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The Effects of Vibration Frequency on Oxidative Stress, Digestive Enzymes and ATPases of Crimson Snapper (*Lutjanus erythropterus*) Fry during Transport

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Abstract: In this study, we sought to characterize the effect of water vibration frequency stress on crimson snapper (*Lutjanus erythropterus*) survival to determine an optimal transportation speed. To achieve this, we used a transport tank (25 cm × 17 cm × 16 cm) to simulate the transport process. After 8 h at five different vibration frequencies (D1 = 75 rpm, D2 = 105 rpm, D3 = 135 rpm, D4 = 165 rpm, and D5 = 195 rpm), the pH and dissolved oxygen (DO) levels in the tanks decreased; ammonia nitrogen levels (NH_4-N) and temperature (T) increased with increasing density; and significant changes in oxidative stress biomarkers, digestive enzymes, and ATPase levels were observed in crimson snapper fry. The enzyme activity increased and reached the maximum value at 195 rpm. The experimental results suggested that during the actual transport, when using transport tanks, the length of the transport time was less than 8 h, and setting the vibration frequency for transportation at 135 rpm was more appropriate, that is, a speed of 50 km/h for transporting crimson snapper fry.

Keywords: vibration frequency; water quality indicators; immunological enzymes; digestive enzymes; crimson snapper (*Lutjanus erythropterus*)

Key Contribution: The transportation process was simulated using transport boxes, and a thermostatic oscillator was used to simulate the actual transport conditions. Finding the optimal vibration frequency to reduce fry stress levels was essential.

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1. Introduction

Fish are aquatic metazoans that are in direct contact with water and susceptible to factors in the surrounding water environment (temperature, salinity, etc.) [1]. Fish of different species or at different stages of growth and development, or perhaps even fish of the same species and at the same stage of growth, still have very marked differences in individual resistance to the environmental stresses to which they are subjected [2]. Environmental stresses largely impact the survival of fish, impacting their growth and development, and fish show a significant stress response to flashes, noise, vibration, transport, etc., from the larval, juvenile, and later life history stages [3–5]. During transport, hatcheries or fish seedling farms are usually located far away from fishponds, nets, and farms, and it takes a long time, from a few hours to a few days, for them to reach their destinations by car. Moreover, during transport, fish fry will be affected by collision, extrusion, and other external forces, and excessive external force will lead to damage and physiological

disorders of the fish, affecting their quality and even leading to death in serious cases [6–8]. Therefore, fish welfare needs to be improved during transportation to ensure survival rates. For example, it has been found that placing zeolites in the water column effectively removes ammonia nitrogen and that the removal of ammonia nitrogen from the water increases with the number of zeolites [9]. Crimson snapper fry must be kept at the right temperature during transportation; thus, a complete set of water temperature regulation techniques can maintain the normal survival of the fish, reduce the chances of being infected by pathogens, and greatly reduce the mortality rate of the fish. For example, in one study, a chiller was used to reduce the transportation water temperature, and at 12 °C, the stress response of the organism was basically eliminated, the tolerance to environmental stimuli was improved, the survival rate during transportation was increased, and the final survival rate was 98% after 72 h of transportation [10].

Celi Monica found that exposure to simulated ship noise with a hydrophone for 10 d had a significant effect on nine parameters, including adrenocorticotropic hormone (ATH), cortisol, and blood glucose, in sea bream (*Sparus aurata*) [11]. It has also been found through the process of net-pen culture of sea bass that fish will actively increase their swimming speed to get away from the noise source. Studies have shown that fish feel sound and vibration in the water mainly through the inner ear, lateral line organs, and air bladder, where the inner ear is the main sound receptor, and the lateral line is the sensory system through which the fish feel tiny disturbances in the water [12]. Low-frequency vibration affects fish mainly by generating changes in water flow, which is then sensed by the lateral line organs and causes cerebral nerve excitation to produce stress, and the physiological and ecological characteristics of different fish cause them to respond differently to vibration [13,14].

Crimson snapper (*Lutjanus erythropterus*) belongs to the order Perciformes, family Lutjanidae, and genus Lutjanus and is a warm-water, near-bottom fish found in the Indo-West Pacific, the Indian Ocean, and the South China Sea [15]. Crimson snapper is an important marine economic fish, and its meat is delicious and rich in protein and other nutrients [16,17]. Due to its high nutritional value, tasty meat, short breeding cycle, and relatively high survival rate, crimson snapper has become an important aquaculture species along the southern coast of China and in Southeast Asia [18,19].

However, no studies have reported the effects of shaking on crimson snapper fry, and there are no relevant studies that indicate whether vibration or noise is the cause of fish stress during transport. Therefore, we sought to measure the water quality and biochemical and digestive indices of crimson snapper fry under different onboard vibration conditions to reveal the patterns and commonalities from the variations, determine the optimal vibration frequency, and confirm the optimal vehicle speed.

