



**PLASTIC
BUSTERS**



IMPACT OF MARINE LITTER ON MEDITERRANEAN BIODIVERSITY

- Provide a thorough analysis on plastics and microplastics as vectors of chemical pollutants
- Overview of tested protocols on monitoring microplastics in biota



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1240

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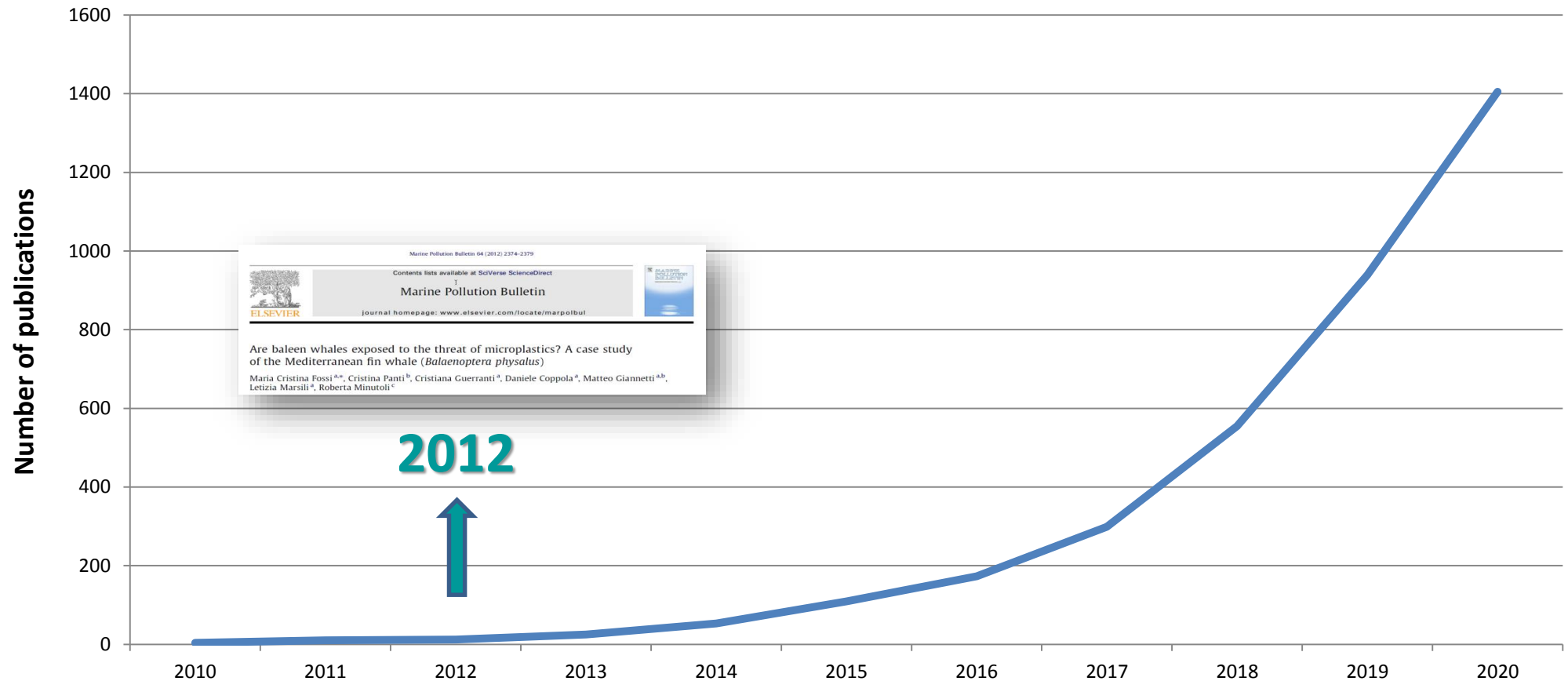


Mediterranean





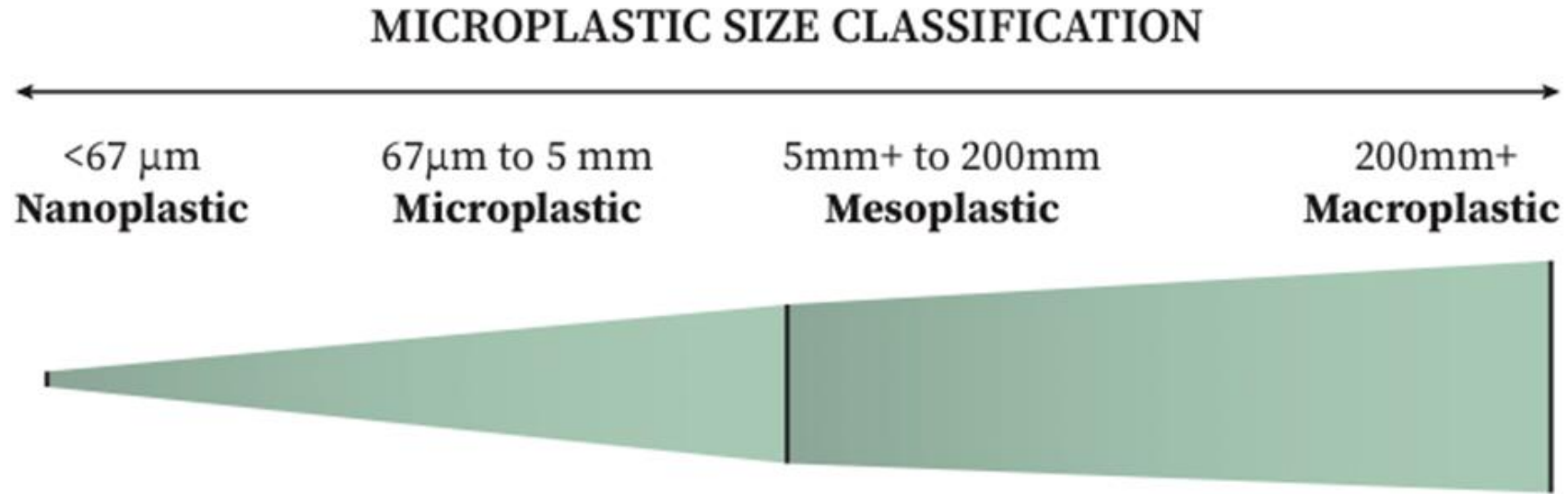
Microplastics publications in the last 10 years



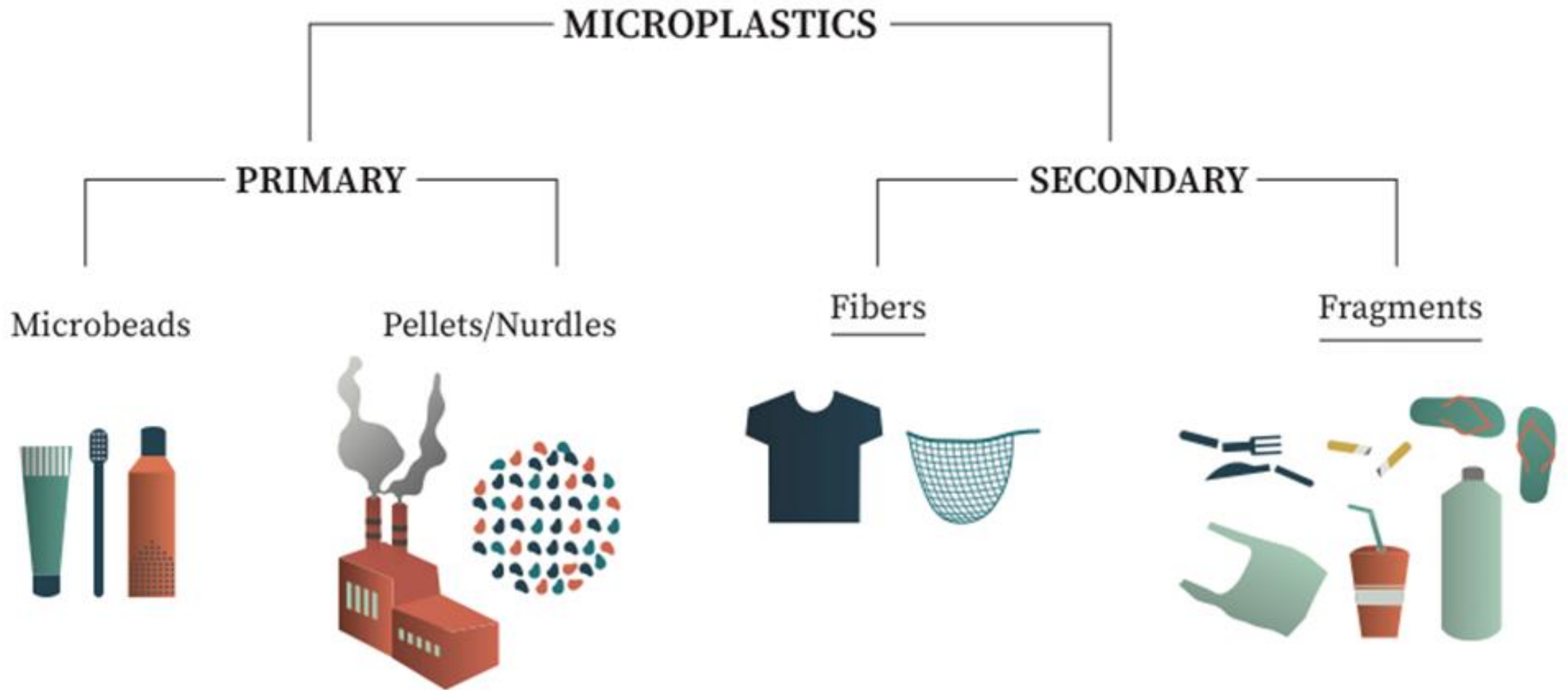
Document results (Scopus) from 2010 to 2021



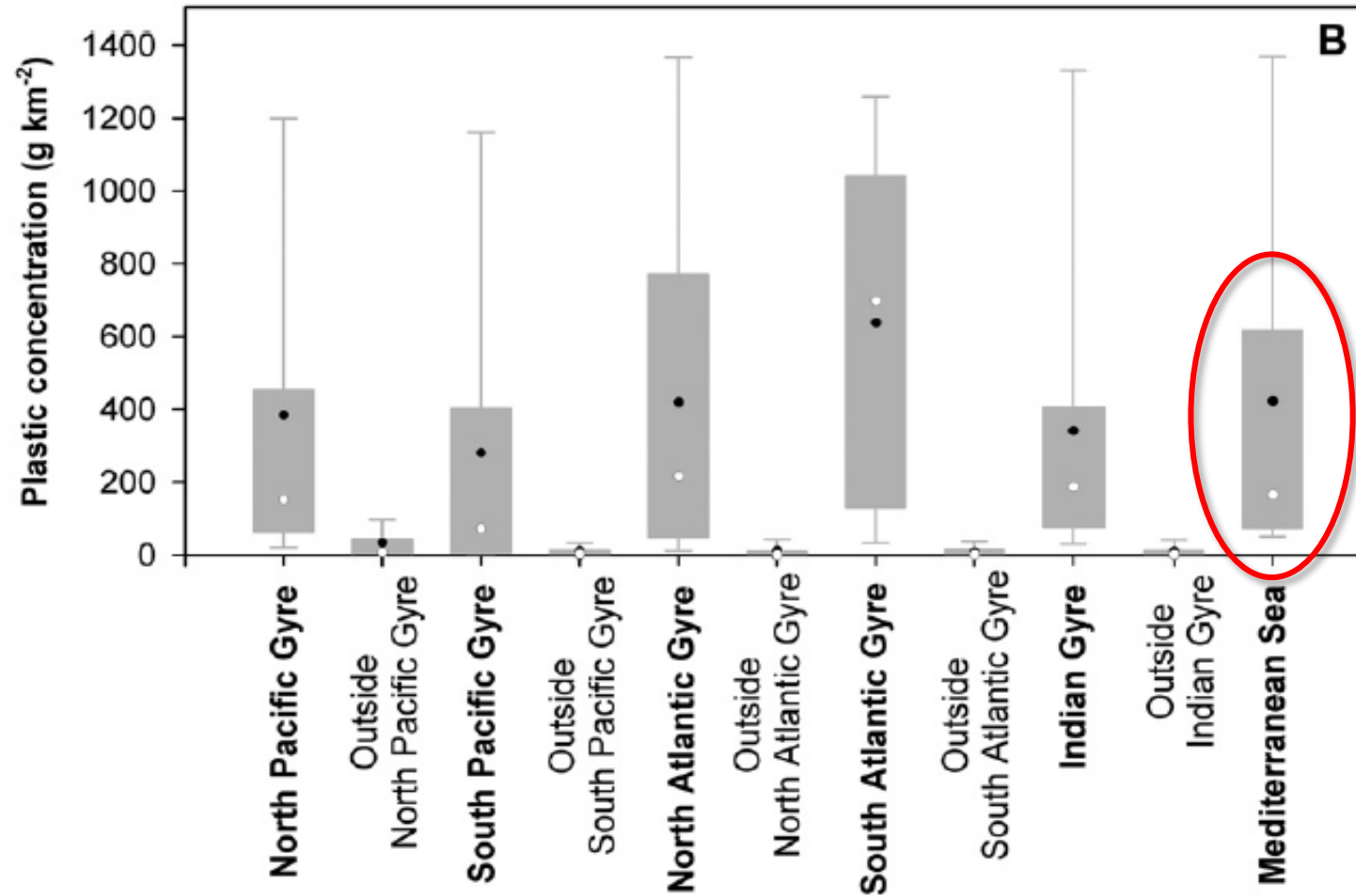
Microplastics



Microplastics

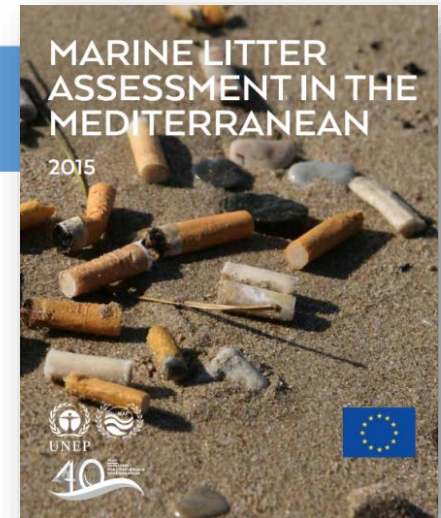


Word microplastics distribution: Mediterranean sea is one of the “hot spot areas”



MARINE LITTER IN THE MEDITERRANEAN SEA

- An highly urbanized and developed coastline
- A closed basin
- 30% of the maritime traffic
- A touristic destination
- Large rivers (Rhône, Nile, Po)



THE MOST AFFECTED AREA WORLDWIDE FOR MARINE LITTER

- Some of the largest amounts of Municipal Solid Waste (MSW) are generated annually per person in the Mediterranean Sea (208 – 760 kg/Year)
- An estimated 731 tons of plastic is littered every day, with important differences depending on country
- Cigarette butts may reach 40% of stranded litter
- the highest densities of marine litter stranded on the sea floor, up to 100,000 items / km² (French Coast) are found in the Mediterranean Sea
- the highest densities of floating microplastics , up to 4680,000 items / km² (Southern Adriatic) are found in the Mediterranean Sea

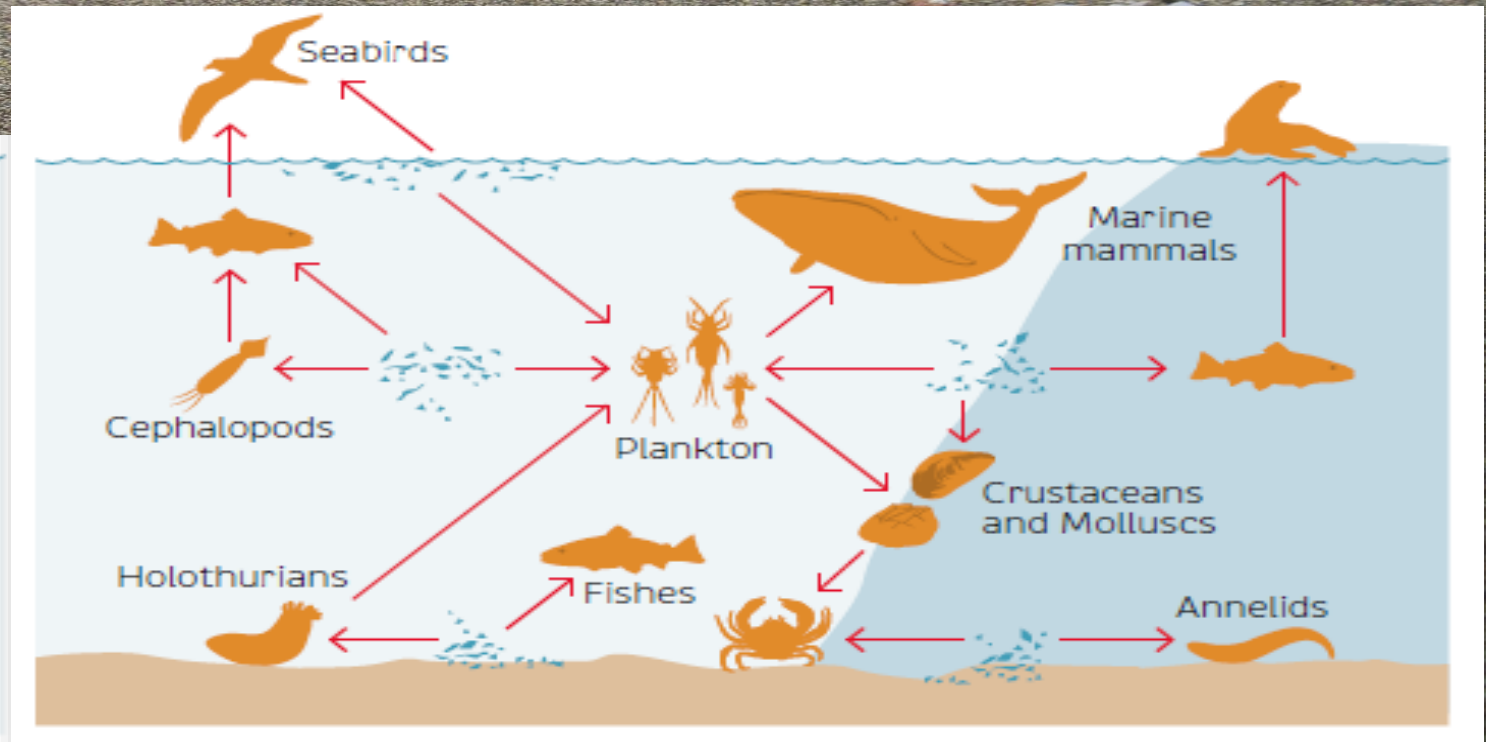
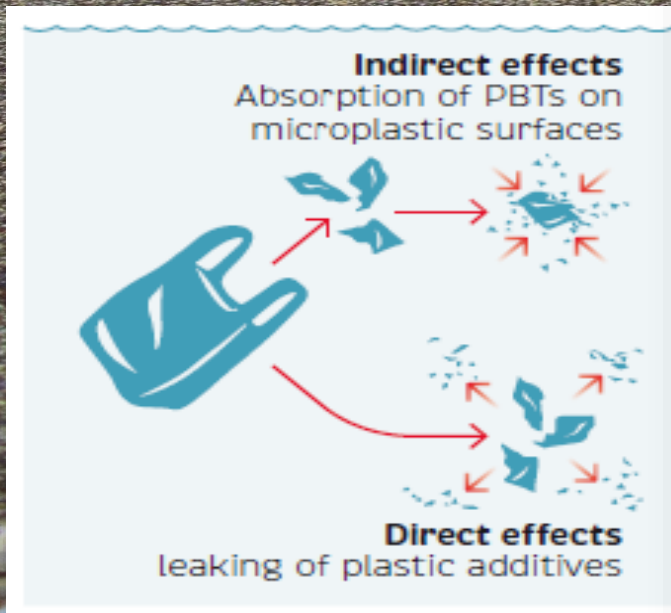
A close-up photograph of a petri dish filled with a large number of small, irregularly shaped fragments of plastic and microplastics. The fragments are scattered across the dark surface of the dish and come in a wide variety of colors, including red, orange, yellow, green, blue, and grey. Some fragments are thin and elongated, while others are more chunky or flake-like. The lighting highlights the textures and colors of the plastic pieces.

