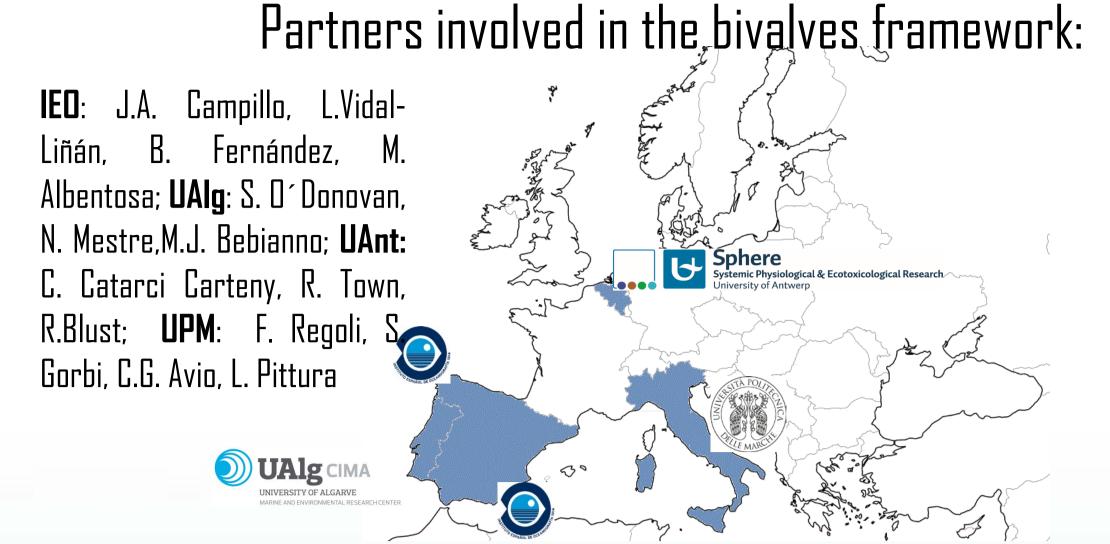


TOXICITY ASSESSMENT WP3 Organism Level WP4 Cellular and Mollecular levels **BIVALVES**



