Interreg Italy - Croatia NET4mPLASTIC EUROPEAN UNION

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NEw **T**echnologies for **m**acro and micro**plastic** Detection and Analysis in the Adriatic Basin

NET4mPlastic

ADRIatic Sea and **PLASTi**c and microplastic pollution from freshwaters – an Adrion territorial challenge

AdriPlast

PhD Corinne Corbau – cbc@unife.it

Conference on Microplastics in drinking water – from source to tap
EU Strategy for the Danube Region Water Quality Priority Area

3 October 2024

NET4mPLAST
GENERAL INFORMATION

2014 - 2020 Interreg V-A
Italy - Croatia CBC Programme
Call for proposal 2017 Standard

Priority Axis: Environment and cultural heritage

Specific Objective 3.3 - Improve the environmental quality conditions of the sea and coastal area by use of sustainable and innovative technologies and approaches

€ 2.478.640,00
Start date: 01/01/2019
End: 30/06/2022

Partnership

- University of Ferrara, IT (LP)
- University of Trieste, IT
- Marche Region, IT
- HYDRA SOLUTIONS SRL, IT
- PROSOFT LTD, HR
- Istituto Zooprofilatico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', IT
- Teaching Institute for Public Health, Primorje-Gorski Kotar County, HR
- Public Institution RERA, HR
- University of Split, HR

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Programme output indicator

3.3O1 Environmental friendly technological solutions (and approaches) implemented

Project main outputs

- Open access common platform coupled with an Early Warning System allowing the sharing of real time and forecast data and the planning of common actions
- On-board unit for field activities and installed on marine drones for activities along the coast and on ships for offshore activities

Programme output indicator

3.3O3 Microplastic waste collected in marine areas

Project main outputs

- Definition of a Joint methodology for MP sampling, monitoring and analysis

Target groups

- Local, regional and national public authorities (municipalities for instance)
- Regional and local development agencies
- Associations, innovation and environmental agencies
- Education and training organizations as well as universities and research institutes

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Interreg IPA Adrion 2021-2027
First call for proposal 2023

Programme priority: Supporting a greener and climate resilient Adriatic -Ionian region

SO 2.3: Supporting environment preservation and protection in the Adriatic - Ionian region
LP: CURSA

€ 1.428.477,65
Start date: 01/09/2024
36 months

Just started
Open for
collaboration

Partnership

- CURSA (LP, IT)
- University of Ferrara Italia (IT)
- Ruđer Bošković Institute Hrvatska (HR)
- National institute of biology Slovenija (SI)
- Inst. for Biological Research "Siniša Stanković" - National Institute of the Rep. of Serbia (RS)
- Institute of Public Health of Montenegro Crna (ME)
- National Center of Environmental Movement Shqipëria (AL)

Associated Partner:
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del
Molise "G. Caporale" (IZS Teramo)

EU part of cooperation area
Non-EU part of cooperation area

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Main objectives

Enhancing transnational collaboration
Identifying and addressing key challenges
Developing sustainable solutions.

Just started
Open to
collaboration

Key Outputs

- Enhanced comprehension of plastic pollution dimensions within pilot sites
- A roadmap for monitoring and mitigating plastic
- Transnational networks and knowledge sharing
- Awareness-raising and capacity-building activities

Work package 1: Preliminary activities and initial state
Work package 2: Plastic and microplastic pollution (PmPP) from source to sea
Work package 3: Waste managment and possible solutions

Target groups

Local public authority - Regional public authority - Interest groups including NGOs - Higher education and research organisations - General public - Cross-border legal body - Education/training center and school

EU part of cooperation area
Non-EU part of cooperation area

Preliminary activities

Data collection for selected Macro-Areas:
- Morphological, sedimentological, and hydrodynamic characteristics
- MP occurrence (beach, sea surface, sea bottom and biota) from literature and EU projects' results
- Characteristics of other relevant water parameters (transparency, temperature, salinity, dissolved oxygen (% and mg/l), pH, depth, flow, etc.)
- Meteo-marine data record

Application of a 3D **transport model** for MP accumulation/deposition areas

Development of the **Integrated Platform's** structure for data analysis and sharing

Field activities

Water, sediment and biota **sampling** (traditional /innovative) for laboratory analysis in areas of MP accumulation and MP non-accumulation

Real-time analysis of parameters relevant to MP (transparency, temperature, salinity, dissolved oxygen (% and mg/l), pH, depth, localization, flow, etc.)

Laboratory activities

Cost-effective lab. analysis procedures for **reducing time consumption**:
- MP content in all the marine compartments
- Occurrence of related co-contaminant sorbed by MP
- other relevant parameters

Analysis of time of permanence of MP and co-contaminant in sampled biota

Analysis of human health risks

Implementation of the **Integrated platform**

Setting and calibration of the **Early-Warning System**

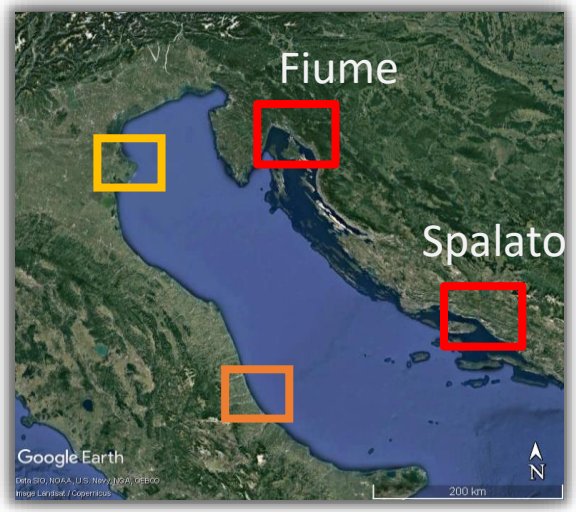
Outputs:

- **Validated model for MP accumulation areas prediction**
- **Potential scenarios forecasting of MP accumulation area**s (MP-vulnerability map creation)
- Early Warning System for MP accumulation areas forecasted after storms/ river overflows
- **Indication to aquaculture and fishing operators,** local authorities, stakeholders for safety food production
- Indication to local authorities for MP vulnerability mitigation
- Shared GIS database
- Cost-effective procedures standardisation
- Formation of operators and technicians

Demonstration activities: test sites

Monitoring plastic and microplastic wastes on coastal and marine environments

***In situ* surveys** have been carried out twice/year and before and after an extreme event (i.e. flooding or sea-storm).
These activities provided a **large pool of samples regarding plastic contamination**.



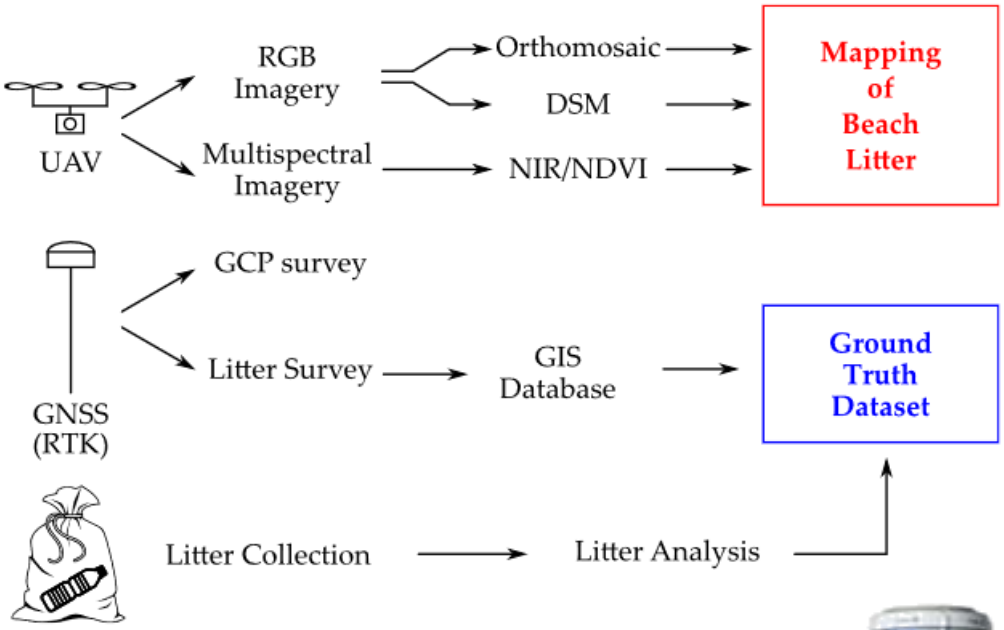
The surveys have been performed adapting the **De FishGear protocols***

Beach Sampling:

- area identification (100 m perpendicular to the coastline)
- subdivision into 10 transepts (~ every 10 m)
- Marine Litter sampling (2,5cm < x < 50cm)
- LMP sampling (mesoplastic) (<2,5cm) on 1mq: 3l within the first 3 cm (x6)
- SMP sampling (microplastics) (0.1 µm <x <5 mm): 250ml (x6)

Monitoring of marine litter

- **Field surveys** (using innovative and traditional approach) for morphology monitoring and plastic detection:
  - **GPS** (topography and bathymetry)
  - **UAV** based monitoring system and remote sensing (multispectral data, ocean colour, phytoplankton, chemistry, atmospheric water vapour, temperature, etc.)

