JCIA research update

Takashi Mori (JCIA)
Yoshifumi Horie (Kobe University)

JCIA-LRI Microplastics research projects

Ongoing

- Assessing sources, emissions and environmental risk of microplastics in support of effective risk reduction strategies (2023-)
- **Ecotoxicological risk assessment of microplastics –as a model case of Osaka Bay- (2023-)**
- Characterization of microplastics for their effects on aquatic organisms and proposal of new guidelines for ecotoxicity testing (2024-)
- Quantitative assessment of the pharmacokinetics of MP as vectors in fish: Implementation of a novel digestive tract administration technique (2024-)

Completed

- Elucidation of the mechanism and rate of microplastic formation leading to risk assessment and preparation of standard microplastics (2022-2024)
- Construction of kinetic model to microplastic-vector effect using artificial bioconcentration device (2022-2024)

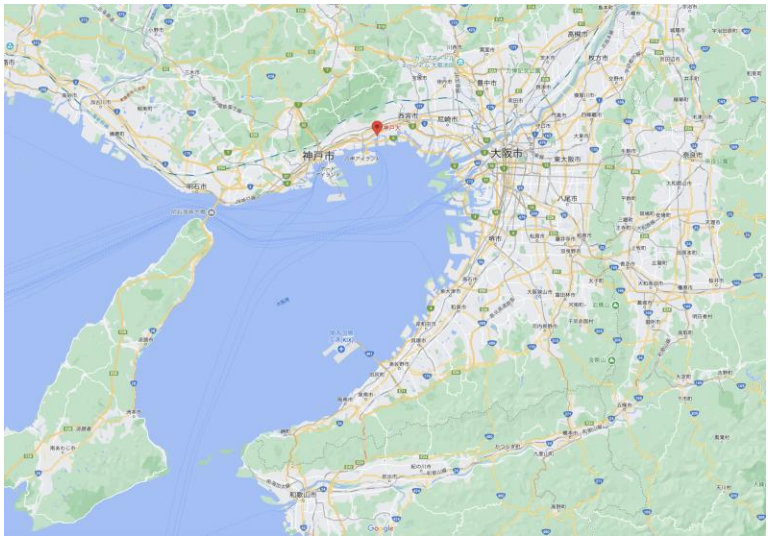
Others

- Microplastics session to be held at the 53rd annual meeting of Japanese Society of Toxicology (July 2026)
- New research project for human health is being discussed.

Ecotoxicity of Microplastics Using Fish

Kobe University
Research Center for Inland Seas
Professor
Yoshifumi Horie

Kobe University （Faculty of Oceanology）



Ship harbor on campus

Small training vessel, Hakuoh

Training ship, Kaijinmaru

The Faculty of Oceanology employs many ship crew members, which makes it easy to conduct surveys of Osaka Bay using research vessels

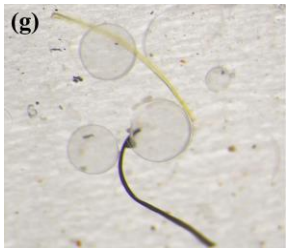
Purpose of study（LRI project period: 2023-2025）

This work was supported by a grant from the Long-range Research Initiative (LRI) of the Japan Chemical Industry Association(JCIA)

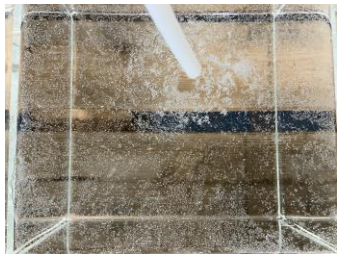
- There are many reports about microplastic survey, development new analysis, harmful for aquatic organisms.
- Increasing number of articles about combined effect of microplastics and chemicals.

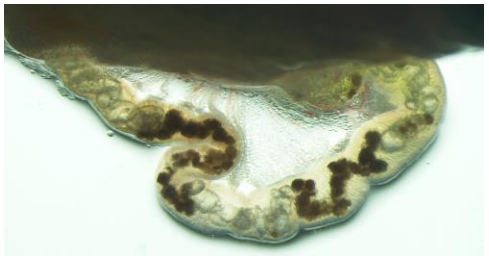
Are microplastic in the water environment harmful to the ecosystem?

MP survey at Osaka bay



MP ecotoxicity by using fish

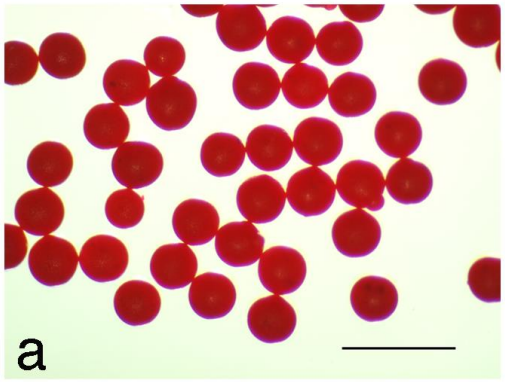


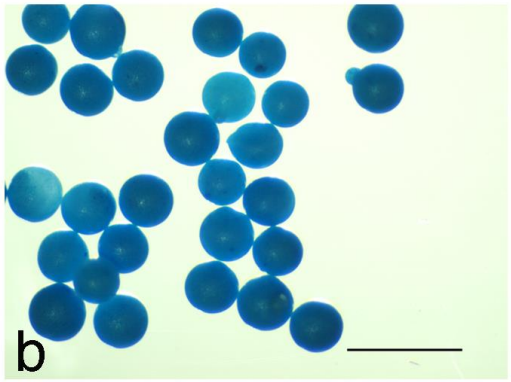


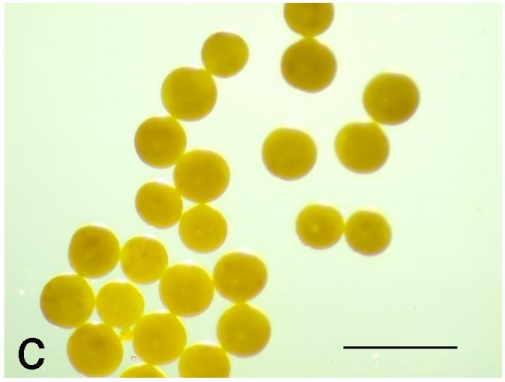
Background

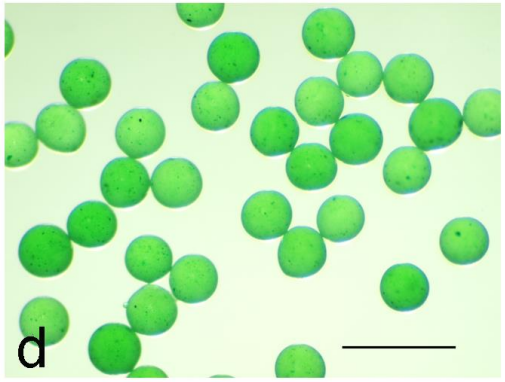
Does fish have color preferences of MP?

