water

MDPI

Article

Short-Term Toxicity of Lanthanum to Embryonic and Yolk-Sac Stage Larvae of the Rare Minnow *Gobiocypris rarus* Ye & Fu, 1983

Liangxia Su [1,†], Dong Hua [1,†], Jun Liu [1,*], Bing Hu [2] and Jianwei Wang [3]

[1] School of Animal Science and Nutritional Engineering, Wuhan Polytechnic University, Wuhan 430023, China; suliangxia027@126.com (L.S.); huadong@126.com (D.H.)
[2] Fujian Key Laboratory of Special Aquatic Formula Feed, Fuzhou 350308, China; hubing@126.com
[3] Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China; wangjw@ihb.ac.cn
* Correspondence: liujunihb@aliyun.com; Tel.: +86-027-83956175
† These authors contributed equally to this work.

Abstract: The wide use of rare-earth elements in China for aquacultural purposes and many other applications has resulted in their accumulation in the aquatic environment and has caused concern about their safety. In this study, we tested the toxicity of lanthanum (La (III)) to the early life stages (embryonic and sac-fry stages) of the rare minnow *Gobiocypris rarus* Ye & Fu, 1983. We exposed fertilized eggs to 0, 0.06, 0.13, 0.25, 0.50, 1.00, and 1.92 mg/L of La (III) until the yolk sac was exhausted in any group (at about 168 h of exposure). Exposure to 1.00 and 1.92 mg/L La (III) had obvious lethal effects on embryos, La (III) exposure also accelerated the development of embryos and had a significant inhibitory effect on the hatching rate after 96 h. As the exposure time increased, the larvae exhibited obvious yolk-sac edema, pericardium edema, spinal curvature, tail bending, and other symptoms of poisoning, including deflated swim-bladder. In general, these results clearly indicate that La pollutants hinder the development of rare minnow embryos and are acutely toxic to rare minnow larvae. Our finding would provide a theoretical basis for further research of relevant feed additive criteria for this fish.

Keywords: lanthanum pollutant; *Gobiocypris rarus*; toxicity test; development; growth

Citation: Su, L.; Hua, D.; Liu, J.; Hu, B.; Wang, J. Short-Term Toxicity of Lanthanum to Embryonic and Yolk-Sac Stage Larvae of the Rare Minnow *Gobiocypris rarus* Ye & Fu, 1983. *Water* **2022**, *14*, 2084. https://doi.org/10.3390/w14132084

Academic Editor: Heiko L. Schoenfuss

Received: 16 May 2022
Accepted: 28 June 2022
Published: 29 June 2022

1. Introduction

Seventeen chemical elements in the periodic table, including lanthanum (La), constitute the rare earth elements (REEs) [1]. Generally, the concentration of rare earth elements in natural seawater is only 0.01 μg/L [2]. But, due to their ability to improve meat quality, nutrient digestibility, and intestinal digestive enzyme activity in organisms, REEs are used on a large scale and in agriculture as feed additives for poultry, livestock, and aquaculture [3,4]. This wide use results in REE accumulation in the environment, especially in aquatic ecosystems [5]. For example, the total dissolved REE concentration in the Wojiang river, China's Jiangxi Province mining area ranges from 363 to 117,520 μg/L [6]. In rivers near mining areas in Shanxi Province, the La (III) content varies between 0.116 and 8.260 μg/L due to wastewater discharge and surface runoff, and leakage of REEs [7]. These reports show that the water around mines in China has been contaminated with REEs, and the lack of remediation in these areas has led to REE pollution spreading to water bodies far removed from mining areas. For example, researchers recently reported that the contents of total dissolved REEs (14 elements) in Taihu Lake ranged from 46.86 to 112.50 ng/L [8].

Data clearly show that REEs pollution exists, and its toxicity to aquatic organisms requires further study. Chen et al. [9] reported that exposure to La (III) could induce oxidative stress and DNA damage in the liver of rats, and Zarros et al. [10] found that La exposure impaired cognitive function in children. Chronic exposure to 20 μg/mL La

inhibited growth of the Cyanobacterium (*Anabaena azollae*) and decreased its chlorophyll a synthesis and oxygen-production activity [11]. Exposure to La induced nuclear anomalies, blebs, and notches in erythrocytes of the rare minnow (*Gobiocypris rarus*) [12] and carp (*Cyprinus carpio* Linnaeus) [13]. These reports indicate that aquatic organisms exposed to La may experience physiological toxicity, cytotoxicity, and genotoxicity. The early life stages of fish are more sensitive to contaminants than the juvenile and adult stages. Thus, studies of the effect of La on the early development of fish species are critical to understanding the toxicity of this element and to provide a theoretical basis for further research and development of relevant feed additive criteria for this fish.

The rare minnow is a freshwater cyprinid fish, with a short generation time (3 or 4 months), tolerance to a range of temperatures (0 to 30 °C), and sensitivity to environmental pollutants [14]. Currently, it is the only native Chinese fish species recommended by the National Standard of China (GB/T29763-2013) for use in biological tests of the aquatic environment. To date, only two studies of the chronic toxic effect of La on its adult species have been reported [12,15], and whether La exposure adversely influences its early development was unknown prior to our study.

