ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 16, Issue, 05, pp.28204-28207, May, 2024
DOI: https://doi.org/10.24941/ijcr.47215.05.2024

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ECOTOXIC EFFECTS OF LOW CONCENTRATIONS OF POLYETHYLENE AND POLYLACTIC ACID MICROPLASTICS ON JUVENILE MARINE MEDAKA (*ORYZIAS MELASTIGMA*)

Lili Zhu, Shihuo Wang, Cuiyu An, Yalin Xie, Yue Yin and Shaobai Wen*

NHC Key Laboratory of Tropical Disease Control, School of Tropical Medicine, Hainan Medical University, Haikou, Hainan, 571199, China

ARTICLE INFO

Article History:
Received 10th February, 2024
Received in revised form
15th March, 2024
Accepted 24th April, 2024
Published online 20th May, 2024

Key words:
Marine medaka, Juvenile Fish, Ecotoxicity, Gut Microbiota.

***Corresponding author:** *Shaobai Wen*

ABSTRACT

The ecotoxicological impacts of microplastics (MPs) on aquatic organisms have garnered considerable attention; however, the toxic effects of MPs on aquatic organisms at environmentally relevant concentrations have been underreported. In this study, juvenile marine medaka were exposed to polyethylene and polylactic acid at concentrations of 20 μg/L for a duration of 60 days. The findings revealed that prolonged exposure to different types of MPs at low concentrations did not exhibit significant detrimental effects on the growth and intestinal tissues of juvenile fish. Nonetheless, long-term exposure led to alterations in gut microbial composition in juvenile fish, potentially influencing their functional roles. PICRUSt2 prediction analysis demonstrated a significant down-regulation in Isoleucine Biosynthesis function within the PE-exposed group at Level 3. Overall, further attention is warranted regarding the adverse consequences associated with prolonged exposure to environmental MPs on aquatic organisms.

Citation: ***Lili Zhu, Shihuo Wang, Cuiyu An, Yalin Xie, Yue Yin, Shaobai Wen.*** **2024.** "Ecotoxic effects of low concentrations of polyethylene and polylactic acid microplastics on juvenile marine medaka (*Oryzias melastigma*)". *International Journal of Current Research*, 16, (05), 28204-28207.

INTRODUCTION

Microplastics (MPs) are widely distributed in various environments and have become persistent pollutants due to their stability and resistance to degradation[1]. Studies have demonstrated the ingestion of MPs by zooplankton, fish, birds, and other marine organisms[2], with MP particles found in the digestive tracts of 233 marine organisms worldwide[3]. Polyethylene (PE) plastic is extensively used in daily production and life, representing one of the most frequently detected plastic fragments in marine environments. Polylactic acid (PLA), a bio-based plastic considered as an alternative to conventional plastics for reducing plastic waste persistence in the environment [4].In 2022, the global plastic production is 400.3 million tons, of which the production of PE is 105 million tons, and the production of bio-based plastics is 0.02 million tons [5]. Biodegradable plastics can only be completely decomposed by biological agents under specific conditions and for a specific period of time. thus, they may persist for extended periods under inappropriate degradation conditions [6]. Moreover, both petroleum-based and biodegradable plastics generate MPs or nanoplastics during aging under natural environmental conditions [7,8].Although previous studies reported toxic effects associated with PLA-MPson zebrafish [6], nematode (*Caenorhabditis elegans*) [9], and earthworms (*Eisenia fetida*)[10], these concentrations were significantly higher than environmental levels and could not accurately reflect the impact of PLA contamination on organisms. In this study, we investigated the effects of long-term exposure to low concentrations of PE and PLA-MPson the growth, intestinal tissue damage, and intestinal microbial community structure of juvenile marine killifishes. To assess the toxic effects of petroleum-based MPs and bio-based MPs on organisms at environmental concentrations.

MATERIALS AND METHODS

Material: The two MPs used in the experiments were purchased from Dongguan Honorable Plastic Raw Material Co, Ltd. The MPs were characterized by Fourier infrared spectroscopy (Thermo Fisher, USA) and scanning electron microscopy (ZEISS EIGMA), and their compositions were determined to be polyethylene (PE) and poly(lactic acid) (PLA) in the shape of lumps, with particle sizes ranging from 1-10 μm for PE and 10-100 μm for PLA.