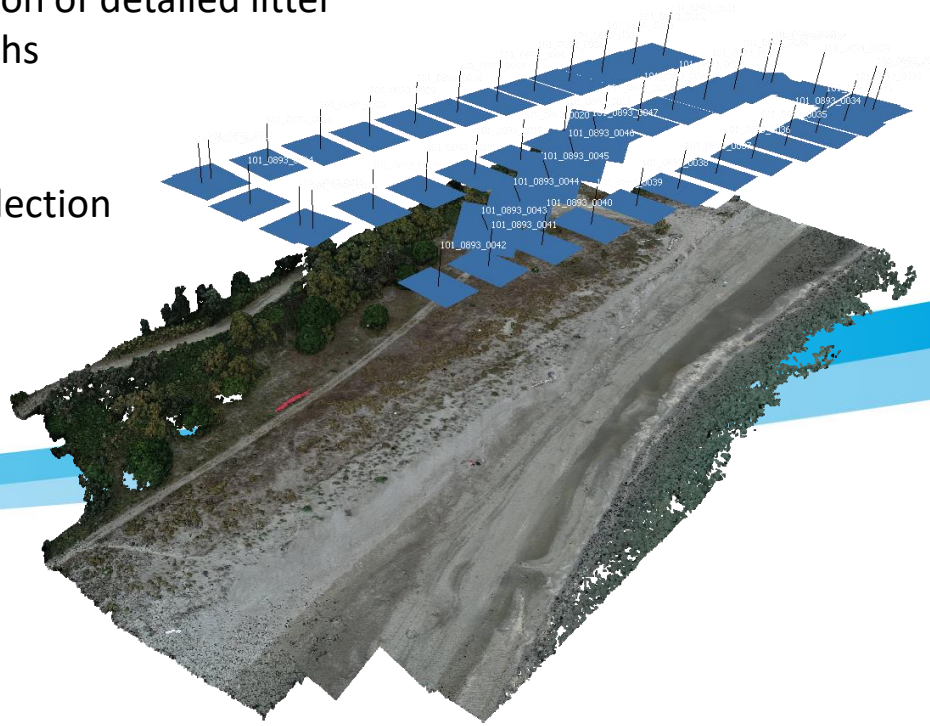


- Execution of flight missions with drone (UAV)

- GNSS survey of marine litter

- Acquisition of detailed litter photographs

- Litter collection



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Monitoring marine litter in coastal waters

Sea Surface Sampling:

- area identification (sampling beach area-facing)
- 4 transects - manta trawl towed by boat along the transepts for ~1/2 hour each
- collection of trapped material and storage in glass jars with alcoholic solution

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Monitoring in Biota - mussels

- commercial size: 4-7 cm
- Global sample consisting of 3 elementary samples that will be carried out at three depth levels:
  - greater depth
  - in the middle
  - at 50 cm from the surface

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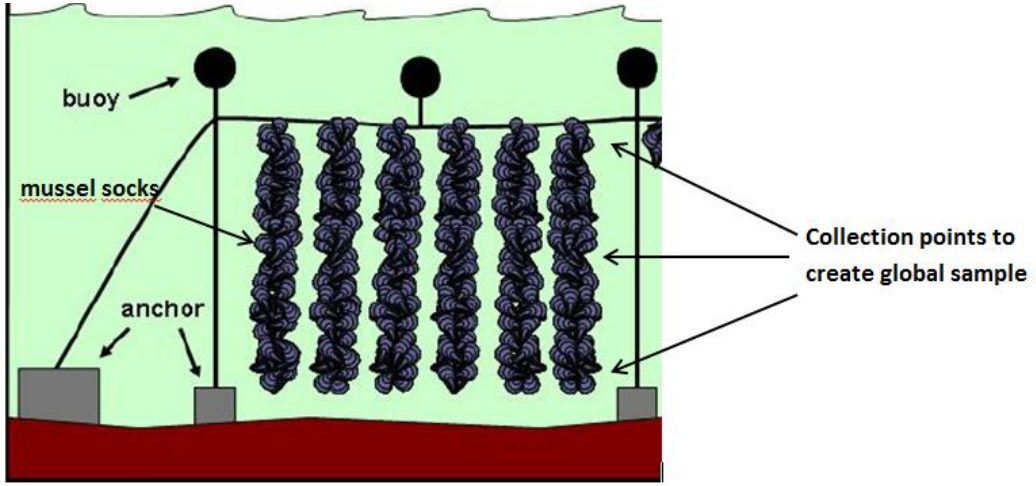
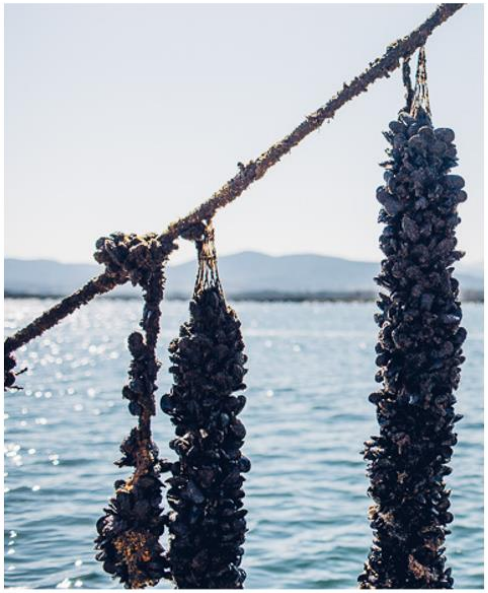
New Technologies for macro and Microplastic Detection and Analysis in the Adriatic Basin

Modulo di campionamento del BIOTA

Partner/Organizzazione	Data del campionamento	Paese - Macroarea	Specie campionata	Condizioni metereologiche (piovoso - soleggiato - nuvoloso)	*Osservazioni*

ID campione		Parametri colonna d'acqua			Quantità di ciascun campionamento:
Ora inizio/fine		Superficie	T (°C)		8-10 kg di mitili (taglia 4-7 cm)
GPS latitudine			Ph		
GPS longitudine			Salinità (ppt)		☐ Per la quantificazione delle MP: circa 2 kg
Habitat (Banchi naturali o allevamenti)			O_2 (mg/l)		
Profondità		Intermedia	T (°C)		☐ Per le analisi chimiche: circa 4-5 kg
Metodo di raccolta			Ph		
T° media dell'aria			Salinità (ppt)		
Vento (direzione e velocità)			O_2 (mg/l)		☐ Per gli esperimenti di depurazione: circa 2 kg
Data e ora di arrivo in laboratorio		Fondo	T (°C)		
			Ph		
			Salinità (ppt)		
			O_2 (mg/l)		





- *Aluminum foil*
- *Balance*
- *Blotting paper*
- *Caliper*
- *Latex gloves without powder*
- *Multi-parameter probe*
- *Portable refrigerator*
- *Scissors*
- *Scalpels*
- *Water resistant pens*

Lab's analysis

Macro Litter

2.5 – 50 cm

Artificial polymer materials	• bags, bottles, food containers incl. fast food containers, crates and containers, plastic caps and lids, cigarette butts and filters, gloves, synthetic rope, fishing net, other plastic/polystyrene items(identifiable)
Rubber	• balloons and balloon sticks, rubber boots, tires and belts, bobbins (fishing), condoms (incl. packaging), other rubber pieces
Cloth/ Textile	• clothing, shoes, bags, carpets, furnishing, ropes, string and nets etc.
Paper/Cardboard	• cardboard (boxes & fragments), cigarette packets, drink containers, newspapers etc.
Processed /worked wood	• pallets, corks, processed wood etc.
Metal	• cans (food, aerosol, drinks), refrigerators, washers, fishing related, car parts, batteries, cables, Large metallic objects, etc.
Glass/ ceramics	• bottles incl. pieces, jars incl. pieces, glass or ceramic fragments >2.5cm, large glass objects (specify), other glass items.

$$CCI = \frac{\text{Total number of plastic items (items)}}{\text{Total area of sampling units (m2)}} \times 20$$

