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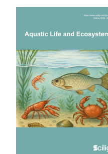

Article

Effects of Long-Term and Low-Concentration Exposure to Hydrogen Peroxide on Growth, Survival of Larval and Juvenile *Larimichthys crocea*

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Abstract: High mortality during the early developmental stages of the large yellow croaker (*Larimichthys crocea*) poses a significant challenge to aquaculture. To assess the effects of low-concentration hydrogen peroxide exposure on larval and juvenile survival and growth, 13-day-old fry were exposed to six nominal concentrations of hydrogen peroxide (0.0, 0.5, 1.0, 2.5, 5.0, and 10.0 μL/L) for 50 days under controlled hatchery conditions, with daily monitoring of seawater quality parameters. Results indicated no significant differences in growth performance or survival between the 0.5 μL/L treatment and the control group. In contrast, juveniles exposed to 1.0, 2.5, and 5.0 μL/L hydrogen peroxide exhibited significantly higher survival rates than the control. Although the survival rate of juveniles in the 10.0 μL/L group was significantly elevated compared to the control group, all measured growth indices—including average body length, body weight, specific growth rate (SGR), and weight gain rate (WGR)—were significantly suppressed. Regarding water quality, ammonia nitrogen concentrations were significantly reduced during the first 7 days in the 5.0 and 10.0 μL/L groups, while nitrite concentrations remained significantly lower from day 13 onward compared to the control. Throughout the experiment, culturable *Vibrio* counts in hatchery tank water exhibited pronounced temporal fluctuations, with no consistent suppression trend attributable to hydrogen peroxide exposure. Collectively, these findings suggest that hydrogen peroxide at concentrations of 1.0–5.0 μL/L enhances juvenile survival without compromising growth performance.

Keywords: *Larimichthys crocea*; hydrogen peroxide; water quality; growth; larvae

1. Introduction

The large yellow croaker (*Larimichthys crocea*) is a commercially important mariculture fish species in China. In 2024, its aquaculture production reached 292,615 metric tons [1], ranking the highest among all mariculture fish species in the country. With the continuous expansion of large yellow croaker farming, the demand for high-quality seedlings has been on the rise. Currently, the seedlings used for aquaculture are mainly obtained through controlled artificial propagation [2], and he number of artificially propagated large yellow croaker fry exceeded 2.7 billion in 2024 [1]. However, marine fish larvae commonly suffer from high mortality during the transition from the larval to the juvenile stage [3,4]. In large yellow croaker, particularly high mortality rates are observed during three critical early developmental phases: (i) the early larval stage, immediately following yolk-sac absorption and the onset of exogenous feeding; (ii) the late larval stage, coinciding with the dietary shift from

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live prey (e.g., copepods) to formulated feed; and (iii) the early juvenile stage [5,6]. Therefore, enhancing survival during the larval and early juvenile stages is essential not only for improving the efficiency of artificial propagation but also for sustaining the economic viability of the industry.

In addition to egg quality and diets, water quality is a crucial factor affecting survival of larval and juvenile fish [4,7]. In natural aquatic environments, concentrations of ammonia and nitrite are typically maintained at low levels. However, in high-density aquaculture systems, water is prone to accumulate substantial amounts of ammonia and nitrite derived from residual feed and fish excretion, including feces [8,9]. Ammonia serves as the primary nitrogenous waste excreted by teleost fish, invertebrates used as live feed, and heterotrophic bacteria metabolizing nitrogenous substrates [10]. Excessive accumulation of ammonia can exert adverse effects on fish, such as growth inhibition, disruption of energy metabolism, and compromised immune function [9]. For instance, long term ammonia exposure to 32 μg/L NH_3-N was found to reduce the growth rate of Atlantic salmon (*Salmo salar* L.) parr [11]. Studies have reported that high concentration of ammonia significantly decrease the hatch rate of obscure puffer (*Takifugu obscurus*) eggs and lowered the larval survival [12]. As an intermediate product of nitrification, nitrite converts hemoglobin into methemoglobin, rendering it incapable of oxygen transport. This process leads to tissue anoxia in fish [13]. It was observed that the growth rate of juvenile channel catfish (*Ictalurus punctatus*) was reduced at NO_2^--N levels of 1.60 mg/L and above, and mortality was increased significantly at concentrations of 3.71 mg/L and above [14]. Both ammonia and nitrite can induce tissue lesions in fish, including epithelial cell hyperplasia, hypertrophy of chloride cells, and lamellar lifting [15]. Additionally, *Vibrio* species represent another significant cause of fingerling mortality. Vibriosis is one of the most common diseases leading to mass mortality in cultured fish, with juveniles being at the greatest risk [16]. Vibriosis outbreaks often linked to physico-chemical changes in seawater [17]. Vibriosis threatens marine hatcheries globally, causing significant economic losses [18]. Therefore, it is crucial to purify and disinfect seawater during fish culture to enhance juvenile survival rate.