In the present study, we tested the toxicity of La (III) to the early life stages (embryo and sac-fry stages) of the rare minnow. The aims of this study were to assess the toxicity of La (III) to rare minnow embryos and to provide a scientific basis for establishing relevant feed additives levels for this fish.

2. Materials and Methods

2.1. Chemicals and Reagents

The test compound used in this study was lanthanum chloride heptahydrate ($LaCl_3 \cdot 7H_2O$, analytical grade 99.999% purity), which was produced by Sigma-Aldrich Corporation (St. Louis, MO, USA). A 100 mg/L La (III) stock solution was prepared in double-distilled water at the beginning of the experiment and stored in the dark at 4 °C until use in this experiment.

2.2. Embryo Collection and Culture

Eggs were obtained from artificial spawning of rare minnow broodstock cultured at the National Aquatic Biological Resource Center, NABRC. Fertilized eggs were examined at 8 h post-fertilization using a dissecting microscope (Olympus B41, Hamburg, Germany), and we selected 630 eggs that appeared to be developing normally (i.e., without coagulation or signs of deformation) for use in this study. These eggs were transferred to 21,600 mL (12 cm diameter) cylindrical glass containers (Huaou Industry, Yanchang, China), each containing 30 eggs. During the experimental period, the photoperiod was maintained at 12L:12D from 08:00 to 20:00. The water temperature, dissolved oxygen content, and pH were monitored daily (DZB-718L, Leici, INESA scientific instrument Co., Ltd., Shanghai, China) and maintained at 22.78 ± 0.98 °C, 6.62 ± 0.36 mg/L, and 7.22 ± 0.06, respectively. All of the experimental procedures were conducted following the guidelines set by the Animal Care and Use Committee of Wuhan Polytechnic University (Approval protocol No. WUPU-20160701) and were in line with the National Guiding Principles for the Care and Use of Laboratory Animals.

2.3. Experimental Design

2.3.1. Chronic Toxicity Assay of Embryonic and Sac-Fry Stages

The chronic toxicity assay of embryonic and sac-fry stages was carried out according to the Organisation for Economic Cooperation and Development (OECD) guideline 212 [16]. The embryos were randomly divided into seven groups of 30 embryos per container with three replicates. The previous study reported that the median lethal concentration (LC_{50}) value at 96 h for rare minnow was 1.92 mg/L [15]. Thus, we set up a control group (0.00 mg/L) and six La (III) test concentration groups at 0.06, 0.13, 0.25 mg/L, 0.50, 1.00, and 1.92 mg/L. Ninety percent of the exposure solutions was renewed daily in order to

maintain the test concentrations. The embryos or larvae were monitored every 24 h for 168 h, and dead individuals were removed (Figure 1). Deceased embryos were identified as appearing cloudy and opaque under the microscope due to a large amount of white floc caused by protein coagulation or precipitation. Deceased larvae were identified as those that lacked a heartbeat, failed to move, and lacked a response to a physical stimulus (touch with the pipette tip). Food was not provided, and the experiment was discontinued when the yolk sac was consumed in any group. At the end of the experiment, the mortality of embryos/larvae was recorded.

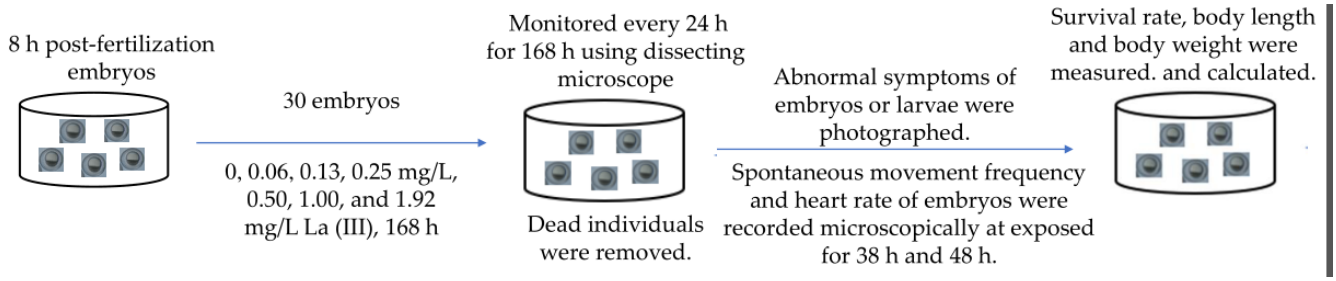

Figure 1. The experimental design of the chronic toxicity assay of embryonic and sac-fry stages.

