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Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA

NATIONAL BIODIVERSITY FUTURE CENTER

CONTAMINANTI EMERGENTI ED ECOTOSSICOLOGIA: PRIME VALUTAZIONI DAI PROGETTI PNRR-CN-BIODIVERSITÀ

Francesco Regoli
Università Politecnica delle Marche

IIIa Giornata di Ecotossicologia Applicata
Metodi ecotossicologici: verso criteri di valutazione comuni per ambiti diversi

Livorno 22-23 Novembre 2023

ISPRA Istituto Superiore per la Protezione e la Ricerca Ambientale

ECOTOX LDS

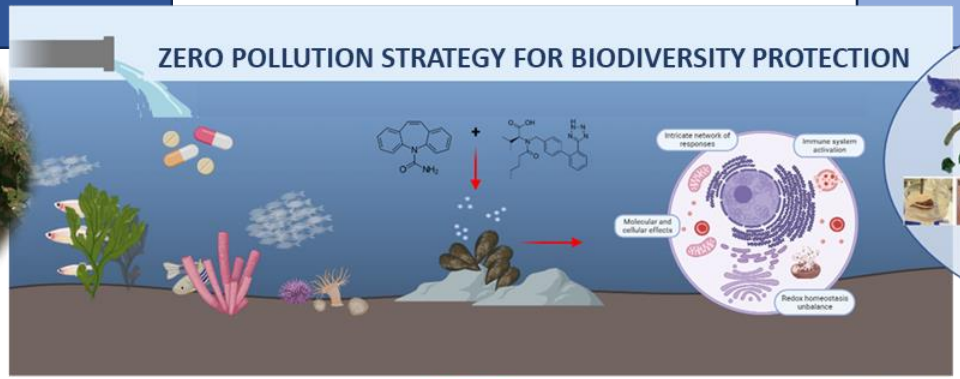
Sistema Nazionale per la Protezione dell'Ambiente

SPOKE 2
Activity 1.2

WP1. MAPPING PRIORITIES AND FILLING KNOWLEDGE GAPS ON EMERGING POLLUTANS IN THE MEDITERRANEAN

emerging pollutants

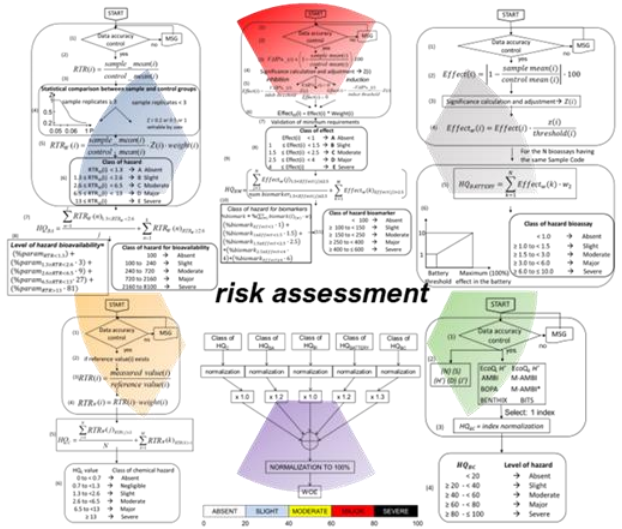
ZERO POLLUTION STRATEGY FOR BIODIVERSITY PROTECTION



WP2. DEVELOPMENT OF INNOVATIVE EFFECT-BASED MONITORING TOOLS

diagnostic health tools

iconic species and biotopes



risk assessment

innovative biosensors

WP3. SUSCEPTIBILITY TO POLLUTION-MEDIATED LOSS OF BIODIVERSITY IN MEDITERRANEAN AND INTERACTIONS OF EMERGING POLLUTANTS WITH CLIMATE CHANGE

WP4. SOCIO-ECONOMIC-INDUSTRIAL INNOVATION: ZERO POLLUTION VISION

WP5. SETUP OF LIGHTHOUSE DEMONSTRATORS FOR INNOVATIVE TECHNOLOGIES TO COUNTERACT POLLUTION THREAT TO BIODIVERSITY

UNIVERSITÀ DEGLI STUDI DI PADOVA

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

UNIVERSITÀ DI SIENA 1240

ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA

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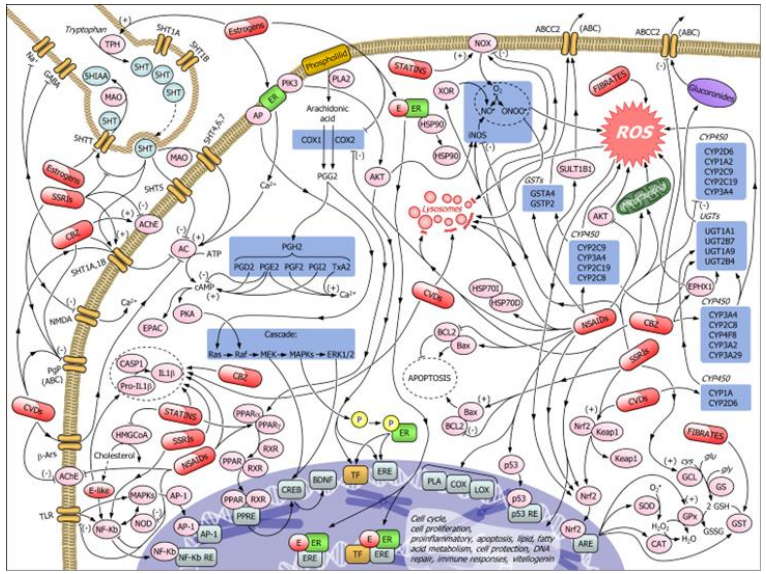
WP1. MAPPING PRIORITIES AND FILLING KNOWLEDGE GAPS ON EMERGING POLLUTANTS IN THE MEDITERRANEAN

Task 1.2.1 – Prioritizing risks from emerging pollutants for Mediterranean biodiversity



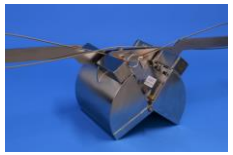
PRIORITIZATION

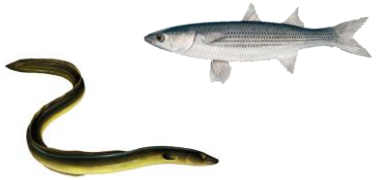
~600 MOLECULES in 10 CATEGORIES

- 112 molecules in ***"priority 1a"*** <u>not included in normative guidelines</u>

Pharmaceuticals/metabolites/alkaloids
Plasticizers and additives
Perfluorinated
Pesticides
Flame Retardants
Fragrances

- 73 molecules in ***"priority 1b"***
- 260 molecules in ***"priority 2"***
- 155 molecules in ***"priorIty 3"***

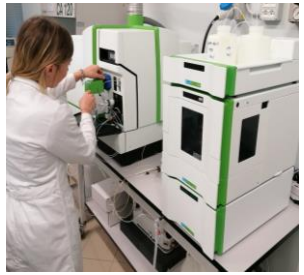




Selected for WP1-WP2-WP3

Analytical methodologies for seawater, sediments and biota have been developed and validated, analyses are currently ongoing

- Extraction
- Purification
- LC-MS, ICP-MS, TOFMS



WP1. MAPPING PRIORITIES AND FILLING KNOWLEDGE GAPS ON EMERGING POLLUTANTS IN THE MEDITERRANEAN

Task 1.2.2 – Mapping sources and distribution of emerging pollutants in selected case studies

Selected case studies:

- **2 rivers (Po, Tevere)** and 4 coastal cities (Ancona, Genova, Palermo, Trieste)

- **Sediment** sampling sites identified along Po mainstream (n=4)
- **Water** sampling site identified far from saltwater intrusion (n=1)
- **Biota** (European eel, n=6; manila clam, n=5) collected along Po mainstream near mouth

Samples collected in **March, June (water after intensive raining episodes), September 2023**

- **Sediment** sampling sites identified at sea nearby the Tevere mouth along three transects (n=8)
- **Water** sampling sites identified **nearby Roma Sud WWTP** and **along stream**, sampled twice per day (morning and evening, n=4 x 2)
- **Biota** (European eel, n= 35; thinlip mullet, n= 15; mussels, n=3) collected along mainstream near mouth

Samples collected in **April-May 2023**

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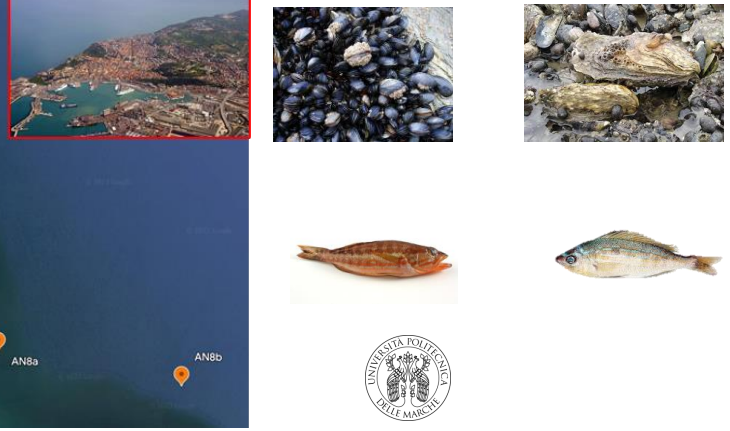

- **Sediment** sampling sites at Genova's "**Punta Vagno" WWTP** outfall (n=5)
- **Water** sampling at the Genova's "**Punta Vagno" WWTP** (in and out) and in the nearby marine area (n=11)
- **Additional water sampling** sites identified at **Rapallo's WWTP** (in and out, n=2)
- **Biota** (22 fish specimens) collected in the marine area near Punta Vagno WWTP by local fisherman. Specimens belong to *D. vulgaris, D. sargus*, *D. annularis*, *C. julis*, *S. cabrilla, S. maena* (n=2)

Samples collected in **March 2023**

- **Sediment** sampling sites identified at sea nearby the **Ancona's "Z.I.P.A." WWTP** (in and out) in the nearby harbour area and along coastal touristic sites (n=8)
- **Water** sampling sites identified at Ancona's **"Z.I.P.A." WWTP** (in and out), in the nearby harbour area and along coastal touristic sites (n=8)
- **Biota** (mussels, oysters, mullets) collected nearby the Ancona's "**Z.I.P.A." WWTP** outfall

Samples collected in **April-May** and **July 2023**

WP1. MAPPING PRIORITIES AND FILLING KNOWLEDGE GAPS ON EMERGING POLLUTANTS IN THE MEDITERRANEAN

Task 1.2.2 – Mapping sources and distribution of emerging pollutants in selected case studies

Selected case studies:

- 2 rivers (Po, Tevere) and **4 coastal cities (Genova, Ancona, Palermo, Trieste)**

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- **Sediment** sampling sites identified in the marine in area front of Palermo's **"Acqua dei Corsari" WWTP** outfall (n=8)
- **Water** sampling sites identified at the Palermo's "**Acqua dei Corsari" WWTP** outfall (in and out) and in the nearby marine area (n=10)
- **Local biota**

Samples will be collected in **June 2023**

OGS

- **Sediment** sampling sites identified at **Trieste's "Servola" WWTP outfal**l and in the nearby marine area (n=6)
- **Water** sampling sites identified at the **Trieste's "Servola" WWTP** (in and out) and in the nearby marine area (n=8)
- **Biota** (mussels, n=1) collected from a buoy nearby the Trieste "Servola" WWTP outfall, sampling of vertebrates in autumn

Samples collected in **April 2023**

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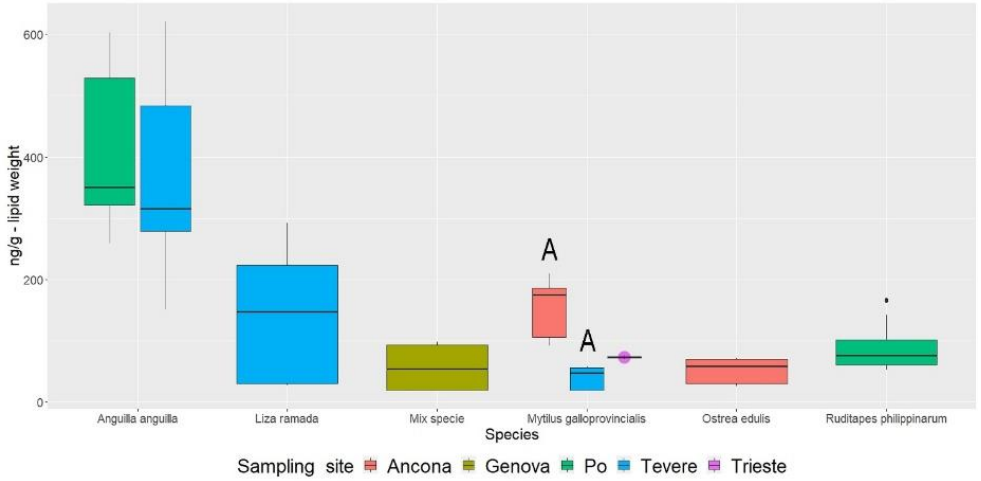
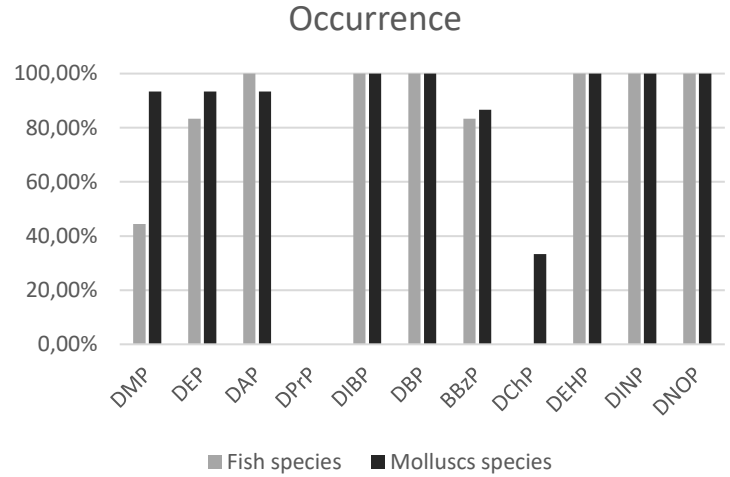
WP1. MAPPING PRIORITIES AND FILLING KNOWLEDGE GAPS ON EMERGING POLLUTANTS IN THE MEDITERRANEAN