Plastics and microplastics as vectors of
chemical pollutants

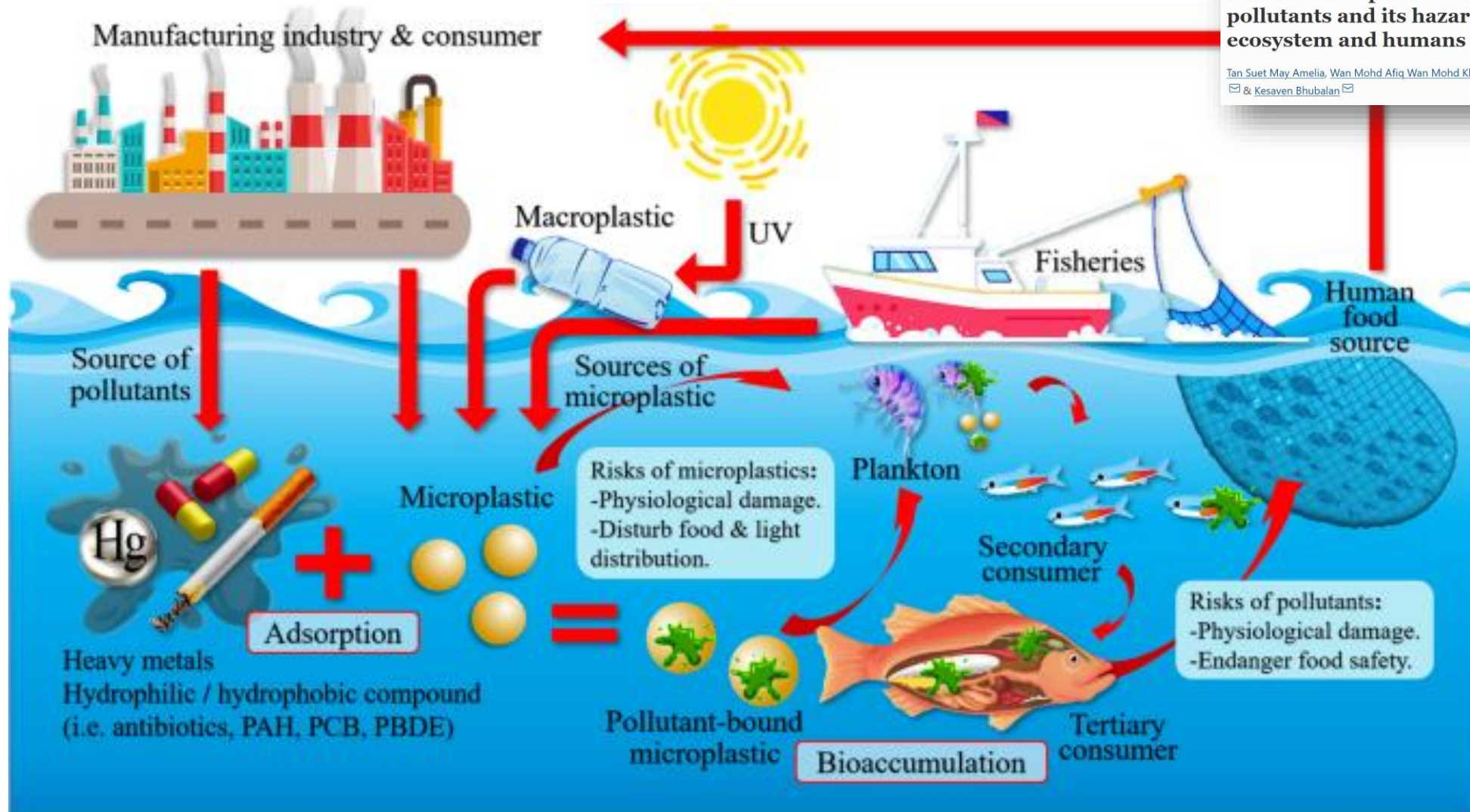
Impact of microplastics on marine organisms

- 1 - transport of persistent, bioaccumulating toxic (PBT) substances from plastics
- 2 - leaching of additives from the plastics such as phthalates
- 3 - physical harm
- 4 - virus and bacteria ?

**Multiple
Stress**



Plastics and microplastics as vectors of chemical pollutants

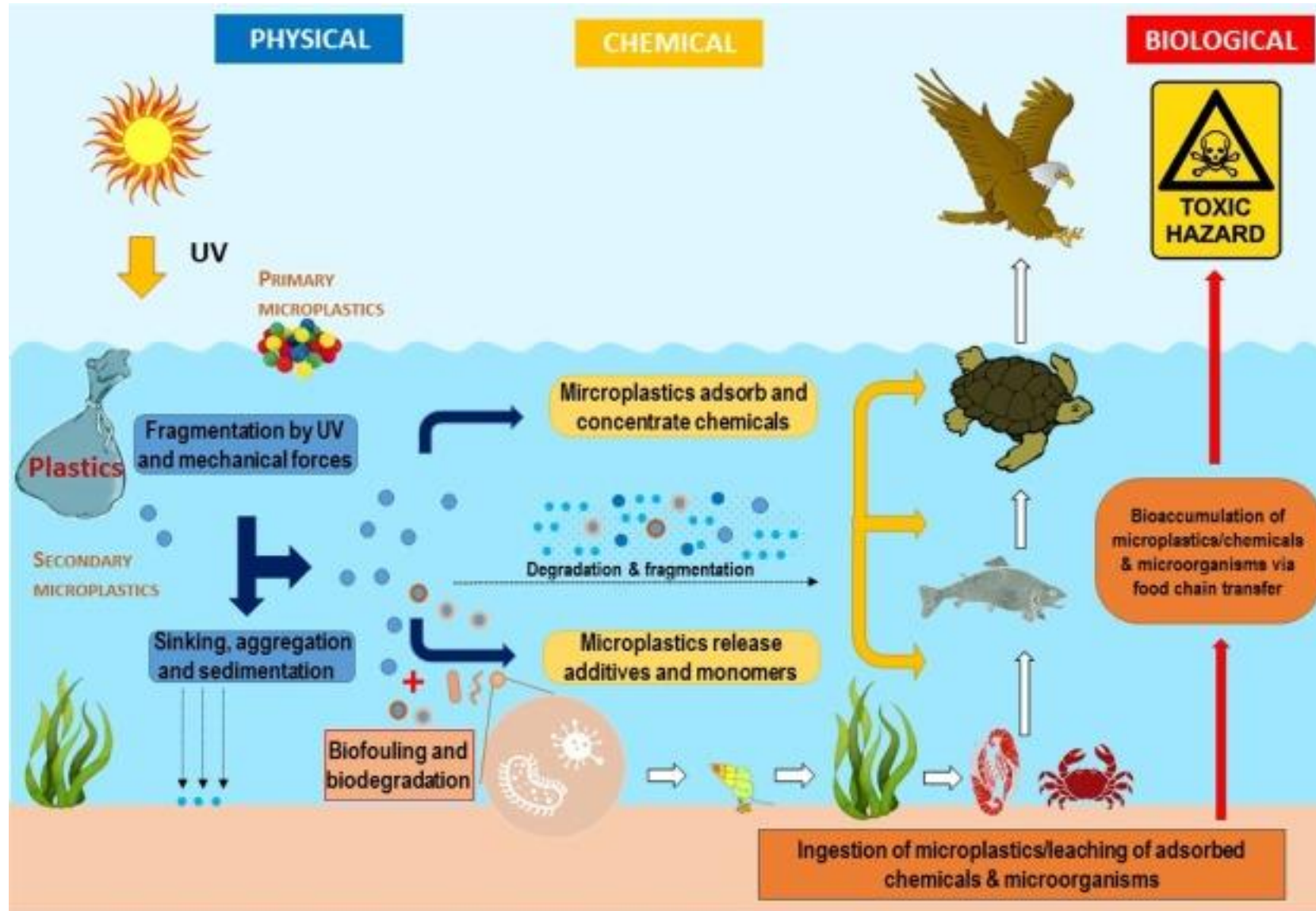


Review | Open Access | Published: 22 January 2021

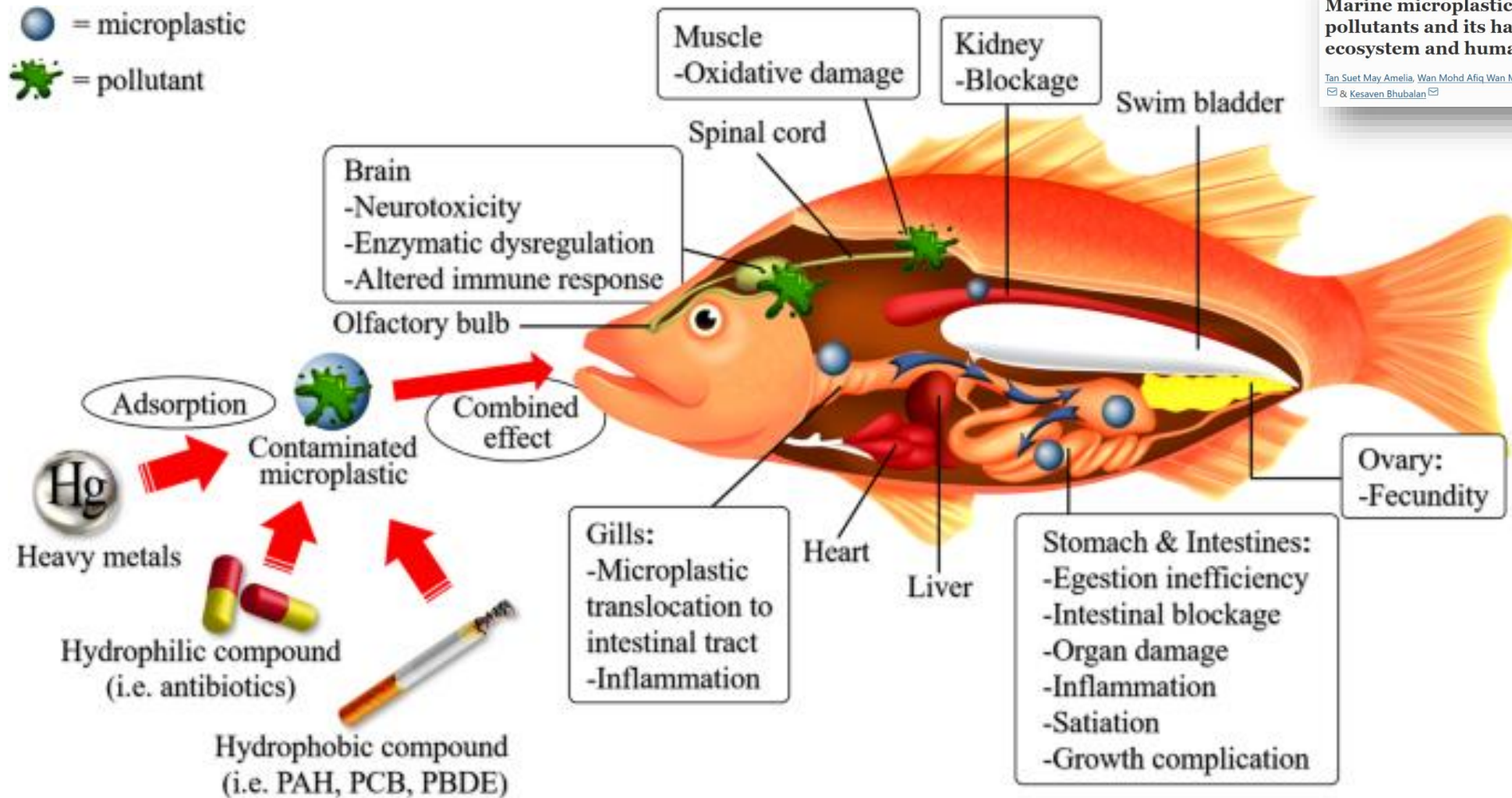
Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans

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

Plastics and microplastics as vectors of chemical pollutants



Plastics and microplastics as vectors of chemical pollutants



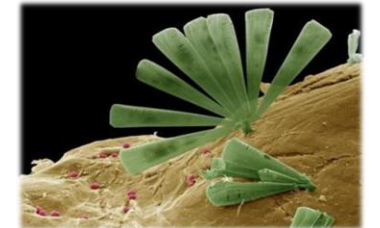
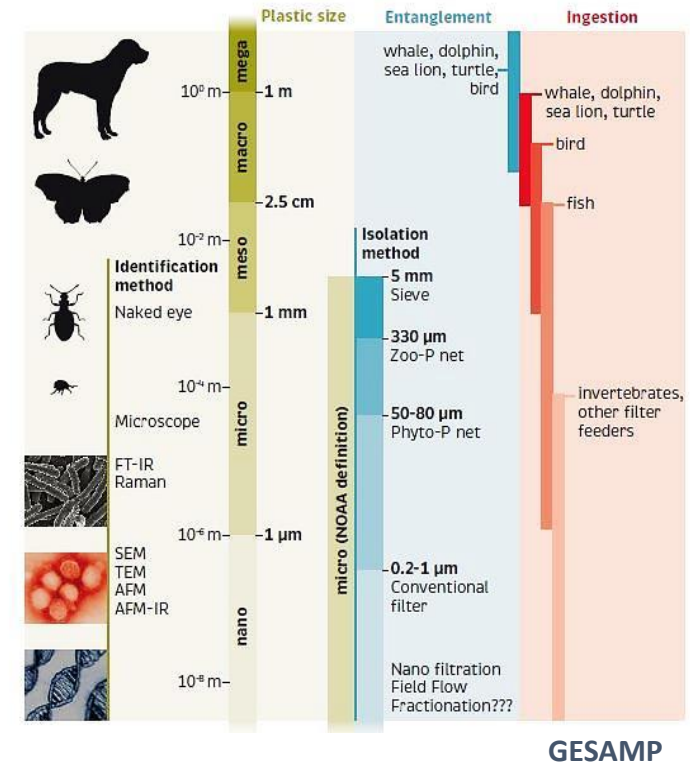
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Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans

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ECOLOGICAL IMPACT OF PLASTIC LITTER

- Habitat destruction
- Introduction and spread of invasive species
- Entanglement/entrapment
- Ingestion
- Transport of chemicals



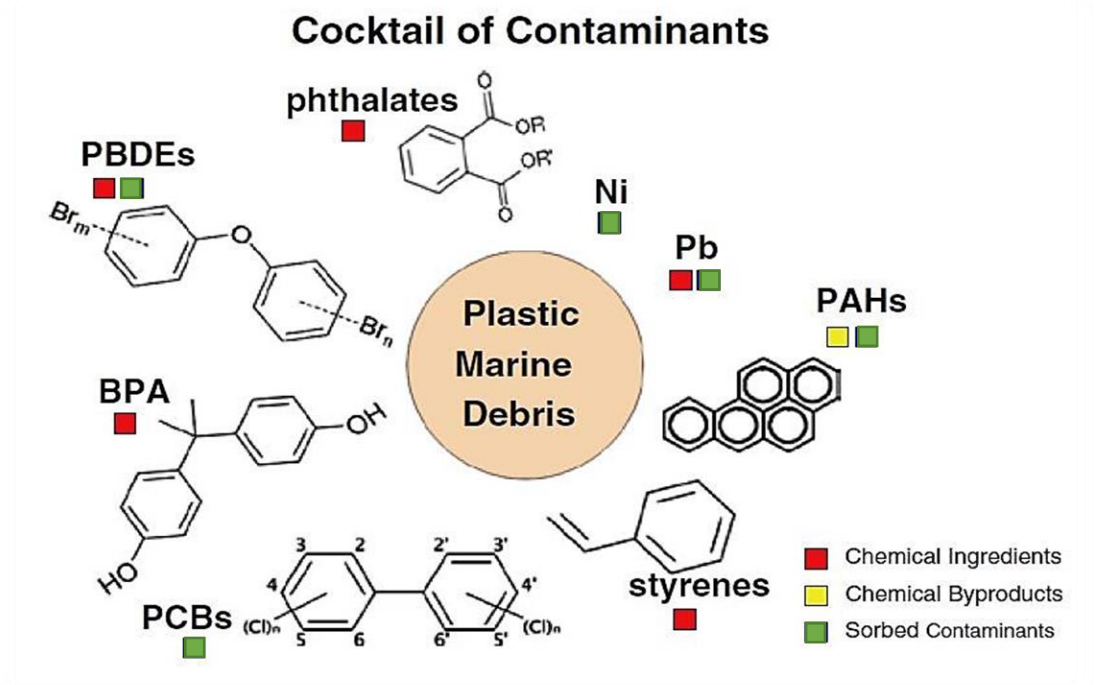
TRANSPORT OF CHEMICALS

This raises concerns regarding:

- ✓ the **complex mixture of chemical** substances associated with marine plastic debris;
- ✓ the **environmental fate** of these chemicals **to and from plastics** in our **oceans**;
- ✓ how this **mixture affects wildlife**, as hundreds of species ingest this material in nature.

TRANSPORT OF CHEMICALS

Because of their **physical** and **chemical properties** marine **plastic** debris are associated with a ‘**cocktail of chemicals**’, including those that are **ingredients of the plastic material** (e.g. **monomers and additives**) and those **present** in the **marine environment** that **absorbs on plastic** when it becomes marine debris (e.g. persistent, bioaccumulative and toxic substances (PBTs)).