To mitigate bacterial loads and suppress blooms of potentially pathogenic microorganisms in water supplies, traditional seawater disinfection strategies primarily encompass physical sterilization and chemical disinfection, including filtration, heat, UV irradiation, ozone, and antibiotics [19]. Sand filtration is the most commonly used and economical treatment method; however, its efficacy in removing bacteria and nitrogenous waste diminishes over time [20,21]. Ozone has also been widely used in a range of aquaculture applications for disinfection and water quality improvement, such as in recirculating aquaculture systems (RAS) to reduce fish diseases. Nevertheless, to prevent harm to juvenile fish, ozone residual levels must be maintained at extremely low concentrations (<0.01 mg/L) [22], which limits its application in fish hatcheries. To further improve water quality, many hatcheries and farms employ chemical disinfectants, including formaldehyde, glutaraldehyde ($C_5H_8O_2$), hydrogen peroxide (H_2O_2), chlorine dioxide (ClO_2), and copper sulphate ($CuSO_4$) [23]. Due to its strong oxidizing capacity, hydrogen peroxide has proven highly effective in treating fish protozoa and fungal infections [24,25]. Compared with other disinfectants, hydrogen peroxide is less harmful and does not generate harmful by-products during decomposition [26]. Hydrogen peroxide has been approved by the US Food and Drug Administration (FDA) for use in farmed fish and is classified as a low regulatory priority for controlling fungus on fish and fish eggs [27]. Previous studies on hydrogen peroxide have predominantly focused on the efficacy of short-term, high-concentration immersion treatments against pathogens and parasites residing on the skin or gills of cultured fish [27–29]. Exposure to high concentrations of hydrogen peroxide can induce mortality in fish. For instance, exposure to 500 μL/L hydrogen peroxide for 15 min led to a fish mortality rate of 11.1%. The tolerance threshold of walleye (*Stizostedion vitreum*) to hydrogen peroxide is as low as 100 μL/L [30,31]. In contrast, relatively few investigations have assessed the impacts of long-term hydrogen peroxide exposure on the growth performance and survival of cultured fish.

In this study, 13-day-old large yellow croaker fry were exposed to seawater supplemented with graded concentrations of hydrogen peroxide for a period of 50 days. To evaluate the effects of hydrogen peroxide on the development of larval and juvenile large yellow croaker and to determine the optimal treatment concentration, ammonia nitrogen, and nitrite levels as well as *Vibrio* counts were monitored, and the growth and survival rates of the juvenile fish were measured.

2. Materials and Methods

2.1. Experiment Animals

The experiment was conducted at the hatchery of Ningde Fufa Fisheries Co., Ltd. (Ningde, China). Fertilized eggs of the large yellow croaker were collected from spawning tanks using an 80-mesh plankton net to remove debris. The eggs were subsequently transferred into a rectangular concrete incubation tank (5 m × 3 m × 1.5 m; total volume:

22.5 m^3). Incubation was conducted under controlled environmental conditions: water temperature was maintained at 25 ± 0.5 °C, salinity at 30 ± 1 ppt, and pH at 8.0 ± 0.1. Continuous aeration was provided throughout the incubation to ensure adequate dissolved oxygen (>6 mg/L) and gentle water circulation, which prevented egg sedimentation while minimizing mechanical stress. To avoid physical damage to the developing embryos, no water exchange was performed during the embryonic stage. A photoperiod of 14 h light and 10 h dark was applied to simulate natural conditions and facilitate visual feeding development. Larval rearing followed the feeding protocol described by Cai et al. [32]. Larvae were fed rotifers (*Brachionus plicatilis*; 0.5–1.5 × 10^4 ind/L) from 3 to 8 days after hatching (DAH), *Artemia nauplii* (1.0–1.5 × 10^4 ind/L) from 6 to 11 DAH, and live copepods starting from 10 DAH. At 13 DAH, upon reaching the juvenile stage and exhibiting active swimming and feeding behaviors, healthy individuals of similar sizes were randomly selected for subsequent experimental procedures. The selected fry had an average body length of 0.439 ± 0.079 cm and an average body weight of 0.009 ± 0.001 g.

