ADDITIVES FROM DIFFERENT POLYMERS AND CONNECTION TO AQUATIC TOXICITY

Anna Rotander

General aims of RESPONSE

Toward a risk-based assessment of microplastic pollution in marine ecosystems

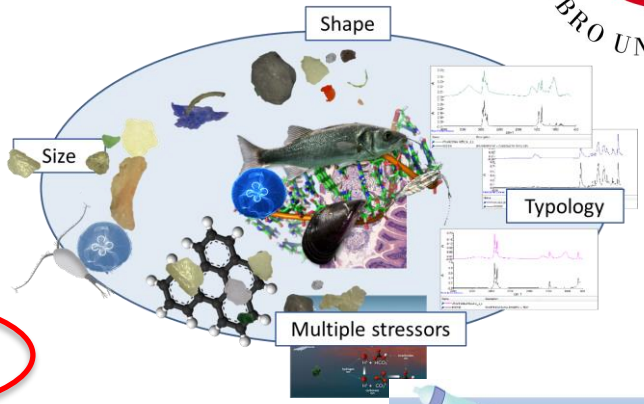
ÖREBRO UNIVERSITET

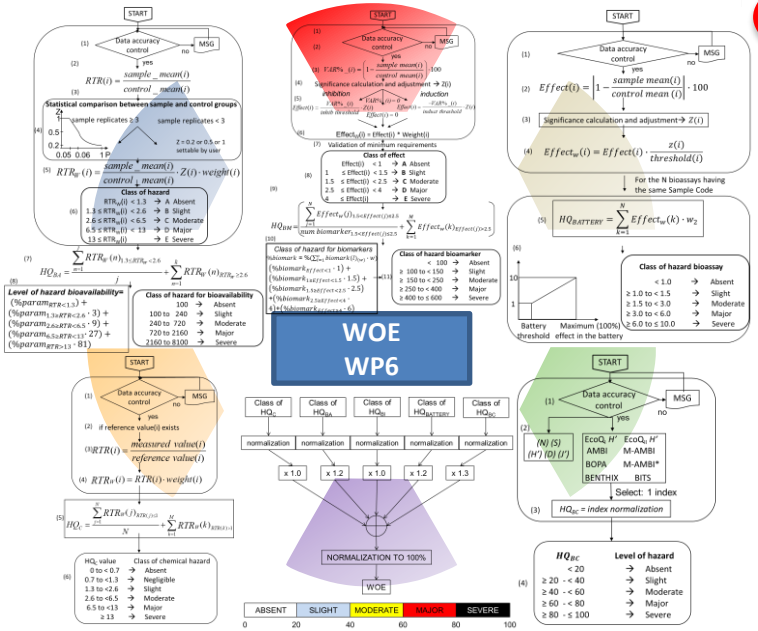
Spatial and temporal distribution of MPs and NPs
WP1

Ingestion/Excretion /Translocation
WP2

Molecular/Cellular Biomarker
WP3

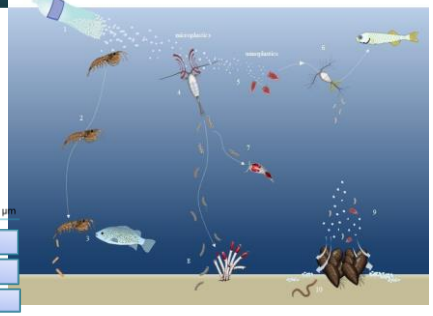
Organism Toxicity
WP4

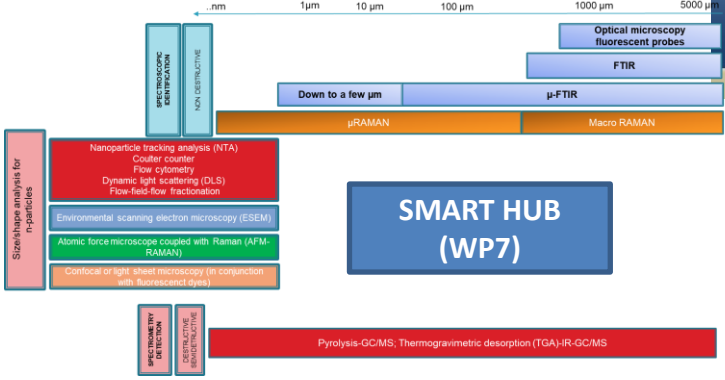




WOE
WP6

Ecology
WP5





SMART HUB
(WP7)

