

Stranding, Necropsy and Sampling:

Collection data, sampling level & techniques



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Stranding response

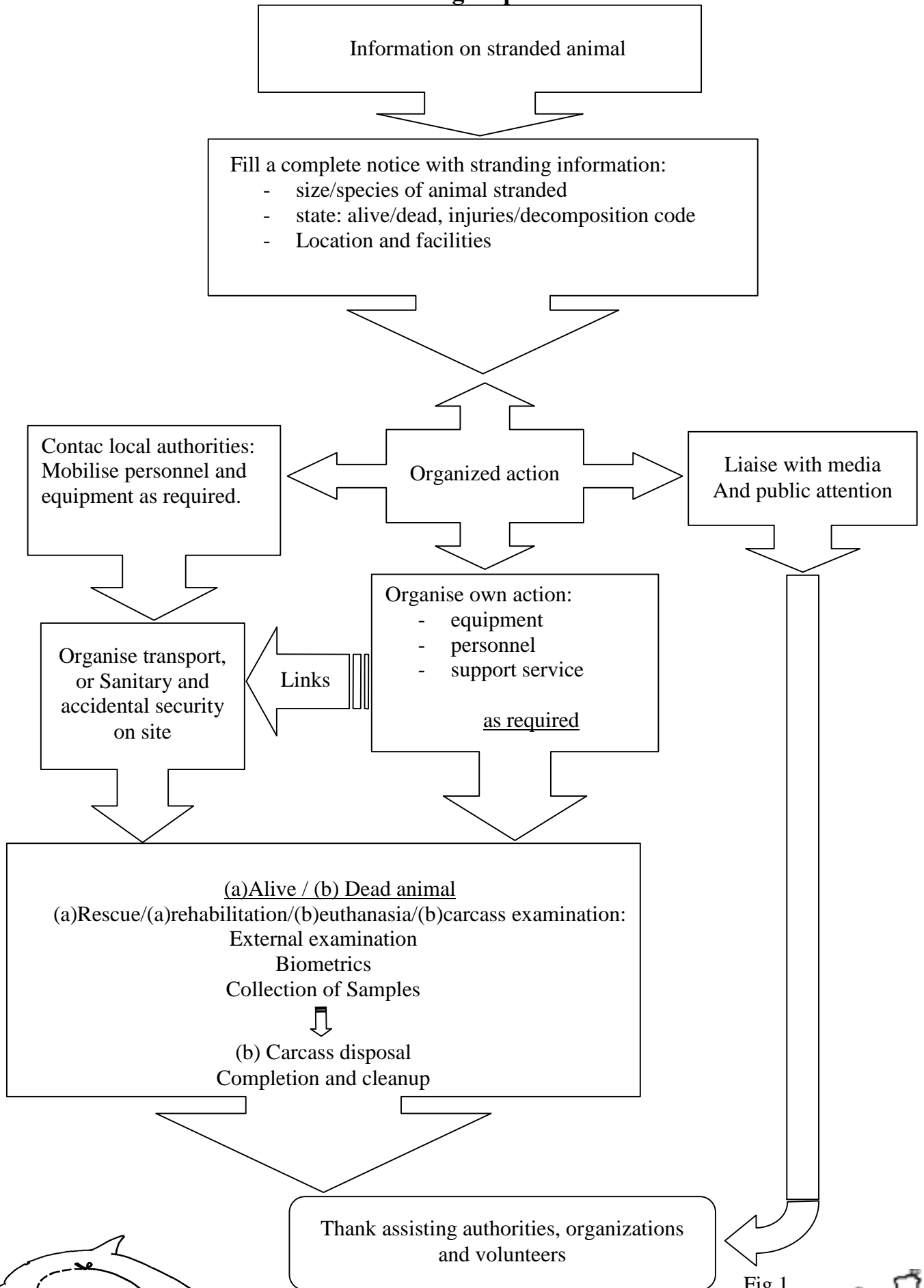


Fig.1



Basic Stranding information

Stranding data

Identification number:

Each animal should get a unique identification number (ID-number).