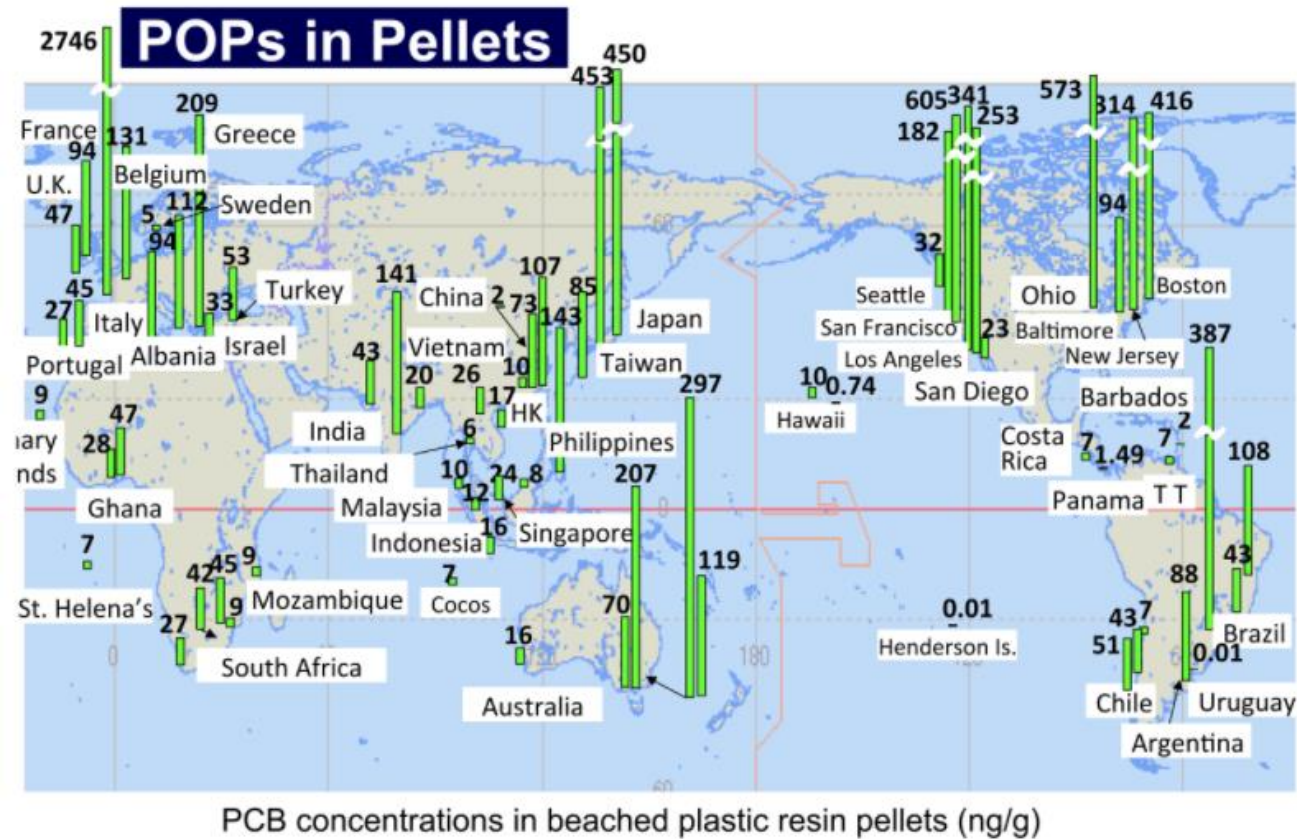
TRANSPORT OF CHEMICALS: PBTs

- Because of their **physical and chemical properties**, plastics accumulate a complex **mixture of chemical contaminants present in the surrounding seawater** adding to the cocktail of chemicals already present from manufacturing.
- As a result of **widespread global contamination of chemical contaminants** and plastic debris, **marine plastic debris** are recovered globally with **measurable amounts of persistent bioaccumulative and toxic substances (PBTs)**: e.g. **polychlorinated biphenyls (PCBs)**, **PAHs** and **PBDEs**. PBTs generally have a **low water-solubility** thus, when PBTs encounter plastic debris they **tend to sorb to this material**.
- The risks of plastic contributing to availability and transfer of chemicals are **still not clear**.



TRANSPORT OF CHEMICALS: PBTs

Plastic pellets, a recognizable component of marine debris, are now **used to examine the global pattern of PBTs, acting as passive samplers** and providing baseline information **regarding PBT contamination in the ocean**. International Pellet Watch leads this effort, collecting plastic pellets globally and measuring the concentrations of various PBTs sorbed to plastic debris.



TRANSPORT OF CHEMICALS: PLASTIC ADDITIVES

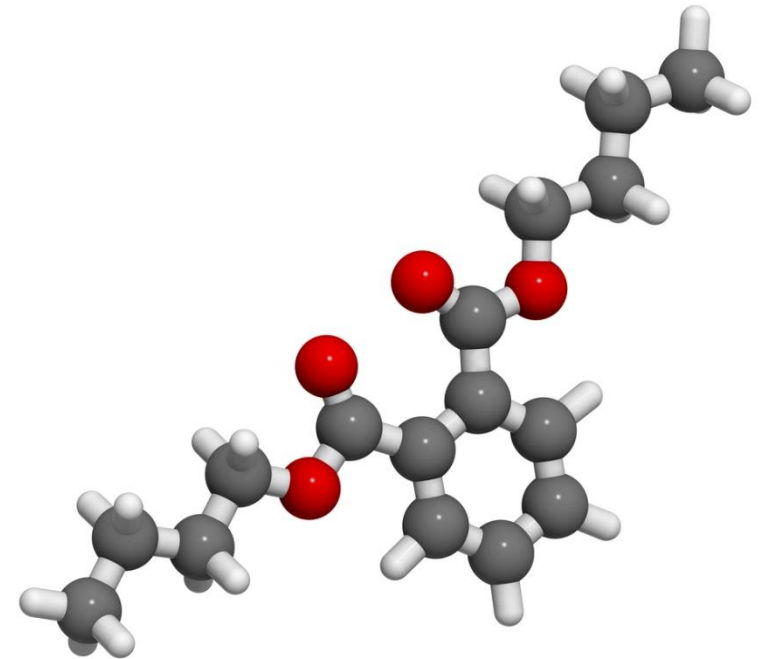
- There are several **different types of plastics** manufactured into a **diversity of products**. These are **made using solvents and other chemicals** that may be used as initiators and catalysts.
- In addition, **several additives** (e.g. flame retardants, stabilizers, pigments and fillers) are included to give the plastic certain characteristics (e.g. flexibility, strength and color).
- Such **chemicals** can potentially migrate from plastic products to the medium in contact with them and **be released during production, use and disposal** of the product, **several** of which can be **harmful**.



TRANSPORT OF CHEMICALS: PLASTIC ADDITIVES

PHTHALATES

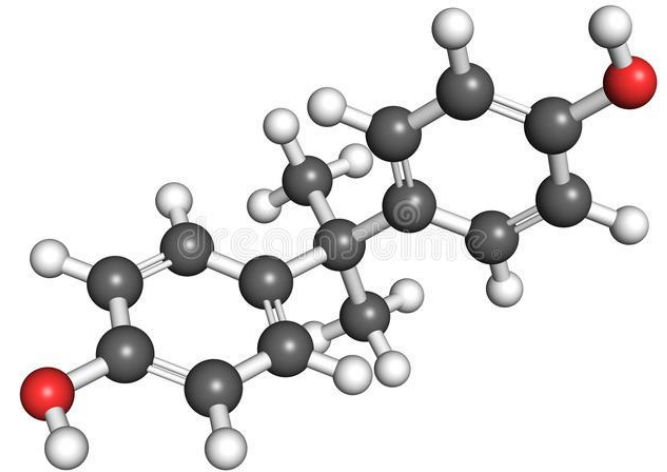
- Phthalic acid esters or phthalates are a family of additives used as **plasticisers**, mainly in PVC production. They **add fragrance to products and make them more pliable**. PVC can contain 10%-60% phthalates by weight. They can **easily leach into the environment during manufacturing, use and disposal**.
- Some **phthalates** have been defined as **endocrine disruptors, even at low concentrations** since they interfere with the production of androgen (testosterone), a hormone critical in male development and relevant to females as well.
- **Di(2-ethylexyl) phthalate (DEHP)** was the most commonly used representing 37% of the global plasticiser market. However, DEHP has gradually been replaced by diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP) and di(2-Propyl Heptyl) phthalate (DPHP), which represented 57% of plasticiser consumption in Europe in 2015.



TRANSPORT OF CHEMICALS: PLASTIC ADDITIVES

BISPHENOLS

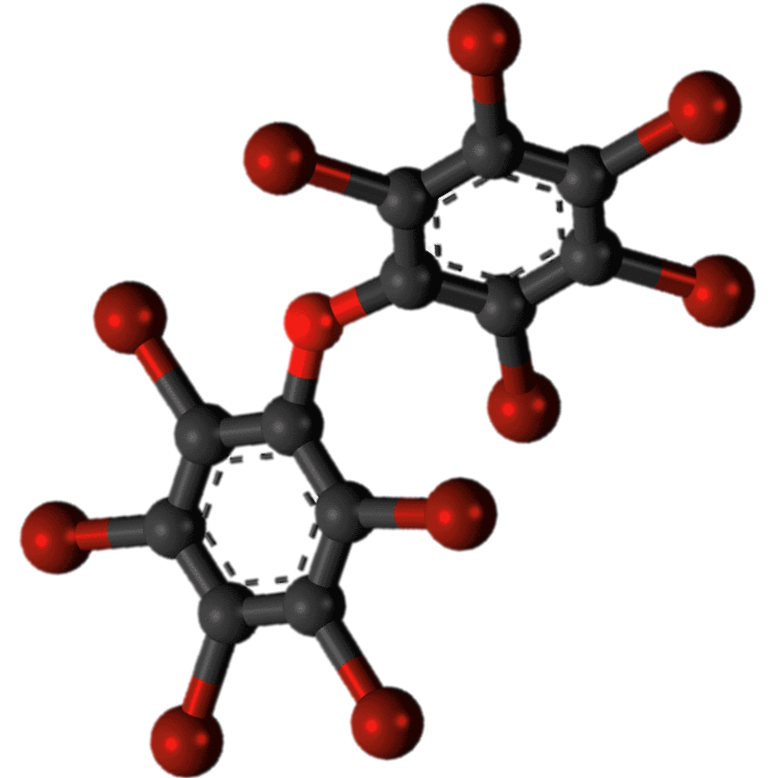
- Bisphenols are a group of chemical compounds with two hydroxyphenyl functionalities. They are present in **many polycarbonate plastic products** (including **water bottles, food storage containers and packaging, sports equipment**), **epoxy resin liners of aluminium cans**, and also bisphenols are frequently used as a developer in thermal paper such as cash register receipts.
- **Bisphenol A (BPA)** is the most representative chemical of the bisphenol group and is one of the most commonly produced chemicals worldwide, with over **three million tons produced annually**.
- BPA can be used as an **antioxidant** or as a **plasticiser** in some polymers such as **PP, PE and PVC**. **Leaching of BPA** can occur, leading to **release from food and drink packaging, a source of exposure for humans**.



TRANSPORT OF CHEMICALS: PLASTIC ADDITIVES

FLAME RETARDANTS

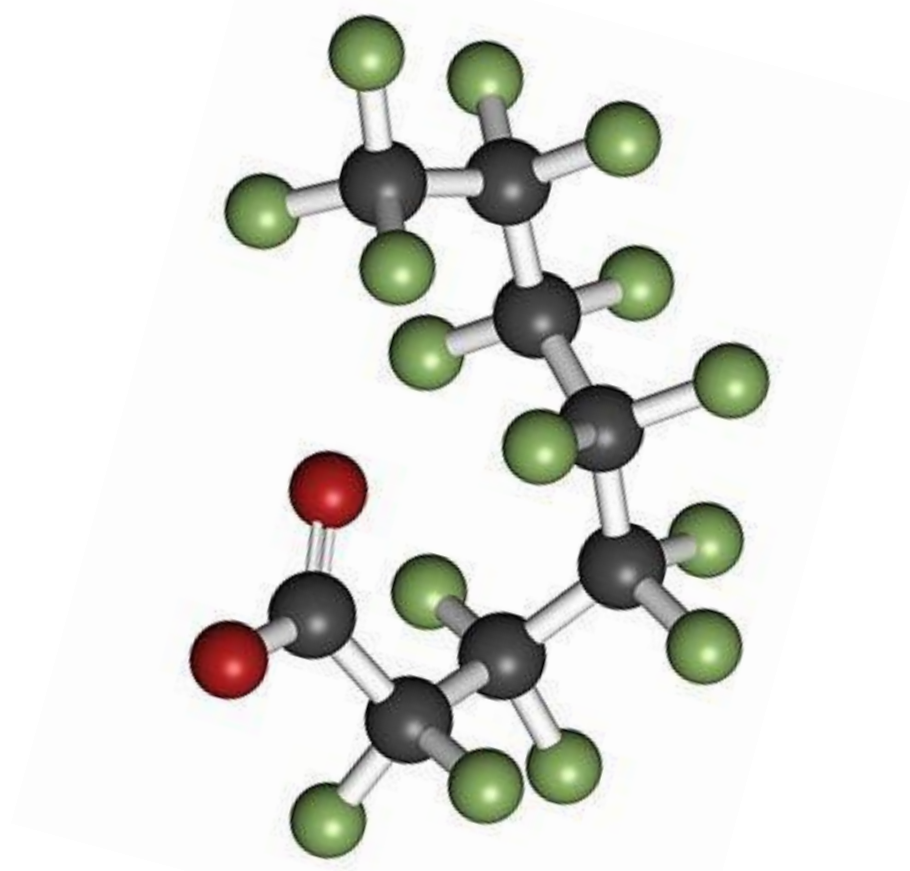
- Flame retardants are a class of additives used in plastic and other polymer products to **reduce flammability and to prevent the spread of fire**. The main retardants used in plastics include **brominated flame retardants (BFRs)** with antimony (Sb) as synergist (e.g. polybrominated diphenyl ethers (PBDEs)).
- PBDEs are **hydrophobic substances** that were produced as three commercial formulations (**commercial penta-BDE, commercial octa-DBE and commercial deca-BDE**). They are **ubiquitous, toxic, and persistent, they bioaccumulate, and they are of great concern for human health**.



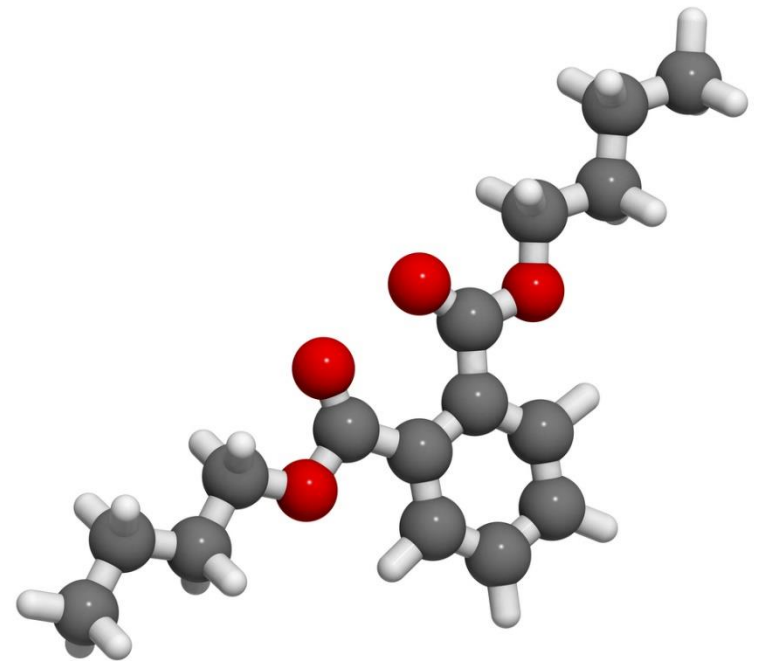
TRANSPORT OF CHEMICALS: PLASTIC ADDITIVES

PERFLUORINATED CHEMICALS

- PFOS and related substances have been **listed under the Stockholm Convention since 2009**, and PFOA and related substances are suggested for listing in the current COP. PFOS and PFOA **do not follow the pattern of a classic POP** — they do **not accumulate in fatty tissues but instead binds to proteins**.
- They therefore accumulate mainly in organs such as the liver, kidney, brain and spleen. In animal studies **PFOS causes cancer, neonatal mortality, delays in physical development, and endocrine disruption**.



TRANSPORT OF CHEMICALS: PLASTIC ADDITIVES



Plastic Additives Analysis: Phthalates

MECHANICAL SHAKING



ULTRASONIC EXTRACTION



PURIFICATION
Dispersive SPE



CONCENTRATION
Stream of nitrogen



Agilent 5977B GC/MS

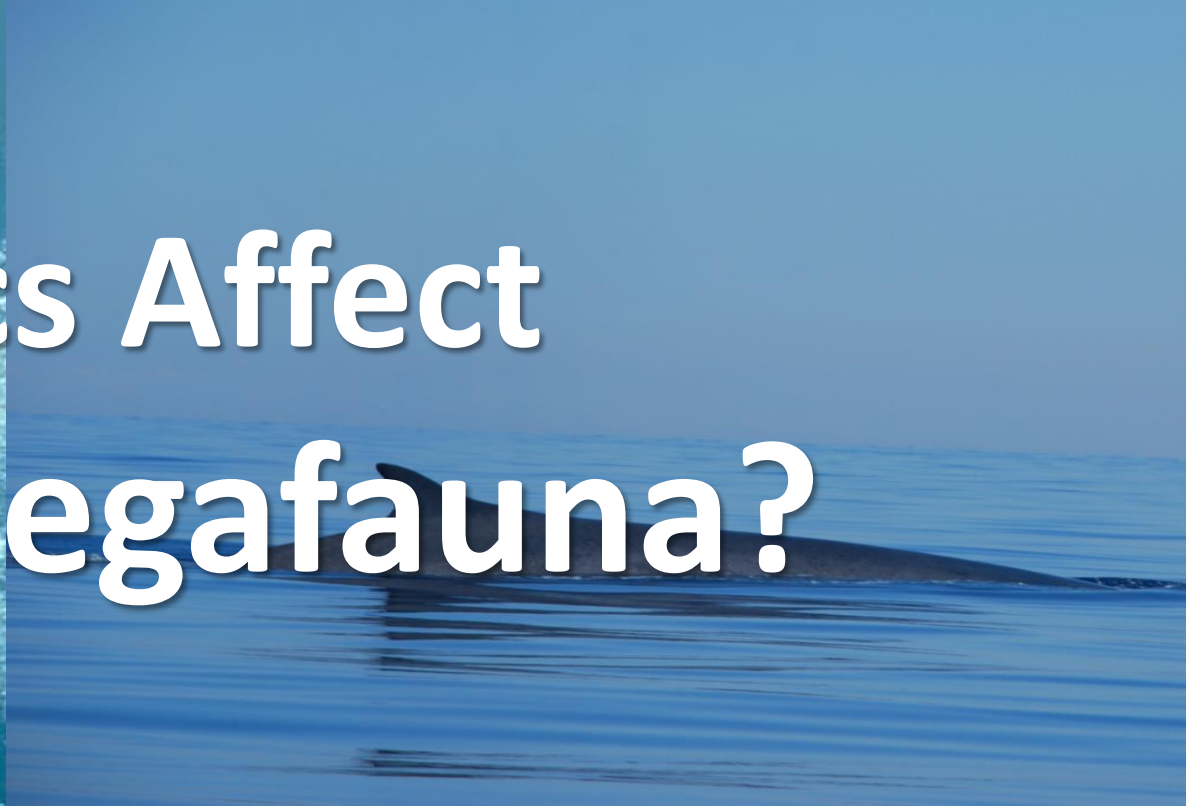
11 phthalates	CAS
Dimethyl phthalate	131-11-3
Diethyl phthalate	84-66-2
Diallyl phthalate	131-17-9
Dipropyl phthalate	131-16-8
Diisobutyl phthalate	84-69-5
<u>Dibutyl phthalate</u>	84-74-2
<u>Benzyl butyl phthalate</u>	85-68-7
Dicyclohexyl phthalate	84-61-7
<u>Bis(2-ethylhexyl) phthalate</u>	117-81-7
<u>Diisononyl phthalate</u>	28553-12-0
<u>Di-n-octyl phthalate</u>	117-84-0

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

Interreg
Mediterranean

PLASTIC BUSTERS
MPAs

Project co-financed by the European
Regional Development Fund

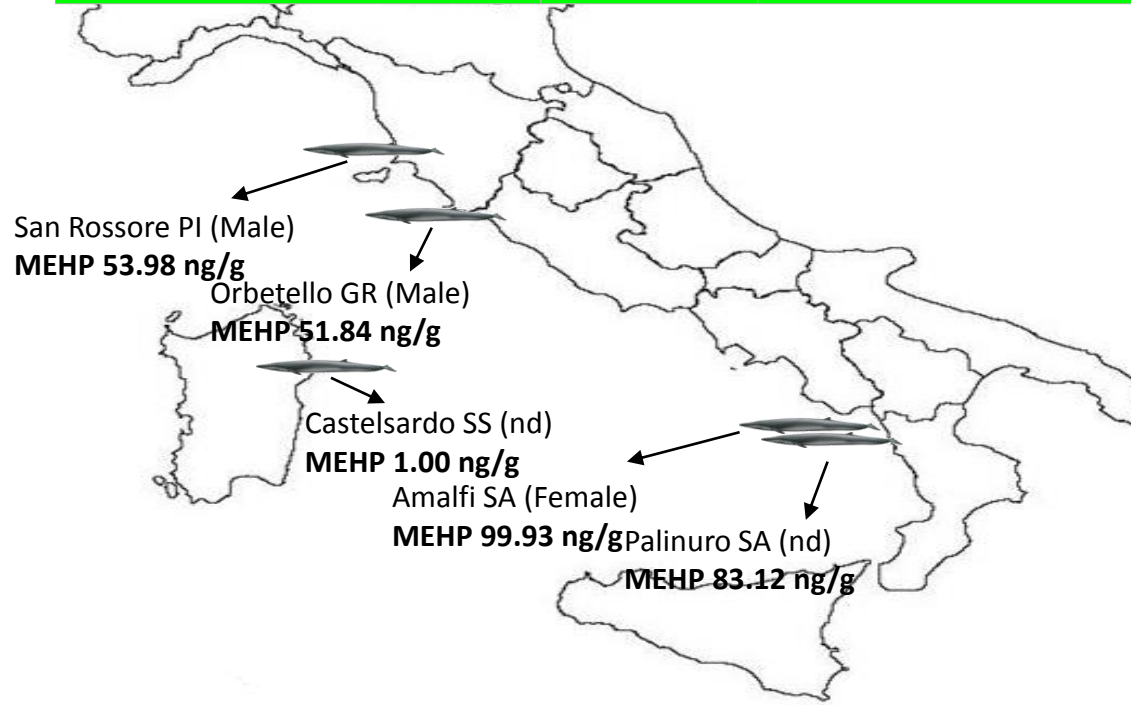


**Could Microplastics Affect
Filter-Feeding Megafauna?**

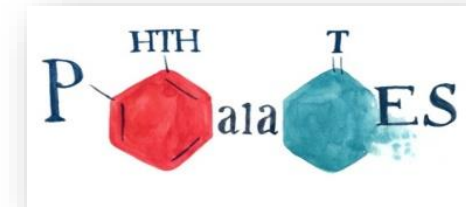
Plastic additives in fin whales




MEHP concentration in stranded fin whales		
SPECIES	TISSUE	Mean MEHP (ng/g)
<i>Balaenoptera physalus</i>	Blubber	57.97



MEHP concentrations (ng/g) in blubber samples of five **stranded fin whales** collected along the Italian coasts during the period July 2007 – June 2011 in five different locations.