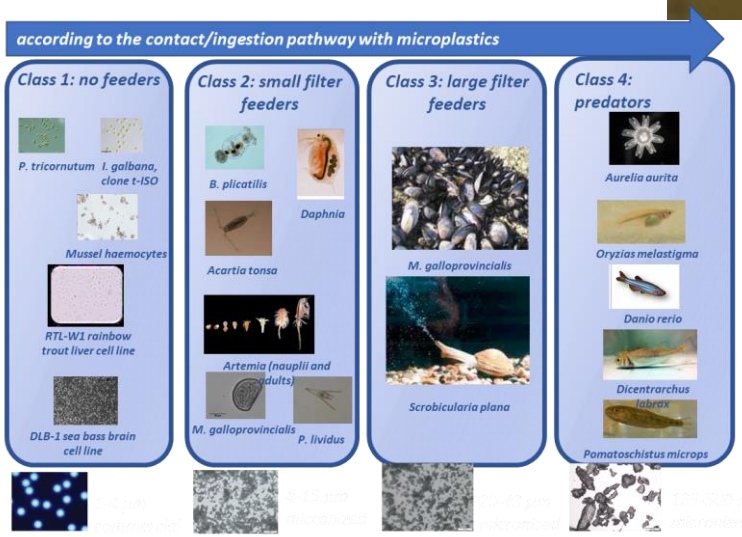
Communication and stakeholder engagement WP8

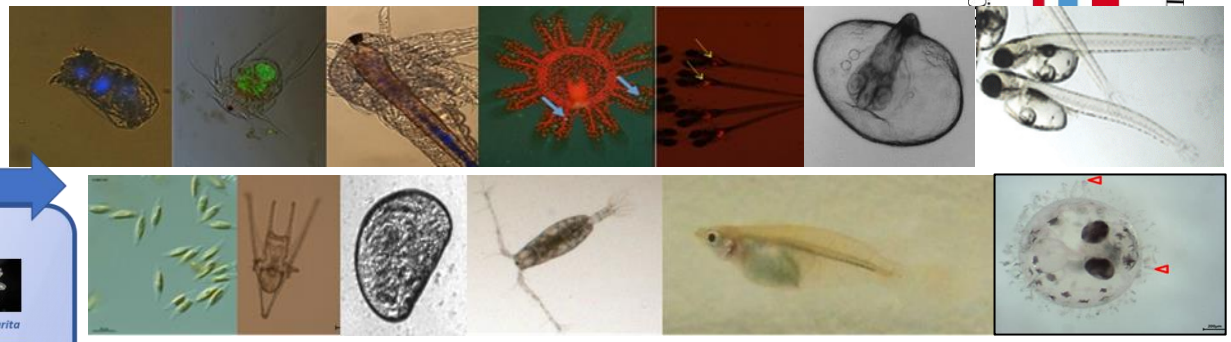
14 Institutions from 11 European countries:
Italy, Belgium, Denmark, Estonia, France, Germany, Ireland, Norway, Portugal, Spain and Sweden

PI: Francesco Regoli

UNIVERSITÀ POLITECNICA DELLE MARCHE

RESPONSE-WP4
Organism toxicity





Objectives:

- to **characterize chronic and long-term effects at organism level** under ecologically relevant exposures to leachates of field-collected plastics and biodegradable polymeric material;

- to **provide insight into biological pathways and mechanisms of action underlying the adverse effects of MPs and NPs at organism level,** including chemical characterization of leachates

- to **assign specific thresholds and weights to individual bioassays and to define standardized batteries of ecotoxicological tests** that are geographically relevant and useful for quantification of hazard quotients (HQs) and integration into the WOE framework.