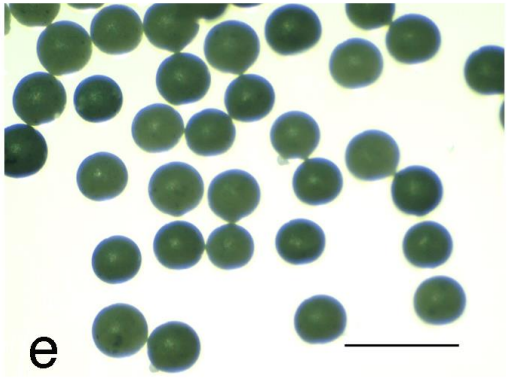
Okamoto, Horie et al, Environmental Pollution, 2022

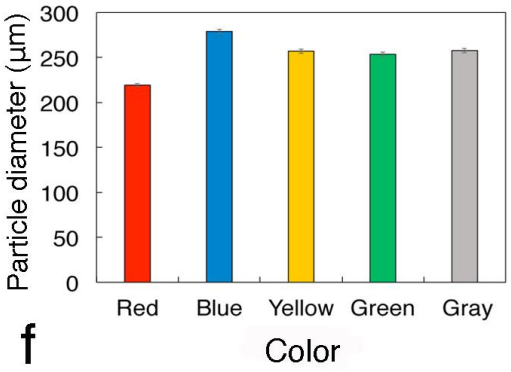


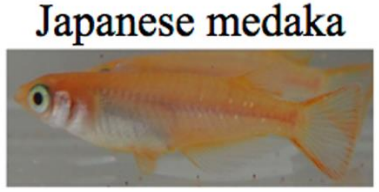


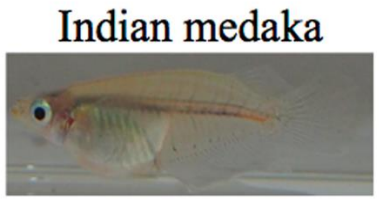


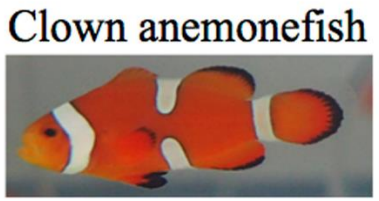


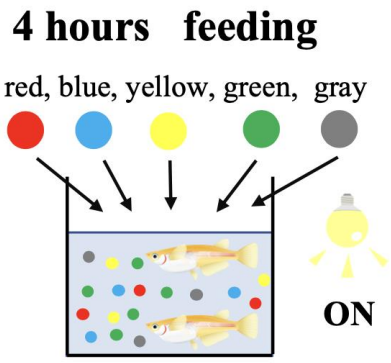


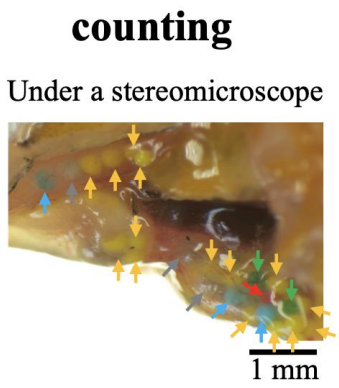


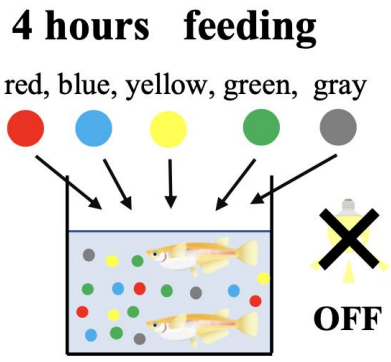