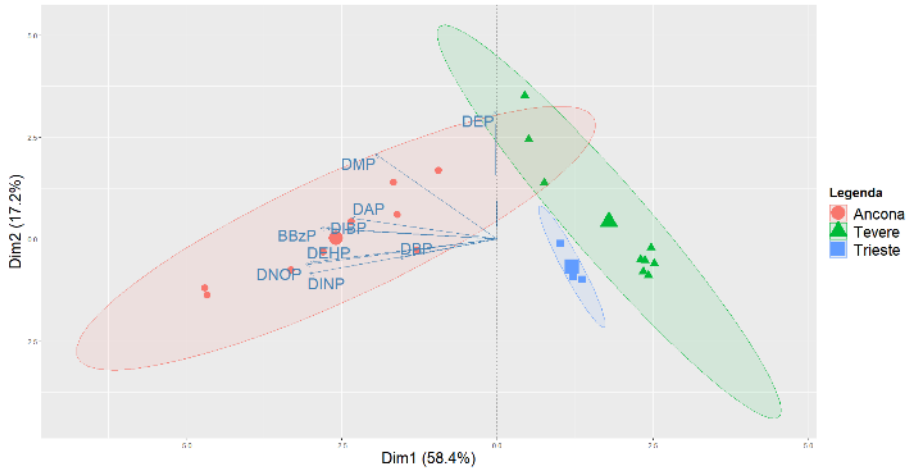
Task 1.2.2 – Mapping sources and distribution of emerging pollutants in selected case studies

Detection of phthalates in the selected Bioindicator species



Concentration of **Σ phthalates in different areas and species** – Letters indicate a statistically significant difference (Kruskall-Wallis test < 0.05 and Dunn-Bonferroni post-hoc test < 0.05).





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WP1. MAPPING PRIORITIES AND FILLING KNOWLEDGE GAPS ON EMERGING POLLUTANTS IN THE MEDITERRANEAN

Task 1.2.2 – Mapping sources and distribution of emerging pollutants in selected case studies

Guidelines for harmonised management of data on emerging pollutants to be integrated in EMODnet Chemistry infrastructure

- **Metadata**: where data were collected, when, how, by whom, why,...
- **Data**: the values
- **Ancillary parameters**: any parameters that help in assessing a specific variable
- **QA/QC information**: all information related to the whole process of assuring the quality of data
- **Data policy**: a description of the data access and usage conditions
- **DOI**: alphanumeric string assigned to uniquely identify an object with all the details accessible.

6. Guidelines for metadata management (Annex 1: Metadata template)

Required metadata are indicated in the template provided as Annex 1.

7. Guidelines for data management (Annex 2: Data template)

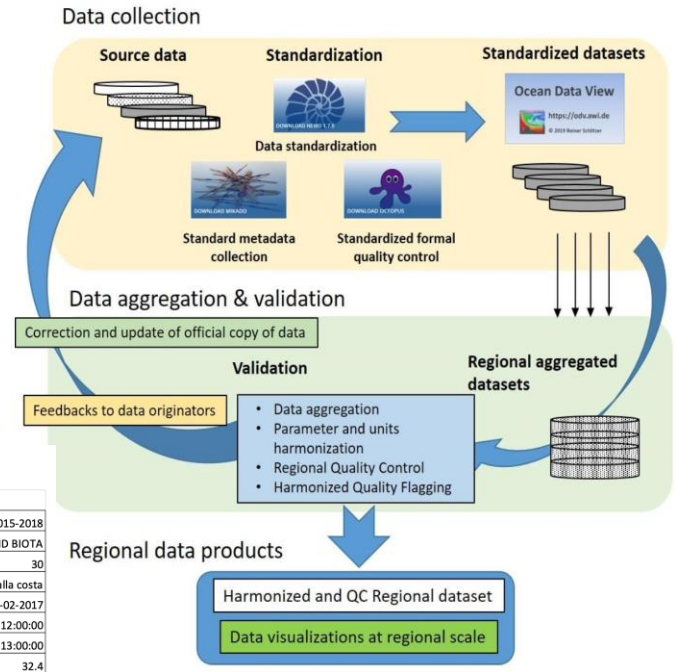
The dataset should be structured according to the template provided as Annex 2. Please include the Quality Flags (QF) according to **Table 1**.

8. Guidelines for QA/QC information management (Annex 3: QA/QC template)

Information on laboratory protocols and QA/QC should be provided using the template provided as Annex 3. Mandatory fields are marked with *, but it is strongly suggested to provide all available information.

Annex 1: Metadata template)

Metadata required:	Example:
dataset_name	MSFD_Monitoring-2015-2018
abstract	CONTAMINANTS 2015-2018 IN SEAWATER, SEDIMENTS AND BIOTA
platforms (L06 vocab)	30
station_name	RIMINI a 20 Km dalla costa
station_short_name	RIMINI-02-2017
station_start_date (dd/mm/yyyy hh:mm:ss)	18/05/2017 12:00:00
end_date (dd/mm/yyyy hh:mm:ss)	18/05/2017 13:00:00
water_depth	32.4
min_depth	0.5
max_depth	32.4
lat	44.2090
lon	12.8121
policy (L08 vocab)	UN
edmerp	12294
Data originator (EDMO)	3009



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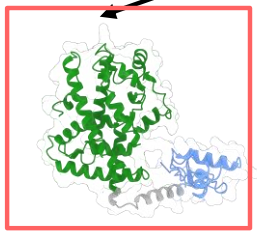
WP2. DEVELOPMENT OF INNOVATIVE EFFECT-BASED MONITORING TOOLS

Task 1.2.3 -Innovative biosensors for early detection of pollutants threatening biodiversity

Receptor Selection

We select from various receptors that play a central role in regulating cell function and serve as targets for drugs.

- PPARs (Peroxisome Proliferator-Activated Receptors)
- AhR (Aryl hydrocarbon Receptor)
- ERs (Estrogen Receptors)



PPAR

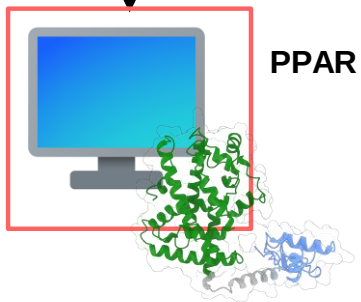
ER

AHR

Computational Analysis

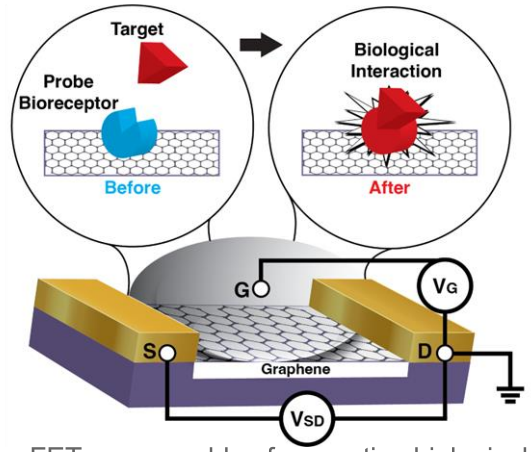
Based on the computational study, the receptor PPAR-γ, which is targeted by obesogens, appears to be the best candidate.

The PPAR-γ plasmid was purchased for the production of recombinant protein.

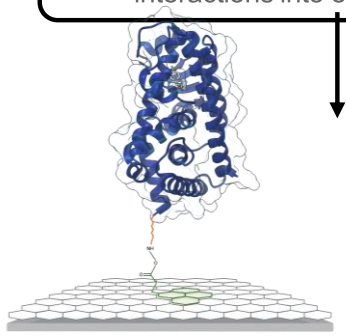


PPAR

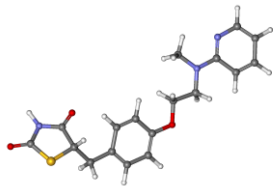
gFET Sensing Platform



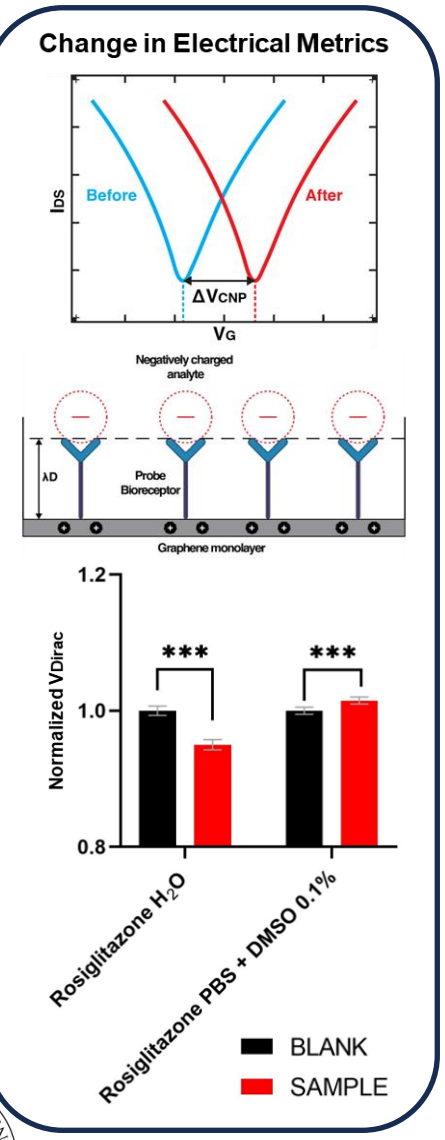
gFETs are capable of converting biological interactions into electrical signals



gFET Functionalization



Rosiglitazone



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WP2. DEVELOPMENT OF INNOVATIVE EFFECT-BASED MONITORING TOOLS

Task 1.2.4 -Health Toolbox for measuring status of marine organisms
Task 1.2.5 Effects of emerging pollutants and interactions with multiple stressors

BIOLOGICAL MODELS

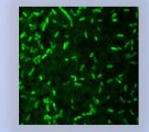
in vivo

Mytilus galloprovincialis

Perinereis aibuhitensis

Balanophyllia europaea

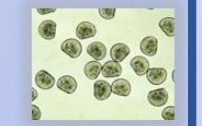
Aliivibrio fischeri



Ciona robusta

P. tricornutum

C. gigas



Paracentrotus lividus

in vitro

Organotypic cell lines obtained from skin biopsies

- *Striped dolphin: Stenella coeruleoalba*
- *Fin whale: Balaenoptera physalus*
- *Sea turtle: Caretta caretta*
- *Mammalian cell lines*

ex vivo

PRECISION CUT TISSUE SLICES (PTCS)
M. galloprovincialis

BIOLOGICAL ENDPOINTS HEALTH TOOLS

Gene to cellular level

- *transcriptomics*
- *gene expression:*
  - *oxidative stress*
  - *biotransformation*
  - *behavioural traits*
  - *immunity*
  - *reproductive traits*
- *Microbiota*
- *ATACseq to assess genome-wide chromatin accessibility*
- *enzymatic biomarkers:*
  - *oxidative stress*
  - *neurotoxicity*
  - *biotransformation, EROD*
- *immunology:*
  - *humoral & cellular activities*
  - *morphological alterations*
  - *lysosomal membrane stability*
  - *in vitro responses in haemocytes*
- *genotoxicity:*
  - *micronucleus*
  - *comet assay*
- *endocrine disruption*

Physiology and organism level

- *survival*
- *growth rates*
- *behaviour*
- *reproduction:*
  - *reproduction performance*
  - *sexual differentiation*
  - *sexual maturation*
- *embryologic development:*
  - *hatching success*
  - *time to hatch*
  - *abnormality rate*
  - *biometrics*
- *energetic physiology:*
  - *feeding rate*
  - *metabolic rate*
  - *absorption of food*

