



International Workshop

“MONITORING MARINE LITTER IN ENVIRONMENT AND BIODIVERSITY”

COMMON and Plastic Busters CAP projects - Manfredonia (Italy), 13th - 14th July 2022

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

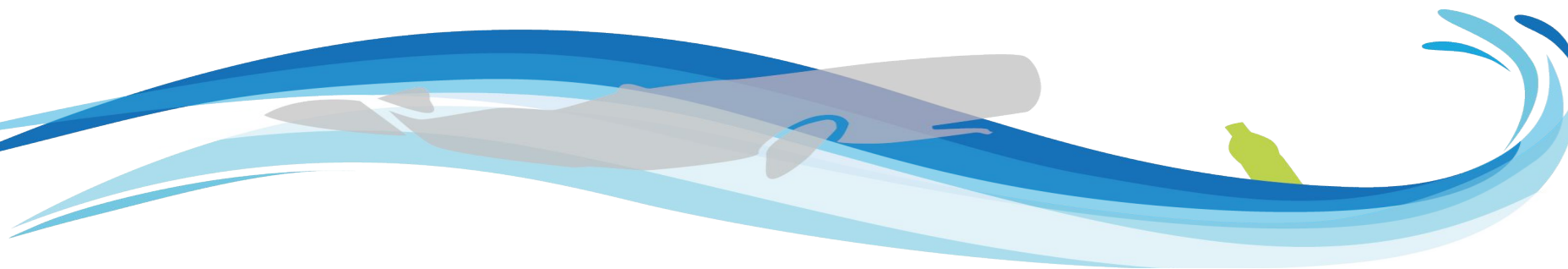
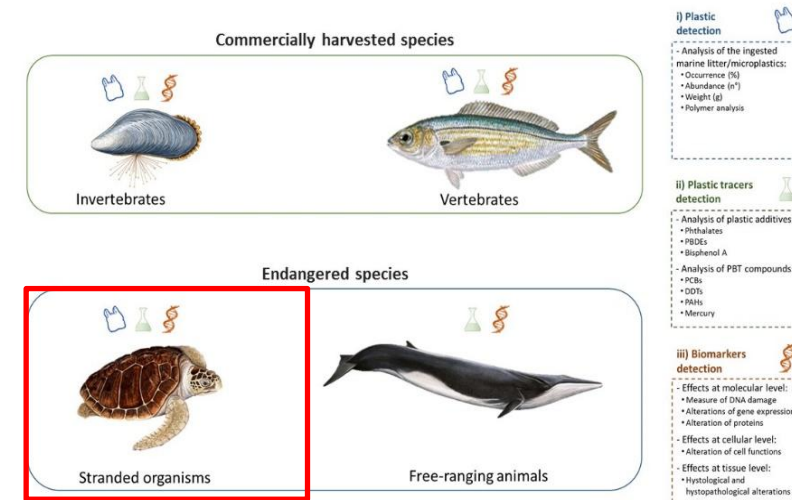
Toolkit for monitoring ML and its impacts on biodiversity in Med MPAs



Experimental design: Selected Biondicators

Target species – Secondary species

	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>Caretta caretta</i> <i>Balaenoptera physalus</i> <i>Physeter macrocephalus</i> <i>Xiphias gladius</i> <i>Thunnus thynnus</i> <i>Chelonia mydas</i> <i>Dermochelys coriacea</i>		
MEDIUM-SCALE (Mediterranean UN Environment/MAP sub-regions)			<i>Caretta caretta</i> <i>Thunnus alalunga</i> <i>Coryphaena hippurus</i> <i>Euthynnus alletteratus</i> <i>Stenella striata</i> <i>Ziphius cavirostris</i>		
SMALL-SCALE (FAO GSA)	<i>Isopods</i> <i>Jellyfish (Pelagia)</i>	<i>Boops boops</i> <i>Trachinotus ovatus</i>	<i>Engraulis encrasicolus</i> <i>Sardina pilchardus</i> <i>Trachurus sp.</i> <i>Sardinella aurita</i> <i>Myctophids</i>	<i>Mullus surmuletus</i> <i>Diplodus sp.</i> <i>Pagellus sp.</i> <i>Spondylusoma</i> <i>Lithognathus mormyrus</i> <i>Galeus melastomus</i> <i>Merluccius merluccius</i>	
LOCAL SCALE				<i>Paracentrotus lividus</i> Holothurians	<i>Decapods (Pachygrapsus marmoratus)</i> <i>Mytilus galloprovincialis</i> (cages?)



