

Mediterranean e-course on marine litter monitoring & mitigation 17 & 19 January 2023, 10.00 – 14.00 CET









REGIONE AUTONOMA DE SARDIGNA REGIONE AUTONOMA DELLA SARDEGNA

Plastic Busters CAP

MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER ON BIOTA

MARIA CRISTINA FOSSI

BIOMARKER LABORATORY, UNIVERSITY OF SIENA, ITALY FOSSI@UNISI.IT

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EFFECTS OF MARINE LITTER ON BIOTA ?

MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER ON BIOTA: STATE OF ART



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REGIONE AUTONOMA DELLA SARDEGN

MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER ON BIOTA: STATE OF ART

Number of species with documented records of marine debris ingestion



Source: Kühn, S., et al., Deleterious Effects of Litter on Marine Life, in Bergmann, M., et al., Marine Anthropogenic Litter, Springer, 2015





MARINE LITTER IMPACT: WHAT HAPPENS IN THE MEDITERRANEAN SEA?



7 plastic items in the stomach



145 plastic items in the stomach





22 Kg of plastic in the stomach

More than 130 marine species affected by marine litter in the Mediterranean basin



PLASTIC BUSTERS MPAs: SIMULTANEUS MONITORING



MONITORING MACRO-AND MICROLITTER IN THE ENVIRONMENT



PREPARED BY

THE INTERREG MED PLASTIC BUSTERS MPAs PROJECT



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MONITORING MACRO-AND MICROLITTER IN BIOTA









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CAPITALIZATION STRATEGY

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MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER ON BIOTA: THE NOVELTY







BIOINDICATORS SELECTION IN RELATION TO HABITAT & HOME RANGE



HARMONIZED MARINE LITTER DIAGNOSIS IN MEDITERRANEAN BIODIVERSITY



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46 Species

MARINE LITTER INGESTION: MEDITERRANEAN SEA



The Marine Strategy Framework Directive defines Criteria 3 of Descriptor 10:

DIO.C3 as "The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned"

D10.C4 as "the number of individuals of each species which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects."



United Nations Environment Programme

Mediterranean Action Plan Barcelona Convention

The Integrated Monitoring and Assessment Guidance defines the indicator for Ecological Objective 10 on marine litter (EO10) (known as Candidate Indicator 24) as "Trends in the amount of litter ingested by or entangling marine organisms, focusing on selected mammals, marine birds, and marine turtles".







MARINE LITTER INGESTION: MEDITERRANEAN SEA



The Marine Strategy Framework Directive defines Criteria 3 of Descriptor 10: D10.C3 as "The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned"

DOES ML ADVERSELY AFFECT THE HEALTH OF THE SPECIES ?



The quantification of marine litter ingestion is not enough... we need to investigate ecotoxicological effects !





PLASTICS AND MICROPLASTICS AS VECTORS OF CHEMICAL POLLUTANTS



Review Open Access Published: 22 January 2021

Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans

Tan Suet May Amelia, Wan Mohd Afiq Wan Mohd Khalik, Meng Chuan Ong, Yi Ta Shao 🖾, Hui-Juan Pan 🗠 & Kesaven Bhubalan 🖂







TRANSFER OF CHEMICALS FROM PLASTIC TO BIOTA



THE BIOMARKER APPROACH



Responsiviness, detectability

THE THREEFOLD MONITORING APPROACH









The simultaneous investigation in bioindicator species of:

A) the analysis of **gastro-intestinal content** to evaluate the **marine litter** ingested by the organisms;

B) the analysis of **plastic additives** and PBT compounds used as plastic tracers;

C) the analysis of the effects by **biomarkers responses** at different level of biological organization

... will allow a more complete assessment of the real impact related to plastic debris ingestion by marine organisms.



