Chronic Toxicity of Silver Nanoparticles to *Tigriopus Japonicus*

Xinwei Wang* and Jiaying Zhao
Key Laboratory of Marine Environment and Ecology, Ministry of Education (Ocean University of China), Qingdao, P. R. China

Keywords: Silver Nanoparticles, Chronic Toxicity.

Abstract: Silver nanoparticles (Ag NPs) have been widely used because of their excellent bactericidal properties, and with them comes their massive discharge, which may pose potential risks to marine ecology and the environment, but relatively few studies have been conducted on the chronic toxicity of Ag NPs to marine organisms. Here, the marine copepod *Tigriopus japonicus* was used to investigate the effects of Ag NPs on the survival, development, and reproduction under two generations. The results showed that Ag NPs significantly increased the mortality of *T. japonicus* at 0.2 mg/L. In the F_0 generation, 0.3 mg/L Ag NPs significantly prolonged the developmental time of *T. japonicus*, while in the F_1 generation, it was significantly prolonged at only 0.1 mg/L. And Ag NPs significantly inhibited the hatching number of *T. japonicus* at F_0 generation while it was alleviated at F_1 generation.

1 INTRODUCTION

Silver nanoparticles (Ag NPs) are widely used in a variety of consumer products, including textiles, care products, and food packaging, due to their physicochemical properties, especially their excellent bactericidal effect. It was estimated that the global production of Ag NPs was 500 tons/year. and more than 60 tons of Ag NPs were released into the water environment each year (Handy, 2012). Several studies have shown that Ag NPs can have acute toxic effects on a variety of organisms, including oxidative stress, genotoxicity, and behavioral effects, but their possible chronic toxicity to organisms was less well studied.

As a key link in the marine food web, zooplankton plays an important role in the process of material cycling and energy transfer and influences the transport of pollutants (Batel, 2016). *Tigriopus japonicus* belongs to Arthropoda, Crustacea, Harpacticoida, is a common species in the estuaries of the western Pacific Ocean, with short generation time, strong reproduction, and easy cultivation, widely used in the detection of microplastics, heavy metals, organic matter, and other pollutants toxicity (Juan, 2020). It has been classified as a standard organism for toxicity testing by OECD.

In this paper, we investigated the effects of Ag NPs on the growth, development, and reproduction of *T. japonicus* under two generations. The chronic toxicity data of Ag NPs were supplemented to provide information and methods for assessing the effects of Ag NPs on marine invertebrates and to provide a reference for understanding the reproductive toxicity of Ag NPs.

2 MATERIAL AND METHODS

2.1 Ag NPs and Organisms

Silver nanoparticles (PVP - Ag NPs, < 100nm) were obtained from Sigma-Aldrich (Germany). *Tigriopus japonicus* and natural seawater were taken from the sea near Qingdao, China, and have been continuously cultured in the laboratory for many generations. The culture conditions were: 32‰ of filtered and sterilized natural seawater as culture medium, 2100 lx of light, 12 h: 12 h of light to dark ratio, and 24℃. During the culture period, the algae were fed with a 1:1 mixture of *Phaeodactylum tricornutum* and *Isochrysis galbana* every 3 days at a feeding density of 1×10^6 cells/mL.

2.2 Experimental Methods

Before the formal experiment, the acute toxicity of Ag NPs to *T. japonicus* was tested, and the 96 h - LC_{50}

Wang, X. and Zhao, J.
Chronic Toxicity of Silver Nanoparticles to Tigriopus Japonicus.
DOI: 10.5220/0012011900003633
In *Proceedings of the 4th International Conference on Biotechnology and Biomedicine (ICBB 2022)*, pages 12-16
ISBN: 978-989-758-637-8

was 0.892 mg/L (0.778 - 1.079 mg/L). Before starting the experiment, *T. japonicus* females with eggs were transferred to 6-well culture plates, one per well, and fed with bait. The nauplius with hatching time <24h were randomly selected for the experiment. Four concentration groups (0.1, 0.2, 0.3, 0.4 mg/L) and control groups were set up, with three parallels in each group. The nauplius (F_0) were transferred to 12-well plates with 10 per well, and the culture conditions were as above. After 12 h of exposure, baits were fed and the experimental solution was changed after 24 h. The development of *T. japonicus* (nauplius - copepodite - adult) and the number of dead individuals were observed and recorded, and the dead individuals were removed. For the nauplius stage, 5 mL of experimental solution was added to each group, and 10 mL was added to the copepodite and adult stage. After the females of *T. japonicus* held eggs, the females were transferred to 12-well culture, one in each well, and the same concentration of Ag NPs was added. Similarly, fed after 12 h of exposure and changed the solution after 24 h. Females were observed for reproduction, and the number of eggs carried, incubations, and hatching were recorded for 10 d. At the peak of reproduction in each group of females, nauplius hatchlings with an incubation time <24 h were randomly selected for the second generation (F_1) experiment. The experimental methods were the same as those for F_0.