Methods

Lixviation 72 h

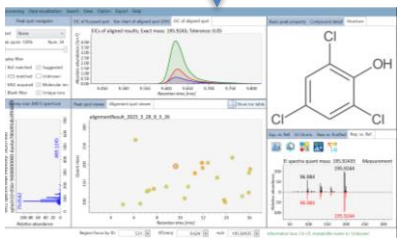
SPE extraction

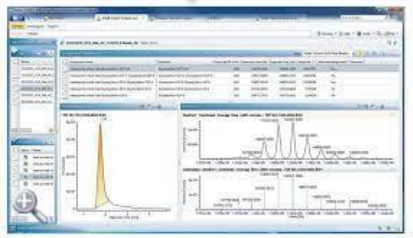
GC fraction

LC fraction

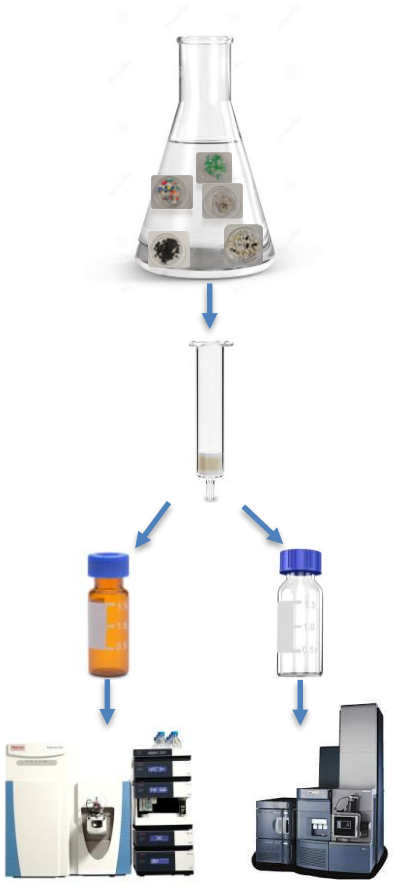
Target/suspect screening

Target/suspect screening





Almeda et al. 2023



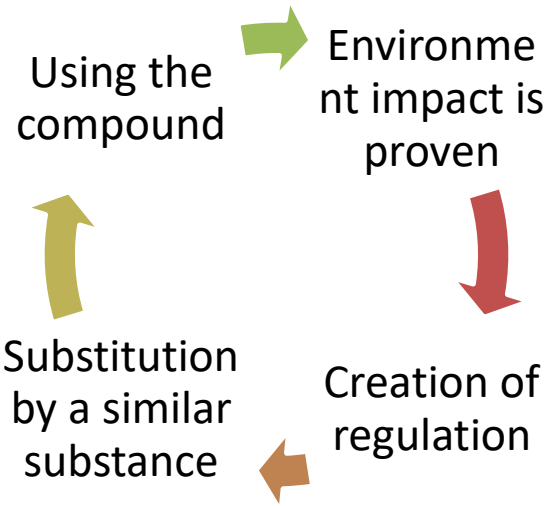
- Challenges with low concentrations of additives
- Challenges with low extracted volumes

Compostable bags and additives

Compostable products, risk of additive release

Some plastic additives present for example endocrine disruption properties

Science diagnostic is slower than industry



Cyprinodon variegatus transcriptomic alterations caused by compostable plastic bag lixiviates

Lixiviate

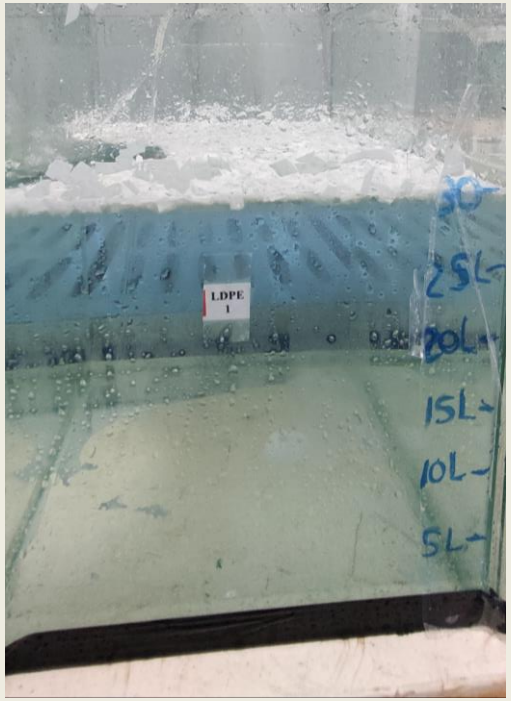
≈ 5 mm plastic pieces
24 h in water with intense aeration