COastal Management and MOnitoring Network



A NEW APPROACH FOR MONITORING MARINE LITTER INGESTION & IMPACT IN MEDITERRANEAN BIODIVERSITY









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THE THREEFOLD MONITORING APPROACH

Commercially harvested species



invertebrates

invertebrates

fish species





THE THREEFOLD MONITORING APPROACH

Endangered species



cetaceans

sea turtles

seabirds





MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER IN INVERTEBRATES

MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER ON BIOTA: <u>INVERTEBRATES</u>





DIAGNOSTIC MARKERS FOR CONTAMINANTS

- Markers of genotoxicity
- (DNA strand break, micronuclei)
- Markers of neurotoxicity
- (inibition of esterases enzymes, transcriptomics)
- Markers of oxidative stress
- (quantification of oxidative enzymes, transcriptomics)
- Markers of xenobiotic metabolism and biotransformation
- (enzymes induction and trascriptomics)

ANALYSIS OF ANTHROPOGENIC CONTAMINANTS

OCs

80°c

- PAHs
- Mercury
- Plastic additives (phthalates, bisphenol A, PBDEs)



Markers of immunotoxicity

DIAGNOSTIC MARKERS FOR REPRODUCTION FUNCTIONALITY

Markers for reproductive system
 (expression and quantification of proteins
transcriptomics)

DIAGNOSTIC MARKERS FOR INFLAMMATION AND MORPHOLOGY

Transcriptomics and histology and histopathology

MARINE LITTER ANALYSIS

Analysis of microplastics and ML in the whole organism





MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER ON BIOTA: <u>INVERTEBRATES</u>



Plastic D detections	Plastic tracers detections	Biomarkers detections
 Analysis of the gastro intestinal (GI) contents: Occurrence (%) Abundance (n*) Weight (g) Polymer analysis 	 - Analysis of plastic additives: • Phthalates • PBDEs • Bisphenol A - Analysis of PBT compounds: • PCBs • DDTs • PAHs • Mercury 	 Effects at molecular level: Measure of DNA damage Alterations of gene expression Alteration of proteins Effects at cellular level: Alteration of cell functions Effects at tissue level: Hystological and hystopathological alterations

	CHEMICAL COMPOUND	TISSUE/SAMPLE	ANALYTICAL METHOD
	Phthalates	Muscle, whole organism	Baini et al., (2017), Fossi et al., (2016), Savoca et al., (2018), Avisar et al., (2019), Lo Brutto et al., (2021),
PLASTIC ADDITIVES	Bisphenol A	Muscle, whole organism	Ballesteros-Gómez et al., (2009), Lo Brutto et al., (2021)
	Polybrominated diphenyl ethers	Muscle, whole organism	Muñoz-Arnanz et al., (2016), Cruz et al., (2019), Cruz et al., (2020)
	Polycyclic aromatic hydrocarbons	Muscle, whole organism	Marsili et al., (2001), León et al., (2013), Benedetti et al., (2014)
ADSORBED CONTAMINANTS	Organochlorine contaminants	Muscle, whole organism	Marsili and Focardi, (1997), León et al., (2021)
	Mercury	Whole organism, muscle	Correa et al., (2013), Fattorini et al., (2008), Besada et al., (2011), León et al., (2021)





MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER ON BIOTA: <u>INVERTEBRATES</u>



Plastic detections	Plastic tracers detections	Biomarkers detections
 Analysis of the gastro intestinal (GI) contents: Occurrence (%) Abundance (n°) Weight (g) Polymer analysis 	- Analysis of plastic additive • Phthalates • PBDEs • Bisphenol A	- Effects at molecular level: Measure of DNA damage Alterations of gene expression Alteration of proteins
	- Analysis of PBT compound • PCBs • DDTs • PAHs • Mercury	 Effects at cellular level: Alteration of cell functions Effects at tissue level: Hystological and hystopathological alterations