2.2. Experiment Design

An 8% (*v*/*v*) hydrogen peroxide solution was obtained from Fujian Xingdechuang Biotechnology Co., Ltd. The experiment was conducted in 0.6 m^3 tanks. For ease of use, hydrogen peroxide was administered based on specific volume ratios relative to the water volume of each tank. Six experimental groups were established, including a control group (T0, no hydrogen peroxide added) and five treatment groups with concentrations of 0.5 (T1), 1.0 (T2), 2.5 (T3), 5.0 (T4), and 10.0 (T5) µL/L. Each group was performed in triplicate. Prior to the experiment, all tanks were disinfected with 50.0 µL/L calcium hypochlorite for 24 h. The *L. crocea* fry were collected from hatchery ponds and placed into a 100 L plastic bucket. Water was aerated and slightly stirred. The density of fry in the bucket was calculated by weighing the total mass in the bucket, after which the fry were evenly distributed into 18 portions and transferred into 18 experimental tanks. To minimize potential confounding factors such as crowding stress and intraspecific competition, the rearing density in the present study was lower than that in commercial hatcheries, and each tank had approximately 750 fry. After a 24-h acclimatization period, the designated concentrations of hydrogen peroxide were introduced to each tank. The fry were fed four times daily (08:00, 11:00, 15:00, and 19:00). For the first 10 days, copepods were provided at a density of 0.2–1 ind./mL. From days 11 to 13, the feeding regime was adjusted to three daily rations of copepods and one ration of commercial compound feed pellets. Starting from day 14, fry were fed exclusively with compound feed pellets at a dosage of 5 g per tank, 4 times daily. Floating feed was adopted throughout the experiment. The amount of feed was adjusted according to the food intake of the fry. The uneaten feed floating on the water surface was collected one hour after feeding. Seventy percent of the water in each tank was replaced daily. The hydrogen peroxide concentration was replenished accordingly after each water change. The actual concentration of hydrogen peroxide in each tank was determined using the cerium sulphate spectrophotometric method [33].

2.3. Growth Performance

During the experiment, 10 fry were randomly selected from each tank for body length and weight measurements each time. Body weight (g) was measured using an electronic balance. Standard body length (SL, cm) was measured using a caliper. The experiment lasted for 50 days. Before the last sampling, the fish were fasted for 24 h.

The total weight of fry in each tank was measured. The final number of live fish was counted and survival rate was calculated. Thirty fish were randomly selected from each tank for individual body weight and body length measurements. The fish were dissected on ice and the hepatopancreas was weighed.

$$\text{Survival rate (\%)} = 100 \times (\text{final fish number}/\text{initial fish number})$$

$$\text{Feed conversion ratio (FCR)} = \text{feed consumed}/\text{wet weight gain}$$

$$\text{Condition factor (CF, \%)} = 100 \times \text{body weight}/\text{body length}^3$$

$$\text{Rate of weight gain (RWG, \%)} = 100 \times (\text{final weight} - \text{initial weight})/\text{initial weight}$$

$$\text{Specific growth rate (SGR, \%)} = (\ln(\text{final weight}) - \ln(\text{initial weight})) \times 100/t$$, of which t is the days of experiment

$$\text{Hepatosomatic index (HPI, \%)} = 100 \times \text{hepatopancreas weight}/\text{body weight}$$

2.4. Water Quality Determination

Throughout the experimental, salinity in the experimental tanks fluctuated within a range of 28.8–31.6 ppt, while pH was maintained between 7.5 and 8.0. The average water temperature ranged from 21.3 °C to 26.1 °C. To monitor nitrogenous waste accumulation, total ammonia nitrogen (NH_4^+-N) and nitrite (NO_2^--N) concentrations were measured every 4 days. Approximately 50 mL of water was collected from each tank prior to the daily 70% water exchange. Samples were immediately filtered through 0.45 μm glass fiber filters and analyzed within 2 h to prevent chemical alterations. Ammonia concentrations were determined using the hypobromite oxidation method [34], which involves the oxidation of ammonia to nitrogen gas in an alkaline medium, with detection limits reaching 0.01 mg/L. Nitrite levels were measured using the diazo-azo photometric method [35], which forms a reddish-purple azo dye measurable at 543 nm, with a sensitivity of 0.005 mg/L. All spectrophotometric measurements were performed using a 752G spectrophotometer (INESA, Shanghai, China).

The total abundance of *Vibrio* spp. in each experimental tank was measured every 3 days. Water samples were collected prior to the daily 70% water change. Preliminary tests were conducted to determine the optimal dilution factor, aiming for a target range of 30–300 colonies per plate. A 100 µL aliquot of the diluted sample was spread onto Thiosulfate-Citrate-Bile salts-Sucrose (TCBS) agar plates using a sterile inoculation loop. The plate was incubated at 30 °C for 24 h in a constant temperature incubator. Based on the chromogenic properties of TCBS medium, *Vibrio* colonies were categorized into two major types: yellow and green. The total number of *Vibrio* spp. in the water samples was calculated based on the colony counts [36].

2.5. Statistical Analysis

All data are presented as mean ± standard deviation (SD). Statistical analyses were performed using SPSS 20.0 statistical software (IBM Corp., Armonk, NY, USA). Percentage data were arcsine-transformed prior to analysis to meet the assumption of homogeneity of variance. Differences among groups were assessed using one-way analysis of variance (ANOVA) with Duncan's multiple range test used for post hoc comparisons. A p-value < 0.05 was considered statistically significant.

3. Result

3.1. Effect of Hydrogen Peroxide Exposure on the Growth and Survival of L. crocea Fry

During the initial 28 days, there was no significant difference in body weight between the control and treatment groups (Figure 1). On day 35, the body weight of juveniles in the T4 and T5 groups was significantly lower than that of the control group ($p < 0.05$), while no significant differences were found among the other groups. By day 50, only the T5 group showed a significantly lower body weight compared to the control ($p < 0.05$).

