Accumulation of microplastics in medaka fish and their effects on reproduction

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（メダカにおけるマイクロプラスチックの蓄積とその繁殖影響）

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論　文　内　容　の　要　旨

Plastics are the most abundant type of marine artificial debris. Pollution of the plastic litter in marine environments has been reported at the sea surface, shorelines and seafloor. There is an increasing scientific concern about microplastics, which is defined as small plastic fragments less than 5 mm in size (microplastics, MPs). They can potentially be ingested by aquatic animals, including marine invertebrates, fish, seabirds and mammals. They may cause some adverse effects such as intestinal blockage, decreased feeding, energy reserves depletion, alteration of behavior, and reproduction. Thus, this study was performed to elucidate accumulation of microplastics in fish and their effects on reproduction.

First, the abundance, and the types of MPs in the water column and sediments of inland sea in Kyushu, Japan was investigated. 127 water column samples and 27 sediment samples were collected from Ariake Sea (May 2015 and December 2017). The collected samples were applied to count the number of MPs and identify its quality. Before counting, MPs were floated from the sediment samples pretreated with H_2O_2. The separated particles were identified as plastics using Fourier transform-infrared spectroscopy. The average number of MPs particles was 1.12 particles/m^3 in water column and 11.3 particles/kg in sediment. The types of MPs in water column were polypropylene (PP), polyethylene (PE), polystyrene (PS), and fiber. The MPs found in sediment samples were PP. PE, polyethylene terephthalate (PET), and resin. These results suggest that MPs pollution in Ariake Sea is the almost same level as other sites in Japan.

In the second, bioaccumulation of polystyrene microplastics (PS-MPs) in Japanese medaka, *Orysias latipes*, was elucidated. And its impacts on survival, reproduction, and gene expression were assessed. Japanese medaka was exposed to 2-µm fluorescent PS-MPs (10^7 particles/L) for 3 weeks. PS-MPs accumulated in the intestine, and the average concentrations in the intestines were $1.43 \pm 2.1 \times 10^9$ particles/L for 14 days of exposure. The bioaccumulation factor (BCF) of PS-MPs to medaka whole body was calculated to 1×10^2. No significant effects on survival and reproduction were observed. However, the mRNA sequencing analysis showed an increase of expression in genes involved in the cell adhesion, xenobiotic metabolic process, brain development, and other functions in medaka intestines after MPs exposure. These results suggest that PS-MPs can accumulate in intestines but have limited toxicity to Japanese medaka at a concentration up to 10^7 particles/L.

In the third experiment, the marine medaka, *Oryzias javanicus* was exposed to PS-MPs (2 µm, 10^7 particles/L) for 3 weeks, and the depuration period was for 2 weeks. The impacts of PS-MPs on the

survival and bodyweight of Java medaka were also investigated. The MPs exposure had no significant effects on survival and reproduction. MPs were rapidly accumulated in fish bodies and saturated in the intestine during the exposure period and then rapidly eliminated when fish was transfer to clean water. The BCF of MPs was estimated to about 4×10^2, which was ca. 4-fold higher than that of freshwater medaka fish. One compartment model analysis was adapted, and its elimination constant, the uptake constant, the biological half-life time and the mean residence time were estimated to be 0.8, 324, 0.855, and 1.2, respectively. These uptake and elimination value of PS-MPs supported the BCF value.

In summary, MPs were detected in water column and sediment samples of Ariake Sea with concentration of 1.12 particles/m^3 in water and 11.3 particles/kg in sediment. The MPs could accumulate in the intestines of fresh water and marine water medaka but had limited adverse effects. Further study is necessary to investigate the impacts of MPs mixed with chemicals on different life stages of aquatic organisms, including the embryonic, juvenile, and adult stages.