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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[1,2], exacerbated by the climate change related stressors[3].

AIMS OF THE RESEARCH PROJECT

Given the background, this project aims to:

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

PRESENCE OF MICROPLASTICS IN MARINE SPECIES

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.
MPs were extracted from the gastrointestinal tracts of organisms following a standardized protocol and MPs physically and chemically characterized through visual sorting and micro-Fourier-transform infrared (µATR-FTIR) spectroscopy, respectively.

TARGET SPECIES

Mullus barbatus

Merluccius merluccius

Solea solea

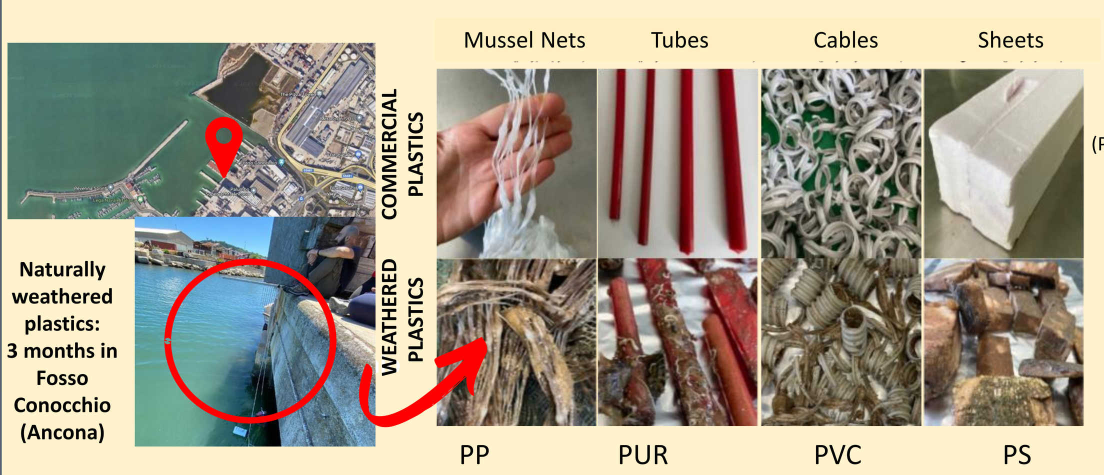
RESULTS

- Overall, the average frequency MPs ingestion exceeds 50% across all sampled organisms.
- *Solea solea* exhibits 100% of ingestion frequency, both in summer and winter, with the highest rate among the three species.
- No significative differences in ingestion frequency were observed among the different sampling sites.
- The mean number of particles detected was 2.5 per individual, regardless the sampling site, period or species.

INTEGRATED APPROACH TO EVALUATE THE BIOLOGICAL EFFECTS OF PLASTIC LEACHATES IN MARINE ORGANISMS

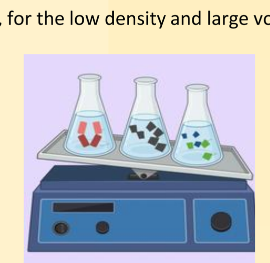
An integrated approach combining chemical and biological analyses was employed to assess the chemical composition of plastic leachates and their ecotoxicological effects at multiple levels of biological organization, aiming to provide a comprehensive assessment of the associated environmental risk.

TARGET MATERIALS and LEACHATES PREPARATION



PP polypropylene | PUR polyurethane | PVC polyvinyl chloride | PS polystyrene

Plastic fragments (1 cm²) / Water ratio: 100 g/L
(PS: 30 g/L, for the low density and large volume occupied)



Leaching Time: 72 h

Plastic items have been naturally weathered in the marine environment and after 3 months were recovered. Both commercial and naturally weathered plastics have been processed to prepare leachates.

CHEMICAL ANALYSES of PLASTIC LEACHATES

Leachates were chemically characterized through GC-MS and LC-MS to identify chemical compounds including brominated flame retardants (BFR), phthalates, Polycyclic Aromatic Hydrocarbons (PAHs), Per- and polyfluoroalkyl substances (PFASs), bisphenol A (BPA), and heavy metals.