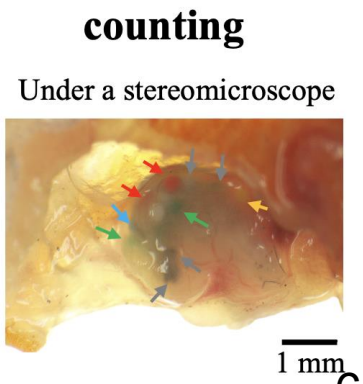






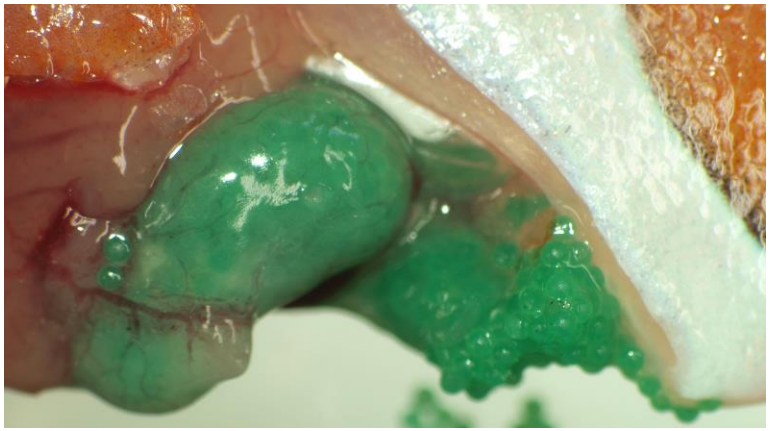


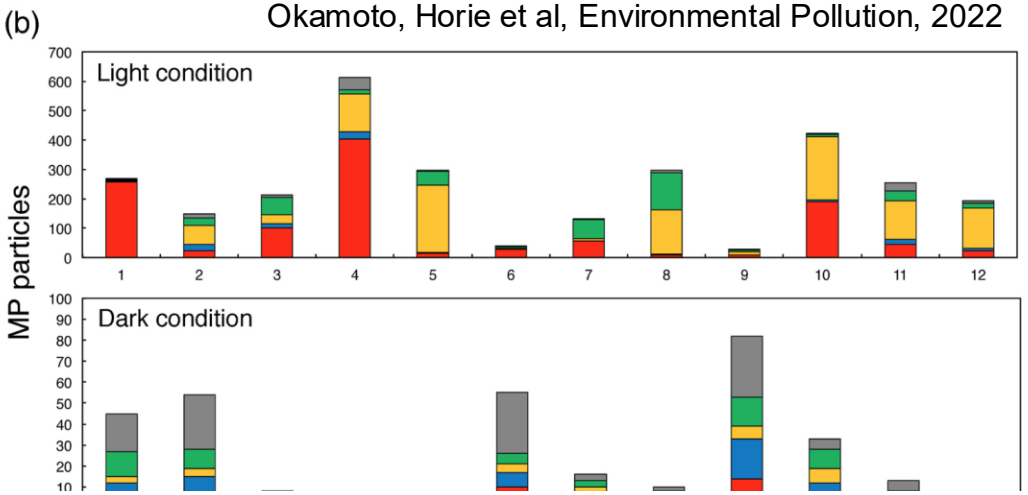


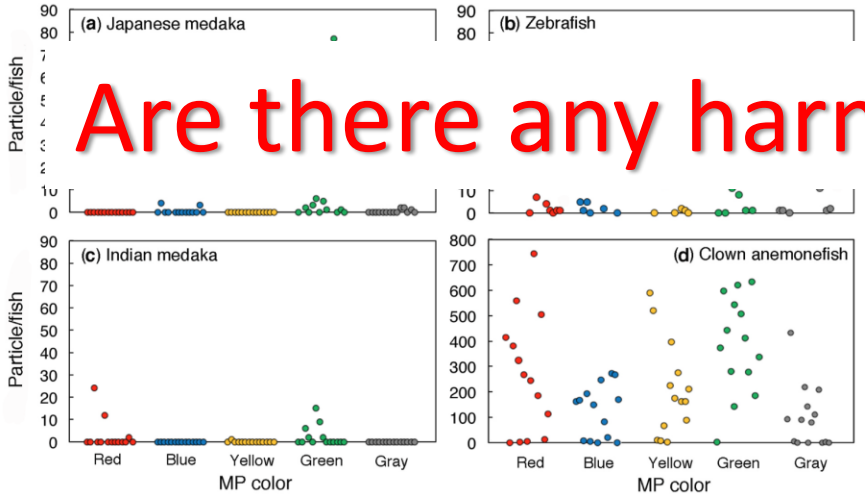


Background

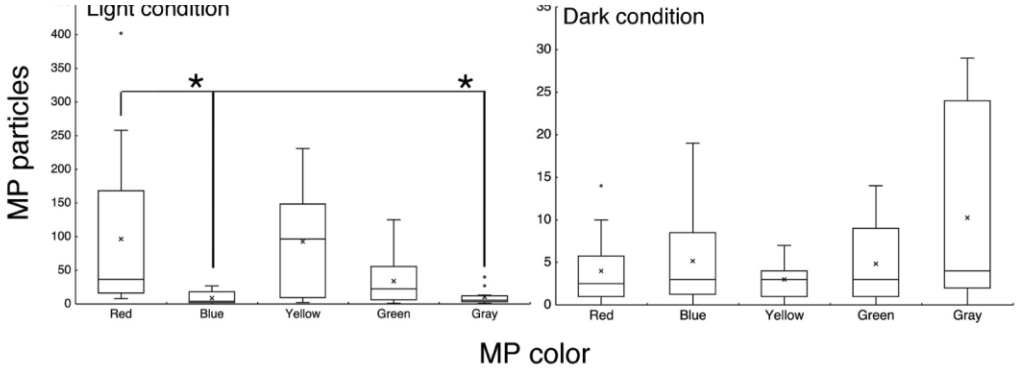
Clown anemonefish preferred red, yellow, and green







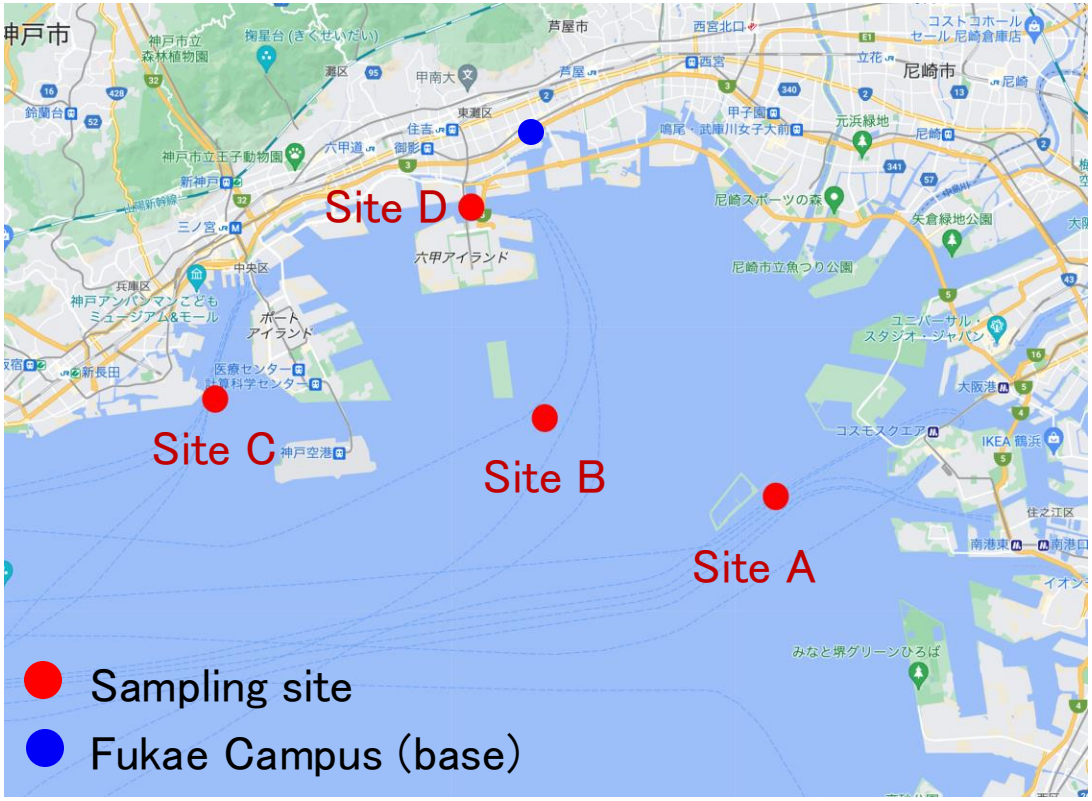
Are there any harmful effects if ingested?



