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Guidelines for Developing Marine Turtle Strandings Networks and Protocols for Data Collection

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Executive summary

The five species of marine turtle found in the Mediterranean: the loggerhead *Caretta caretta*, the green turtle *Chelonia mydas*, the lute turtle *Dermochelys coriacea*, the imbricated turtle *Eretmochelys imbricata* and the Kemp's turtle *Lepidochelys kempii* are listed in Annex II, the List of Endangered or Threatened Species, to the Protocol on Specially Protected Areas and Biological Diversity in the Mediterranean (Barcelona, 1995). These species are indeed subjected to different threats, mainly an increasingly great mortality due to fishing activities and loss of habitats that are vital for these reptiles at sea (wintering and feeding areas) and on land (nesting beaches).

Faced with this situation, and as a result of increasingly great international concern as to the status of marine turtles in the Mediterranean, in 1989 the Parties to the Barcelona Convention adopted the Action Plan for the Conservation of Mediterranean Marine Turtles. Among the priorities of this Action Plan, adopted in 1989 and revised in 1999 and 2007, it is stated in Paragraph III.2 on research and monitoring that it is necessary to improve knowledge about data collection via strandings networks.

A stranded turtle indeed would constitute a mine of information on biology (growth, reproduction, sex ratio, etc.), ecology (biogeography, population, diet, etc.) and health (toxicity, parasites, etc.). Furthermore, the presence of stranded animals on the beach would indicate that incidental fishing was happening in the region, and the study of strandings could give an idea about the level of bycatch. Such information is of great importance for conservation and avoids the sacrifice of such threatened species.

To this end, the Plan provides for the crafting by RAC/SPA of protocols for data collection on strandings of marine turtles, for the Contracting Parties to set up strandings networks.

The present report falls within the framework of implementing the Action Plan for the Conservation of Marine Turtles, and aims at:

- helping the countries in developing marine turtle strandings networks
- Drafting protocols for data collection via stranded turtles.

The main aims of a strandings network lie in collecting information and samples from the greatest possible number of strandings, and making these available to the national or regional scientific community. This requires the publishing of regular reports and the crafting of tissue banks, but first and foremost standardised protocols and skills. A strandings network should also help develop public awareness.

To attain such objectives, the collection of appropriate information from living or dead stranded turtles requires team organisation for a quick, efficient response using the appropriate means. To work well, a strandings network should possess:

- equipment to examine and transport the animals when necessary
- facilities for treating and rehabilitating live animals
- facilities for doing autopsies on carcasses
- Several involved institutions: research institutes, universities, NGOs, fisheries administrations, rescue centres, tissue banks etc.

It should however be said that setting up, developing and managing strandings networks should not give rise to enormous expenses and should not be subordinated to this economic aspect. In the same way, a strandings network on marine turtles could concern other predator species at the top of the food chain and that are endangered, like cetaceans.

In the Mediterranean, few networks concern marine turtles. Some are local and do not cover all the country's coast (Italy, Spain), Greece and recently Tunisia, have national strandings networks.

In several other countries each researcher collects his/her data on the beaches where s/he works. However, there are sometimes scientific reports now and then from local or public NGOs. As a result, information on stranded turtles is rather sketchy, either between scientists or local NGOs. Networking such activity on a national or even regional scale is necessary.

As for cetaceans, it should be said that recently much has been done to study them and create cetacean strandings networks. These networks (six or seven in the entire Mediterranean) could develop to deal in parallel with turtle strandings, or even strandings of other predator species at the top of the food chain, like sharks. The Valencia (Spain) and Tunisian networks handle the first two groups of species.

Considering:

- the importance of studies on marine turtle strandings at the level of conservation biology
- the presence of sometimes very old strandings networks in the Mediterranean, and thus of experience
- the scarcity of information on marine turtle strandings in the Mediterranean
- the interest shown by scientists in this kind of study, even in the absence of a specific strandings network
- the development of strandings networks on cetaceans in the Mediterranean that could also extend to turtles or even other marine vertebrates
- the possibility of involving several teams (several disciplines) and several institutions
- the possibility of not spending a lot of money,

It is recommended that national strandings networks be developed in all the Mediterranean countries according to the Guidelines that are the subject of this work. This makes an enormous contribution to the implementing of the Action Plan for the Conservation of Mediterranean Marine Turtles. RAC/SPA could contribute alongside these Guidelines especially by training teams on the spot. Developing strandings networks is in fact very easy to do when there are the necessary skills to hand.

I. INTRODUCTION

Strandings constitute a very important source of information and knowledge on marine turtle populations and also on other groups of threatened animals such as cetaceans and some elasmobranchs. Such knowledge is of great importance for conservation. A stranded animal is in fact a mine of information on biology (growth, reproduction, etc.), ecology (migration, population, diet etc.) and health (toxicity, parasites, etc.). Furthermore, the presence of stranded animals on the beach would indicate that incidental fishing was happening in the region and the study of strandings could give an idea about the level of bycatch. However, the method remains rather unreliable. Research on stranding activities should be done in all Mediterranean countries. But much has to be done to set up more homogeneous strandings networks on all national coasts to improve communication, the flow of information and the collection of stranded specimens in a centralised site, to provide regular reports on strandings and to promote the scientific use of the biological material obtained within the network.

Among the priorities of the Action Plan for the Conservation of Mediterranean Marine Turtles, adopted in 1989 and revised in 1999 and 2007, it is stated in Paragraph III.2 on research and monitoring that it is necessary to improve knowledge about data collection via the strandings networks.

To this end, the Plan provides for the crafting by RAC/SPA of protocols for data collection on strandings of marine turtles, for the Contracting Parties to set up strandings networks.

The present report falls within the framework of implementing the Action Plan for the Conservation of Marine Turtles, and aims at:

- Helping the countries in developing marine turtle strandings networks alongside other networks, on cetaceans, for example
- Drafting protocols for data collection via stranded turtles.

The present report first takes stock of the state of knowledge on the strandings networks that exist in the Mediterranean, in particular on marine turtles, and analyses their weak and strong points.

II. STRANDINGS NETWORKS EXISTING IN THE MEDITERRANEAN

To get an idea about the strandings networks existing in the Mediterranean we have mainly used books, contacts with some experts at the Third Conference on Marine Turtles (Yasmine Hammamet, Tunisia, 20-23 October 2008) followed by a request for information on strandings networks in the Mediterranean using a questionnaire (Annex 1). However, we received few replies.

II.1. Importance of strandings networks and groups of species concerned

Marine turtles are seriously threatened throughout the Mediterranean region. Human-origin and natural mortality is great. A recent upwards trend of dead stranded turtles has even been noted in several parts of the Mediterranean. There were more strandings in 2007 and 2008 than have been recorded since 1990 (Aliki Panagopoulou, medturtles). Similarly, in the northern Adriatic, over 220 km. of coast, there were 144 strandings in 2007 (134 dead individuals), the highest figure since 1993. Usually 100 specimens are recorded each year (Marco Affronte, medturtles). On the Mediterranean coast of Morocco, an increase in strandings has been noted for lute turtles (Alvaro G. de los Rios y Loshuertos, medturtles). However, there is very little information on marine turtle strandings in the Mediterranean, especially when compared with the data collected in the context of the marine turtle strandings and rescue networks in the United States.