- PFAS, PAHs, BAP and BFRs: no significant levels were found in commercial and weathered leachates, for all the polymers, except for few congeners of phthalates reaching 1 ng/mL.
- Commercial PUR leachates showed the highest phthalates levels, with the presence of 11 congeners, 7 of which reaching at least 1.48 ng/mL

EFFECTS at ORGANISM LEVEL: BATTERY of ECOTOXICOLOGICAL BIOASSAYS

Each commercial and weathered plastic leachate was tested at different concentrations (0.1, 0.5, 1, 10, 30 and 100 g/L) to assess the toxicity of contaminants leached from plastic fragments by measuring the bioluminescence inhibition in *Aliivibrio fischeri*, the algal growth inhibition in *Phaeodactylum tricornutum* and the embryotoxicity in *Crassostrea gigas.*

Results obtained from the individual bioassays were elaborated and integrated through a *Weight of Evidence* (WOE) model for risk assessment revealing higher toxicity for leachates from commercial plastics compared to those from naturally weathered ones, with the concentration of 30 g/L eliciting a moderate – major toxicity for all the four polymers.

WOE ELABORATION: LEVEL OF HAZARD

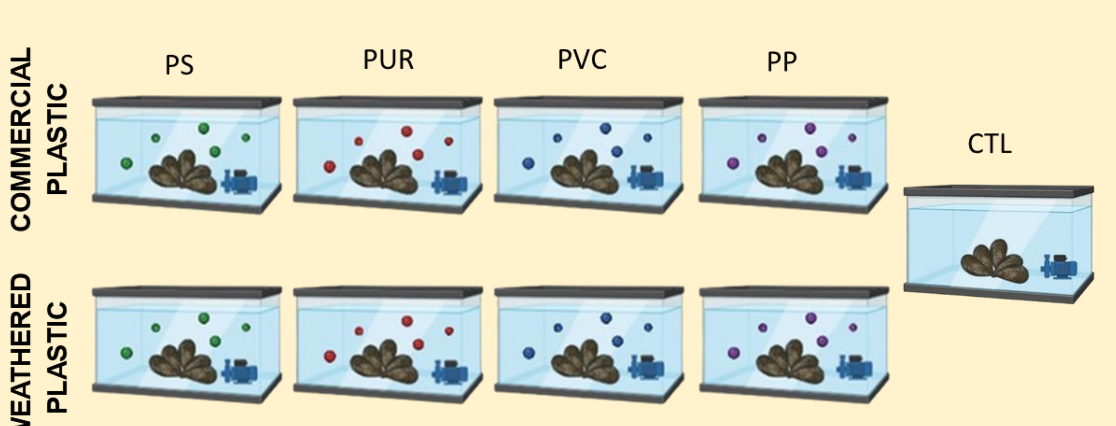
	Commercial				Naturally Weathered			
g/L (d.w.)	PP	PUR	PVC	PS	PP	PUR	PVC	PS
0.1	1.14	1.76	0.29	0.38	0	0.01	0.36	0
0.5	1.31	1.62	0.69	0.16	0.3	0.48	0.7	0.29
1	1.07	1.76	0.87	0.06	0.33	0.57	0.31	0.48
10	1.25	1.32	1.01	1.96	0	0.03	0.15	0.59
30	2.44	4.59	3.65	2.53	0.03	0.07	0.44	2.19
100	5.01	6.29	4.1	\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

