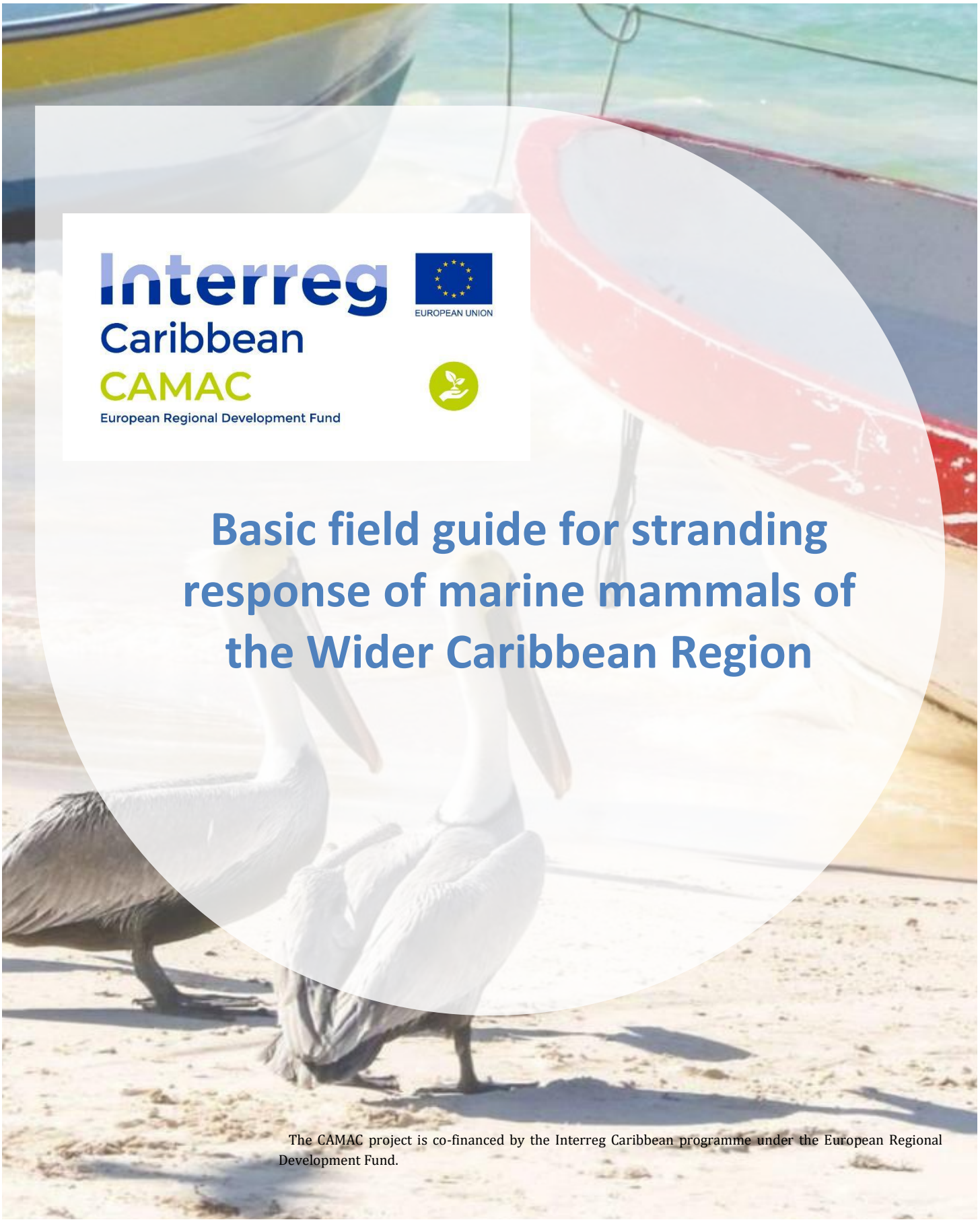


Interreg Caribbean CAMAC European Regional Development Fund

EUROPEAN UNION

Basic field guide for stranding response of marine mammals of the Wider Caribbean Region

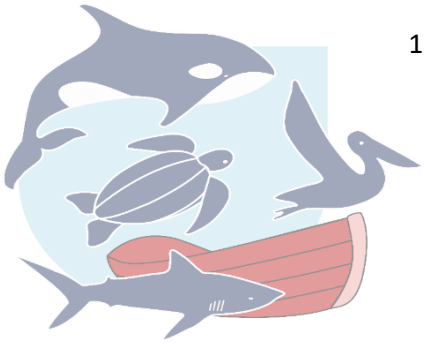
The CAMAC project is co-financed by the Interreg Caribbean programme under the European Regional Development Fund.

RÉPUBLIQUE FRANÇAISE
Liberté
Égalité
Fraternité

OFB

AGOA

CAR-SPAW-RAC



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1 GENERAL RECOMMENDATIONS FOR STRANDING RESPONSE PROCEDURE

1.1 Stranding incident

Marine mammals strandings (i.e. animals that cannot go back to water on their own) can occur with a single individual, or with several individuals simultaneously (the latter are known as mass strandings except when these involve a mother with her calf), or with multiple single strandings within a short period of time. Animals may be discovered stranded dead or alive on the beach or in the surf. When dead, evaluations are needed by the Stranding Response Network parties for the best course of action for data sample collection and removal or burial of the carcass, depending on the location and size of the animal. If alive, complex evaluations are needed to decide the best course of action, depending on the size and condition of the stranded individual(s), the species and age, if socially dependent or independent, the stranding location's safety and accessibility, and availability of needed equipment and expertise.

Marine mammals in principle beach themselves accidentally, when healthy, due to coastal topography (for example sloping shores with a soft sediment base), disorientation due to magnetic anomalies, when chasing prey, escaping from threat (predators/loud noise), and due to tide changes. The animal also can strand "purposefully" or by weakness due to medical distress, caused for example by collision injuries or disease. Mass strandings usually occur by toothed whales and dolphins due to group cohesion and family bonds with a sick or injured animal. In most cases, the "due to medical distress" live stranded cetaceans may not survive even with assistance. Rehabilitating dolphins and whales is often logistically and financially not feasible and the rate of success is low. However, any attempts to return them to sea should be done swiftly to limit stress on the animals and follow human safety measures. In some cases, humane euthanasia should be considered to optimize the welfare of the animal.

The main objectives of a marine mammals stranding response network are to provide rapid and effective action for the wellbeing of the stranded animal (s), to protect the public while handling a response, and to gain maximum scientific information.

1.2 Legal concerns

As marine mammals are protected by the law in many countries, but also for biosafety reasons, a trained cetacean stranding response network needs to be well established, within compliance of the country's legal framework, with: a clear description of the authority responsible for marine mammals incidents and their partners within a "chain of command"; the operating procedures and role of all parties involved; a register of contacts for expertise and equipment; a list of laboratories and scientists to send the collected bio-samples to; a protocol for marine mammals handling and for bio-sample collection and transport

following the national procedures and legislations, including the legal permit procedure to transport marine mammals bio-samples out of and into the country.

1.3 Biosafety

When handling a marine mammal during collection, transportation, and postmortem examination, the potential biological, chemical, and residue risks to environmental and public health need to be assessed and mitigated including risks of fluid leakage, noxious odors or aerosols from the carcass.

There are also safety precautions and biosafety procedures to protect the responder against injury, zoonotic diseases, and infections by pathogenic organisms through aerosol, ingestion, or direct contact transmission (particularly through pre-existing or induced breakage in skin barriers).

When handling a live marine mammal, always avoid the tail swing area, and the bite (beak area), and turn your head away from the blowhole when the animal exhales. Always wear protection equipment: (mask, gloves, glasses, disposable clothes and shoes). Have adequate drinking water, and protection from sun, wind, cold, and rain. Wash hands quickly with soap and freshwater (not sea water) in the event of any cuts or punctures related to handling or necropsy.

Basic biosafety guidelines for postmortem data collection are:

- Have a list of all chemicals (e.g., fixatives, preservatives) used and their safety measures, and a chemical spill treatment kit.
- Have a disposal receptacle for knives, blades, and needles.
- Wear double gloves and apply barrier cream to skin, face mask, goggles or splash shield, overalls and booties.
- Practice good hygiene, wash well with detergent, or 70% alcohol.
- Disinfect all cuts with iodine.
- Cover sharp bone fragments.
- After use, disinfect all instruments, equipment, tables and floor with broad-spectrum disinfectant, for example Virkon, or with the newer non-corrosive broad-spectrum low pH phenolic disinfectants, like **Vesphene®** IIIse (Steris) and **LpH®** III (Steris) or with alcohol at least 70%.