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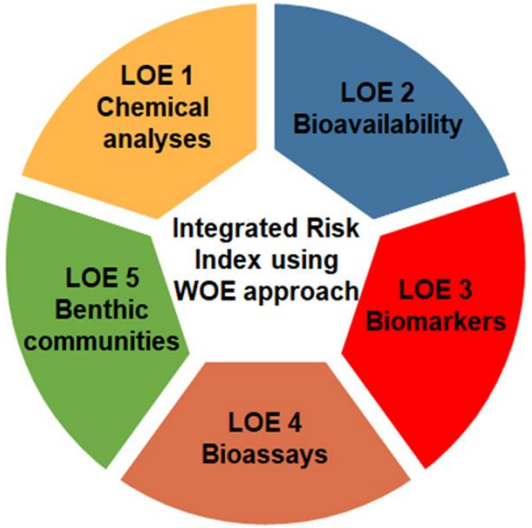
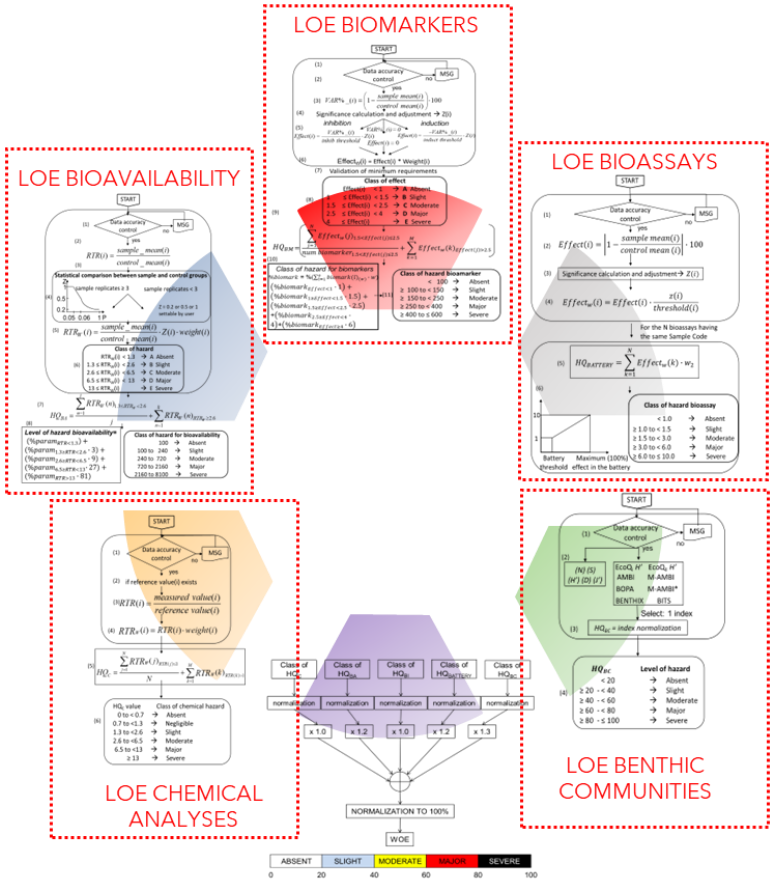
Task 1.2.4 -Health Toolbox for measuring status of marine organisms
Task 1.2.5 Effects of emerging pollutants and interactions with multiple stressors

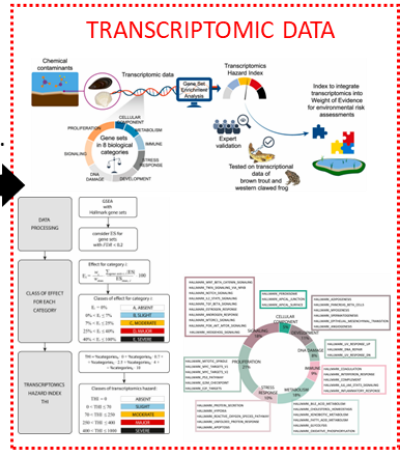
WEIGHT OF EVIDENCE ELABORATION

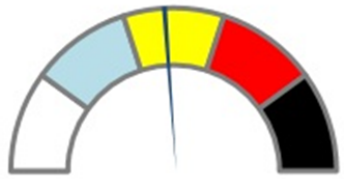




NEW LOEs

e.g.





SYNTHETIC RISK INDEX

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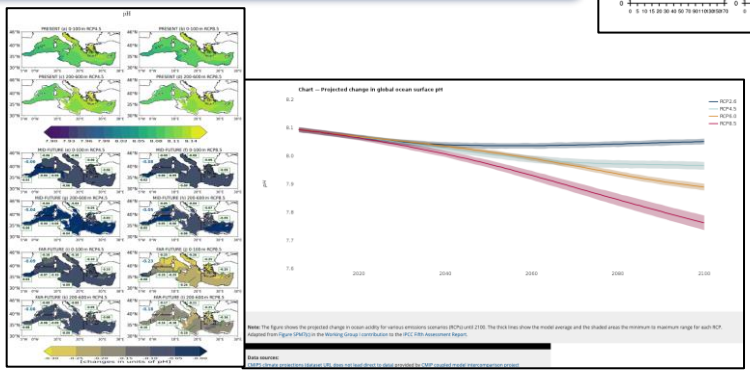
Task 1.2.4 -Health Toolbox for measuring status of marine organisms
Task 1.2.5 Effects of emerging pollutants and interactions with multiple stressors

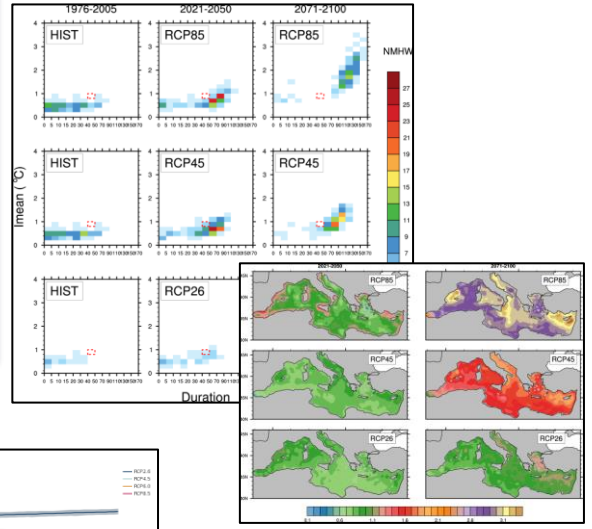
***PERFORMED in vivo* EXPERIMENTS, *Mytilus galloprovincialis*:**

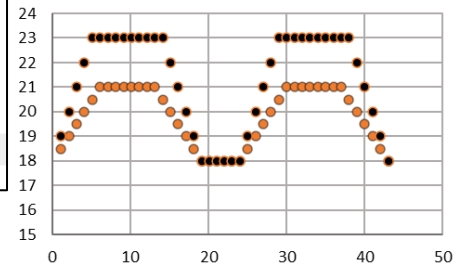
Exp 1- Carbamazepine, Valsartan, Mixture
Exp 2- Ibuprofen, Paroxetine, Mixture
Exp 3- Exposure to **PE-fragments** of different size classes: 20-50 µm, 50-100 µm, 100-250 µm, 250-500 µm, 500-1000 µm
Exp 4- Mixtures of **micronized plastic fragments**: micronized commercial items (PE: 43%, PET: 32.4%, PP: 23.1%, PS: 1.12%, PVC: 0.38%), micronized environmental items (PE: 43%, PET: 32.4%, PP: 23.1%, PS: 1.12%, PVC: 0.38%), micronized rubber items
Exp 5 -Venlafaxine, Gemfibrozil, Ramipril, Metformin, Mixture
Exp 6-Cocaine, Caffeine, Mixture
Exp 7-Glyphosate, AMPA, Mixture

Exp 8 - **Pharmaceuticals mixtures** under **climate change scenario**: MIXTURE of Carbamazepine, Ibuprofen, Venlafaxine, Metformin, Ramipril, Gemfibrozil combined with **future projection of seawater pH decrease** and **cyclic Marine Heatwaves**



RCP 4.5 – Target pH = -0.10 – far future (2080 – 2099) + coastal acidification, Wallace et al., 2014





RCP 4.5 2071-2100 more but shorter MHWs

ENVIRONMENTAL DOSES: 0.1-1.5µg/L
EXPOSURE PHASE: 14 or 30 days
DEPURATION PHASE: 14 days

WP2. DEVELOPMENT OF INNOVATIVE EFFECT-BASED MONITORING TOOLS

Task 1.2.4 -Health Toolbox for measuring status of marine organisms
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-DRUGS BIOACCUMULATION:
Carbamazepine, Valsartan, Ibuprofen, Paroxetine. Venlafaxine, Gemfibrozil, Ramipril, Metformin, Citalopram, Bezafibrate; Cocaine; Caffeine; Glyphosate; AMPA

-MICROPLASTICS INGESTION

- **MOLECULAR CHANGES**: New transcriptome assembly, RNA Seq
- **MICROBIOTA:** 16S Amplicon Sequencing
- **GENOME-WIDE CHROMATIN ACCESSIBILITY** (ATACSec)

-IMMUNOLOGICAL PARAMETERS:
Lysosomal membrane stability , Granulocytes/Hyalinocytes, Phagocythosis rate

-NEUROTOXIC DAMAGE: Acetylcholinesterase in hemolymph and gills

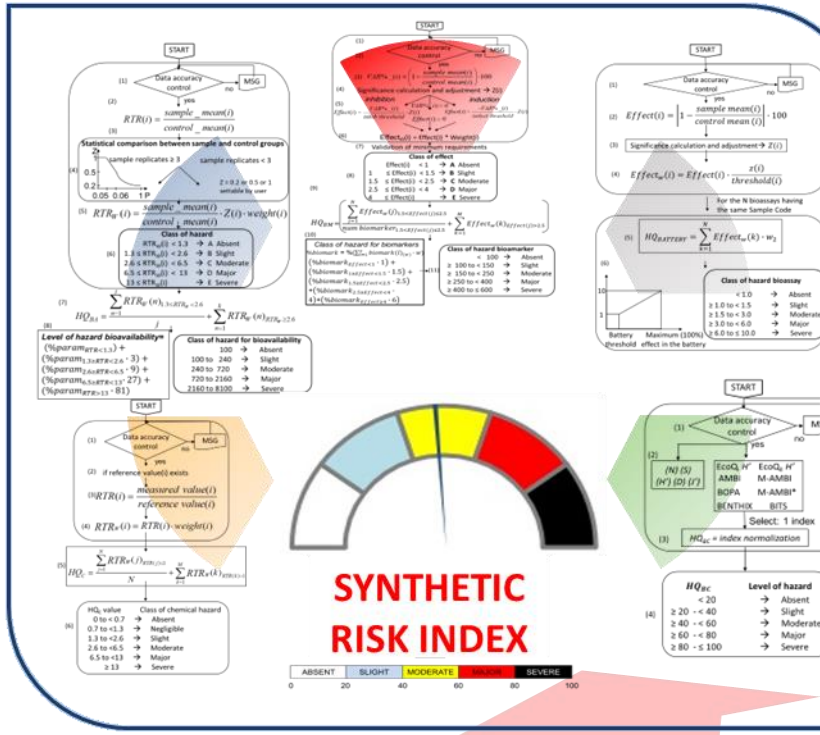
-OXIDATIVE STRESS:
Catalase, glutathione reductase, gluthatione peroxidases, glutathione S transferases, total glutathione, Total oxiradical scavenging capacity toword hydroxyl and peroxyl radicals

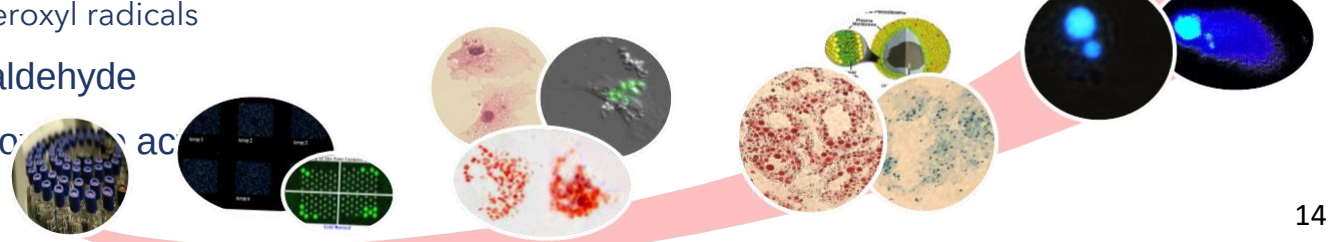
-CELLULAR EFFECTS: Accumulation of lipofuscin and malondialdehyde