FIRST NOTES ON THE DISCOVERY SITE

1. CONTACT

Name, contact (phone, mail) and institution of the observer(s) (data collector).

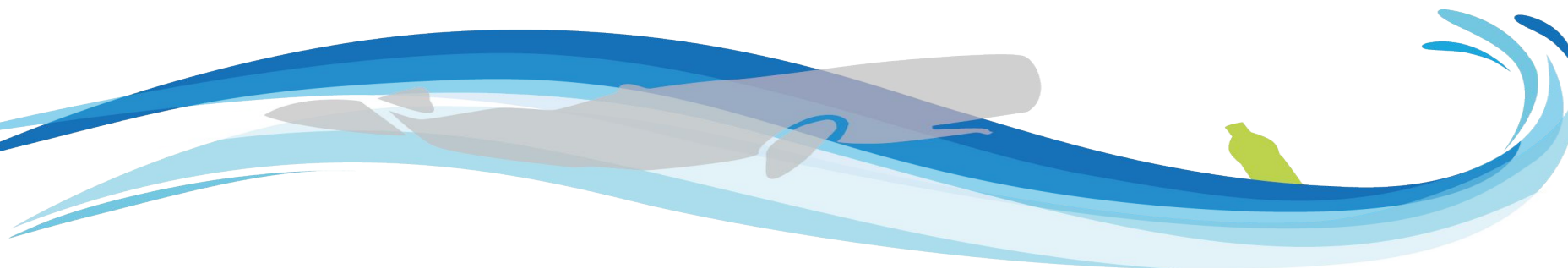
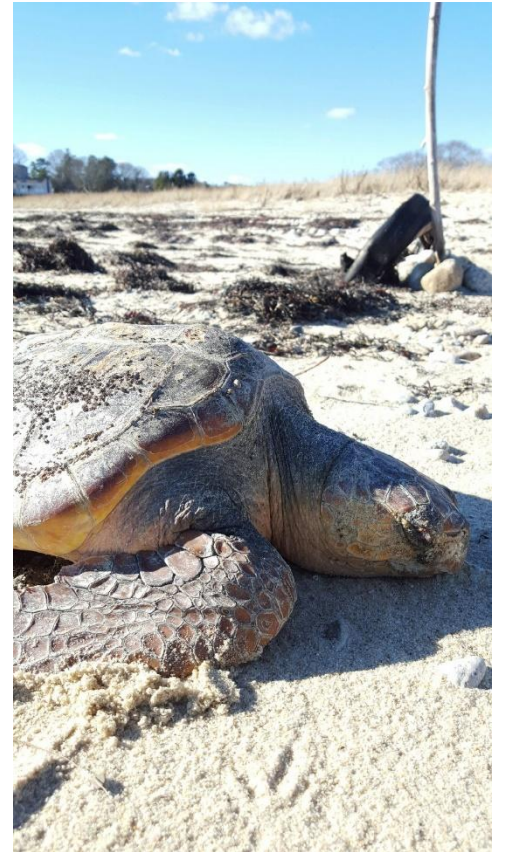
2. ON THE SITE

Note the date of discovery (dd/mm/yyyy), the location of discovery and the coordinates if available (X, Y: in decimal degrees, or specify the coordinate system);

3. ON THE INDIVIDUAL

Species identification:

Cc (*Caretta caretta*); Cm (*Chelonia mydas*); Dc (*Dermochelys coriacea*)