The zebrafish and clown anemonefish both ingested large numbers of MP particles compared to medaka.

The clown anemonefish rely on color vision to recognize MP colors and express color preferences.

Sampling site and day

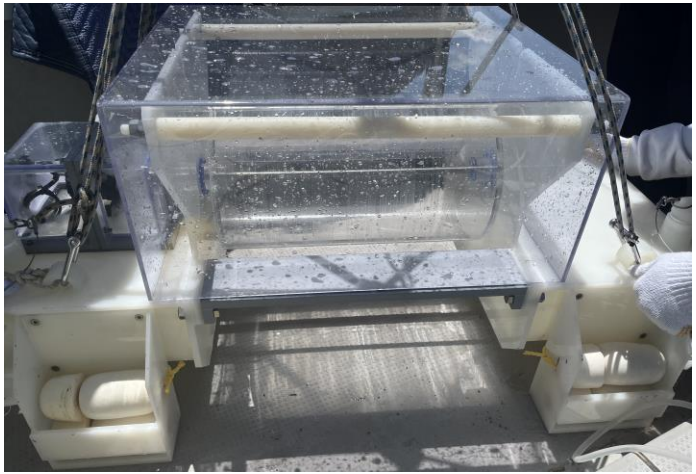


Sampling day

24 th May 2023
18 th July 2023
16 th October 2023

Small training vessel, Hakuoh

Approximately 10 L of surface water was collected by using the custom-made rotating-drum water sampler



Laboratory analysis

10 L of surface water

digested with 30 %–H_2O_2

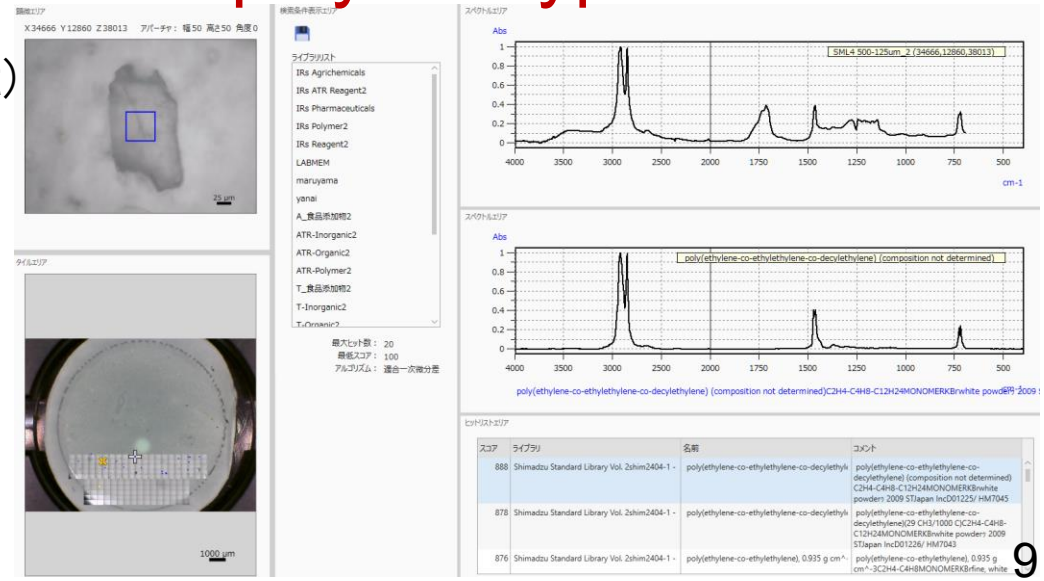
Before digestion

After digestion

categorized to “shape” and “color”

categorized to “size” and “polymer types”