1.4 Actions when receiving a stranding alert

<u>Inform the person reporting the stranding of the:</u>

- Health risks.
- Benefit of reporting;
- Role of the stranding network;
- Actions that will be taken; and thank the person reporting the incident.

Ask the person reporting the stranding for the following information:

- The informant's contact details.
- The location of the stranding: in water or on land, town, name of the beach, landmarks on the shore and access route.
- The number of animals, description of the animal(s): alive/dead, cetacean/manatee,size, pigmentation, morphology and state of decomposition.
- Request photographs of the entire animal from which to identify the species and to assess the general condition of the animal.
- Description of the site: sandy or rocky coast, accessibility on foot or for vehicles, exposure to the tide.

Prepare for field intervention:

- Evaluate field conditions and organize the intervention accordingly (time, equipment, response team, necropsy team, vehicle,etc.
- Mobilize the response team, according to your needs. An optimal response team for one stranded animal should include at least: one stranding trained expert that will coordinate the intervention, a vet in case of live stranding, one person to support the coordinator and one "clean person" that will communicate to the public and external support, fill in forms, etc
- Make sure that you have the necessary personal protective equipment, tools for data and sample collection (see next chapter) and material for dealing with alive animals (if needed).
- Report the event to local, state and/or federal authorities with legal competence on the matter.
- Get external help when necessary: police/military services (to handle public), services in charge of the carcass disposal, alive facilities, maritime services (if a boat is needed), stranding experts for advice.

1.5 Field intervention

1.5.1 When the animal is alive

The evaluation of the condition of the live animal for the course of action to be taken is determined through visual external assessment (1), visual bio-health assessment (2) and when possible, blood sample collection (3).

For the visual external assessment see Annex 1. Some considerations of the animal is if it is entanglement with fishing gear, emaciated (underfed/sick see chart), propeller strike, vessel or predator strike (hematomas), predator attack (shark, orca bite mark), bleeding, old or fresh scars, skin lesions.

Visual bio health assessment:

- When in water: Movement manner, drifting horizontal/sideways, drifting vertical, sinking, repetitive circle formation, repetitive beach approaches.
- Beached: breathing intervals, mucus at blowhole, blood in blowhole, mouth or anus (see Annex 1).

Prepare for intervention

- Verify whether the animal is alive (puffs of air from the blowhole, and movements).
- Evaluate the feasibility of a refloating operation: accessibility of the site, accessibility of the sea, sea conditions, available resources, condition of the animal (if it can be assessed by a vet or experienced individual).
- Determine and mobilize the needed resources (response team, medicines, vehicles, swimming pool, equipment, and machinery).
- Regardless of the decision made, the stranded animal must be handled and transported in the best way, ensuring that measures are applied for its well-being.

Get the animal in a comfortable position

- Act calmly, limit crowding and noise pollution (if the event takes place or extends overnight, avoid the use of direct lights and flashes towards the animal).
- Place the animal on its stomach, keep its skin moist by gently pouring water avoiding the blowhole and protect it from the sun with a wet towel, shading devices.
- Do not use sunscreen's of any brand. Zinc oxide can be use it
- Do not cover the blowhole, and protect it from sand and water splashes
- Dig water trenches under the pectoral fins
- On hard substrates, isolate the animal using a tarpaulin, towels or a mattress

Transport

- Pay attention to sudden movements, be aware of the flippers, the head, and restrain the tail to avoid injuries.
- Move the animal preferably using a soft stretcher or a tarpaulin.
- Remember to systematically protect it from the sun with wet towels, shading devices like beach umbrellas.
- Make sure that the animal does not tip over by stabilizing the body using rolled towels or foam blocks.
- Do not tow or drag the animal by its tail or handle it by the pectoral fins.
- Consider moving the animal to another site that is more suitable for refloatin

Refloating

- Carry the animal into water deep enough to support its weight and keep it at the surface, with the blowhole and dorsal fin in upright position, until it re-acclimatizes (e.g., when it breathes normally and its tail moves)
- Try to be calm so as not to stress the animal. All personnel should be quiet and any onlookers also instructed to remain at a safe distance and quiet. This maneuver should

ideally be performed with the minimum number of people possible and associated with the weight, size and experience of the individuals involved in handling the specimen.

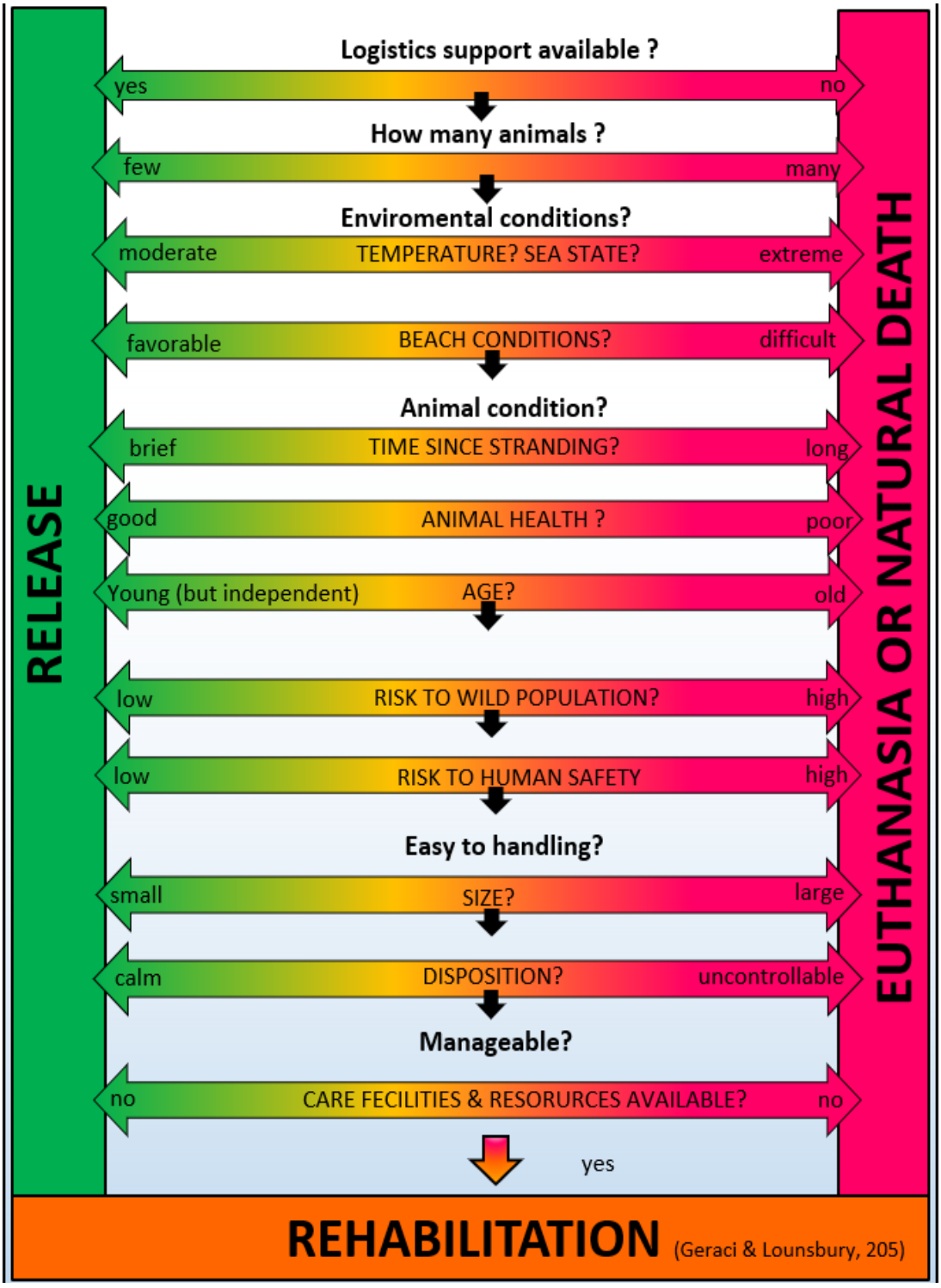
- If a boat is available, take the animal to an opensea access area if needed, for example when trapped in a harbor area (problematic area). As long as the size of the animal allows it and it demonstrates that it is strong enough to be able to swim on its own, the animal can be carried away from the coast; otherwise, take the animal beyond the surf area.
- Once it re-acclimatizes, release the sling once it is clear that the animal can keep itself upright and swim.
- In case of a cow-calf pair or other small groups (2 to 3 individuals), refloat them simultaneously.
- If the animal strands again, it is probably too weak and will die soon. In this case, a choice needs to be made between letting nature take its course or euthanasia intervention. Whenever this option is legally viable, euthanasia intervention must always be assessed and performed by a veterinarian.
- Some euthanasia medications result in prolonged agony or increased excitement in cetaceans more so than in terrestrial animals, and therefore approved medications determined after consultations with a marine mammal expert is necessary.