2. Materials and Methods

2.1. Experimental Materials

Crimson snapper fry fish were purchased from Hainan Qingli Aquatic Breeding Co., Ltd., Hainan, China, and temporarily reared at the Tropical Aquatic Research and Development Centre of the South China Sea Fisheries Research Institute of the Chinese Academy of Fisheries Sciences, where simulated transport experiments were conducted.

The fry fish were temporarily reared in 1.5 m × 1.2 m round tanks with 1 m^3 of water, and the density of rearing was 1 kg/m^3. The water quality parameters for the respite were salinity (33.00 ± 0.80), temperature (27.00 ± 0.50) °C, an NH_4-N mass concentration less than 0.01 mg/L, a nitrite mass concentration less than 0.04 mg/L, and a dissolved oxygen (DO) mass concentration greater than 6.50 mg/L.

2.2. Simulation of Transport Experiments

Disease-free, healthy, uniformly sized fry (body length (12.00 ± 0.60) cm/tail, body mass (10 ± 0.50) g/tail) were randomly placed in plastic tanks (25 cm × 17 cm × 16 cm) for live fish transport. Then, 3 L of fully aerated water was added to each bucket; the

experiment was divided into 5 groups of 3 parallels each, which started at 8:00 a.m.; and the transport was simulated until 4:00 p.m. The salinity, dissolved oxygen, ammonia nitrogen, and pH of the water in the water column were measured at the beginning of the experiment to maintain the same conditions, and a thermostatic oscillator was used to simulate the actual transport conditions. The shaker vibration frequency was set to D1 = 75 rpm, D2 = 105 rpm, D3 = 135 rpm, D4 = 165 rpm, and D5 = 195 rpm. The shaker was turned on and shaken for 3 min every 1 h during the experiment to simulate water movement during transport, as well as to increase dissolved oxygen levels. The temperature in the experiment was set at 30 °C, and oxygenation was maintained throughout the period. The behavior of juvenile fish in the box was observed every 2 h during the experiment, and the experiment ended after 8 h.

2.3. Sample Collection and Analysis

According to the actual transport situation for reference, transportation from Ledong City, Hainan Province, to Lingshui County, Hainan Province, took approximately eight hours, with an average speed of 30 km/h; therefore, 75 rpm was set as the speed for the control group, and 105, 135, 165 and 195 rpm were set as the speed for the experimental groups. Immediately after the 8 h transport experiment, the water temperature (T), dissolved oxygen (DO) content, and pH were measured and recorded for each experimental group using a HACH HQ40d (Wuhan Puluosi Technology Co., Ltd., Wuhan, China) portable water quality analyzer for the transported water bodies. The ammonia nitrogen levels (NH_4-N) were determined using an ammonia nitrogen meter. Next, six fish were removed from each transport bucket as whole fish samples. After the fish were anesthetized with an appropriate amount of eugenol, they were dissected on crushed ice, and samples of liver, gill, and intestinal tissues were removed and weighed with a universal balance. Then, 0.86% saline was added 9 times at a ratio of 1:9 (g/mL), and the mixture was milled with a DREAMEL grinder (Hangzhou Allsheng Instruments Co., Ltd., Hangzhou, China) and placed in the EXPERT 18K-R freezing centrifuge (Hunan Gilson Technology Development Co.,Ltd., Changsha, China) at 2500 r/min centrifugation for 10 min. The supernatant was collected and placed at −80 °C for freezing.

Malondialdehyde (MDA) (the TBA method), total superoxide dismutase (SOD) (the WST-1 method), catalase (CAT) (the ammonium molybdate method), total antioxidant capacity (T-AOC) (the ABTS method), lactate dehydrogenase (LDH) (the microplate method), and total protein (TP) (the BCA microplate method); ATPase (Na^+k^+-ATP and $Ca^{2+}Mg^{2+}$-ATP) (the microplate method) in the gills; and lipase (LPS) (the methyl carnitine substrate method) and α-amylase (AMS) (starch–iodine colorimetry) in the intestine and liver were detected and analyzed using the method developed by Nanjing Jianjian Bioengineering Institute, Nanjing, China.

2.4. Data Analysis

Experimental data were obtained using the mean ± standard deviation (mean ± SD), and one-way ANOVA (one-way ANOVA) was performed using SPSS 22.0 (International Business Machines Corporation, New York, NY, USA) to compare the significance of differences in tissue enzyme activity between transport water vibration frequency groups after transport stress using Tukey's method. The results were expressed as the mean earth standard deviation (mean earth SD). The significance level was set at $p < 0.05$.