For example: year code/month/number of individual in year => first dolphin stranded in year 2005, in February: collection number = 105 02 001. All documents, samples, should be labelled with this identification number.

Species:

Species identification needs to be accurate; in case of doubt (hybrids, rare species, etc.)? take good photos and additional measurements.

Stranding location and date:

The stranding location should be identified by its (local) name, as well as exact details on geographic latitude and longitude. The stranding date is defined as the date of finding, if sometime differs from the examination date, these dates should be noted. Always note all persons responsible for discovery, logistics, transport...in case of lack of information and acknowledgements of assisting people, authorities and volunteers.

Photos:

Document the animals outside and inside by good quality photos as well as possible.

Specimen data

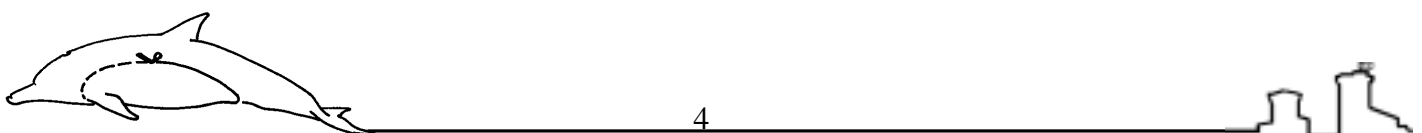
Sex:

Unpractised workers or any doubts, take accurate measurements from tip to anal and genital hole, additional photos of urea-genital region.

Weight:

Should be determined by weighing with an accurate scale. In the case where no weighing possibility exists (larger dolphins), the two girths immediately before (girth 1) and behind (girth 2) the front flipper should be taken.

Foetus: Weight should be taken from any foetus including 10 cm umbilical cord. When possible the entire foetus should be taken as a sample and with measurements and samples in the laboratory or stored as a whole.



Body measurements:

Take all body measurements (fig.3), be careful for accurate measurements, use perpendicular line from limits (e.g. tips to caudal fins) for linear measurements (fig.2)

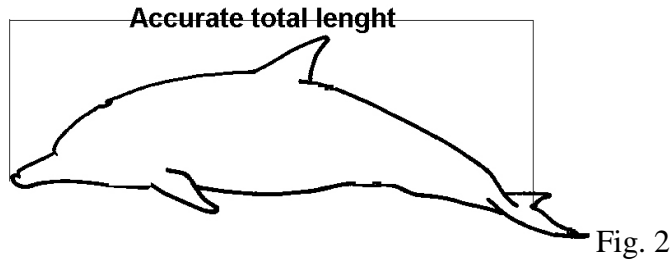


Fig. 2

The blubber thickness is measured on three pieces of blubber (extracted dorsal, ventral and on the median) extracted on the girth-line immediately behind the cranial of the dorsal fin. Care should be taken to measure perpendicular to the skin surface. The limits of the blubber are: the end of the (dark) epidermis, and the beginning of the reticulated subcutis.

From the same pieces of extracted blubber used for the blubber measurement, the skin thickness should be measured perpendicular to the skin surface (three measurements)

