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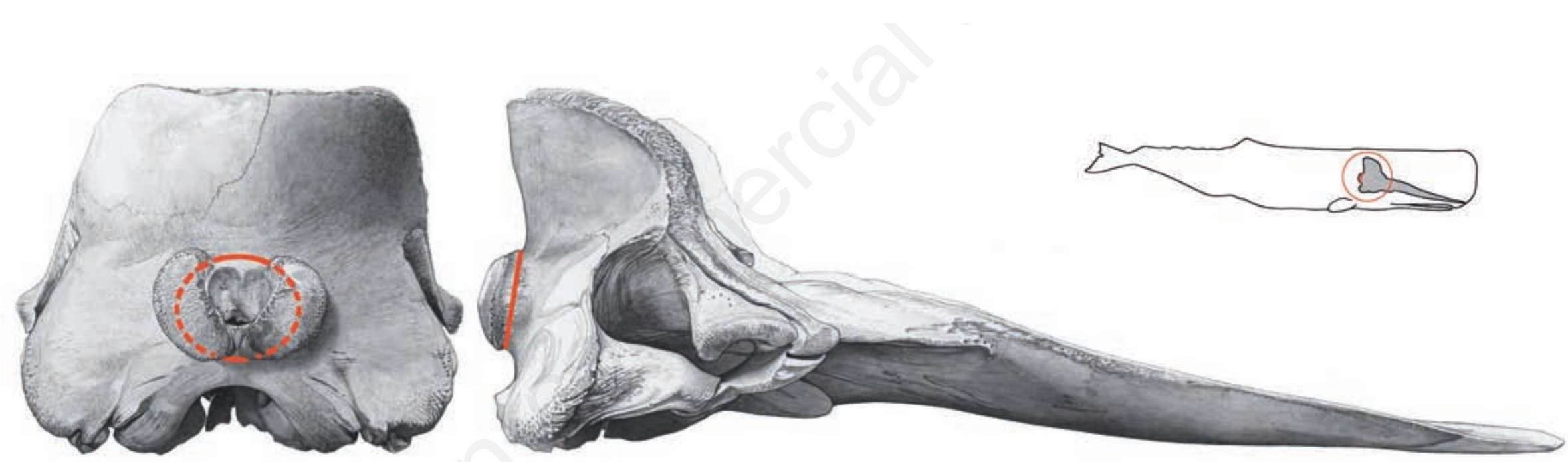
ACCOBAMS training on necropsies

Part I - Online, 28 - 29 June 2021

Introduction to the Best Practices on cetacean postmortem investigation and tissue sampling resulted from the harmonization process in ACCOBAMS and ASCOBANS



Sandro Mazzariol - University of Padova

ACCOBAMS Training on necropsies, Part I - 28 – 29 June 2021, Online

Background

During the VIII ASCOBANS MoP (2016), the AC and Secretariat were requested to engage actively in the work on best practice guidelines for response to stranding events and in the establishment of an updated post- mortem protocol within the frameworks of the International Whaling Commission (IWC), ACCOBAMS and the European Cetacean Society (ECS) - Resolution 8.10.

In the same year, ACCOBAMS endorsed the document on common best practices for a basic post mortem examination of stranded cetaceans under the Resolution no. 6.22 during the VI MoP. In the same Recommendation, an approach to ASCOBANS, ECS and IWC was requested to the Scientific Committee (SC) to review the common definitions, common data collections and common post-mortem protocols during the triennium.

In 2018, during the 24th ASCOBANS AC and 12th ACCOBAMS SC a joint workshop was proposed to harmonize the existing initiatives. This meeting was organised in Padua (Italy) in June 2019 involving 24 experts from different countries of the two regional Agreements and from Macaronesia area representing the MARCET project.

