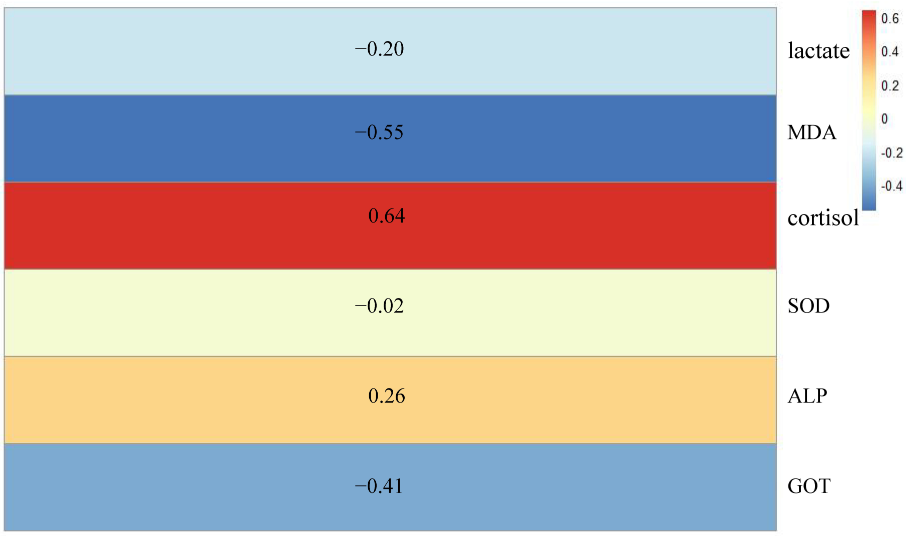

Figure 4. Results of correlation analysis between mortality rate and physiological indicators. The right bar shows the correlation closeness between 0.6 represented by red and −0.4 represented by light blue.

4. Discussion

4.1. Changes in Water Quality

Changes in water quality during transport are a critical abiotic factor contributing to mortality in aquatic animals [21]. The water quality monitoring in this study showed that NH_4^+-N levels increased with transport duration and density, while pH and DO levels decreased significantly. NH_4^+-N is a primary metabolic byproduct during live transportation that is usually diffused into the water through fish gills [22]. However, excessively high concentrations of NH_4^+-N in the water can inhibit the normal ammonia excretion process in fish, thereby influencing their other physiological functions and potentially leading to death [23]. Thus, NH_4^+-N level reflects water quality. The significant increase in NH_4^+-N with higher transport intensities (both duration and density) is in line with those reported for other fish species, including large yellow croaker (*Larimichthys crocea*) [24], goldfish (*Carassius auratus*) [25], topmouth culter (*Culter alburnus*) [18], and Yangtze catfish (*Silurus meridionalis*) [26], and confirms the pattern that fish metabolic burdens increase with transport intensity.

Previous studies have shown that the optimal pH range for *S. marmoratus* survival rate is between 7.0 and 9.0 [27]. In this study, the pH values of the oscillated water ranged from 7.34 to 7.71, which, although within the suitable range, still showed a declining trend under high-intensity transport stress. A decline in pH can have adverse effects, particularly under conditions of prolonged stress. Specifically, nitrifying bacteria in the water convert harmful ammonia into harmless nitrates [28]; however, the growth and reproduction of these bacteria are highly sensitive to water pH levels [29]. Transport-induced increase in metabolic activity results in an accumulation of CO_2, resulting, in turn, in reduced water pH and the inhibition of the activity of nitrifying bacteria [30]. This may explain why NH_4^+-N levels increased significantly during transport.

Sufficient DO is essential for maintaining the survival and health of aquatic organisms. It is generally accepted that seawater DO levels must exceed 5 mg/L to sustain normal life activities in both fish species and nitrifying bacteria [31]. In this study, only the 120 kg/m^3 group after 8 h of transport had DO levels below 5 mg/L, and other groups with mortality had DO levels above 5 mg/L. However, relying on DO levels measured at the end of the oscillation period may not directly correlate with the mortality rate. In the high-intensity transport groups, the higher mortality rates may have reduced the overall oxygen consumption as individual fish died, allowing DO levels to recover. Therefore, the existing measurement methods may underestimate the impact of DO fluctuations on fish mortality and experimental results. To address this, we will continue to monitor the DO levels during transport to enhance the results in future research.