FIRST NOTES ON THE DISCOVERY SITE



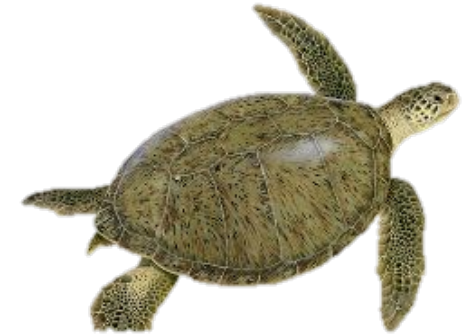
Caretta caretta

It has 2 pairs of pre-frontals scutes and the nuchal scale (the marginal just dorsal to the neck) is in contact with the first rib



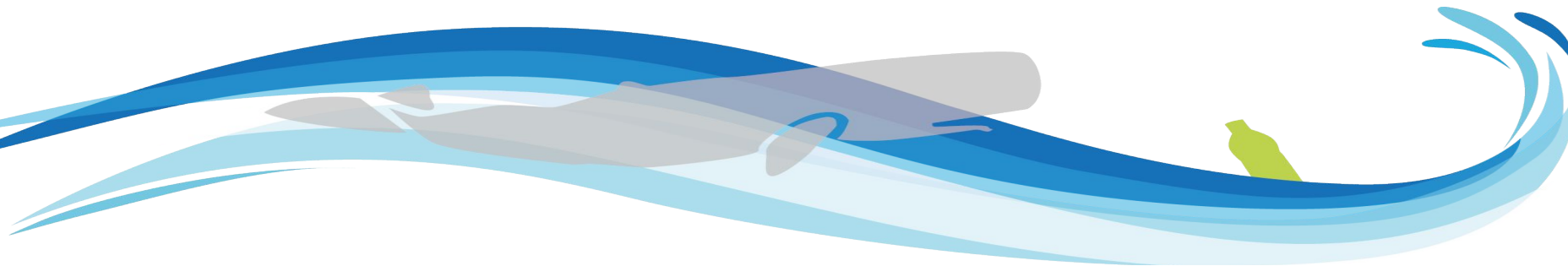
Dermochelys coriacea

It is characterized by the absence of keratinized scutes and the presence of 'leather' and ridges.



Chelonia mydas

It has 1 pair of pre-frontals scutes and the nuchal scale not in contact with the first rib

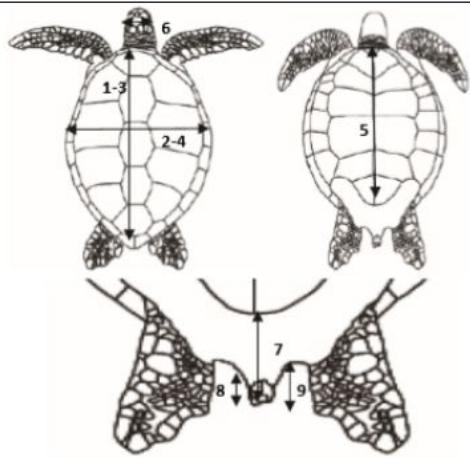


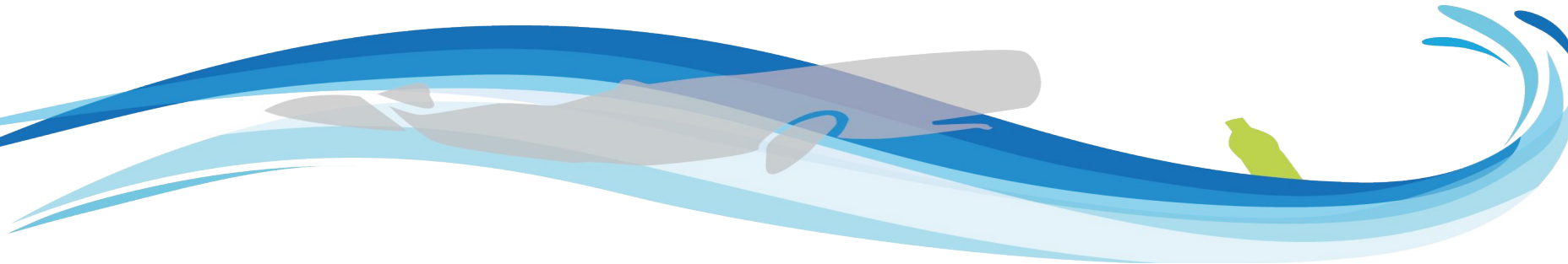
BIOMETRIC MEASUREMENTS

7. Several **basic and optional body lengths** should be measured (in centimetres, precision 0.01 cm), as well as the **weight** (in kilograms, precision 0.01 g).