EFFECT	TISSUE	TEST
GENOTOXICITY	Hemolymph, digestive gland	Comet assay (Revel et al., 2019) (*) Mn test (Avio et al., 2015) (*)
OXIDATIVE STRESS	Digestive gland	LPO, CAT, SOD, GST, GSH, GR, GPX (Revel et al., 2019) (*) qPCR GPX, SOD, CAT (Ravel et al., 2019)
IMMUNOTOXICITY	Gills, Mantle, Digestive gland	CASP, TRAF, Transcriptomics (Avio et al., 2015; Revel et al., 2019) (*) Transcriptomics (Gardon et al., 2020) qPCR LYS, CASP3 (Paul- Pont et al, 2016)
REPRODUCTION	Gonads	Gamete Quality and Larval Development (Sussarellu et al., 2016) (*)
HISTOPATHOLOGY INFLAMMATION AND MORPHOLOGY	Digestive gland	Histopathology, histology (Avio et al., 2015) (*)
XENOBIOTIC METABOLISM AND BIOTRANSFORMATION	Digestive gland, whole organism	Porphyrins (Grandchamp et al. 1980; Guerranti et al. 2014) (*) EROD (Zhang et al., 2019) Transcriptomics (Gardon et al., 2020) (*)
NEUROTOXICITY	Whole organisms, muscle, gills	AChE activity (Magni et al., 2018) (*)
CELLULAR STRESS	Whole organisms, muscle, hemolymph, digestive gland	Lysosomal membrane stability-LMS (Canesi et al 2015) (*) IDH (Oliveira et al., 2013) (*) Transcriptomics (Détréé et al. 2018) qPCR IDH, HSP70 Détréé e al. 2017)







MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER IN FISH

MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER ON BIOTA: <u>FISH</u>



Plastic D detections	Plastic tracers detections	Biomarkers detections
 Analysis of the gastro intestinal (GI) contents: Occurrence (%) Abundance (n°) Weight (g) 	 Analysis of plastic additives: Phthalates PBDEs Bisphenol A Analysis of PBT compounds: 	- Effects at molecular level: • Measure of DNA damage • Alterations of gene expression • Alteration of proteins - Effects at cellular level:
Polymer analysis	PCBs DDTs PAHs Mercury	 Alteration of cell functions Effects at tissue level: Hystological and hystopathological alterations

DIAGNOSTIC MARKERS FOR CONTAMINANTS Markers of genotoxicity

(DNA strand break, micronuclei and nucle abnormalities)

Markers of neurotoxicity

(inibition of esterases enzymes, transcriptomics)

Markers of oxidative stress

(quantification of oxidative enzymes, transcriptomics)

• Markers of xenobiotic metabolism and biotransformation

rphyrins)

ANALYSIS OF ANTHROPOGENIC CONTAMINANTS

• OCs

• PAHs

- Mercury
- Plastic additives (phthalates, bisphenol A, PBDEs)



DIAGNOSTIC MARKERS FOR IMMUNE

• Markers of immunotoxicity

Tis	sues:
\checkmark	Blood
\checkmark	Muscle

- ✓ Gills
- ✓ Liver✓ Kidney
- ✓ Gonads
- ✓ GI tract

MARINE LITTER ANALYSIS

Analysis of microplastics and ML in the GI tract



DIAGNOSTIC MARKERS FOR REPRODUCTION

DIAGNOSTIC MARKERS FOR INFLAMMATION AND MORPHOLOGY

• Markers for reproductive system



MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER IN SEA TURTLES

MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER ON BIOTA: <u>SEA TURTLES</u>



Plastic detections	Plastic tracers detections	Biomarkers detections
- Analysis of the gastro intestinal (GI) contents: • Occurrence (%) • Abundance (n°) • Weight (g) • Polymer analysis	 - Analysis of plastic additives: • Phthalates • PBDEs • Bisphenol A - Analysis of PBT compounds: • PCBs • DDTs • PAHs • Mercury 	Effects at molecular level: •Measure of DNA damage •Alterations of gene expression •Alteration of proteins •Effects at cellular level: •Alteration of cell functions •Effects at tissue level: •Hystological and hystopathological alterations



Project funded by th FUROPEAN UNIO

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MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER IN SEA BIRDS

MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER ON BIOTA: <u>SEA BIRDS</u>





MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER IN MARINE MAMMALS

MONITORING MARINE LITTER IMPACTS IN CETACEANS

i) Plastic 🛛	ii) Plastic tracers detection	iii) Biomarkers S
Analysis of the ingested marine litter/microplastics: •Occurrence (%) •Abundance (n°) •Weight (g) •Polymer analysis	Analysis of plastic additives: •Phthalates •PBDEs •Bisphenol A • Analysis of PBT compounds: •PCBs •DDTs •PAHs •Mercury	Effects at molecular level: • Measure of DNA damage • Alterations of gene expression • Alteration of proteins • Effects at cellular level: • Alteration of cell functions • Effects at tissue level: • Histological and histopathological alterations



FIGURE 6.3 The threefold monitoring approach to detect marine litter presence and impact in cetacean species (stranded and free-ranging organisms).