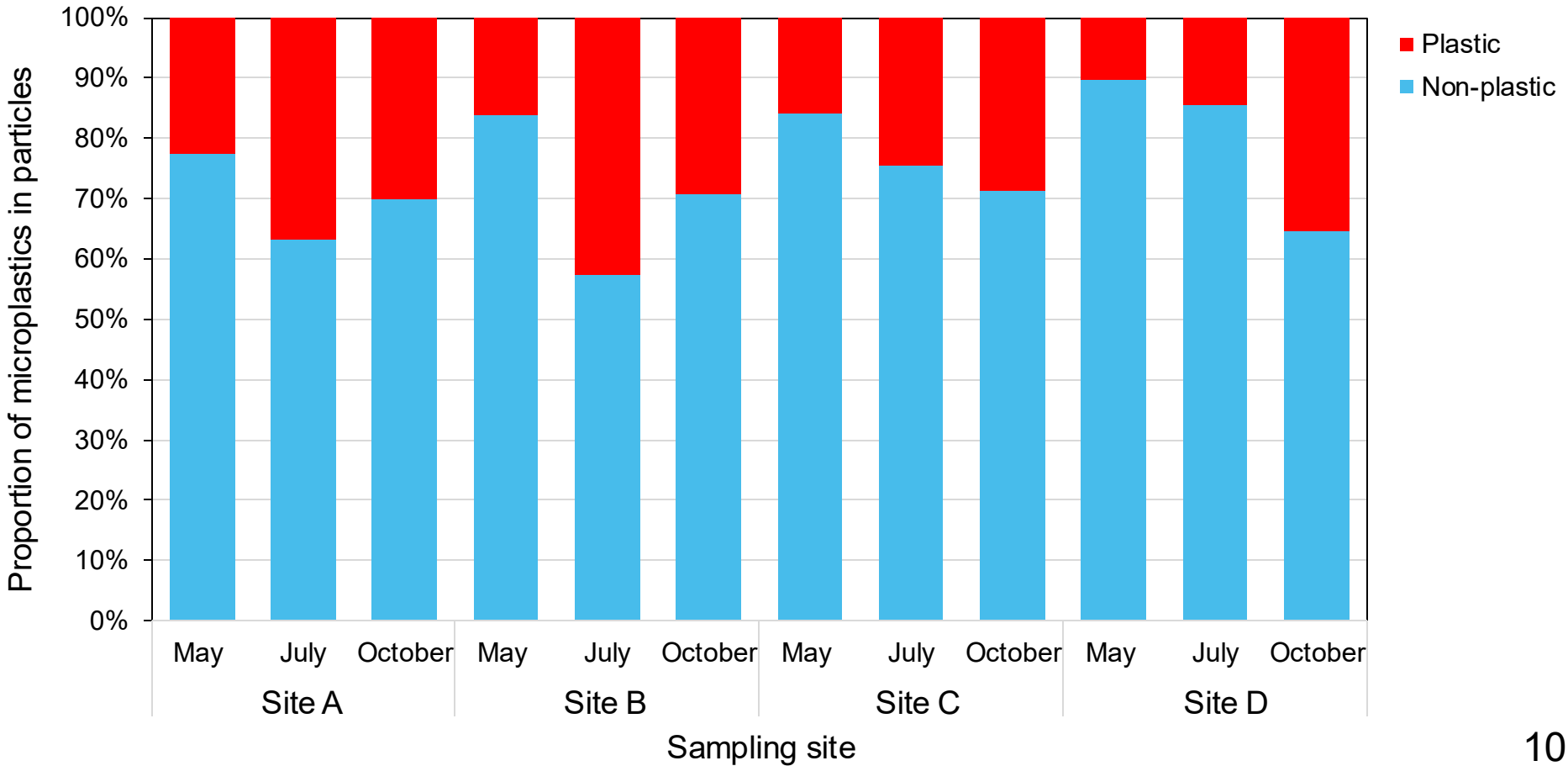
FT-IR microscopy（Shimadzu AIMsight）



Proportion of Microplastics in relation to non-plastic

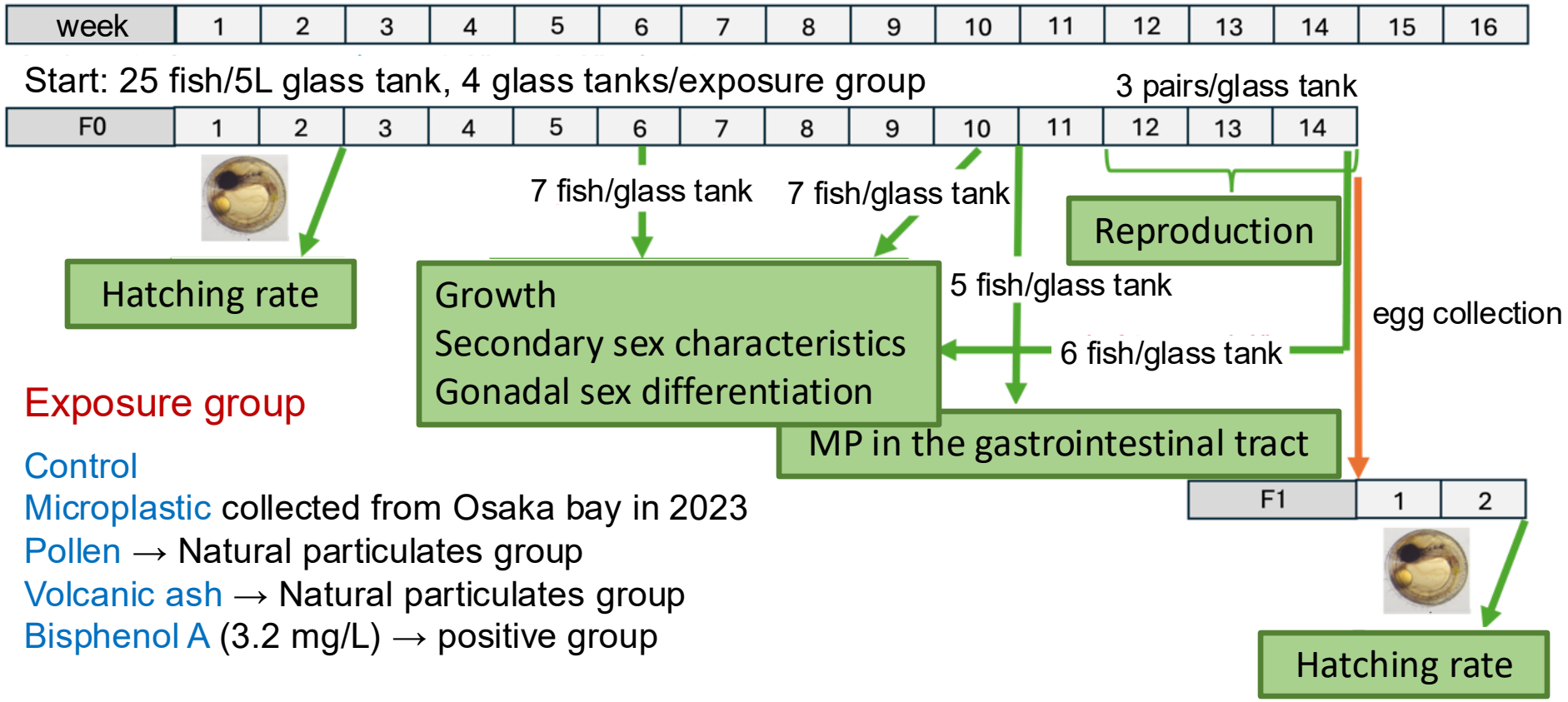
Uaciquete, Horie et al., Environ Sci Pollut Res Int. 2024

	Particles/10 L											
	Site A			Site B			Site C			Site D		
	May	July	October	May	July	October	May	July	October	May	July	October
Non-plastic	329	418	389	548	425	472	400	591	365	669	999	1121
Plastic	96	243	167	105	315	197	76	191	147	77	169	614
Total particles	425	661	557	653	740	668	476	782	512	746	1168	1735



Ecotoxicity testing of MPs collected from Osaka Bay

「The test period and observation endpoints were based on OECD TG 240 (modified MEOGRT)」