In the Mediterranean, few national networks deal with marine turtles. However, from time to time sketchy, sparse studies on stranded turtles by small teams are published, but these rarely make full use of a stranded turtle or put the information and samples at the disposal of scientists. In many countries each researcher collects his/her data on the beaches where s/he works. There are sometimes scientific reports now and then from local or public NGOs. As a result, information on stranded turtles is rather sketchy, either between scientists or local NGOs. Networking such activity on a national or even regional scale is necessary.

Marine turtle strandings networks on a national or local scale have been developed in certain countries and work relatively well. We cite in particular Greece, Spain, Italy and Tunisia. In the last country the network concerns both marine turtles and cetaceans.

Some networks are now very old and have been improved over time with the collection of data. Already in 1988 Valencia University (Spain) set up its network on the Spanish coast of the central Mediterranean. The ARCHELON association developed a national network in Greece in 1990 (300 stranded turtles/year are on average sighted by 236 stations involved in signalling strandings along the Greek coast). In 1992 the Naples Zoological Station (Italy) set up a local marine turtle strandings network along Italy's south-western coast.

The situation is not much better for cetaceans but it should however be said that much has been done recently for cetacean studies and the setting up of cetacean strandings networks. These networks (six or seven for the entire Mediterranean) could develop to simultaneously handle turtle strandings or even strandings of other predator species at the top of the food chain, such as sharks.

Since the remains of the world *Monachus monachus* monk seal population is mainly found in Greece, monitoring of strandings of this species is mentioned there.

No shark network exists in the Mediterranean. Some sightings have however been recorded in the MEDLEM (MEDiterranean Large Elasmobranchs Monitoring) database.

II.2.Institutions involved and cooperation with other networks

Usually, when it exists, a strandings network is an initiative of research or university institutions or of a NGO, supported by the authorities.

The strandings networks set up enjoy close collaboration with

- Especially, marine turtle rescue and care centres that have now been developed in many countries
- Tissue banks. In the Mediterranean, two tissue banks are known:

  - The Padua Bank
    A tissue bank for Mediterranean marine mammals
    Department of Experimental Veterinary Science, University of Padua
    Vialle dell'Università 16 35020 Legnaro – Agripolis (PD) – Italy
    Web site: http://www.sperivet.unipd.it/tissue bank/

  - The Barcelona Bank
    GRUMM-GBC, Department of Animal Biology (Vertebrates), Faculty of Biology, University of Barcelona 08028 Barcelona – Spain

- other strandings networks like MEDACES (Mediterranean Database of Cetacean Strandings). This database was set up to coordinate all national and regional efforts for countries bordering on the Mediterranean. This project was created in accordance with the Barcelona Convention, extended to the ACCOBAMS area. It is currently funded by the Spanish Ministry of the Environment, the rural world and maritime business. (http://medaces.uv.es/home_eng.htm)

II.3.Data collection and presentation

All the networks or even individual researchers have a data collection file. Standardisation of the data collection file was deemed necessary for the Mediterranean region. A database was set up as a result. It should be said, furthermore, that few of these structures bring out a regular report on marine turtle strandings.

II.4.Pertinent results

Marine turtle strandings happen along the entire Mediterranean coast and principally concern the *Caretta caretta* loggerhead turtle, which is anyway the most common in the Mediterranean, with known major nesting and feeding areas. Strandings of the *Chelonia mydas* green turtle are regularly observed, mainly in Greece and Turkey. The lute turtle is rarely seen on the Mediterranean coast and a stranding of this species can be seen from time to time. Anyway, there are more strandings of the lute turtle on the Moroccan coast near to the Atlantic (Alvaro, 2008), since this coast represents the most important wintering area in the Mediterranean for the species (Alvaro, 2005).

About a quarter of the strandings are the result of fishing activities and as many or more die from boat accidents, which peak in the summer, especially on the northern shore of the Mediterranean. Fishing problems are observed throughout the year and in all the Mediterranean countries.

Several turtles die from bad health conditions, conditions that prevent them feeding before being stranded, or after ingesting human-origin debris. Compared to captured turtles, prey is infrequent and not abundant and debris is abundant in the contents of the stomach, composed of benthic and pelagic prey. The abundance of pelagic prey and floating debris shows that the turtle feeds in the water column when health conditions do not allow it to dive and feed on benthic prey (Tomas *et al*., 2008).

The size classes that are most represented in strandings are those of juveniles and sub-adults (CCL between 50 and 70 cm.).

We can also note several other results:

- Collection of historical data (data over 20 years old)
- Important knowledge on parasitology, feeding ecology, epibionta, cetacean and marine turtle genetics
- The rehabilitation and liberation of dozens of marine turtles and dolphins in certain rescue centres
- Success in public awareness on species and the need for conservation
- Detection of the most important human-origin threats (including fishing) that affect marine turtles and cetaceans
- A marking/tagging programme is usually grafted onto the network's activities.

A list, which is not exhaustive, appears in Annex 2 to this report.

III.GUIDELINES FOR DEVELOPING MARINE TURTLE STRANDINGS NETWORKS

III.1. Aims

A strandings network's general aims should mainly focus on:

- the efficiency and speed of the stranding report
- signalling the most strandings possible. Besides the declared strandings systematic prospections should be planned (choose the spring tide period…and the place and weather)
- putting at the disposal of scientists as much information and as many samples as possible, to develop knowledge on these species' conservation biology
- Setting up a tissue bank
- Regularly publishing a report on strandings in a scientific journal
- Providing expertise to the local and national authorities, local communities and various partners on managing strandings
- Developing public awareness

III.2.The necessary means

To attain such aims, collecting the appropriate information from a stranded live or dead turtle requires team organisation for quick and effective response with the appropriate means. For this to work well, a strandings network should possess:

- a warning mechanism (24/24 hour phone service) to quickly signal the stranding of live, wounded or dead turtles
- an action team on the spot to report the event
- equipment to examine and transport the animals when necessary
- a data collection protocol
- facilities for treating and rehabilitating live animals
- facilities for doing autopsies on carcasses
- staff (veterinary biologists) who are qualified and trained for such intervention (determining species, measuring, autopsy, rehabilitation, etc.) and/or working with specialist institutions
- several involved institutions: research institutes, universities, NGOs, fishing administrations, ministries of the environment, of defence and of the interior, rescue centres, tissue banks

But it must be said that setting up, developing and managing strandings networks should not give rise to enormous expense and should not be subordinated to this economic aspect. In the same way, a strandings network on marine turtles could concern other predator species at the top of the food chain and that are endangered, like cetaceans, elasmobranchs and even seabirds.