The water was filtered and placed into the experimental containers

A. Martínez and R. Beiras, UVIGO

GC-HRMS screening

LDPE



369 features

Mater-Bi

844 features

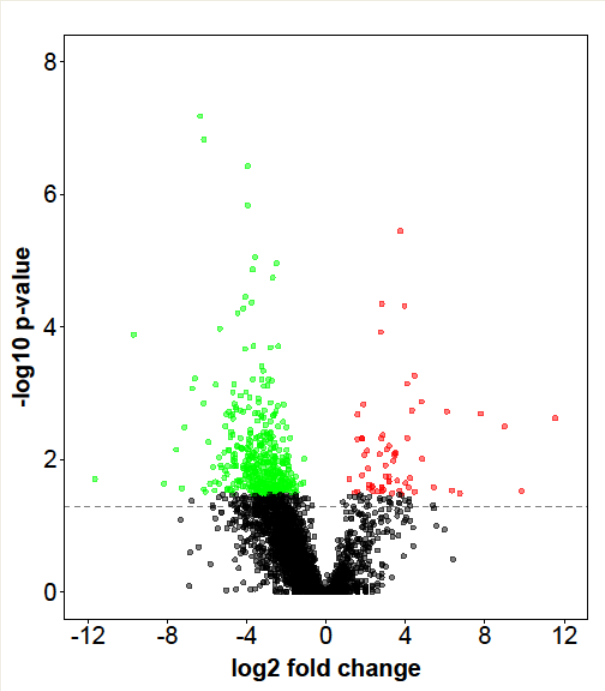
Bag Brown

1489 features

After 5 fold blank filtration

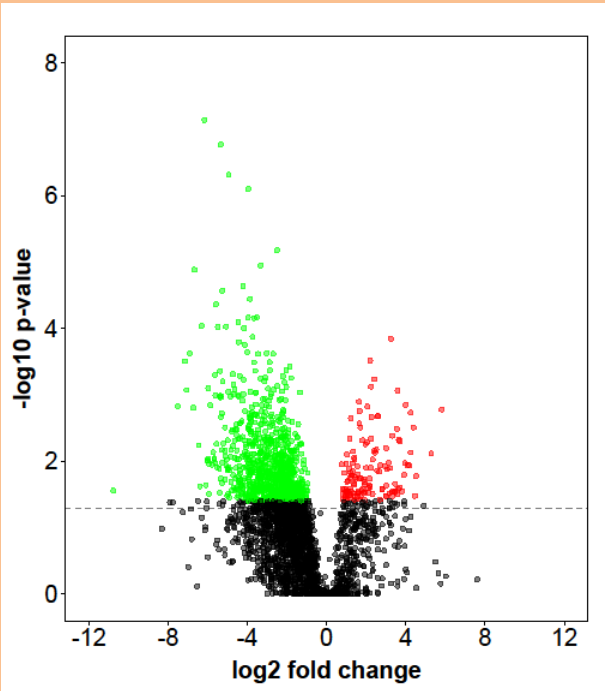
Transcriptomics alterations in *Cyprinodon variegatus*