-LIPID METABOLISM: Accumulation of neutral lipids, Acyl-CoA o… ac…

-GENOTOXICITY: Micronuclei frequency, DNA fragmentation

WEIGHT OF EVIDENCE ELABORATION





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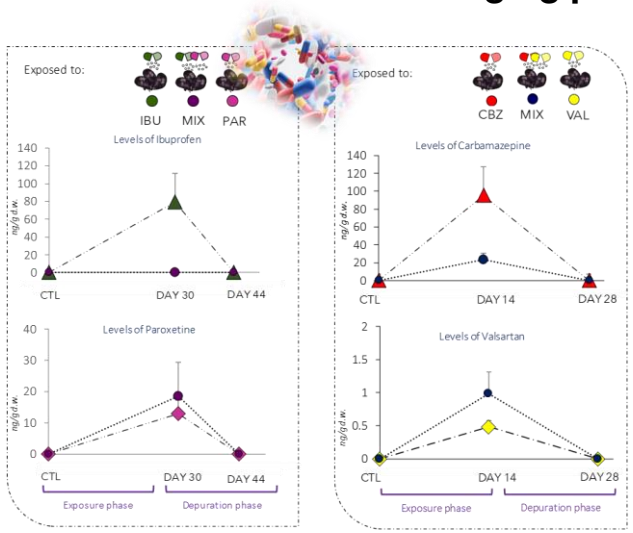

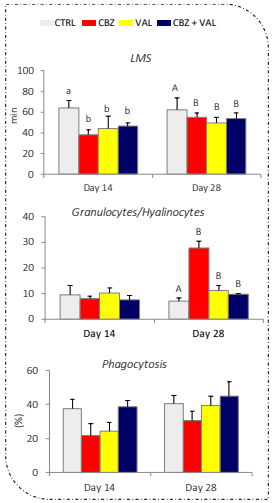

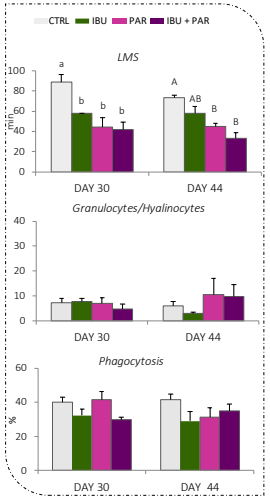

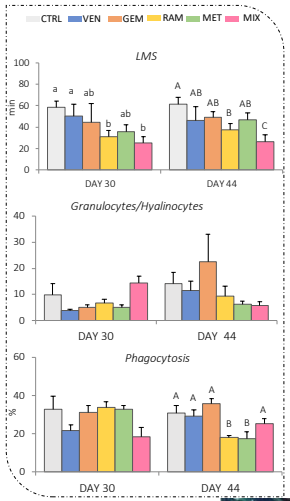

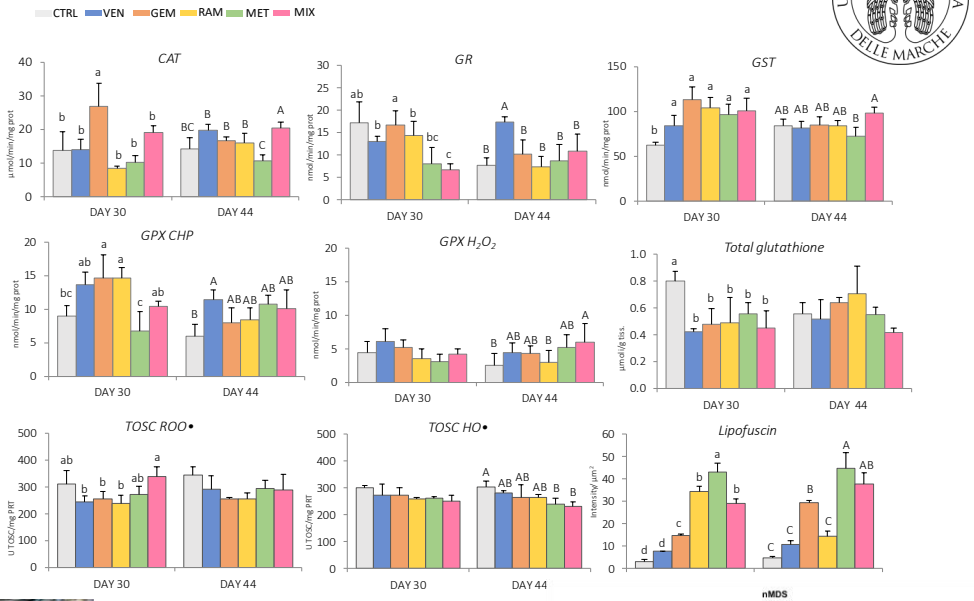

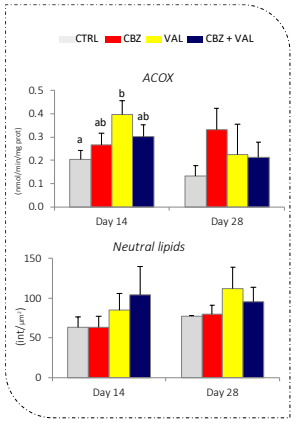

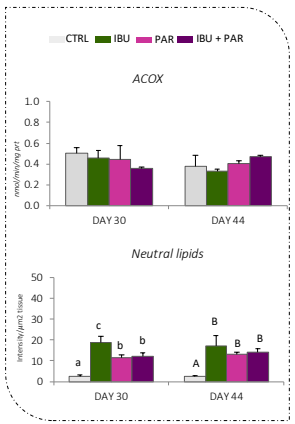

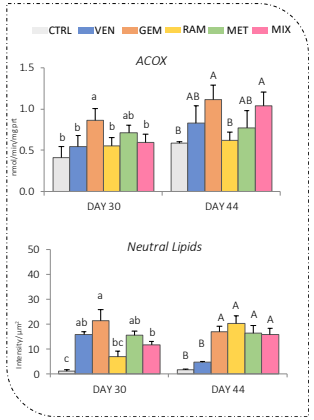

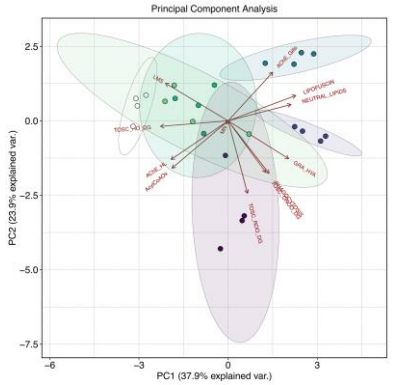

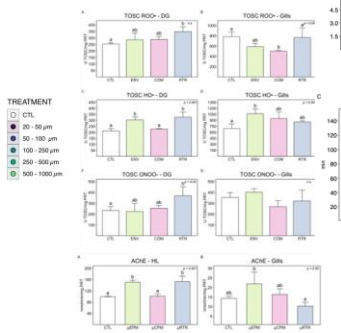

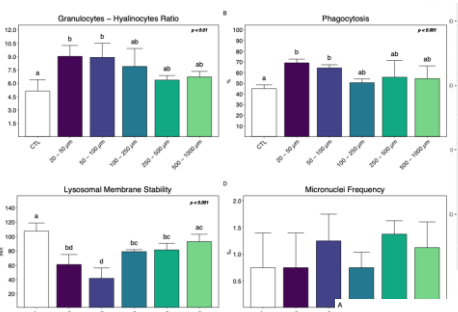

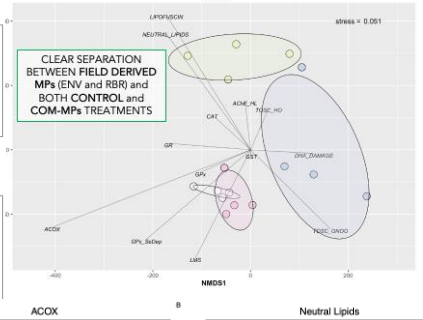

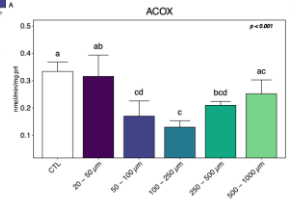

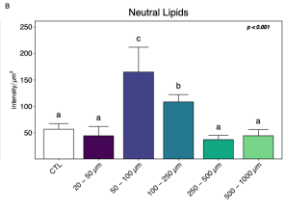

Francesco Regoli| Università Politecnica delle Marche | Spoke 2, Activity 1.2 | **ZERO POLLUTION STRATEGY FOR BIODIVERSITY PROTECTION**

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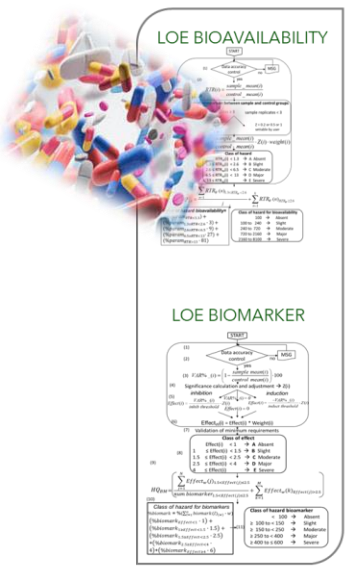
WEIGHT OF EVIDENCE ELABORATION



EXPOSURE PHASE

Treatment	Loe 2 Bioavailability	Loe 3 Biomarker	WOE INTEGRATION	Level Of Risk
Cbz	Severe	Moderate	MAJOR	
Val	Moderate	Moderate	SLIGHT	
Cbz + Val	Major	Slight	SLIGHT	
Ibu	Major	Moderate	MODERATE	
Par	Major	Moderate	MODERATE	
Ibu + Par	Major	Moderate	MODERATE	
Ven	Ongoing	Moderate		
Gem	Ongoing	Moderate		
Ram	Ongoing	Moderate		
Met	Ongoing	Moderate		
Mixture	Ongoing	Moderate		

DEPURATION PHASE

Treatment	Loe 2 Bioavailability	Loe 3 Biomarker	WOE INTEGRATION	Level Of Risk
Cbz	Absent	Moderate	SLIGHT	
Val	Absent	Slight	ABSENT	
Cbz + Val	Absent	Slight	ABSENT	
Ibu	Absent	Moderate	SLIGHT	
Par	Absent	Moderate	SLIGHT	
Ibu + Par	Absent	Absent	SLIGHT	
Ven	Ongoing	Moderate		
Gem	Ongoing	Moderate		
Ram	Ongoing	Moderate		
Met	Ongoing	Moderate		
Mixture	Ongoing	Moderate		

All biomarker responses

Treatment	LEVEL OF HAZARD
MPs_20-50 µm	MODERATE
MPs_50-100 µm	MODERATE
MPs_100-250 µm	MODERATE
MPs_250-500 µm	SLIGHT
MPs_500-1000 µm	SLIGHT

Immune system responses

Treatment	LEVEL OF HAZARD
MPs_20-50 µm	MAJOR
MPs_50-100 µm	MODERATE
MPs_100-250 µm	SLIGHT
MPs_250-500 µm	ABSENT
MPs_500-1000 µm	ABSENT

WEIGHTED ELABORATION

EXP TREATMENT	PARAMETERS IN EACH CLASS				Level of hazard for biomarkers
	SLIGHT	MODERATE	MAJOR	SEVERE	
ENV	(TOSC HO•)	(LMS) (AChE)	(LIPOFUSCIN)	0	SLIGHT
COM	(LMS)	(LIPOFUSCIN) (NEUTRAL LIPIDS) (GPx TOT)	0	0	SLIGHT
RBR	(TOSC ROO•) (DNA DAMAGE)	(AChE) (TOSC HO•) (TOSC ONOO-) (ACOX)	0	0	MODERATE

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MicroCO₂sme

Microplastics in a CO_2-rich ocean: using micro-mesocosms to assess impacts on a temperate ecosystem

Exposure for **1 year** to:
- **Microplastics** (mixture of polymers)
- Ocean acidification and warming: **+2°C pH -0,3**
- Multistress (**OAW+MP**)

biocenosis

Planctonic biodiversity, epibionths richness, bentic associations, functional analyses

Ostrea edulis

Digestive gland
Gills

Growth, survival, reproduction; Ecophysiology; Immunity; Energy metabolism

Molecular Analyses

Microbiota characterization

Gene expression profiles

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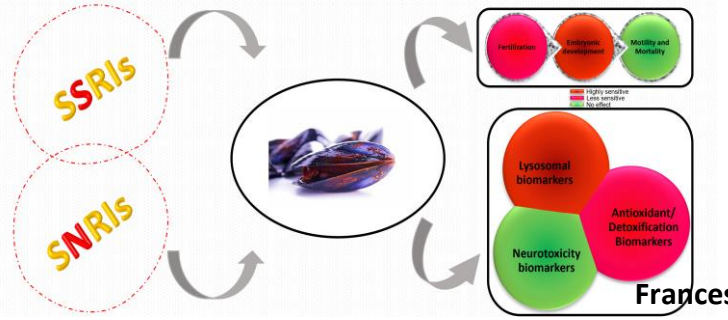
Selective serotonin reuptake inhibitors (SSRI) Antidepressants

1. Fluoxetine
2. Metabolite: nor-fluoxetine
3. Citalopram
4. Sertraline

Serotonin and norepinephrine reuptake inhibitors (SNRI)
Antidepressants

1. Venlafaxine
2. Metabolite: O-desmethylvenlafaxine (ODV)



EARLY LIFE STAGES
(10 concentrations, 0.5-500 ng/L)

% FERTILIZATION:
Small (20%) but significant effects by all compounds in the range 25-500 ng/L, including metabolites; no effect by venlafaxine (SNRI).

EMBRYOTOXICITY:
Small (20-25%) but significant embryotoxicity by SSRIs (parent and metabolite) in the range 25-500 ng/L. No effect by SNRIs, venlafaxine and its metabolite ODV.