III.2.1. Team organisation and the sighting of strandings

The strandings network to be set up should concern the country's entire coast. However, according to the length and features of the coast, the general context of the country and the status of the marine turtles, several work teams could be envisaged. Each team is coordinated by a leader; a national coordinator coordinates the activities of all the teams (Bradai *et al*., 2008).

An awareness effort and requests for aid and collaboration from the various users of the sea and the authorities would be necessary so that information reach the work groups. Information on the importance of studying strandings and the names of the various actors with their respective cellular phone numbers (a non-paying green number for this is advisable) should be widely circulated to the target administrations and people.

III.2.2. Rules of intervention in the field

The action of experts in the field must bear the following in mind:

- quick action by the experts after a stranding is signalled (make sure the necessary material for the terrain is available and ready for use)
- coordination with the authorities, volunteers and institutions involved in the network
- respect for public health
- avoid stress for live animals
- scientific decision-making

III.2.3. Basic field equipment

- latex gloves
- data collection files
- 'waterproof' markers
- measuring equipment (tape measure, calliper rule) and weighing equipment (scales, dynamometer)
- knives, scissors, scalpel, plastic knives, string
- appropriate bottles for the various samples
- aluminium foil and unused plastic bags
- coolboxes
- chemical products (alcohol, formalin, etc.)
- first aid kit
- photo and video cameras

III.2.3. Basic data collection

The basic information to be collected after a marine turtle stranding, and that should be the subject of a file (Annex 3) if as follows:

- name, address and phone number of the observer
- code of the region where the stranding happened when there are several teams in the national network
- date and hour of the stranding or of the observing of the carcass
- exact location (latitude/longitude, place)
- exact identification by a qualified person and description of the animal (size, weight, sex, colour, etc.). A photo is highly desirable. A key for determining Mediterranean marine turtle species appears in Annex 4
- condition and state of the turtle (live, recently dead, moderately decomposing, severely decomposed, dried carcass, remains of skeleton) (Annex 5). If the carcass already smells bad, the turtle is not recently dead.

The stranding report must also mention and locate on the body any anomaly, wound, collision accident, pollution by hydrocarbon, presence of marks, epibionta, remains of fishing gear – nets, hooks etc.

The historical data gathered here would constitute an important database and mainly serve to determine:

- the distribution in time and space of the strandings (include stranding on egg-laying sites)
- the stranded species
- the causes of death
- the sex ratio
- the size structures

a. Causes of death

Although many causes of death are uncertain after examination of the stranded animal, some deaths can be easily attributed to the following causes:

Natural causes

- disease
- depredation
- nesting

Human-origin causes

- fishing activity (presence of hook, turtle entangled in nets or ropes)
- collision with boats and propellers/fractures
- ingestion of foreign matter (plastic bags, etc.)

The data gathered must be analysed and checked against existing data on the fishing effort, the size of the fleet, the fishing gear used and the interaction with fishing.

Taking samples of parts of the body and organs where the stranding happened or after autopsy in a laboratory is also to be anticipated for possible studies on the life cycle and health of marine turtles. Work protocols for the taking and conserving of samples of tissues and other things must be crafted beforehand (see below).

III.2.5. Autopsies and the taking of samples

The first examination is the inventorying of the event, the describing of the species and the acquiring of biometric data. Necropsies (autopsies) aim at assessing the causes of death, the pathologies and parasitism of the stranded animals, and any other memorable fact. The information obtained supplies information about the state of health of the animal and populations, age classes, reproductive health, etc. The samples allow biological material to be acquired that is needed for various analyses, especially toxicological ones. Moreover, they enable a bank of tissues available to be built up later, particularly during specific national or international research programmes.

According to the case, autopsies could be done *in situ* or in the laboratory (Annex 6).

a. Taking and storing samples

Taking samples for additional analyses and examinations that are specific to the pathologies and life cycle of turtles is preferably only done on animals that are in a good state of freshness (firm, intact skin, animal that has not swollen up, viscera that are not distended by putrefying gases, etc.).

When the animal is in a state of fairly advanced putrefaction (lacerated skin, viscera distended by putrefying gases, very rotten smell, etc.), the sampling would be restricted as far as possible to the digestive contents after opening up the oesophagus, and to the muscles.

Two labels should be placed on the samples of tissue, liver, spleen, gonads, stomach contents and parasites, one on the inside and the other on the outside of the container. Each label should show:

- the reference of the autopsy or the animal
- the date of sampling
- identification of the tissue
- the destination of the sample (histology, microbiology, parasitology, toxicology, biology, genetics).

The epibionta attached to the animal's body and the humerus of the carcasses are also retained for respectively studies of migrations and of age.

The precautions to be taken for the different samplings, the fixatives and the storage techniques are set out in detail below.

III.2.6. Tissue bank

Strengthening a strandings network and achieving its objectives involves *inter alia* setting up a tissue bank on a national scale where specimens and samples from the network are stored and made available to the scientific community on request. Where means are lacking, collaboration with Mediterranean tissue banks is desirable.

Ideally, each Mediterranean country that is a Party to the Protocol on Specially Protected Areas and Biological Diversity would set up its own bank. However, a coordinated Mediterranean network should be set up to act as a link between the various tissue banks.

Contacts, exchanges and research programmes should follow the CITES protocols and national and international legislation on threatened species.

The tissue bank aims at receiving and freely distributing samples of animal tissue and information on these animals. The bank should represent a link between research groups that are active in the strandings networks and scientists in that country or the entire Mediterranean.

The bank's aims are the following:

- Collecting and storing tissues systematically and in well documented fashion
- Providing histology samples for retrospective or new analyses of interest
- Comparing the results over time
- Conserving tissues for genetic studies
- Storing biological liquids.

The bank takes samples of all the organs of stranded animals and keeps them in 10% neutral buffer formalin, DMSO, alcohol or frozen; the bank also keeps biological liquids for biochemical studies.

III.2.7. Treating and rehabilitating stranded live animals

These facilities are found in marine turtle rescue centres, now developed in many Mediterranean countries. Such centres could make an effective contribution to the work of the national turtle strandings network, mainly by:

- autopsy of some dead turtles in the laboratory to determine the cause of death
- treating and rehabilitating live or tired stranded turtles that have been brought in when incidentally captured.

Transporting a live (sick or wounded) turtle should be done in a controlled environment and extremes of heat and cold should be avoided. Ideally, the turtle must be protected from dehydration during the journey by the application of a thin layer of Vaseline, for example, over the shell and the soft tissues (except for the eyes). If damp towels are used to avoid dehydration, the turtle must not be allowed to become very cold. In this case, the turtle covered by damp towels must not be transported in an air-conditioned environment.

The transporting or moving of live turtles, carcasses or samples within or outside the country usually requires permits from the appropriate authority.