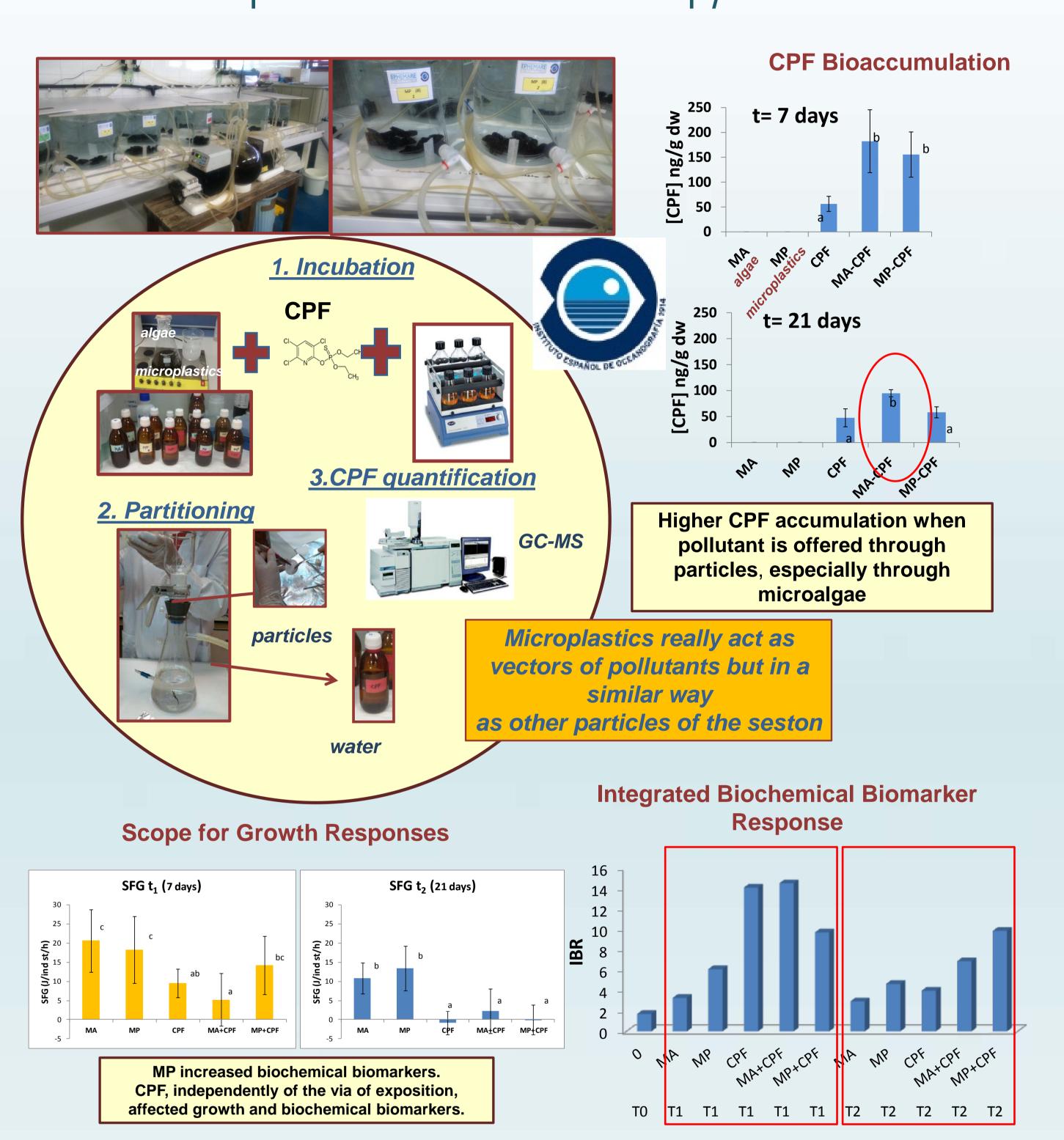




Test Organisms Scrobicularia plana Mytilus galloprovincialis

Bivalves experiments under the WP3 & WP4 frameworks were directed to evaluate the effect of MPs and adsorbed PPs using a variety of biological responses, at cellular, biochemical, molecular and physiological levels in two species: the mussel Mytilus galloprovincialis, the worldwide sentinel species used for monitoring pollution and the clam Scrobicularia plana, a benthic bivalve used in sediment pollution studies.

Microplastics loaded with Chlorpyrifos



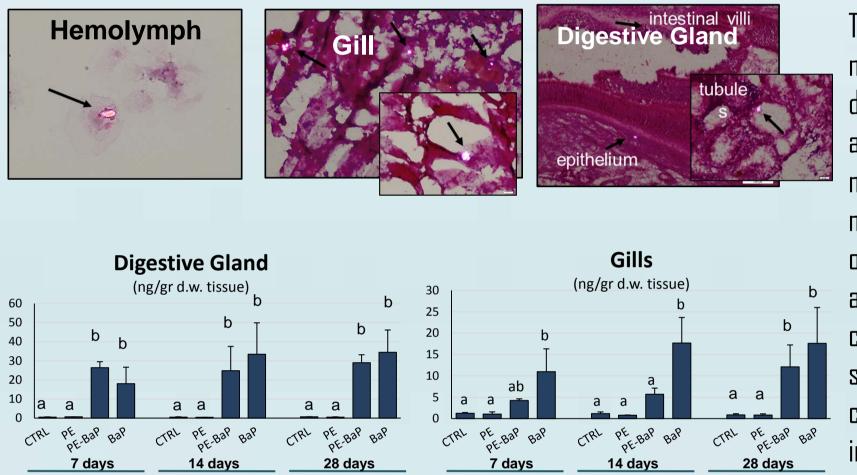
Microplastics loaded with BaP



Mytilus galloprovincialis Filter feeder LD-PE Heterogeneous shape LDPE dose: 10mg/L Exposure 20-25 µm in size BaP adsorbed to PE: 15 µg/g BaP dose: 150 ng/L 7 days 14 days 28 days

> After 7, 14 and 28 days, organisms were collected; analyses included: Histological Examination (occurrence and localization of microplastics), Chemical Analyses (BaP accumulation), Biomarkers (immunological parameters, antioxidant defences, oxidative stress, DNA damages, neurotoxicity, peroxisomal proliferation).