MOTILITY AND MORTALITY:
No significant effects.

ADULT MUSSELS (3 concentrations, 0.5-5-10 ng/L, 7 days)

LYSOSOMES AS MAIN TARGET OF ALL TESTED ANTIDEPRESSANTS:

- 50% reduction of haemocytes LMS,
- increase of lysosomal/cytosol ratio (up to 200% by all compounds),
- increase of neutral lipid (max 200% by flx, vfx and odv) and
- lipofuscin (max 400% by venlafaxine and odv) contents.

ANTIOXIDANT/DETOXIFICATION ENZYMES:
Significant modulation by SSRIs, but lack of effects caused by SNRIs.

PEROXIDATION PRODUCTS AND NEUROTOXIC EFFECTS:
No significant effects on MDA and AChE.

DRUG METABOLITES: NFL and ODV, are as effective as the parent compounds.

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Ciona robusta (Phlebobranchia, Cionidae)

Percentages of normal, **malformed, depigmented and dead** *C. robusta* **larvae** and **juveniles** after 22h or 24-48h-6 days exposure to **pharmaceuticals**.

	Larvae (%)				Juveniles survival (%)		
	Normal	Malformed	Depigmented	Dead	24h	48h	6 days
CTRL	83.33	8.86	0	7.82	91.28	91.28	91.28
0.01% DMSO	83.24	8.38	0	8.38	94.94	94.94	94.94
BPA 10 µg/L	36.02	5.42	52.28	6.28	94.92	94.92	94.92
BPA 100 µg/L	0	0	0	100	0	0	0
CBZ 1 µg/L	88.94	5.53	0	5.53	94.3	94.3	94.3
CBZ 10 µg/L	82.68	6.72	0	10.59	95.05	95.05	31.46
IBU 1 µg/L	86.11	8.94	0	4.95	62.74	0	0
IBU 10 µg/L	85.33	5.56	0	9.11	94.57	33.53	0
VAL 1 µg/L	90.24	5.39	0	4.38	93.06	93.06	93.06
VAL 10 µg/L	87.42	3.85	0	8.73	89.9	89.9	30.12

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Balanophyllia europaea

(Scleractinia, Dendrophylliidae)

Percentages of **mortality** and **metamorphosis** and amount of **photosynthetic pigments** (chlorophyll a and c2) for **larvae** exposed to single **pharmaceuticals** and their **mixture with BPA**

	Larvae			
	Mortality (%)	Metamorphosis (%)	Chl a/larva (µg/L)	Chl c_2/larva (µg/L)
CTRL	9.1	75.146	0.144	0.147
DMSO 0.1%	27.3	61.176	0.165	0.261
DMSO 0.35%	0	---	---	---
CBZ 1 µg/L	0	63.2	0.143	0.145
CBZ 10 µg/L	0	26.3	0.175	0.194
IBU 1 µg/L	0	80.0	0.160	0.169
IBU 10 µg/L	18.2	35.0	0.213	0.235
VAL 1 µg/L	0	42.9	0.137	0.233
VAL 10 µg/L	9	50.0	0.146	0.245
MIXL	0	---	---	---
MIXH	8.3	---	---	---

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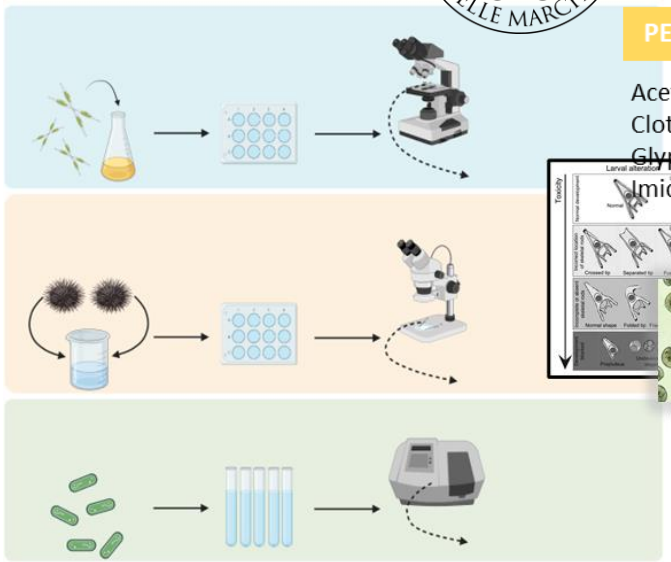
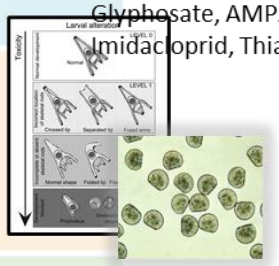
IN VIVO MODELS (ORGANISM LEVEL) – BATTERIES OF ECOTOXICOLOGICAL BIOASSAYS

Performed experiments

- 38 SINGLE CECs
- 6 CECs Mixtures
- 2 doses: 1-10µg/L
- 4 Species





MUSCULOSKELETAL

NSAIDs: Diclofenac, Ibuprofen, Ketoprofen, Naproxen, Nimesulide

ADDITIVES

Bisphenol A

PESTICIDES & HERBICIDES

Acetamiprid, Bifenthrin, Clothianidin, Deltamethrin, Glyphosate, AMPA, Imidacloprid, Thiamethoxam

NERVOUS SYSTEM

SSRIs: Fluexetine, Paroxetine, **NSRIs:** Duloxetine, Venlafaxine, Desmethylvenlafaxine **Antiepileptics**: Carbamazepine, 10,11,diH-10, 11diOH carbamazepine. **Anxiolitics:** Lormetazepam, Lorazepam **Stimulants:** Caffeine **Illicit drugs:** Cocaine

CARDIOVASCULAR

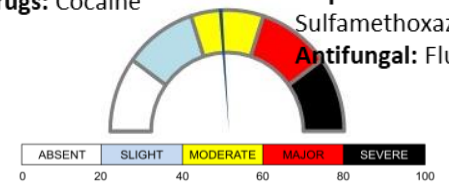
β- blockers: Atenolol, **ACE-inhibitors**: Ramipril, **Sartans:** Valsartan **Antidiabetics:** Metformine, Guanylurea

LIPID REGULATORS

Fibrates: Gemfibrozil

ANTIBIOTICS

Macrolides: Azythromycine, Clarithrom Erythromycin, **Sulphonamides:** Ofloxacine, Sulfamethoxazole. **Antimicrobials:** Tricl **Antifungal:** Fluconazole



Mixtures

1. **Mixture 1:** IBU + PAR
2. **Mixture 2:** CBZ + VAL
3. **Mixture 3:** VEN + NAP
4. **Mixture 4:** IBU + PAR + CBZ + VAL
5. **Mixture 5:** IBU + PAR + CBZ + VAL + LORM + DIC + NIM
6. **Mixture 6:** VAL + ATE + GEM + RAM

PSYCHIATRIC DRUGS

Sample	Level of hazard
VEN 1 µg/L	ABSENT
VEN 10 µg/L	ABSENT
O-DESVEN 1 µg/L	ABSENT
O-DESVEN 10 µg/L	ABSENT
PAR 1 µg/L	ABSENT
PAR 10 µg/L	SLIGHT
FLU 1 µg/L	ABSENT
FLU 10 µg/L	ABSENT
LORM 1 µg/L	ABSENT
LORM 10 µg/L	ABSENT
CBZ 1 µg/L	ABSENT
CBZ 10 µg/L	SLIGHT
CBZ-EP 1 µg/L	ABSENT
CBZ-EP 10 µg/L	SLIGHT

CARDIOVASCULAR DRUGS

Sample	Level of hazard
VAL 1 µg/L	ABSENT
VAL 10 µg/L	ABSENT
ATE 1 µg/L	ABSENT
ATE 10 µg/L	ABSENT
RAM 1 µg/L	ABSENT
RAM 10 µg/L	ABSENT
GEM 1 µg/L	ABSENT
GEM 10 µg/L	MODERATE

Sample	Level of hazard
MIX 1 1 µg/L	ABSENT
MIX 1 10 µg/L	SLIGHT
MIX 2 1 µg/L	ABSENT
MIX 2 10 µg/L	MODERATE
MIX 3 1 µg/L	SLIGHT
MIX 3 10 µg/L	MODERATE
MIX 4 1 µg/L	SLIGHT
MIX 4 10 µg/L	MODERATE
MIX 5 1 µg/L	ABSENT
MIX 5 10 µg/L	SLIGHT
MIX 6 1 µg/L	ABSENT
MIX 6 10 µg/L	ABSENT

- MOA-DEPENDENT TOXICITY OF SINGLE TESTED CHEMICALS
- 5 MIXTURES ON 6 SHOW DOSE-DEPENDENT EFFECTS
- WEAK ACUTE TOXICITY COMPARED TO SUB-CHRONIC EFFECTS

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DRUGS

Fluoxetine
Propranolol
Diclofenac
Carbamazepine
Acyclovir

UV-FILTER

Benzophenone-3
(BP3)

PHTHALATES

Diethyl Phthalate (DEP)
Butyl Benzyl Phthalate (BBP)
Bis(2-ethylhexyl)
Phthalate(DEHP)

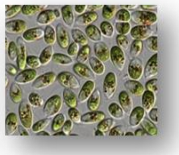
	End-point	Fluoxetine	BP3
		EC50 (mg/L)	
D.tertiolecta 72h	Growth inhibition	0.16	1.031
P.tricornutum 72h	Growth inhibition	0.04	1.313
S.pseudocostatum 72h		0.01	0.17
A.amphitrite 48h	Immobility	0.11	2.33
	Behaviour	0.04	0.77
P.lividus 72h	Larval development	0.05	4.11
	Behaviour	0.17	-

Phytoplankton

S. pseudocostatum

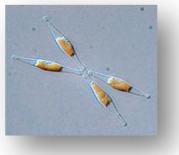
72 h
20 °C
Light condition

D. tertiolecta



72 h
20 °C
Light condition

P. tricornutum

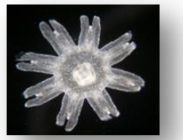


72 h
20 °C
Light condition

Growth inhibition

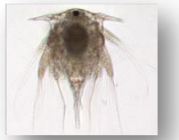
Zooplankton

Aurelia sp.



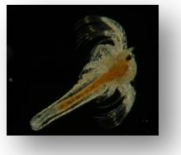
48 h
20 °C
Dark condition

A. amphitrite



48 h
20 °C
Dark condition

A. franciscana



48 h
25 °C
Dark condition

Immobility
Behaviour

P.lividus

72 h
20 °C
Dark condition

Larval development
Behaviour

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

Fluoxetine affects algal growth, crustacean behaviour and sea urchin larval development and behaviour at **environmental concentration**

BP-3 affects algal growth and crustacean behaviour at **environmental concentration**

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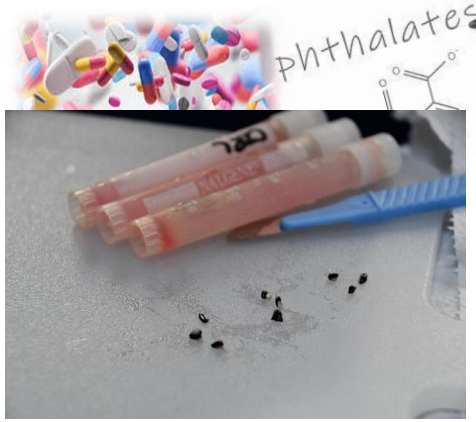
cima RESEARCH FOUNDATION

Exposures with organotypic cetaceans cell lines



76 Organotypic cell lines obtained from skin biopsies - collected from target cetaceans species (*S. ceruleoalba, B. physalus, P. macrocephalus*) in the Pelagos Sanctuary - were treated with the following emerging chemicals (single or in mixture – 24 h exposure)

CARBAMAZEPINE (CBZ) - CTRL; 1µg/l; 10µg/l; 100µg/l *

VALSARTAN (VAL) - CTRL; 1µg/l; 10µg/l; 100µg/l *

IBUPROFEN (IBU) - CTRL; 1µg/l; 10µg/l; 100µg/l *

MIX PHARMACEUTICALS (MIX_F) - CTRL; 1µg/l (CBZ + VAL + IBU); 10µg/l (CBZ + VAL + IBU); 100µg/l (CBZ + VAL + IBU) *

DI(2-ETHYLHEXYL) PHTHALATE (DEHP) - CTRL; 10µg/l; 100µg/l

MIX PHTHALATES (MIX_PT) - CTRL; 10 µg/l; 100 µg/l

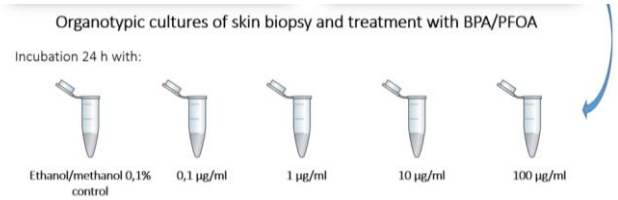
BISPHENOL A (BPA) - CTRL; 10 µg/ml; 100µg/ml

MIX EMERGING CONTAMINANTS (Super MIX)- CTRL; Low ; High

Biological pathways identification

Transcriptomics Development of a set of molecular biomarkers to be applied to in vitro studies (cells, organotypic cultures).