<u>Marine mammal euthanasia</u>

According with the American Veterinary Medical Association and the guidelines for euthanasia marine mammals (2020): “Selecting a method of euthanasia for free-ranging marine mammals can be a substantial challenge because of large body size, environmental constraints, and concerns for the safety of personnel. It can also be difficult to determine when stranded marine mammals are unconscious or dead. Currently available euthanasia methods generally have significant limitations that fail to meet conventional standards for euthanasia of marine mammals under field conditions, particularly for large animals. Nevertheless, the options available must be evaluated to identify the best one under a given set of circumstances. Further research is warranted to identify improved methods of euthanasia”.

Some acceptable methods that can be used under field conditions like injectable agents for overdoses. Also, there is acceptable physical methods such as gunshot, when injectable methods are not practical. Conventional projectile ballistics are not recommended for use in large odontocetes or large mysticetes. Knowledge of anatomy and physiology of these animals is crucial to avoid unnecessary agony. Unacceptable methods included inhaled agents and exsanguination.

Decision guide for alive animals



1.5.2 When the animal is dead

Before the necropsy

- Whenever possible, as long as the size and the weight allow it, evacuate the carcass to a site sheltered from the public, convenient for the necropsy, and accessible to the rendering services.
- Always secure a perimeter around the carcass.
- All members of the necropsy team must wear dedicated protective clothing, impermeable clothing protection (e.g. rain gear), boots, thick gloves, safety glasses or face shield and a mask (ideally a P2/N95 respirator).
- Identify a coordinator for the necropsy team (usually the most experienced at the site) and assign a role to all other team members.
- Coordinate with external services, notably notify the ad hoc services of when the necropsy will be completed so that they can organize the disposal of the carcass.
- Communicate to the public present onsite on the stranding network's objectives and warn about health risks. Ask them to respect a safe distance and keep pets away.
- Be prepared and responsive to the questions of the media, in particular in case of an unusual event. State only facts in non-technical language and avoid speculations to prevent misunderstandings and/or misinformation.
- If the necropsy is done in the field, make sure you have personnel or machinery to make the hole in the sand deep enough for proper burial. In the case of small animals (less than 3 meters) the hole must be at least 1 meter larger than the size of the animal with a depth of 1.5 meters. In the case of larger animals, the hole must be at least 2 meters larger than the size of the animal and with a depth of no less than 3 meters.

After the necropsy

- Leave the site clean and collect the biological waste in bags to be sent for rendering. Ideally the bags should be identified as biohazard.
- Be aware of possible scavengers at the zone that could be attracted during the procedures such as shark and crocodiles.
- Sharp items, like surgical blades, should be stored in a container. For example, that could be a plastic bottle or a specific container for sharp instruments.
- Decision regarding disposal must be made before a necropsy is started. Burial at sea is also an option if a boat can tow it back out. Also consider respecting the cultural traditions and wishes of the traditional owners at the site (e.g. in some cases the heart will need to be buried on site, there may need to be a ceremony, etc.
- The carcass must be eliminated by rendering (be aware that the selected

rendering is not for animal food preparation). If the circumstances do not allow it, the carcass must be buried as described before.

- If it is a rocky beach without sand and machinery cannot enter, the animal must either be taken far enough out to sea so that it does not return to nearby beaches, or the carcass must be left to decompose naturally.
- Thank and inform the informants and the external services.
- Respond to the media through the appropriate channels (in many cases environmental authorities must control the flow of information) and draft a press release. This is very important in case of an unusual event. Do not send photos or stranding information through social media or the media.
- Send the "stranding" and "sample" sheets as well as the photographs to the organization in charge of coordinating the stranding network.

1.5.3. Large dead cetaceans and mass strandings

- It is essential to determine and mobilize the needed resources (people, equipment, and machinery) and coordinate them well.
- Organize an information meeting with all participants before the start of operations and assign a specific role to each person/group.
- Coordinate with external resources, such as the services in charge of the carcass cutting (large animal) and disposal.

- Appoint a field coordinator to be in charge of guiding operations.
- Make sure all interveners wear dedicated protective equipment.
- For mass strandings, the priority is always to refloat all live animals with good condition (with better prognosis), more moribund animals should be considered for euthanasia.
- Once this is done, the standard management and examination of a stranding can be considered and organized.
- Experiences with mass strandings in New Zealand have shown that refloating mothers and calves first is more effective than first refloating males or lone individuals to return the rest of the group.

Therefore, if possible, give priority to mothers and calves.

- For large dead animals, take precautions against exposure to putrefaction gasses when opening up the carcass of a large animal (asphyxiation and explosion), and establish a safety perimeter of at least 100 m for the maneuvering of machinery.

2. DATA AND SAMPLE COLLECTION

2.1 Material

Biosafety equipment (to wear even for data collection):

- ✔ Box of protective masks (N95/P2 respirator preferred)
- ✔ Box of disposable latex gloves
- ✔ One pair of heavy rubber gloves
- ✔ Safety glasses
- ✔ Coveralls, impermeable materials (e.g. rain gear) or Tyvek coveralls
- ✔ Rubber boots (preferably steel-toed) or disposable overshoes
- ✔ First aid kit
- ✔ Soap and freshwater
- ✔ Cones/poles and tape to secure the perimeter

Data collection material:

- ✔ Stranding and sampling forms
- ✔ Names and contact information of regional marine mammal live stranding and necropsy experts available to remotely assist.
- ✔ Kitchen paper
- ✔ Camera
- ✔ Clipboard
- ✔ Pencils with refills and eraser
- ✔ Plastic measuring tape and ruler

Sampling material:

- ✔ Permanent markers
- ✔ Plastic small bags (like Ziploc or whirlpak bags)
- ✔ Sharp stainless steel knives
- ✔ Electric knife sharpener
- ✔ Portable electricity source for large mysticetes like a generator or equivalent so that it can be done on-site)
- ✔ Stainless steel scissors
- ✔ Small metal saw
- ✔ Pruners (large, long-handled)
- ✔ Surgical blades
- ✔ Syringes
- ✔ Large hands saw and/or Electronic bone saw (e.g. snozzle) only to be used by trained personnel with appropriate PPE
- ✔ sterile swabs
- ✔ A large tarp

Critical material for tissue sampling:

- ✔ Garden plastic bags (30L and 100L)
- ✔ Ziploc bags (18*15 cm and 32*23 cm)
- ✔ aluminum foil
- ✔ Permanent markers
- ✔ Labels
- ✔ 1-2 gallon (3-7 liters) with 10% buffered formalin
- ✔ Plastic tubes/vials of different volumes

- ✔ String
- ✔ Ethanol 95% for DNA samples and parasites
- ✔ 70% alcohol

Transportation and storage material:

- ✔ Hard-sided cooler with ice packs (ideally) or ice (since ice melts, tissue samples should be proper sealed and put them in a plastic bag preventing ice water from entering)
- ✔ Access to a freezer (-20°C or less) to store the samples long term

2.2 Data collection

2.2.1 Recommendations

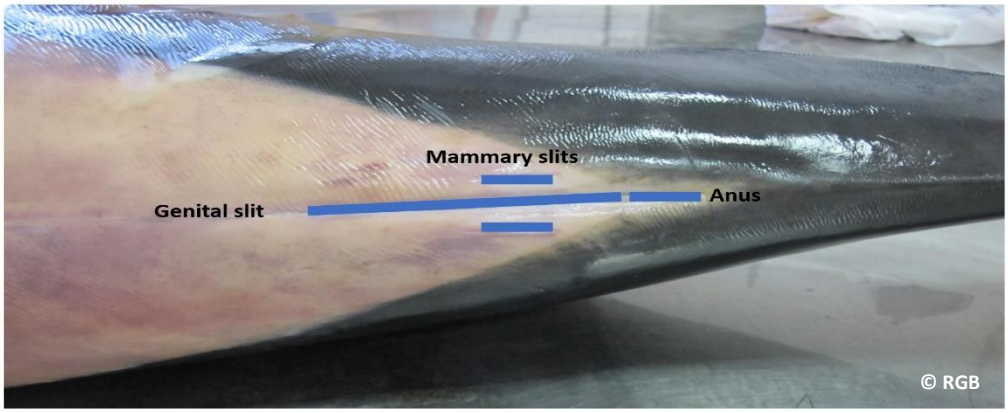
- ✔ Wear protective equipment (mask, gloves, glasses, impermeable clothes and boots), even for data collection. It is essential to have a specific scribe or clean person that never gets dirty as an assigned role
- ✔ Wash the animal before examination and taking photos.