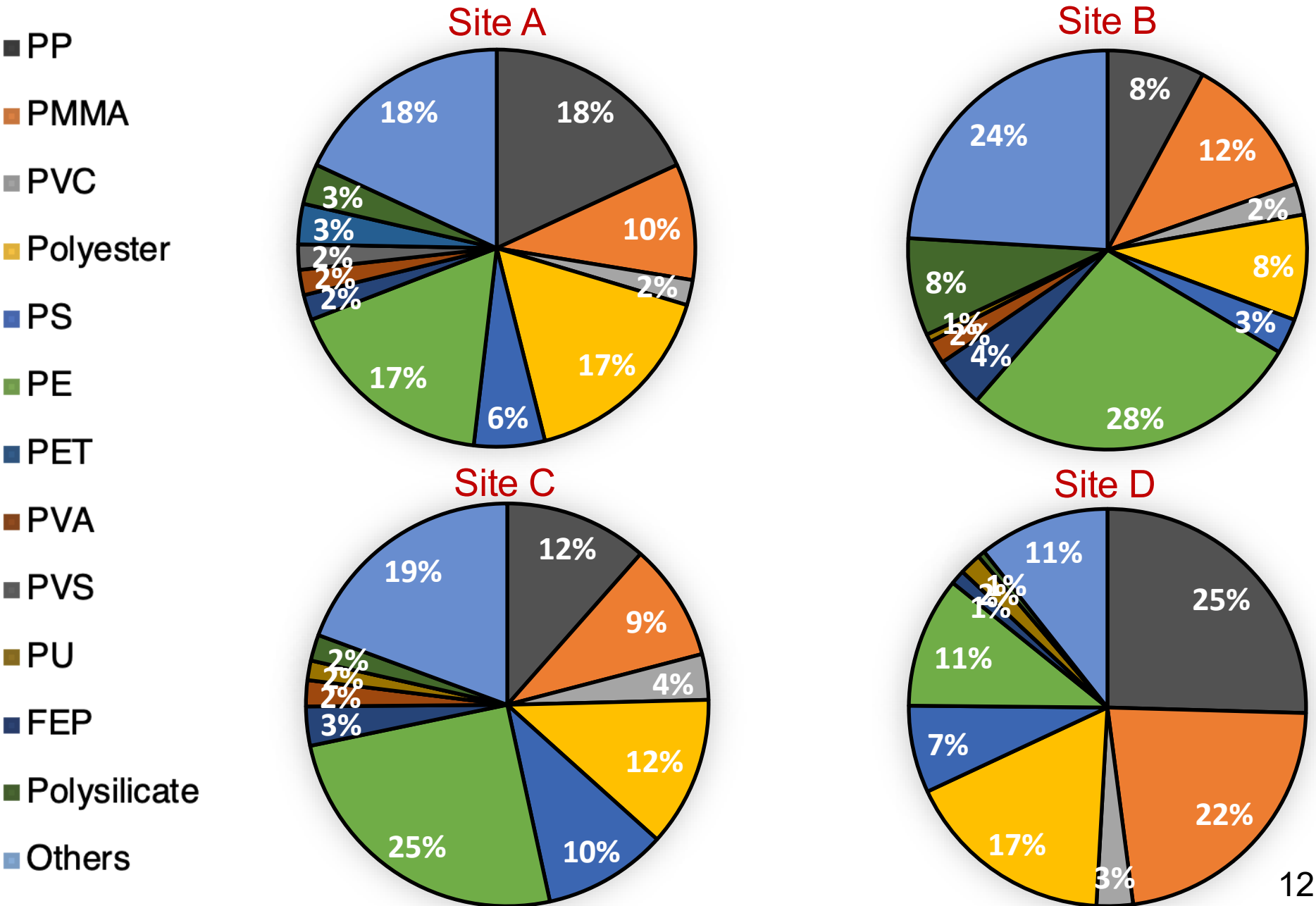
Exposure group

Control
Microplastic collected from Osaka bay in 2023
Pollen → Natural particulates group
Volcanic ash → Natural particulates group
Bisphenol A (3.2 mg/L) → positive group

Measured bishenol A concentration using LC/MS

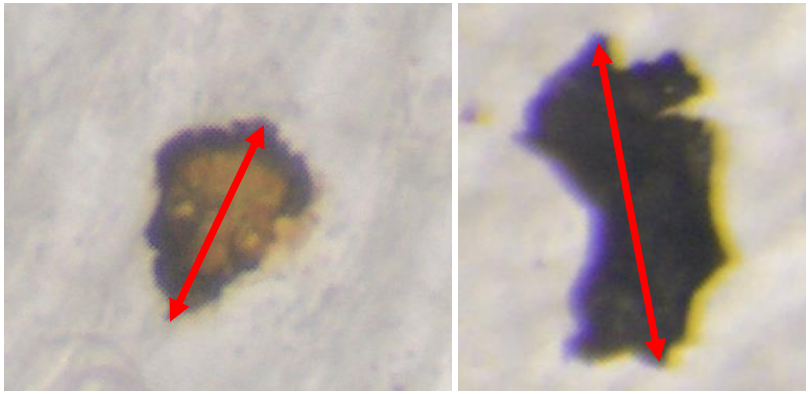
week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Average
BPA level(mg/L)	2.26	2.32	2.34	2.35	2.33	2.33	2.32	2.23	2.26	2.27	2.25	2.37	2.38	2.41	2.32

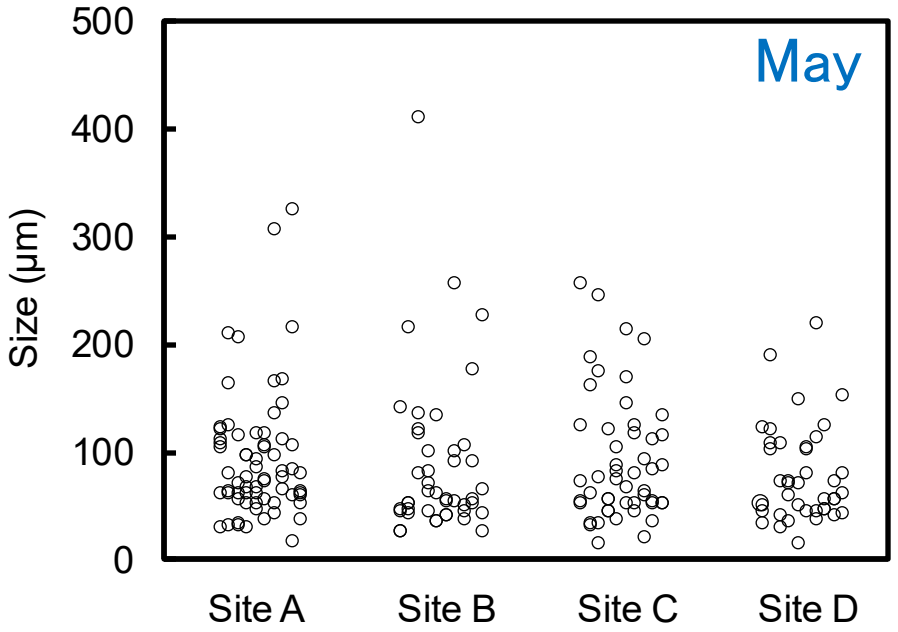
Composition of microplastics _Osaka bay July survey