CLEAN COAST INDEX	
Values	**Cleanliness**
0 – 2	**Very clean** (no litter is seen)
2 - 5	**Clean** (no litter is seen over a large area)
5 – 10	**Moderate** (a few pieces of litter can be detected)
10 – 20	**Dirty** (a lot of waste on the shore)
20+	**Extremely dirty** (most of the shore is covered with plastic debris)

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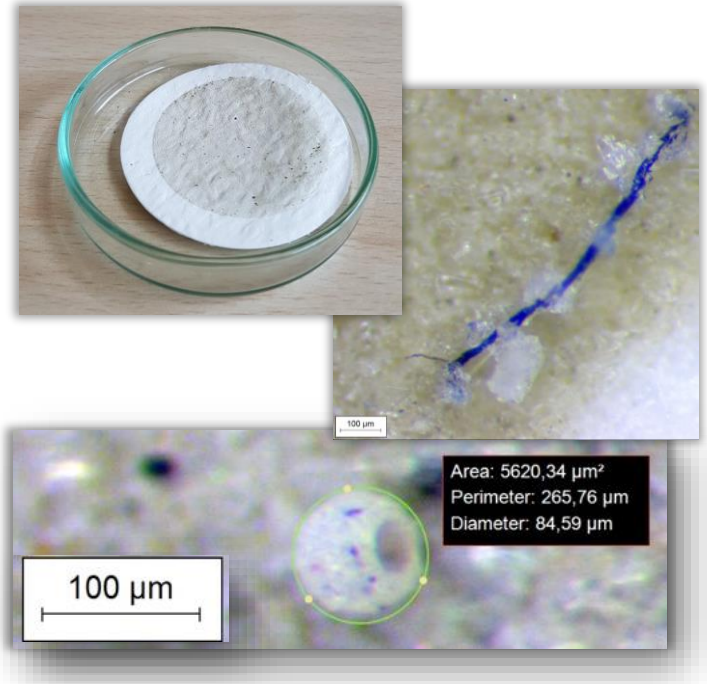
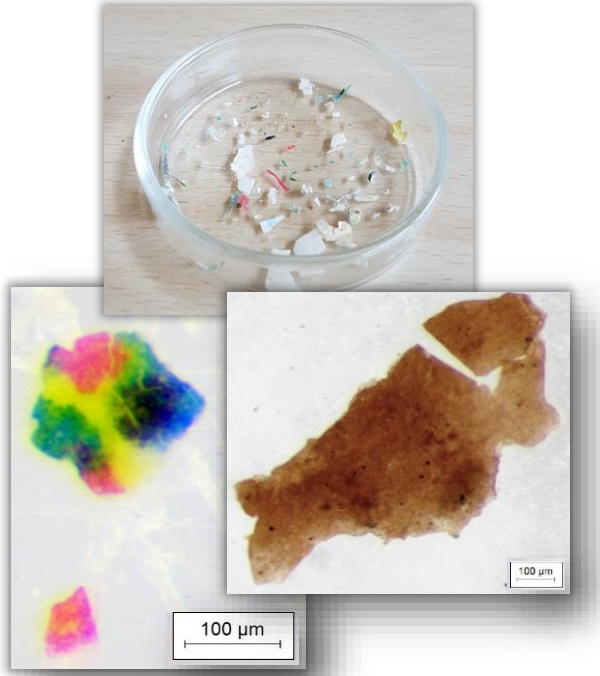
Lab's analysis

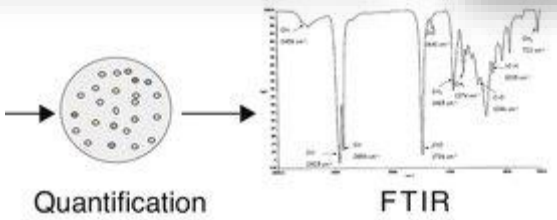
LMP - Large Micro Plastics
(1 - 5 mm)

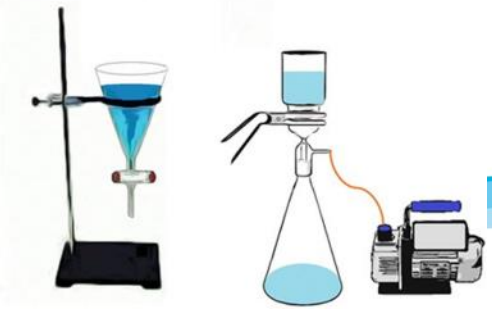
SMP - Small Micro Plastics
(20 µm - 1 mm)



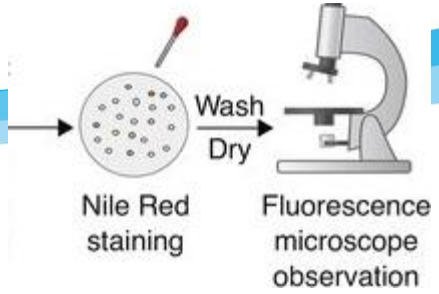
SSL items
(300 µm - 5 mm)







Vacuum filtration



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Lab's analysis

1) SAMPLE PRETREATMENT

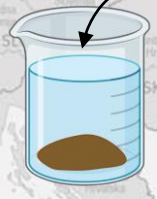
WATER

H_2O_2 30% + Ethanol

- 50°C
- Orbital stirring
- 24-48 h

Pretreament for eliminating organic matter

WET SEDIMENT



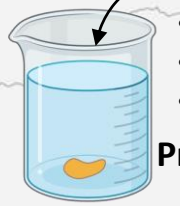
NaCl

- Pre-drying sediment (2g, 60°C, 24h)
- Mix sediment with saline solution, allow to settle, collect supernatant (repeat for 3 times)

Pre-treatment with saturated salt solution to float microplastics to the surface

MOLLUSK

- Separating soft tissue from shell
- Weighing the soft tissue



H_2O_2 30% + Ethanol

- 50°C
- Orbital stirring
- 1-7 days

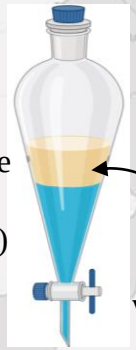
Pretreament for eliminating organic matter

2) PURIFICATIION WITH OIL

For greater sample purification: oil can capture all microplastics in the sample

1) In the funnel: pre-treated sample + 10 ml olive oil> shake > sediment>discard the watery fraction and mantain the oily (repeat 3 thimes)

2) Wash the oily fraction with ethanol and filter



Oily fraction with MP

3) FILTRATION

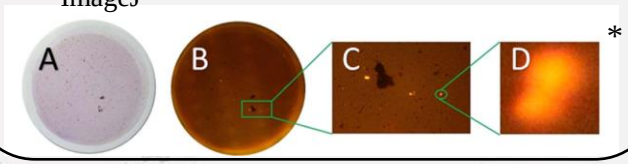
The oily part with microplastics is filtered

Filter: Whatman glass fiber grade GF/F (0,7 µm)

4) QUANTIFICATION

Nile red staining makes MP glow under UV light

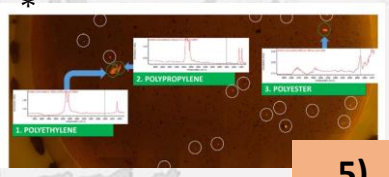
- 10µm/ml in acetone, 30 minutes
- UV Stereomicroscope with orange filter
- ImageJ



*

Micro-FTIR *iN10MX*

*



5) IDENTIFICATION

EU part of cooperation area

* Non-EU part of cooperation area

Maes, T., Jessop, R., Wellner, N. et al. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging

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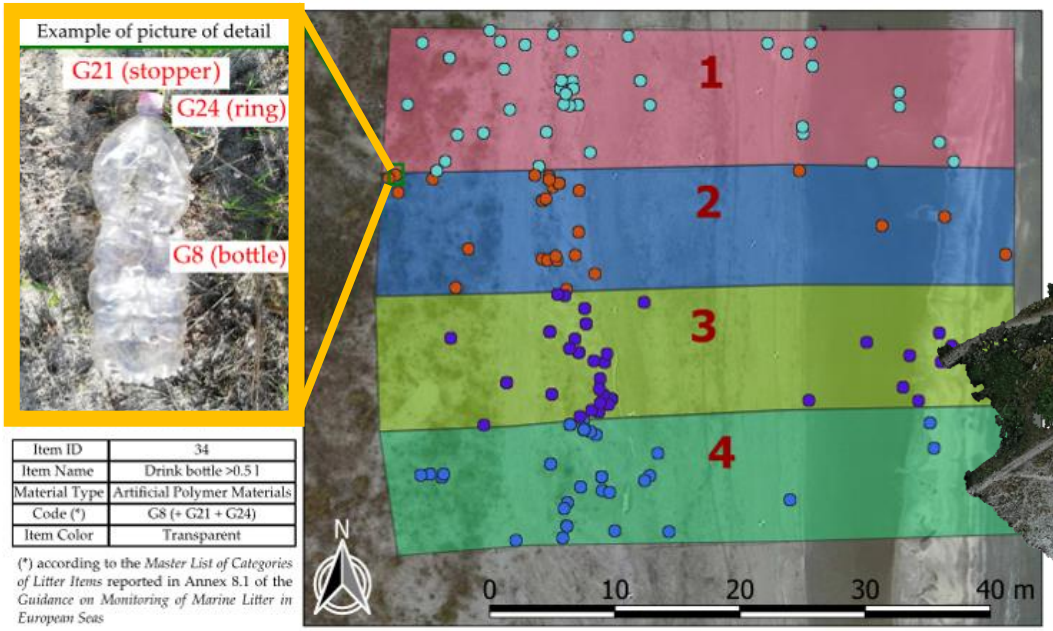
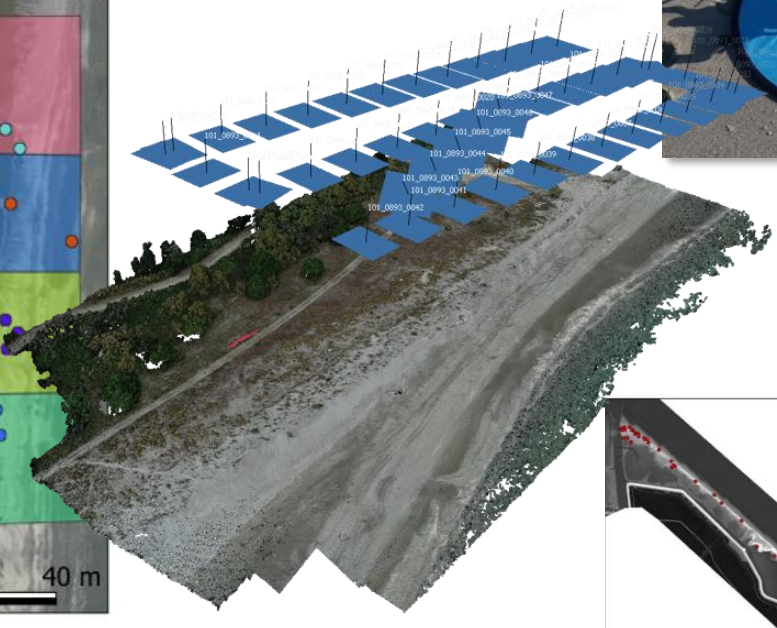
Lab's analysis - UAV

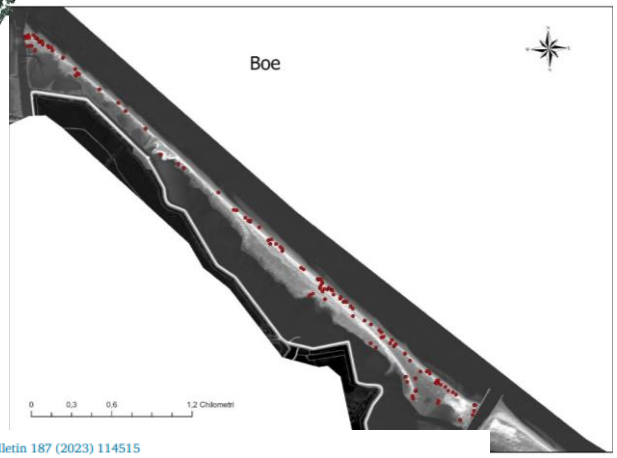
GIS elaboration from drone images



Item ID	34
Item Name	Drink bottle >0.5 l
Material Type	Artificial Polymer Materials
Code (*)	G8 (+ G21 + G24)
Item Color	Transparent

(*) according to the *Master List of Categories of Litter Items* reported in Annex 8.1 of the *Guidance on Monitoring of Marine Litter in European Seas*





Open Access | Editor's Choice | Article

drones

UAV Approach for Detecting Plastic Marine Debris on the Beach: A Case Study in the Po River Delta (Italy)

by Yuri Taddia [1,*], Corinne Corbau [2], Joana Buoninsegni [2], Alberto Pellegrinelli [1]

Marine Pollution Bulletin 187 (2023) 114515

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Understanding through drone image analysis the interactions between geomorphology, vegetation and marine debris along a sandy spit

Corinne Corbau [a,b,c,*], Joana Buoninsegni [a], Elisabetta Olivo [a], Carmela Vaccaro [a], William Nardin [b], Umberto Simeoni [c]

[a] University of Ferrara, Ferrara, Italy
[b] HPL – UMCES, Cambridge, MD, USA
[c] CURSA, Roma, Italy

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Protocol for quali-quantitative analysis of microplastics in biota

4 STEP

Delivery and storage of the sample

analyzed within 24 ours after collection or stored in freezer at -20° +/- 5°C

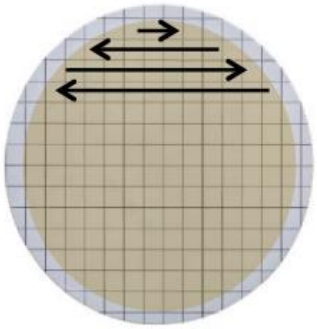
Oxidizing digestion

50 organisms -> dissection soft tissue + Hydrogen Peroxide at 30% (20ml / each gram of soft tissue) -> incubation at 55-60° for 4-7 days

Filtration

Cover the Petri dishes with the appropriate lid and leave the filters to dry at room temperature for at least 24 hours before reading with the stereomicroscope.

Reading with the stereomicroscope



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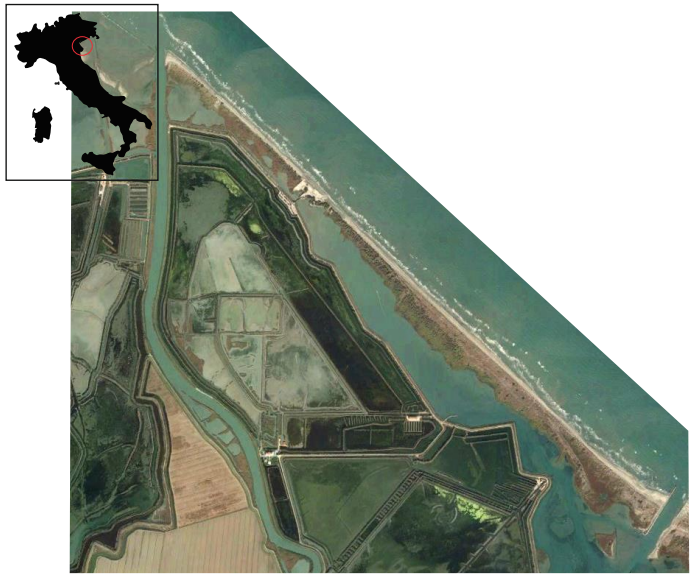
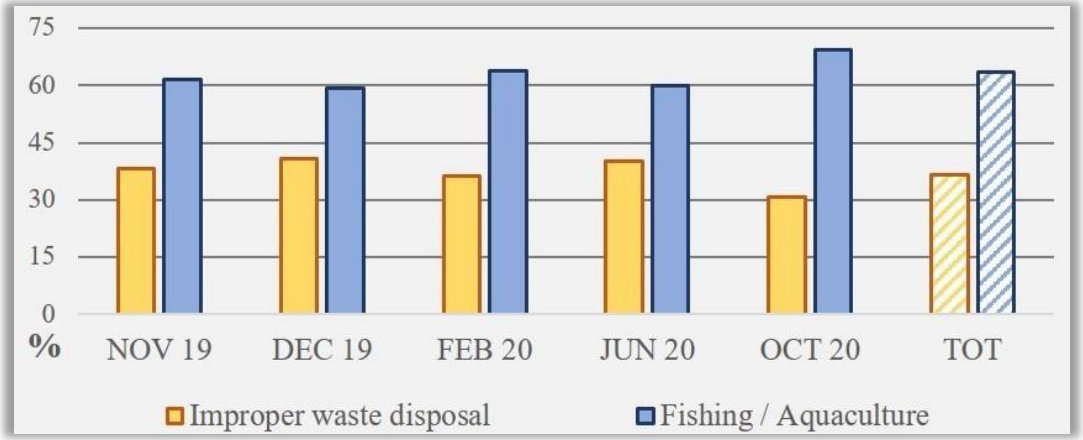
Results



MARINE LITTER SURVEYS ON BOCCASETTE BEACH (ROVIGO, ITALY)