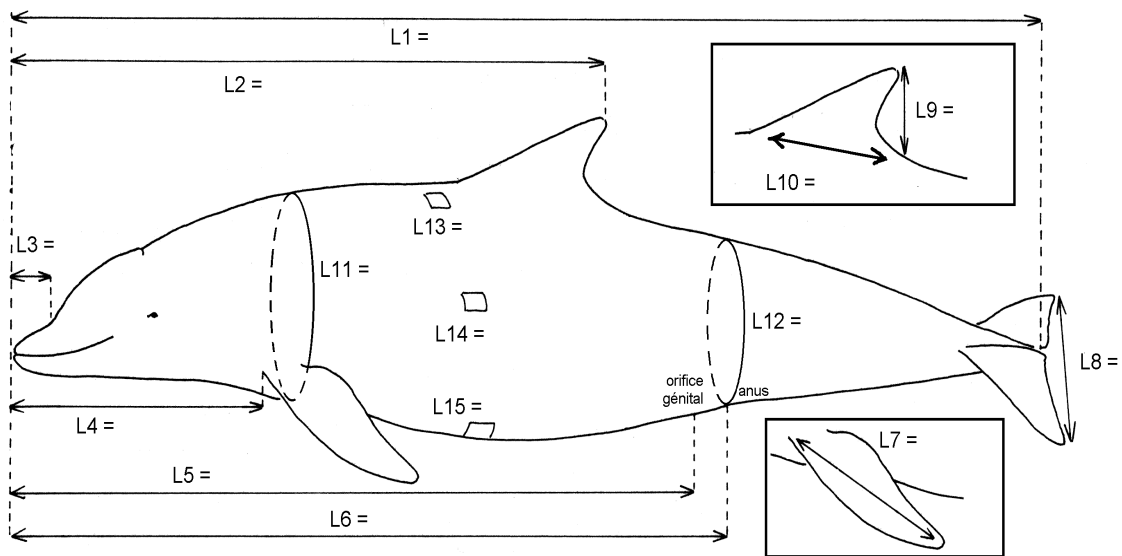


Fig.3

Teeth or plate counts can help in species identification if doubts persist, specially for hybrids, rare species, etc....

Teeth or plates ^(a)	
Sup. Left	Sup. Right
Inf. L	Inf. R

Weight :

real estimation ^(a) /



DCC (Decomposition condition code), and sampling levels:

The „decomposition condition code“ (DCC) is based on the external and internal decomposition signs of the carcass. It is the same as the „condition code“ (CC) defined in the ECS proceedings (Kuiken & García Hartmann, 1993)

DCC 1:

Very fresh, less than 48 hours dead, may show signs of rigor mortis (< 24 h), blood still separates serum (24-48 h), rigidity of eyes is diminished but not very flacid, cornea is not cloudy

DCC 2 (equal to former CC2):

Fresh, first signs of decomposition visible, eyes and surface quality of the skin reveal decomposition, otherwise good state, organs look intact, blood does not separate the serum, no smell of decomposition.

DCC3 (equal to former CC3):

Skin peeling, moderate but clear signs of decomposition (changes in colour and consistency [flacid]) of skin and organs, not suitable for bacteriology because of overgrowth, moderate smell of decomposition.

DCC4 (equal to former CC4):

Advanced decomposition, skin and organs clearly altered, the loss of consistency changes the organ's shapes (liver!), clear smell of decomposition, not suitable for any tissue analysis, even gross pathology is very unclear and can hardly be interpreted at all.

DCC5 (equal to former CC5):

Completely useless for pathological examination, organs are beyond clear recognition or absent, may be mummified, etc.

Based on the DCC of a carcass, the sampling will need to be adapted:

	DCC 1	DCC 2	DCC 3	DCC 4	DCC 5
Genetics	x	x	x	x	x
Ageing	x	x	x	x	x
Reproduction	x	x	x	ovaries	ovaries
Diet	x	x	x	stomach	stomach
POPs	x	x	(x)		
HM	x	x	(x)		
Bacteriology	x	(xx)			
Virology	x	(xx)			
Histopathology	x	x	(x)		
Parasitology (stomach)	x	x	x	x	

x = collect

(x) = collect if possible

(xx) = collect if suitable



The sampling level is also adapted in function of stranding events, animal size, numbers (mass die-off), foetus presence, stranding site (tide, accessibility....). Operate within those limits, and do not expect more than your resources allow.

NCC (Nutritive condition code):

The nutritive state of the animal should be evaluated immediately before the necropsy, as a general „impression“ gained from several details which often are not written down by the pathologists.