2.3.2. Signs of Embryonic and Sac-Fry Stage Toxicity

Daily observations of abnormal development, including yolk sac edema and pericardial edema, and mortality were performed based on the lethal and sublethal toxicological endpoints provided by OECD guidelines [16] and on previously published methods. Spontaneous movement frequency of embryos was recorded microscopically at exposed for 38 h, for six randomly selected embryos from each replicate in a room with constant temperature (22.78 ± 0.98 °C). The number of heartbeats within 20 s of each group of surviving embryos was detected to calculate heart rate when exposed for 48 h. Newly hatched larvae were monitored under a dissecting microscope. After hatching, larvae were observed every 24 h after being anesthetized in 100 mg/L tricaine methanesulfonate (MS-222, Sigma Aldrich, St Louis, MO, USA) buffered with 10 mg/L sodium bicarbonate ($NaHCO_3$). The number of individuals with abnormal symptoms (yolk-sac edema, pericardium edema, deflated swim bladder, weak pigmentation, spine curvature, and body shortening) was determined under the dissecting microscope. During the experimental period, the observed embryos/larvae were returned to the cylindrical glass containers for further observation. After 168 h of exposure, the survival rate was calculated, and the body length of surviving larvae was measured (Figure 1). Ten larvae per replicate were placed together in one weighing bottle, dried to a constant weight at 105 °C, and dry weight was recorded using a Mettler Toledo XS 105DU balance. All observations were made by the same investigator.

2.4. Statistical Analysis

All data are shown as mean ± standard deviation (SD). Data were checked for normality and assumptions of homogeneity of variance using the Kolmogorov-Smirnov and Levene tests, respectively. Due to normality and homogeneity of variance, data were analyzed by one-way analysis of variance (ANOVA). Statistically significant differences between the control group and exposure groups were confirmed by the least significant difference test using SPSS 19.0 (IBM, Armonk, NY, USA). $p < 0.05$ and $p < 0.01$ were considered to be statistically significant and highly statistically significant, respectively. All figures were created using OriginPro 8.0 (OriginLab, Northampton, MA, USA) and Photoshop CS 7.0 (Adobe, San Jose, CA, USA).

The parameters were calculated as follows:

$$\text{Hatchability (\%)} = (\text{number hatching/the total number of eggs}) \times 100$$

$$\text{Survival rate (\%)} = [\text{survival number (at end of test)/number of hatching}] \times 100$$

3. Results

3.1. Hatchability and Survival Rates

The hatchability in the control group was 2.22 ± 1.92% at 72 h and 57.78 ± 3.85% at 96 h. Compared to the control group, the 0.06 mg/L La (III) exposure group had a slightly higher hatchability ($p > 0.05$), and a significant increase was observed as the La (III) concentration increased ($p < 0.01$). However, none of the embryos in the groups exposed to 1.00 and 1.92 mg/L La (III) survived after 72 h. After 96 h of exposure, the hatchability of the 0.06–0.50 mg/L exposure groups were significantly lower than that of the control group ($p < 0.01$) (Figure 2).

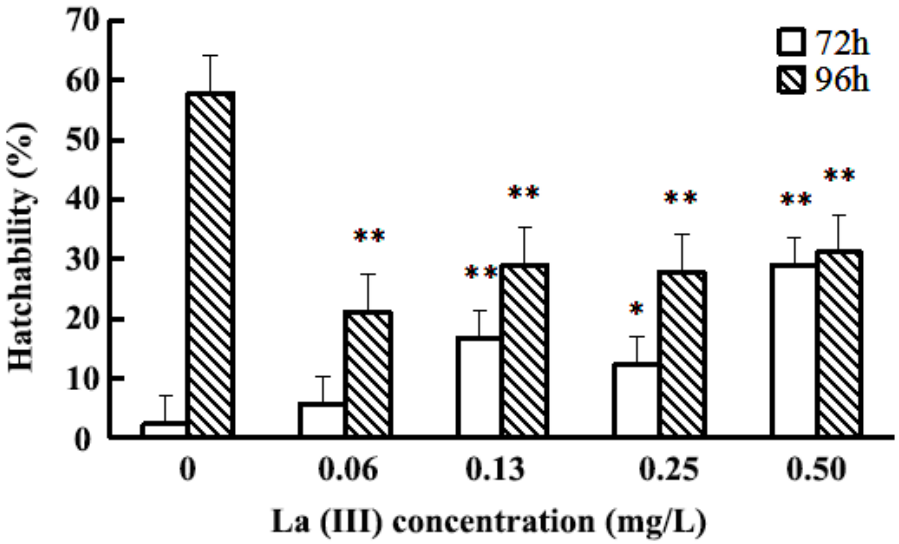

Figure 2. Effects of La (III) on hatchability of rare minnow embryos. Error bars represent standard deviation. * Significant difference from the control group ($p < 0.05$) and ** highly significant difference from the control group ($p < 0.01$).

We also detected a concentration-time-dependent decrease in the survival rate of rare minnows exposed to La (III). The survival rate in the control group was 64.45 ± 5.09% at 168 h of exposure, but it was significantly lower in the 0.06 and 0.13 mg/L La (III) exposure groups ($p < 0.01$). At 120 h, all larvae in the higher concentration group (>0.13 mg/L) had died (Table 1).

Table 1. Survival rates following exposure of rare minnows to different levels of La (III) for varying durations.