Contents lists available at ScienceDirect

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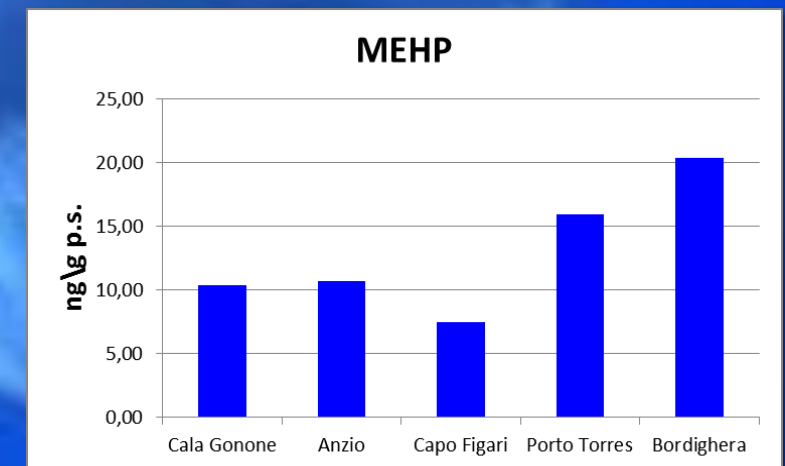
Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: The case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*)

Maria Cristina Fossi ^{a,*}, Daniele Coppola ^a, Matteo Baini ^a, Matteo Giannetti ^{a,b}, Cristiana Guerranti ^a, Letizia Marsili ^a, Cristina Panti ^a, Eleonora de Sabata ^c, Simona Clò ^{c,d}

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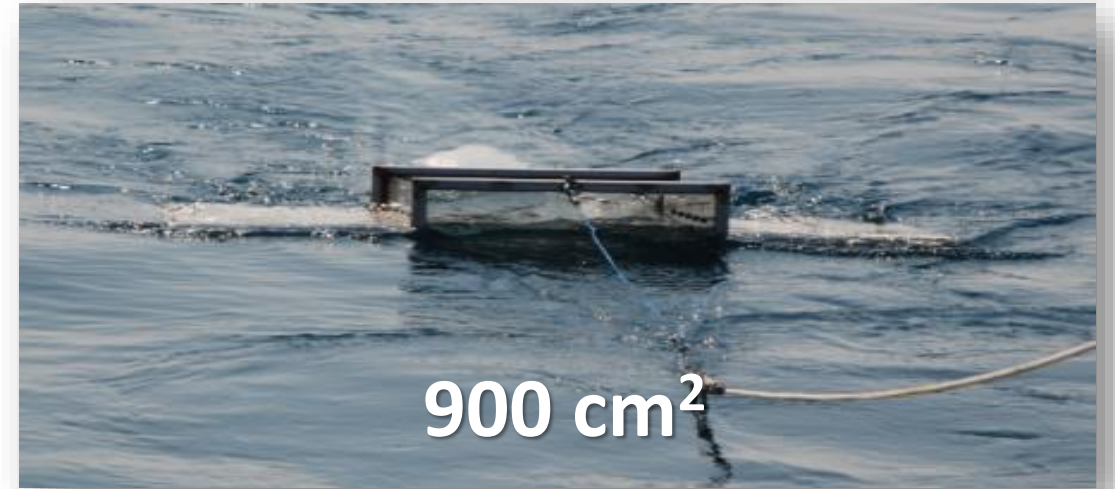
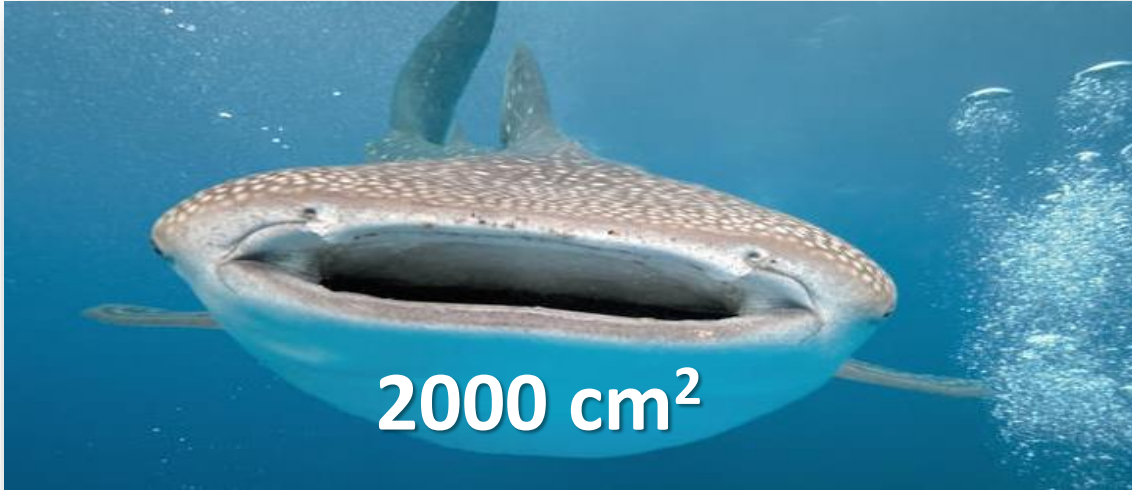
MEHP concentration in basking sharks

SPECIES	TISSUE	Mean MEHP (ng/g)
<i>Basking sharks</i>	muscle	12.97



Phthalates as plastic tracer ?

...one of the largest filter feeders in the sea



8 h/day feeding at the surface

Based on open mouth internal heights, the estimated total open mouth area was **2035 cm²**
for shark A (622cm TL), **1841 cm²** for shark B (593cm TL),



PCB levels in skin biopsy

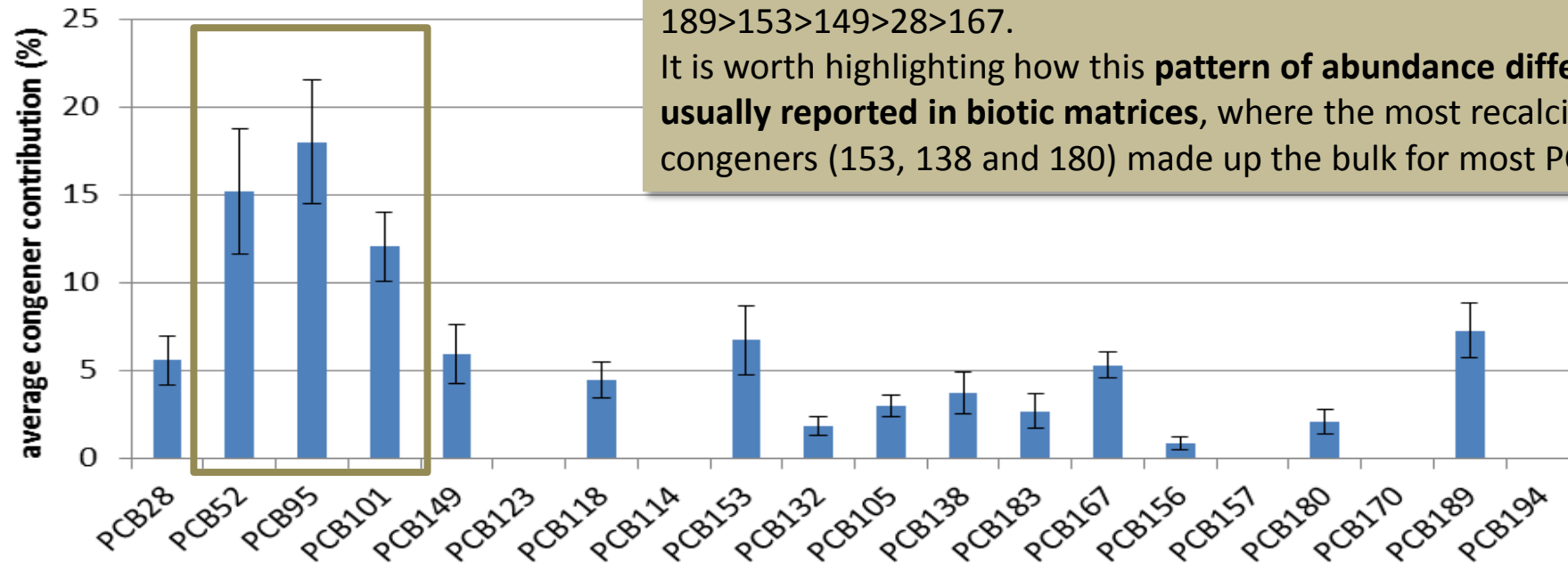


Surface oceans are enriched in this type of congeners (Jurado et al., 2004).

PCB content was mostly dominated by congeners with medium-low chlorine content such as **PCB 95, 101** and **52** with contributions **>10%**.

Other relevant contributions (>5%) were presented by congeners 189>153>149>28>167.

It is worth highlighting how this **pattern of abundance differs from what is usually reported in biotic matrices**, where the most recalcitrant PCB congeners (153, 138 and 180) made up the bulk for most PCB burdens.



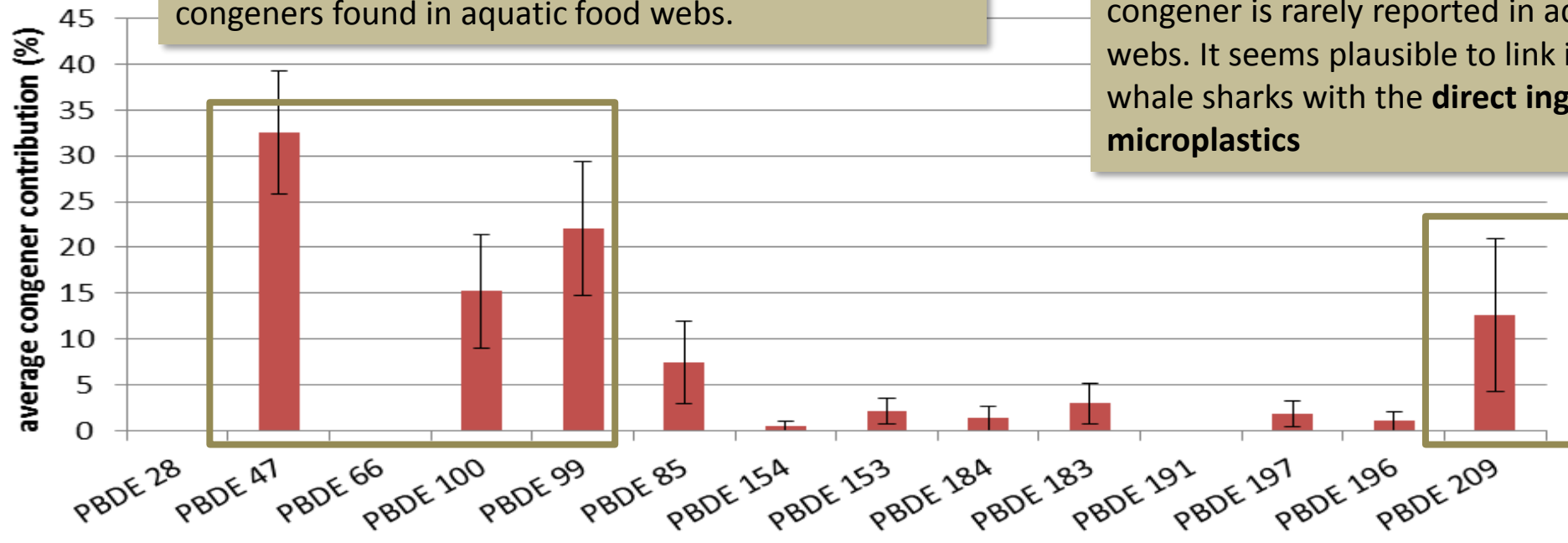
Average PCB congener profile in skin biopsies of whale sharks. Error bars represent standard errors (SE).



PBDE levels in skin biopsy

As with PCBs, **PBDE content was dominated by lower-medium brominated congeners such as 47 > 99 > 100**. Not surprisingly, these are examples of predominant congeners found in aquatic food webs.

Unexpected, was the important contribution found for **BDE-209** accounting for an average of **12.6%** of the total PBDE burden. This congener is rarely reported in aquatic food webs. It seems plausible to link its presence in whale sharks with the **direct ingestion of microplastics**



Average PBDE congener profile in skin biopsies of whale sharks. Error bars represent standard errors (SE).



Plastic Litter: POPs and EDCs

Gallo et al. *Environ Sci Eur* (2018) 30:13
<https://doi.org/10.1186/s12302-018-0139-z>

Environmental Sciences Europe

DISCUSSION

Open Access



Marine litter plastics and microplastics and their toxic chemicals components: the need for urgent preventive measures

Frederic Gallo^{1*}, Cristina Fossi², Roland Weber^{3*}, David Santillo⁴, Joao Sousa⁵, Imogen Ingram⁶, Angel Nadal⁷ and Dolores Romano⁸

2018



- Persistent plastics, with an estimated lifetime for degradation of hundreds of years in marine conditions, can break up into micro- and nanoplastics over shorter timescales, thus facilitating their uptake by marine biota throughout the food chain.
- **These polymers may contain chemical additives and contaminants, including some known endocrine disruptors that may be harmful at extremely low concentrations for marine biota, thus posing potential risks to marine ecosystems, biodiversity and food availability.**
- Although there is still need to carry out focused scientific research to fill the knowledge gaps about the impacts of plastic litter in the marine environment (Wagner et al. in *Environ Sci Eur* 26:9, 2014), **the food chain and human health**, existing scientific evidence and concerns are already sufficient to support actions by the scientific, industry, policy and civil society communities to curb the ongoing flow of plastics and the toxic chemicals they contain into the marine environment.
- This document was prepared by a working group of **Regional Centres of the Stockholm and Basel Conventions** and related colleagues intended to be a background document for discussion in the 2017 Conference of the Parties (COP) of the Basel Convention on hazardous wastes and the Stockholm Convention on persistent organic pollutants (POPs).



Monitoring Microplastics in Biota

How can
Marine litter
impact on
Mediterranean
marine
life?



IMPACTS OF MARINE DEBRIS



INGESTION

Animals mistakenly eat plastic and other debris.



ENTANGLEMENT & GHOSTFISHING

Marine life gets caught and killed in ghost nets, trapped in derelict gear, and entangled in plastic bands and other marine debris.



HABITAT DAMAGE

Heavy marine debris crushes sensitive habitat, such as coral reefs and sea grass.



NON-NATIVE SPECIES

Marine debris transports alien and invasive species from one region to another.

Marine litter impact: what happens in the Mediterranean sea?



7 plastic items in the stomach



145 plastic items in the stomach

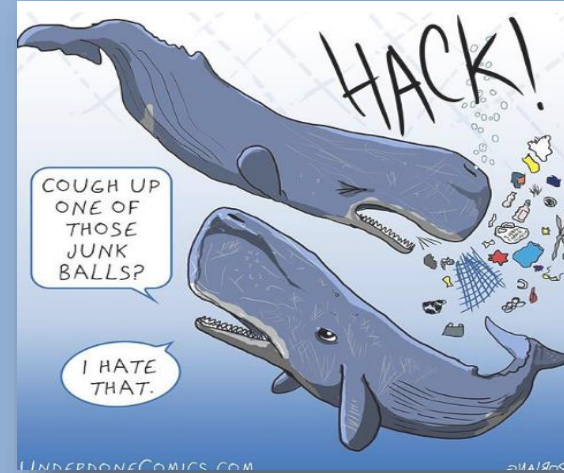


22 Kg of plastic in the stomach

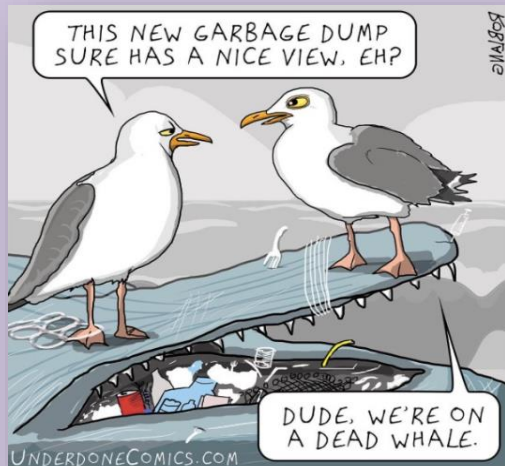
More than 130 marine species affected
by marine litter in the Mediterranean basin

Marine Litter impact: two points of view...

**Detect the impact of
marine litter
on Mediterranean species**



..and/or



**Bioindicators of impact of
marine litter on
Mediterranean sea**

Impact on whales?