2.2.2 List of information to collect

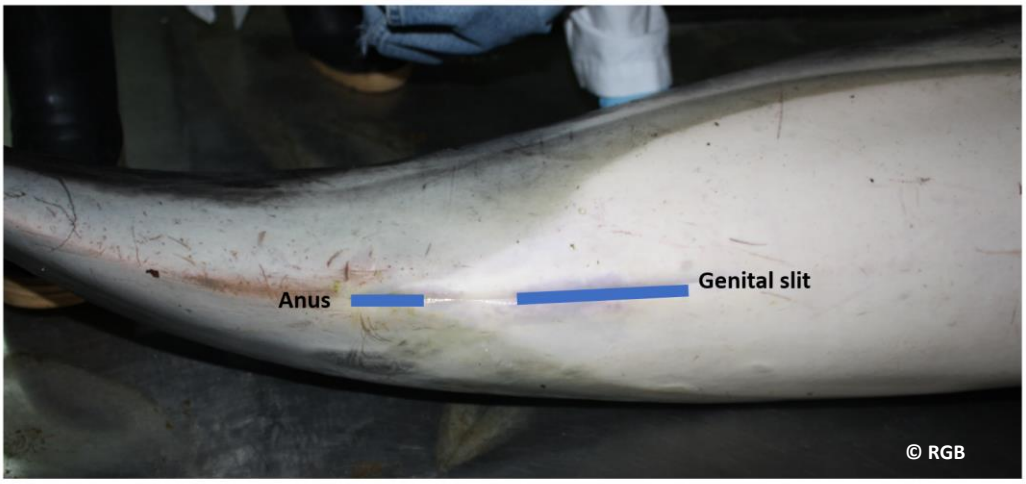
- ✔ Field stranding ID
- ✔ Name and agency of the field data documenter and their phone and email
- ✔ Discovery date of specimen and by whom (contact)
- ✔ Discovery location description and coordinates
- ✔ Type of stranding: single stranding, incidental stranding, boat collision, mass stranding, mother and calf, capture, mass stranding (number of animals)
- ✔ Information on site: accessibility, how was the weather, offshore human or predator activity, tide conditions
- ✔ Identified species of the specimen and certainty of identification

✔ Sex (female, male)

FEMALE



MALE



✔ Body condition: emaciated / not emaciated

Emaciate

Not emaciated

✔ For dead animals: Code of condition (2=Fresh dead, 3=Moderate decomposition, 4=Advanced decomposition, 5=Mummified carcass)

Code 1: Alive

Code 2: Fresh dead organs in good condition

Code 3: Decomposed basically intact organs

Code 4: Advanced decomposition of non-recognizable organs

Code 5: mummified or skeleton only

- ✔ For animals stranded alive:
  - Describe how was the behavior before it stranded: the animal was weak? the animal could swim? the animal was disoriented? Arching? Seizures?
  - Was the animal refloated, or did it die: before refloating attempt, during refloating attempt, after refloating attempt, it was euthanized

- ✔ External observation, note:
  - Presence of wounds or marks: line, rake, puncture, flipper of fluke removed, broken jaw, scavenger damage, hematoma, skin disease, indicate where
  - Presence of fishing gear
- ✔ Teeth and baleen count: upper left, upper right, lower left, lower right
- ✔ Sample collection performed: none, basic, comprehensive

2.2. 3 List of photos to collect (see figure 2)

Use tags (with the name, date, or number) and scale (put a ruler or an object of known dimensions)

- ✔ Anything interesting in the environment or on the carcass
- ✔ Entire carcass at discovery location
- ✔ Right and left flank
- ✔ Dorsal side
- ✔ Ventral side
- ✔ Dorsal fin (both sides)
- ✔ Genital area
- ✔ Front and right and left side of head
- ✔ Right and left flipper
- ✔ Dorsal and ventral side of fluke
- ✔ Scars, wounds, entanglements

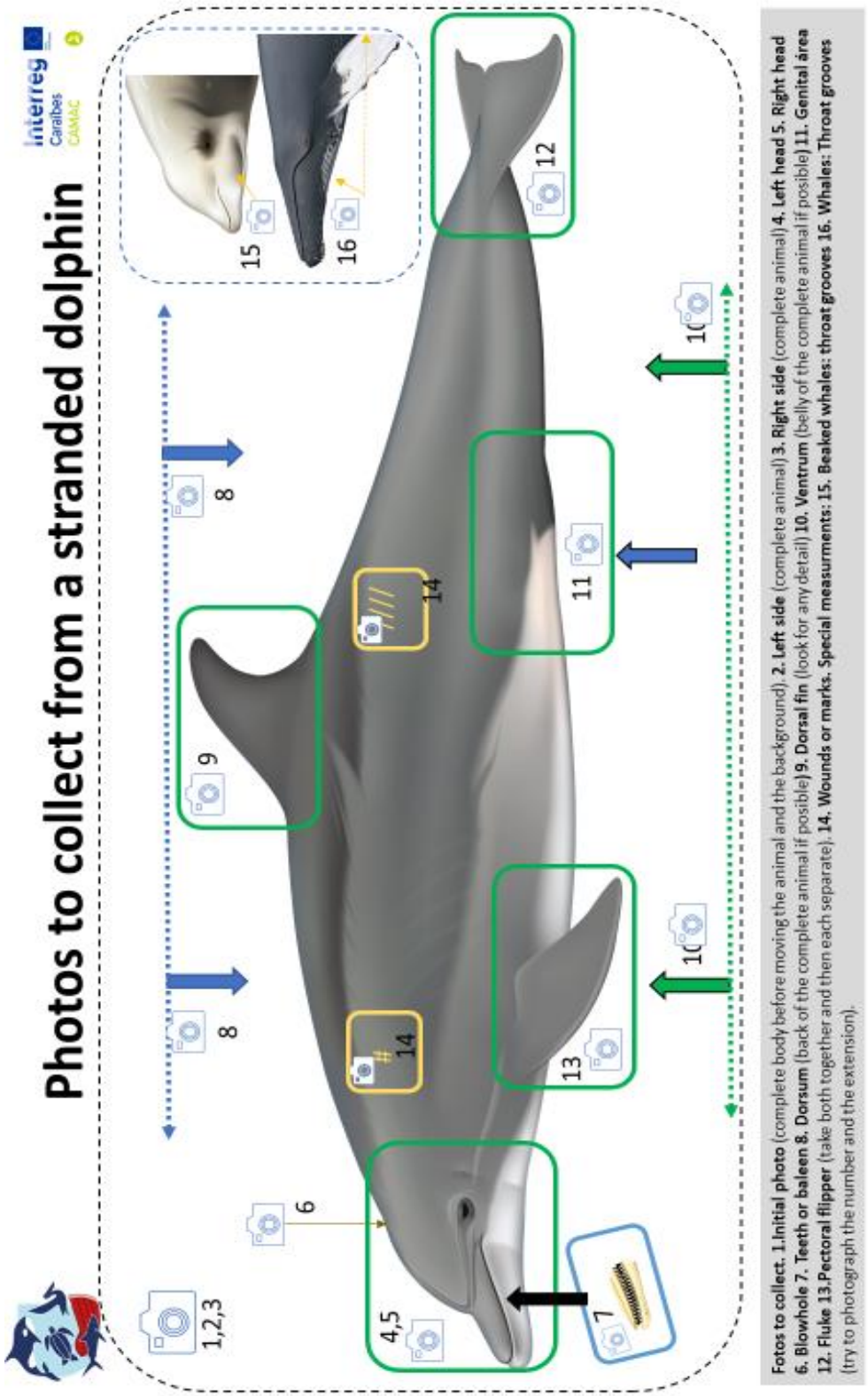


Figure 2. Photos to collect (Adapted from Geraci and Loundsbury. 2005)