- Standard Curved Carapace Length (CCLst)
- Standard Curved Carapace Width (CCWst)
- Standard Straight Carapace Length (SCLst)
- Straight Carapace Width (SCWst)
- Curved Plastron Length (CPL)
- Head Width (HW)
- Plastron Tail Length (PTL)
- Cloaca Tail Length (CTL)
- Carapace Cloaca Length (CaCL)

Biometric measurement			
1. CCLst	cm	6. HW	cm
2. CCWst	cm	7. PTL	cm
3. SCLst	cm	8. CTL	cm
4. SCWst	cm	9. CaCL	cm
5. CPL	cm		
Weight (kg)			
Sex			





ADDITIONAL INFORMATIONS

5. Discovery circumstances

Note the circumstances among the 4 categories:

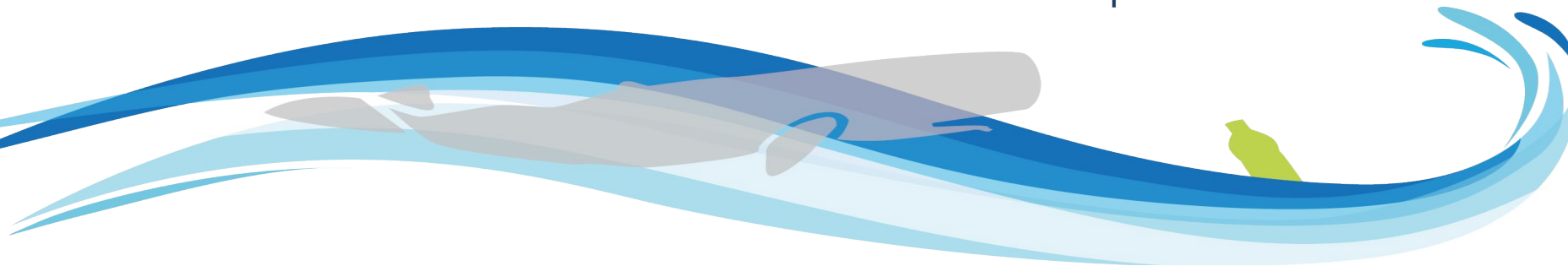
- **Stranding:** animal found stranded on the beach or in the shoreline.
- **By-catch/fisheries:** animal accidentally captured by fishermen (e.g. ingestion of a hook, entangled in a net, brought back by fishermen, etc.) during fishing operations.
- **Found at sea:** dead animal discovered on sea surface.
- **Dead at the recovery center:** the animal arrived alive but died during its hospitalization.

6. Possible cause of morbidity and mortality, type of impact

If possible, the type of interaction with human activities and impact observed or suspected on dead or live stranded individuals should be deduced from external or organs observations during the necropsy and complemented with veterinarian examinations.

7. Main injuries

In case of injuries, the **main type of injury** (fracture, amputation, sectioning, abrasion or other) and the **affected body part** should be reported. If the individual has been found entangled in litter, the type of material in which the sea turtle was found should be specified.



DESCRIPTION OF THE ANIMAL'S BODY CONDITIONS

8. Note the conservation status or decomposition level according to these 5 levels



Level 1

Live animals: biological samples (blood, carapace, plasma, faeces) can be removed for biomarkers and chemical analyses.