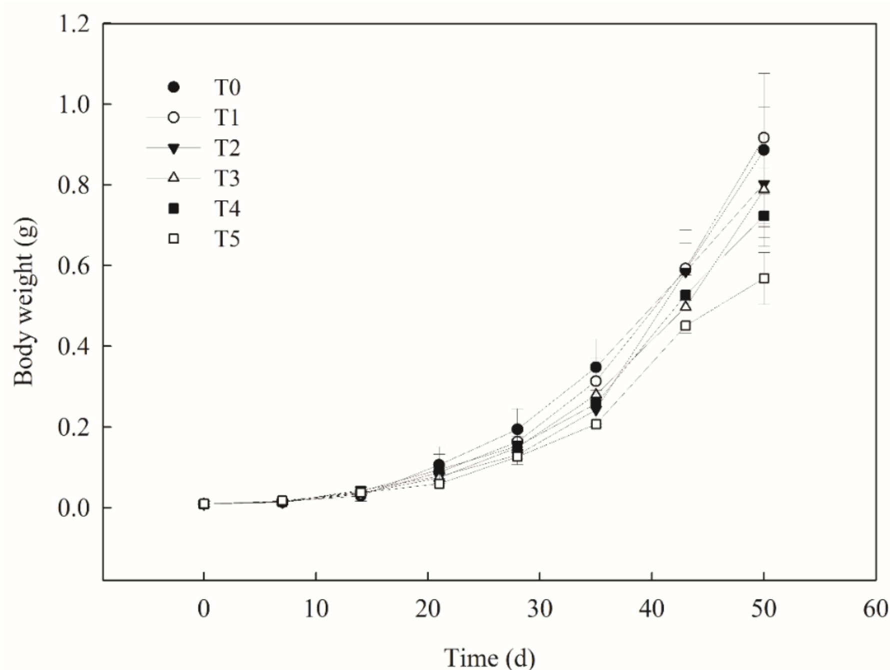

Figure 1. Body weight of juvenile *L. crocea* exposed to different concentrations of hydrogen peroxide.

No significant differences in body length were observed among the groups during the first 43 days (Figure 2). On day 50, the body length of the juvenile fish in the T5 group was significantly lower than that of the control ($p < 0.05$).

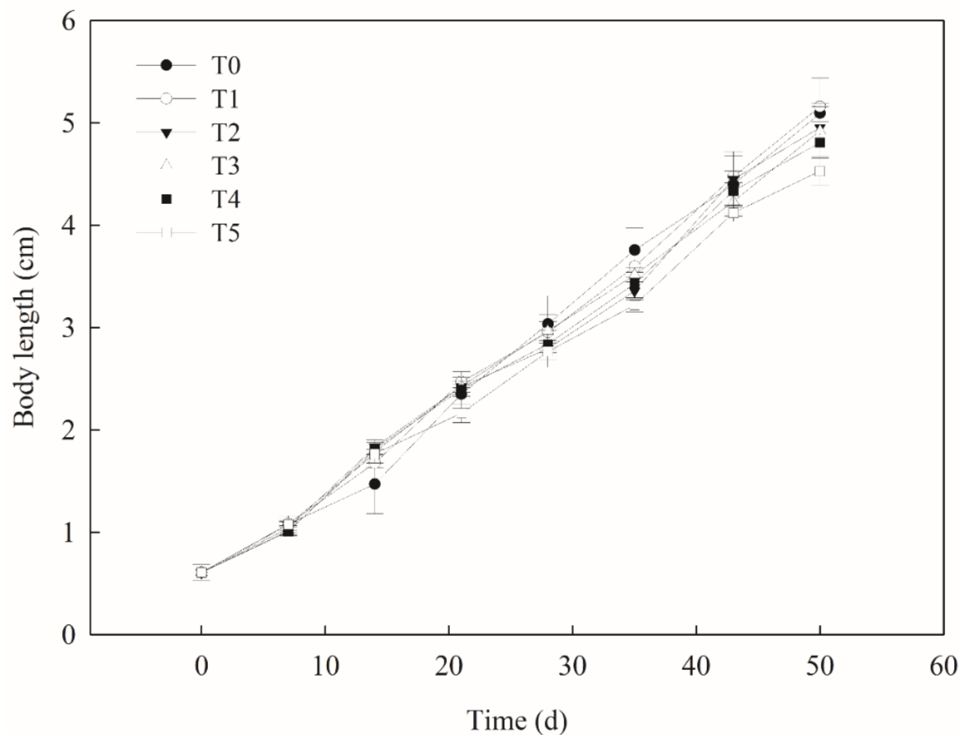

Figure 2. Body length of juvenile *L. crocea* exposed to different concentrations of hydrogen peroxide.