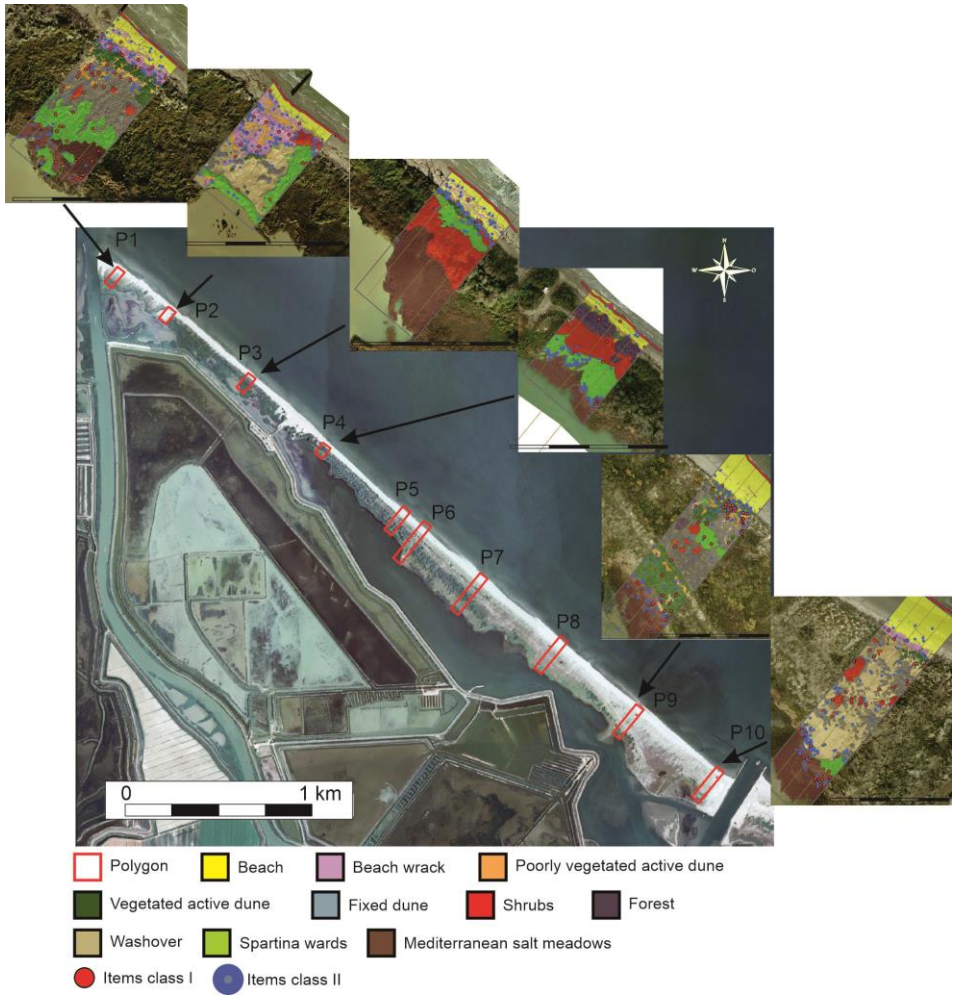
- Collected items: **5.578 -** 96% artificial polymer materials
- Items Density: **0.35 items/m²** (± 0.13 SD)
- Clean Coast Index:
  - **Moderately clean** (Nov/Dec-19; Oct-20)
  - **Clean** (Feb/Jun-20)

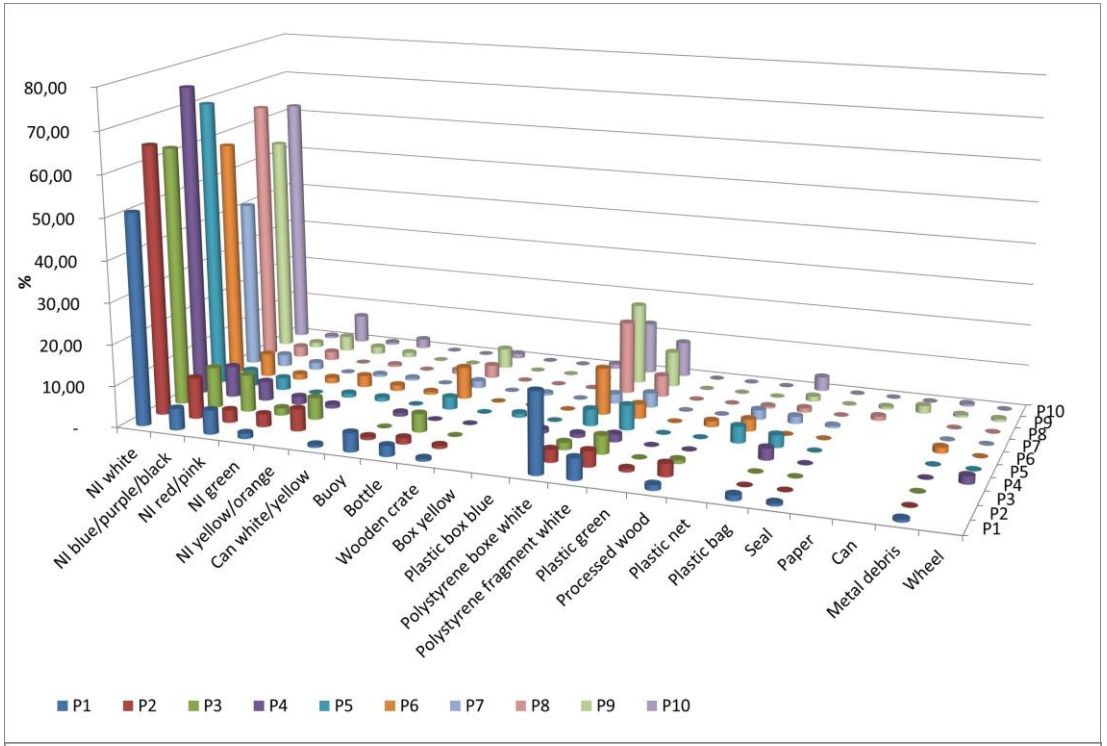
Date	Event notes
Nov 2019	Beginning of 1st sea-storm, high-water and flooding of Po
Dec 2019	After 1st sea-storm; wane high-water and flooding of Po
Feb 2020	After 2nd sea-storm
Jun 2020	Restart of fishing activity and tourism after Covid-19
Oct 2020	After tourist season