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

EFFECTS at CELLULAR LEVEL: *IN VIVO* APPROACH

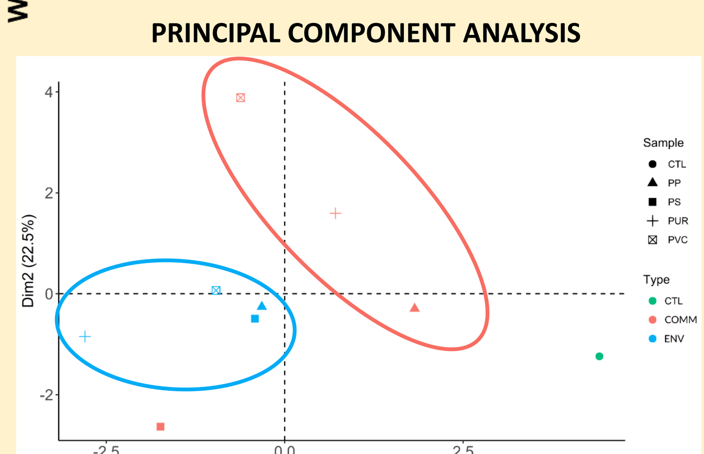
Organisms of *Mytilus galloprovincialis* were exposed to fragments of the commercial and weathered target materials to assess the effects of chemical compounds leached directly in the exposure tanks by measuring a battery of cellular and biochemical biomarkers related to immune and cholinergic systems, lipid metabolism, antioxidant defenses as well as neurotoxic and genotoxic damage.

EXPERIMENTAL PLAN



- 1 week of acclimation + 3 weeks of exposure.
- Exposure concentration: 10 g/L for PUR, PVC and PP; 3 g/L for PS.
- 15 L tanks with artificial seawater, 35‰, pH 8.20. 45 mussels per tank (3 individuals/L).
- Aquarium pump to mimic the action of marine currents and to promote a homogeneous distribution of fragments throughout the whole water volume.
- Partial water changes once a week to preserve a part of the contaminants leached by plastic until that moment.

PRINCIPAL COMPONENT ANALYSIS



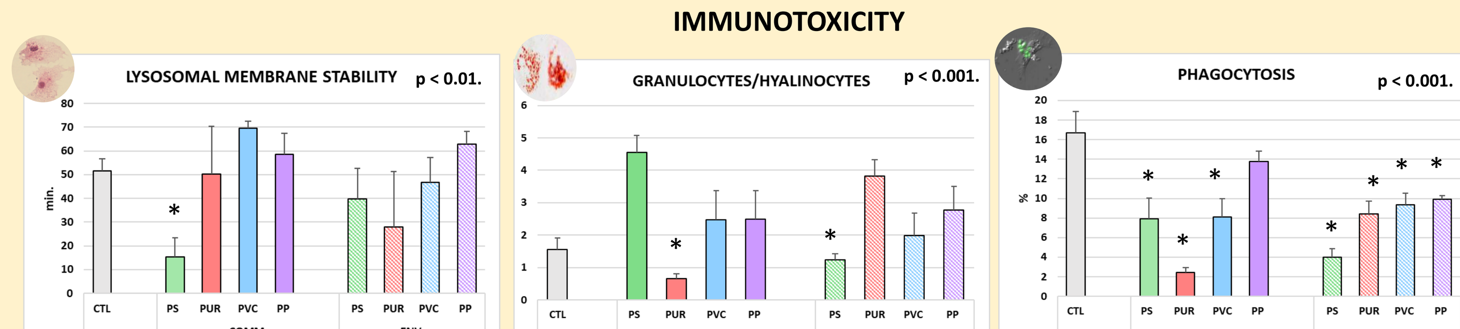
WOE ELABORATION: LEVEL OF HAZARD

	Treatments	N. param in class ABSENT	N. param in class SLIGHT	N. param in class MODERATE	N. param in class MAJOR	N. param in class SEVERE	Level of hazard for biomarkers
	CTL	15	0	0	0	0	ABSENT
COMMERCIAL PLASTIC	PS	7	1	2	3	2	MAJOR
	PUR	10	1	3	1	0	MODERATE
	PVC	8	1	4	1	1	MODERATE
	PP	14	0	1	0	0	SLIGHT
NATURALLY WEATHERED	PS	11	0	2	1	1	MODERATE
	PUR	7	2	3	2	1	MODERATE
	PVC	11	0	4	0	0	SLIGHT
	PP	9	1	4	1	0	MODERATE

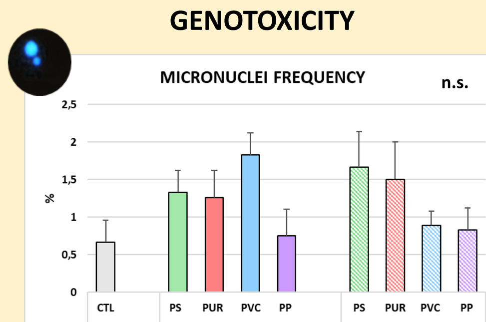
RESULTS ON BIOMARKERS ANALYSES

* Indicates statistically significant differences between the control (CTL) and the exposed groups.