To reduce NH_4^+-N accumulation and DO depletion during transport, certain devices or additives are considered effective. For example, microbubble diffusers can efficiently increase DO level, ensuring stable DO conditions throughout the transport process [32]. Adding pH buffers such as sodium bicarbonate can effectively stabilize water pH, thereby reducing the inhibition of nitrifying bacteria. Additionally, zeolite, as an adsorbent material, can be used to lower NH_4^+-N concentrations in the water [33]. These strategies will be tested in future studies to enhance the effectiveness and practicality of live fish transportation management.

4.2. Changes in Lactate, MDA, and Cortisol Levels

Changes in lactate, MDA, and cortisol levels are typically the secondary physiological responses of fish under stress. Lactate is a key byproduct of anaerobic metabolism, and its levels generally increase when fish are hypoxic or stressed [34]. Although the fish samples in this study were stressed under different transport conditions, the difference of their lactate levels was not significant. This result suggests that *S. marmoratus* possess a strong metabolic compensation ability that allows it to adjust its metabolic pathways to cope with lactate stress to some extent. Li et al. (2023) reported that when oscillation frequency was kept at or below 100 r/min, stress and anaerobic metabolism in barred knifejaw

(*Oplegnathus fasciatus*) remained low; however, once the frequency exceeded this threshold, lactate levels significantly increased [35]. In the current study, the oscillation frequency was set at 80 r/min, indicating that the controlled oscillation frequency may have contributed to preventing a significant increase in lactate levels in the *S. marmoratus* samples.

MDA is a marker of lipid peroxidation in fish and reflects the level of oxidative stress [36]. During transport, MDA levels in the fish samples were significantly higher than that in the control group, indicating that the transport process markedly increased oxidative stress. This stress may generate large amounts of free radicals, trigger lipid peroxidation, accelerate metabolism, and lead to the accumulation of metabolic byproducts [37]. Notably, the MDA levels in the 90 kg/m^3 group were the highest across all durations. This may be related to the competitive effects induced by transport density [38]. Compared to the lower density group (60 kg/m^3) and the higher density groups (120 and 150 kg/m^3), the intermediate transport density of 90 kg/m^3 may have created a more challenging environment. In this scenario, fish may have had neither sufficient space to avoid frequent conflicts, nor been crowded enough for the reduced activity required to lower their stress levels. Consequently, the samples in this group may have experienced oxidative stress earlier, with the cumulative effect of MDA becoming more pronounced over longer transport duration. Furthermore, during extended transport, high-density groups may experience reduced competition pressure as some individuals die, thereby decreasing overall oxygen demand and alleviating some of the stress on the remaining fish.

Cortisol is a critical hormone in fish for responding to stress. In this study, cortisol levels in the *S. marmoratus* juveniles subjected to transport stress were significantly higher than those in the control group, which is consistent with findings from other studies. For instance, after transport stress, cortisol levels in *C. alburnus* increased by 1.9 to 2.2 times [18], cortisol levels in the serum of African catfish (*Clarias gariepinus*) rose by 4 to 5 times [39], and serum cortisol levels in silver pomfret (*Pampus argenteus*) also increased with higher transport densities [40]. In this study, cortisol levels in the 150 kg/m^3 group were significantly higher than those in the other density groups, indicating that high-density transport exacerbated the stress response in *S. marmoratus*.

4.3. Changes in Enzyme Activities of S. marmoratus

The liver is a crucial organ for maintaining health and adapting to environmental changes in fish, particularly when they are under stress [41]. ALP and GOT are commonly used biomarkers in the evaluation of liver function. ALP, a key enzyme involved in liver metabolism, is primarily responsible for the metabolism of phospholipids in liver cell membranes and maintaining membrane stability. Elevated ALP activity is typically associated with liver and biliary system damage. GOT, on the other hand, is a key indicator for assessing liver damage [42]. When liver cells are damaged or destroyed, GOT is released into the bloodstream, resulting in increased activity. In addition to liver function, maintaining a dynamic balance between the antioxidant system and free radicals is essential for the organism's stability, particularly when under stress [43]. SOD is a critical enzyme in the fish antioxidant system that is primarily responsible for scavenging superoxide radicals generated during metabolic processes [18]. Changes in SOD activity reflect the organism's ability to respond to oxidative stress. In this study, the activities of ALP, GOT, and SOD in the transport groups were generally higher than those in the control group, indicating that increased oxidative stress and liver damage occurred in *S. marmoratus* during transport.