Exposure experiment: Marine medaka larvae of uniform size (15 days after hatching) were selected and exposed to artificial seawater with PE of 20 μg/L and PLA of 20 μg/L, respectively.

The exposure vessel was a 2 L glass beaker containing 1 L of exposure solution. 6 juvenile fish were placed in each beaker, with clean artificial seawater as a blank control and three replicates per treatment. Commercial diets were fed twice daily during the exposure period and the exposure solution was changed once every 48 hours. The exposure experiments were conducted in an air-conditioned room at a room temperature of 26±1°C with light/darkness=14h/10h, and all artificial seawater used for aquaculture was filtered through a 0.45 μm filter membrane.

Length, weight and intestinal pathology in juvenile fish: On the 60th day of exposure, six fish were collected from each treatment, and their length and weight were determined using vernier calipers and a ten-thousandths balance (Mettler Toledo, ML204T), respectively. After the juvenile fish were place on ice for freezing and execution, their intestines were dissected and removed (all operations followed the relevant ethical requirements of the Ethics Committee of Hainan Medical College)and fixed with paraformaldehyde fixative (Wuhan Xavier Biotechnology Co., Ltd.) for 24 h. Subsequently, sections were embedded, stained, sealed and examined under a microscope.

Nucleic acid extraction, amplification and sequencing: For each treatment, 15 intestinal tissues from marine medaka were collected and divided into three equal parts, with five fish intestines in each aliquot. Total DNA from the intestinal samples was extracted using HiPure Stool DNA Kit (model D3141, Guangzhou Meiji Biotechnology Co., Ltd., China).DNA purity was detected using a Nanorop microspectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, Inc., USA), and DNA quality was assessed by electrophoresis. To construct the library, total DNA was amplified using the forward primer CCTACGGGG NGG CWG CAG and the reverse primer GGACTACH VGGGGTATCTAAT in the V3-V4 region of 16S rRNA. The products of the second round of amplification were purified using AMPure XP Beads, quantified using the ABI Step One Plus Real-Time PCR System (Life Technologies, USA), and sequenced online according to the PE250 pattern pooling of Novaseq 6000.

Data processing: Data were analyzed and plotted using SPSS 26 and Origin 2021 to compare the differences between treatments. Results are expressed as mean ± standard deviation (Mean ± SD). $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The influence of body length and weight: Marine medaka larvae were exposed to PE and PLA at a concentration of 20 μg/L for 60 days, and none of the treatments resulted in larval mortality. Exposure to PE and PLA had no significant effect on the length and weight of the larvae when compared with the blank control (Fig. 1 A, B). Their body lengths were 1.8274 ± 0.093, 1.7941 ± 0.042, and 1.7409 ± 0.22 cm, respectively, and their body weights were 0.05465 ± 0.011, 0.05155 ± 0.0063, and 0.05345 ± 0.013 g, respectively, which were statistically analyzed and showed no significant differences. The effect of MPs on the growth and development of organisms is mainly related to their concentration, shape (size, diameter, surface functional groups, etc.), and polymer composition [11,12]. In the present study, different MPs had no effect on the growth of juvenile fish, indicating that the mass concentration of MPs is a key factor affecting their toxicity [12] length, B: body weight

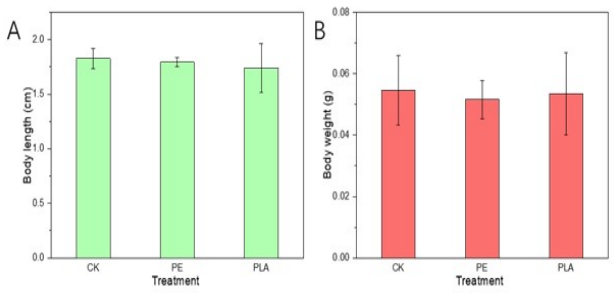

Figure 1. Changes of body length and weight of juvenile fish exposed to different MPs. A: body length, B: body weight

Intestinal tissue damage: The intestine is an important organ of an organism, and many studies have reported that MPs accumulate in the intestines of organisms, causing satiety, damage to intestinal tissues, oxidative stress, and a series of other adverse effects [13]. After 60 days of exposure to the different types of MPs, the intestines of juvenile fish were sectioned and stained to observe that PE and PLA-MPs (20 μg/L) had no significant effect on the intestinal tissues of juvenile fish (Fig. 2), and the villi of the small intestines grew normally without shedding, atrophy, or other obvious damage. The most important reason may be that because of the low concentration of MPs in the water column, the MPs ingested into the body by juvenile fish were very small, which was not enough to cause the accumulation of MPs in the intestinal tissues, which could block and damage the intestinal tissues.