3. Results

3.1. Changes in the Treatment Groups after Transportation

After 8 h of transport, as shown in Figure 1, the DO content and pH of the water in the transported tanks showed a decreasing trend with the increase in vibration frequency; there was no significant difference ($p > 0.05$) in the DO content and pH between the D2 and control groups, and those of the D3, D4, and D5 groups were significantly ($p < 0.05$) lower than those of the control group. The changes in NH_4-N content and T showed a general

upward trend, with no significant ($p > 0.05$) difference in the NH_4-N content between the D2, D3, and control groups, but it was significantly higher in the D4 and D5 groups. While T in D2 was not significantly different from that of the control group, it was significantly lower than in the D3 and D4 groups. T was significantly higher in the D5 group than in all other groups.

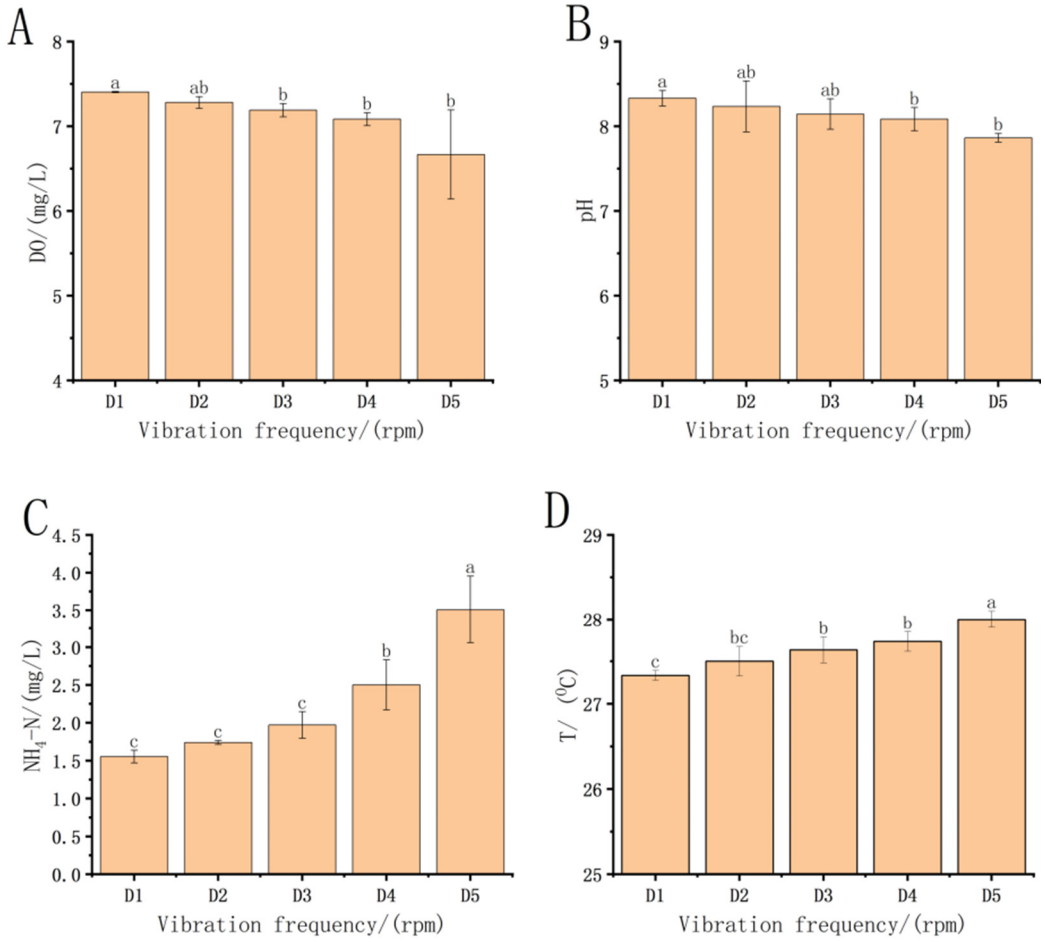

Figure 1. Changes in (**A**) DO, (**B**) pH, (**C**) NH_4-N, and (**D**) T of water quality at five transport frequencies, with different letters above the bars indicating significant differences at the 0.05 level following Tukey's post hoc test.

3.2. Effect of Vibration Frequency Stress on Oxidative Stress Biomarkers in Fish Fry in Each Treatment Group

As shown in Figures 2 and 3, after 8 h of transport, the SOD and T-AOC activity of the transported fry was not significantly ($p > 0.05$) different between the D2, D3, and D4 groups. The CAT activity of the transported fry was also not significantly ($p > 0.05$) different between the D2, D3, and control groups; by contrast, the SOD activity was significantly ($p < 0.05$) lower in the D2, D3, and D4 groups than in the control group. The activity of the three enzymes was significantly ($p < 0.05$) higher in the D5 group than in the other groups, and an overall increase was observed with an increase in the frequency of vibrations.

