# Corso di Dottorato Nazionale in Sustainable Development and Climate Change - Cycle XXXIX

Istituto Universitario di Studi Superiori, Pavia

PhD SDC SUSTAINABLE DEVELOPMENT AND CLIMATE CHANGE

IUSS Scuola Universitaria Superiore Pavia

# Impacts of climate change related stressors and anthropogenic pollution on marine biodiversity


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## BACKGROUND

Climate change related-stressors represent one of the most relevant threats for marine ecosystems, along with introduction of relevant loads of contaminants, including Contaminants of Emerging Concern (CECs). CECs include substances and materials part of everyday life such as plasticizers (PFAS), pesticides, flame retardants, microplastics (MPs), nanoparticles, endocrine disruptors, pharmaceuticals and personal care products whose toxicity is still scarcely explored, representing a relevant threat for marine ecosystems. These stressors represent the major challenge to the marine biodiversity in coastal environments, especially in enclosed shallow basins, like the Mediterranean Sea. This area is characterized by a consistent anthropogenic footprint (fishery, aquaculture, tourism, oil and gas exploitation, riverine effluents), in addition, during the last decade, this basin was influenced by increasingly intense heatwaves, both in terms of frequency and intensity, along with other extreme events such as floods. Overall, co-occurring contaminants can interact with each other, causing additive, synergistic or antagonistic effects on organisms[2 - 3], exacerbated by the climate change related stressors[1].

## AIMS OF THE RESEARCH PROJECT

Given the background, this project aims to:

1. **describe the presence of CECs in aquatic species of ecological and commercial value[2];**
2. **analyse the possible role of microplastics as vectors for chemical contaminants in marine organisms[2-3];**
3. **detect the biological effects caused by possible interactions between climatic stressors, microplastics and other CECs on marine biodiversity[1].**

In the first six months of PhD research, experimental activities and preliminary analyses have been carried out to evaluate the presence of MPs in aquatic species and their possible role as carriers for contaminants in marine biota.

## PRESENCE OF MICROPLASTICS, PHTHALATES AND FLAME RETARDANTS IN MARINE SPECIES

### SAMPLING PLAN

As part of this project, 3 species were selected and collected in two sites (Ancona and Civitanova Marche – Central Adriatic Sea) and seasons (summer and winter) to detect the presence of MPs and contaminants in their tissues.


SAMPLING SITE: Ancona and Civitanova (Central Adriatic Sea).

SAMPLING PERIOD: Summer and Winter 2022

TARGET SPECIES:

*Merluccius merluccius* *Solea solea* *Mullus barbatus*

SAMPLE SIZE: 10-20 organisms per each sampling site and period.

### ANALYSES

- Bioaccumulation of Brominated Flame Retardants (PBDE, HBCD, e BFRs for a total of 22 analytes), and per- and polyfluoroalkyl substances (PFAS including 19 congeners) in muscle.
- Ingestion of Microplastics (MPs) in gastro-intestinal tract.

### METHODOLOGIES FOR CHEMICAL ANALYSIS

GC-MS/MS (PBDE/HBCD) and LC-MS/MS (HBCD) allow the qualitative and quantitative determination of Persistent Organic Compounds (PFAS, PBDE, HBCD, and BFR).

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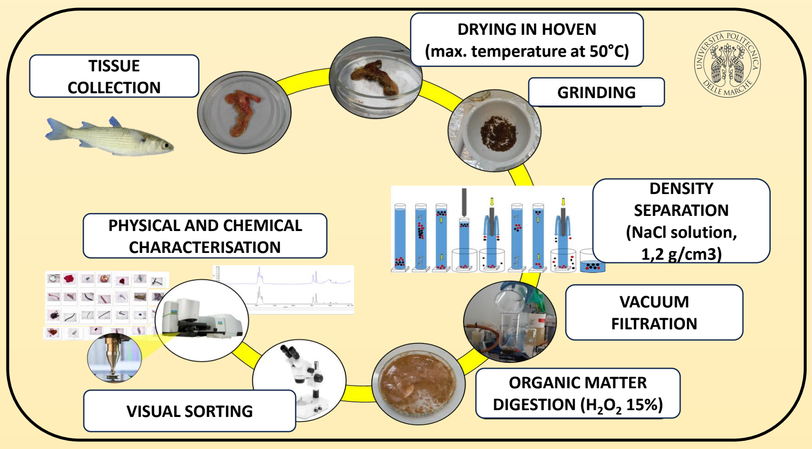
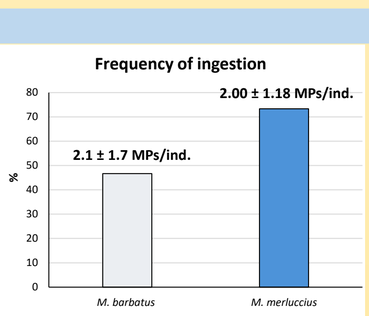
### METHODOLOGIES FOR MPs EXTRACTION AND CHARACTERISATION