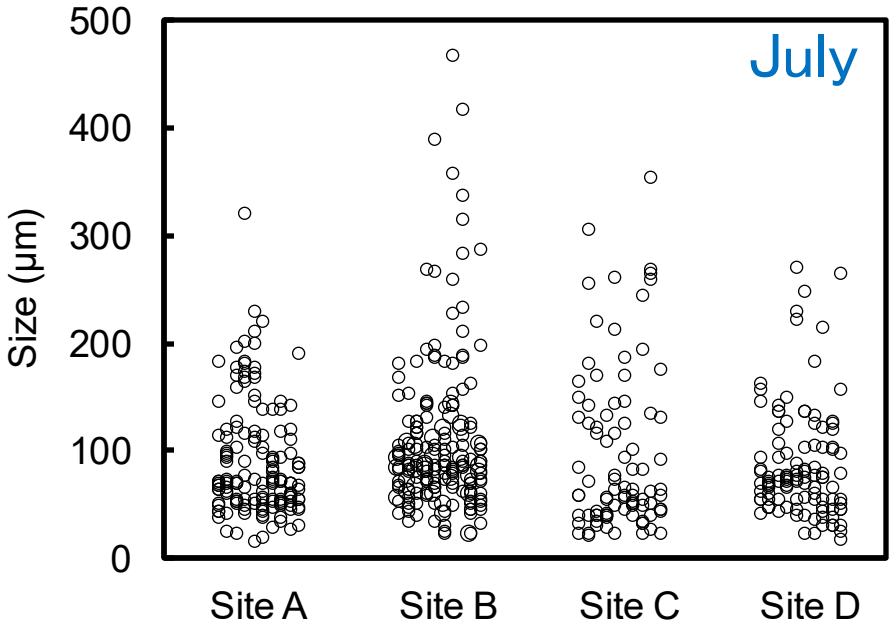


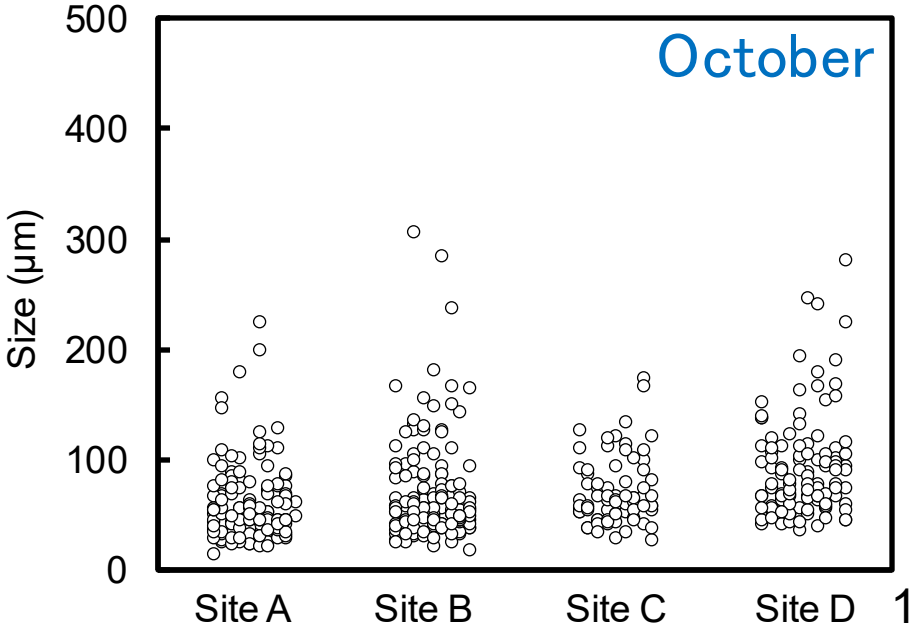
Size of microplastics _Osaka bay

Measurement (Fragment)