The LDH activity and MDA and TP contents in the livers of crimson snapper fry tended to increase and then decrease. The LDH activity and MDA contents in the control group were significantly ($p < 0.05$) lower than those in the D2 group, and those in the D5 group were significantly ($p < 0.05$) higher than those in the other groups, but the MDA content was significantly ($p < 0.05$) higher in the D2 group, and the TP content in the D5 group was significantly ($p < 0.05$) lower than those in the other groups.

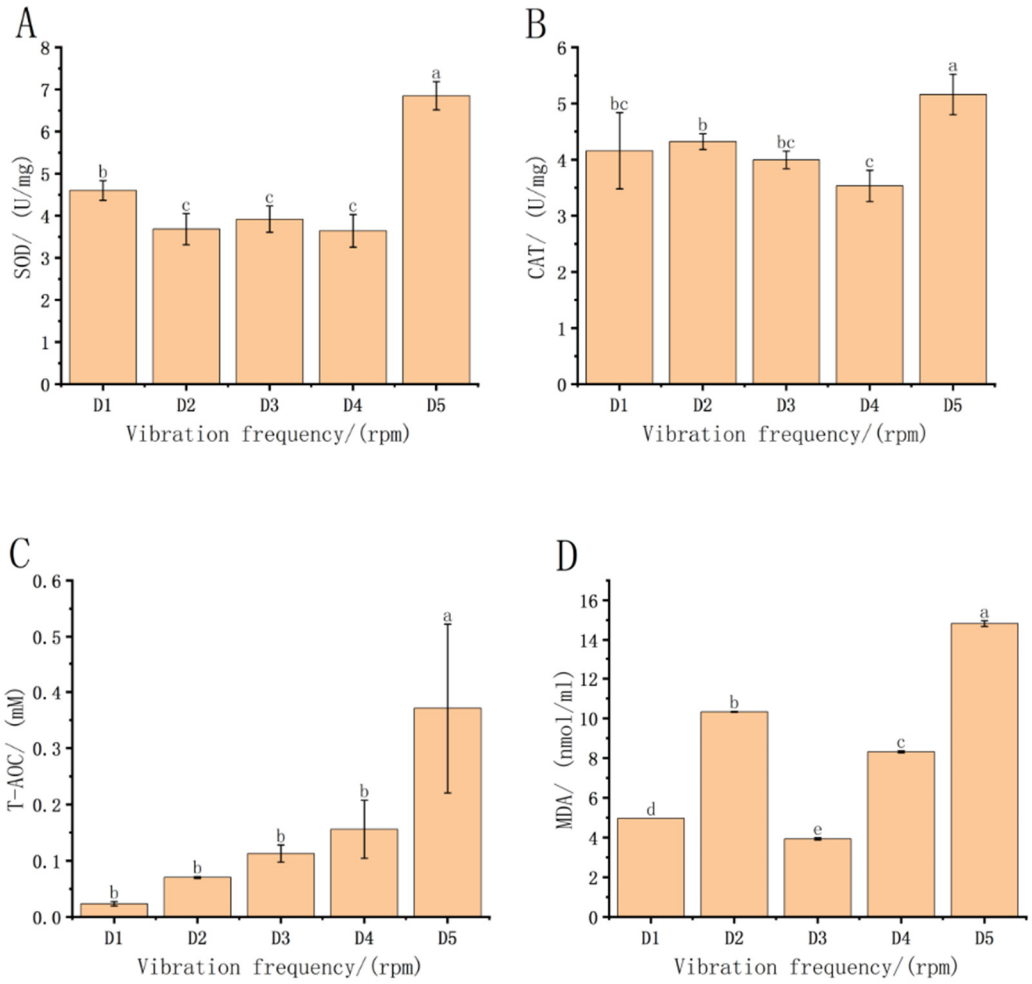

Figure 2. Relative expression levels of (**A**) SOD, (**B**) CAT, (**C**) T-AOC, and (**D**) MDA in the liver at five transport frequencies. Different letters above the bars indicate significant differences at the 0.05 level following Tukey's post hoc test.