2.2.4 Morphometrics measurements in cetaceans (figure 3) and manatees (figure 4)

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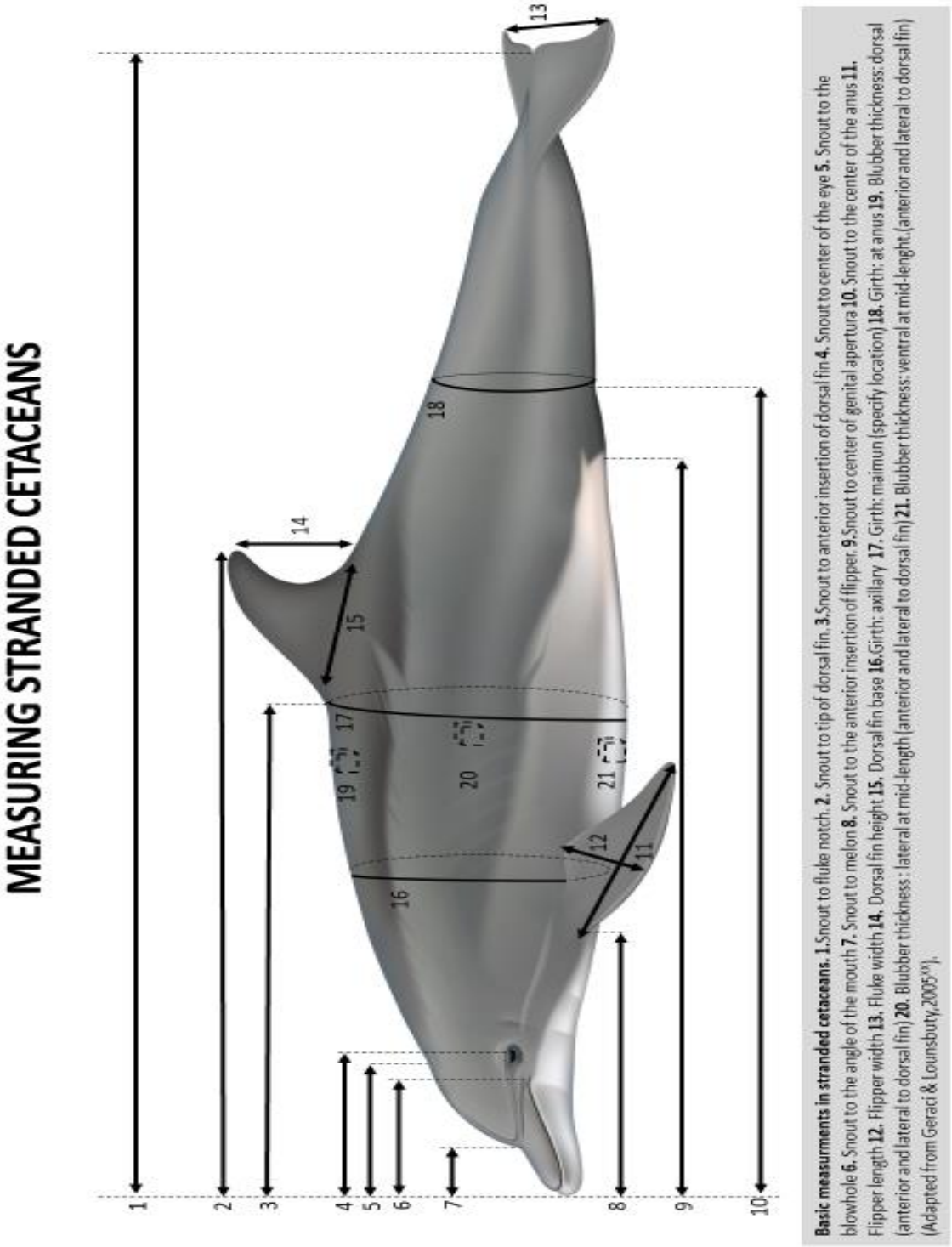


Figure 3. Measurements of stranded manatee (Adapted from Geraci and Loundsbury. 2005)

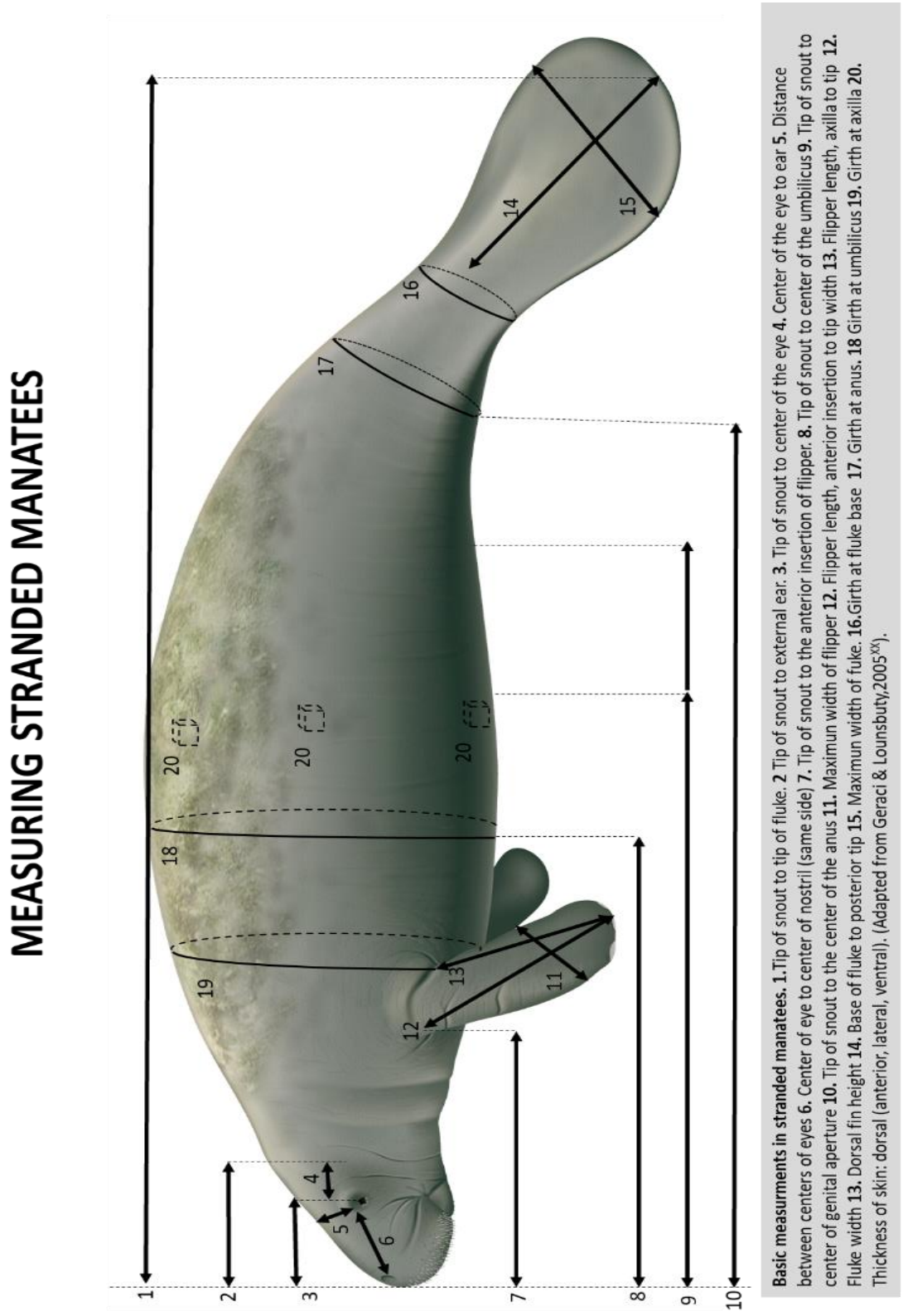


Figure 4. Measurements of stranded manatee (Adapted from Geraci and Loundsbury. 2005)