On its arrival in the centre, the turtle will be given a full examination and appropriate therapy, when necessary. Several pathological and other problems, frequently found, are effectively treated in a rescue centre: pneumopathy, superficial and deep wounds, removal of hooks.

The water temperature during the care treatment must not be less than 17°C. Individuals kept in a good condition will later be marked and released.

III.2.8. Training staff

The team involved in the strandings network must be qualified and experienced. To this end, participating in training courses is vital for recognising species, conservation biology and doing autopsies on marine turtles. Similarly, participating in seminars and workshops on such subjects is called for. The training courses RAC/SPA organises or supervises, and which are thus beneficial for the staff in question, are:

- A course on monitoring marine turtle egg-laying beaches at the Lara hatchery (Cyprus)
- A course on marine turtle care and rescue at the Naples Zoological Station (Italy)
- The Biology and Conservation European course on Marine Mammals in Valencia (Spain).

IV.BIOLOGICAL, ECOLOGICAL AND HEALTH STUDIES

Once a national network is in place and running, an additional effort must be made to make sure the information from the biological material is available for the study of the pathological and genetic causes of death, and the general biology of local populations. This action is vital; a strandings monitoring network, even when effective, will restrict its efficiency if it only provides basic data.

a.Stomach contents

Analysis of the stomach contents allows the species' diets to be described, and the ecological niche in which they evolve to be determined. It also permits the ways of parasitic and toxicological contamination to be assessed.

They must be kept at -20°C to be identified in the laboratory. Alternatively, 70% alcohol can be used to preserve the stomach contents. Use of formalin should be avoided. Formalin attacks the skeletons of bony fishes.

Beforehand, all the unattached parasites must be extracted.

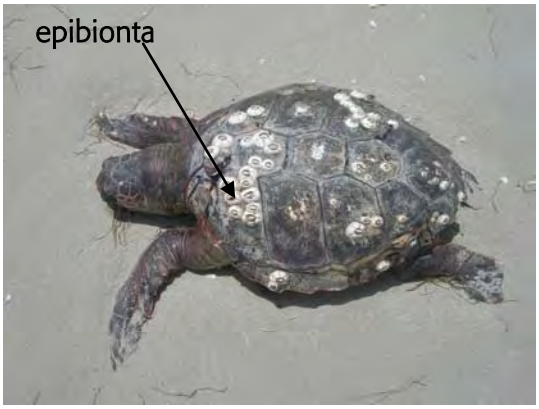
b.Genetic studies

Fragments of tissue of about 1 cubic centimetre are removed, immediately frozen and kept at -30/-80°C or fixed in 70% alcohol or in a 20% dimethyl sulphoxide (DMSO) solution saturated with NaCl.

C.Studies of epibionta

A great number of epibionta attach themselves to marine turtles, especially *Caretta caretta* (L.) (Dodd,1988). These organisms and their relationship with their hosts could reveal bio-geographical differences and provide interesting ecological information.

The epibionta are carefully removed from the live or dead stranded turtles and then fixed and conserved in 70% ethanol for them to be determined and counted in the laboratory.



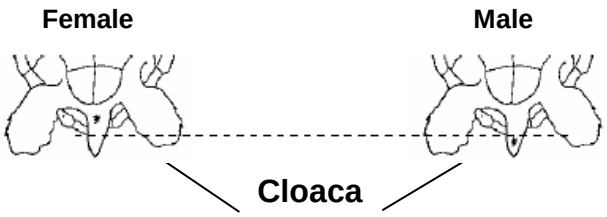
e.Determining the sex ratio

The sex ratio is a very important parameter in population dynamics studies. That of newly born animals could be very easily assessed on the egg-laying beaches, directly by sampling newly born turtles or indirectly by monitoring the temperature of the nests/sand or the period of incubation. For juveniles, since these do not present external sexual dimorphism, the sex ratio can be directly assessed by observing the gonads (necropsy or laparoscopy) or indirectly by using hormonal levels. Adults' sex ratio can be assessed by external observation. In fact, they present sexual dimorphism.

Monitoring strandings could make an enormous contribution to the knowledge of this parameter for juveniles and adults, especially since the latter are very rare.

Adult *Caretta caretta* loggerheads (the most commonly found in the Mediterranean) are over 75 cm. long on the LCC (curved shell length). According to the Casale *et al*. (2005) method, sexing stranded turtles is done in the following way:

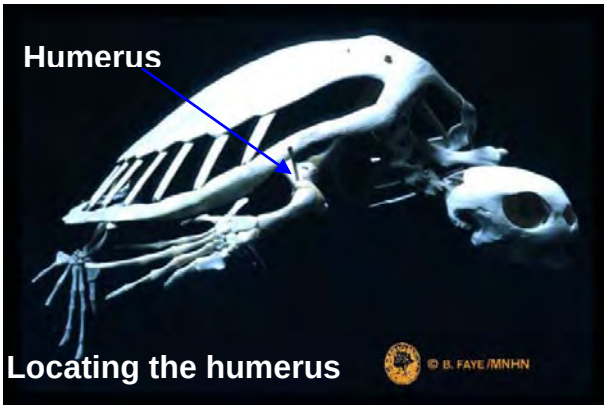
- turn the turtle upside down, with its back against the soil
- align the tail along the body axis
- check the position of the cloaca compared to the hind edge of the shell. The turtle is female if the cloaca is inside this, male if it is outside this (see the sketch below).



f. Determining age

Estimating the age and biological parameters associated with this (age at sexual maturity, growth, longevity, etc.) is vital for demographic studies of natural populations. Skeleto-chronology is a credible way of determining the age of turtles. Its principle is based on the counting of skeletal growth marks annually recorded on different bony structures of poikilothermic animals like turtles, whose rhythm of growth is interrupted or discontinuous. These interruptions of growth are expressed by stria showing a halt in growth on certain parts of the skeleton.

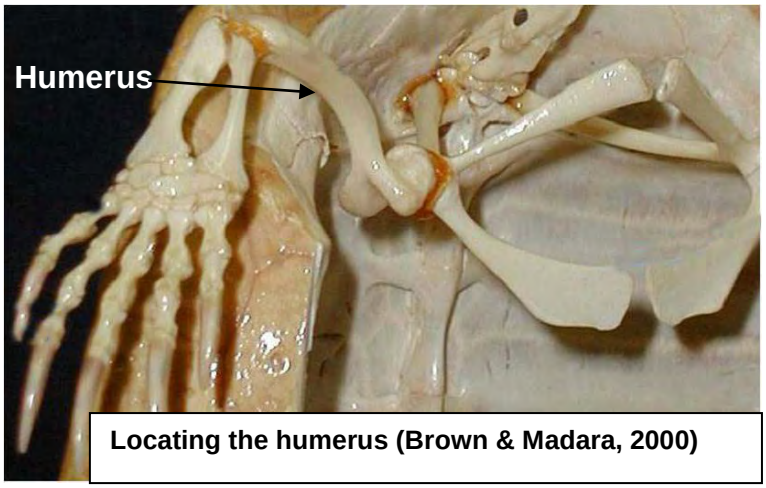
In the case of marine turtles, the humerus is used for this, and can easily be recuperated from dead stranded turtles, avoiding any sacrifice of these threatened species.