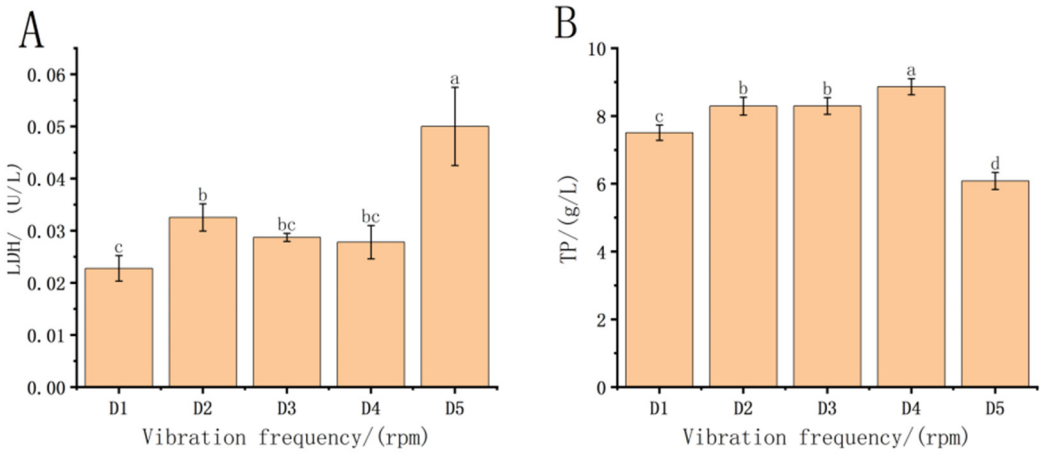

Figure 3. Relative expression levels of (**A**) LDH and (**B**) TP in the liver at five transport frequencies. Different letters above the bars indicate significant differences at the 0.05 level following Tukey's post hoc test.

3.3. Effect of Vibration Frequency Stress on Digestive Enzymes in Fish Fry

According to Figure 4, the AMS activity of D2, D3, and D5 was significantly ($p < 0.05$) higher than that of the control group, with that of the D3 group being significantly ($p < 0.05$) higher than that of the other groups and that of the D4 group being significantly ($p < 0.05$) lower in activity than that of the control group; however, the difference in the LPS activity between each group was not significant ($p > 0.05$).

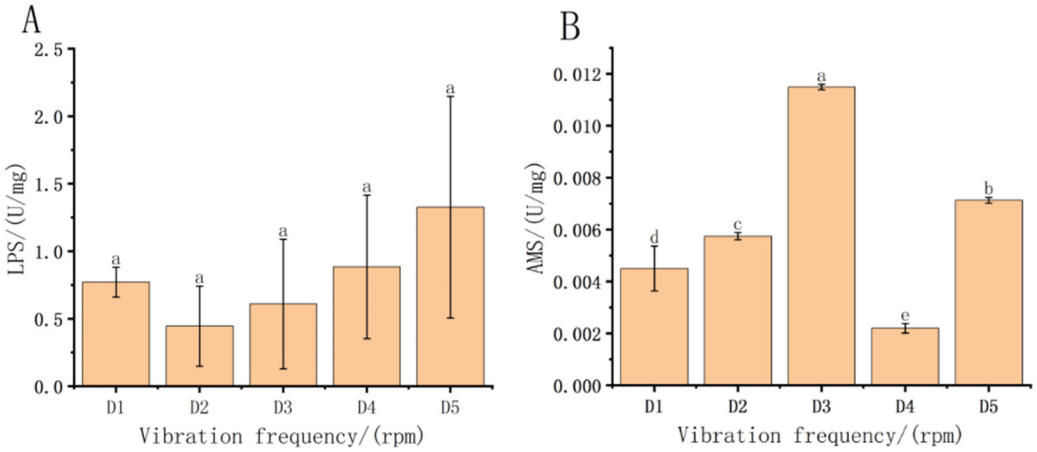

Figure 4. Relative expression levels of (**A**) LPS and (**B**) AMS in the gut at five transport frequencies. Different letters above the bars indicate significant differences at the 0.05 level following Tukey's post hoc test.

3.4. Effect of Vibration Frequency Stress on ATPase in Fish Fry

The Na^+k^+-ATPase and $Ca^{2+}Mg^{2+}$-ATPase activities of the fry after 8 h of transport tanks are shown in Figure 5, with an overall gradual decreasing trend. The Na^+k^+-ATPase activities of groups D2 and D3 were significantly ($p < 0.05$) higher than those of the control group, while the $Ca^{2+}Mg^{2+}$-ATPase activities of groups D3, D4, and D5 were significantly ($p < 0.05$) lower than those of the control group, and the enzyme activity of group D5 was significantly ($p < 0.05$) lower than that of all the other groups.