Balaenoptera physalus



THE MEGAFaUNA AND MPs STORY



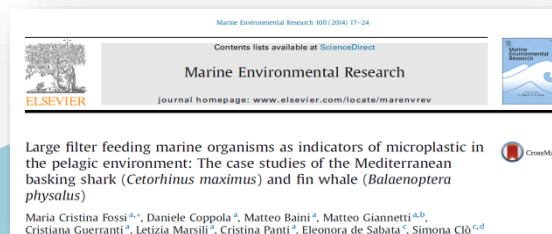
2012



2018



2014



Whale and shark species at increasing risk from microplastic pollution - study

Large filter feeders, such as baleen whales and basking sharks, could be particularly at risk from ingesting the tiny plastic particles, say scientists



2016



2017

Plastic Debris Occurrence, Convergence Areas and Fin Whales Feeding Ground in the Mediterranean Marine Protected Area Pelagos Sanctuary: A Modeling Approach

Maria Cristina Fossi¹, Teresa Romeo², Matteo Bains¹, Cristina Panti^{1*}, Letizia Marsili¹, Tommaso Campan¹, Simonepietro Canese², François Galgani³, Jean-Noël Druon⁴, Sabina Airoldi⁵, Stefano Taddei⁶, Maria Fattorini^{6,7}, Carlo Brandini^{6,7} and Chiara Lapucci^{6,7}

2017



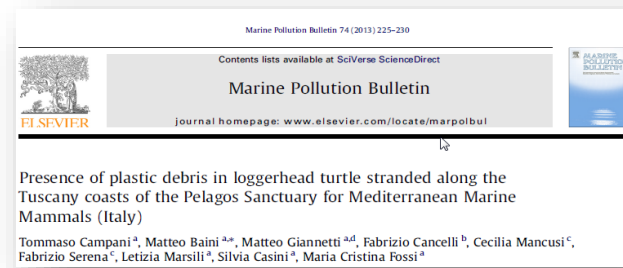
PLASTIC BUSTERS

Impact on sea turtles?



Caretta caretta

MSFD-D10 Sentinel species for marine litter in Mediterranean sea



2013



2017



Found 483 pieces of marine litter with a total mass of 62.37g



22 loggerhead turtles out of 31 animals had ingested marine debris (71%)

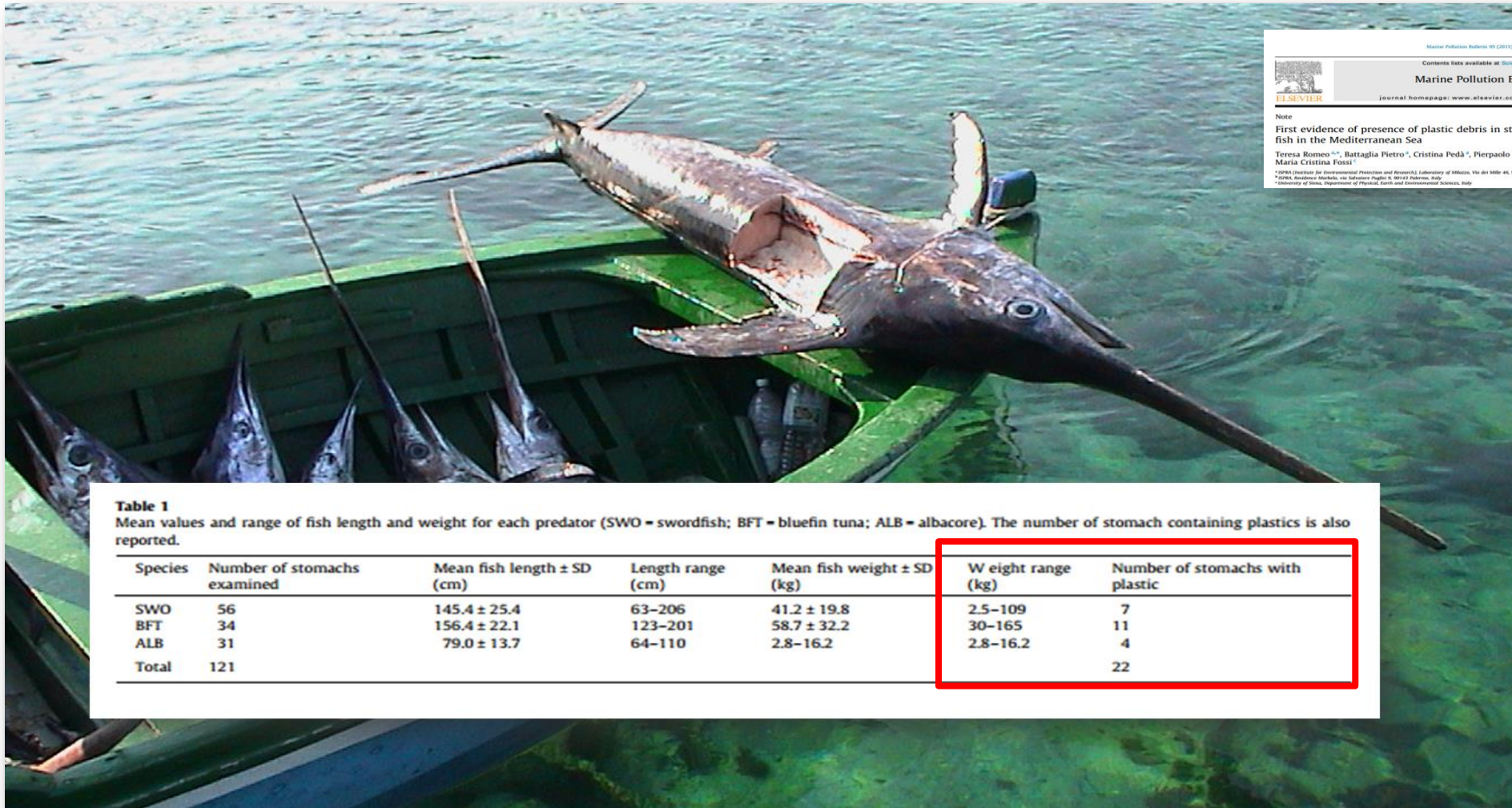
Caretta caretta									
NE	EW	LO	NT	VI	EN	CR			
NOT ENLIGHTENED	ENLIGHTENED	LAST CONCERN	WELL THREATENED	VULNERABLE	ENDANGERED	CRITICALLY ENDANGERED			





Impact on fish species?

Microplastic impact in top predator fish



2015

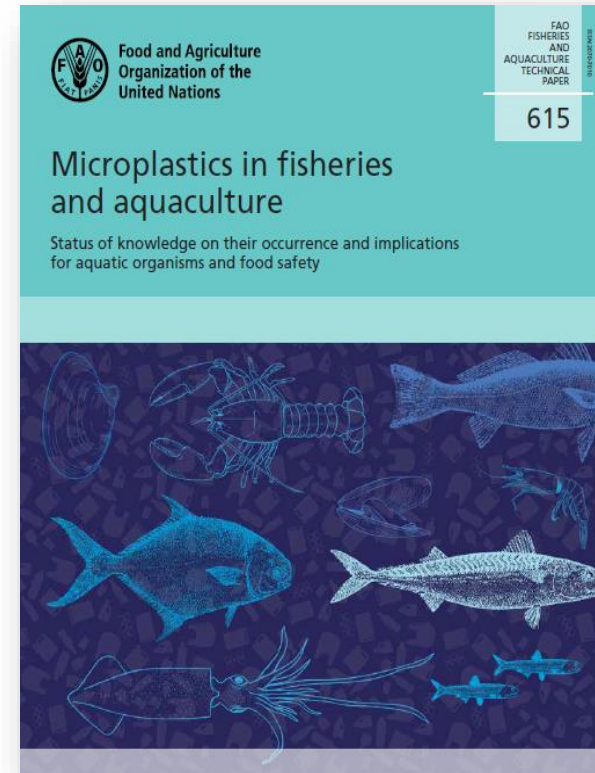
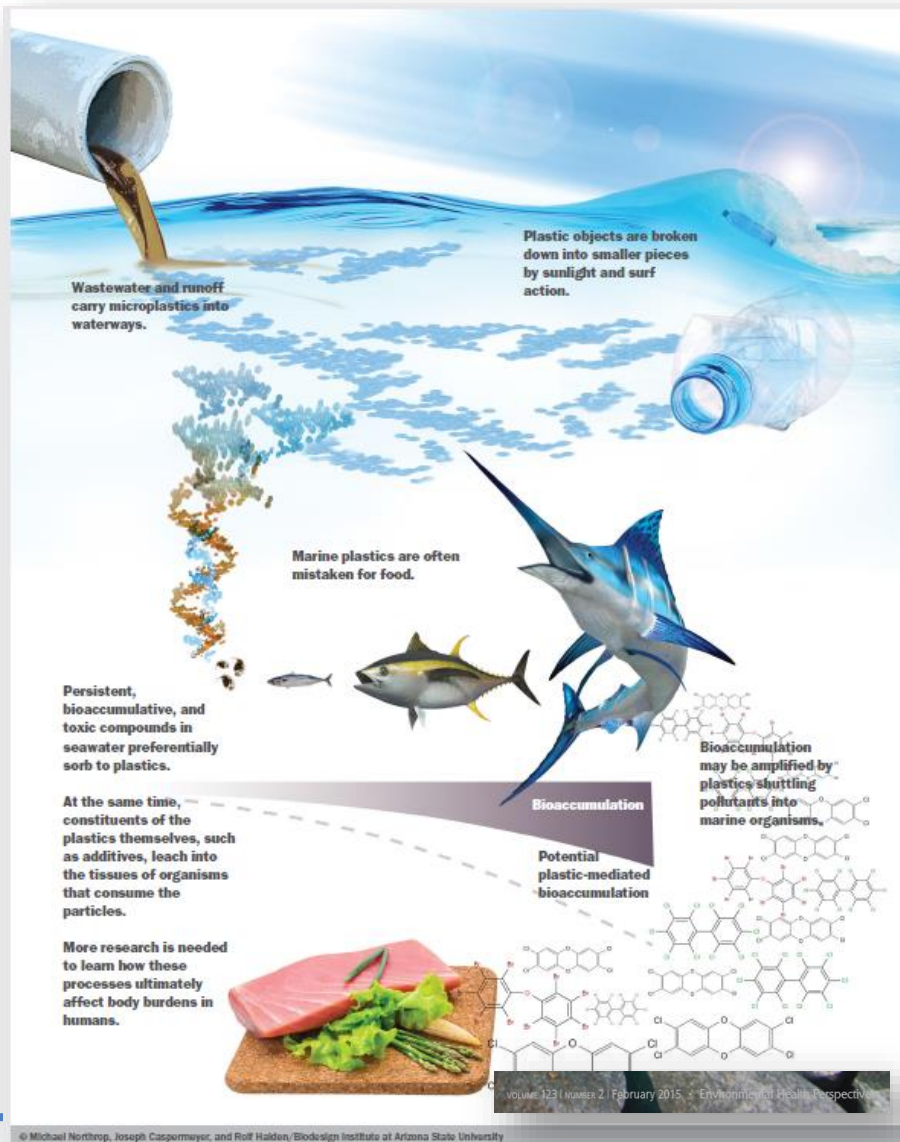
Table 1
Mean values and range of fish length and weight for each predator (SWO = swordfish; BFT = bluefin tuna; ALB = albacore). The number of stomach containing plastics is also reported.

Species	Number of stomachs examined	Mean fish length \pm SD (cm)	Length range (cm)	Mean fish weight \pm SD (kg)	Weight range (kg)	Number of stomachs with plastic
SWO	56	145.4 \pm 25.4	63–206	41.2 \pm 19.8	2.5–109	7
BFT	34	156.4 \pm 22.1	123–201	58.7 \pm 32.2	30–165	11
ALB	31	79.0 \pm 13.7	64–110	2.8–16.2	2.8–16.2	4
Total	121					22



Microplastic impact in the food chain?

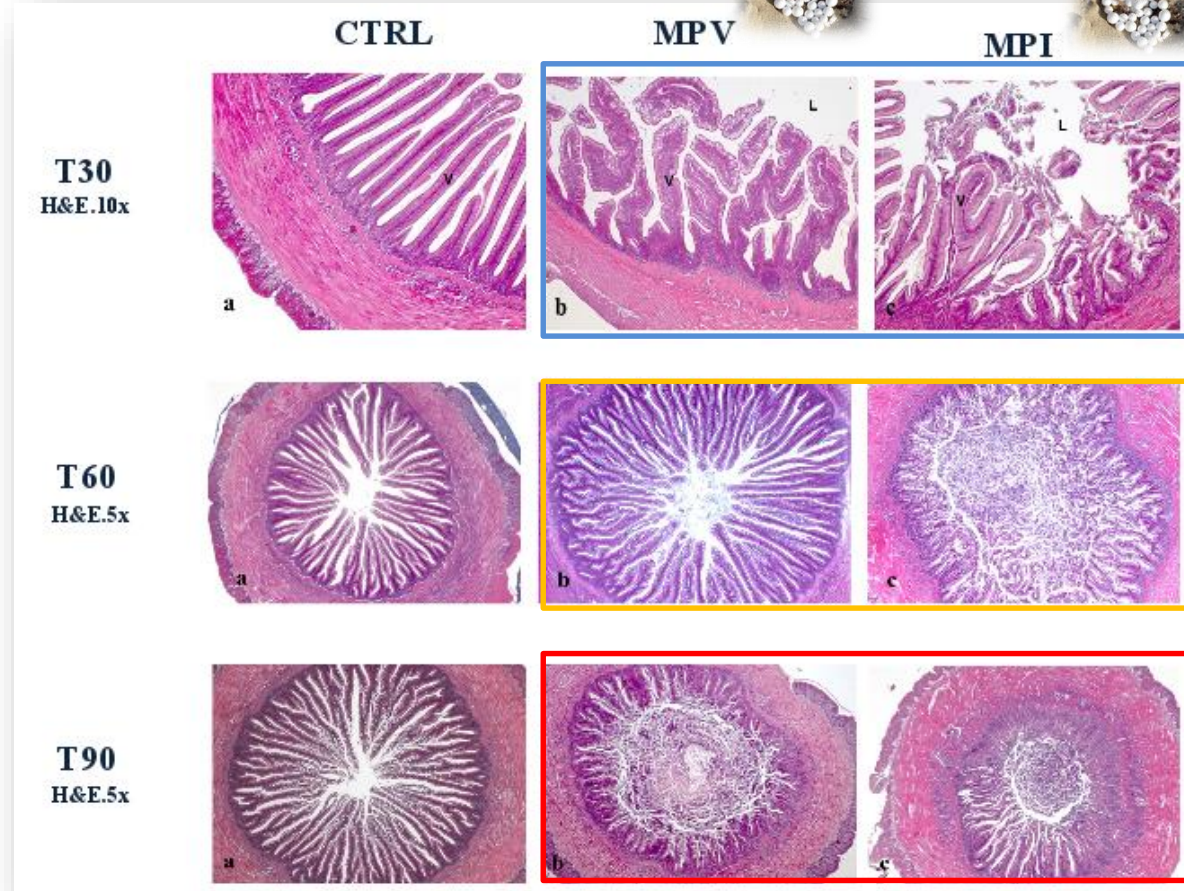
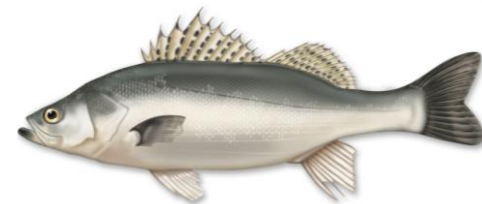
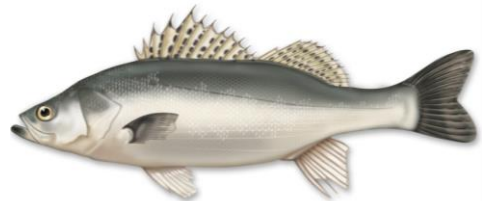
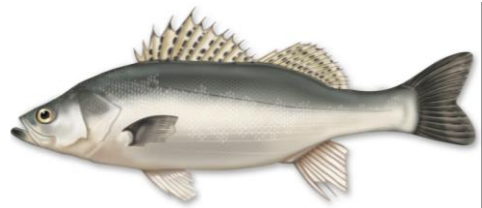
Marine Plastic and seafood safety



...Whereas the overall human health risks posed by microplastics in seafood at present appear to be low, it is important to consider the unavoidable increase of micro- and nanoplastics in the future as a result of degradation of plastics already released in the environment as well as future inputs.



Microplastic impact in edible fish species



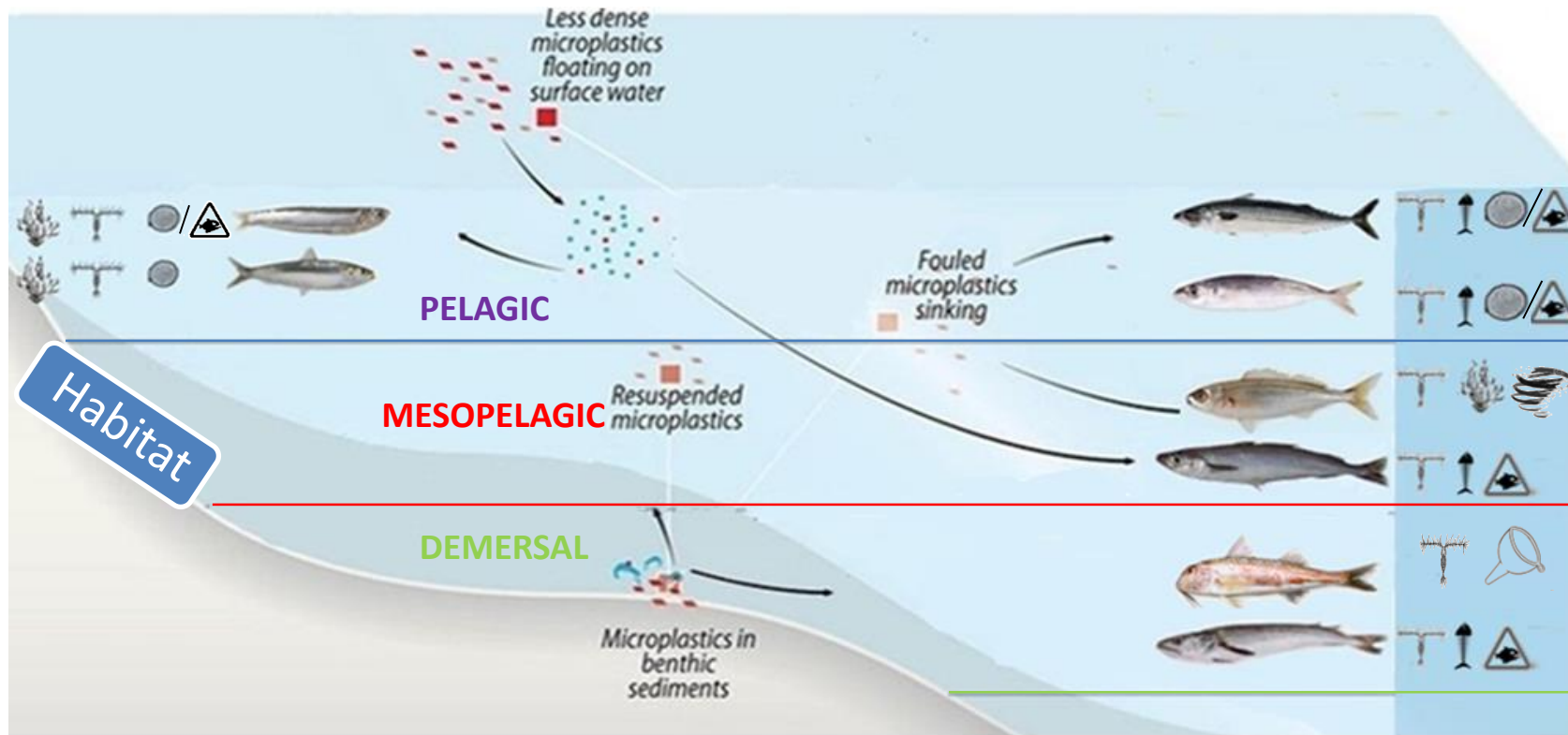
2016

L
Intestinal
alteration

M
Intestinal
alteration

H
Intestinal
alteration

MPs in edible species: species selection criteria



- ❖ European anchovy (*Engraulis encrasicolus*)
- ❖ European pilchard (*Sardina pilchardus*)
- ❖ Atlantic mackerel (*Scomber scombrus*)
- ❖ Atlantic horse mackerel (*Trachurus trachurus*)
- ❖ Bogue (*Boops boops*)
- ❖ Blue whiting (*Micromesistius poutassou*)
- ❖ Red mullet (*Mullus barbatus*)
- ❖ European hake (*Merluccius merluccius*)