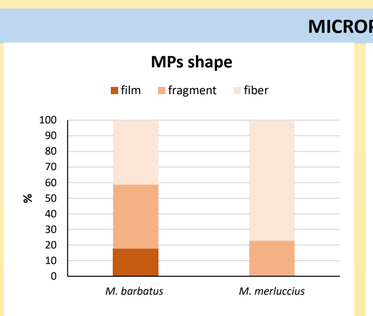


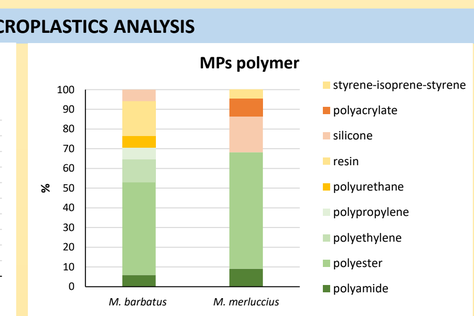

### PRELIMINARY RESULTS

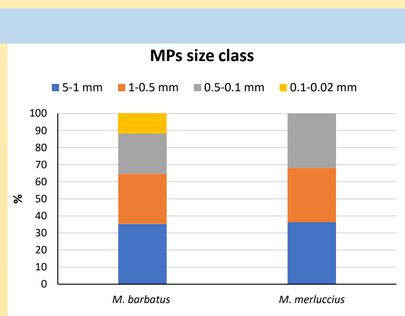
Although analyses are still going on, preliminary results demonstrate the presence of MPs in 2 of the 3 selected species (*M. barbatus* and *M. merluccius)*, with a frequency of MPs ingestion of 2.1 ± 1.7 and 2.00 1.18 respectively. Preliminary analyses revealed that the most common shape is fragment, followed by fibers and film while the most abundant polymer is polyester in both of the selected species. Preliminary chemical analyses highlighted higher levels for PFAS in the fish muscle.

#### MICROPLASTICS ANALYSIS

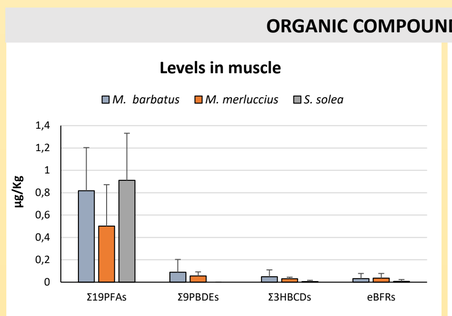


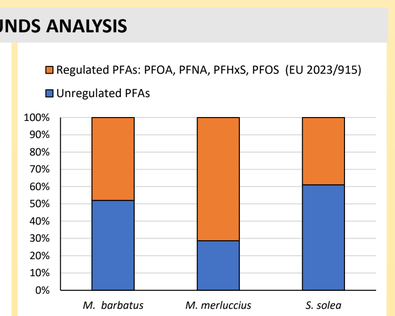




#### ORGANIC COMPOUNDS ANALYSIS




## POSSIBLE ROLE OF MICROPLASTICS AS VECTORS FOR CHEMICAL CONTAMINANTS IN MARINE ORGANISMS

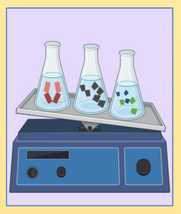
Plastic items have been naturally weathered in marine environment (Fosso Conocchio, Ancona harbour) and after 3 months were recovered. Both pristine and naturally weathered plastics have been processed to prepare leachates.


**Naturally weathered plastics: permanence in Fosso Conocchio (Ancona) for 3 months.**

### TARGET MATERIALS


### LEACHATE PREPARATION

**Cutting pristine and weathered plastics into smaller pieces (1 cm²)**



**Plastic / Water ratio 100 g/L**

**Leaching Time**

**3 DAYS**

**LEACHATE FILTRATION**
FILTER 0.22µm
+
**MPs collection**

#### CHEMICAL ANALYSIS

- Phthalates
- Polyclic Aromatic Hydrocarbons
- Brominated Flame Reatardants (PBDE, HBCD, e BFRs)
- Bisphenol A
- Per- and polyfluoroalkyl substances

#### ECOTOXICOLOGICAL BIOASSAYS

6 concentrations have been tested for each commercial and naturally weathered polymer:

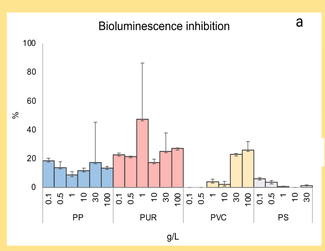
- 100 g/L;
- 30 g/L;
- 10 g/L;
- 1 g/L;
- 0,5 g/L;
- 0,1 g/L;