Survival rates increased in a dose-dependent manner with rising hydrogen peroxide concentrations (Table 1). Specifically, groups T2, T3, T4, and T5 exhibited significantly higher survival rates compared to the control group ($p < 0.01$). On day 50, no significant difference was found in body length, body mass, feed conversion ratio, condition factor, specific growth rate and hepatosomatic index between the control group and the T1, T2, T3, T4 groups. However, juveniles in the T5 group displayed significantly lower body length, body weight, and specific growth rate than the control group ($p < 0.05$).

Table 1. Effects of hydrogen peroxide exposure on survival rate and growth performance of *L. crocea* fry.

Growth Index	Groups					
	T0	**T1**	**T2**	**T3**	**T4**	**T5**
Survival rate %	36.08 ± 3.87 [b]	41.59 ± 0.44 [b]	55.91 ± 7.88 [a]	56.19 ± 9.43 [a]	55.98 ± 6.71 [a]	60.34 ± 4.21 [a]
FCR	2.48 ± 0.20	2.24 ± 0.22	1.95 ± 0.42	1.94 ± 0.75	2.02 ± 0.45	2.08 ± 0.36
CF %	1.59 ± 0.13	1.62 ± 0.05	1.56 ± 0.01	1.61 ± 0.02	1.59 ± 0.02	1.58 ± 0.07
RWG %	4504.34 ± 662.62 [a]	4306.44 ± 747.80 [a]	3744.766 ± 534.29 [a]	3761.78 ± 1133.45 [a]	3509.92 ± 677.93 [a]	3179.17 ± 466.35 [b]
SGR %	9.73 ± 0.38 [a]	9.64 ± 0.20 [a]	9.32 ± 0.16 [a]	9.40 ± 0.48 [a]	9.27 ± 0.18 [a]	9.03 ± 0.25 [b]
HPI %	1.70 ± 0.29	1.81 ± 0.17	1.53 ± 0.01	1.42 ± 0.08	1.55 ± 0.27	1.53 ± 0.43

Note: Different superscripts ([a], [b]) within the same row indicate significant differences between groups ($p < 0.05$). Values sharing the same superscript are not significantly different.

3.2. Effect of Hydrogen Peroxide Treatment on the Water Quality

In the present study, ammonia concentration in groups T2 through T5 exhibited a downward trend during the initial 20-day period (Figure 3). However, for the majority of the experimental period, no statistically significant differences in ammonia-nitrogen concentrations were observed between the hydrogen peroxide treatment groups and the control group ($p > 0.05$).

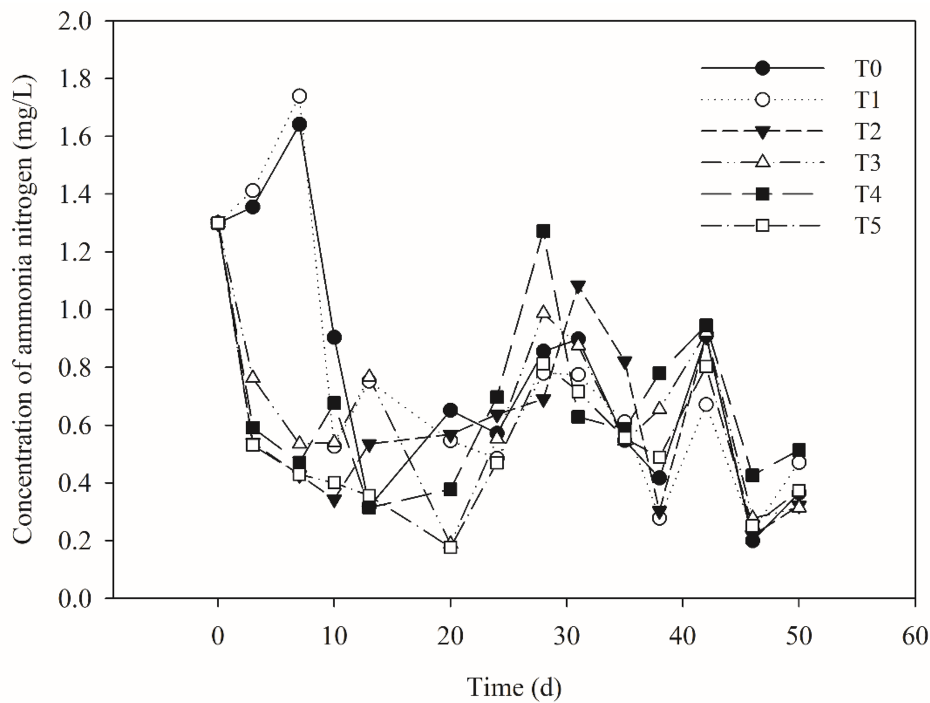

Figure 3. Changes of ammonia nitrogen concentration under different concentrations of hydrogen peroxide.

Nitrite concentrations gradually increased over time across all groups (Figure 4). Compared with the control group, the T1, T2 and T3 groups exhibited significantly lower nitrite levels on days 20 and 24 ($p < 0.05$). Groups T4 and T5 showed significantly lower nitrite concentrations than the control group ($p < 0.05$) throughout the experiment, with the exception of day 3 and day 10, respectively.