2.3 Sample collection

2.3.1 Recommendations

- ✓ Use gloves, mask (ideally N95/P2 respirator), and cover your body as much as possible.
- ✓ Remember that each animal has invaluable information on its population therefore if you are willing to perform a necropsy please be as detailed as possible
- ✓ All the samples should be placed in separate plastic bags and should be labeled, in permanent marker, with a stranding ID on the container plus a label inside the container.
- ✓ Choose one person with legible and good handwriting. This person ideally should be the "clean" person through the process
- ✓ Prepare the sampling bags before starting sample collection
- ✓ The knife sharpness is lost with sand and the animal skin, therefore try to find one person that sharpens the knives throughout the process (ideally with an electric sharpener) and particularly in large whales

In the next paragraphs are presented:

- ✔ A summary of the internal anatomy of a cetacean (Figure 5)
- ✔ A summary on how to start a necropsy (Figure 6)
- ✔ A summary table for basic sample collection, with almost no carcass opening
- ✔ A summary table for an extensive sample collection

You will find in these tables some recommendations on: the number of samples to be collected for each organ, their size, and the way they should be conditioned and stored.

If you do not have access to a freezer, note that two samples can still be collected:

- ✔ The teeth for age analysis can be kept at room temperature
- ✔ The skin for DNA analysis can be kept in 95% ethanol

Some important diseases to consider as a cause of the stranding in cetacean includes: Morbillivirus, brucellosis, influenza, toxoplasma, herpesvirus among others.

For brucellosis, the screening can be performed using serum and rose Bengal test or rapid test for smooth brucellae. For confirmation, cerebro spinal fluid (CSF), brain, placenta, milk, lung nematodes should be collected and send them to a specialized laboratory.

Brain can be collected not only for Brucella but for Morbillivirus, Herpes virus, and Toxoplasma analyses. In the case of Influenza, rectal and blowhole swabs can be collected for detection of the agent by rapid tests, and also brain can be send for later analysis. Lungs are important samples for morbillivirus, influenza a toxoplasma.