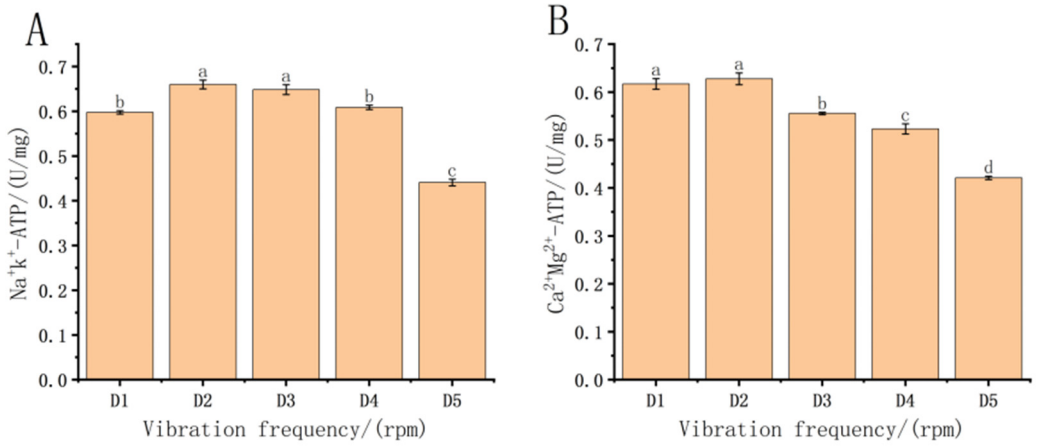

Figure 5. Relative expression levels of (**A**) Na^+k^+-ATPase and (**B**) $Ca^{2+}Mg^{2+}$-ATPase in the gut at five transport frequencies. Different letters above the bars indicate significant differences at the 0.05 level following Tukey's post hoc test.

4. Discussion

During transport, the transport vehicle will usually maintain a certain speed while traveling. When the road conditions change to cause the vehicle to take emergency braking measures, the movement of the water tank will produce a strong back-and-forth vibration or road bumps will cause the vehicle to swing left and right, which will also cause the

water to oscillate from left and right [20,21]. Fish in the water will be frightened by the fluctuation of the water, swim around, and even rush against the sidewall of the tank, causing damage to the body or even death [22]. Although this cannot be avoided, the effects can be minimized only by studying vibrational stress in crimson snapper fry. In this study, we provide a more suitable transport method to alleviate fish stress reactions and increase the survival rates of fish; thus, our study has high research value and economic benefits. The results of this study will also be very important to help ensure the soundness and perfection of the aquatic product circulation system to effectively meet the needs of most consumers and to promote the healthy development of aquaculture in terms of breeding and releasing products [23].

4.1. Impact of Transport on Water Quality in Each Treatment Group

Water is not only the main medium of gas exchange and ion exchange for fish but also the purifier of fish metabolites. Good water quality is necessary for the survival of healthy fish but also affects the efficiency and key factors of the fish transport survival rate [24]. Different species require different suitable water conditions for transport, and attention should be given to the dissolved oxygen, pH, and ammonia–nitrogen substances in the water body. Fish use water as a medium to consume oxygen to carry out metabolism, and it is necessary to ensure that there is not a lack of oxygen or too much oxygen [25].

Dissolved oxygen in the water body is an important environmental factor for the growth of fish, especially during the long-distance transport process. When the water supply of oxygen is insufficient, it will cause hypoxic stress in tissues, affecting normal physiological functions and biochemical processes [26]. These include changes in tissue morphology and structure, oxygen consumption rate, respiratory metabolism, individual behavior, and immune response, which affect the normal functioning of collective systems [27,28]. Ze-feng found that hypoxic stress can have a very significant effect on the physiological metabolism of the hybrid grouper [29] (*Epinephelus fuscoguttatus*♀× *Epinephelus lanceolatus*♂), with severe damage to the liver and gills. The dissolved oxygen content in the water decreased with increasing vibration frequency, the water body needed to be kept in the oxygen-saturated state during the transport of crimson snapper fry, and the oxygen saturation value of the water was 7.82 mg/L. In this experiment, the dissolved oxygen content of the control group and the D2 group was significantly higher than that of the other groups, and it showed a continuous decreasing trend. The dissolved oxygen content in the D5 group was at its lowest value, and although there was no significant difference compared to the D3 and D4 groups, it was not suitable for fish fry transportation.

During transport, fry fish will produce more carbon dioxide (CO_2) and ammonia nitrogen due to stress, and CO_2 will lead to an abnormal pH value in the water; an abnormal pH value and excessive ammonia nitrogen content in the water will have more effects on the physiological condition and disease resistance of the fry [29,30]. Ammonia nitrogen will damage gill epidermal mucous cells, intestinal mucous cells, and the neuroendocrine system, which will in turn damage the function of the liver and kidneys and lead to death [31,32]. After the fish undergoes metabolism, the CO_2 produced will react with the water to form weak acids; the acidification of the water body will corrode the gills of the fish, leading to respiratory and metabolic disorders of the organism; and the increased acidity in the blood will lead to the acidosis of the organism. Goss found that electrolytes were disrupted under acidic conditions and that sodium levels in fish were much lower than normal, while the oxygen-carrying capacity of erythrocytes, as well as the immunological capacity, phagocytosis, and activity of SOD in liver tissue, were also affected [33]. Ammonia nitrogen levels and pH in this experiment decreased with increasing frequency, but all were within the normal range; however, the ammonia nitrogen level of the high-frequency group was significantly higher than that of the control group, and the D5 group recovered poorly from the experiment. These results indicate that there is a clear trend of ammonia nitrogen increase and pH decrease is obvious, and if the transportation time is extended, it will significantly impact the fish fry.