Trophic level (main prey) Stergiou&Karpouzi 2002

Ichthyophagous Fitoplantophagous Zooplantophagous

Feeding habits

Selective predator

Filter-feeding "slurp" mode



Giani et al. (2018)

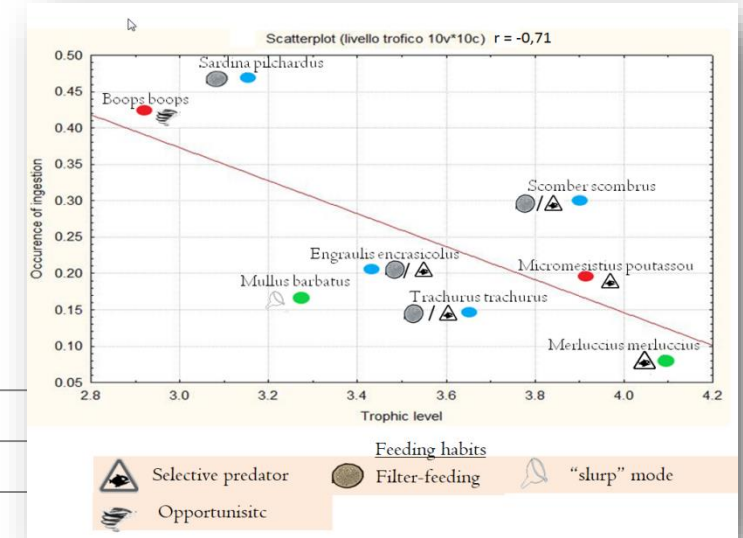
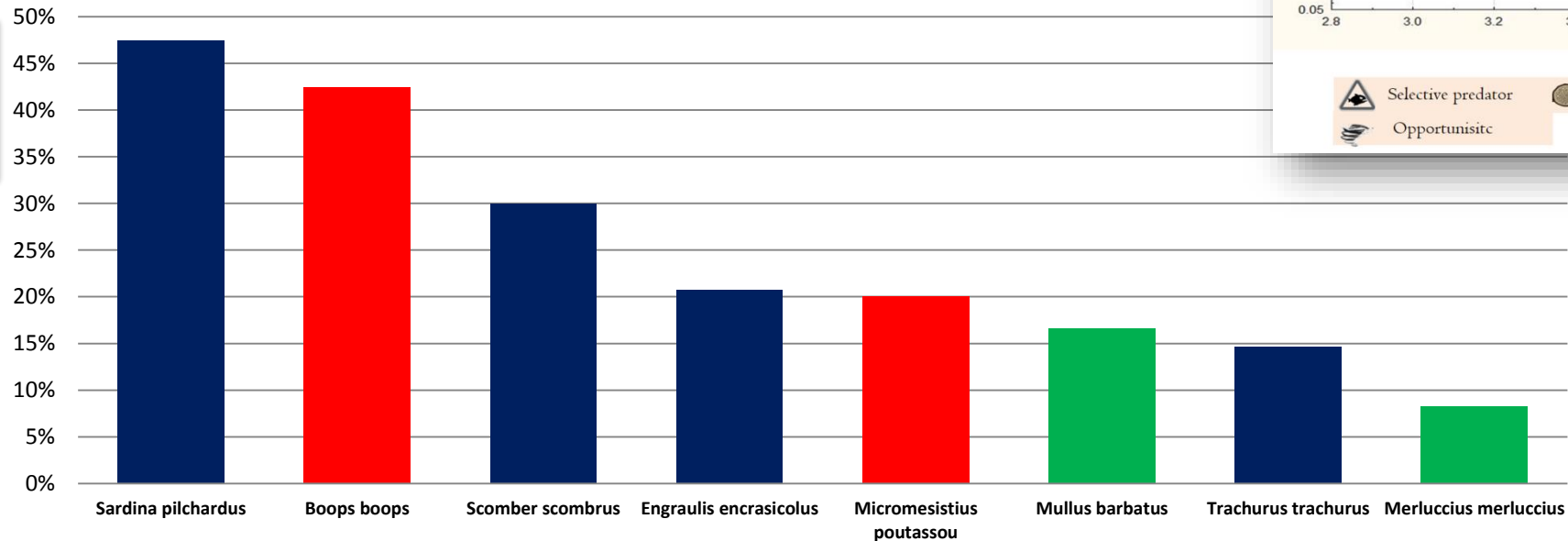


MPs in edible species: % of MPs ingestion



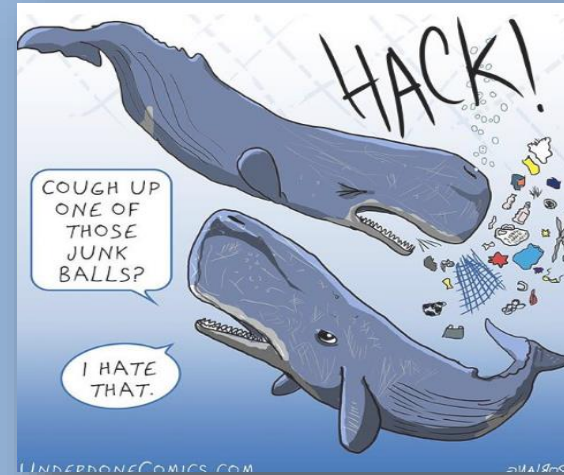
- ✓ Sample analyzed in GSA9 → 330
- ✓ Total plastic items → 118
- ✓ Number of fish with MPs → 87 (25.2%)

PELAGIC
MESOPELAGIC
DEMERSAL

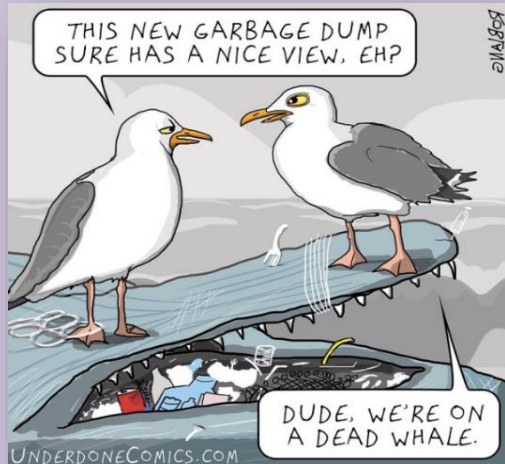


Marine Litter impact: two points of view...

**Detect the impact of
marine litter
on Mediterranean species**



..and/or



**Bioindicators of impact of
marine litter on
Mediterranean sea**

Plastic Busters initiative

Plastic Busters on basin scale



A crucial aspect of the marine litter issue, underlined by the **Barcelona Convention within the Regional Plan for Marine Litter** (Istanbul 2013) is that: "Marine pollution knows no border, pollution in one country affects all other 21 countries, hence there is a need for a regional approach".

Plastic Busters is the first project at basin scale that binds the Southern and Northern Mediterranean countries on the issue of Marine Litter under the umbrella of UNEP/MAP and UfM, with 10 countries already involved in the project and 12 countries endorsing the project.



Diagnosis of the problem to identified specific solutions

- Impact on Biodiversity?
- Impact on Fisheries?
- Identification of Hot spot areas?
- Impact on Human?

Project coordinator
Maria Cristina Fossi

Biomarker Laboratory, University of Siena, Italy



SDSN-MED Flagship project



2013

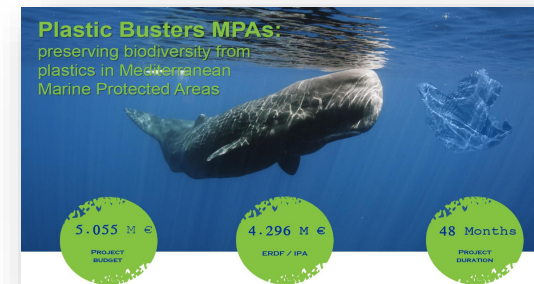
UfM Labelling



Union for the Mediterranean
Union pour la Méditerranée
الإتحاد من أجل المتوسط

2016

MED-Interreg



2018

ENI -CBC



2019 - 21

Plastic Busters MPAs:

preserving biodiversity from
plastics in Mediterranean
Marine Protected Areas



MINISTERO DELL'AMBIENTE
E DELLA TUTELA DEL TERRITORIO E DEL MARE

5.055 M €

PROJECT
BUDGET

4.296 M €

ERDF / IPA

48 Months

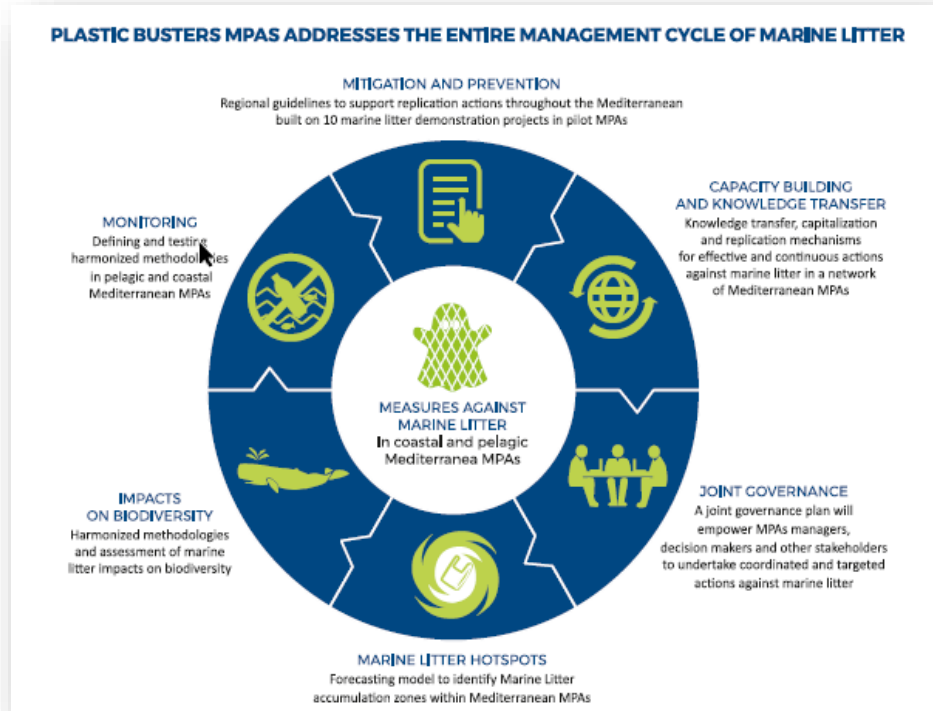
PROJECT
DURATION

Interreg
Mediterranean
PLASTIC BUSTERS
MPAs

Project co-financed by the European
Regional Development Fund

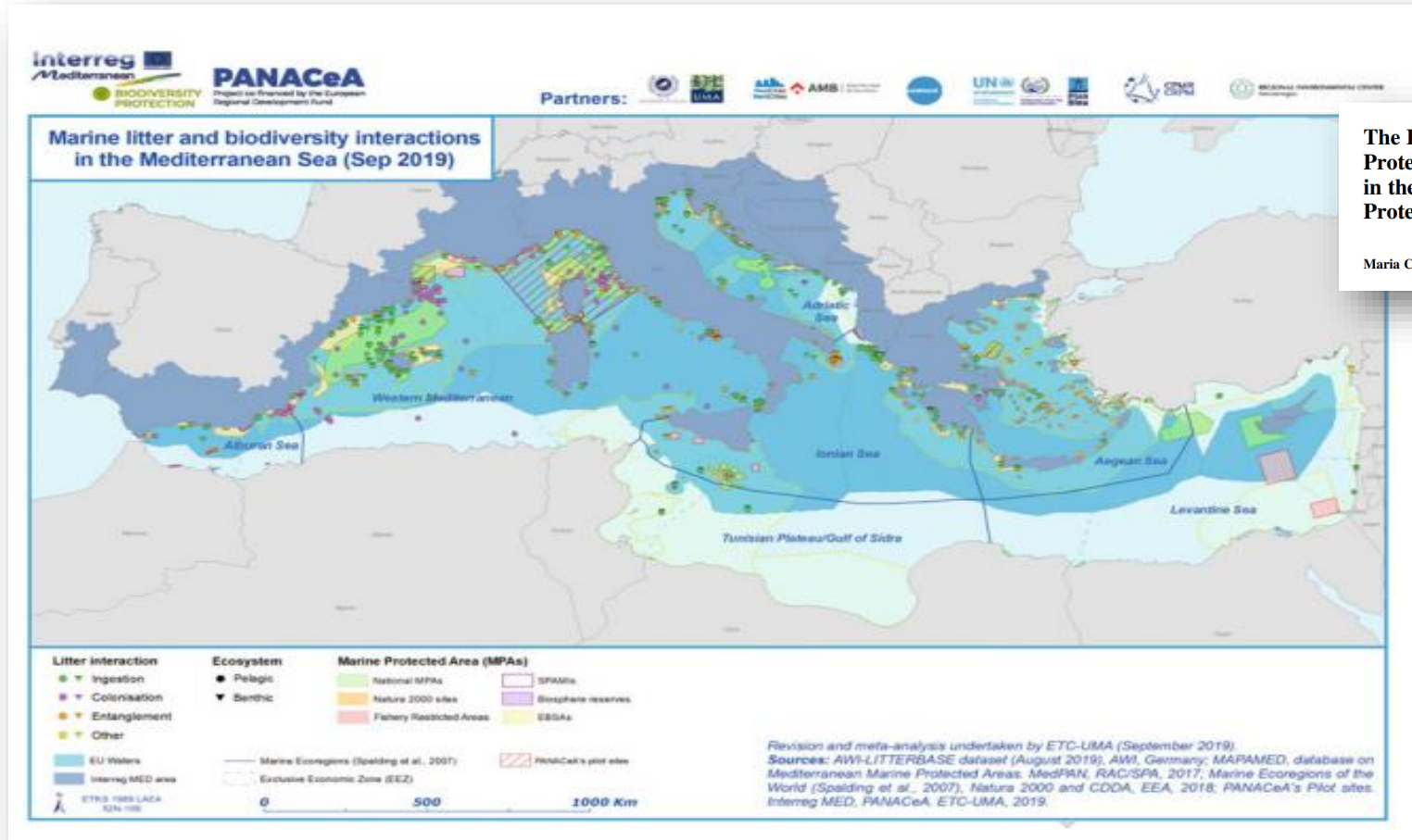
Plastic Busters MPAs general objectives

Whole Management Cycle Of Marine Litter



- **PlasticBusters MPAs**, is a 4-year-long project Interreg Mediterranean funded project aiming to contribute to **maintaining biodiversity and preserving natural ecosystems** in pelagic and coastal **marine protected areas (MPAs)**, by defining and implementing a **harmonized approach against marine litter**.
- The project entails actions that address the **WHOLE MANAGEMENT CYCLE OF MARINE LITTER**, from **monitoring and assessment** to **prevention and mitigation**, as well as actions to **strengthen networking** between and among pelagic and coastal MPAs located in Italy, France, Spain, Croatia, Albania and Greece.
- The project will support the **implementation** of the **MSFD** and the **Barcelona Convention Regional Plan on Marine Litter Management** in the Med.

The Impact of Marine Litter in Marine Protected Areas (MPAs) in the Mediterranean Sea



The Impact of Marine Litter in Marine Protected Areas (MPAs) in the Mediterranean Sea: How Can We Protect MPAs?

Maria Cristina Fossi and Cristina Panti



In the whole Mediterranean basis 1231 MPAs and OECMs (Other Effective area- based Conservation Measures) cover 179,798 km for a total surface of 7.14% under a legal designation. Many of these areas are heavily subjected to marine litter pressure.