Identification of **target genes** sensitive to endocrine disrupting chemicals and persistent organic chemicals.



GENE OF INTEREST	MAIN BIOLOGICAL FUNCTION
P2RX4	Energy metabolism /Energy metabolism
KYAT3	Energy metabolism/Lipid
AGTR1	Endocrine signaling/xenobiotic
COX-1	Inflammation/Immune responses
VAMP4	Energy metabolism/ Lipid
AKT	Energy metabolism/xenobiotic
CHRNA5	Neurotransmitter
ACLY	Energy metabolism/ ATP citrate synthase
ERα	Endocrine signaling/xenobiotic
AR	Endocrine signaling/xenobiotic
PPARα	Signaling receptor activity/ DNA-binding transcription factor activity
PPARγ	Chromatin binding/ DNA-binding transcription factor activity
CYP1A1	Energy metabolism/ Endocrine signaling/ Immune responses/ marker of Xenobiotic exposure

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WP2. DEVELOPMENT OF INNOVATIVE EFFECT-BASED MONITORING TOOLS

Task 1.2.4 -Health Toolbox for measuring status of marine organisms
Task 1.2.5 Effects of emerging pollutants and interactions with multiple stressors

UNIVERSITÀ DI SIENA 1240

Exposures with organotypic sea turtle cell lines

Organotypic cell lines obtained from skin biopsies and of target species (***Caretta caretta***) collected in a rescue center have be treated with a mixture of emerging chemicals

A set of **cellular biomarkers**: **oxidative stress** (respiratory burst), **genotoxicity** (comet assay; Erythrocytic Nuclear Abnormalities assay-ENA assay), **immunotoxicy** (lysozyme, differential count of white cells) and **neurotoxicity** (acetylcholinesterase, AChE; butyrylcholinesterase, BChE; carboxylesterase, CaE) and...

gene expression biomarkers (PRα, ACADL, HSP60, THRα, CD83, Erα, RXRα, Lys, CYP1a, GST, PPARα, CCR7), as well as **proteomics**, were developed and **harmonized** to test toxicity of **plastic additives and pharmaceuticals** to sea turtles

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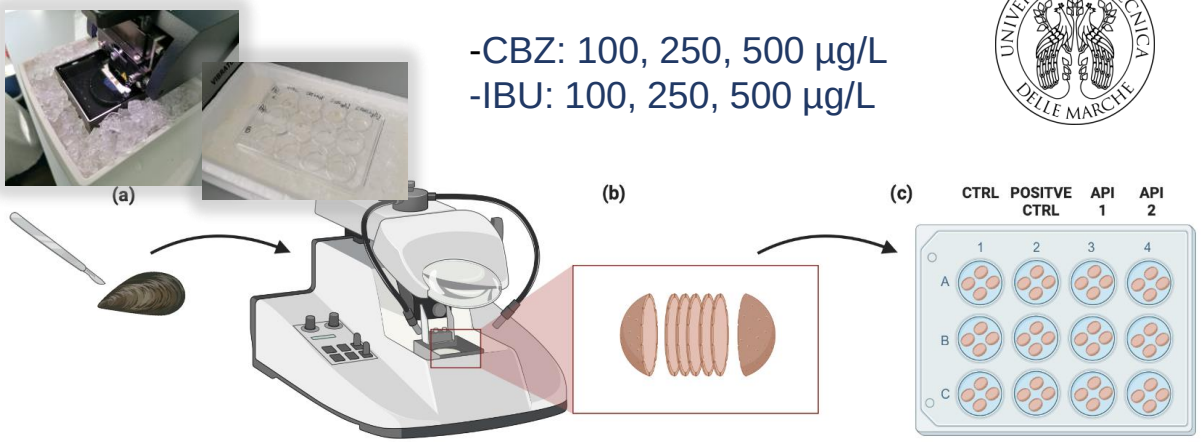
EXPERIMENTS ON EX VIVO MODELS

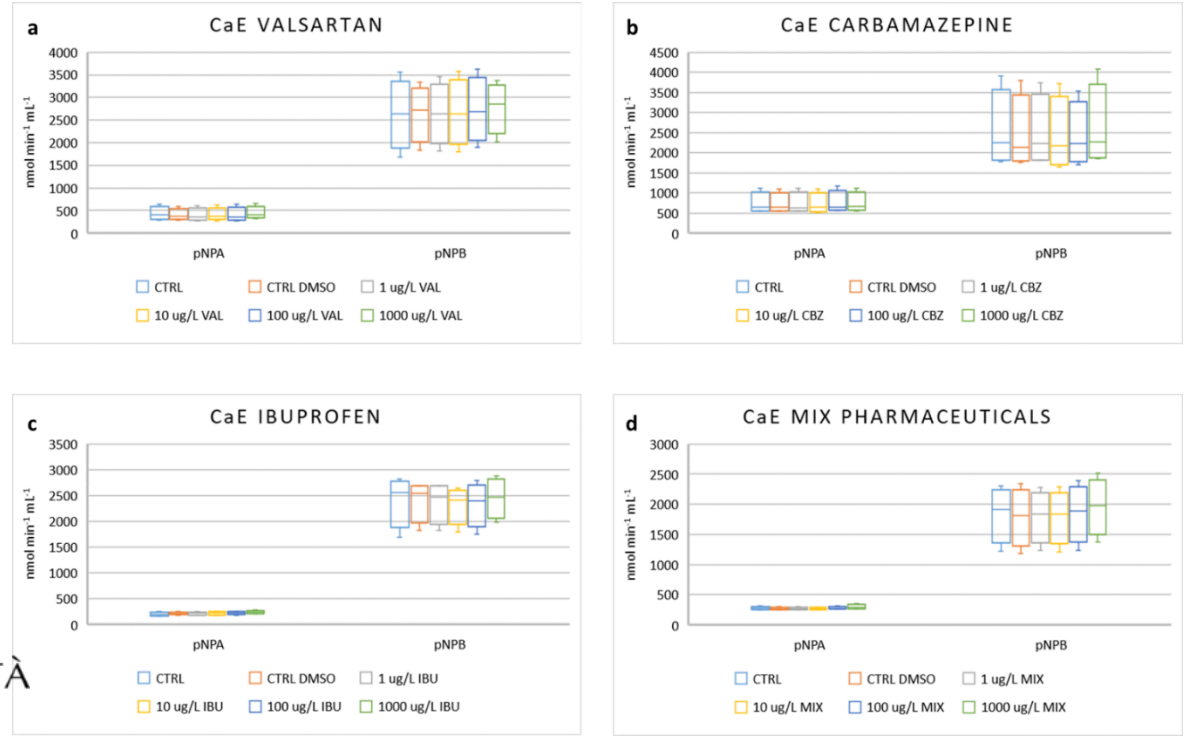
Precision Cut Tissue Slices (PTCS) in digestive glands of *M. galloprovincialis*

-CBZ: 100, 250, 500 µg/L
-IBU: 100, 250, 500 µg/L



TYPOLOGIES OF ANALYSES:
- viability test
- Selection of biomarkers
- RNAseq*

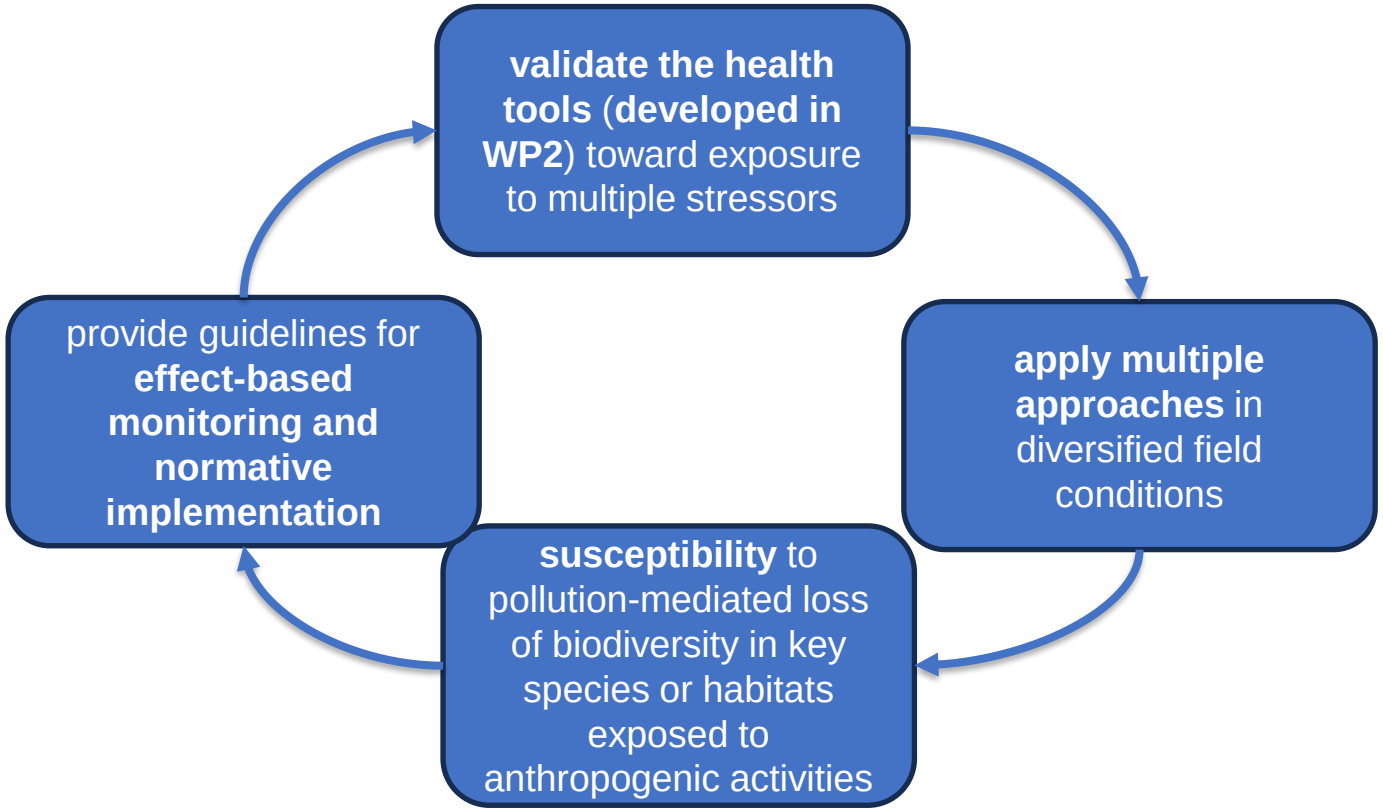
Exposure of sea turtle plasma samples to emerging contaminants and evaluation of neurotoxicity



WP3. SUSCEPTIBILITY TO POLLUTION-MEDIATED LOSS OF BIODIVERSITY IN MEDITERRANEAN AND INTERACTIONS OF EMERGING POLLUTANTS WITH CLIMATE CHANGE

Task 1.2.6 -Susceptibility to pollution-mediated loss of biodiversity in key Mediterranean field scenarios

Task 1.2.7 Dynamic risk maps based on future scenarios of emerging pollutants and climate change



Key Areas/Species:

- ✓ **a) iconic species** in field conditions (cetaceans in Pelagos Sanctuary, sea turtles in Ionian and Adriatic Sea);
- ✓ **b) pristine** vs **impacted marine areas**,
- ✓ **c)** areas of biodiversity significance and subjected to the influence of future scenarios (**CO_2 emissions and acidification with emerging pollutants at Panarea**)
- ✓ **d) harbour areas** and natural environments exposed to **disposal of dredged sediments**.

