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Evaluation of Effects of Environmental Pharmaceuticals on Fish Reproduction

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Environmental pharmaceuticals prescribed in Japan and expected to be detected at high concentrations in environmental water include G-protein-coupled receptor inhibitors (GPCR inhibitors) and antidepressants. Since these pharmaceuticals act on nerve cells, it is highly likely that they will affect fish via the central nervous system and neuroendocrine system, especially the brain, thereby interfering with their normal behavior and physiological functions. Therefore, this study was conducted to elucidate the dynamics of environmental pharmaceuticals in bodies of water and relate these chemicals to cellular, molecular and individual responses, as well as to evaluate their effects on the ecosystem. This research was conducted from the perspectives of sub-theme 1, "Analysis of the effects of environmental pharmaceuticals on fish behavior and reproduction," sub-theme 2, "Understanding the actual presence of environmental pharmaceuticals in aquatic environments," and sub-theme 3, "Identification and evaluation of fish biomolecules that are targets of environmental pharmaceuticals."

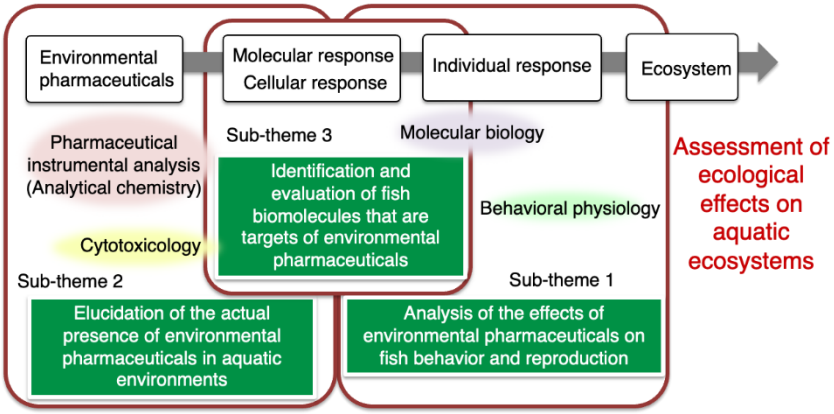

Fig. 1 Understanding the linkage between actual contamination of environmental pharmaceuticals and their effects on individual organisms and ecosystems.

The results were 1) creation of an in vitro assay for quantifying the concentration of environmental pharmaceuticals in bodies of water (equivalent quantity) by using a cell line expressing fish receptors that are a target of pharmaceuticals (Fig. 2), and 2) development of simultaneous analysis using a mass spectrometer, which makes it possible to measure the concentrations of environmental pharmaceuticals and some metabolites in bodies of water. These enable efficient analysis of the actual state of environmental pharmaceuticals present in bodies of water. In addition, analyzing the effects of

environmental pharmaceuticals on fish behavior and reproductionclarified that 3) swimming and spawning behavior are inhibited by pharmaceuticals targeting monoamine transporters (Fig. 3), and 4) reproduction (next-generation production) is affected through a reduction in the number of spawned eggs. In addition, a comprehensive analysis of response genes using pharmaceutical-exposed individuals revealed that 5) some genes increase or decrease as a result of exposure to pharmaceuticals, and that circadian rhythm-related genes, including central clock molecules, in particular may be affected. These results are indispensable for understanding the extent of environmental-pharmaceutical contamination of rivers, treated wastewater and other bodies of water, and the biological effects caused thereby, including the mechanisms of action. The foundation has been completed for establishing an adverse outcome pathway (AOP) that includes effects on the ecosystem.

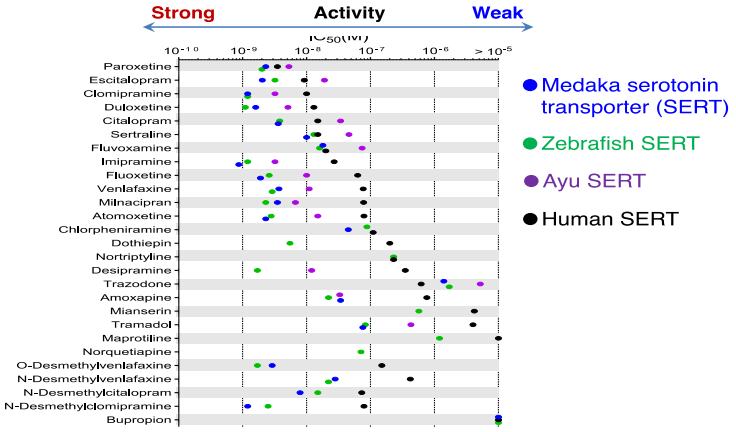

Fig. 2 Determination of pharmacological activity of antidepressants.
An in-vitro assay was performed to quantify the concentration (equivalent quantity) of environmental pharmaceuticals in bodies of water using a cell line expressing the fish receptor targeted by the pharmaceutical (Ihara et al., 2021). The fish monoamine transporter was proven to be strongly inhibited by environmental pharmaceuticals. Some environmental pharmaceuticals have a greater impact on fish than on humans.

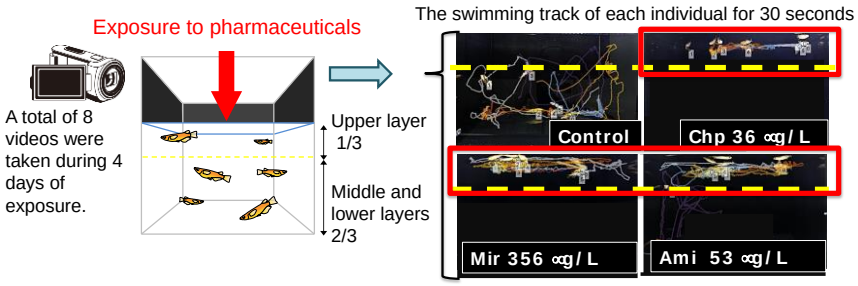

Fig. 3 Analysis of the effects of environmental pharmaceuticals on the behavior of *medaka*.
The behavioral effects of mirtazapine (Mir), chlorpromazine (Chp), and amitriptyline (Ami) were tested. The behavior was recorded on video and analyzed using tracking software. Antidepressants and antipsychotics act on the central nervous system of fish, inducing behavioral effects (surface swimming). Drugs that do not primarily target the central nervous system, such as antiallergic drugs, have no effect.

<Journal articles>

Ihara, M., Zhang, H., Ihara, M. O., Kato, D. and Tanaka, H. (2021) Proposal for fluorescence-based in vitro assay using human and zebrafish monoamine transporters to detect biological activities of

antidepressants in wastewater. *Science of the Total Environment*, 770: 144665.

Zhang, H., Ihara, M.O., Nakada, N., Tanaka, H. and Ihara, M. (2020) Biological-activity-based prioritization of pharmaceuticals in wastewater for environmental monitoring: G protein-coupled receptor inhibitors. *Environmental Science and Technology*, 54(3): 1720-1729.