NCC1:

very good nutritive condition, very well nourished, abundant blubber, significant other subcutaneous fat present in the dorsal neck and -sometimes- on the lateral thorax, Longuisimus dorsi and neck are convex, the whole animal makes a "round, barrel-like" body shape



NCC2:

good nutritive condition, well nourished, abundant blubber, some subcutaneous fat, Longuisimus dorsi and neck are straight or slightly convex



NCC3:

normal nutritive condition, blubber is normal thickness, no subcutaneous fat present, neck and Longuisimus dorsi are straight, on movement of the animal sometimes slightly convex.



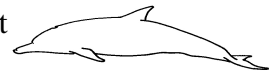
NCC4:

bad nutritive condition, blubber is on the thin side, sometimes skin thickness increased, neck and Longuisimus dorsi visibly concave



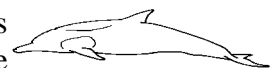
NCC5:

very bad nutritive condition, blubber is thin, skin thickness most often increased, Longuisimus dorsi and neck clearly concave



NCC6:

extremely bad nutritive condition, severely emaciated, blubber is very thin, neck and Longuisimus dorsi are severely concave, the contour of the scapula (especially the Spina scapulae) may be visible



General sampling

Genetics:

Samples for genetic analyses should be taken in a sterile manner, making sure that no direct or indirect contact with other DNA is possible. This includes direct touch with unprotected fingers, or contact with any object which has been directly touched with unprotected fingers.

Two samples need to be taken: 10 g of kidney, and 10 g of skin (without blubber). Samples should be stored in 70% alcohol. A minimum size of sample should be taken to allow to extract a part of the sample without other DNA , furthermore without contamination with unprotected fingers.

Teeth for aging:

Five (5) teeth should be extracted from the left lower mid jaw and be stored frozen.

Reproduction:

For the collection of reproductive tracts, if it's a very fresh female, or with the presence of a foetus , or evidence of pathology, remove entire reproductive tract with gonads and complete vagina, and then fix in 10% buffered formalin. In other cases, ovaries and part of uterine horn should be taken and fixed in 10% buffered formalin.

For the male, testes must be removed entirely or sample should be taken at mid-length in which case weight must taken before sampling and stored in formalin.

Note the weight of each gonad in grams: ovaria, testes, epididymis

Storage in formalin:

Put at least 10 times more formalin than sample in the sample container (1:10). Higher ratios (1:20) lead to better fixation quality.

Diet analysis

Stomach

The stomach should be taken after ligatures and finally stored frozen (-20° C).

If stomach content only can be taken, weigh the closed stomach before opening, then at the end weigh the empty stomach. Stomach content could be stored in 70% alcohol before analysis.

Blubber fatty acid analysis

Fifty (50) grams of blubber should be sampled and stored frozen (-20° C). The sample should be taken from the left middle girth sample used for measuring blubber thickness.

Milk analysis

Two (2) ml of milk should be taken for fatty acid analysis whenever possible.



POP Toxicology:

Blubber & liver

A blubber sample of twenty (20) grams should be taken from the dorsal blubber thickness sampling site (dorsal sample on the girth cranial to the dorsal fin). Similarly, a sample of 50 g of liver should be taken. Both samples should be stored in hexan-washed aluminium foil and frozen (-20° C).

Blood & milk

Ten (10) ml of blood and –if present- 2 ml of milk should be sampled with a glass syringe and stored in hexan-washed containers.

Foetus

In the case of finding a foetus in DCC 1-2, a liver sample of 50 grams should be taken and stored in hexan-washed aluminium foil. Foetus could be stored frozen (-20° C) *in Toto* and sampling later in laboratory

Heavy Metal Toxicology:

All heavy metal samples should be stored in a clean plastic bag (or alternatively: in a plastic container washed with nitric acid) and frozen (-20° C).