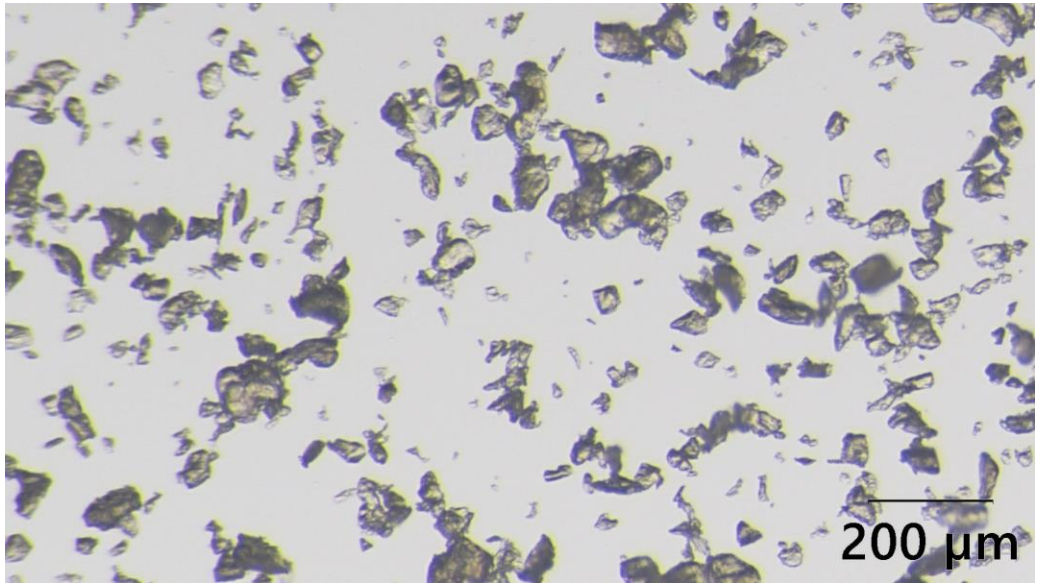




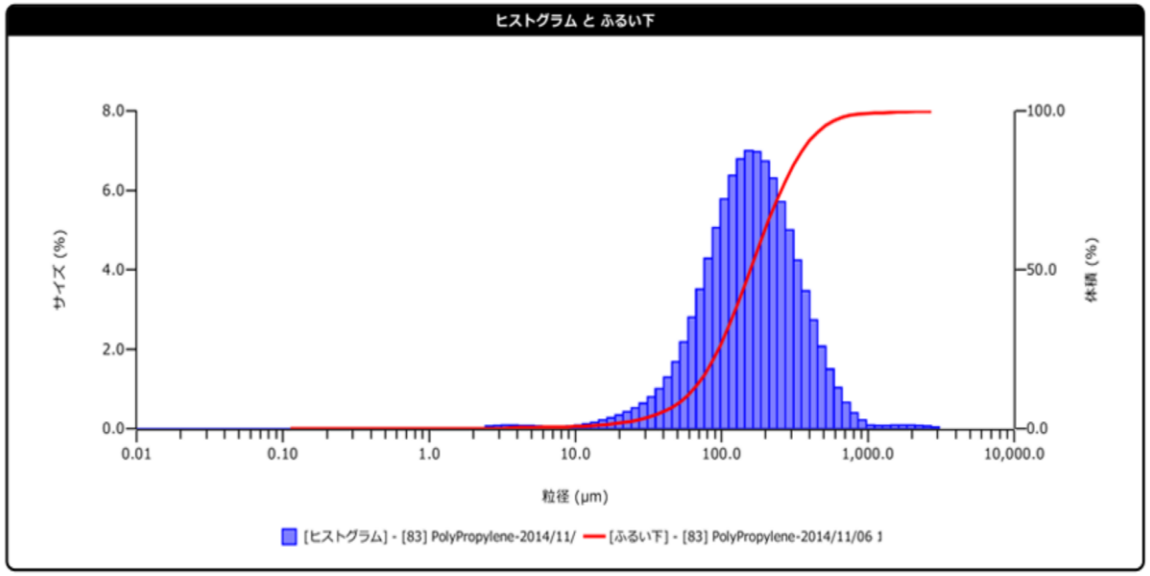




Ecotoxicity testing of polypropylene



Particle size

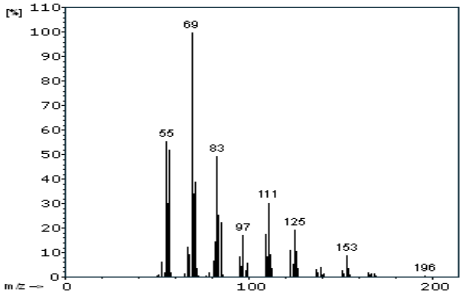


PY-GCMS analysis

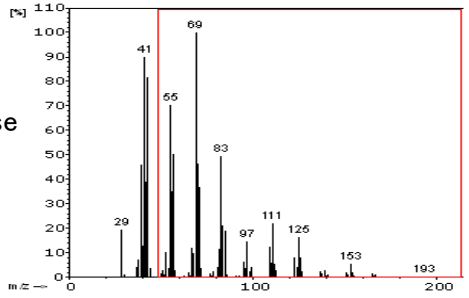
Polypropylene ; PP

Preliminary analysis

Measured data (consistent with the database)



Reference data from the database



【 Analytical conditions 】

PY-3030D

Pyrolysis temp.: 600 ℃

Interface temp.: 300 ℃

GC

Pre column:Ultra ALLOY-50, Main column:Ultra ALLOY+-5

INJ : 300℃, Split raitio : 1/100, Constant pressure : 75kPa

Oven temp. : 50℃-(20℃/min.)-320℃(5min.)

MS(QP-2050)

Interface temp.: 300 ℃, Ionsource temp. : 230 ℃

SCAN mode(m/z : 50-350)

Sample amount : 0.26mg

Test period and endpoints were determined according to OECD TG 240 (modified MEOGRT)

week 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

4 pairs/glass tank

F0 1 2 3

Reproduction

F1 generation: 30 fish/5L glass tank, 5 glass tanks/exposure group

採卵

4 pairs/glass tank

F1 1 2 3 4 5 6 7 8 9 10 11 12 13 14

8 fish/glass tank

8 fish/glass tank

Hatching rate

Growth
Secondary sex characteristics
Gonadal sex differentiation

Reproduction

6 fish/glass tank

8 fish/glass tank

egg collection

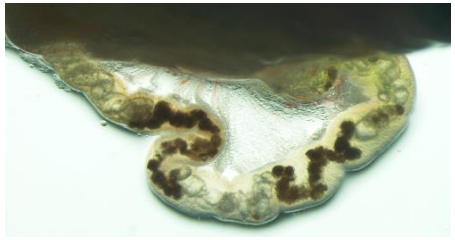
MP in the gastrointestinal tract

F2 1 2

Hatching rate

Exposure group

Control
1 mg/L (5 mg/400 cm^2)
3.2 mg/L (16 mg/400 cm^2)
10 mg/L (50 mg/400 cm^2)
32 mg/L (160 mg/400 cm^2)
100 mg/L (500 mg/400 cm^2)



The qualitative analysis was conducted with PY GC–MS

Summary results

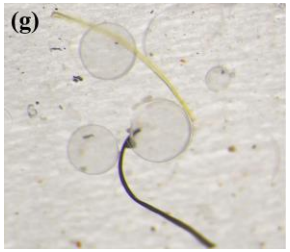
	Microplastic collected from Osaka bay in 2023	Polypropylene
Ingestion	Confirmed	Confirmed
Lethal effect	No effect	No effect
Embryo development	No effect	No effect
Gonadal sex differentiation	No effect	under analysis
Secondary sexual characteristics	No effect	under analysis
Reproduction	No effect	No effect

No adverse effects were observed in Japanese medaka after long-term exposure to microplastics collected from Osaka Bay

The final goal of this project

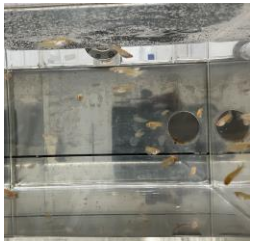
Are microplastic in the water environment harmful to the ecosystem?

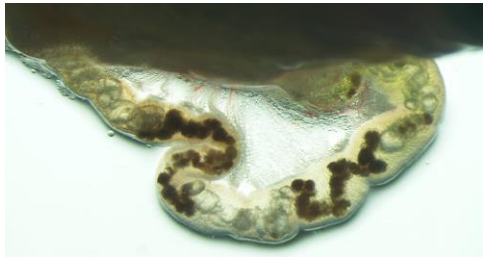
MP survey at Osaka bay



Q1. The residual concentration of microplastics will be determined using PY–GC–MS.

MP ecotoxicity by using fish





Q2. What are the effects of long-term exposure (modified MEOGRT) to microplastics collected from Osaka Bay in Japanese medaka (*Oryzias latipes*)?

Q3. What concentration of polypropylene causes harmful effects on Japanese medaka in the modified MEOGRT test?