La (III) Concentration (mg/L)	Survival Rate (%)						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
0	98.9 ± 1.93	98.9 ± 1.93	90.0 ± 3.33	84.4 ± 1.93	74.4 ± 1.93	67.8 ± 3.85	64.4 ± 5.09
0.06	98.9 ± 1.93	95.6 ± 5.09	94.4 ± 1.93	71.1 ± 3.85 *	53.3 ± 12.62 **	15.6 ± 8.39 **	2.2 ± 3.85 **
0.13	97.8 ± 1.93	97.8 ± 1.93	71.1 ± 10.72 **	53.3 ± 12.02 **	34.4 ± 12.62 **	23.3 ± 6.67 **	6.7 ± 5.77 **
0.25	96.7 ± 3.33	96.7 ± 3.33	53.3 ± 8.82 **	17.8 ± 8.39 **	0.0 ± 0.00 **	0.0 ± 0.00 **	0.0 ± 0.00 **
0.50	98.9 ± 1.93	97.8 ± 1.93	12.2 ± 6.94 **	0.0 ± 0.00 **	0.0 ± 0.00 **	0.0 ± 0.00 **	0.0 ± 0.00 **
1.00	97.8 ± 1.93	31.1 ± 6.94 **	0.0 ± 0.00 **	0.0 ± 0.00 **	0.0 ± 0.00 **	0.0 ± 0.00 **	0.0 ± 0.00 **
1.92	94.4 ± 1.93 *	0.0 ± 0.00 **	0.0 ± 0.00 **	0.0 ± 0.00 **	0.0 ± 0.00 **	0.0 ± 0.00 **	0.0 ± 0.00 **

Data are expressed as means ± SD. * $p < 0.05$ and ** $p < 0.01$.

3.2. Body and Heart Development

We measured spontaneous movements to evaluate body development in rare minnow embryos. In the control group, the spontaneous movement frequency of embryos was 8.7 ± 2.34 time/30 s at 38 h of exposure. The spontaneous movement frequency of embryos in the low-concentration La (III) exposure groups (0.06, 0.13, and 0.25 mg/L) was lower

than that of the control at 38 h ($p > 0.05$), and it was significantly lower in the 0.50 mg/L exposure group ($p < 0.01$) (Figure 3).

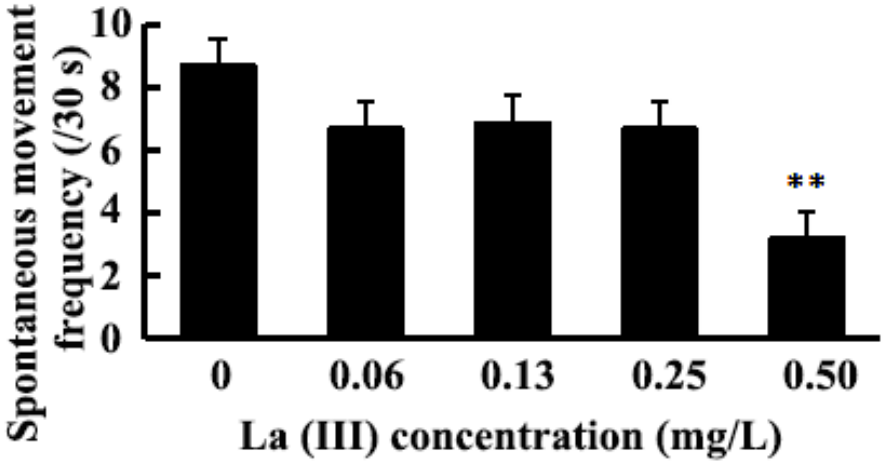

Figure 3. Effects of La (III) on spontaneous movement frequency of rare minnow embryos at 38 h. Error bars represent SD. ** Highly significant difference from the control group ($p < 0.01$).

We used heart rate to evaluate heart development in the embryos. In the control group, the heart rates of embryos at 40 h and 60 h of exposure were 40.3 ± 1.86 and 46.8 ± 1.72 beat/30 s, respectively. The average heart rate of rare minnow embryos increased after exposure to the lower concentrations of La (III) ($p > 0.05$), but it significantly decreased at higher concentrations of La (III) (>0.25 mg/L, $p < 0.01$) (Figure 4).

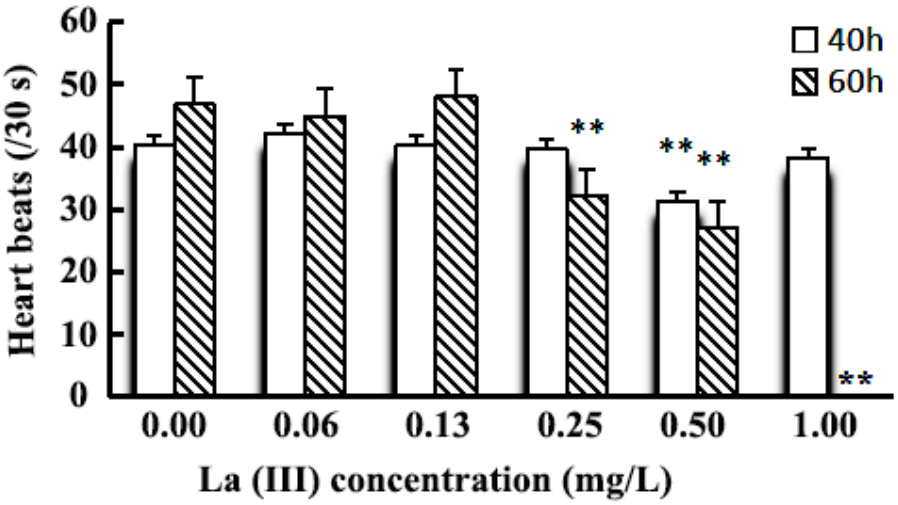

Figure 4. Effects of La (III) on the heart rate of rare minnow embryos. Error bars represent SD. ** Highly significant difference from the control group ($p < 0.01$).