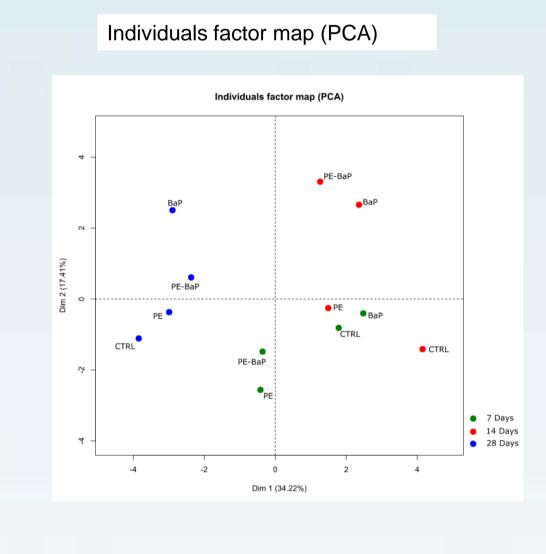
QUALITATIVE ANALYSIS OF MICROPLASTICS UPTAKE AND BAP BIOACCUMULATION



Benzo(a)pyrene Organic pollutant

The uptake of PE particles was revealed by polarized-light microscopy, showing microplastics in the hemolymph, gills and digestive gland. Chemical analyses highlighted a marked accumulation of BaP both in mussels exposed to contaminated microplastics and to pollutant alone. In the digestive gland the maximum of bioaccumulation was observed already after 7 days of exposure, while in the gills the same condition was obtained after 28 days. Levels of BaP measured in the water of contaminated MPs group is below detection limit, thus excluding a significant desorption of BaP from plastics to seawater and confirming that bioaccumulation occurred after microplastics' ingestion.

PRINCIPAL COMPONENT ANALYSIS (PCA) AND ELABORATION WITHIN THE WOE MODEL



Results of the PCA analysis on the whole set of biomarker responses indicated that separation of groups at the initial stage of the exposure (7 days) is related to the polymer itself. Over the time the separation is more evident for both BaP groups (14 days). After 28 days separation is clearly caused by exposure to the chemical alone.

Results on the WOE model indicated that contaminated MPs cause a "MAJOR" risk in respect to the virgin ones mostly related to BaP bioaccumulation the risk associated to treatments is not time-dependent.

BaP, BP3 and PFOS* **Acclimation Exposure (days)** 7 days **Treatments:** LDPE (11-13 µm) LDPE (11-13 µm) LDPE (11-13 µm) LDPE (11-13 µm) Control

Scrobicularia plana exposure to

LDPE microplastics with adsorbed

Gills, Digestive gland, Hemolymph

UAlg CIMA

Biomarkers with elevated levels when compared to controls, with time:

CAT **GPx** GST

GST AChE LPO **DNA** damage

BaP (17 μg g⁻¹)

SOD AChE LPO

BP3 (82 ng g⁻¹)

SOD LPO

PFOS (70 μg g⁻¹)

14

Adsorbed contaminants present in microplastics affect clams; Synergy between LDPE and BaP after 14 days for GST; Tissue specific response

*For further details check poster by Mestre et al., entitled: Ecotoxicological effects of microplastics with adsorbed contaminants in the clam Scrobicularia plana

		Class of hazard		Class of risk	
TREATMENT	TIME	BIOAVAILABILITY	BIOMARKERS	WOE	
PE	7 DAYS	Absent	Slight	SLIGHT	
PE-BaP		Severe	Slight	MAJOR	
ВаР		Severe	Slight	MAJOR	
PE	14 DAYS	Absent	Slight	SLIGHT	
PE-BaP		Severe	Slight	MAJOR	
ВаР		<u>Severe</u>	Moderate	MAJOR	
PE	28 DAYS	Absent	Slight	SLIGHT	
PE-BaP		<u>Severe</u>	Slight	MAJOR	
ВаР		<u>Severe</u>	Slight	MAJOR	







