Level 2

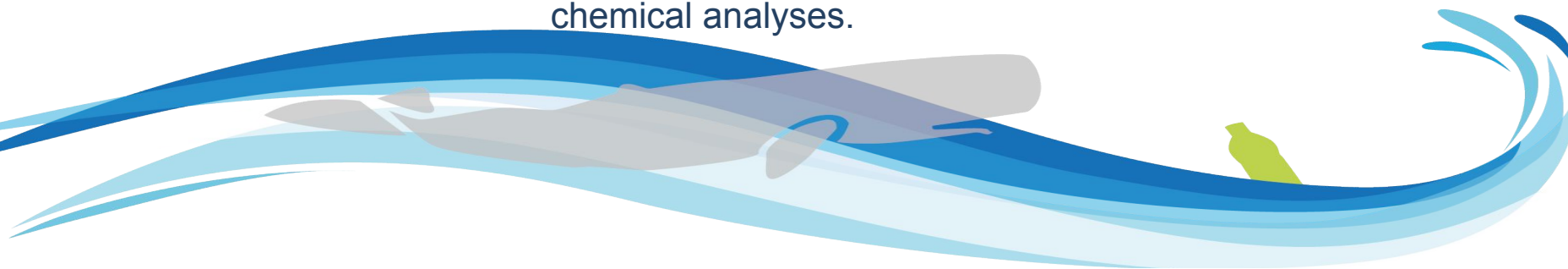
Animals that have just died (< 2 hours post mortem), gastrointestinal system is adequate for litter ingestion analysis, and other tissues (muscle, liver) can be used for biomarker and chemical analyses.

Levels 3 and 4

Animals are adequate litter ingestion analysis (in GI) from necropsies and chemical analysis.

Level 5

Individuals have usually lost their gastro-intestinal material and thus the analysis of litter ingestion is not possible.



Laboratory analysis in stranded endangered species

Level 1: Specimen found alive or deceased at most by 2 hr

Tissue:

- Blubber
- Skin
- Liver
- Kidney
- Muscle
- Gastro intestinal tract
- Blood

ANALYSIS OF ANTHROPOGENIC CONTAMINANTS

- OCs
- PAHs
- PBDEs and other HAHS
- Plastic additives



DIAGNOSTIC MARKERS FOR CONTAMINANTS

- Markers of exposure (CYP1A, CYP2b, AhR)
- Markers of general stress (HSPs, Cortisol and other stress hormones)
- Markers of reproductive alterations (Ers, ARs, steroid sex hormones)
- Markers of genotoxicity (Lipid peroxidation, DNA Adducts, DNA strand break, Micronucleous, apoptosis)
- Markers of susceptibility (AhR)

ANALYSIS OF THE INGESTED MARINE LITTER/MICROPLASTICS:

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

Laboratory analysis in stranded endangered species

Levels 2: Fresh carcass;

Levels 3: Moderate decomposition;

Level 4: Advanced decomposition.

Tissue:

- Blubber
- Skin
- Liver
- Kidney
- Muscle
- Gastro intestinal tract

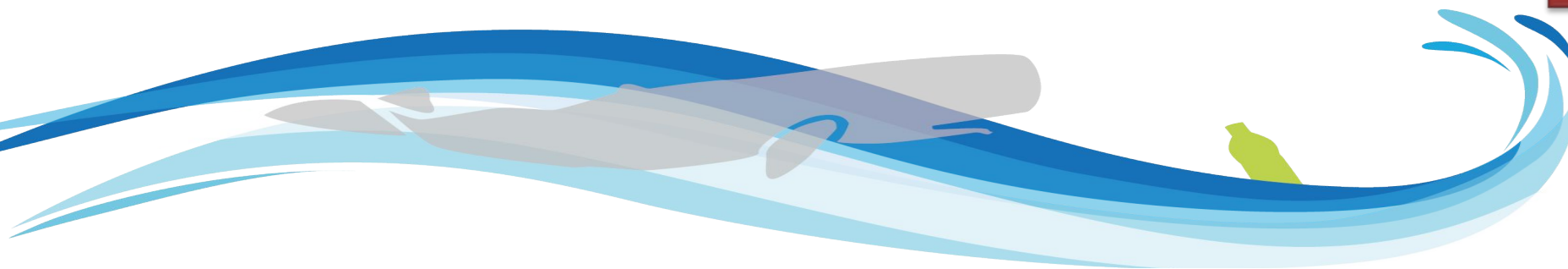
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- Plastic additives