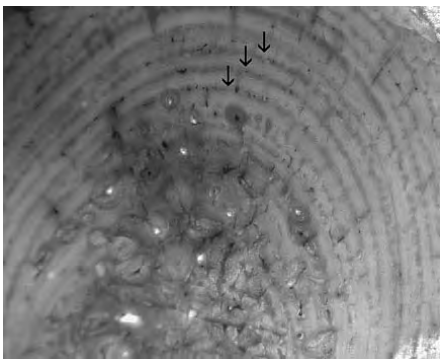
Recuperating and preparing the humerus to read the growth halt stria require the following stages (Snover, 2002):

1. Dissecting the dead turtle and recuperating the humerus
2. Cleaning it, boiling it and then drying it outdoors for about 2 weeks
3. Cutting a section of part of the humerus, about 2-3 mm., using an appropriate microtome
4. Fixing the section by (10%) formalin to make the bone harder during decalcification and cutting
5. Decalcifying the section by a decalcifying agent. The decalcification time depends on the size of the bone (humerus) and the power of the decalcification solution (12-36 hours). The aim of decalcification is to eliminate as much of the calcium as possible to get a better view of the growth halt stria
6. Making the section thinner by using a freezing microtome (the section thus obtained will be about 25µm)
7. Adding a solution of haematoxylin diluted in distilled water (1:1) to be able to see the growth halt stria
8. Mounting the section in (100%) glycerine for reading under a low power stereo microscope

It should moreover be said that this age study must be done by an expert in the subject, but at the level of the strandings network the basic thing is to collect samples and data for the scientific community especially since the material (humerus) can be kept for a long time.



Locating the humerus (Brown & Madara, 2000)



Growth halts stria on a humerus cross-section

g. Toxicology

Samples taken according to standardised protocols of various animal organs allow contaminants to be classified and measured. There are many contaminants – heavy metals (cadmium, mercury, lead, etc.), POPs (persistent organic pollutants (PCB and its like, pesticides, etc.)), dioxins, etc. The aim is to determine the relationship between the toxicity of certain human-origin pollutants and these predators that are high in the trophic chain. The samples must either be sent quickly to the laboratory or frozen at -20°C. A minimum sample of 10 g of tissue (muscle, liver, kidney) should be taken.

The tissue must be cut with a plastic knife and placed in a plastic bag; any metal is to be avoided.

h. Microbiology

The samples should be taken in sterile conditions from lesions (using the sterile Pasteur pipette or the sterile bud) from very fresh animals kept at 4°C to be sent quickly to the concerned laboratory. If they are not analysed in time, the samples must be frozen at -20°C for bacteriology and at -80°C for virology.

i.Parasitology

Unattached parasites are fixed in a 10% formalin solution or in a solution of 70% alcohol with 5% glycerine. Tissues with parasites and parasitic cysts must be refrigerated at +4°C and sent to the laboratory within the space of the following 24 hours to be identified; if not, they must be frozen at -20°C.

k. Histopathology

Tissues from the organs (stomach, intestine, liver, heart, kidney) must be fixed in a 10% formalin solution (preferably buffered at pH 5). Thin tissue cuts must be made (maximum 1 cm thick); one must make sure that the volume of the fixative is at least 10 times the volume of the tissue. In the case where there is a lesion, fix a piece of healthy tissue and another of the affected tissue.

V.ELIMINATING THE CARCASS

For reasons of health and cleanliness of beaches, the carcass should be eliminated either by incineration (to be avoided on the beach for public health reasons) or by burial. But it is advisable to mark the carcass in any case with paint, for example, to show that it has been examined.

The shell or skeleton could be recuperated to be put in a museum or for teaching purposes.

VI.EXTENDING THE TURTLE NETWORK TO OTHER SYSTEMATIC GROUPS

The network could concern other systematic groups: cetaceans, sharks and seabirds. Also, other existing networks, like those for cetaceans, could be extended to handle turtles. Indeed, actors in the field could easily report strandings of big vertebrates. The means implemented and the precautions to be taken during the various samplings, the fixatives and storing techniques are pretty much the same. Marine turtles, cetaceans and sharks could be targeted by a national strandings network.

VI.1. Cetaceans

For cetaceans, see the guidelines for developing national networks for monitoring cetacean strandings crafted by ACCOBAMS. Other guidelines could be useful in this context, e.g.:

- guidelines for setting up a tissue bank system with ACCOBAMS
- guidelines for returning cetaceans to the natural environment

All these documents can be downloaded from the ACCOBAMS site http://www.accobams.org/

VI.2. Elasmobranchs

The system for signalling strandings, the *in situ* action team, and the necessary means are practically the same as for other groups of animals. However, the team involved must include people with knowledge of this systematic group. The main data and samples to be considered when faced with a stranded elasmobranch appear in the file in Annex 7.

As with the other systematic groups, the main aim of a strandings network would be to provide scientists with as much information and as many samples as possible, to develop knowledge on the conservation biology of these, mostly threatened, species.

The strandings network to be developed could restrict itself to some chondrichthyans that are already protected on a regional scale in the context of the Barcelona Convention: mainly the great white shark (*Carcharodon carcharias*), the basking shark (*Cetorhinus maximus*) and the Mediterranean giant Manta ray (*Mobula mobular*) (Annex 8).

Other chondrichthyans also deserve to be monitored and are considered as priority species in the Action Plan for the Conservation of Cartilaginous Fishes (Chondrichthyans) in the Mediterranean Sea; they are listed on the IUCN's Red List, in the Annexes to the Berne and Bonn Conventions, and some have been listed in the CITES Annexes.

These priority species are: the sawfishes *Pristis* spp. (considered as "in critical danger of extinction" (CR) by the IUCN's Red List), the bull-shark *Carcharias taurus*, the tiger shark *Odontaspis ferox* (considered as "in critical danger of extinction" (CR) at Mediterranean level by the IUCN), and the grey pochetau *Dipturus batis* (considered as "endangered" (EN) at Mediterranean level by the IUCN).

The information and measurements to be done in the presence of a shark would be the subject of a file (Annex 7) inspired by that crafted by the MEDLEM programme.