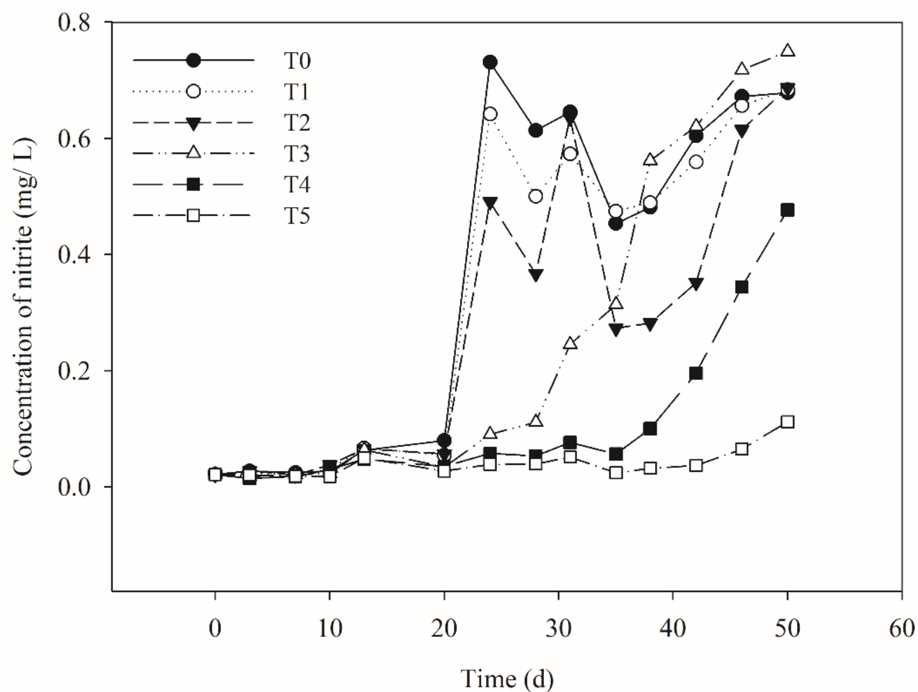

Figure 4. Changes of nitrite concentration under different concentrations of hydrogen peroxide.

The abundance of *Vibrio* spp. in the rearing water exhibited significant temporal fluctuations throughout the experimental period (Figure 5). For the majority of the experimental period, no statistically significant differences in *Vibrio* counts were observed between the hydrogen peroxide treatment groups and the control group ($p > 0.05$).

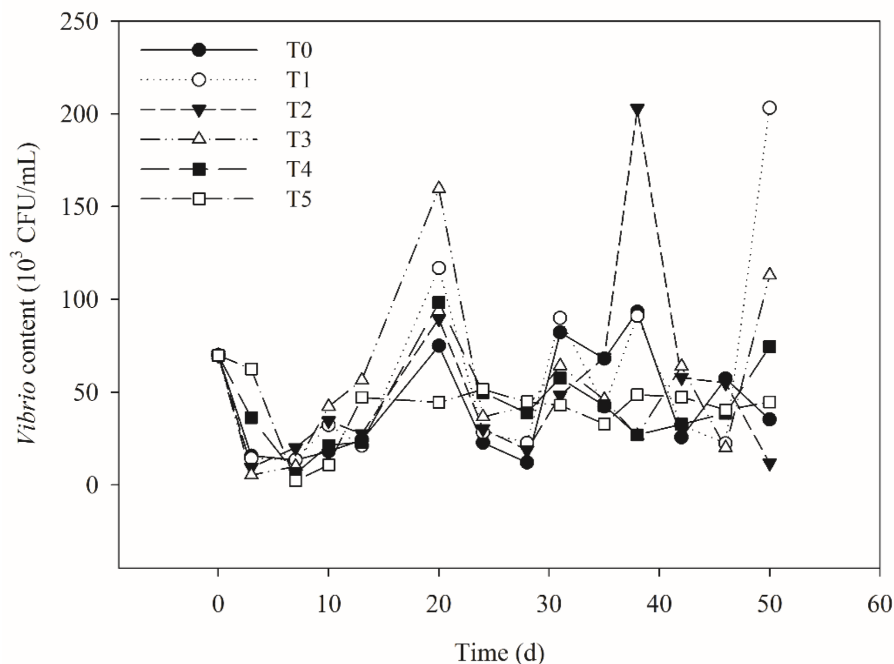

Figure 5. Number of *Vibrio* in the water treated with different concentrations of hydrogen peroxide.