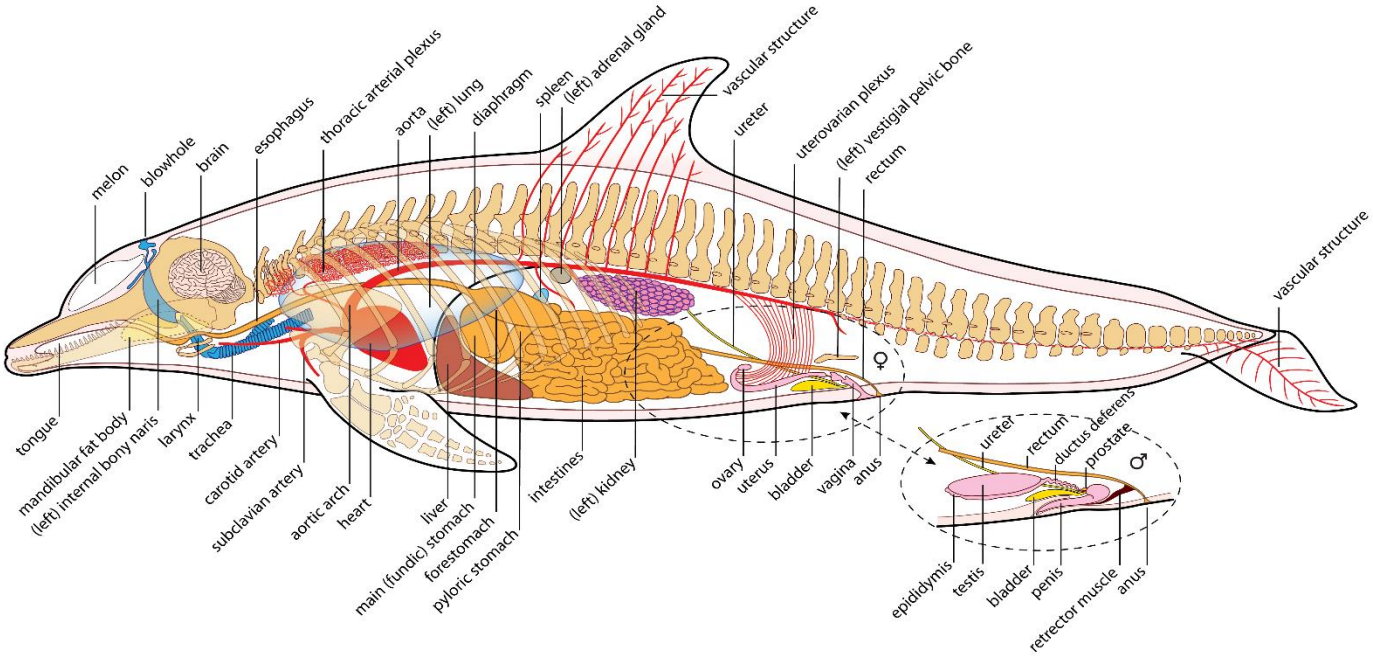


Figure 5. Basic internal anatomy of a cetacean

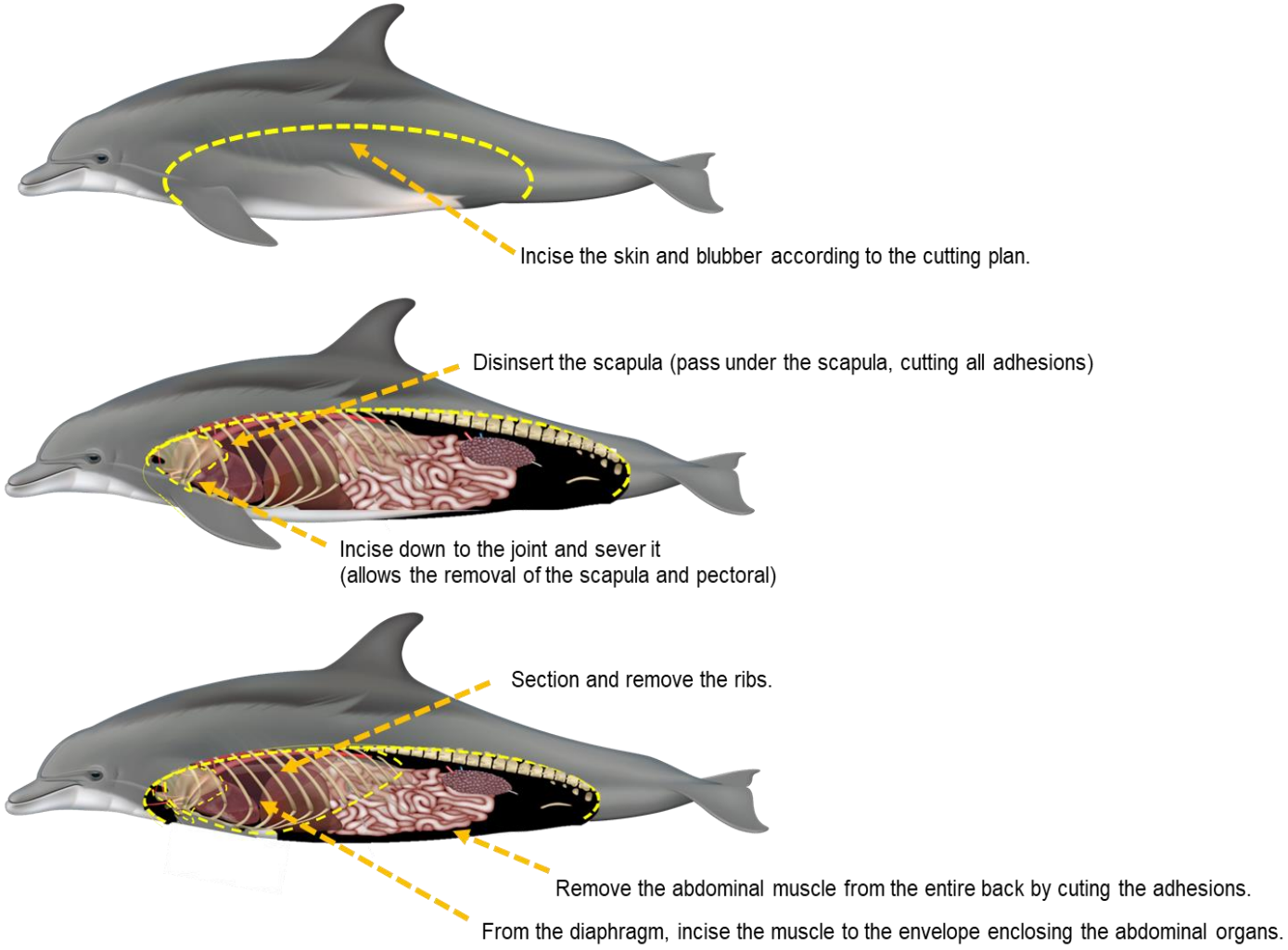
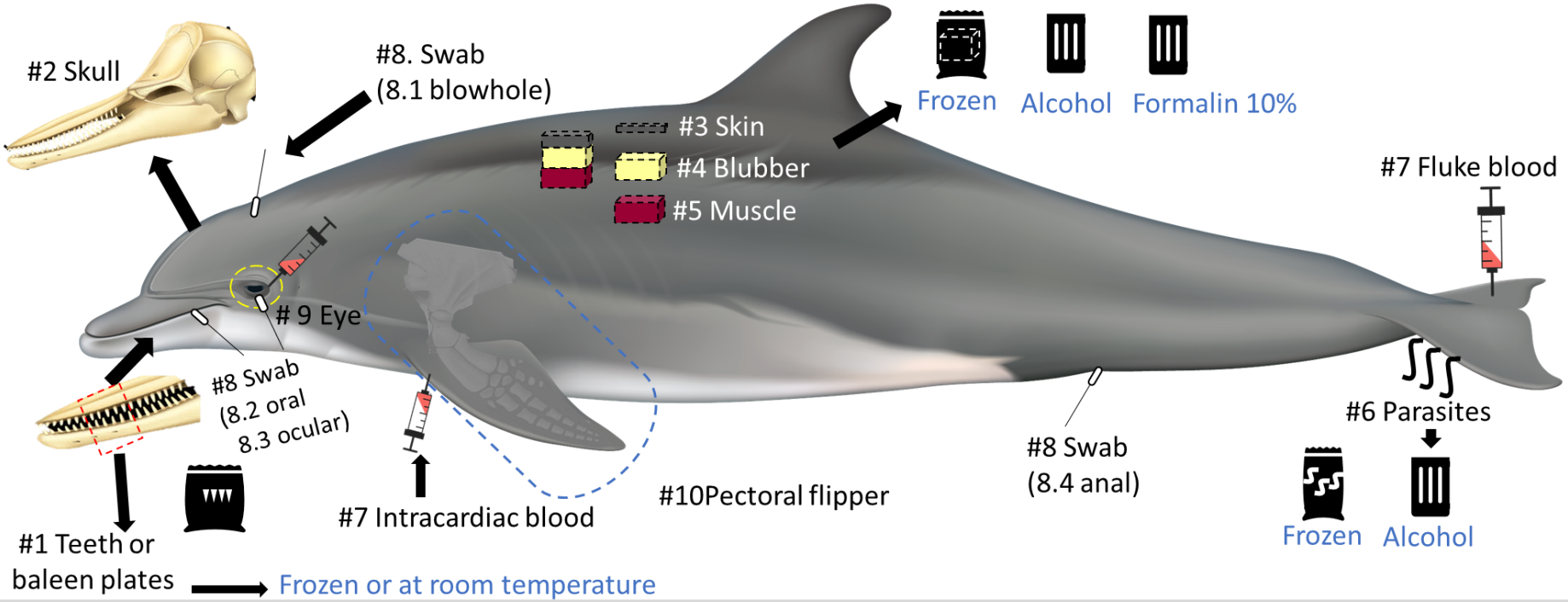


Figure 6. How to start a necropsy on a stranded cetacean

2.3.2 List of samples to collect for basic sampling protocol* *(Adapted from Ijsseldijk et al., 2019)**

Organ to sample **(cause of death cannot be determined!)*	**Analyses:** ✓ life history **(Max DDC=5)** ✓ DNA **(Max DDC=3)** ✓ Biomarkers **(Max DDC=1)** ✓ microbiology (virus, bacteria, biotoxins) **(Max DDC=2-3)** ✓ stable isotope &fatty acids **(Max DDC=5)** ✓ serology **(Max DDC=2)** **Storage:** plastic bag in Freezer (-20 to -80°C)				**Analyses**: contaminants **(Max DDC=3)** **Storage:** wrapped in aluminum foil and put in plastic bags in freezer (-20 to -80°C)		**Analyses**: histology (pathology & reproduction) **(Max DDC=3) (no more than 1 cm thick!)** **Storage:** plastic container with 10% buffered formalin (9 times liquid versus tissue), at room temperature		**Analysis:** parasitology **(Max DDC=4)** **Storage:** Plastic vial with alcohol 10% in freezer (-20 to -80°C), and one vial at room temperature	
	What for	Container	Sample Size	Number of samples	Sample Size	Number of samples	Sample Size	Number of samples	Sample size	Number of samples
Teeth	Life history	Sterile plastic bag	5 teeth collected in the middle of the jaw							
Skull	Life history	Plastic bag	All							
Skin	DNA Microbiology	Sterile plastic bag	5x5 cm	2 ☐			3x3 x **0.5 cm**	1 ☐		
Blubber	Stable isotopes & fatty acids Microbiology	Sterile plastic bag	5x5x5 cm	2 ☐	5x5x5 cm	1 ☐	5x5x**1 cm**	1 ☐	Several parasites	2 ☐
Muscle	Stable isotopes & fatty acids Microbiology	Sterile plastic bag	5x5x5 cm	2 ☐	5x5x5 cm	1 ☐	5x5x**1 cm**	1 ☐	Several parasites	2 ☐
External parasites (mites & lice)									Several parasites	2 ☐
Complete blood (if less than 16 hours of death)	Microbiology Serology	Blood collector tube (red cap)	5-10 ml	2 ☐						
Eye-fluid (alternative to blood if carcass not fresh)	Serology	Sterile vial 1.5 ml	3 ml	1 ☐						
Eye and ocular swab	Microbiology Histology	Sterile plastic bag	All organ (1 eye)	1 ☐ 1 from eye ☐			(2^{nd} eye) (inject formalin)	1 ☐		
Respiratory swab	Microbiology	Sterile swab stick	1 from blowhole ☐							
Digestive swabs	Microbiology	Sterile swab stick	1 from anus ☐							
Scapula	Life history	Plastic bag	All organ	2 ☐						

Basic samples to collect and preserve (DDC 2-3)



Basic samples to collect from a stranded cetacean 1. Teeth sample: Count the total teeth (or baleen) and empty sockets and collect 4-5 teeth or baleen (from middle part of the jaw), store frozen or dry in a bag. **2. Skull** can be preserved by different methods (if collected try to get a brain and cerebro espinal fluid samples). **Samples #3 skin, #4 blubber, # 5 muscle** cut a square of 5x5 cm using a knife or blade and tweezers and separate them. The samples for formalin should have maximun 1 cm thick. The alcohol could be 70% ideally at 95% (even vodka and gin works!). They can be frozen directly. Blubber ideally should be wrap first with aluminium paper foil and then put it in a bag.**6.External parasites.** Storage in alcohol at room temperature and/or frozen. **7. Complete blood** can be collected ideally intracardiac untill 16 hours after death. For serum deposit it in red cap blood tubes. **8.Swabs:** Sterile swabs from the blowhole, oral, ocular and anal can be collected for disease diagnoses .**9. Eye:** both eyes can be collected for serology, and histopathology **10.Pectoral flipplers**: can be collected and frozen for later interpretation of age class by Xrays. **IMPORTANT: remember to label each bag with the case identification and date!**