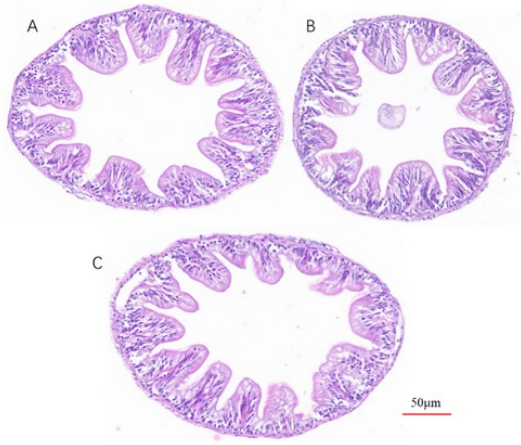

Figure 2. Intestinal tissue sections of juvenile fish with different MPs exposures.A: Blank control, B: PE exposure, C: PLA exposure

Changes of intestinal microbial community: The microbial species signature sequences of 16S rDNA amplified by PCR were examined using high-throughput sequencing to study the effects of exposure to different MP on the gut microbial community of marine medaka after 60 days. At the phylum level, PLA exposure led to an increase in the relative abundance of Proteobacteria compared with the blank control, whereas PE exposure led to a decrease in the relative abundance of Proteobacteria and an increase in the

abundance of Verrucomicrobia (Fig. 3A). At the genus level, different MPs exposures led to changes in the gut microorganisms of juvenile fish, as shown in Figure 3B, the top three genera in relative abundance in the PLA treatment were *Nautella* (80.42%), *Ruegeria* (4.91%), and *Roseibacillus* (3.95%), whereas the top three genera in relative abundance in the PE treatment were *Nautella* (47.21%), Vibrio (13.22%) and *Roseibacillus*(10.96%); while for the CK treatment it was *Nautella* (60.82%), *Ralstonia* (9.39%) and *Rubritalea* (6.28%). VENN plots of species by OUT classification level showed that PE exposure had 99 unique OUT numbers compared to the blank control, whereas PLA exposure was much less with only 55 unique OUT numbers (Fig. 3C, D). Many studies have shown that MPs exposure can lead to dysbiosis of gut microflora, accompanied by changes in community function [6]. The mechanisms related to the effect of MPs on the intestinal flora are less reported, whether it is the direct effect of MPs or the indirect effect of additives in MPs, and what kind of molecular mechanism exists needs to be further investigated.

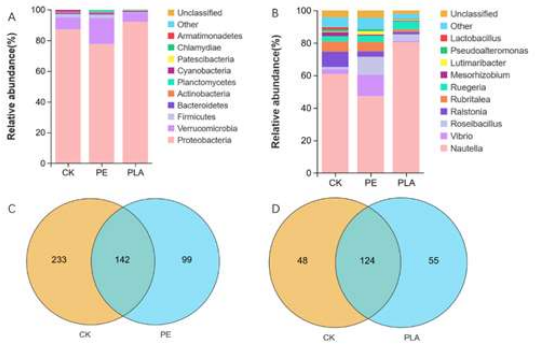

Figure 3. Changes in gut flora of juvenile fish with different MPs exposures. a: species stacked plot at phylum level, b: species stacked plot at genus level, c, d: species VENN plot at OUT taxonomic level