The results of this study showed that in the present experimental situation, the transport vibration frequency had a significant effect on pH, DO, and ammonia nitrogen content, but they were all within the adaptive range of the crimson snapper fry; however, it had a small effect on the water temperature, so the vibration frequency of the water body should be kept at approximately 135 rpm during transport.

4.2. Effect of Transport on Oxidative Stress Biomarkers in Each Treatment Group

Fish produce reactive oxygen species (ROS) during metabolism, and in a suitable environment, they can normally remove ROS without causing damage to the organism. However, fish produce excessive ROS under adverse conditions such as high temperature, low oxygen, and high-frequency shaking. If the body accumulates too much ROS and is unable to remove it, it will cause oxidative stress to the organism, leading to cell damage and causing adverse reactions such as lipid peroxidation [34,35].

SOD and CAT are important components of the body's antioxidant system, and T-AOC is a comprehensive indicator of the functional status of the body's antioxidant system. Among them, SOD is the most sensitive substance to oxidative stress in living organisms and can control the lipid peroxidation of cell membranes and protect the organism. When an organism generates ROS, SOD catalyzes the conversion of superoxide (O_2^-) radicals into oxygen (O_2) or hydrogen peroxide (H_2O_2) [36,37]. CAT further catalyzes the production of H_2O_2 to H_2O and O_2, which in turn protects the organism [38]. The T-AOC includes the body's antioxidant enzyme system and nonenzymatic system, which respond to the compensatory capacity of external stimuli and the state of the body's free radical metabolism, and its main role is still to scavenge the ROS produced during metabolism [39,40]. In this experiment, both SOD and CAT enzyme activities were the highest in the high-frequency group, indicating that the body has a strong response to high-frequency shaking stress, and the body secretes more SOD and CAT enzymes to scavenge ROS to ensure that the body is protected from antioxidant damage. The vigor of group D1 was significantly higher than that of groups D2–D4, indicating that the vibration frequency of 75 rpm did not have a greater effect on the fry and that they did not need to regulate the vigor of the SOD enzyme to eliminate the effect of ROS on the body. The T-AOC enzyme activity of the yellow tail (*Seriola aureovittata*) liver tended to decrease and then increase after hypoxic stress, suggesting that the T-AOC increases during stress to protect the organism from oxidative damage [41]. This experiment still showed that only the high-frequency group had significantly higher activities than the other groups, suggesting that high-frequency shaking indeed produces a strong stress response in crimson snapper fry, generating more T-AOC to protect the organism, which is consistent with the trend of changes in the enzyme activities of SOD and CAT.

LDH plays a role in oxidizing intracellular catalytic lactate to pyruvate in fish and is also involved in cellular metabolism, where an increase in enzyme activity indirectly reflects an increase in the proportion of metabolizable energy and where an elevated LDH indicates damage to the cellular structure of tissues such as the liver, kidneys, and muscles in fish [42,43]. When oxidative free radicals react with antioxidant lipids such as polyunsaturated fatty acids, lipid peroxidation is triggered, and lipids are ultimately degraded to produce MDA, which reacts with a wide range of amino acids in the organism, thereby damaging the structure of the proteins and differentially damaging mitochondrial respiration and related dehydrogenases, leading to cellular damage [44,45]. LDH increased with time in GIFT Nile tilapia juveniles (*Oreochromis niloticus*) [46] under high-temperature stress, while MDA markedly increased in juvenile yellow drum (*Nibea albiflora*) [47] under low-salt stress, and the body had difficulty clearing it slowly. In this experimental study, the LDH activity and MDA content increased with increasing vibration frequency, and they were significantly higher in the high-frequency group than in the control group, indicating that the crimson snapper fry seedlings were severely exposed to external stimuli, the antioxidant system was unable to scavenge ROS in time, and the liver had already been damaged due to shaking stress. However, the vigor of the D2 group was significantly

higher than that of D3, D4, and the control group, probably due to the violent shaking up and down at 105 rpm vibration, which may also cause stress in the fish. The vigor in the D3 group was significantly lower than that of the D1 and D2 groups, indicating that neither side-to-side nor up-and-down swaying occurred under the influence of the vibration frequency of the water body at 105 rpm and that the water body was kept in a relatively smooth state; thus, ROS in the fish were removed in time.

TP is the main substance that provides energy to the organism, and it can also play a role in maintaining the balance of plasma colloid osmotic pressure and pH in fish and has coagulation, immunity transport, and energy supply functions. When subjected to external stimuli, the content of TP is significantly altered [48,49]. Half of the tongue sole (*Cynoglossus semilaevis*) suffered liver damage after 0–12 h of acute stress, with a decrease in the protein content and an increase in the concentration of TP, which can be used as an indicator of liver damage in fish [50]. In this experimental study, the TP content in the 75 rpm–165 rpm group increased, indicating that the fish had been subjected to external stimuli and thus produced a stress response, and the organism needed a large amount of energy; however, the TP content of the high-frequency group was significantly lower than that of the other groups, suggesting that the organism suffered damage to the liver, resulting in a decrease in the protein content.