LDPE



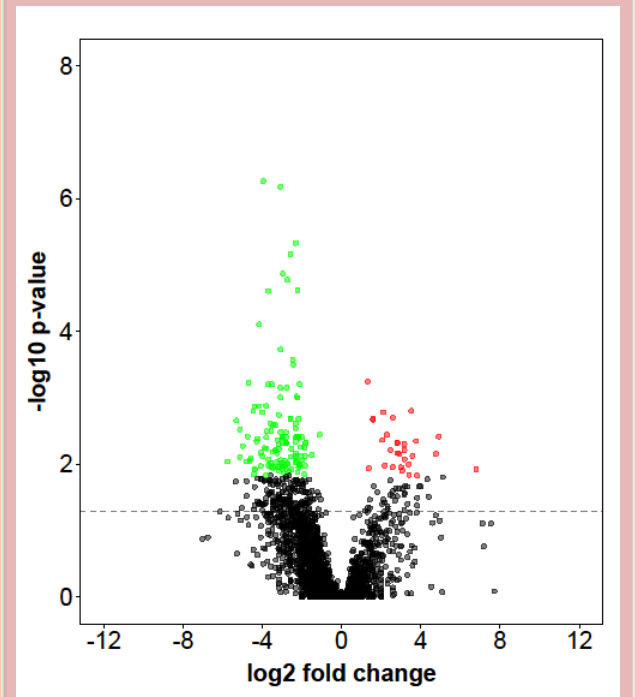
LDPE 1 g/L
(lixiviated)
480 transcripts

Mater-Bi



Materbi 1 g/L
(lixiviated)
987 transcripts

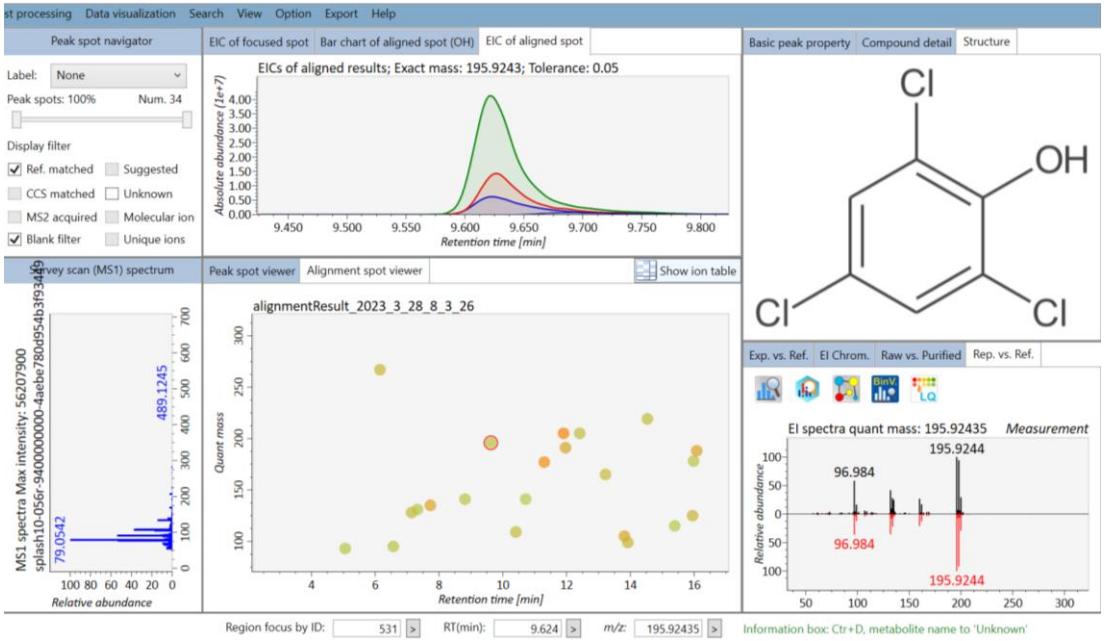
Bag Brown



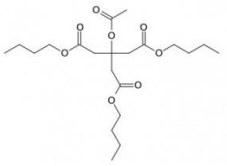
BagBrown 1 g/L
(lixiviated)
158 transcripts

Identification using in-house GC HRMS library

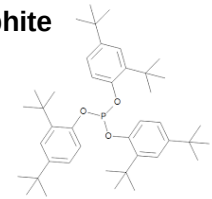
Example: Identification (probable structure) of **2,4,6-Trichlorophenol** in leachates of Mater-Bi biodegradable plastic.