2.3 Statistical Analysis

The experimental data were analyzed by one-way ANOVA and LSD multiple comparison analysis using SPSS 16.0 to compare the significance of differences between concentrations, with $P < 0.05$ indicating a significant difference.

3 RESULTS

3.1 Effects of Ag NPs on the Growth and Development of T. Japonicus

The mortality rate of *T. japonicus* F_0 and F_1 generations after 21 days of exposure to Ag NPs was shown in Fig. 1. The mortality rate in the control group was 3.33%, and 13.4%, 33.33%, 70%, and 86.7% for each concentration of the F_0 generation, while 23.3% and 36.7% for the F_1 generation, respectively. The results showed that the mortality rate of *T. japonicus* increased gradually with the increase of Ag NPs concentration. Compared with F_0, the mortality rate increased in all groups in F_1. It can be seen that Ag NPs had a greater effect on the survival of *T. japonicus* at higher concentrations.

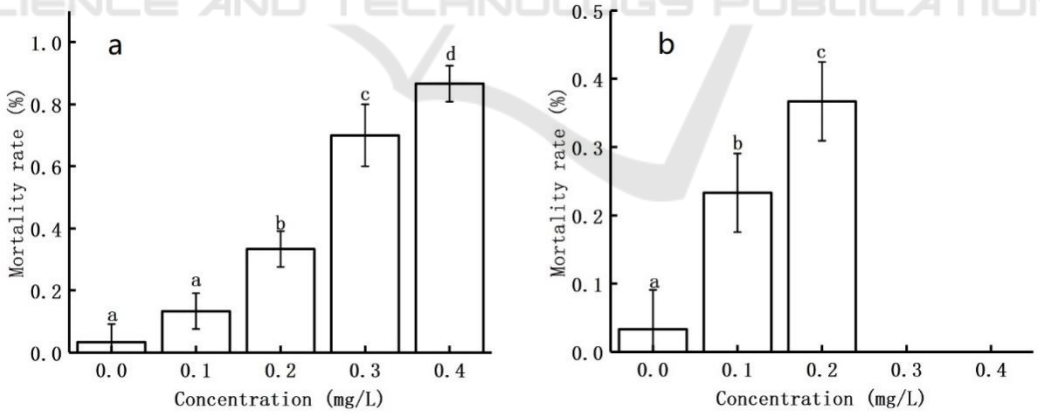

Figure 1 21 - day mortality of *T. japonicus* F_0 (a) and F_1 (b) generations exposed to Ag NPs. The data of 0.3 mg/L and 0.4 mg/L in (b) were missing because *T. japonicus* in F_0 did not incubate enough nauplius to continue the experiment. Values are shown as mean ± S.D., different letters: $P < 0.05$.

Effect of Ag NPs on the time from nauplius to copepodite (N-C) and from nauplius to adults (N-A) in the F_0 and F_1 generations of *T. japonicus* are shown in Fig. 2. In the F_0 generation, the duration of N-C and N-A in the control was 4.67 and 12.33 days, respectively, and 5.33, 5.67, 6.33, and 7.67 days for each concentration of N-C; and 12.33, 13.33, 14.67, and 16.33 days for N-A, respectively. In the F_1 generation, the duration of N-C and N-A in the control group was 4.67 and 12.67 days, respectively.

The duration of N-C and N-A for each concentration group was 7.33 days, 8.67 days, and 15 days, 18.33 days, respectively. The results showed that the N-C, N-A of both *T. japonicus* F_0 and F_1 increased with the increase of Ag NPs concentration, and the growth time of F_1 was longer than F_0. There was no significant difference in N-C and N-A of F_0 generation compared to the control at concentrations of 0.1 mg/L and 0.2 mg/L.