4. Discussion

4.1. Effect of Hydrogen Peroxide Exposure on Survival and Growth Performance of L. crocea Fry

The survival rate of *L. crocea* during large-scale production remains relatively low. Zhu [37] reported that the fry survival rate was only 33.5%, while Jiang [38] reported a survival rate of 32.0%, and Xu [39] found an average fry survival rate of 28.4%. Additionally, we consulted with the technical staff at *L. crocea* hatcheries, who reported a fry survival rate of approximately 30% (unpublished data). In the present study, the survival rate of *L. crocea* fry in the control group was consistent with the findings of these previous studies. However, the survival rate of fry treated with 1.0–5.0 μL/L hydrogen peroxide increased by more than 50%, indicating that hydrogen peroxide treatment can effectively reduce the mortality of *L. crocea* fry. Rach et al. reported that hydrogen peroxide treatments can reduce mortality in salmonids associated with bacterial gill disease, and hydrogen peroxide exposure could prevent gill damage [40]. However, this effect is dose-dependent. In this study, the significant increase in the survival rate of *L. crocea* fry may also be attributed to the ability of hydrogen peroxide to kill or inhibit pathogenic bacteria, thereby improving water quality in the aquaculture system.

The application of chemical agents in aquaculture necessitates a critical balance: achieving effective disinfection and pathogen control while simultaneously ensuring that these treatments do not compromise the survival or normal growth of the cultured organisms. In the present study, no statistically significant differences were found in the specific growth rate and rate of weight gain between the control group and the treatment groups (T1, T2, T3, T4), indicating that the applied concentrations of hydrogen peroxide were well-tolerated by the large yellow croaker fry, exerting no detectable adverse effects on their metabolic efficiency or somatic growth. However, high concentrations of hydrogen peroxide can significantly affect growth parameters, as evidenced by the reduced body weight, body length and SGR observed in juveniles, suggesting the potential toxicity of hydrogen peroxide to *L. crocea* fry. Therefore, in this study, the concentration of 10 μL/L hydrogen peroxide may have caused damage to the juvenile yellow croakers.

During the decomposition of hydrogen peroxide, various reactive oxygen species (ROS) are released, which have the capacity to oxidize essential biomolecules, including proteins, lipids, carbohydrates, and nucleic acids. For instance, the bactericidal efficacy of hydrogen peroxide is primarily mediated through the generation of highly reactive hydroxyl radicals [41]. When present in excessive amounts, hydrogen peroxide can induce cellular damage. Previous studies have reported that when exposed to hydrogen peroxide for 15-min or 45-min every other day for four consecutive treatments, the tolerated concentration is 1000 μ/L or greater for brown trout (*Salmo trutta*), lake trout (*Salvelinus namaycush*), fathead minnow (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), and bluegill (*Lepomis macrochirus*) [30]. Furthermore, exposure to hydrogen peroxide via bath treatment (1000–1500 mg/L for 20 min) was found to adversely affect the growth performance of rainbow trout (*Oncorhynchus mykiss*), resulting in a substantial 22–36% decline in specific growth rate [42]. In a related study,

Henriksen et al. found that exposure to 150 mg/L of hydrogen peroxide for 60 min significantly altered the expression of immune-related genes and caused tissue damage in rainbow trout, manifesting as gill filament lysis and columnar epithelial cell necrosis [43]. At the high dosage (1,250 mg/L for 20 min), particularly under specific temperature conditions, hydrogen peroxide exposure induced moderate gill histopathology and triggered a significant elevation in plasma electrolyte concentrations (Na^+, Cl^-, and K^+) [44].

In the present study, the growth retardation observed in large yellow croaker fry is likely attributable to hydrogen peroxide-induced gill pathology, which compromised feeding efficiency, metabolic rate, and overall physiological function [45]. This inference is corroborated by the absence of significant alterations in other indicators, such as feed conversion ratio, condition factor, and hepatosomatic index. These findings suggest that the gills were the primary target of toxicity, rather than hepatic or somatic tissues.

4.2. Effect of Hydrogen Peroxide Treatment on Water Quality

Ammonia nitrogen represents a significant environmental stressor in aquaculture systems, exerting detrimental effects on the growth, survival, and physiological homeostasis of fish [10,46]. Hydrogen peroxide has been utilized for the removal of ammonia nitrogen from wastewater [47]. In the present study, a significant reduction in ammonia concentration was only observed during the initial stages of treatment. Subsequently, no significant changes in ammonia levels were detected across any of the treatment groups, indicating that low-concentration hydrogen peroxide treatment was ineffective in removing ammonia nitrogen from the hatchery water. It is important to note that the comprehensive biological nitrogen removal process typically involves two distinct and sequential stages: nitrification under aerobic conditions and denitrification under anoxic (or hypoxic) conditions. These transformations are mediated by specific functional microbial communities, namely autotrophic nitrifying bacteria, which oxidize ammonia to nitrate, and heterotrophic denitrifying bacteria, which subsequently reduce nitrate to nitrogen gas [48]. Due to its non-selective bactericidal nature, hydrogen peroxide can significantly disrupt the stability of nitrifying bacterial communities, leading to a temporary loss of their metabolic viability. In recirculating hatchery water experiments with rainbow trout, the addition of elevated concentrations of hydrogen peroxide inhibited ammonia removal by the biofilm. This inhibition is likely attributable to the toxic effects of residual H_2O_2 on the nitrifying bacterial community within the biofilm [29]. In the bioflocs of a shrimp (*Litopenaeus vannamei*) culturing system, the addition of hydrogen peroxide negatively influenced the nitrification process leading to an increase in ammonia concentration [49]. The present experiment was conducted in a non-recirculating water system, where the denitrification process was significantly influenced by factors such as the natural microbial community in the water, water change frequency, and stocking density. In addition to the bactericidal effect of hydrogen peroxide, regular large-scale water changes also achieved physical dilution of ammonia nitrogen. These combined factors resulted in relatively limited and unstable denitrification efficiency in the non-recirculating water system.

