



## Monitoring of microplastics toxicity analysis on fish ongoing at the UMR CNRS 5805 EPOC Laboratory, University of Bordeaux (following 13<sup>th</sup>-16<sup>th</sup> June 2016 visit)



As a reminder, the aim of this analysis is to investigate toxicity of environmental microplastics (< 5 mm), collected during the Race for Water Odyssey, in living model.

This study is due to the collaboration between the UMR CNRS 5805 EPOC Laboratory of Bordeaux University and Race for Water Foundation.

Pauline Pannetier, who is currently doing a thesis at Bordeaux University include this microplastics toxicity analysis on fish in her research

([https://www.researchgate.net/profile/Pauline\\_Pannetier](https://www.researchgate.net/profile/Pauline_Pannetier)).

Jérôme Cachot, Professor at University of Bordeaux, supervises this study

([https://www.researchgate.net/profile/Jerome\\_Cachot](https://www.researchgate.net/profile/Jerome_Cachot)).

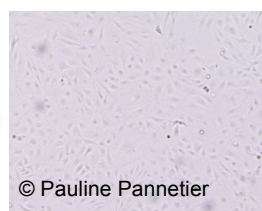
Microplastics samples used on this study were firstly sorted by categories, size and typology at the GR-CEL laboratory of the EPFL before being sent to the EPOC laboratory.

Two main tests have then been assessed, one on Rainbow trout (*Oncorhynchus mykiss*) fish cell line and another on Japanese Medaka (*Oryzias latipes*) fish embryos.

### 1-Test on fish cell line of Rainbow trout (*Oncorhynchus mykiss*) liver (RTL-W1)



Microplastics samples



Cell lines (RTL-W1)

#### ANALYSIS

- Cytotoxicity (MTT assay)
- EROD activity
- Genotoxicity (Comet assay)

All microplastics samples sent by EPFL (Azores, Bermuda, Easter Island, Hawaii and Guam) were tested on fish cell line of rainbow trout liver (RTL-W1) to investigate the toxicity of these environmental microplastics collected on the different beaches

visited during the Race for Water Odyssey ([Annex](#)). It was chosen to test on rainbow trout cell line instead of Japanese medaka cell line, as these last one does not express EROD activity.

Three different toxicity assays were then realised on rainbow-trout cell line: MTT assay (a colorimetric assay for assessing cell survival), EROD activity (protein involved in organic pollutant detoxification and elimination, used as a biomarker of organic pollutant exposure) and Comet assay (a single cell gel electrophoresis assay for evaluating DNA damage in cells).

A part of these first results have been already presented as a poster at Micro 2016 in Lanzarote at the end of May 2016.

## 2-Test on fish embryos of Japanese Medaka (*Oryzias latipes*)



Japanese Medaka embryos at 5 days post-fertilization (EPOC Laboratory)

Following these first results on fish cell line, it has been chosen to use one of the previously shown toxic environmental microplastic sample for testing on fish embryos of Japanese Medaka (*Oryzias latipes*).

This sample comes from Hawaii, Big Island (Kawa Bay): Ha13.

From this microplastic sample, three concentrations have been tested:

- 0.01% (3 µg/L = environmental concentration)
- 0.1% (30 µg/L)
- 1.0% (300 µg/L = maximum concentration reported in the North Pacific Subtropical Gyre<sup>1</sup>)

Extraction in organic solvent (DMSO) by stirring during 16 hours has previously been realised (extraction kinetics tested before).

1% of this extract was used for the fish eggs incubation during less than 10 days (Japanese medaka fish eggs develop at 26°C).

During the growth of these fish eggs different parameters was measured as: mortality, egg development, viability, biometry, hatching rate and developmental abnormalities.

As for test on fish cell lines, two cellular analyses on fish embryos were realised in well-defined date: EROD activity and Comet assay.

Behaviour study was also analysed three days after the hatching (some may dye with the desorption of the yolk sac).

No embryonic mortality, hatching success decline and malformations were observed. In the contrary, a marked increase of EROD activity in liver and a slight decrease of head to body length ratio at the two highest tested concentrations of Ha13 extract were noted.

This first experimental study on fish cell line of Rainbow trout and fish embryos of Japanese Medaka is almost finished. Further experimental studies will be realized on fish embryos with another previously shown toxic environmental microplastic sample, probably coming from Bermuda and/or Guam at the beginning of 2017. However, all these results are still being treated, interpreted and will remain confidential until a first publication expected at the end of 2017.