Blubber:

Sample 100 g of blubber from the same place as for POP analysis (from the dorsal blubber thickness sampling site, dorsal sample on the girth cranial to the dorsal fin).

Kidney:

Sample 100 g of kidney by cutting the kidney transversally in slices and place one cranial, one medial and one caudal slice (all three samples in one bag).

Muscle & Liver:

Sample 100 g each of muscle and liver and place them each in one clean plastic bag.

Milk:

If present, collect 5 ml of milk.

Blood:

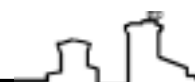
Sample 5 ml of blood and place it in a heparine container.

Urine:

Store 10 ml urine in a plastic container and store it at –20° C.

Foetus:

In a foetus, sample liver, kidney and muscle. Or in an alternative way, foetus could be stored frozen (-20° C) *in Toto* and sampled later in the laboratory, for either heavy metal and/or POP samples.



Histopathology:

The dissection and gross pathology should follow the rules established in the ECS protocol (Kuiken & García Hartmann, 1993).

General:

Collect both standard tissue samples as well as samples of all lesions (lesions are: any tissue abnormalities in colour or consistency, neoplasms, foreign bodies, etc.). Make notes on any lesion found, including giving details about changes in colour, consistency. Please provide a size of the lesion in your description (in absolute measures! Words like „large“, „big“, „small“ etc. should be avoided).

All samples for histopathology should not exceed 2 cm in thickness (1 cm is better). Samples of lesions should be taken from representative areas, and should include a sample which has the border between pathological and normal tissue.

Store all samples in neutrally buffered formalin, the relation of sample to formalin must be > 1:10

For the brain:

Use a relation of sample to formalin of at least 1:20. A good method of brain preservation is to place it in a large volume of formalin for 3 days, then turn the container upside down for better perfusion. After 7 days, change the formalin.

Alternatively, take small subsamples of the fresh organ, but still follow the recommendations above.

Bacteriology:

General:

Bacteriology analysis requires only very fresh animals and needs sterile sampling conditions. If such conditions do not exist, store suspected bacterial lesions as a tissue block of 5x5 cm at -70° degrees. The tissue sample should be wrapped in cling film (household), and then stored in a plastic bag .

A duplo of all lesions plus some standard samples need to be stored in 10% formalin. Label and store all lesions individually.

Brucellosis:

All sample with any lesion which is suspected to be caused by *Brucella* sp.. Such lesions can be: abscesses under the blubber, inflammation of the testes, epididymis, prostata, penis, pyometra, abortions, inflammation of the placenta, etc.

Sample the lesion and divide the tissue in two: one part for histopathology (see above), one part for culture .Additionally, provide 1 ml of blood in a clean container. This blood can be stored frozen (-20° C).

With or without any lesion, always taken part or entire spleen and stored in plastic bag at -20° C.



Virology:

General:

Store all lesions of suspected viral origin and a list of standard tissues at -70° degrees (put the tissue sample in [household] cling film, then in a plastic bag).

Blood:

As much blood as possible should be collected and stored for serology in a plastic container at -20° C.

Abnormal content of the mammary gland:

Abnormal content of the mammary gland should be stored at -70° C in a plastic bag or container.

Parasitology:

General:

Store all relevant parasites in 70% alcohol, and all lesions caused by parasites in 10% formalin (see histopathology).



References:

Jauniaux T., Garcia Hartmann M., Haelters J., Tavernier J., Coignoul F. (2002), Echouage de mammifères marins : guide d'intervention et procédures d'autopsie ; Annales de Médecine Vétérinaire, 146, 261-276.

Kuiken, M. & García Hartmann, M. (1993): Proceedings of the First ECS Workshop on Cetacean pathology: Dissection Techniques and Tissue Sampling. *ECS Special Newsletter*

Geraci, J. R. and Loundsbury, V. J. (1993) Marine mammals ashore, a field guide for strandings. Texas A&M Sea Grant Publication, USA. pp261.