Nitrite is a pervasive and toxic intermediate in aquaculture systems, primarily accumulating as a consequence of the microbial mineralization of organic nitrogenous wastes. These wastes originate from a variety of sources, including uneaten feed, fecal matter, metabolic excretions (predominantly ammonia) from the cultured species, and the decomposition of detritus and mortalities [50,51]. Elevated nitrite concentrations are lethal to aquatic animals, disrupting essential functions such as ion regulation, respiration, and endocrine balance. This toxicity leads to systemic hypoxia and asphyxiation, while simultaneously weakening host immune defenses and precipitating disease outbreaks [52]. Throughout the duration of the experiment, nitrite concentrations in all treatment groups exhibited a progressive accumulation, characterized by a pronounced surge following the transition to commercial feed. This distinct temporal correlation strongly suggests that residual uneaten feed serves as a primary driver of nitrite contamination within the large yellow croaker aquaculture systems. This study demonstrates that the effectiveness of hydrogen peroxide in nitrite removal exhibits a distinct dose-dependent effect: low concentrations (0.5–2.5 μL/L) were ineffective, whereas treatments with 5.0 and 10.0 μL/L hydrogen peroxide sustained significant efficacy. These findings demonstrate that hydrogen peroxide is an effective agent for mitigating nitrite accumulation in the large yellow croaker aquaculture system. Hydroxyl radicals generated by the decomposition of hydrogen peroxide can oxidize nitrite to nitrate [53]. The reduction in nitrite concentration observed in this study may be attributed to the oxidation effect of hydrogen peroxide. The correlation between survival rate and nitrate concentration indicates that hydrogen peroxide can improve water quality, and nitrate might serve as an important factor affecting fry survival rate.

Vibriosis is characterized by a short incubation period, fulminant progression, and high contagiousness, which poses a severe threat to aquaculture productivity. The disease often leads to acute mortality with rapid onset, causing significant economic losses in farmed fish populations [16]. The present study revealed that low

concentrations of hydrogen peroxide have only a very limited effect on killing *Vibrio* spp. in water. Experiments on *Vibrio* isolated from *L. crocea* found that the lowest inhibitory concentration of hydrogen peroxide against *V. alginolyticus* and *V. parahaemolyticus* was 16 µL/L and the lowest bactericidal concentration was 32 µL/L [54]. *Vibrio* numbers in the treatment groups may be because the hydrogen peroxide concentration in this study did not reach the inhibitory or bactericidal threshold concentration. On the other hand, pelleted feeds are rich in essential nutrients, particularly proteins. The accumulation of uneaten feed residues and fish feces significantly increases the organic load in the water column, thereby deteriorating water quality. Oxygen radicals derived from decomposition of hydrogen peroxide can react rapidly with organic pollutants or organic matter in water [49]. This competitive consumption led to a rapid depletion of residual hydrogen peroxide concentrations, thereby limiting its sustained antimicrobial activity. Concurrently, the combination of continuous feed input and rising water temperatures creates favorable conditions for rapid microbial proliferation.

5. Conclusions

The results of this study suggest that long-term and low-concentration hydrogen peroxide treatment could significantly improve the survival rate of *L. crocea* fry and reduce nitrite concentrations in the water in a dose-dependent manner; however, hydrogen peroxide had little effect on the removal of ammonia and *Vibrio* numbers in the water. Low-concentration hydrogen peroxide treatment had no negative effect on fry growth, while hydrogen peroxide concentrations of 10.0 µL/L suppressed fish growth. We recommend treating *L. crocea* hatchery water with 1.0–2.5 µL/L hydrogen peroxide.

Author Contributions

Q.C. and B.T.: Conceptualization; Q.C., X.S. and M.Z.: Methodology; X.S. and M.Z.: Validation; Q.C. and M.Z.: Investigation, Data Curation; Q.C.: Writing—Original Draft Preparation; B.T. and H.Z.: Writing—Review and Editing; H.Z.: Supervision; B.T.: Project Administration; H.Z.: Funding Acquisition. 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 Animal Care Welfare Committee of East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The ethical code is 20210016, which was approved on 5 May 2021.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data supporting the results of this study can be obtained from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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