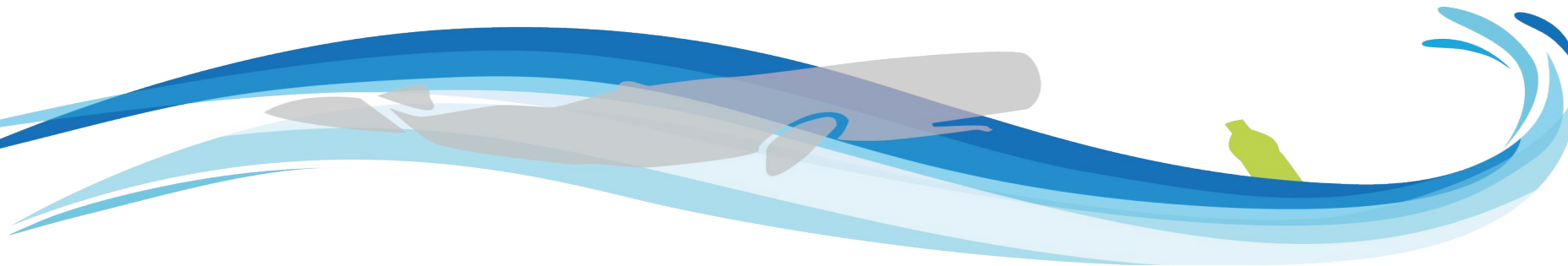
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- Occurrence (%)
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PROTOCOL FOR NECROSCOPY

The carcass should be placed on its back, trying to wedge it with an object so that it does not wobble from side to side. The plastron should be removed and separated from the carapace through an incision on the outside edge (yellow line). The incision should be made with special attention, with the use of a short blade or by cutting with a horizontal tilt to avoid affecting the integrity of the interior organs. Once the inside of the plastron is accessed, cut the ligament attachment to the pectoral and pelvic girdle to pull back the plastron and reach the muscles and then the internal organs.

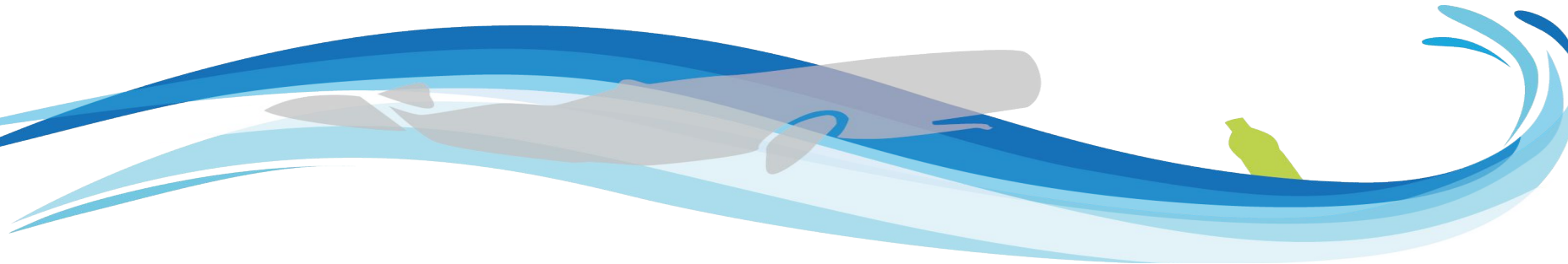
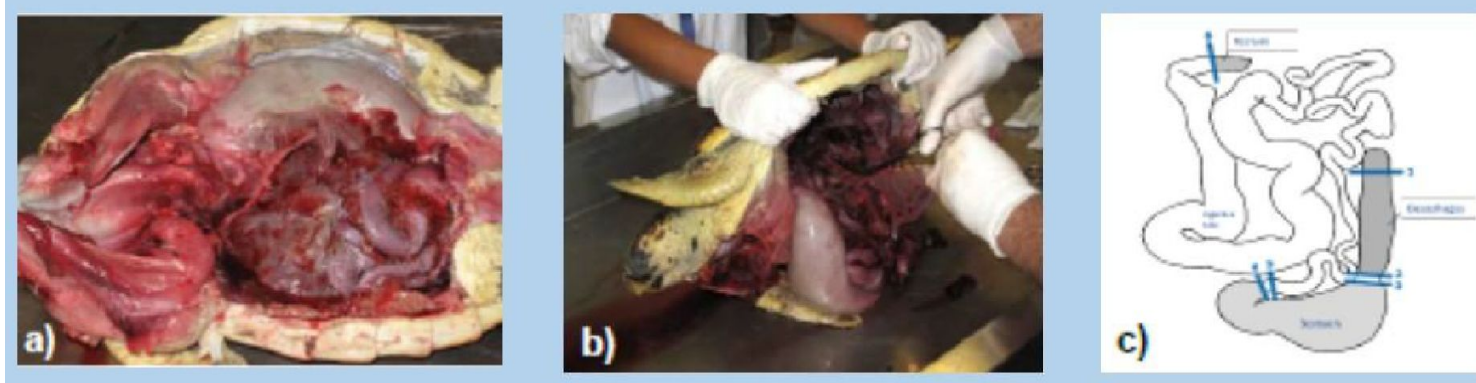