3.3. Signs of La (III) Toxicity in Rare Minnow Embryos/Larvae

The development of rare minnow embryos/larvae was severely affected by La (III) exposure. Abnormal symptoms in embryos were primarily egg condensation, melanin depletion, embryo autolysis, and eyespot disappearance (Figure 5A–D). Abnormal symptoms in larvae were mainly yolk sac edema, pericardium edema, deflated swim bladder, weak pigmentation, spine curvature, and body shortening (Figure 5E–L).

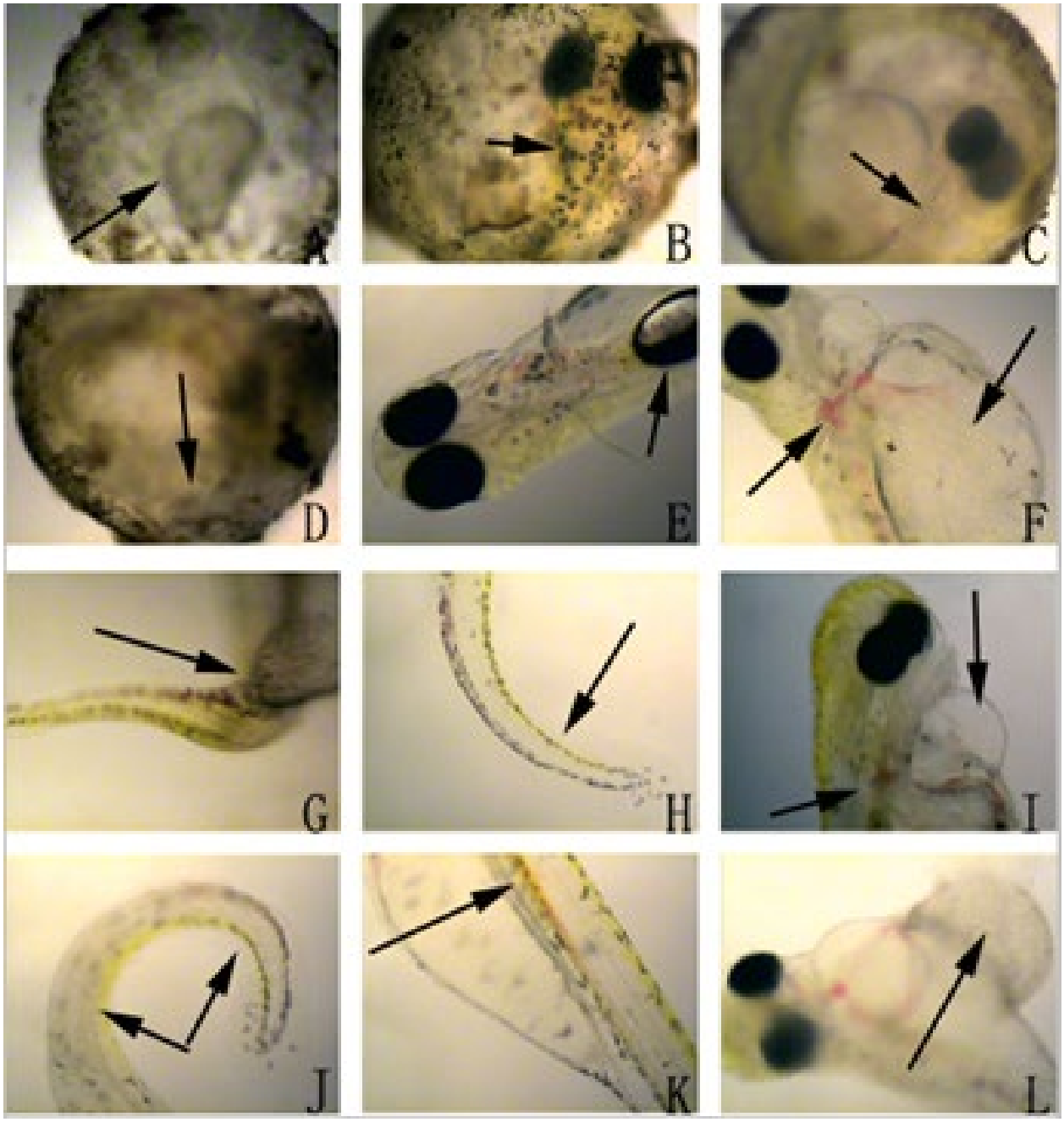

Figure 5. Effects of exposure to La (III) on developing embryos/larvae of rare minnows. The black arrows represent the following: (**A**) embryonic eggs condensation; (**B**) normal embryos; (**C**) melanin depletion; (**D**) embryo autolysis and eyespot disappearance; (**E**) normal larva; (**F**) yolk-sac edema and blood circulation disorder; (**G**) spinal curvature; (**H**) tail ending; (**I**) pericardium edema and blood circulation disorder; (**J**) body bending; (**K**) no functional swim-bladder; (**L**) edema rupture.