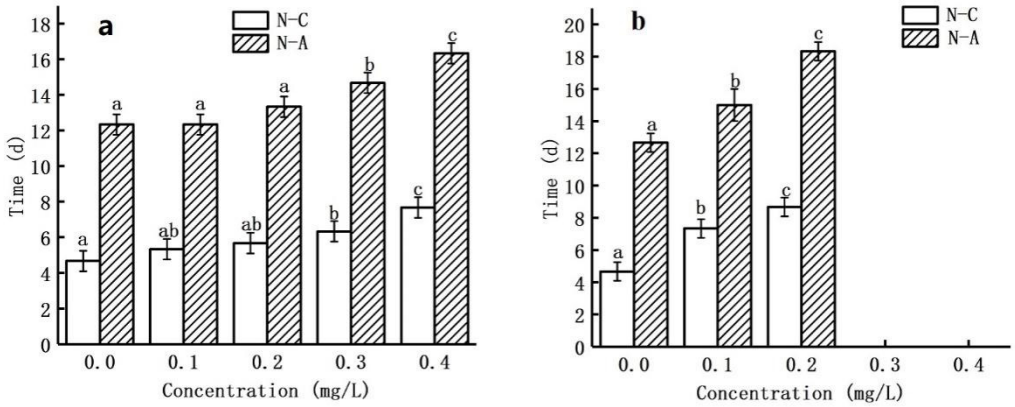

Figure 2 Effect of Ag NPs on the time from nauplius to copepodite (N-C) and from nauplius to adults (N-A) in the F_0 (a) and F_1 (b) generations of *T. japonicus*. The data of 0.3 mg/L and 0.4 mg/L in (b) were missing because *T. japonicus* in F_0 did not incubate enough nauplius to continue the experiment. Values are shown as mean ± S.D., different letters: $P < 0.05$.

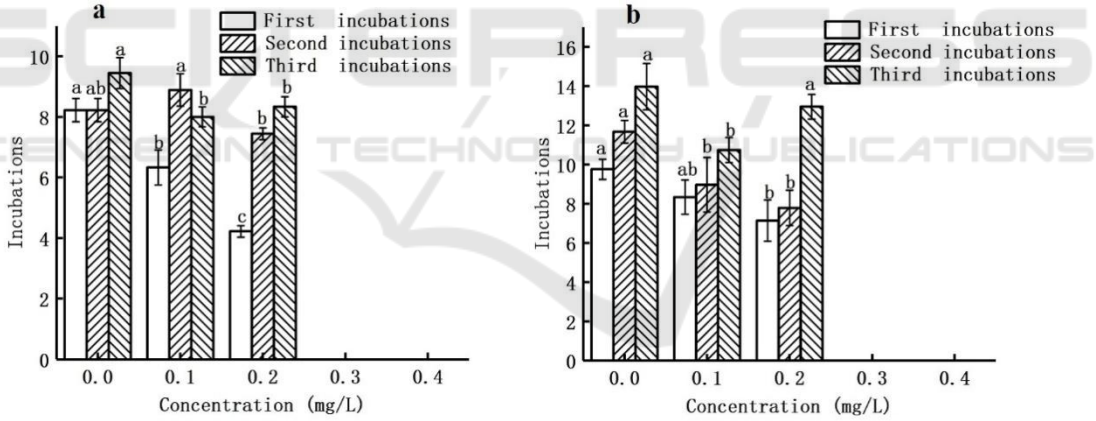

Figure 3 Effect of Ag NPs on the number of three hatchings in *T. japonicus* females F_0 (a) and F_1 (b) generations. The data of 0.3 mg/L and 0.4 mg/L were missing because the mortality rate was too high for the hatchings to be known. Values are shown as mean ± S.D., different letters: $P < 0.05$.

3.2 Effects of Ag NPs on the Reproduction of T. japonicus

The effect of Ag NPs on the number of three hatchings of *T. japonicus* females for 10 days was shown in Fig. 3. In F_0 generation, the total number of hatchings in 10 days of the control group was 25.89, and 22.00 and 14.46 at 0.1 mg/L and 0.2 mg/L, respectively; in the F_1 generation, the total number of hatchings in 10 days of the control group was 29.40, and 24.18 and 20.28 at 0.1 mg/L and 0.2 mg/L, respectively. The results showed that the hatching number of *T. japonicus* gradually decreased with the increase of concentration and the number of hatchings increased with the number of incubations at the same concentration in both F_0 and F_1 generations.