Plastic Busters MPAs: *Harmonized Diagnosis in Biota*

4.1 – Coordinating WP 4

4.2

Piloting harmonized ML monitoring in Med MPAs to assess ML (macro- and micro-plastics) in the coastal and pelagic environment

4.3

Piloting harmonized ML monitoring approaches in Med MPAs and hotspots to establish the impacts on biota, including endangered species and fishery resources

4.4

Testing the ML forecasting model

4.5

Preparation of the demo projects

4.6

Piloting ML prevention and mitigation measures



i) Plastic detection



Analysis of the ingested marine litter/microplastics:

- Occurrence (%)
- Abundance (n°)
- Weight (g)
- Polymer analysis

ii) Plastic tracers detection



Analysis of plastic additives:

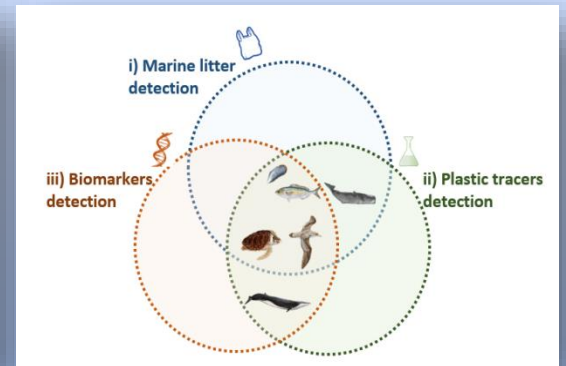
- Phthalates
- PBDEs
- Bisphenol A
- Analysis of PBT compounds:
- PCBs
- DDTs
- PAHs
- Mercury

iii) Biomarkers detection



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



MARINE LITTER IMPACTS ON BIOTA

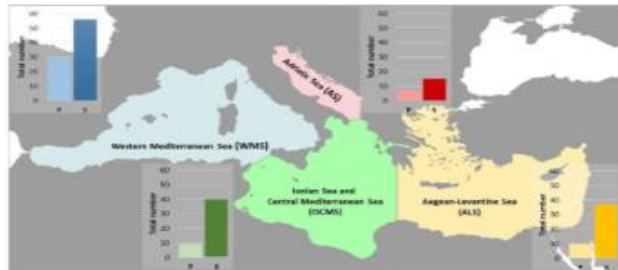
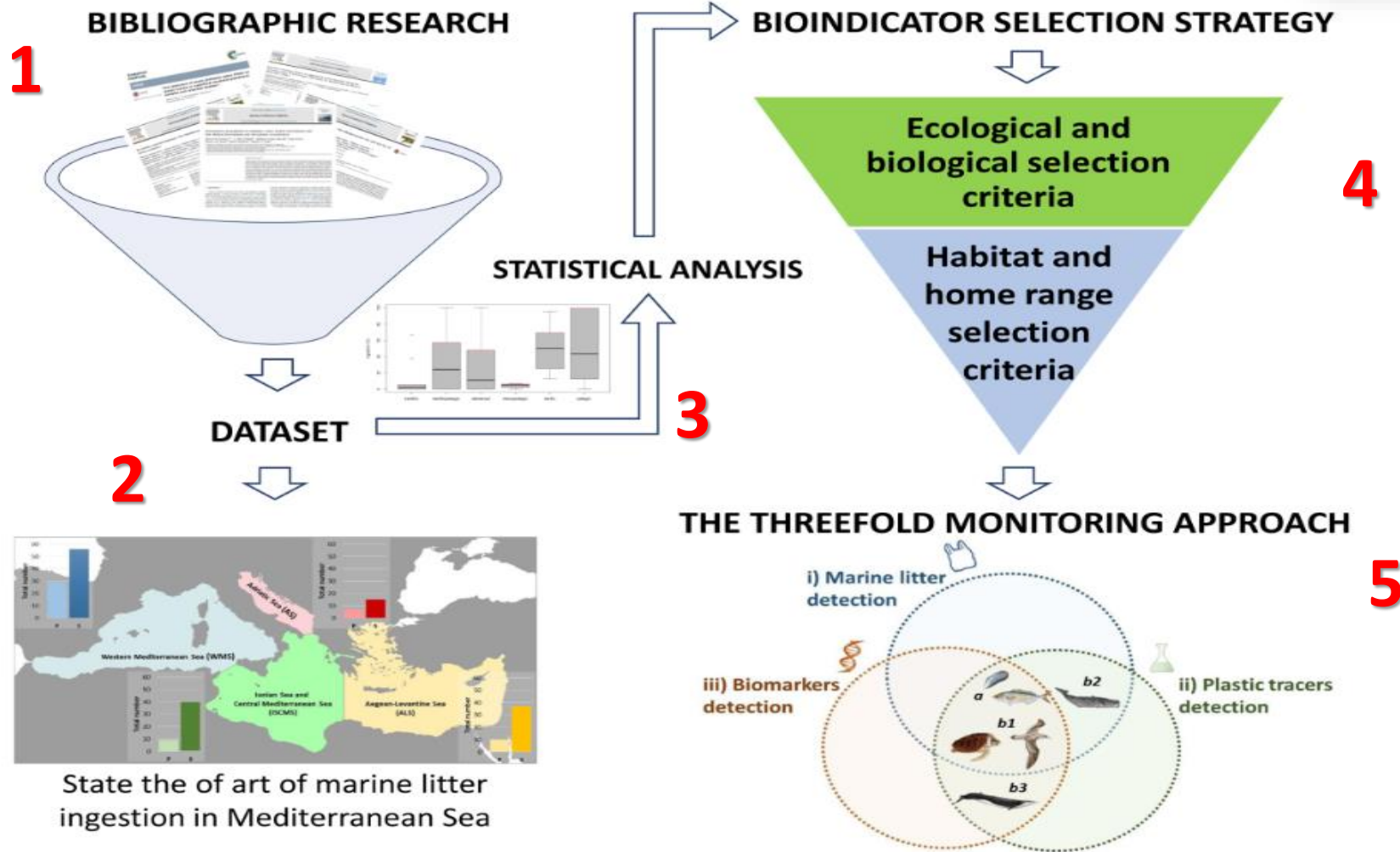
Interreg
Mediterranean



PLASTIC BUSTERS
MPAs

Project co-financed by the European
Regional Development Fund

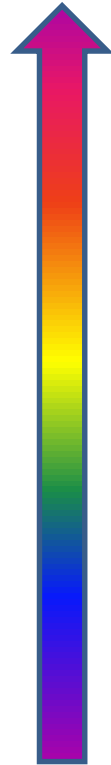
Identification of marine litter bioindicators



State the of art of marine litter ingestion in Mediterranean Sea



Percentage and ranking of marine litter ingestion in the Med species calculated on the data present in literature



% of ingestion

Species	% of ingestion
<i>Balaenoptera physalus</i> (Linnaeus, 1758)	100.00%
<i>Calonectris diomedea</i> (Scopoli, 1769)	95.92%
<i>Siganus luridus</i> (Rüppell, 1829)	86.67%
<i>Cetorhinus maximus</i> (Gunnerus, 1765)	83.33%
<i>Pagrus pagrus</i> (Linnaeus, 1758)	77.78%
<i>Physeter macrocephalus</i> Linnaeus, 1758	77.78%
<i>Argyrosomus regius</i> (Asso, 1801)	74.51%
<i>Puffinus yelkouan</i> (Acerbi, 1827)	70.97%
<i>Mullus surmuletus</i> Linnaeus, 1758	70.59%
<i>Puffinus mauritanicus</i> Lowe, 1921	69.57%
<i>Diplodus annularis</i> (Linnaeus, 1758)	68.75%
<i>Boops boops</i> (Linnaeus, 1758)	67.71%
<i>Pagellus acarne</i> (Risso, 1827)	67.31%
<i>Serranus cabrilla</i> (Linnaeus, 1758)	66.67%
<i>Pelates quadrilineatus</i> (Bloch, 1790)	65.19%
<i>Trachurus mediterraneus</i> (Steindachner, 1868)	62.13%
<i>Saurida undosquamis</i> (Richardson, 1848)	55.56%
<i>Pomadasys incisus</i> (Bowdich, 1825)	55.17%
<i>Nemipterus randalli</i> Russell, 1986	54.81%
<i>Dermochelys coriacea</i> (Vandelli, 1761)	50.00%
<i>Scomber japonicus</i> Houttuyn, 1782	47.73%
<i>Upeneus moluccensis</i> (Bleeker, 1855)	44.44%
<i>Sparus aurata</i> Linnaeus, 1758	43.64%
<i>Liza aurata</i> (Risso, 1810)	43.59%
<i>Upeneus pori</i> Ben-Tuvia & Golani, 1989	41.03%

Species	% of ingestion
<i>Trigla lucerna</i> Linnaeus, 1758	37.50%
<i>Mullus barbatus</i> Linnaeus, 1758	36.03%
<i>Lithognathus mormyrus</i> (Linnaeus, 1758)	34.78%
<i>Larus michahellis</i> J.F. Naumann, 1840	33.33%
<i>Trachyrincus scabrus</i> (Rafinesque, 1810)	33.33%
<i>Polyprion americanus</i> (Bloch & Schneider, 1801)	32.35%
<i>Caretta caretta</i> (Linnaeus, 1758)	31.10%
<i>Dentex gibbosus</i> (Rafinesque, 1810)	28.57%
<i>Schedophilus ovalis</i> (Cuvier, 1833)	28.57%
<i>Trachinotus ovatus</i> (Linnaeus, 1758)	24.35%
<i>Trachurus trachurus</i> (Linnaeus, 1758)	24.00%
<i>Pagellus erythrinus</i> (Linnaeus, 1758)	22.39%
<i>Squalus acanthias</i> Linnaeus, 1758	21.05%
<i>Sardina pilchardus</i> (Walbaum, 1792)	20.41%
<i>Naucratis dactor</i> (Linnaeus, 1758)	18.00%
<i>Nettastoma melanurum</i> Rafinesque, 1810	16.67%
<i>Coryphaena hippurus</i> Linnaeus, 1758	14.34%
<i>Balistes caprisicus</i> Gmelin, 1789	14.00%
<i>Larus audouinii</i> Payraudeau, 1826	13.33%
<i>Thunnus alalunga</i> (Bonnaterre, 1788)	12.90%
<i>Thunnus thynnus</i> (Linnaeus, 1758)	12.67%
<i>Morus bassanus</i> (Linnaeus, 1758)	12.50%
<i>Xiphias gladius</i> Linnaeus, 1758	12.28%
<i>Stenella coeruleoalba</i> (Meyen, 1833)	11.67%
<i>Tursiops truncatus</i> (Montagu, 1821)	11.17%
<i>Cataetys laticeps</i> Koefoed, 1927	10.00%

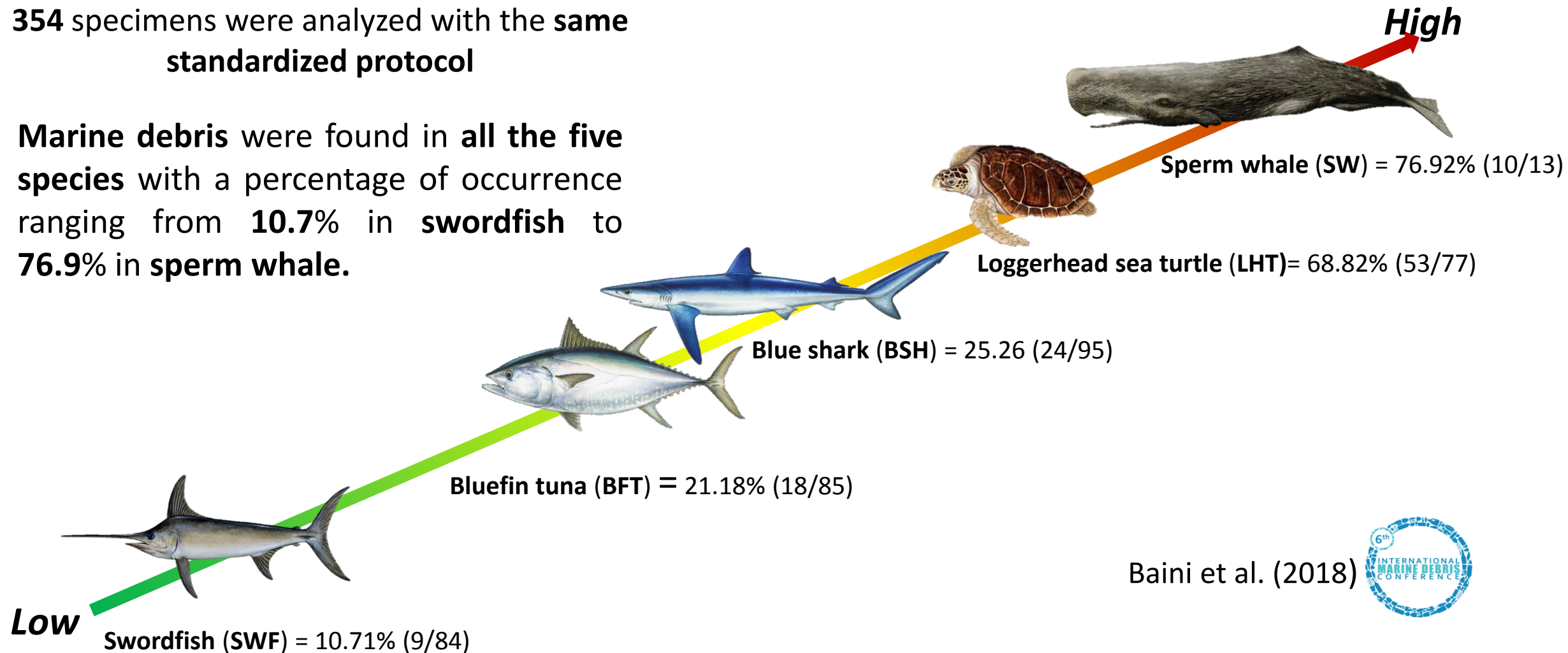
Species	% of ingestion
<i>Mora moro</i> (Risso, 1810)	9.09%
<i>Etmopterus spinax</i> (Linnaeus, 1758)	8.82%
<i>Merluccius merluccius</i> (Linnaeus, 1758)	7.69%
<i>Galeus melastomus</i> Rafinesque, 1810	7.11%
<i>Hygophum benoiti</i> (Cocco, 1838)	6.85%
<i>Electrona risso</i> (Cocco, 1829)	6.10%
<i>Myctophum punctatum</i> Rafinesque, 1810	4.23%
<i>Centroscymsus coelolepis</i> Barbosa 1864	2.99%
<i>Solea solea</i> (Linnaeus, 1758)	2.27%
<i>Seriola dumerili</i> (Risso, 1810)	2.00%
<i>Citharus linguatula</i> (Linnaeus, 1758)	1.92%
<i>Pagellus bogaraveo</i> (Brünnich, 1768)	1.67%
<i>Squalus blainville</i> (Risso, 1827)	1.33%
<i>Trachurus picturatus</i> (Bowdich, 1825)	1.00%
<i>Helicolenus dactylopterus</i> (Delaroche, 1809)	0.42%
<i>Diaphus metopoclampus</i> (Cocco, 1829)	0.34%
<i>Alepocephalus rostratus</i> Risso, 1820	0.00%
<i>Brama brama</i> (Bonnaterre, 1788)	0.00%
<i>Conger conger</i> (Linnaeus, 1758)	0.00%
<i>Molva macrophthalma</i> (Rafinesque, 1810)	0.00%
<i>Phycis blennoides</i> (Brünnich, 1768)	0.00%
<i>Raja oxyrinchus</i> Linnaeus, 1758	0.00%



Percentage and ranking of marine litter ingestion in Med species calculated on the field data

354 specimens were analyzed with the same standardized protocol

Marine debris were found in all the five species with a percentage of occurrence ranging from 10.7% in swordfish to 76.9% in sperm whale.



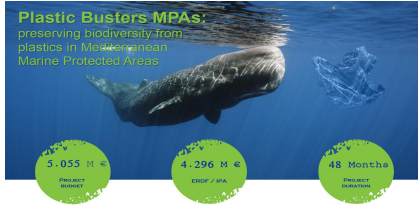
Baini et al. (2018)



Bioindicator selection in relation to habitat and home range



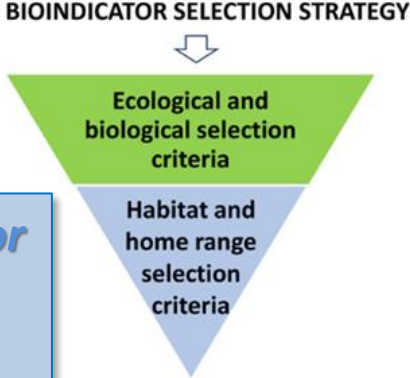
Basin Scale



	SEA SURFACE	COASTAL WATERS	OPEN WATERS	SEAFLOOR	COAST LINE AND BEACH SEDIMENT
BASIN SCALE (Mediterranean Sea)	<i>Calonectris diomedea</i> , <i>Puffinus yelkouan</i>	<i>Calonectris diomedea</i> , <i>Puffinus yelkouan</i>	<i>Balaenoptera physalus</i> ; <i>Cetorhinus maximus</i> <i>Xiphias gladius</i> ; <i>Thunnus thynnus</i> <i>Xiphias gladius</i> ; <i>Thunnus thynnus</i> <i>Caretta caretta</i> <i>Physeter macrocephalus</i>		
MEDIUM-SCALE (Mediterranean UN Environment/MAP sub-regions)			<i>Thunnus alalunga</i> <i>Trachurus trachurus</i> <i>Caretta caretta</i> <i>Thunnus alalunga</i>		
SMALL-SCALE (FAO GSA)		<i>Boops boops</i> <i>Trachinotus ovatus</i>	<i>Maurolicus muelleri</i> <i>Engraulis encrasicolus</i> <i>Sardina pilchardus</i> <i>Myctophids</i>	<i>Mullus barbatus</i> <i>Merluccius merluccius</i> <i>Merluccius merluccius</i> <i>Solea spp.</i> <i>Galeus melastomus</i> <i>Scyliorhinus canicula</i>	
LOCAL SCALE				Holoturians	<i>Mytilus galloprovincialis</i> <i>Arenicola marina</i> Decapods (e.g. <i>Carcinus sp.</i>)

BLUE: bioindicator for macrolitter

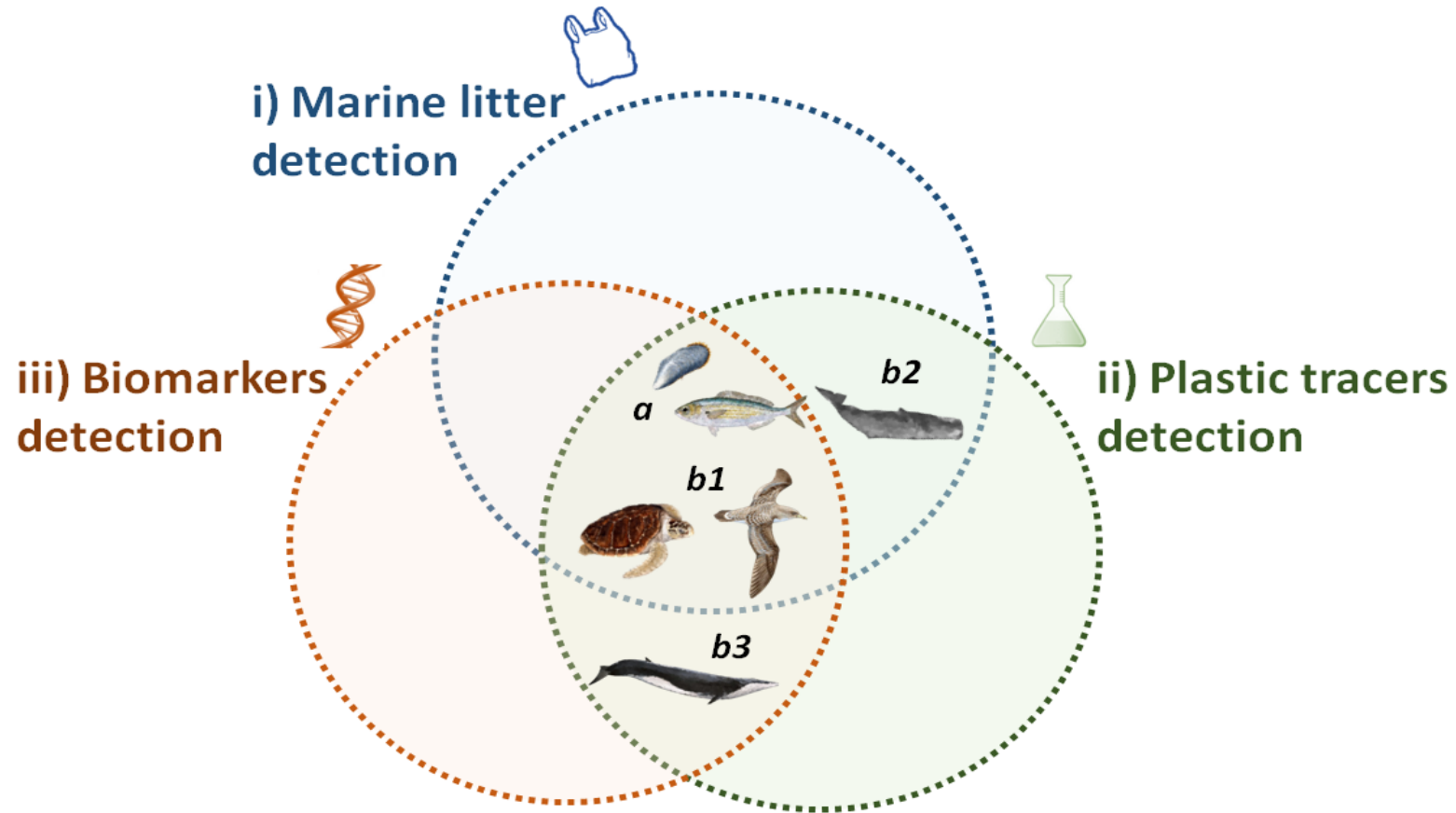
RED: bioindicator for microplastics



Local Scale



THE THREEFOLD MONITORING APPROACH



COMMISSION DECISION (EU) 2017/848
of 17 May 2017

laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing Decision 2010/477/EU

D10C3 -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



Descriptor 10

Properties and quantities of marine litter do not cause harm to the coastal and marine environment