Acetyl tributyl citrate



Tris(2,4-di-tert-butylphenyl) phosphite



Comparative assessment of the acute toxicity of commercial bio-based polymers on marine plankton

Laranjeiro et al. submitted

Bioassay	Particle size	Leachate plastic load	Dilutions tested	Endpoint recorded
Microtox	<250 µm	10 g/L	100% / 80% / 40% / 20%	Bioluminescence inhibition
Algae test	<250 µm	1 g/L	100% / 33.3% / 10% / 3.3%	Growth inhibition
Sea Urchin embryo test	<250 µm	1 g/L	100% / 33.3% / 10% / 3.3%	Growth inhibition
Mussel embryo test	<250 µm	10 g/L	100% / 50% / 25% / 12.5%	Larval normality
Copepod nauplii test	<250 µm	1 g/L	100% / 33.3% / 10% / 3.3%	Survival

TU = toxic unit (1/EC50)

< 1 No effect
1-2.5 slight
2.5-5 moderate
> 5 high

80% of leached substances in PHBv,
15% in PLA and 5% in PP

2,4,6-trichlorophenol in PHBv

Other chlorinated substances tentatively
Identified in PHBv using the NIST spectral library

Item	Species	EC_{10} (mg/L)	EC_{50} (mg/L)	TU
PP	A. fischeri	4960.7 (3379.8 – 7281.27)	n.c.	< 1
	R. salina	102.3 (86.5-120.9)	376.9 (338.0-420.3)	2.7
	P. lividus	728.6 (556.3-954.1)	n.c.	< 1
	M. galloprovincialis	2637.3 (2201.1-3159.9)	n.c.	< 1
	A. tonsa	n.c.	n.c.	< 1
PHBv	A. fischeri	684.8 (467.1-1003.7)	3942.3 (3488.5-4455.0)	2.5
	R. salina	6.5 (4.1-10.4)	73.6 (60.6-89.2)	13.6
	P. lividus	392.0 (331.5-463.6)	931.8 (840.7-1032.6)	1.1
	M. galloprovincialis	1557.6 (1457.6-1664.6)	2647.0 (2536.0-2762.8)	3.8
	A. tonsa	421.6 (385.2-461.4)	657.0 (618.0-698.5)	1.5
PLA	A. fischeri	4429.3 (2935.3 – 6683.7)	n.c.	< 1
	R. salina	42.1 (30.5-58.0)	538.2 (30.5-58.0)	1.9
	P. lividus	808.73 (723.8-903.6)	n.c.	< 1
	M. galloprovincialis	1888.89 (1720.7-2073.5)	4624.3 (4362.0-4902.3)	2.1
	A. tonsa	n.c.	n.c.	< 1

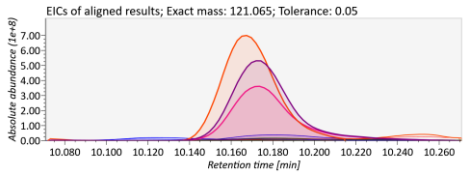
Mesocosm studies at UMF using bio-based polymers June 2023

- Three weeks exposure to PHBv, PLA and PP
- Plankton communities
- Fish
- Mussels
- Biofilm formation

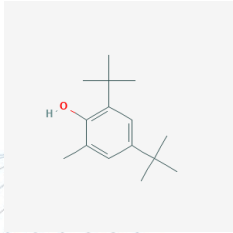
…..

- Preliminary results: PHBv has negative effects on Zooplankton communities

In the three mesocosms with PHBv on day 21

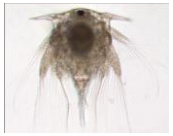


2,4-ditert-butyl-6-methylphenol



Physiological and behavioral bioassays

Ecotoxicological Endpoints



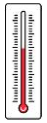
A. amphitrite

B. plicatilis

P. lividus

Aurelia sp.