3.4. Growth

La (III) had severe toxicity effects on the growth of rare minnow larvae. In the control group, the mean body length and body weight of larvae were 4.36 ± 0.37 mm and 1.23 ± 0.25 mg, respectively. La (III) exposure significantly reduced the body length of rare minnow larvae ($p < 0.05$), but there was no significantly difference in body weight ($p > 0.05$) (Figure 6).

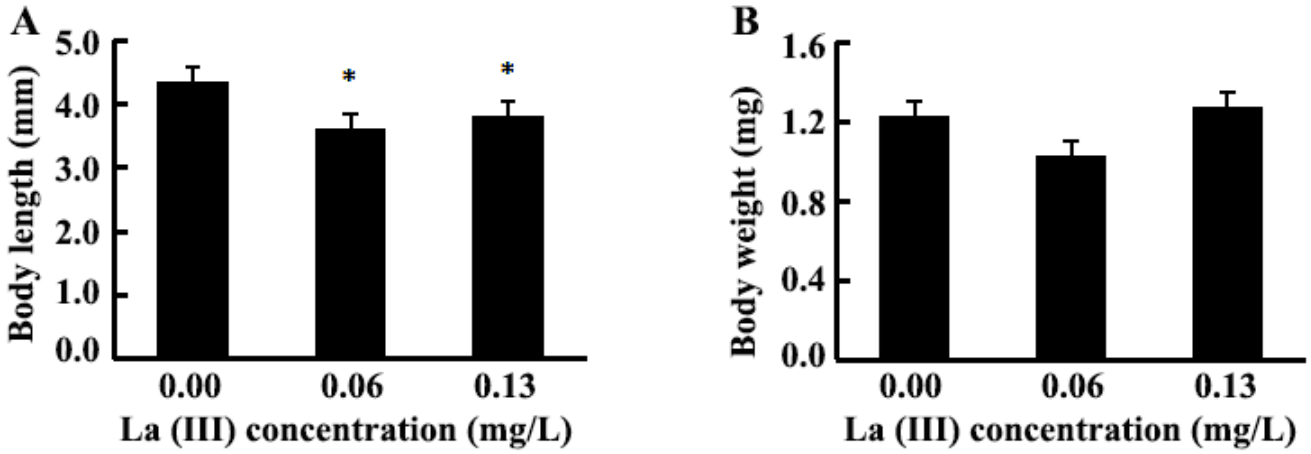

Figure 6. Effects of La (III) on body length (**A**) and body weight (**B**) of rare minnow larvae at 168 h. Error bars represent SD. * Significant difference from the control group ($p < 0.05$).

4. Discussion

REEs are widely used as feed additives because they can improve meat quality, nutrient digestibility, and intestinal digestive enzyme activity in organisms. However, little is known about their toxicity [3,4]. We previously reported that La caused erythrocytes with nuclear anomalies, blebs, and notches in rare minnow [12]. Yong et al. [17] found that La exposure could increase micronucleus frequency, DNA single-strand breaks, and unscheduled DNA synthesis in cultured human lymphocytes. Thus, REE-associated toxicity is an important research focus.

Chemical substances can be absorbed by fish embryos and eventually be transported into the yolk sac and body, which suggests that these toxic substances could persist throughout embryonic development [18,19]. Due to their relative ease of culture and sensitivity, fish species at the embryo and yolk-fry stages are often used in toxicity tests [20]. In this study, we used rare minnow embryos to test the effects of chronic exposure to La (III) to evaluate the developmental toxicity of this REE on aquatic organisms.

La (III) exposure had severe toxic effects on the growth and development of rare minnow embryos/larvae, including decreased survival rate and body length, reduced spontaneous movement frequency and heart rate, increased the symptoms of malformation, such as yolk sac edema, pericardial edema, spinal curvature, and deflated swim bladder. Remarkably, no obvious difference in weight was detected between the control group and the exposure groups. A similar phenomenon was reported for other chemical pollutants [21,22], which suggests that survival rate, malformation, and body length are the best and most easily assessed indicators to evaluate the toxicity effects of La pollutants on rare minnows. For the symptoms of malformation, Chen et al. [23] found that heavy mental could inhibit the entry of calcium (Ca) into fish, resulting in abnormal development. In addition, Dubé et al. [24] indicated that La (III) had an ionic radius close to that of Ca^{2+} (100 pm), which could promote the displacement of calcium at various binding sites. Thus, the symptoms of malformation in exposed rare minnow larvae may be due to the fact that La (III) in an ionic state could inhibit Ca^{2+} from entering rare minnow larvae, which would result in malformation.