However, with prolonged transport duration, certain indicators, such as SOD and GOT activities, began to decrease. The change in SOD activity aligns with findings from other studies in which SOD activity initially increased under mild stress to counteract the accumulation of free radicals but began to decrease under severe stress, indicating that the organism's antioxidant capacity has weakened, likely because oxidative stress has surpassed the body's self-regulatory ability [19,44]. Unlike SOD, GOT activity typically increases with escalating stress. In this study, however, the observed decline in GOT activity suggests that liver function in the experimental samples had already deteriorated severely. This reduction in GOT activity may indicate that liver damage had progressed to the point where liver cells could no longer efficiently release GOT, marking significant liver dysfunction. However, the specific extent of liver damage caused by transport stress remains unclear. Future research will utilize histopathological examinations to provide a more comprehensive assessment.

4.4. Correlation Between Mortality Rate and Physiological Indicators

In this study, the mortality times of the samples were concentrated between 6 and 8 h of transport, particularly in the 90, 120, and 150 kg/m^3 density groups, with the later onset of mortality coinciding with lower transport density. Spearman correlation analysis revealed a significant positive correlation between the mortality rate and cortisol levels. As the transport duration and density increased, the cortisol levels gradually rose, leading to a higher mortality rate. This result aligns with the defense mechanisms observed in *L. crocea* [24] and *C. auratus* [25] under transport density and salinity stress. Additionally, a significant negative correlation was detected between mortality rate and MDA content as well as GOT activity. This finding is consistent with changes observed in adult bigfin reef squid (*Sepioteuthis lessoniana*) during transport [19]. Because MDA reflects oxidative stress and GOT signals liver function, lower levels of these indicators suggest advanced physiological deterioration, correlating with increased mortality.

In recent years, the MINE algorithm has provided a new strategy for analyzing correlations between nonparametric variables. Unlike traditional regression models based on specific functions, the MINE algorithm can detect complex relationships driven by multiple factors. Its primary advantage is the ability to assess the strength of correlations on a standardized scale, allowing for the identification of the strongest variable pairs [45,46]. The MINE algorithm has been widely applied in studies of the relationships between biological and environmental parameters and provides a standardized data-mining solution for analyzing various types of variables [47,48]. In this study, the MINE algorithm identified cortisol as the physiological indicator most strongly correlated with mortality, which was consistent with the results from the Spearman correlation analysis. This result further underscores the importance of cortisol in influencing the mortality rate of *S. marmoratus*. Although cortisol is a stress hormone that helps fish to adapt to stress and enhances their resistance, prolonged elevated cortisol levels can accelerate metabolic rates, reduce disease resistance, and impair tolerance to hypoxia, ultimately leading to a significant decline in survival rates [19,49]. This is why high-density transport conditions are associated with increased fish mortality rates.

Although significant correlations can reveal potential associations, they cannot directly establish causal relationships between physiological indicators and mortality. Moreover, fluctuations in hormone levels and enzyme activities during different stages of transport may be driven by multiple mechanisms. Relying solely on endpoint data for correlation analysis may fail to capture the dynamic processes of physiological changes induced by transport stress in fish. Future research will incorporate time-series data monitoring combined with histopathological examinations to further elucidate the complex mechanisms and dynamic processes of transport stress on fish health.

5. Conclusions

Our results addressed that as duration and density increased, NH_4^+-N levels in the water significantly rose, while DO content and pH levels decreased. The fish also exhibited a series of physiological and biochemical responses to cope with transport stress. Prolonged elevated cortisol levels and liver damage might be the factors contributing to the high mortality rates of *S. marmoratus* juveniles during transport. Based on these findings, a transport duration of >6–8 h is not recommended. If the transport duration must be extended, fish density should be within the range of 60 to 90 kg/m^3.

Author Contributions: J.W. and K.X. contributed to methodology, formal analysis, and writing—original draft preparation. X.C. and H.W. contributed to resources and data curation. K.X. contributed to writing—review and editing, visualization, supervision, and project administration. Z.L. contributed to writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, China) and approved by the Institutional Animal Care and Use Committee of Zhejiang Ocean University (ZJOU-AQU-2022-090).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

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Conflicts of Interest: The authors declare no conflicts of interest.

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