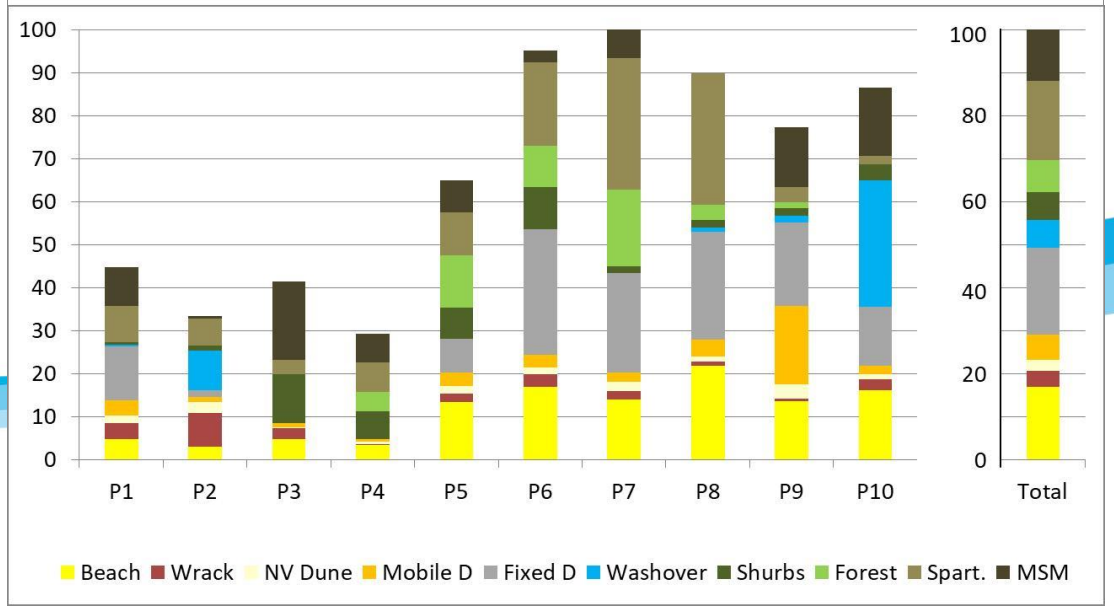


Fishing and Aquaculture > Improper Waste Disposal

Results







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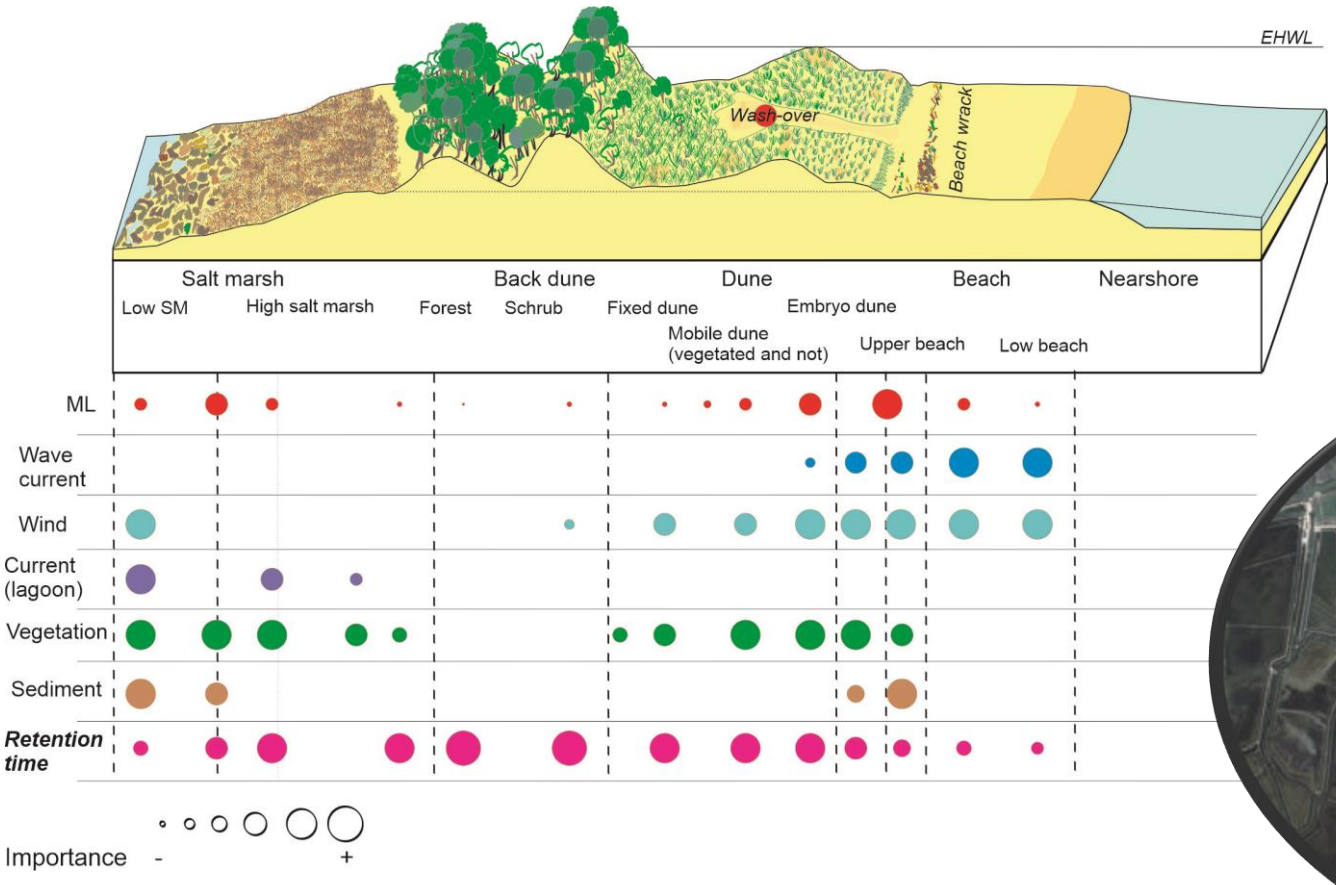
Results

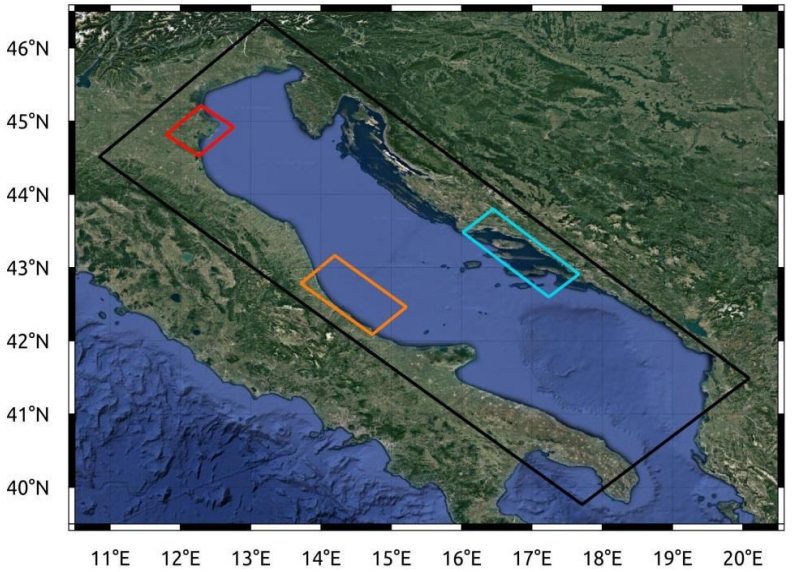


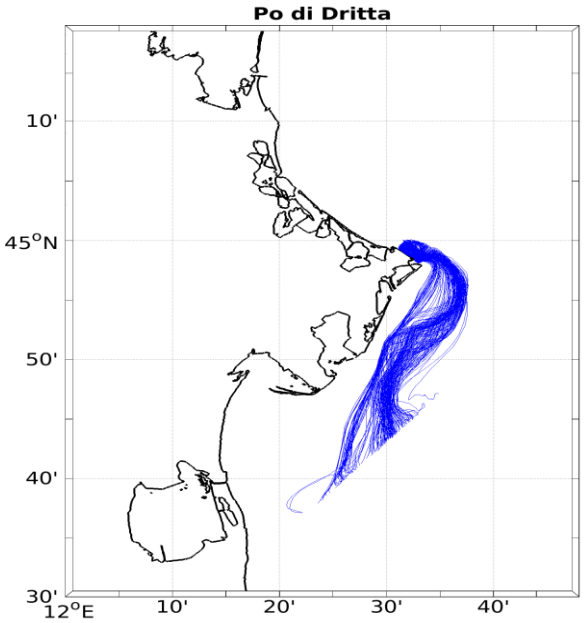
Presence of EPS fishing Boxes
-> Actions are needed

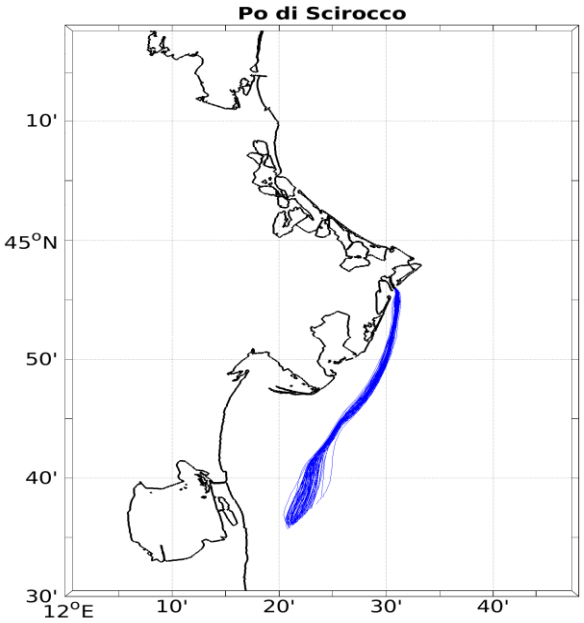
Results

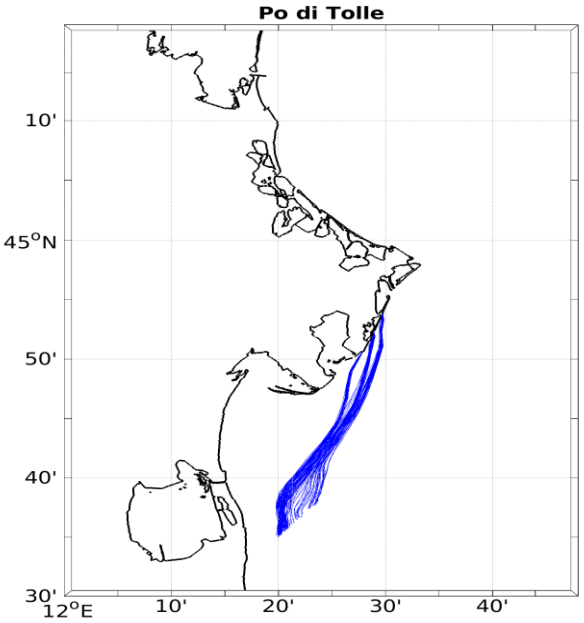
For each site a **4 years long simulation** have been carried out. The analysis of the modeling results has given the possibility to identify the percentage of **potential stranding for fluvial micro-plastics** for each pilot site and to calculate **impact hazard maps for the coasts** both on a daily and climatological basis (yearly, seasonal and monthly).