We found 1.00 mg/L La (III) exposure prevented the embryo from hatching. It is well known that adenosine cells secrete high levels of chorionic enzymes during hatching and low levels of chorionic enzymes during embryo incubation [25]. Fish hatching is completed by breakage of the egg membrane under the action of chorionic enzymes and twisting of the embryo [26]. However, chorionic enzymes are only present for a short period of time during the embryo hatching process, and they are susceptible to external pollutants [27]. During embryonic development, La may enter the embryo and affect the development of the yolk, and this change may directly reduce the hatching rate and increase yolk sac edema. On the

other hand, the survival rate of La (III) exposure groups significantly decreased compared with the control group. This result may be related to the absence of egg membrane, which directly exposed rare minnow larvae to La (III), thus resulting in their death.

We also found that 1.00 mg/L La (III) exposure was acutely toxic to rare minnow embryos, and malformation occurred at 48 h in this exposure group. Oral et al. [28] found that 1.389 mg/L La (III) induced 100% developmental defects in sea urchin (*Paracentrotus lividus*) embryo, without causing embryonic mortality. But, Balusamy et al. [29] showed that 1000 mg/L La (III) had an absence of toxic effect on *Chlorella* sp. This considerable difference in La toxicity to embryos and sac-fry suggests that rare minnows are more sensitive to La (III) pollutants. Thus, different standards are needed for different species when La is used as a feed additive.

5. Conclusions

In the present study, we found that exposure to 1.00 mg/L La (III) was acutely toxic to rare minnow embryos. Additionally, the La (III) concentrations tested had a significant inhibitory effect on embryonic development. The larvae showed obvious pericardium edema, yolk-sac edema, spinal curvature, tail bending, and other poisoning symptoms, including a deflated swim-bladder. Thus, La pollutants pose a threat to this species.

Author Contributions: Conceptualization, L.S.; methodology, D.H.; software, L.S. and D.H.; formal analysis, L.S. and J.L.; investigation, D.H.; resources, J.L. and J.W.; data curation, D.H. and J.L.; writing—original draft preparation, L.S.; writing—review and editing, L.S., J.L., B.H. and J.W.; supervision, B.H.; project administration, J.L. and L.S.; funding acquisition, J.L. and L.S. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Scientific Research Project of the Hubei Provincial Department of Education (NO. D20131705) and the Fujian Key Laboratory of Special Aquatic Formula Feed (No. TMKJZ2102).

Institutional Review Board Statement: The experimental protocols of the study were approved by the Animal Care and Use Committee of Wuhan Polytechnic University, Approval Code: WUPU-20160701, Approval Date: 20160701.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We would like to express our gratitude and appreciation to those who have taken the time to critically review this article.

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

Abbreviations

Lanthanum	La (III)
rare earth elements	REEs
median lethal concentration	LC_{50}
standard deviation	SD
one-way analysis of variance	ANOVA
calcium	Ca

References

1. Hou, Z.; He, Y. Overview and application prospect of rare earth resources in China. *Sci. Technol. Econ. Inner. Mongol.* **2017**, *7*, 28–30.
2. Elderfield, H.; Greaves, M.J. The rare earth elements in seawater. *Nature* **1982**, *296*, 214–219. [CrossRef]
3. Shahraki, S.; Shiri, F.; Majd, M.H.; Dahmardeh, S. Anti-cancer study and whey protein complexation of new lanthanum (III) complex with the aim of achieving bioactive anticancer metal-based drugs. *J. Biomol. Struct. Dyn.* **2019**, *37*, 2072–2085. [CrossRef]
4. Ma, J.J.; Ren, Y.J.; Yan, L.Y. Effects of Spray Application of Lanthanum and Cerium on Yield and Quality of Chinese Cabbage (*Brassica chinensis* L.) Based on Different Seasons. *Biol. Trace Elem. Res.* **2014**, *160*, 427–432. [CrossRef]
5. Bai, X.; Yang, Z. Protection of Resources and Environmental of China in Context of Globalization. *Ecol. Econ.* **2012**, *10*, 140–142.