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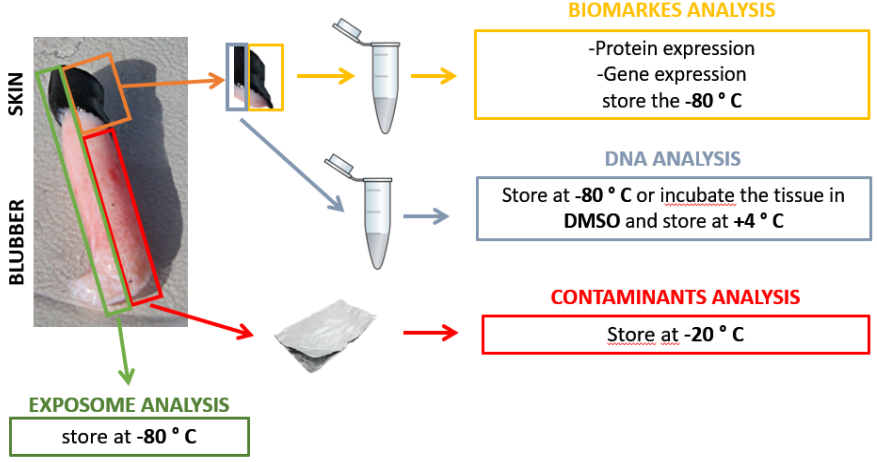
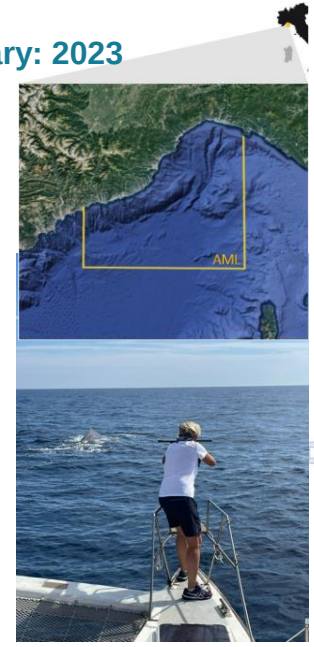
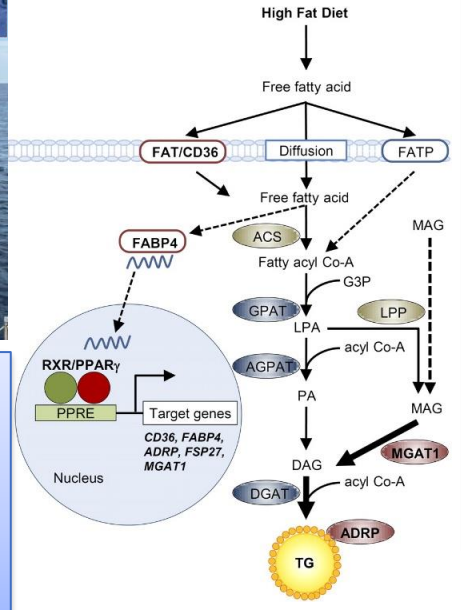


Pelagos Sanctuary: 2023



Application of diagnostic tools developed in WP2

Target genes sensitive to **endocrine disrupting chemicals** (e.g.thyroid hormone receptors α and β, nuclear receptors PPARα and γ) and **persistent organic pollutants** (e.g. cytochromes 1A, 1B, 3A and aryl hydrocarbon receptor)



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PPARα	Signaling receptor activity/ DNA-binding transcription factor activity
PPARγ	Chromatin binding/ DNA-binding transcription factor activity
CYP1A1	Energy metabolism/ Endocrine signaling/ Immune responses/ marker of Xenobiotic exposure

- **Sperm whale** (n=7) (*Physeter macrocephalus*)
- **Fin whale** (n=10)(*Balaenoptera physalus*),
- **Cuvier's beaked whale** (n=8)(*Ziphius cavirostris*)
- **Striped dolphin** (n=15) (*Stenella coeruleoalba*).

WP3. SUSCEPTIBILITY TO POLLUTION-MEDIATED LOSS OF BIODIVERSITY IN MEDITERRANEAN AND INTERACTIONS OF EMERGING POLLUTANTS WITH CLIMATE CHANGE

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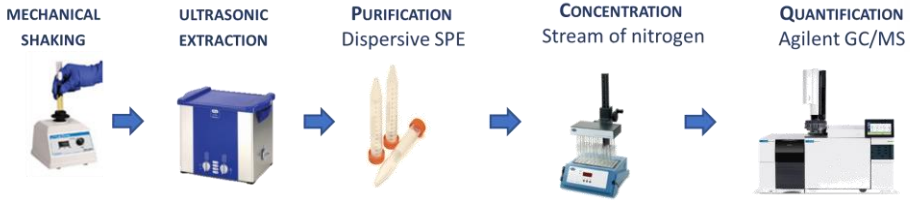
Ongoing activities: PAEs concentration detection in blubber

11 phthalates	CAS
Dimethyl phthalate (DMP)	131-11-3
Diethyl phthalate (DEP)	84-66-2
Diallyl phthalate (DAP)	131-17-9
Dipropyl phthalate (DPrP)	131-16-8
Diisobutyl phthalate (DiBP)	84-69-5
Dibutyl phthalate (DBP)	84-74-2
Benzyl butyl phthalate (BBzP)	85-68-7
Dicyclohexyl phthalate (DCHP)	84-61-7
Bis(2-ethylhexyl) phthalate (DEHP)	117-81-7
Diisononyl phthalate (DiNP)	28553-12-0
Di-n-octyl phthalate (DnOP)	117-84-0

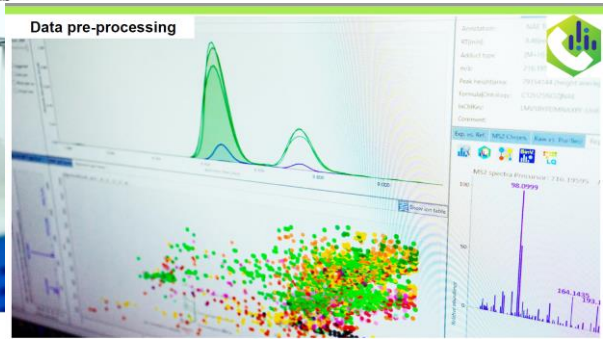
Listed as reprotoxic category 1B substances under EU Regulation (EC) 1272/2008



Exposomic in cetacens

Fin whale samples are analyzed integrating gas and **liquid chromatography (GC, LC) ultrahigh-resolution mass spectrometry (Orbitrap)** for a full coverage of the **EXPOSOME**, which reveal environmental exposures to anthropogenic chemicals **including LEGACY POLLUTANTS (PCBs, DDT, dioxins)** and novel entities **of EMERGING CONCERN (PFAS, bisphenols, phthalates, prescription drugs, and other commercial products).**

Blubber and Skin - Fin Whale biopsies
Sample preparation in clean lab
GC and LC HRMS (Orbitrap) analysis
Raw data pre-processing
Data normalization (IS, sample lipid or weig
Blank filtering & blank subtraction
Chemical identifications (MS1 + MS2) + Pri
Multivariate modelling



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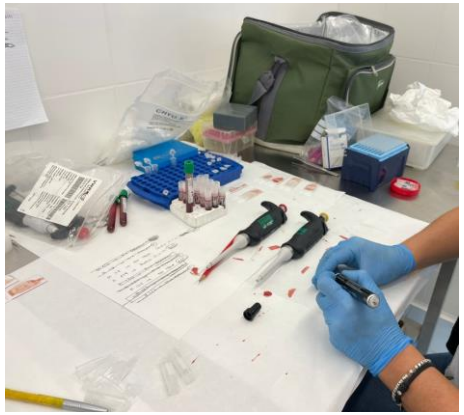
Susceptibility to pollution and validation of harmonized health tools in sea turtle



- ✓ **Sampling** of a significant number of sea turtle specimens (blood, plasma, skin biopsies) in **rescue centers** in the **Adriatic and Ionian Sea** to validate the harmonized health tools.
- ✓ **Field validation** of a **panel of biomarkers** (oxidative stress, genotoxicity, immunotoxicy and neurotoxicity) and **proteomic methods** for sea turtles to test toxicological health status. Selected area: **south Adriatic/Ionian Sea** (Manfredonia)
- ✓ Multi-residue extraction for the evaluation **of emerging contaminants including plastic additives**, will be investigated in whole blood samples.

	Median	Minimum	Maximum
CaE ($nmol*min^{-1}*mL^{-1}$)			
pNPA	1731.66	274.83	3020.27
pNPB	4707.77	249.94	8640
Erythrocytic Nuclear Abnormalities (ENA) assay (‰)			
Lobed	30	11	45
Kidney	0	0	1
Segmented	0		
Micronuclei	0	0	1
Total and differential white blood cells (WBC) count (/200 cells)			
Heterophils	94	56	137
Eosinophils	4	0	11
Basophils	0	0	2
Monocytes	3	1	6
Lymphocytes	64	48	116
Thrombocytes	16	0	71
Lysozyme (µg HEL* mL^{-1})	11.26	9.91	35.74
Comet assay (% tail DNA)	17.56	13.02	21.97

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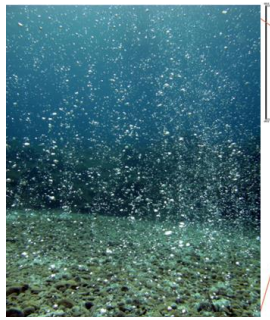
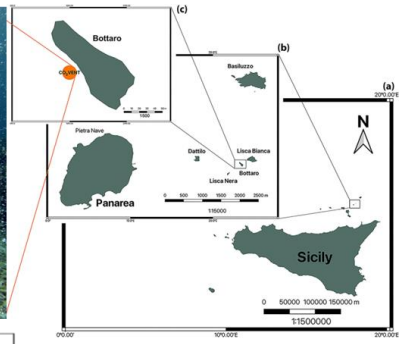
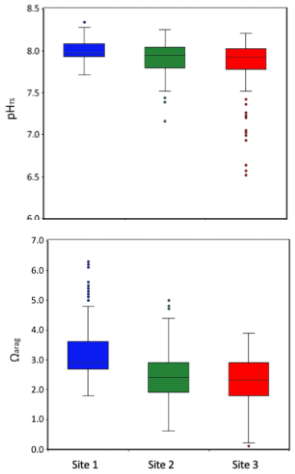
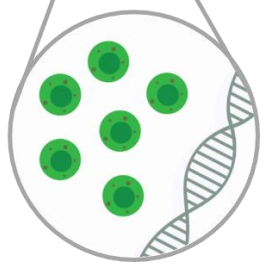
The CO_2 vent **off Panarea Island (Aeolian archipelago) generate a stable pH gradient (**7.4-8.1) reflecting **pH values projected for 2100 under different IPCC scenarios** (14-20 July 2023, https://youtu.be/k_oGhaiZJGY)





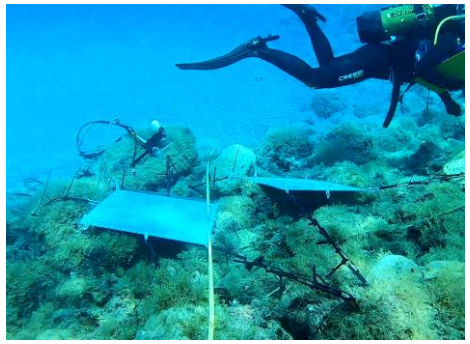


1. **Seawater samples and environmental data** (pH, temperature, salinity) for general characterization and carbonate chemistry analysis (omega aragonite)
2. **Collection** of **vent gas emissions**
3. **Sediment samples** to determine grain-size and bulk chemical composition



4. *Balanophyllia europaea* corals along the pH gradient:

- to evaluate the **accumulation of emerging contaminants** and **PAHs** in its tissues and algal symbionts
- to obtain its **whole transcriptome profiling** and correlate gene modules with **environmental parameters and physiological assessments, including contaminant bioaccumulation**



5. Placement of steel cages with different **bioplastics films** to investigate the **microbial colonization dynamics** and their **biodegradation**, and collection of nearby **sediments** for a comparative characterization of the **microbial community**

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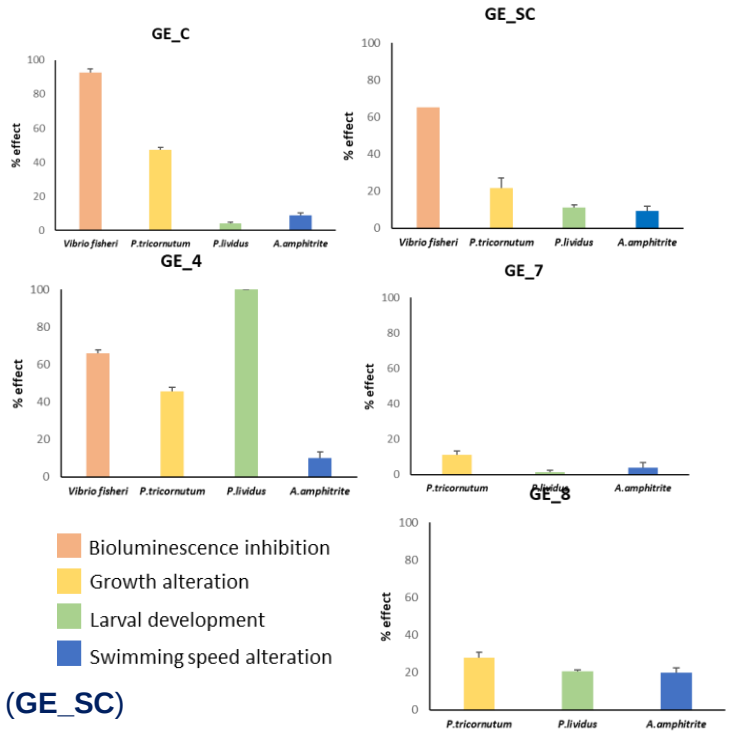
Validation of harmonized health tools in natural environments subjected to the impact of WWTPs outfall for normative implementation

WWTP Area, Genova

MODEL ORGANISMS	END-POINTS
Bacteria *Vibrio fisheri*	Bioluminescence inhibition
Microalgae *Dunaliella tertiolecta* *Phaeodactilum tricornutum*	Algal growth alteration
Cnidarian (jellyfish) *Aurelia sp.* *Sanderia malayensis*	Immobility Alteration of Frequency pulsations Ingestion
Crustaceans *Artemia salina* *Amphibalanus amphitrite* *Tigriopus fulvus* *Acartia tonsa*	Mortality, Immobility Swimming speed alteration Ingestion Hatching
Mollusca *Cassostrea gigas*	% of abnormal larvae (embriotoxicity)
Echinoderms *Paracentrotus lividus*	Fertilization rate Sperm motility % of abnormal larvae (embriotoxicity) Larval swimming speed alteration