Taking samples of parts of the body and organs is also to be anticipated for possible studies of the biology and health of sharks. The main samples and way of conserving them appear in Table 1 below:

Samples to be taken and conservation method (Source: MEDLEM programme)

	Yes1	No	Alcohol 70%	formalin4%	Freezer	Bouin
Contents of stomach			***	*	*	
Contents of intestines			***	*	*	
Gonads				***		
Muscle			***		*	
Liver				***		
Gills and branchiospines2				***		
Eye						***
Vertebra					***	
Skin				***		
Subcutaneous fat				***		
Spermatophors				***		
Parasites			***	***		
Embryos in the uterus					***	
Uterus				***		

*** Recommended method * Alternative method

1: Yes/No – You take samples or not
2: To conserve the gills and branchiospines, it is recommended to inject 10% formalin (formalin and seawater) for a period of 12-24 hours, and then store the sample after rinsing with water in 80% alcohol

The tissue bank to be set up as part of the strandings studies would be common for all these vertebrates studied.

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Annex 1

Information on strandings networks in the Mediterranean

Country……
Compilor……**e-mail**……
Strandings network yes ☐ no ☐
Set up date.....
National ☐ **local** ☐
Region covered by the network……..
Groups of species concerned

Turtles ☐ **Cetaceans** ☐ **Sharks** ☐

Cooperation
With rescue centre Yes☐ No☐
With tissue bank Yes☐ No☐
With other networks Yes☐ No☐

Institutions involved
……
……

Data
Data collection file Yes☐ No☐
Database Yes☐ No☐
Regular report Yes☐ No☐

Strong points-Weak points
……
…… ……
…
Pertinent results (species, size structure, sex ratio, causes of death) (add pages if necessary)
……
……

List of reports and publications (add pages if necessary)
……
……
……
……
……
……

Annex 2

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Standings of Marine turtles

Data collection file

Observer

Name :.....

Adress :.....

.....**Telephone** :.....

E-mail :.....

Date and Place

Date (jj/mm/aaaa) ☐ **Time (hh :mn)** ☐

Place ☐ **Contry** ☐

Latitudes ☐ **Longitudes** ☐

Specific description of the place:.....

.....

Photo ☐ **Yes** ☐ **No** **video** ☐ **yes** ☐ **no**

Loggerhead *Caretta Caretta*
Green turtle *Chelonia mydas*
Lute turtle *Dermochelys coriacea*
Other

State of the turtle (tick a box)

☐ **Live**
☐ **Recently dead**
☐ **Moderately decomposing**
☐ **Severely decomposed**
☐ **Dried carcass**
☐ **Remains of skeleton**

Marking information

Presence of tag ☐ **Yes** ☐ **No**

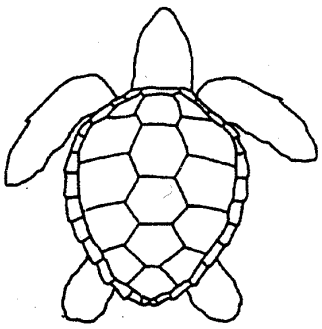
Nombres ☐ **Plastique** ☐ **Métal** ☐ **Couleur** ☐

Position **Right forelimb** ☐ **Left forelimb** ☐

Right hindlimb ☐ **Left hindlimb** ☐

Numéro.....

Adresse.....



Mark the wounds/anomalies on the sketch on the right (also note the presence of oil or tar, the remains of fishing gear, epibionta, damage caused by propellers) etc.

.....

.....

.....

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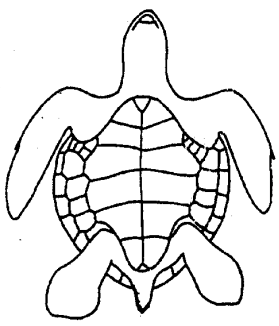


Measuring

Shell curve length (LCC) (cm)……

Shell curve width (lCC) (cm)……

Masse ☐ **Weight** ☐ **Estimate (Kg)**……

Sexing

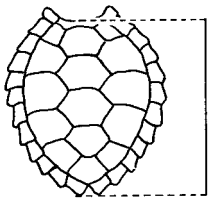
☐ **Immature, undetermined**

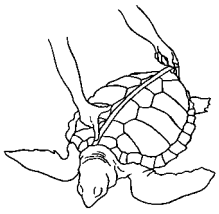
☐ **Female** ☐ **Male**

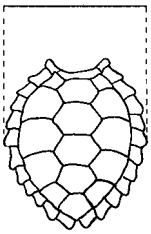
Sex determined by:

☐ **Autopsy**

☐ **Length of tail (for adults)**







Samples taken

☐ **Yes** ☐ **No**

If Yes ☐ **Blood** ☐ **Skin** ☐ **Muscle** ☐ **Epibionta**

☐ **Stomach contents** ☐ **Skeleton** ☐ **Liver** ☐ **Kidney**

Other ☐

State aim of the sampling……

Future of the stranded turtle (tick a box)

☐ **Left where it is on the beach** **Apinted ?** ☐ **Yes** ☐ **No** **Color** ☐

☐ **Buried** ☐ **On the beach** ☐ **Elsewhere**

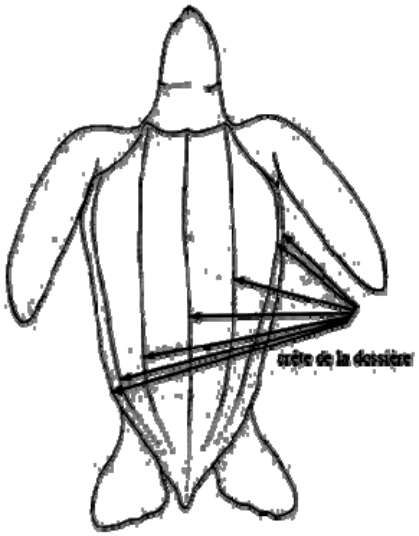
☐ **Recuperated** ☐ **Entirely** ☐ **Partially**

Why ?……

☐ **Live, realeased** ☐ **Live, Kept for rehabilitation**

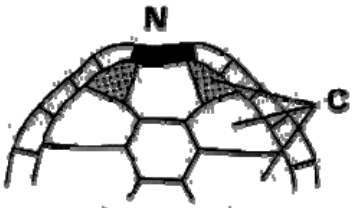
Identification des Tortues marines

sans écailles

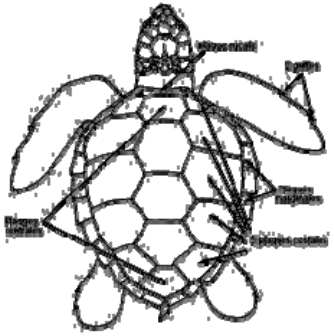


Tortue luth
Dermochelys coriacea
Peau lisse, ressemblant à du cuir ; bleue ou noire avec des taches bleues ou roses

avec écailles



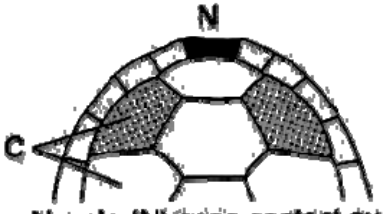
Nucale (N) en contact avec les premières costales (C)
5 paires de costales