MONITORING APPROACHES FOR ASSESSING THE EFFECTS OF MARINE LITTER ON BIOTA: <u>CETACEANS</u>



Plastic

- detections
- intestinal (GI) contents:
 Occurrence (%)
 Abundance (n°)
- Weight (g)
 Polymer analysis

Plastic tracers detections - Analysis of plastic additives: • Phthalates • PBDEs • Bisphenol A - Analysis of PBT compounds: • PCBs • DDTs

PAHs

Mercury

	Biomarkers detections
	 Effects at molecular level: Measure of DNA damage Alterations of gene expression Alteration of proteins
s: ¦	 Effects at cellular level: Alteration of cell functions
	- Effects at tissue level: • Hystological and

hystopathological alterations

DIAGNOSTIC MARKERS FOR IMMUNE DIAGNOSTIC MARKERS FOR CONTAMINANTS Markers of immunotoxicity • Markers of genotoxicity • Markers of neurotoxicity DIAGNOSTIC MARKERS FOR REPRODUCTION Skin biopsy • Markers of oxidative stress Epidermis-Dermis-Blubbe FUNCTIONALITY • Markers for reproductive system • Markers of xenobiotic metabolism and biotransformation 2 DIAGNOSTIC MARKERS FOR INFLAMMATION AND MORPHOLOGY ANALYSIS OF ANTHROPOGENIC CONTAMINANTS • OCs PAHs Mercury MARINE LITTER ANALYSIS Plastic additives (phthalates, Analysis of microplastics and ML in GI tract

bisphenol A, PBDEs)



Analysis of microplastics and ML in faeces



MONITORING MARINE LITTER IMPACTS IN CETACEANS: STRANDED ORGANISMS



ii) Plastic tracers detection

- Analysis of the ingested marine litter/microplastics:

•Occurrence (%)

- Abundance (n°)
- Weight (g)

Polymer analysis

Analysis of plastic additives:
Phthalates
PBDEs
Bisphenol A
Analysis of PBT compounds:
PCBs
DDTs
PAHs
Mercury



Ziphius cavirostris



A new prototype to isolate macro and microplastics in the gastrointestinal tract of stranded cetaceans







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MONITORING MARINE LITTER IMPACTS IN CETACEANS: FREE-RANGING CETACEANS



Skin biopsy sampling

 1987
 • BDDE

 • Markers of Reproduction Alterations (EARAS Service) hormonol
 • Debe

 • Markers of Reproduction Alterations (EARAS Service) hormonol
 • Debe

 • Markers of Reproduction Alterations (Debe provise)
 • Debe

 • Markers of Susceptibility (Mark)
 • Debe

 • Mark
 • Debe

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LESSONS LEARNED FROM ML MONITORING IN MED













This resource has been developed within the framework of the Interreg Med Plastic Busters MPAs project. Find more info here The Plastic Busters MPAs protocols on how to monitor the presence and effects of marine litter in Mediterranean MPAs

This document features a compilation of all the protocols that should be applied in order to elaborate a comprehensive diagnosis of the presence and effects of marine litter in the Mediterranean. It takes stock of all recent advances made by the European Marine Strategy Framework Directive Technical Group on Marine Litter and the Barcelona Convention Correspondence Group on Monitoring.



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The Plastic Busters MPAs e-learning module on how to monitor the presence and effects of marine litter

This self-paced e-learning module is designed specifically for the busy practitioner, conservationist, researcher, professional and student working for litter-free coasts and seas. By following this module, participants will learn how to design and implement marine litter monitoring campaigns and how to generate fitfor-purpose, reliable and comparable data on the presence and effects of marine litter, thus contributing with essential knowledge for a targeted response to address the urgent marine litter threat in the Mediterranean.







This resource has been developed within the framework of the Interreg Med Plastic Busters MPAs project.

Find more info here









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Thank you شکرا Merci Grazie

G For a litter FREE Mediterranean