Sampling activity (march 2023)

No acutely toxic, only moderate effects at the discharge point (**GE_SC**)
High toxicity at **GE_4**
No toxic effects for **GE_7** and **GE_8** samples

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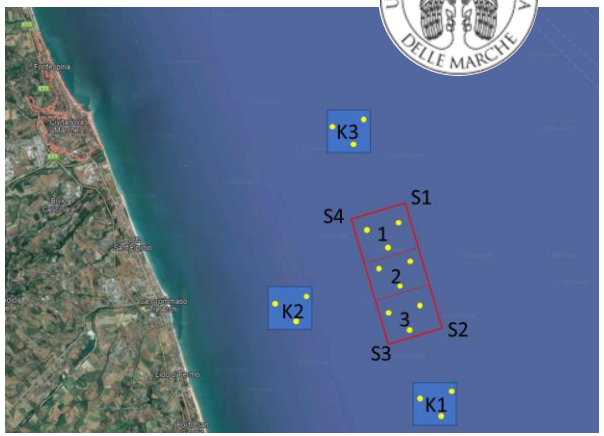
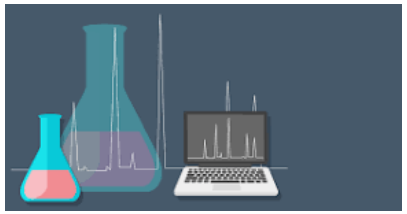
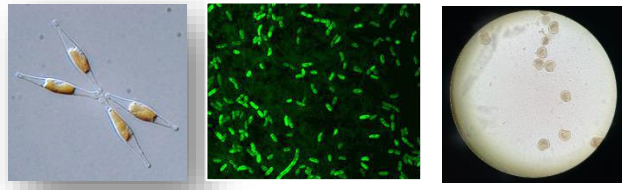
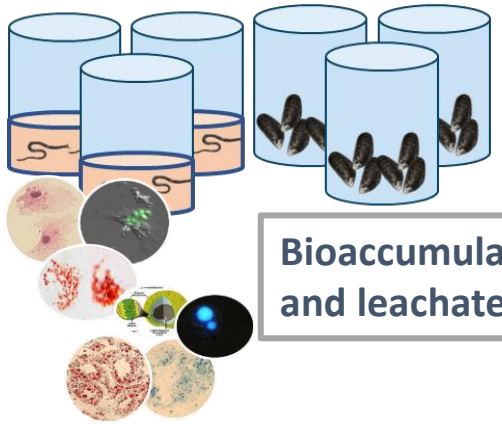




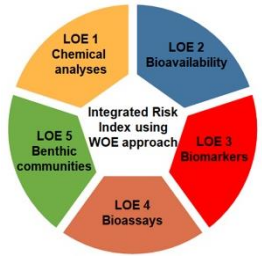
Chemical analyses in sediments

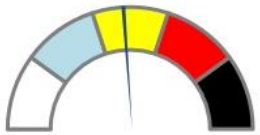
Ecotoxicological characterization of sediments





Bioaccumulation and biomarkers from sediments and leachates to polychates and mussels





SYNTHETIC RISK INDEX

Weight of Evidence Integration for future normative implementation

ABSENT	SLIGHT	MODERATE	MAJOR	SEVERE

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CEC modeling: which kind of data and information?

CEC modeling: data to do what?

Physico-chemical properties

Pollutant	Short name	Class	Molecular Weight	K_{ow}	K_{oc}	K_H	pKa	acid/base
Ibuprofene	IBU	Pharmaceutical						
Carbamazepine	CBZ	Pharmaceutical						
17β-estradiol	E2	Pharmaceutical						
Sulfamethoxazole	SMX	Pharmaceutical						
Venlafaxine	VLF	Pharmaceutical						
Metformin	Met	Pharmaceutical						
Glyphosate	GLY	Herbicides						
Aminomethylphosphonic acid	AMPA	Herbicides						
Bisphenol A	BPA	Plasticizers and additives						
Bis (2-ethylhexyl) phthalate	DEHP	Plasticizers and additives						
PerFluoroButanoic acid	PFBA	PFAS						

To understand environmental fate and parameterize it in model tools

Bioaccumulation and impacts

Short name	Effects on non-target organisms	Biota concentrations and/or BCF/BAF	PNEC
IBU			
CBZ			
E2			
SMX			
VLF			
Met			
GLY			
AMPA			
BPA			
DEHP			
PFBA			

To understand environmental concern and estimate possible hazards/impacts

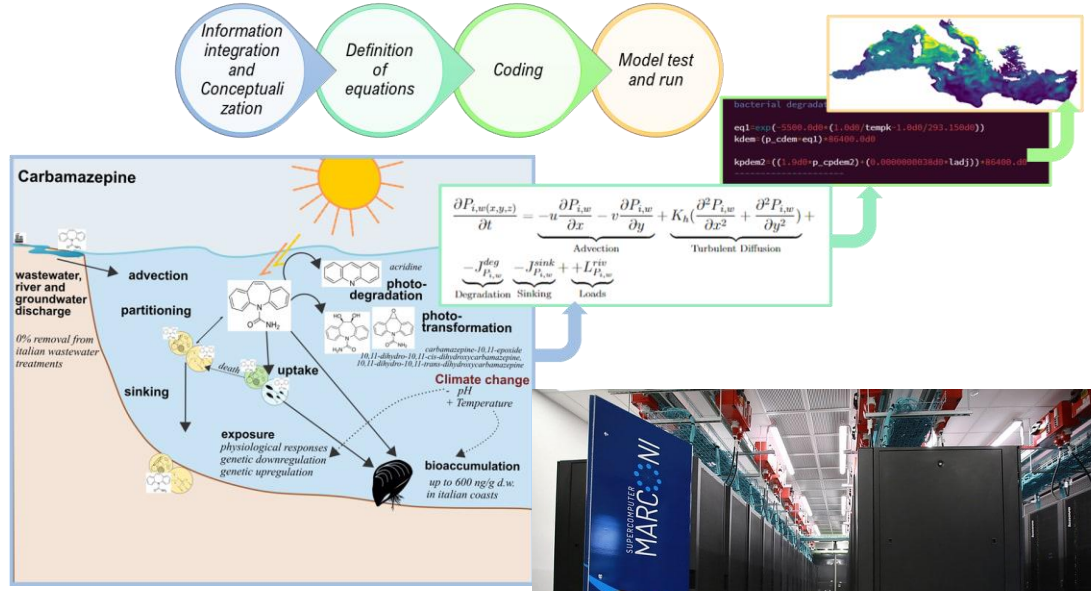
Fate and Transport

Short name	River levels	SeaWater levels	Sediment levels	WW Removal Efficiency	Photo-degradation	Biological degradation	Measured Climate Change impact
IBU							
CBZ							
E2							
SMX							
VLF							
Met							
GLY							
AMPA							
BPA							
DEHP							
PFBA							

To parameterize and validate models

Information reliable, quantitative, and ready to be used
Information reliable, qualitative and/or requires assumptions
Information uncertain, qualitative and/or requires assumptions
Information seem to be scarcely available
Information not yet collected, but possibly available

CEC modeling: workflow



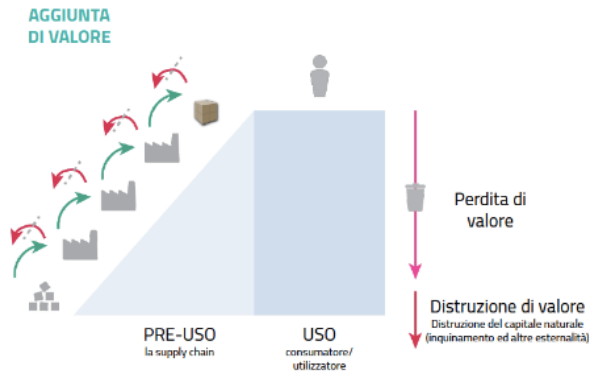
OGS

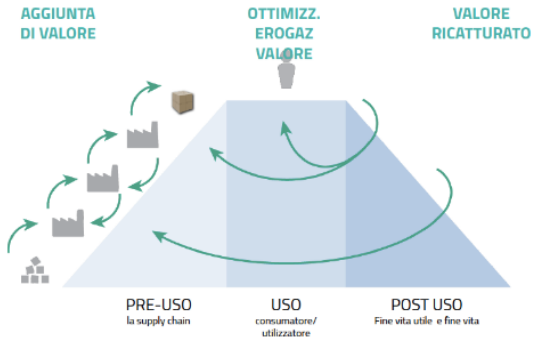
WP4. SOCIO-ECONOMIC-INDUSTRIAL INNOVATION: ZERO POLLUTION VISION

Task 1.2.8 New business models and socio-economic-industrial toward zero pollution

- Provide **new business models and socio-economic-industrial vision** toward zero pollution.
- **Rethink** the way goods and services are **designed, produced, delivered, performed and/or used and disposed**
- Sustain biodiversity protection and economic prosperity **transforming production and consumption modes** and **directing investments** towards zero pollution.
- Enhance **public awareness** on the importance of healthy oceans and biodiversity protection, **adoption** of new business models, scientifically sound ***recommendations***, and behavioral **practices**.

Deliverable name	Deliverable description	Deliverable Quantification	Date of Release	Link to NBFC Milestones
Workplan of activities for preparing socio-economic transition toward zero pollution	Report	1	feb. 2023	14.1
Preliminary identification of sustainable business models and good industrial practices	Report	1	ott. 2023	14.3
Benchmarking and cost-benefit analyses for selecting viable sustainable business models	Report	1	feb. 2024	14.4
Presentation of new business models (stakeholder engagement)	Report	1	giu. 2024	14.5
Testing of the proposed solutions (through pilot showcases)	Report	1	ott. 2024	14.6
Policy recommendations and guidelines for implementing sustainable business models at EU and Mediterranean levels	Report	1	feb. 2025	14.7





WP4. SOCIO-ECONOMIC-INDUSTRIAL INNOVATION: ZERO POLLUTION VISION

Task 1.2.8 New business models and socio-economic-industrial toward zero pollution

Modelli di valutazione degli impatti economici, sociali e ambientali

L'obiettivo è la definizione di uno **schema di analisi (framework)** che fornisca un metodo per *valutare i costi* e *i benefici* in termini *economici*, *ambientali* e *sociali* **per i blocchi che caratterizzano il business model**.

Sono stati valutati i potenziali **stakeholder** da coinvolgere per sviluppare un prototipo di modello di valutazione che tenga conto di molteplici e differenziate esigenze dei potenziali utilizzatori che possono incidere sulle scelte e quindi, la valutazione.

Partendo dallo **sviluppo di un modello generale** si tenterà poi l'applicazione in ambiti legati alla **protezione della biodiversità marina**.

Metodi di valutazione degli impatti economici, sociali ed ambientali

- **Strumenti**
  Benchmarking, Cost Benefit Analysis, Cost Effectiveness Analysis, Eco-Efficiency Analysis, Environmental Impact Assessment, Exergy Analysis, Life Cycle Assessment, Life Cycle Costing, Multi-Criteria Decision Analysis, Risk Assesment, Statical Entropy Analysis Strategic Environmental Assessment.

- **Analisi critica dei modelli e individuazione punti di forza e di debolezza**

- **Metodo in via di sviluppo: combinazione dei metodi**
  Cost Benefit Analysis (CBA) e Multi-Criteria Decision Analysis (MCDA).

L'output del modello combinato è composto da **due valori**:

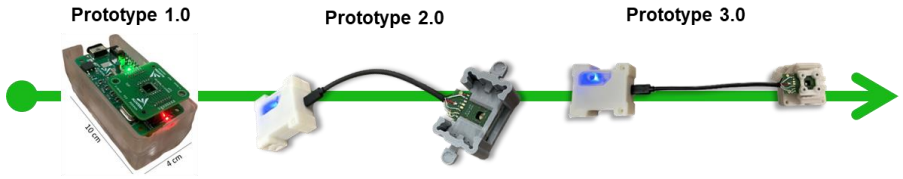
- un **valore economico di sintesi** che emerge come risultato dell'analisi costi-benefici
- un **valore quali-quantitativo** che sintetizza costi e benefici potenziali in ottica **economica (di lungo periodo)**, **ambientale** e **sociale**

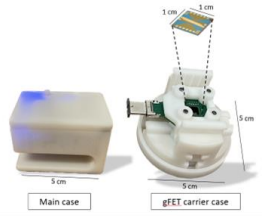
→ **inizio definizione variabili non finacial e somministrazione questionari**

WP5. SETUP OF A LIGHTHOUSE DEMONSTRATOR FOR INNOVATIVE TECHNOLOGIES TO COUNTERACT POLLUTION THREAT TO BIODIVERSITY

Task 1.2.9 Setup of a Lighthouse Demonstrator for innovative technologies to counteract pollution threat to biodiversity

Bio-sensing platform for environmental pollutant detection





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Grazie per l'attenzione

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