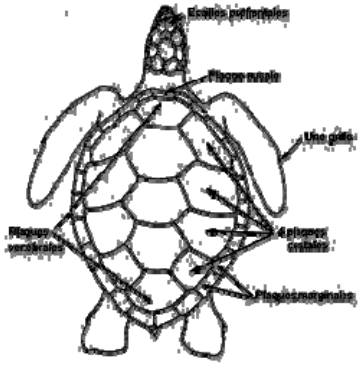
Tortue Caouanne
Caretta caretta

Grosse tête
Carapace plus longue que large
3 paires de plaques inframarginales
2 paires d'écailles préfrontales

1 pore présent sur chaque inframarginale il s'agit de la tortue de kemp *Lepidochelys kempii*



Nucale (N) sans contact avec les premières costales (C)
4 paires de costales



Tortue Verte
Chelonia mydas

Tête moyenne
4 paires de plaques inframarginales
1 paires d'écailles préfrontales

Ecailles se chevauchant souvent comme des tuiles d'un toit il s'agit de la tortue imbriqué ou caret *Eretmochelys imbricata*

State of stranded turtles

Recently dead (firm, intact skin, animal that has not swollen, viscera that are not distended by putrefying gases, etc.)

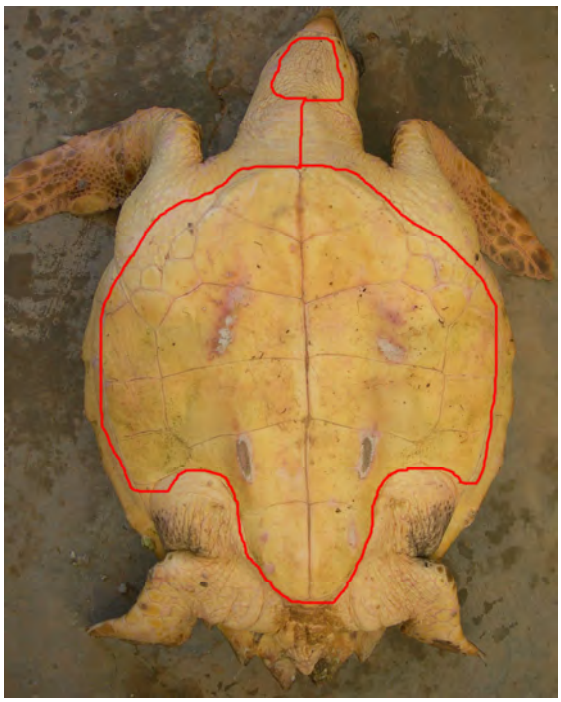
2- Moderately decomposing

3- Severely decomposed (lacerated skin, viscera distended by putrefying gases, very rotten smell, etc.)

4- Dried shell

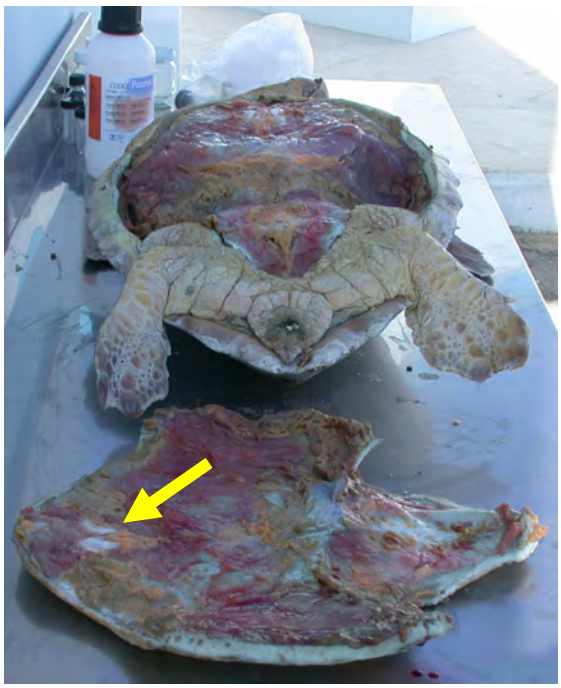
5- Remains of skeleton

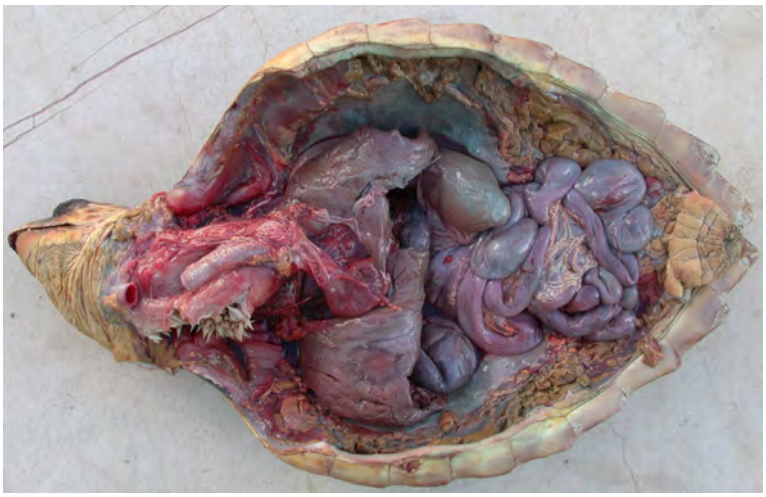
Autopsy



(Red) line to be cut along to remove the plastron

Cutting with a knife or scalpel blade. The tip of the knife should be kept near the plastron so as not to harm the viscera





To free the plastron, the thing that attaches it to the acromion process must be cut (arrow)

STRANDING OF AN ELASMOBRACH
Data collection file

Date ☐ Hour ☐

Place ☐ Country ☐

Latitude ☐ Longitude ☐

Animal: ☐ Live ☐ dead ☐ Putrified

Species:

Scientific Name ☐

Common Name ☐

Photo Yes ☐ No ☐

Observer

Name……

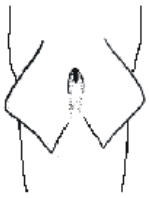
Adress……

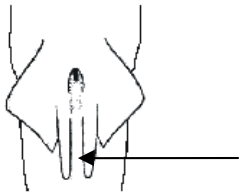
e-mail…… Phone……

Biological Observation:

Sexe

Ventral View

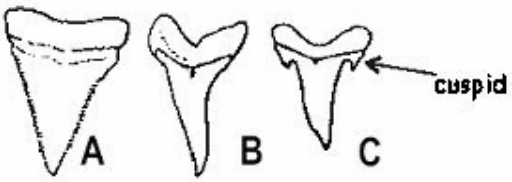




Pterygopods
Copulative organ

Female ☐ **Male** ☐

Form of teeth



Jagged edge ☐
Smooth edge ☐

Photo YES ☐ NO ☐

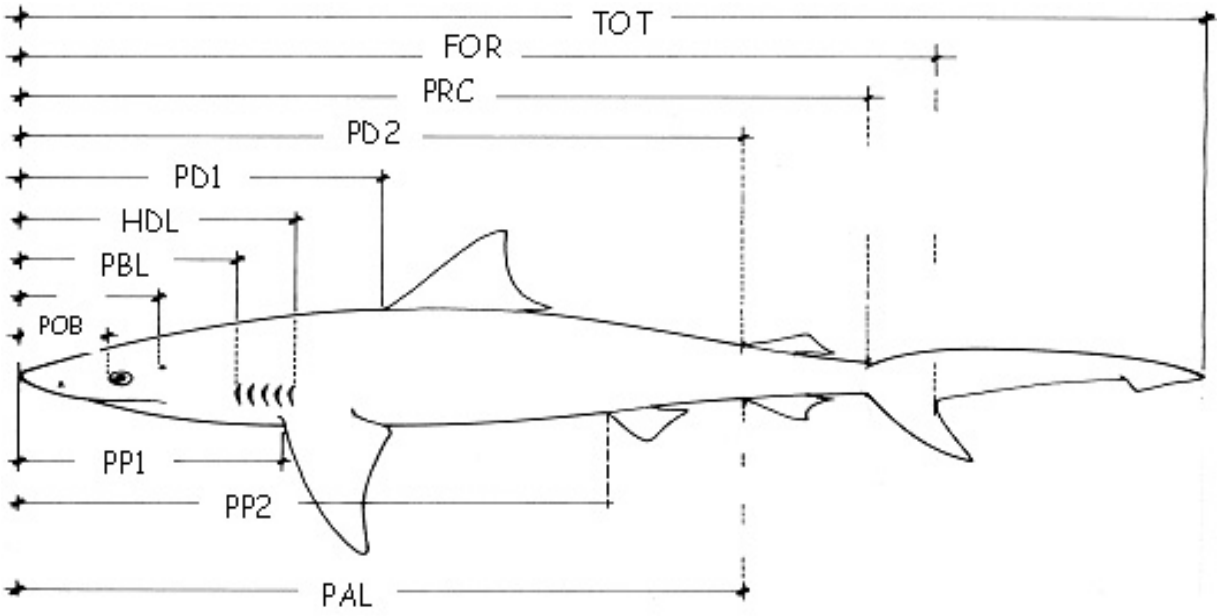
Other information

Stomach contents Yes ☐ No ☐

Presence of embryos in the uterus (If possible, kept them frozen) Yes ☐ No ☐

Measurements to be taken

A – Pleurotremata (sharks)



TOT = Total length (posterior nose-tip of caudal)

FOR = Length of fork (nose-V point)

PRC = precaudal distance (nose-insertion of caudal)

PD1 = predorsal distance 1 (nose-origin of the first dorsal)

PD2 = predorsal distance 2 (nose-origin of second dorsal)

HDL = head length (nose-last branchial slit)

PBL = prebranchial distance (nose-1st branchial slit)

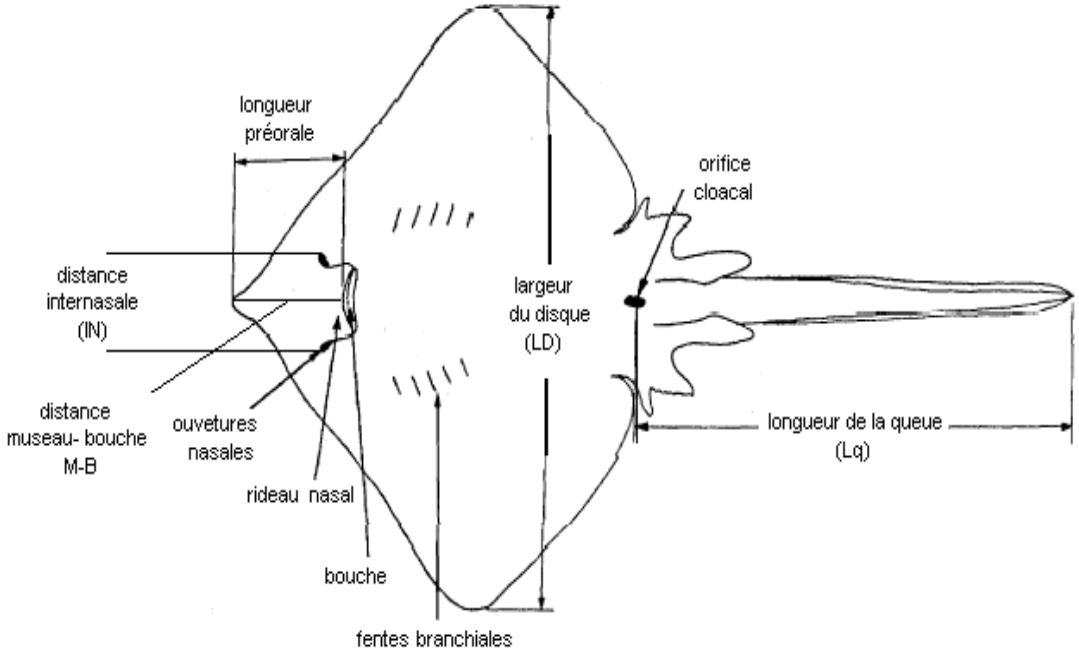
POB = preorbital distance (nose-front edge of the eye)

PP1 = Prepectoral distance (nose-origin of pectoral)

PP2 = prepelvic distance (nose-origin of pelvic)

PAL = preanal distance (nose-origin of anal)

B – Hypotremata (batoides)



Preoral length	
Cloacal orifice	
Internasal distance (IN)	
Disc width (LD)	
Nose-mouth distance (M-B)	
Nasal openings	
Nasal curtain	
Mouth	
Branchial slits	
Length of tail (Lq)	

Other information to be noted (cut fins, remains of fishing gear, wounds, etc.)

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Recognising protected elasmobranchs in the Mediterranean

The basking shark *Cetorhinus maximus* (Gunnerus, 1765)

Features Five very high branchial slits meet in the middle of the head. The caudal is in the form of a crescent, with almost symmetrical lobes, and the caudal peduncle with strong lateral carina. The nose is conical, very long and snub (in young fishes).

The Manta ray or devilfish *Mobula mobular* (Bonnaterre, 1788)

Features : this is the only species of the family that is found in the Mediterranean. A gigantic animal with lozenge-shaped disc, twice as wide as it is long. The anterior part of the head is distinct from the disc. Lower mouth. There are two typical anterior cephalic fins or 'horns'. Back is usually black and stomach white.

The great white shark *Carcharodon carcharias* (Linnaeus, 1758)

Features : A massive fusiform body, with black back and white stomach. The branchial arcs do not have branchiospines; the teeth are very big and point backwards; only one highly denticulated cuspid. A proeminent carina on either side of the caudal peduncle, continuing along the caudal. This is crescent-shaped with almost symmetrical lobes. The second dorsal is in front of the anal.