Predictive analysis of gut microbial community functions. The 16S rRNA sequences of prokaryotes with existing genomes in the KEGG database were analyzed using PICRUSt2 prediction, and the function of intestinal microorganisms changed by MPs was analyzed using KEGG enrichment. The results showed that at Level 3, PE resulted in the downregulation of the synthesis and degradation of ketone bodies, fatty acid degradation, and valine, leucine, and isoleucine degradation. In addition, PE exposure caused Valine, leucine and isoleucine biosynthesis, Lipoic acid metabolism, Bacterial chemotaxis, C5-branched dibasic acid metabolism, Fatty acid biosynthesis, Pyruvate metabolism, Pantothenate and CoA biosynthesis, C5-Branched dibasic acid metabolism, Fatty acid biosynthesis, Pyruvate metabolism, Pantothenate and CoA biosynthesis, Peptidoglycan biosynthesis, D-Glutamine and D-glutamate metabolism, Biosynthesis of ansamycins, One carbon pool by folate, Peptidoglycan biosynthesis, d-glutamine and d-glutamate metabolism, biosynthesis of ansamycins, one carbon pool by folate, Upregulation of Biotin metabolism, Flagellar assembly, Histidine metabolism and Biosynthesis of vancomycin group antibiotic, as opposed to PE, PLA exposure resulted in the downregulation of the above functions. Welch's t test showed that PE exposure significantly reduced isoleucine biosynthesis (p = 0.01078) (Fig. 5). Currently, there are limited reports on the exact role of bacteria in the fish gut, but some are known to be associated with host survival, metabolism, and beneficial functions.The genera Ruegeria and Pseudoalteromonas have fundamental metabolic capabilities that enhance the survival of Atlantic cod larvae against pathogens and are potentially beneficial probiotic organisms for their hosts[14]. Studies have shown that impaired synthesis of Isoleucine Biosynthesis by gut microorganisms is strongly associated with disease severity and inflammatory response [15]. Prolonged exposure of PE at low concentrations affects the gut microorganisms of larval fish, which may lead to down-regulation of relevant functions and cause inflammatory response.

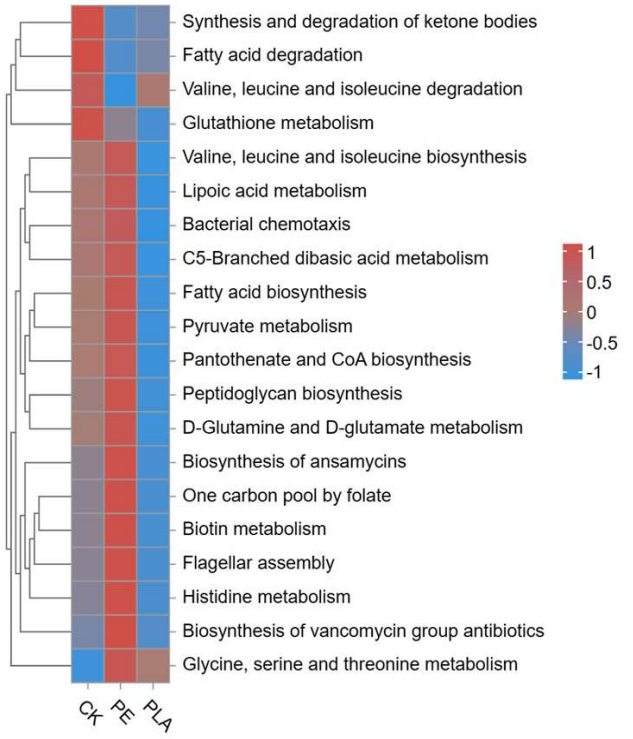

Figure 4. PICRUSt2 predictive analytics functionality difference map

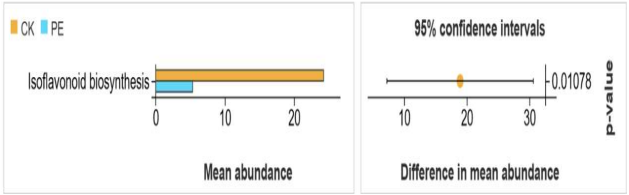

Figure 5. Plot of significant differences in function at level 3 of the PICRUSt2 predictive analysis level

CONCLUSION

In this study, we investigated the effects of different MPs on juvenile marine medaka by analyzing individual growth and development, histopathology, and the gut microbiota. The results showed that exposure to low concentrations of PE and PLA for 60 days did not significantly affect the body length, body weight, or gut organization of juvenile fish, but affected the structure of the gut microbial community, which led to changes in the functional gut flora. PE exposure treatment led to a significant downregulation of isoleucine biosynthesis, suggesting that long-term exposure to PE may have a greater effect on juvenile fish.