On following the transport model has been integrated with an **Early Warning System platform.**





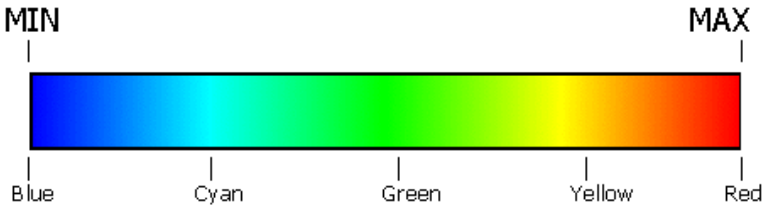




Winter – all fluvial contributions

Accumulation Points

Accumulation Points



Winter – Goro Po river contribution

Experimental protocol "Laboratory uptake and clearance"

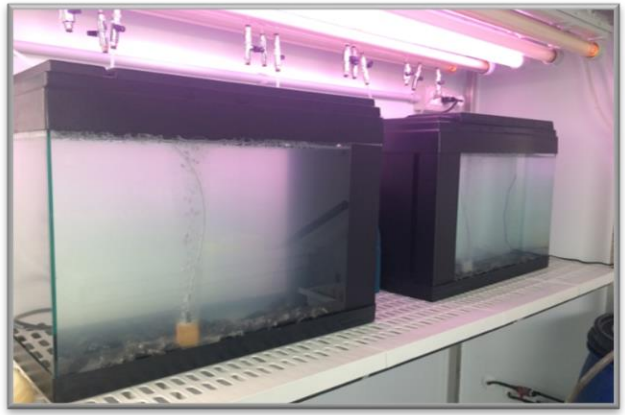
Acclimatization

➔ 7 days in a glass aquarium system (50 l) with filtered artificial seawater aerated at **18 ± 1 °C** and **salinity 35 ‰.**

➔ 12 h light-dark regime, no food

➔ Gut clearing

➔ After 7 days: 20 specimen for blank – remaining -> contamination



Exposure phase

➔ 3 days

➔ A concentration of **2000 MPs/L** with a ratio of **granules, fragments and fibers** of **1: 1: 8** (same proportion found in marine environment according to *Su et al., 2016)*

➔ constant **salinity of 35‰,** T° of **18 °C**, and **12 h light-dark regime**

➔ Water was renewed, Daily feed, monitoring of the mortality

Purification phase

➔ **20 mussels for control**

➔ Two different purification times were applied:

- a **"microbiological" purification** lasting 2 days;
- a **"experimental" purification** lasting 7 days.

Experimental protocol "Laboratory uptake and clearance"

MPs' qualitative and quantitative analysis

For each sampling time
- control group
- 0 time group
- 2 time group7 time group

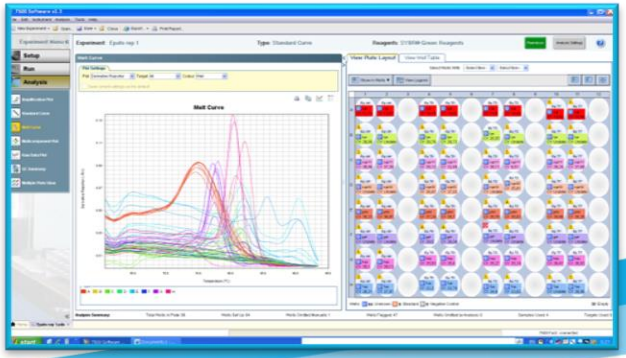
20 organisms were taken and MPs' qualitative and quantitative analysis were performed

Gene expression analysis

For each sampling time
- control group
- 0 time group
- 2 time group7 time group

✓ 5 organisms were taken and from each separatly gills and digestive gland were taken and pools created

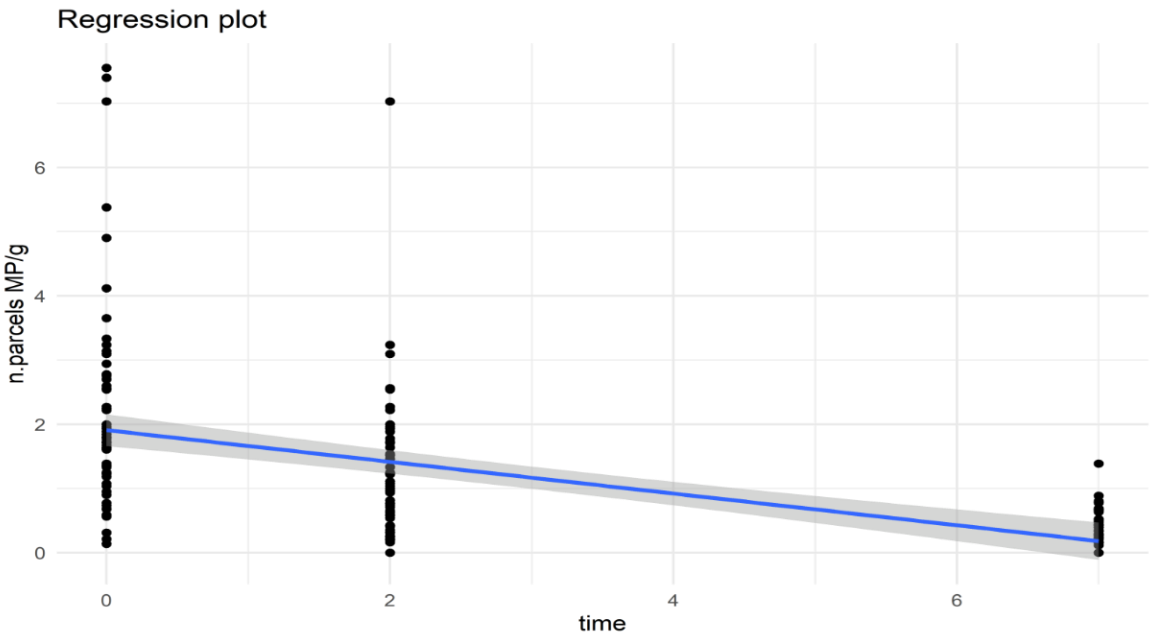
✓ In these pools bio-molecular (qPCR real-time) investigations were performed in order to evaluate a set of target genes related



Experimental protocol "Laboratory uptake and clearance"

Results

The replicates, conducted under the same experimental conditions, **did not show statistically significant variability**, therefore the experimental error did not interfere with the results obtained.



From the regression plot it is possible to highlight that there is a **statistically significant decrease** (p value 2.5E-14) in the presence of the number of microplastic particles found per gram of soft tissue of the analyzed mussels, in fact we have as average values in the 3 experimental groups:

- Group T0: 2.17 MPs/g;
- Group T2: 0.49 MPs/g;
- Group T7: 0.27 MPs/g.

This decrease can be seen already after 2 days of purification and is even more evident after 7 days of purification.

Instead, no statistically significant differences emerged between the T0 and T2 groups indicating that the two-days depuration time may not have been long enough to completely eliminate the microplastic particles or that MP could have been translocated to other tissues, or even to the circulatory system (*Birnstiel et al., 2019; von Moos et al., 2012; Browne et al., 2008*).