4 DISCUSSION

In the ocean, the survival, growth, and reproduction of *T. japonicus* were affected by a variety of factors. For example, seawater acidification and various pollutants (heavy metals, organic matter, microplastics, etc.). It has been shown that 4-methylbenzylidene camphor (4-MBC) (Hong, 2021), Ni (Mohammed, 2010), the microplastics polyethylene (PE), and polyamide-nylon 6 (PA 6) (Yu, 2020) all reduced *T. japonicus* survival in a dose-dependent manner, and that nauplius was more sensitive to pollutants than copepodite and adults, similar to Ag NPs. In addition to its effect on survival, the impact on growth and reproduction was also a major concern. Hong et al. (2021) noted that 4-MBC reduced the developmental time of *T. japonicus* at the N-C stage and the number of female hatchings decreased with increasing concentration. For the incubation number, 4-MBC barely affected the F_0 generation, but at high concentrations (5 and 10 μg/L), the F_1 and F_2 generations were significantly inhibited, and inhibition was relieved at the F_3 generation (Chen, 2018). This differs from Ag NPs, which in this study significantly prolonged the developmental time of *T. japonicus* although they inhibited the hatching number of females. Of course, some pollutants did not adversely affect the survival of *T. japonicus* but inhibit reproduction. For example, dibutyl phthalate (DBP) did not have a lethal effect on *T. japonicus* at the concentrations tested, but prolonged incubation time and inhibited hatching numbers (Li, 2020). Most contaminants reduced the hatching number while prolonging the development of *T. japonicus*. It has been shown that when *T. japonicus* were exposed to oil-contaminated sediments, the growth rate of nauplius decreased, developmental time increased significantly, and the number of egg-bearing females decreased and hatching was significantly reduced (Won, 2018). Seawater contaminated with various metals (containing Cr, Zn, Ni, As, etc.) can significantly inhibit the survival and reproduction rate of *T. japonicus*. And ZnO nanoparticles completely inhibited the reproduction of *T. japonicus* at 0.5 mg/L (Jeong, 2019). Similar to the metal contaminants mentioned above, Ag NPs had adverse effects on the growth, development and reproduction of *T. japonicus*.

5 CONCLUSIONS

Ag NPs inhibited the survival and development of *T. japonicus* at all stages, and the inhibition increased with increasing concentration and generations. In contrast, the inhibition of hatching numbers decreased with increasing generations. In future studies, the chronic toxicity of Ag NPs should continue to be investigated and attention the effects on *T. japonicus* reproduction under multiple generations.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grant No. 41276104), and Public Science and Technology Research Funds Projects of Ocean (Grant No. 201505034-2).

REFERENCES

Batel, A., Linti, F., Scherer, M., Erdinger, L. & Braunbeck, T. 2016. Transfer of benzo[a]pyrene from microplastics to *Artemia nauplii* and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. *Environmental Toxicology and Chemistry*, 35**,** 1656-1666.

Chen, L., Li, X., Hong, H. & Shi, D. 2018. Multigenerational effects of 4-methylbenzylidene camphor (4-MBC) on the survival, development and reproduction of the marine copepod *Tigriopus japonicus*. *Aquatic Toxicology*, 194**,** 94-102.

Handy, R. D., Cornelis, G., Fernandes, T., Tsyusko, O., Decho, A., Sabo-Attwood, T., Metcalfe, C., Steevens, J. A., Klaine, S. J., Koelmans, A. A. & Horne, N. 2012. Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. *Environmental Toxicology and Chemistry*, 31**,** 15-31.

Hong, H., Wang, J. & Shi, D. 2021. Effects of salinity on the chronic toxicity of 4-methylbenzylidene camphor (4-MBC) in the marine copepod *Tigriopus japonicus*. *Aquatic Toxicology*, 232**,** 105742.

Jeong, C. B., Kang, H. M., Lee, M. C., Byeon, E., Park, H. G. & Lee, J. S. 2019. Effects of polluted seawater on oxidative stress, mortality, and reproductive parameters in the marine rotifer *Brachionus koreanus* and the marine copepod *Tigriopus japonicus*. *Aquatic Toxicology*, 208**,** 39-46.

Juan, Y., Yuan, T. J., Rui, X., Yu, Z. Z., Peng, Y. G., Dan, W. X., Guang, L. J. & Rong, C. 2020. Effects of microplastics exposure on ingestion, fecundity, development, and dimethylsulfide production in

Tigriopus japonicus (Harpacticoida, copepod). *Environmental Pollution*, 267.

Li, Z., Zhou, H., Liu, Y., Zhan, J., Li, W., Yang, K. & Yi, X. 2020. Acute and chronic combined effect of polystyrene microplastics and dibutyl phthalate on the marine copepod *Tigriopus japonicus*. *Chemosphere*, 261**,** 127711.

Mohammed, E. H., Wang, G. & Jiang, J. 2010. The effects of nickel on the reproductive ability of three different marine copepods. *Ecotoxicology*, 19**,** 911-916.

Won, E. J., Lee, Y., Gang, Y., Kim, M. S., Kim, C. J., Kim, H. E., Lee, K. W., Chung, C. S., Kim, K., Lee, J. S. & Shin, K. H. 2018. Chronic adverse effects of oil dispersed sediments on growth, hatching, and reproduction of benthic copepods: Indirect exposure for long-term tests. *Marine Environmental Research*, 137**,** 225-233.

Yu, J., Tian, J. Y., Xu, R., Zhang, Z. Y., Yang, G. P., Wang, X. D., Lai, J. G. & Chen, R. 2020. Effects of microplastics exposure on ingestion, fecundity, development, and dimethylsulfide production in *Tigriopus japonicus* (Harpacticoida, copepod). *Environmental Pollution*, 267**,** 115429.