6. Liu, S.W.; Huang, Y.Y.; Zhu, X.H.; Liu, X.R. Environmental effects of ion-absorbed type rare earth extraction on the water and soil in mining area and its peripheral areas. *Environ. Sci Technol.* **2015**, *6*, 25–32.
7. Zhao, F.; Cong, Z.; Sun, H.; Ren, D. The geochemistry of rare earth elements (REE) in acid mine drainage from the Sitai coal mine, Shanxi Province, North China. *Int. J. Coal Geol.* **2007**, *70*, 184–192. [CrossRef]
8. Zhu, Z.; Liu, C.; Wang, Z.; Li, J. Rare earth element geochemistry in waters of Taihu Lake, Eastern China. *J. Chin. Rare Earth Soc.* **2011**, *29*, 233–238.
9. Chen, D.; Liu, Y.; Chen, A.-J.; Nie, Y.-X. Experimental study of subchronic toxicity of lanthanum nitrate on liver in rats. *Nonlinearity Biol. Toxicol. Med.* **2003**, *1*, 15401420390271074. [CrossRef]
10. Zarros, A.; Byrne, A.M.; Boomkamp, S.D.; Tsakiris, S.; Baillie, G.S. Lanthanum-induced neurotoxicity: Solving the riddle of its involvement in cognitive impairment? *Arch. Toxicol.* **2013**, *87*, 2031–2035. [CrossRef]
11. Song, L.Y.; Hu, W.Y.; Zhao, J.Z.; Shao, H.X.; Zhang, Y. Physiological effects of lanthanum on cyanobacterium. *Acta Sci. Nat. Univ. Pekin.* **2000**, *36*, 783–788.
12. Qiu, Y.; Hua, D.; Liu, J.; Wang, J.; Yu, D. Induction of micronuclei, nuclear anomalies, and dimensional changes in erythrocytes of the rare minnow (*Gobiocypris rarus*) by lanthanum. *Environ. Sci. Pollut. Res.* **2020**, *27*, 31243–31249. [CrossRef]
13. Zhang, G.S. Effects of lanthanum on the genetic toxicity and circulating blood corpuscle parameters in *Cyprinus carpio* Linnaeus (C. carpio). *J. Anhui Agric. Sci.* **2008**, *36*, 7269–7270.
14. Wang, J.W.; Cao, W.X. *Gobiocypris rarus* as a Chinese Native Model Organism: History and Current Situation. *Asian J. Ecotoxicol.* **2017**, *12*, 20–33.
15. Hua, D.; Wang, J.W.; Yu, D.H.; Liu, J. Lanthanum exerts acute toxicity and histopathological changes in gill and liver tissue of rare minnow (*Gobiocypris rarus*). *Ecotoxicology* **2017**, *26*, 1207–1215. [CrossRef]
16. Organisation for Economic Cooperation and Development (OECD) guideline 212. OECD Guidelines for the Testing of Chemicals, Section 2: Effects on Biotic Systems. 2013. Available online: https://www.oecd.org/publishing/corrigenda (accessed on 28 June 2022).
17. Yongxing, W.; Xiaorong, W.; Zichun, H. Genotoxicity of Lanthanum (III) and Gadolinium (III) in Human Peripheral Blood Lymphocytes. *Bull. Environ. Contam. Toxicol.* **2000**, *64*, 611–616. [CrossRef]
18. Jezierska, B.; Ugowska, K.; Witeska, M. The effects of heavy metals on embryonic development of fish (a review). *Fish Physiol. Biochem.* **2009**, *35*, 625–640. [CrossRef]
19. Ługowska, K.; Sarnowski, P. Heads or tails—Fish hatching. *Acta Ichthyol. Piscat.* **2011**, *41*, 13–17. [CrossRef]
20. Mohammed, A. Why are Early Life Stages of Aquatic Organisms more Sensitive to Toxicants than Adults? *New Insights Into Toxic. Drug Test.* **2013**, 49–62. [CrossRef]
21. Nikola, M.; Stefan, M.; Darko, P.; Sanjin, K.; Srdja, L.; Jasna, T.; Svetolik, S.; Marko, D.; Jelena, N.O. Cadmium significantly changes major morphometrical points and cardiovascular functional parameters during early development of zebrafish. *Environ. Toxicol. Pharmacol.* **2021**, *87*, 103723.
22. Luo, S.; Wu, B.L.; Xiong, X.Q.; Wang, J.W. Short-term toxicity of ammonia, nitrite, and nitrate to early life stages of the rare minnow (*Gobiocypris rarus*). *Environ. Toxicol. Chem.* **2016**, *35*, 1422–1427. [CrossRef]
23. Chen, Q.C.; Zhang, K.J.; Xu, G.W. A comprehensiveinvestigationon the toxic effects of heavy metals of fish. *J. Fish. China* **1988**, *12*, 21–32.
24. Dubé, M.; Auclair, J.; Hanana, H.; Turcotte, P.; Gagnon, C.; Gagné, F. Gene expression changes and toxicity of selected rare earth elements in rainbow trout juveniles. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2019**, *223*, 88–95. [CrossRef]
25. Inohaya, K.; Yasumasu, S.; Araki, K.; Naruse, K.; Yamazaki, K.; Yasumasu, I.; Iuchi, I.; Yamagami, K. Species-dependent migration of fish hatching gland cells that commonly express astacin-like proteases in common. *Dev. Growth Differ.* **2010**, *39*, 191–197. [CrossRef]
26. Gaspar, I.D.; Blanquez, M.J.; Fraile, B.; Paniagua, R.; Arenas, M.I. The hatching gland cells of trout embryos: Characterisation of N- and O-linked oligosaccharides. *J. Anat.* **2010**, *194*, 109–118. [CrossRef]
27. Xiong, X.Q.; Luo, S.; Wu, B.L.; Wang, J.W. Comparative Developmental Toxicity and Stress Protein Responses of Dimethyl Sulfoxide to Rare Minnow and Zebrafish Embryos/Larvae. *Zebrafish* **2017**, *14*, 60–68. [CrossRef]
28. Oral, R.; Bustamante, P.; Wamau, M.; D'Ambra, A.; Guida, M.; Pagano, G. Cytogenetic and developmental toxicity of cerium and lanthanum to sea urchin embryos. *Chemosphere* **2010**, *81*, 194–198. [CrossRef]
29. Balusamy, B.; Tastan, B.E.; Ergen, S.F.; Uyar, T.; Tekinay, T. Toxicity of lanthanum oxide (La_2O_3) nanoparticles in aquatic environments. *Environ. Sci. Process. Impacts* **2015**, *17*, 1265–1270. [CrossRef]