EXTRACTION OF THE GASTROINTESTINAL TRACT

Expose the gastrointestinal system (GI) by removing the pectoral muscles and the heart of the animal. The blood can be emptied from the abdominal cavity by carefully rolling the turtle onto a side.

Clamp the oesophagus proximal to the mouth and clamp the cloaca, the closest to the anal orifice.

Remove the entire GI and place it on the examination surface. Isolate the different portions of GI (oesophagus, stomach, intestines) by strangling and cutting between the 2 clamps (see the blue solid lines in Figure 1) the gastro-oesophageal sphincter and the pyloric sphincter.

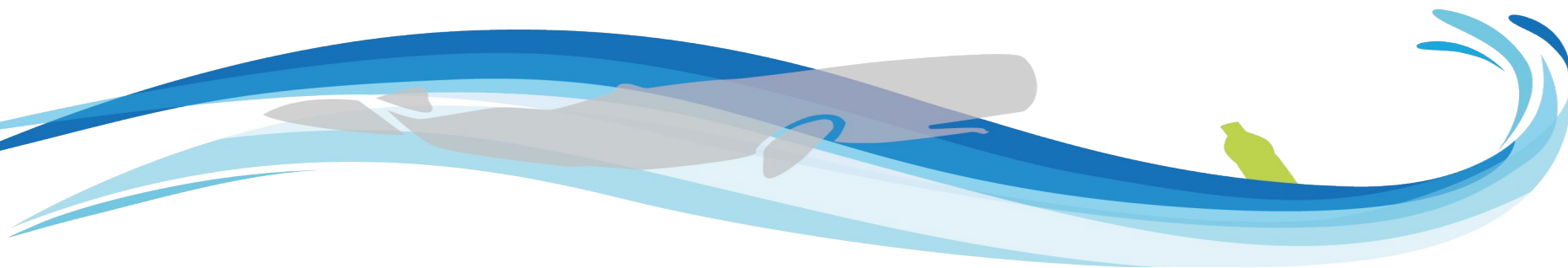


GI TRACT ANALYSIS

Each GI section should be opened lengthways using a scissor and slide the material directly out of the section onto a 1mm mesh sieve. The content should be cleaned with current and abundant tap water to remove the liquid portion, the mucus and the digested unidentifiable matter.

The content should be inspected for the presence of any tar, oil, or particularly fragile material, which should be subsequently removed and treated separately.

All material should be rinsed, collected in the 1mm sieve, and placed in tubes or in zipped bags, reporting the sample code (individual code, respective GI section) and stored at -20°C, pending the laboratory analyses.