ACKNOWLEDGMENT

This work was financially supported by theNational university student innovation experiment project (No. 202311810023).

REFERENCES

Uy, C. A., & Johnson, D. W. (2022). Effects of microplastics on the feeding rates of larvae of a coastal fish: direct consumption, trophic transfer, and effects on growth and survival. Mar Biol, 169(2), 27.

Cui, J., Zhang, Y., Liu, L., Zhang, Q., Xu, S., & Guo, M. Y. (2023). Polystyrene microplastics induced inflammation with activating the TLR2 signal by excessive accumulation of ROS in hepatopancreas of carp (Cyprinus carpio). Ecotoxicol Environ Saf, 251, 114539.

Wilcox, C., Sebille, E. V., & Hardesty, B. D. (2015). Threat of plastic pollution to seabirds is global, pervasive, and increasing. P Natl Acad Sci Usa, 112(38), 11899-11904

Zuo, L. Z., Li, H. X., Lin, L., Sun, Y. X., Diao, Z. H., Liu, S., Xu, X. R. (2019). Sorption and desorption of phenanthrene on biodegradable poly(butylene adipate co-terephtalate) microplastics. Chemosphere, 215, 25-32.

Global plastic waste, 2023. https://www.statista. com/markets/410/topic/960/plastic-rubber/#overview. (Accessed 4 April 2024).

Duan, Z., Cheng, H., Duan, X., Zhang, H., Wang, Y., Gong, Z., Wang, L. (2022). Diet preference of zebrafish (*Danio rerio*) for bio-based polylactic acid microplastics and induced intestinal damage and microbiota dysbiosis. J Hazard Mater, 429, 128332.

Qin, J., Liang, B., Peng, Z., Lin, C., (2021). Generation of microplastic particles during degradation of polycarbonate films in various aqueous media and their characterization.J. Hazard. Mater. 415

Sintim, H.Y., Bary, A.I., Hayes, D.G., English, M.E., Schaeffer, S.M., Miles, C.A., Zelenyuk, A.,Suski, K., Flury, M., (2019). Release of micro- and nanoparticles from biodegradable plastic during in situ composting. Sci. Total Environ. 675, 686–693

Schöpfer L, Menzel R, Schnepf U, et al.(2020). Microplastics effects on reproduction and body length of the soil-dwelling nematode Caenorhabditis elegans [J]. Frontiers in Environmental Science, 8:41.

Zhang S W, Ren S, Pei L, et al (2022). Toxicological effects of polyethylene and polylactic acid microplastics on earthworms [J/OL]. Chinese Journal of Applied and Environmental Biology: 1-9

Botterell, Z. L. R., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R. C., & Lindeque, P. K. (2019). Bioavailability and effects of microplastics on marine zooplankton: A review. Environ Pollut, 245, 98-110.

Castro-Castellon, A. T., Horton, A. A., Hughes, J. M. R., Rampley, C., Jeffers, E. S., Bussi, G., & Whitehead, P. (2022). Ecotoxicity of microplastics to freshwater biota: Considering exposure and hazard across trophic levels. Sci Total Environ, 816, 151638.

Lu, L., Luo, T., Zhao, Y., Cai, C., Fu, Z., & Jin, Y. (2019). Interaction between microplastics and microorganism as well as gut microbiota: A consideration on environmental animal and human health. Sci Total Environ, 667, 94-100.

Fjellheim, A. J., Klinkenberg, G., Skjermo, J., Aasen, I. M., & Vadstein, O. (2010). Selection of candidate probionts by two different screening strategies from Atlantic cod (Gadus morhua L.) larvae. Vet Microbiol, 144(1-2), 153-159.

Fen, Z., Yating, W., Tao, Z., Yun Kit, Y., Qin, L., Lin, Z., Hui, Z., Wenqi, L., Wenye, X., Grace C Y, L., Amy Y L, L., Chun Pan, C., Chun Kwok, W., Paul K S, C., Francis K L, C., & Siew C, N. (2021) Prolonged impairment of short-chain fatty acid and L-isoleucine biosynthesis in gut microbiome in patients with COVID-19., Gastroenterology, 162.2: 548-561.e4.

****