24, 48, 72 h

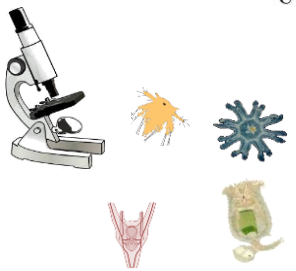


20 - 25 °C

Dark condition

Immobility

Developmental anomalies



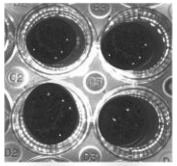
Swimming Speed Alteration

Alteration of the Frequency of Pulsations

Measured by
BEHAVIORAL ALTERATION RECORDER (BAR) system

developed at CNR-IAS

Software



Multiwell plate with organisms

Swimming speed measure (frames/sec)
Or
Frequency of pulsations (pulsation number/min)

Behavioral Alteration → EC50

RESPONSE

CNR IAS
ISTITUTO PER LO STUDIO DEGLI IMPATTI ANTROPICI E SOSTENIBILITÀ IN AMBIENTE MARINO

Gambardella et al. 2023

1 g/L leachate	
TU (1/EC50)	Toxicity
<1	None
1≤TU<2	Slight
2≤TU<5	Relevant
≥5	High

TU (Toxic Units, 1/EC50)

Geographic area	Plastic category	Endpoint	Barnacle	Rotifer	Sea urchin	Jellyfish
Adriatic Sea	Trawl rubber (rapidi)	Mortality, immobility	<1	<1		<1
		Development			3,1	
		Behavior	1,5	<1	<1	2,7
	Hard plastics	Mortality, immobility, behavior	<1	<1	<1	<1
		Development			1,56	
	Fishing nets	Mortality, immobility	<1	<1		<1
		Development			4,3	
		Behavior	<1	<1	<1	2,63
Ligurian Sea	Bottles	Mortality,immobil/develop, behavior	<1	<1	<1	<1
Bay of Biscay	Hard plastic container	Mortality, immobility, behavior	<1	<1	<1	<1
		Development			1,96	
	Pellet	Mortality, immob/develop, behavior	<1	<1	<1	<1
	Fishing nets	Mortality, immobility, behavior	<1	<1	<1	<1
		Development			2,32	

1 g/L leachate	
TU (1/EC50)	Toxicity
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		Development			3,1	
		Behavior	1,5	<1	<1	2,7
	Hard plastics	Morta				
		Devel				
	Fishing nets	Morta				
		Devel				
		Behavior				
Ligurian Sea	Bottles	Mortality,immobil/develop, behavior	<1	<1	<1	<1
Bay of Biscay	Hard plastic container	Mortality, immobility, behavior	<1	<1	<1	<1
		Development			1,96	
	Pellet	Mortality, immob/develop, behavior	<1	<1	<1	<1
	Fishing nets	Mortality, immobility, behavior	<1	<1	<1	<1
		Development			2,32	

ALL CATEGORIES USED IN FISHING THAT REPRESENT THE MAJOR MARINE LITTER FOUND IN THE MARINE ENVIRONMENT.

Characterization of co-contaminants in leachates

Trawl rubber

Benzothiazole

2-(Methylthio)benzothiazole

2,2,4-trimethyl-1H-quinoline

Diphenylamine

nitrogen- containing additives identified with NIST

Fishing nets Adriatic Sea

4-tert-Octylphenol

Fishing nets Bay of Biscay

Diphenylamine

Frequently identified

Dimethyl phtalate
Benzophenone
Triclosan
4-Methylbenzophenone
Tris(nonylphenyl)phosphite
Tris(2-chloroethyl) phosphate (OPFR)
…….

Occasionally identified

UV327
Tributyl phosphate (OPFR)
Triphenyl phosphate (OPFR)
2,4-Diisocyanatotoluene
oxybenzone
……

Main conclusions (so far)

Bio-based plastic leach more additives compared to PP and PE

Toxicity associated with bio-based plastics

Chlorinated substances identified in PHBv and compostable plastic bags

Leachates of beached plastic materials can have effects in physiological and behavioral bioassays

Improve libraries and work flow to identify more substances!

Questions? Thanks for your attention!

Thanks to ORU co-workers: Anna Kärrman, Maria Larsson, Steffen Keiter, Arslan Hashmi, Fredric Södergren Seilitz, Kevin Ugwu Hernández

Thanks to the RESPONSE team