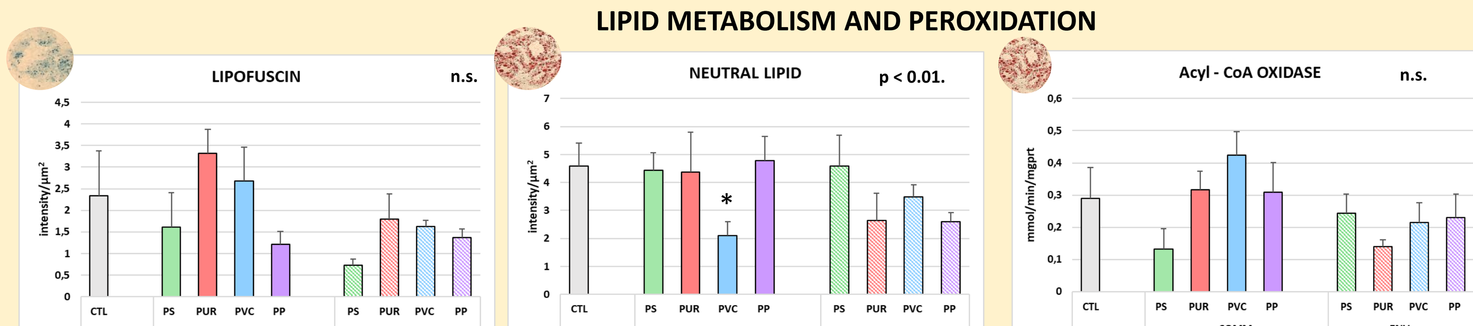
IMMUNOTOXICITY



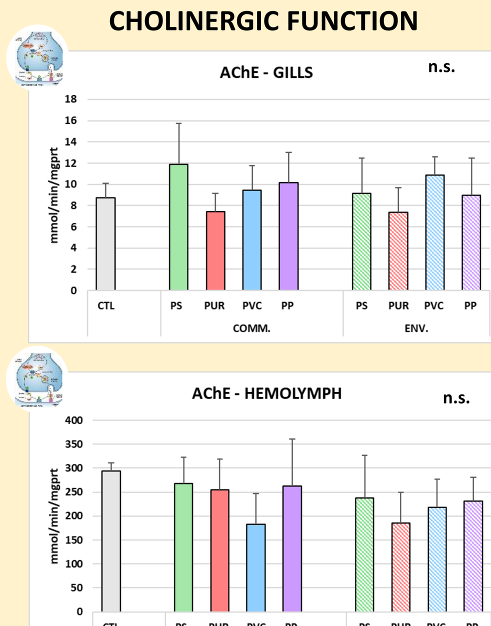
GENOTOXICITY



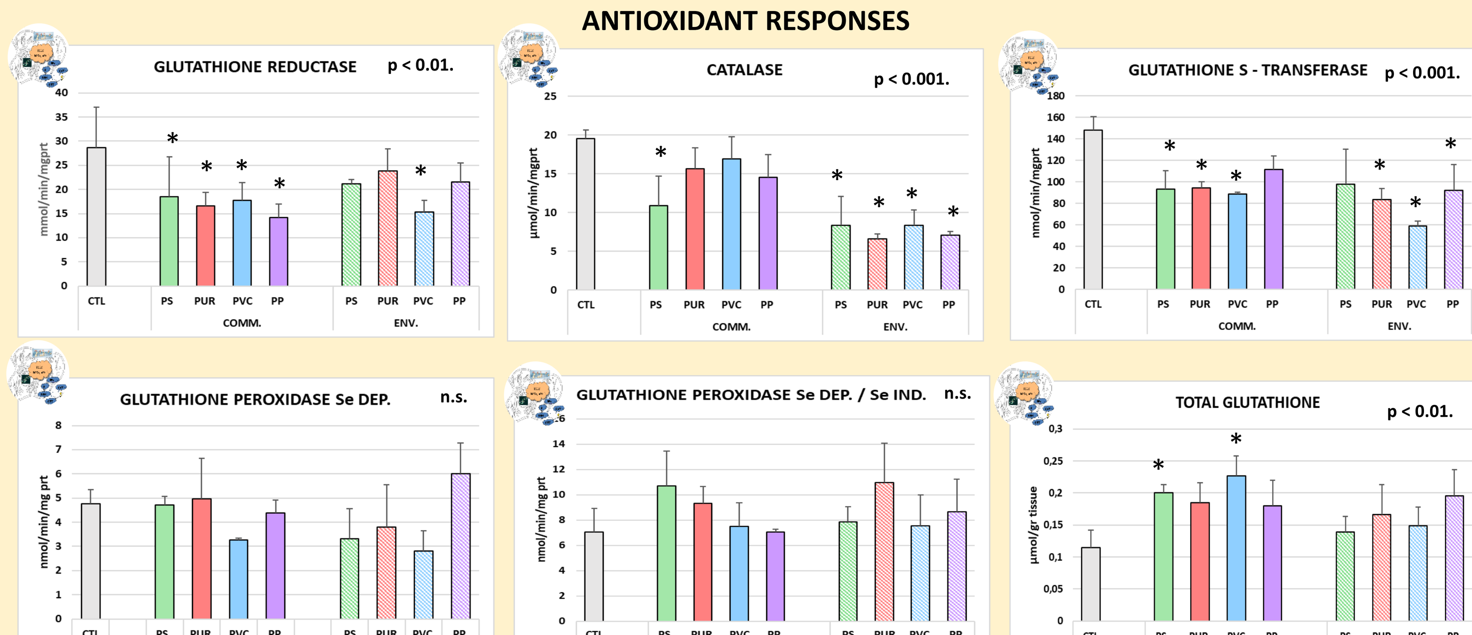
LIPID METABOLISM AND PEROXIDATION



CHOLINERGIC FUNCTION



ANTIOXIDANT RESPONSES



HIGHLIGHTS

- Overall, the heterogeneity of the effects depends both on the type of polymer (PS, PUR, PVC, and PP) and the experimental group (commercial or weathered).
- The immune system and antioxidant defenses showed the most significant alterations.
- In particular, the phagocytic activity of haemocytes is inhibited in mussels exposed to almost all types of commercial and weathered plastics.
- Interestingly, the enzymatic activity of glutathione reductase, glutathione transferase, and the total glutathione content were mainly modulated by commercial plastics, while all the environmental polymers caused a significant inhibition of catalase activity.
- A significant decrease in neutral lipid content was observed only mussels exposed to commercial PVC.
- PCA revealed a clear separation between the exposed groups (COMM. and ENV.) and the control (CTL), as well as a distinct separation between commercial and environmental treatments.
- The Weight of Evidence elaboration confirmed the heterogeneity of responses, revealing a Major hazard level for PS_COMM compared to the Slight or Moderate risks of the other treatments.

FUTURE OUTCOMES

Considering the key role of the immune system on bivalves' health, an *in vitro* approach using haemocytes will be applied to further investigate the immunomodulation and the mechanisms of toxicity of plastics and associated chemicals on marine organisms. Moreover, considering the complexity of environmental scenarios, it would be relevant to assess whether climate-related stressors (e.g., heatwaves, salinity fluctuations) can modulate the responses of organisms exposed to plastic-derived additives, within a multiple stressor framework. By integrating ecotoxicological assays with both *in vivo* and *in vitro* approaches, it would be possible to gain a more comprehensive understanding of the effects induced in marine organisms by plastic-derived contaminants in combination with climate stressors.

AKNOWLEDGMENTS

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REFERENCES

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