2.3.3 List of samples to collect for an extensive data collection (Adapted from Ijsseldijk et al., 2019)

Organ to sample	**Analyses:** ✔ life history **(Max DDC=5)** ✔ DNA **(Max DDC=3)** ✔ Biomarkers **(Max DDC=1)** ✔ microbiology (virus, bacteria, biotoxins) **(Max DDC=2-3)** ✔ stable isotope &fatty acids **(Max DDC=5)** ✔ serology **(Max DDC=2)** **Storage:** plastic bag in Freezer (-20 to -80°C)				**Analyses**: contaminants **(Max DDC=3)** **Storage:** wrapped in aluminum foil and put in plastic bags in freezer (-20 to -80°C)		**Analyses**: histology (pathology & reproduction) **(Max DDC=3) (no more than 1 cm thick!)** **Storage:** plastic container with 10% buffered formalin (9 times liquid versus tissue), at room temperature		**Analysis:** parasitology **(Max DDC=4)** **Storage:** Plastic vial with alcohol 10% in freezer (-20 to -80°C), and room temperature	
	What for	Container	Sample Size	Number of samples	Sample Size	Number of samples	Sample Size	Number of samples	Sample Size	Number of samples
Tonsils	Microbiology	Sterile plastic bag	5x5 cm	1 ☐			3x3x1cm	1 ☐		
Lung		Sterile plastic bag	5x5 cm	1 in upper location ☐ 1 in lower location ☐			3x3x1cm	1 ☐	Several parasites	2 ☐
Lymph nodes		Sterile plastic bag	Half	1 ☐			half	1 ☐		
Heart/ cardiac muscle		Sterile plastic bag	5x5 cm	1 ☐			3x3x1cm	2 in different regions ☐		
Liver		Sterile plastic bag	5x5 cm	2 ☐	5x5 cm	1 ☐	3x3x1cm	1 ☐	Several parasites	2 ☐
Pancreas		Sterile plastic bag	5x5 cm	1 ☐			3x3x1cm	1 ☐		
Kidney		Sterile plastic bag	5x5 cm	1 ☐	5x5 cm	1 ☐	3x3x1cm	1 ☐		
Adrenal glands		Sterile plastic bag	5x5 cm	1 ☐	5x5 cm	1 ☐	3x3x1cm	1 ☐		
Spleen		Sterile plastic bag	half	1 ☐			half	1 ☐	Several parasites	2 ☐
Thyroid gland		Sterile plastic bag	5x5 cm	1 ☐	5x5 cm	1 ☐	3x3x1cm	1 ☐		
1r. Stomach		Sterile plastic bag	5x5 cm	1 ☐			3x3x1cm	1 ☐		
2d. Stomach		Sterile plastic bag	5x5 cm	1 ☐			3x3x1cm	1 ☐		

Organ to sample	**Analyses:** ✔ life history **(Max DDC=5)** ✔ DNA **(Max DDC=3)** ✔ Biomarkers **(Max DDC=1)** ✔ Microbiology (virus, bacterias, biotoxins) **(Max DDC=2-3)** ✔ Stable isotope &fatty acids (**Max DDC=5)** ✔ Serology **(Max DDC=2)** **Storage:** plastic bag in Freezer (-20 to -80°C)				**Analyses**: contaminants **(Max DDC=3)** **Storage:** wrapped in aluminum foil and put in plastic bags in freezer (-20 to -80°C)		**Analyses**: histology (pathology & reproduction) **(Max DDC=3)** **Storage:** plastic container with 10% buffered formalin (9 times liquid versus tissue), at room temperature		**Analysis:** parasitology **(Max DDC=4)** **Storage:** Plastic vial with alcohol 10% in freezer (-20 to -80°C), and room temperature	
	What for	Container	Sample Size	Number of samples	Sample Size	Number of samples	Sample Size	Number of samples	Sample Size	Number of samples
Stomach content	Microbiology	Sterile vial	10 ml	1 ☐					Several parasites	1 ☐
	Diet from prey analysis	Sterile plastic bag	Entire content ☐							
Intestine	Microbiology	Sterile plastic bag	5x5 cm	2-3 regions ☐			5x5x1 cm	2-3 regions ☐	Several parasites	1 ☐
Faeces		Sterile vial	1-10 ml	1 ☐					Several parasites	1 ☐
Testicles/Ovary		Sterile plastic bag	half	1 ☐			half	1 ☐		
Milk		Sterile plastic vial	1-10 ml	1 ☐						
Placenta		Sterile plastic bag	5x5 cm	1 ☐	5x5 cm	1 ☐	5x5x1 cm	1 ☐		
Fetal tissue (liver,kidney,lung, brain and stomach content)		Sterile plastic bag	3x3 cm	1 ☐	3x3 cm	1 ☐	5x5x1 cm	1 ☐		
Mammary gland		Sterile plastic bag	5x5 cm	1 ☐			5x5x1 cm	1 ☐		
Brain		Sterile plastic bag	5x5x5 cm	2-3 regions ☐			Half brain with cuts in the tissue so the formalin can penetrate adequately	1 ☐		
Cerebro spinal fluid	Microbiology	Syringe or 1.5 vial	0.1-5 ml	1-3 ☐						

2.6 REFERENCES

This guide has been developed by the CARI'MAM network, in the framework of the CAMAC project, from the following referent documents:

1. IWC (2016) Report of an IWC Workshop Developing Practical Guidance for the Handling of Cetacean Stranding Events. IWC/66/WKM&WI Rep02
2. SPAW RAC (2007) Protocols and techniques for responding to strandings
3. Van Canneyt O, Dabin W, Dars C, Dorémus G, Gonzalez L, Ridoux V, Spitz J (2015) Guide to marine mammal strandings. Technical booklet of the Observatoire PELAGIS.
4. Geraci J.R. & V.J. Loundsbury. 2005. Marine Mammals Ashore: A field guide for strandings. 2nd ed. National Aquarium in Baltimore, Baltimore, MD.
5. Gulland, Frances MD, Leslie A. Dierauf, and Karyl L. Whitman, eds. 2018. CRC handbook of marine mammal medicine. 3rd ed CRC Press, Boca Ratón, Fl, USA.
6. Pugliares, K. R., Bogomolni, A., Touhey, K. M., Herzig, S. M., Harry, C. T., & Moore, M. J. (2007) Marine mammal necropsy: an introductory guide for stranding responders and field biologists.Woods Hole, MA., Woods Hole Oceanographic Institution, 133pp. (WHOI Technical Report 2007-06). DOI: https://doi.org/10.1575/1912/1823
7. IJsseldijk, L. L., Brownlow, A. C. and Mazzariol, S. (2019) European Best Practice on Cetacean Post-Mortem Investigation and Tissue Sampling. Documentation. ACCOBAMS/ASCOBANS. (Doi: 10.31219/osf.io/zh4ra).
8. Ward, N., Bogomolni, A, and Potter, C. (2013). A Stranding Guide to the Marine Mammals of the Wider Caribbean Region: An introductory field guide for stranding responders, CEP Technical Report 74.can

Additional information and training resources is available on: https://www.car-spaw-rac.org/?Stranding-networks-1306.

If you need support, think link will give you access to the contacts of the caribbean stranding networks https://www.car-spaw-rac.org/?Caribbean-stranding-expert-contacts.

ANNEX1. CHECKLIST FOR VISUAL ASSESSMENT

Assessment checklist

Describe the situation:

☐ Adult ☐ Juvenile ☐ Newborn ☐Male ☐Female ☐ Solitaire ☐ Companion group nearby, ☐ nr

Beached:

☐ Breathing intervals _____
☐ Mucus at blowhole ☐ Blood in: ☐ Blowhole ☐ Mouth ☐ Anus

In water:

☐ In water < waist deep. ☐ In water > waist deep.
☐ Entangled ☐ In distress ☐ Calving ☐ Actively swimming around ☐ Drifts and swims steadily
☐ Drifting sideways ☐ Resting (one eye closed) ☐ Drifting in vertical position ☐ Sinking to bottom
☐ Repetitive circle formation ☐ Repetitive beach approaches

Beached and in water:

☐ Emaciated
☐ Injury: ☐ mild ☐ severe
☐ Cuts ☐ Hematomas ☐ Bite marks ☐ Rake marks ☐Fresh scars ☐Old scars ☐ Bleeding

INTERVENTION checklist:

☐ Protection from crowd/boats ☐ Disentangle ☐ First care on beach ☐ Transport to water
☐ Support in water ☐ Monitor ☐ Leave undisturbed.
☐ Relocate to another sea area ☐ by boat ☐ by vehicle ☐ by walking
☐ In situ medical support (Vet). ☐ Transport to rehab facility
☐ Euthanize

Outcome :

☐ swam away on its own/date
☐ released in the wild/date
☐ died/date