ANNEXE 1



MARIN MAMMALS STRANDING FORM : CETACEAN



Ministry of Ecology and Durable Development, Direction of Nature and Landscape.
(a) : to line useless mention

LOCALITY NAME : _____ **Observation date** : ____/____/____
 Dept./Grid ref^(a) : _____ Beach name : _____
Geographical position : Latitude _____° _____' _____" | **N** | Longitude _____° _____' _____" | **W** |

Specie : _____ **Determination** : probable / certain^(a) **Sex** : _____
Determination criteria : _____

DEAD ANIMAL **Death date** : ____/____/____ **Stranding Date (discovery)** : ____/____/____
 decomposition Code: : 1 very fresh (<48h) : 2 fresh : 3 putrefy : 4 very putrefy : 5 remains
 By catch : probable / certain^(a) By catch marks : _____
Sampling : _____

ALIVE ANIMAL^(a) **Stranding Date** : ____/____/____ **Time** : ____ : ____
 Animal refloating **refloating time** : ____ : ____
 Dead Animal^(a) : Before intervention / during intervention / euthanasia **Death time** : ____ : ____
Comments et observations (describe refloating attempt and care, identify intervening, etc.) : _____

CIRCUMSTANCE OF OBSERVATION : by chance / by informant (name)^(a) : _____
 Photos Other animals observed at proximity : _____
 Other observations : _____

Teeth or plates ^(a)	
Sup. L	Sup. R
Inf. L	Inf. R

Weight : _____
 real / estimation^(a)

RESERVED TO CRMM n° info. : _____ n° coll. : _____ by catch code : _____
 n° photo : _____

OBSERVER Nom : _____ To ask for other forms
 Address : _____

Thanks to send this form (and check-list if necessary) at :
 INSTITUT DU LITTORAL ET DE L'ENVIRONNEMENT
 CENTRE DE RECHERCHE SUR LES MAMMIFERES MARINS
 Avenue du Lazaret - Port des Minimes - 17000 LA ROCHELLE
 TEL : 05 46 44 99 10 - FAX : 05 46 44 99 45
 E-mail : crrm@univ-lr.fr Web : crrm.univ-lr.fr



or at your local correspondent



ANNEXE 3

Check list – Collection number:

	Repro	HP.	Viro.	Bact.	Parasi	HM	POPs	Foods	Age	Genet
Aorta		■								
Heart L/R		■		■						
Rib/bone						▨				
Teeth						3			5a+5c	
Stomach					■			▨		
Liver		■	■		■	▨	2x			
Bronchial node		■	■							
Mesenteric node		■	■							
Gonads	▨									
Hypophysis	■									
Intestine		■	■	■						
Blubber						▨	2x	▨		
Muscle		■				▨				
Oesophagus		■		■						
Pancreas		■								
Skin										▨
Lung		■	■	■	■					
Spleen		■		▨						
Kidney		■				▨				▨
Blood			■			■	■			
Adrenal	■									
SNC		■	■			■				
Thymus	■									
Thyroid	■									
Reproductive tract	■									
Bladder			▨							
♂ Prostate L/R	■									
♀ Placenta, umbilic	■		■	■						
♀ Mammary gland G/D	■		■	■		■	■	■		
Milk						■	■	■		
Other										

Repro : reproduction, formalin
HP. : histopathology : Sample in formalin
Viro. : virology : -80°C ou -40°C
Bact. : bacteriology -80°C ou -40°C
Parasi : parasitology : alcohol 70%
HM : Heavy metals, -20°C
POPs : persistant organics pollutant: aluminium foil in -20°C

Foods : Stomach or food remains, -20°C
Blubber : aluminium foil in -20°C
Age : 5 teeth in alcohol and 5 in -20°C
Genet : genetics alcohol 70 %
Minimum sampling :



Gonads weight (L/R) :