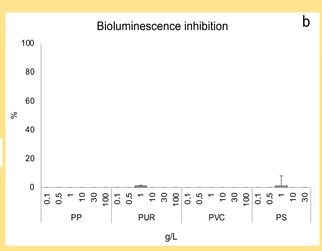
### PRELIMINARY RESULTS

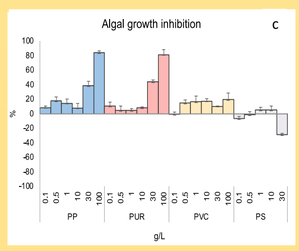
#### CHEMICAL ANALYSES

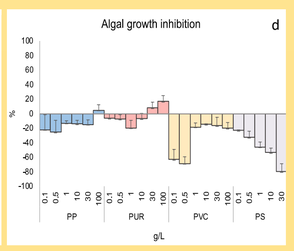
- Concerning PFAS, IPA, BAP and Brominate Flame Retardants no significative levels were found in virgin and environmentally aged plastic leachates, for all the four polymers.
- Concerning Phthalates, no significative levels were found in virgin and environmentally aged plastic leachates, except for few congeners reaching 1 ng/mL.
- PUR virgin leachates showed highest Phthalates levels, with the presence of 11 congeners, 7 of which reaching at least 1.48 ng/mL compared to the environmental weathered plastic ones.

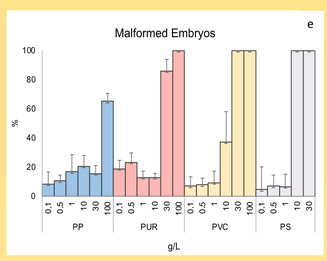
#### ECOTOXICOLOGICAL BIOASSAYS

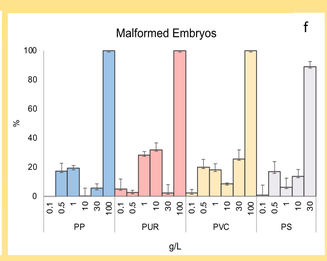



#### WEIGHT OF EVIDENCE ELABORATION

| g/L (d.w.) | Commercial PP | Commercial PUR | Commercial PVC | Commercial PS | Naturally Weathered PP | Naturally Weathered PUR | Naturally Weathered PVC | Naturally Weathered PS |
|---|---|---|---|---|---|---|---|---|
| 0.1 | 1.14 | 1.76 | 0.29 | 0.38 | 0 | 0.01 | 0.36 | 0 |
| 0.5 | 1.31 | 1.62 | 0.69 | 0.16 | 0.3 | 0.48 | 0.7 | 0.29 |
| 1 | 1.07 | 1.76 | 0.87 | 0.06 | 0.33 | 0.57 | 0.31 | 0.48 |
| 10 | 1.25 | 1.32 | 1.01 | 1.96 | 0 | 0.03 | 0.15 | 0.59 |
| 30 | 2.44 | 4.59 | 3.65 | 2.53 | 0.03 | 0.07 | 0.44 | 2.19 |
| 100 | 5.01 | 6.29 | 4.1 | \ | 2.01 | 2.22 | 1.96 | \ |

ABSENT | SLIGHT | MODERATE | MAJOR | SEVERE
0 1 1,5 3 6 10

- Ecotoxicological assays highlighted higher toxicity for pristine plastic leachates than for naturally weathered ones.
- Bioluminescence inhibition test in *Alivibrio fischeri* (a - b) was the less sensitive for all the polymers, with the exception of PUR 1g/L (inhibition rate: 47.36%).
- *Phaeodactylym tricornutum* algal growth inhibition test (c - d) confirmed higher toxicity levels for commercial leachates than in environmental aged ones.
- Embryotoxicity test on *Crassostrea gigas* (e-f) highlighted harmful effects induced by commercial and environmental polymers, especially at the highest concentrations (30 and 100 g/L).
- Weight of evidence elaboration confirmed 30 g/L is the lowest concentration causing moderate – major toxicity for all the four polymers.

## HIGHLIGHTS

Microplastics are bioavailable for many marine species with different ecological and trophic characteristics, depending on their size, shape and composition. Overall, weathering leads to structural modifications of the pristine plastic and to the leaching of related-plastic compounds to the surrounding environment, which may determine harmful effect on the marine biota. In fact, comparing the toxicity of leachates from commercial polymers and the naturally weathered materials, preliminary results highlight how toxicity of environmentally aged plastics decreased probably due to weathering occurring in 3 months. Future analyses may provide further clarification on the different toxicity of these complex matrices.

## FUTURE OUTCOMES

Given the results, the bioaccumulation of additives released by plastics will be assessed in *in vivo* exposure experiments using *Mytilus galloprovincialis* as bioindicator. The biological impact will be evaluated by analysing a panel of biological responses (biomarkers) at biochemical and cellular level. Finally, the possible interaction between these compounds and climate stressors will be assessed through *in vivo* exposure considering emerging chemical contaminants (*i.e.,* PFAS), salinity fluctuations and acidification conditions.


## AKNOWLEDGMENTS

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## REFERENCES


(1) Nardi et al. 2022 , https://doi.org/10.1016/j.envpol.2022.118970;
(2) Parashar et al. 2023, 10.20517/wecn.2023.25;
(3) Pittura et al. 2022 https://doi.org/10.3389/frwa.2022.902885