### **Acknowledgements**

I would like to thank Jérôme Cachot and Pauline Pannetier for allowing me to observe their experiences one week and providing me useful information on their ongoing analysis on fish regarding our microplastics samples collected during the Odyssey.

For more information, you can contact, Jérôme Cachot, University of Bordeaux, EPOC Laboratory, UMR CNRS 5805, [jerome.cachot@u-bordeaux.fr](mailto:jerome.cachot@u-bordeaux.fr).

**Kim VAN ARKEL**

Scientific Advisor

Race for Water Foundation



## References

1. Rochman and *al.*, 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263; DOI: 10.1038/srep03263.
2. Lippiatt and *al.*, 2013. NOAA Technical Memorandum NOS-OR&R-46.

## Annex: To reposition

Microplastics samples tested here, were collected, following the standardized NOAA's protocol<sup>2</sup>, **on 2 to 5 beaches** of each island visited during the **Race for Water Odyssey** (Figure 1).

The Race for Water Odyssey was an environmental expedition initiated by the Race for Water Foundation in 2015 which sailed cross the Atlantic, Pacific and Indian Oceans to assess plastic pollution in all trash vortices in less than a year.

The goals of the expedition were to:

- **Analyze plastic pollution** on beaches of remotes islands,
- **Raise awareness** about water plastic pollution among the population,
- **Identify solutions** by studying waste management processes in significant coastal cities and on islands.

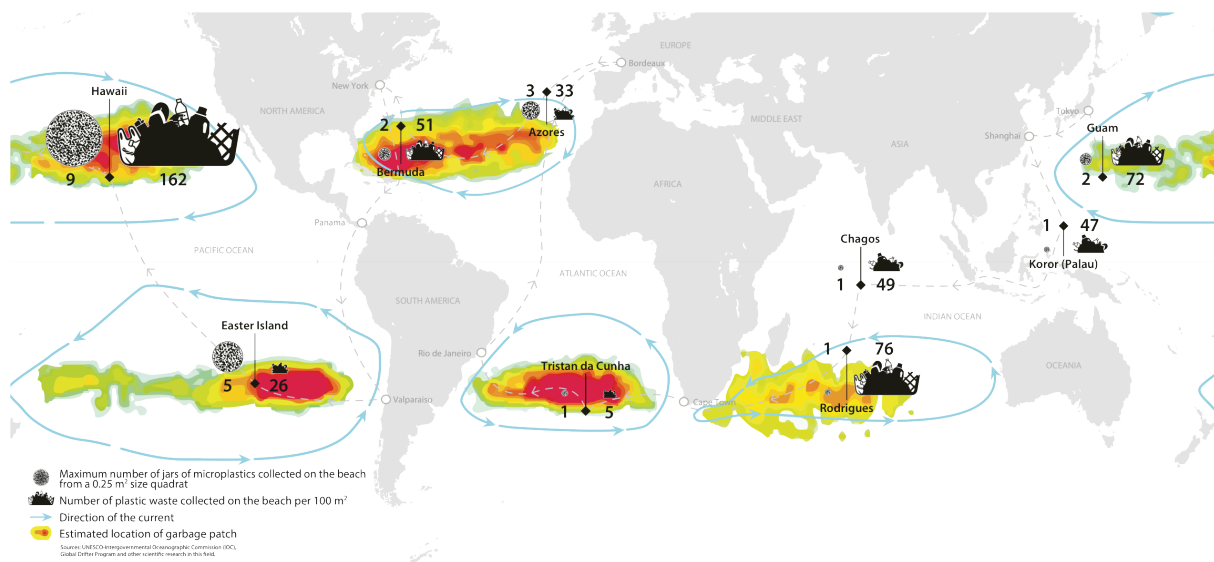


Figure 1: The Race for Water Odyssey, 2015 (**Azores**: March 2015, **Bermuda**: April 2015, **Easter Island**: May 2015, **Hawaii**: June 2015, **Mariana**: July 2015, **Palau**: August 2015, **Mascarene**: September 2015.)

Microplastics samples (<5mm) are currently being analyzed in 3 laboratories:

- **Microplastics typology** at the Central Environmental laboratory (GR-CEL) of the EPFL in Lausanne, Switzerland
- **Analysis of micropollutants adsorbed on microplastics** at the laboratory of HEIA in Fribourg, Switzerland
- **Microplastics toxicity on cell, embryos and fish larvae** at the EPOC laboratory of Bordeaux University, France