4.3. Effect of Transport on Digestive Enzymes in Each Treatment Group

The main role of LPS and AMS is to participate in the absorption and digestion of nutrients, which can reflect the basic physiological characteristics of organisms, with amylase converting starch into monosaccharides and lipase breaking down fats in the organism into fatty acids and glycerol acids to provide energy [51,52]. When the external environment changes, it leads to changes in the digestive enzymes of the fish organism, which in turn affects feeding, growth, and health. Liang found that high-temperature stress affects the digestive enzymes of black sea bream (*Sparus macrcephalus*) and that within a moderate temperature range, the activity of digestive enzymes increases with temperature; beyond the moderate range, the digestive enzyme activity slows with temperature [53,54].

In this experimental study, it was found that the D3 group had significantly higher amylase activity than the control group, and it showed a gradual increase, indicating that the amylase activity was not much affected in the low-frequency group, whereas the decline in the amylase activity in the D4 and D5 groups was due to the insufficient amount of starch in the body, which led to a decrease in the vigor of the enzyme; therefore, the organism needed other substances to provide nutrients. However, although there was a tendency for lipase activity to rise in all five groups, there was no significant difference, indicating that the organism began to consume fat after consuming amylase but did not consume too much fat or cause a large secretion of lipase. However, if the transportation time was prolonged or the fish were subjected to greater stimulation, the lipase activity would be significantly affected.

4.4. Effect of Post-Transport Treatment Groups on ATPase

The main site of respiration, ammonia excretion, and osmotic pressure regulation in fish is the gills, which play an important role in osmoregulation [55–57]. The Na^+k^+-ATPase is an intrinsic protein that spans the plasma membrane and catalyzes the hydrolysis of the terminal phosphate of ATP, a reaction that generates energy and is used by the body to counteract the electrochemical gradient of ions and to achieve the active transport of Na^+ and k^+, which is central to the ionic regulation of the whole organism [26,58]. $Ca^{2+}Mg^{2+}$-ATPase affects muscle contraction, nerve cell action potential conduction, cell secretion, and reproduction. The gill filament Na^+k^+-ATPase activity of black carp (*Mylopharyngodon piceus*) under ammonia stress tended to increase and then decrease, and the active osmoregulation mechanism of the gill was activated in the early stage of stress and then decreased in the late stage due to the gill tissues being damaged by ammonia stress [59].

In this experimental study, it was found that both Na^+k^+-ATPase and $Ca^{2+}Mg^{2+}$-ATPase in the gills of crimson snapper fry showed a gradual decrease, and both were significantly lower in the high-frequency group than in all other groups. The Na^+k^+-ATPase activity of group D1 was significantly lower than that of groups D2 and D3, suggesting that 75 rpm did not induce a stress response in the fry during transportation. This indicates that Na^+k^+-ATPase and $Ca^{2+}Mg^{2+}$-ATPase are activated in the gills and start active osmoregulation when subjected to vibratory frequency stress. The osmoregulation mechanism is initiated faster with an increase in time and frequency, and consequently, gill tissues incur damage, gill respiratory epithelial cells become detached, and gill filaments become congested, leading to a decrease in enzyme activity.

5. Conclusions

Vibration frequency stress affects immune enzymes, digestive enzymes, and ATP enzymes in fish. With the increase in vibration frequency, fry fish produce a large amount of ROS to destroy the cells in a short period. Thus, the load on the antioxidant enzyme system and metabolic system of the body increases, which depletes the body's nutrients and leads to a decrease in seedling health, which affects their survival rate. The mucus and feces secreted during stress affect the quality of the transport water. Combined with the actual situation analysis, for the land transport of crimson snapper fry (body length of 12.00 ± 0.60 cm/tail, body mass of 10 ± 0.50 g/tail), it is recommended that the transport time be kept within 8 h, and the vibration frequency of the transport be kept at approximately 135 rpm, which is equivalent to 50 km/h speed; the minimum should not be lower than 105 rpm, which is equivalent to 40 km/h speed [56]. On this basis, further research can be carried out at the road level, that is, according to different highway grades and transport paths, and a relevant model for the transport of live and fresh aquatic products can be established to quickly provide the appropriate level of dissolved oxygen and transport routes, reduce transport costs, and increase the survival rate [20].

Author Contributions: C.Q. conceived the study and participated in its design. J.L. and X.Z. conducted field experiments. J.L. drafted the manuscript. G.Y., X.W. and Z.M. provided scientific advice. Y.G. and S.Z. sorted the data. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study was reviewed and approved by the Ethical Committee for Animal Experiments of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, China (approval code nhdf2023-09).

Data Availability Statement: All data are available from the corresponding author upon reasonable request.

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