**DOES ML ADVERSELY
AFFECT THE HEALTH OF
THE SPECIES ?**



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



THE THREEFOLD MONITORING APPROACH

Environmentally relevant species



Protected species



Plastic detections

- Analysis of the gastro 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
- Effects at cellular level:
 - Alteration of cell functions
- Effects at tissue level:
 - Histological and histopathological alterations

The simultaneous investigation in bioindicator species of:

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

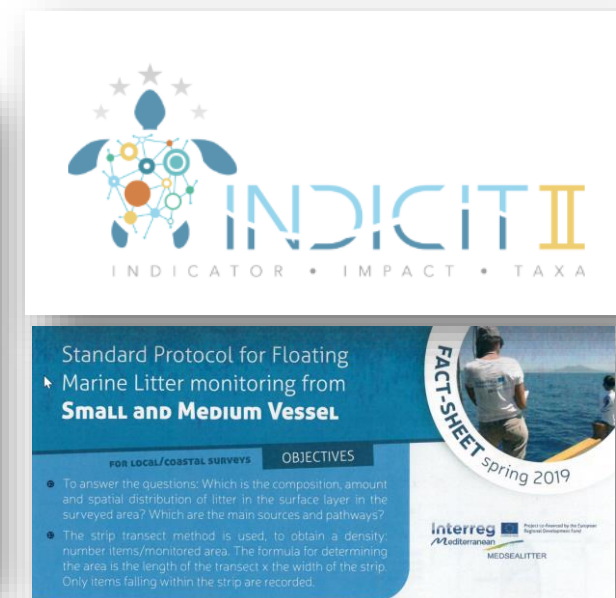
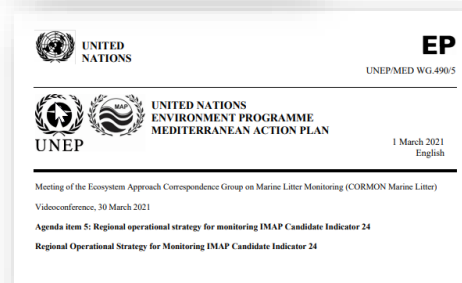
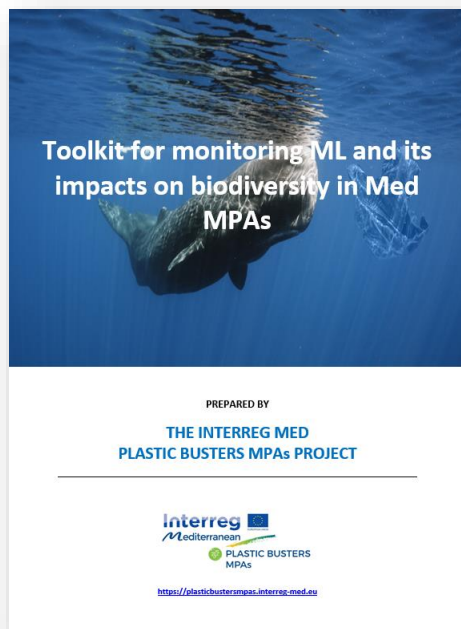
B) the analysis of **plastic additives** and PBT compounds used as plastic tracers;

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.



Plastic Busters MPAs: Toolkit – Supporting documents



...and more



Commercial fish species

Laboratory analysis: *Microplastics ingestion*

- ❖ Use of glass materials washed with micro-filtred water (0,45 µm)

- ❖ One procedural blanks every two samples

Digestion and
filtration

- Digestion method: KOH 10% (1:5 w/v) (Rochman *et al* 2015, modified)
- Filtration with 1.6µ glass-fibre filter

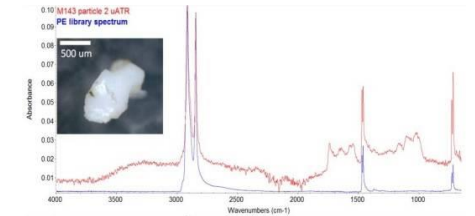
Characterization
of plastics

- Shape, color, size of plastic particles
- Polymer identification (FTIR) (10% total items)



i) Plastic detection

- Analysis of the ingested marine litter/microplastics:
 - Occurrence (%)
 - Abundance (n°)
 - Weight (g)
 - Polymer analysis



Three-Fold Monitoring Approach in Striped Red Mullet



Cabrera - IEO

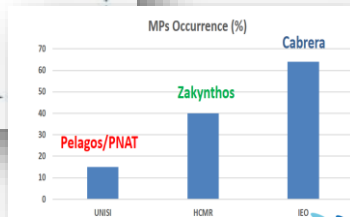
Zakynthos - HCMR

Pelagos/PNAT - UNISI

i) Plastic detection

- Analysis of the ingested marine litter/microplastics:

- Occurrence (%)
- Abundance (n°)
- Weight (g)
- Polymer analysis



ii) Plastic tracers detection

- Analysis of plastic additives:

- Phthalates
- PBDEs
- Bisphenol A

- Analysis of PBT compounds:

- PCBs
- DDTs
- PAHs
- Mercury



iii) Biomarkers detection

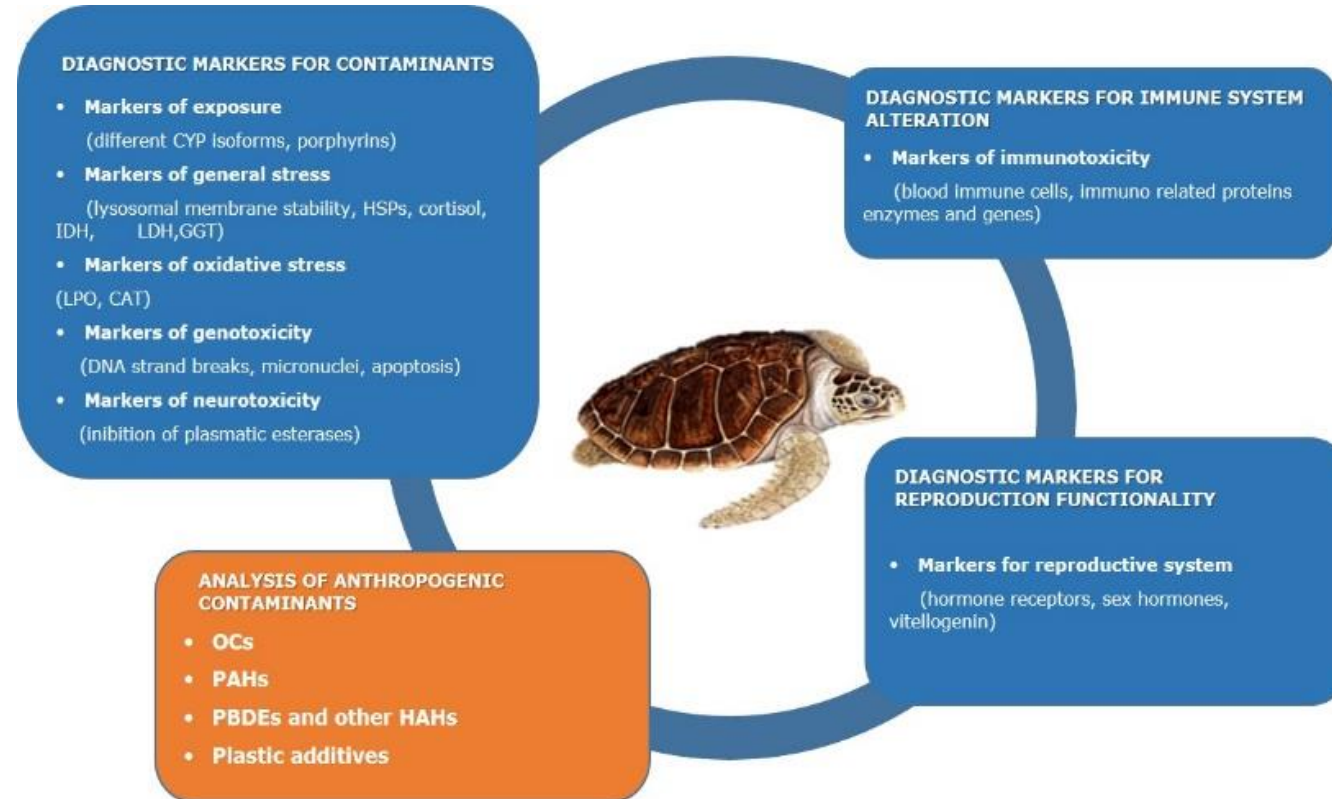
- Effects at molecular level:
 - Measure of DNA damage
 - Alterations of gene expression
 - Alteration of proteins
- Effects at cellular level:
 - Alteration of cell functions
- Effects at tissue level:
 - Histological and histopathological alterations





Monitoring Endangered Hospitalized Sea Turtles

Monitoring MACRO and MICROLITTER in BIOTA: ENDANGERED SPECIES



12. Methodology for monitoring MACROLITTER and MICROLITTER ingestion in biota: sea turtles

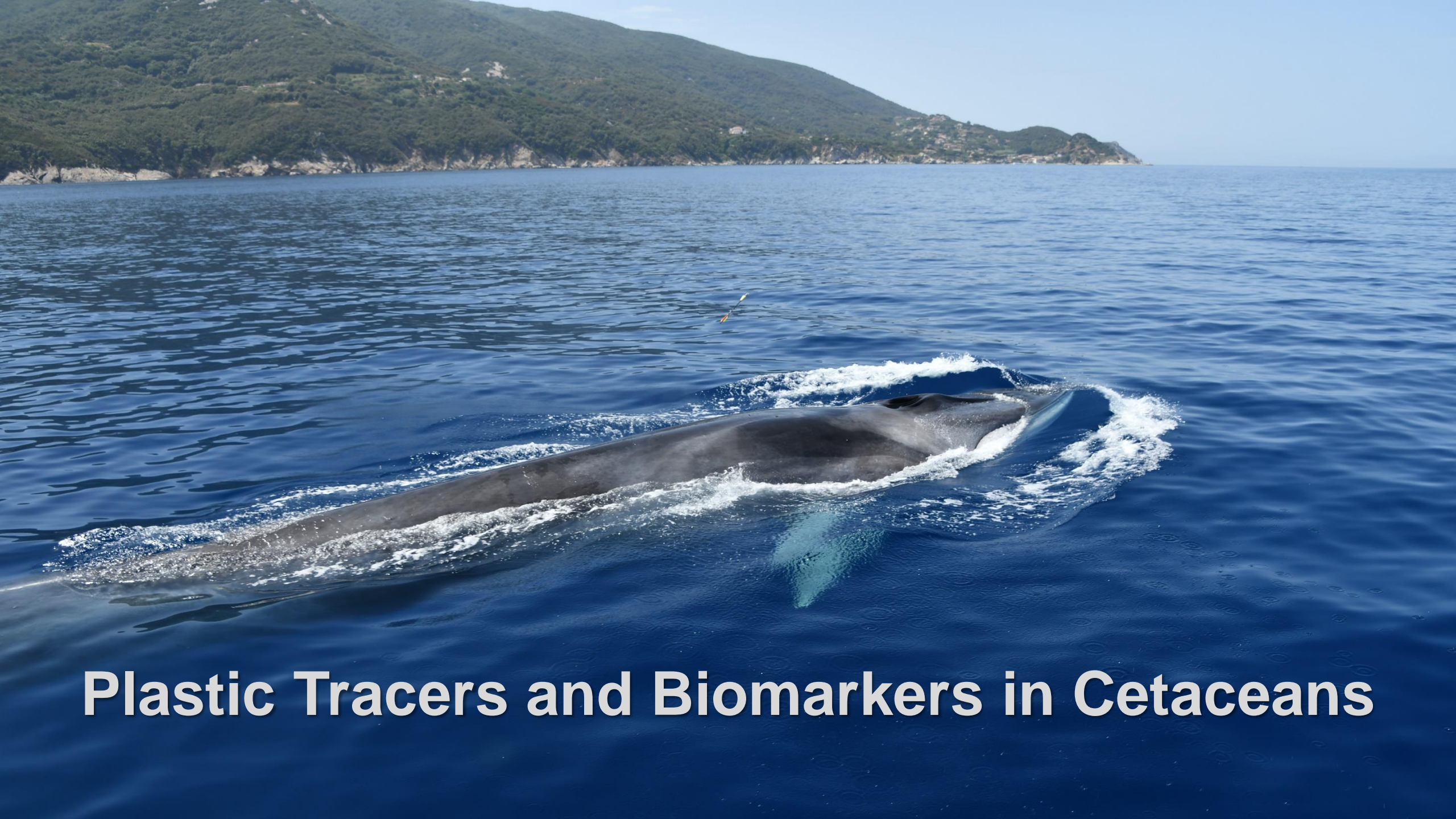
This document describes the methodological approach for monitoring macrolitter and microlitter ingestion in sea turtles. It has been compiled building on the methodologies developed within the EU-funded INDICT I project and the marine litter monitoring guidance documents of the MSFD TS10 and UNEP/MAP and it has been adapted within the framework of the Interreg Med PlasticBusters MPAs project to address the recent advances in the field.

PREPARED BY

THE INTERREG MED
PLASTIC BUSTERS MPAs PROJECT



Project co-financed by the European
Regional Development Fund



Plastic Tracers and Biomarkers in Cetaceans

Monitoring marine litter impacts in Cetacean Species



i) Plastic detection



Analysis of the ingested marine litter/microplastics:

- Occurrence (%)
- Abundance (n°)
- Weight (g)
- Polymer analysis

ii) Plastic tracers detection



Analysis of plastic additives:

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

iii) Biomarkers detection

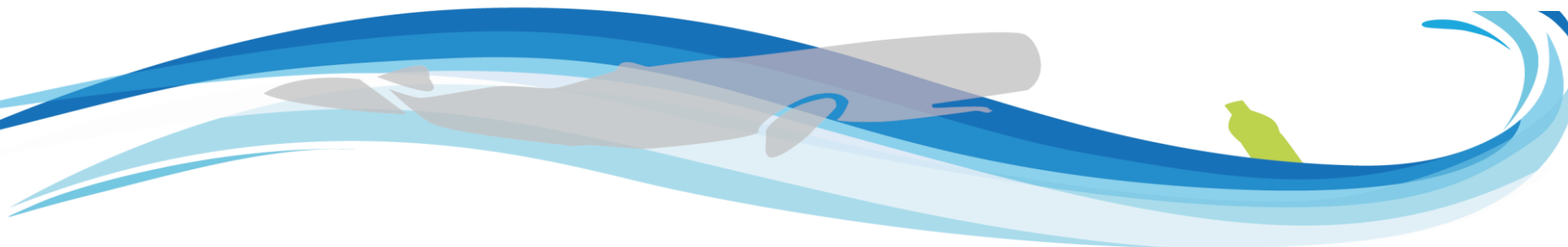


Effects at molecular level:

- 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 Marine Litter Impacts in Cetaceans : *Stranded Organisms*

i) Plastic detection



- Analysis of the ingested marine litter/microplastics:

- Occurrence (%)
- Abundance (n°)
- Weight (g)
- Polymer analysis

ii) Plastic tracers detection



- Analysis of plastic additives:

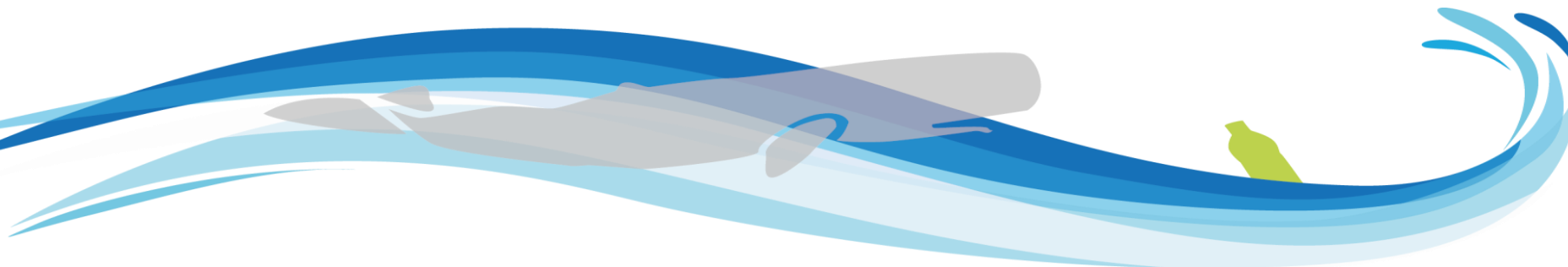
- Phthalates
- PBDEs
- Bisphenol A
- Analysis of PBT compounds:
 - PCBs
 - DDTs
 - PAHs
 - Mercury



Ziphius cavirostris



A new prototype to isolate macro and microplastics
in the gastrointestinal tract of stranded cetaceans
UNIVERSITY of PADOVA, IZS



Monitoring marine litter impacts in Cetacean Species:

Skin Biopsy in Free-ranging Organisms

ii) Plastic tracers detection



- Analysis of plastic additives:

- Phthalates
- PBDEs
- Bisphenol A

- Analysis of PBT compounds:

- PCBs
- DDTs
- PAHs
- Mercury

iii) Biomarkers detection



- Effects at molecular level:

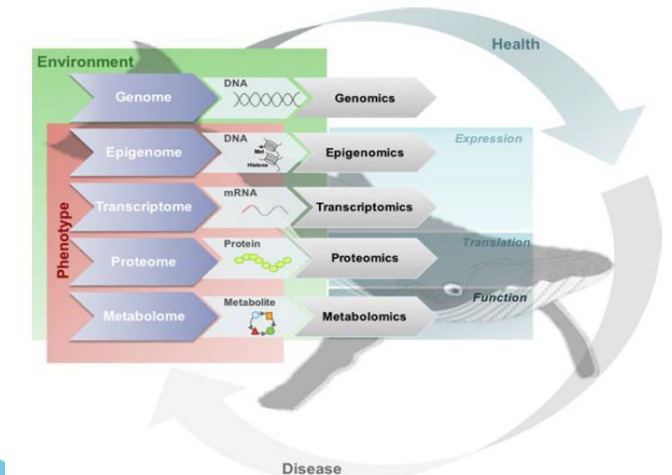
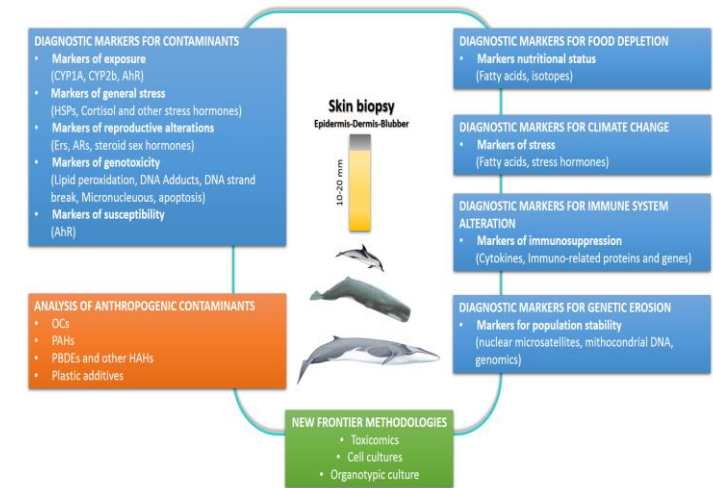
- Measure of DNA damage
- Alterations of gene expression
- Alteration of proteins

- Effects at cellular level:

- Alteration of cell functions

- Effects at tissue level:

- Histological and histopathological alterations





Thank you !



Project co-financed by the European Regional Development Fund



Thank you for your attention!