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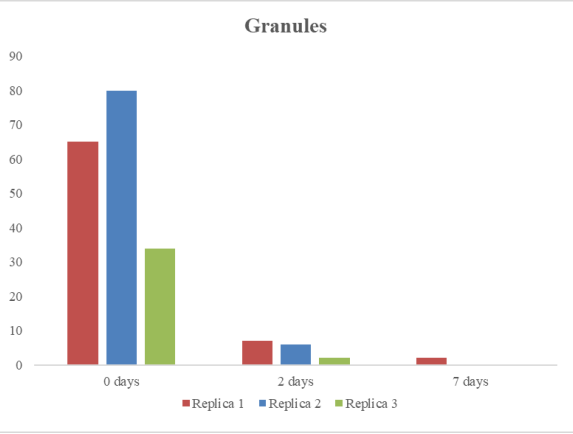
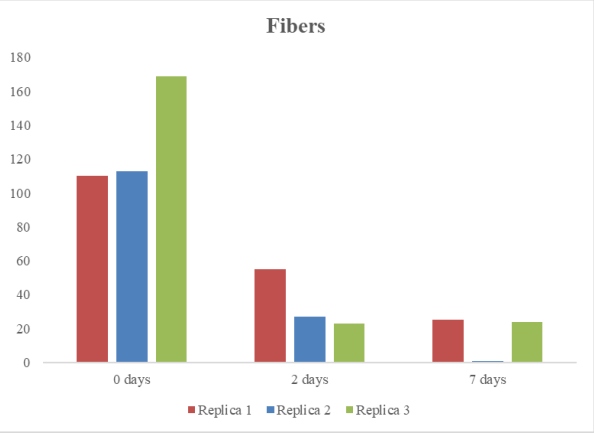
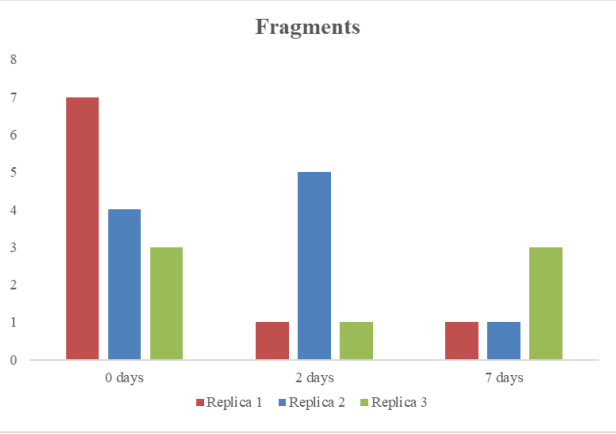
Experimental protocol "Laboratory uptake and clearance"

Results



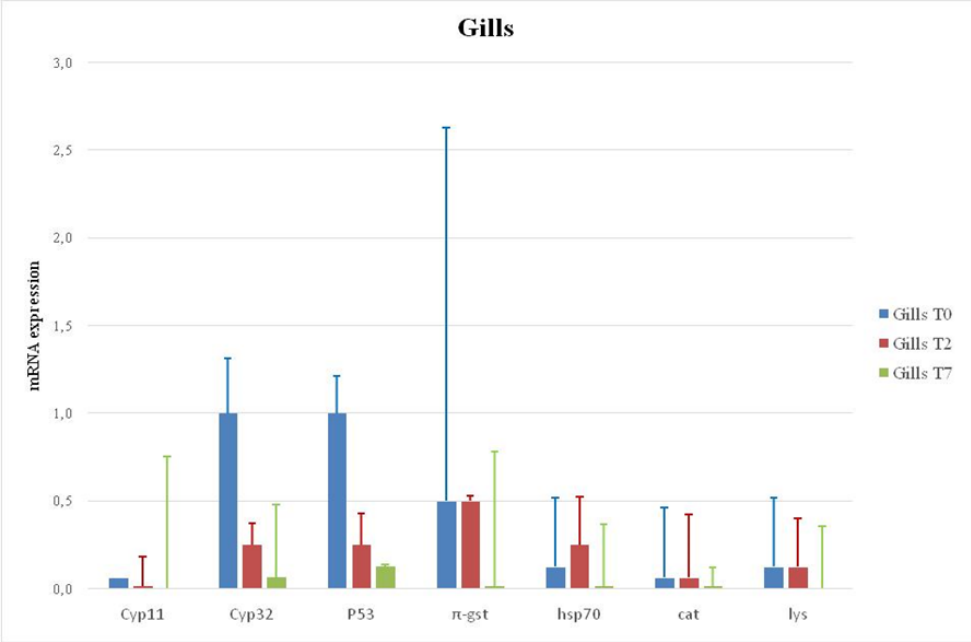




D**ecrease of the number of each type of microplastic** in the three experimental replicas.
More efficient elimination of microplastics : **granules -> filaments -> fragments.**

Experimental protocol "Laboratory uptake and clearance"

Gene expression analysis

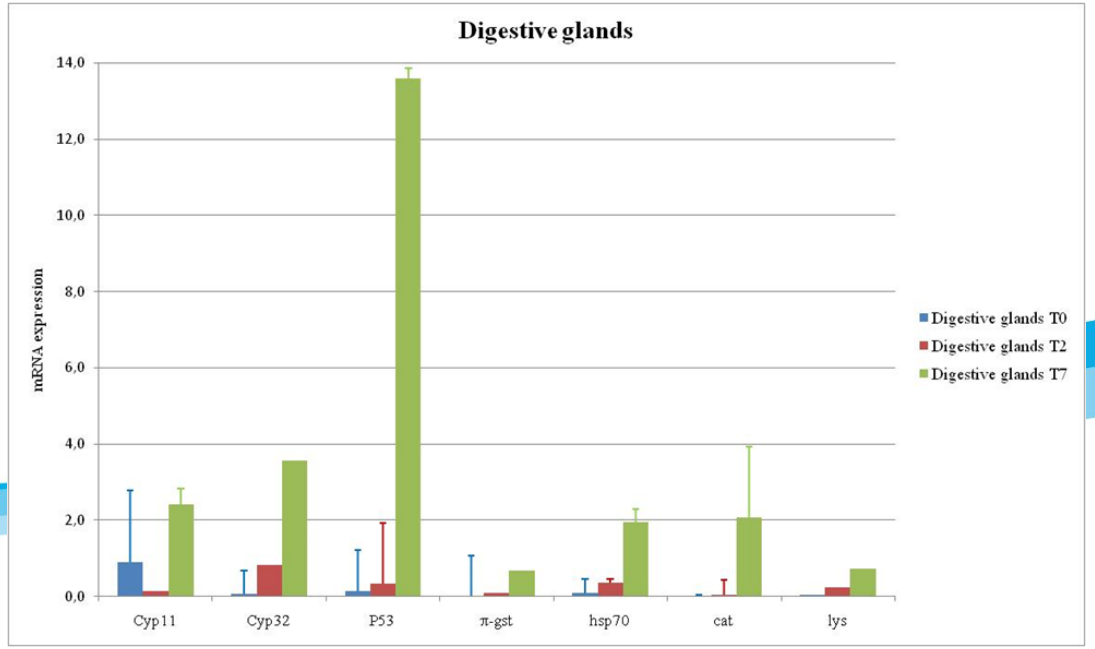


Gene expression data showed that

- In both tested organs we observed differences in contaminated mussels' group or purificated group (T7 group) with respect to the control group.

- In particular, the microplastics altered the expression of genes associated with biotransformation, DNA damage and cell-tissue repair genes

Therefore, the results of the present work confirm that cyp32, π-gst and P53 are transcript biomarker candidates in mussel, in accordance with Lacroix et al. 2014;



Experimental protocol "Laboratory uptake and clearance"

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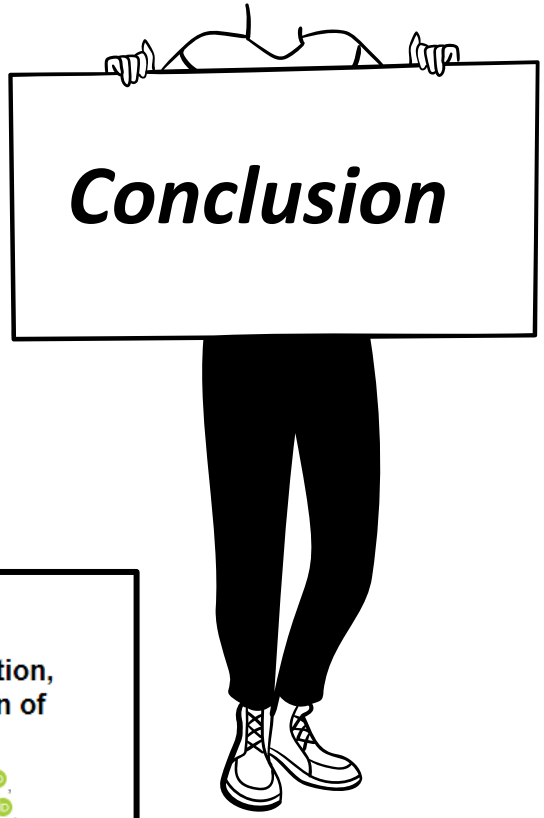

Open Access Article

Exposure of *Mytilus galloprovincialis* to Microplastics: Accumulation, Depuration and Evaluation of the Expression Levels of a Selection of Molecular Biomarkers

by Federica Pizzurro [1], Eliana Nerone [1,*], Massimo Ancora [1], Marco Di Domenico [1], Luana Fiorella Mincarelli [1], Cesare Cammà [1], Romolo Salini [1], Ludovica Di Renzo [1], Federica Di Giacinto [1], Corinne Corbau [2], Itana Bokan [3], Nicola Ferri [1] and Sara Recchi [1]

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