LITTER CLASSIFICATION

PLASTICS	INDUSTRIAL	PELLETS	Industrial plastic granules with different shapes (cylindrical, oval, spherical or cubical)	G112
		SPHERES		G118
	USER PLASTICS	SHEETLIKE	Remains of bag, cling-foil, agricultural sheets, rubbish bags etc	G119
		THREADLIKE	Pieces of nylon wire, net-fragments, woven clothing etc.	G120
		FOAMED	All foamed plastics so polystyrene foam, foamed soft rubber etc.	G121
		FRAGMENTS	Fragments, broken pieces of thicker type plastics, can be bit flexible, but not like sheetlike materials	G122
		OTHER	Any other, incl elastics, dense rubber, cigarette-filters, balloon-pieces, softairgun bullets; objects etc	

RUBBISH	PAPER	Newspaper, packaging, cardboard, includes multilayered material (eg tetrapack pieces) and aluminium foil	G157
	KITCHEN FOOD	Human food remains (galley wastes) like onion, beans, chickenbones, bacon, seeds of tomatoes, grapes, peppers, melon etc	G215
	VARIOUS RUBBISH	Other consumer waste, like processed wood, pieces of metal, metal air-gun bullets; leadshot, painchips.	G216
	FISH HOOK	Fishing hook remains (not for hooks on which longline victims were caught - those under notes)	G183
POLLUTANTS	SLAG/COAL	Industrial oven slags ('looks like non-natural pumice) or coal remains	G212
	OIL/TAR	Lumps of oil or tar (also not n=1 and g=0.0001g if other particles smeared with tar but cannot be sampled separately)	G214
	PARAFFIN/CHEMICAL	Lumps or mash of unclear paraffin, wax like substances (NOT stomach oil!) If needed subsample and estimate mass	G213
	FEATHER LUMP	Lump of feathers from excessive preening of fouled feathers (n=1 with drymass) (NOT for few normal own feathers)	



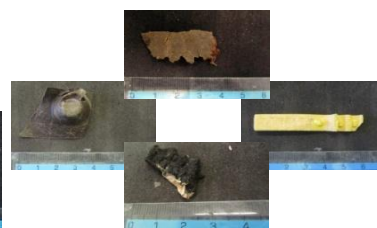
SHEETLIKE



THREADLIKE



FOAMED



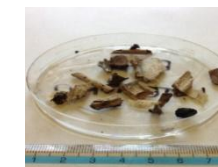
FRAGMENTS



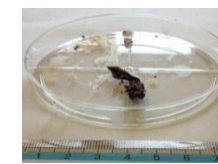
OTHER



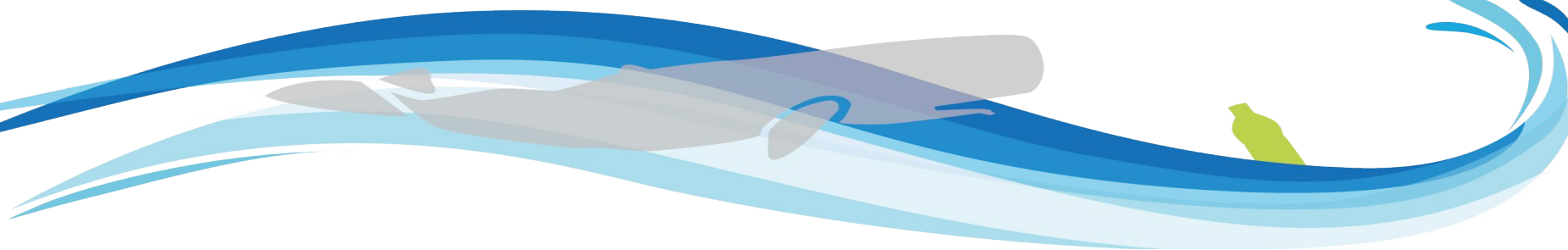
FISH HOOK



VARIOUS RUBBISH



TAR





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Thank you!

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