REPORT OF THE JOINT ACCOBAMS/ASCOBANS/ECS/SPA-RAC WORKSHOP ON MARINE DEBRIS AND CETACEAN STRANDING

Stranding Networking

1.1. Stranding events

- Evaluation of the needs for further development of national stranding networks;
- Promotion of establishment of National Stranding Networks under the national coordination/support;
- Promotion of harmonization of stranding protocols (collection, analysis, etc.) in order to exchange common data, as appropriate* ;
- Assessment of existing stranding protocols. Tiered guidelines- simpler as required: What is the *de minimis* approach? *;
- Addition of tiered marine debris collection protocols to updated ACCOBAMS/ASCOBANS strandings protocols;
- Implementation of relevant Capacity building ;
- Promotion/exchange of best practices in addressing cetacean stranding events*;
- Particular focus in areas of known high density of marine debris (e.g. Adriatic);
- *Special focus on stranding data from low densities and/or data deficient species (e.g. Grampus).*

1.2. Data banks

- Collation of existing data- which species, which regions, etc.;
- Inventory of all stranding information available from stranding data banks;
- Promotion of the establishment of regional tissue databank where there are none (e.g. Black Sea area);
- Improvement of communication between tissue data banks and between possible providers. Improvement also of access in both ways, providing and collection;
- Establishment of the minimum set of samples and the proper way of collection for tissue banks.

** See ASCOBANS Resolution 8.10 (2016) and ACCOBAMS Resolution 6.22 (2016)*

Necropsies - Improve general results from necropsies

- Investigation of pathogens presence;
- Investigation of contaminant levels released by debris ingestion and by prey ingestion (trophic transfer);
- Establishment of a list of the most important pollutants, pathogens, etc. which should be investigated in order to have a starting base line in common studies;
- Investigation of potential impacts of underwater anthropogenic noise;
- Identification of research groups/labs that may be able to analyse material collected by stranding networks;
- Identification of best practices worldwide;*
- Harmonization of pathology sampling methodologies;*
- Consideration should be given in using categorization of debris resulting from the MedSealitter project;
- Establishment of a common approach in interpreting results from postmortem analyses identifying a common language and code for mechanisms, as well as causes of death.

ACCOBAMS-MOP6/2016/Res.6.22

RESOLUTION 6.22

CETACEAN LIVE STRANDING

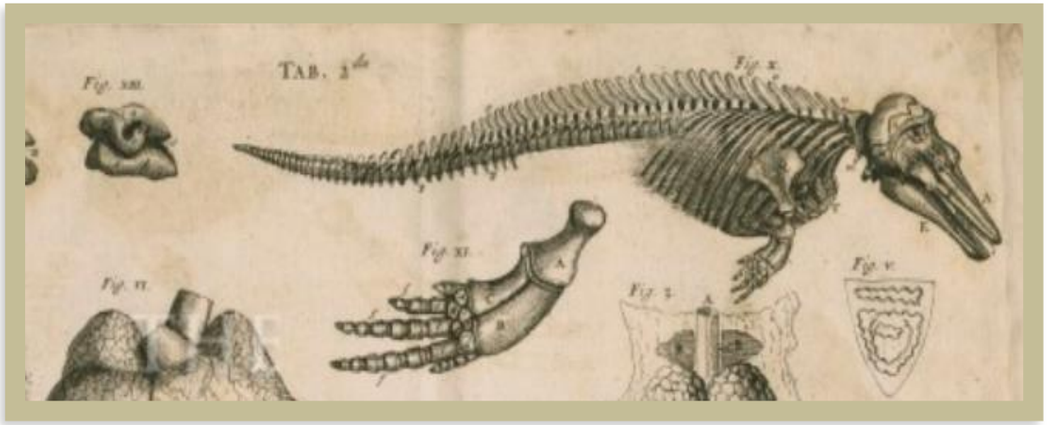
- Necropsies should be routinely carried out according to comparable procedures and approaches for data sharing
- Different situation of the stranding network in different countries
- Consider resources commonly present in each countries
- Support countries without national protocols (procedures, forms and data collection)
- Minimum standard for those countries with an established procedure
- Multilevel

Workshop on harmonizazon of the best practices for necropsy of cetaceans and for the development of diagnostc frameworks

June 24th-25th, 2019 - Legnaro (PD), Italy

Best practice on cetacean post mortem investigation and tissue sampling

Joint ACCOBAMS and ASCOBANS document



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September 2019

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Keywords

- Glossary
- Multi-tier triage approach
- Evidenced based approach
- Cooperation and multidisciplinary approach
- Risks
- Carcass disposal

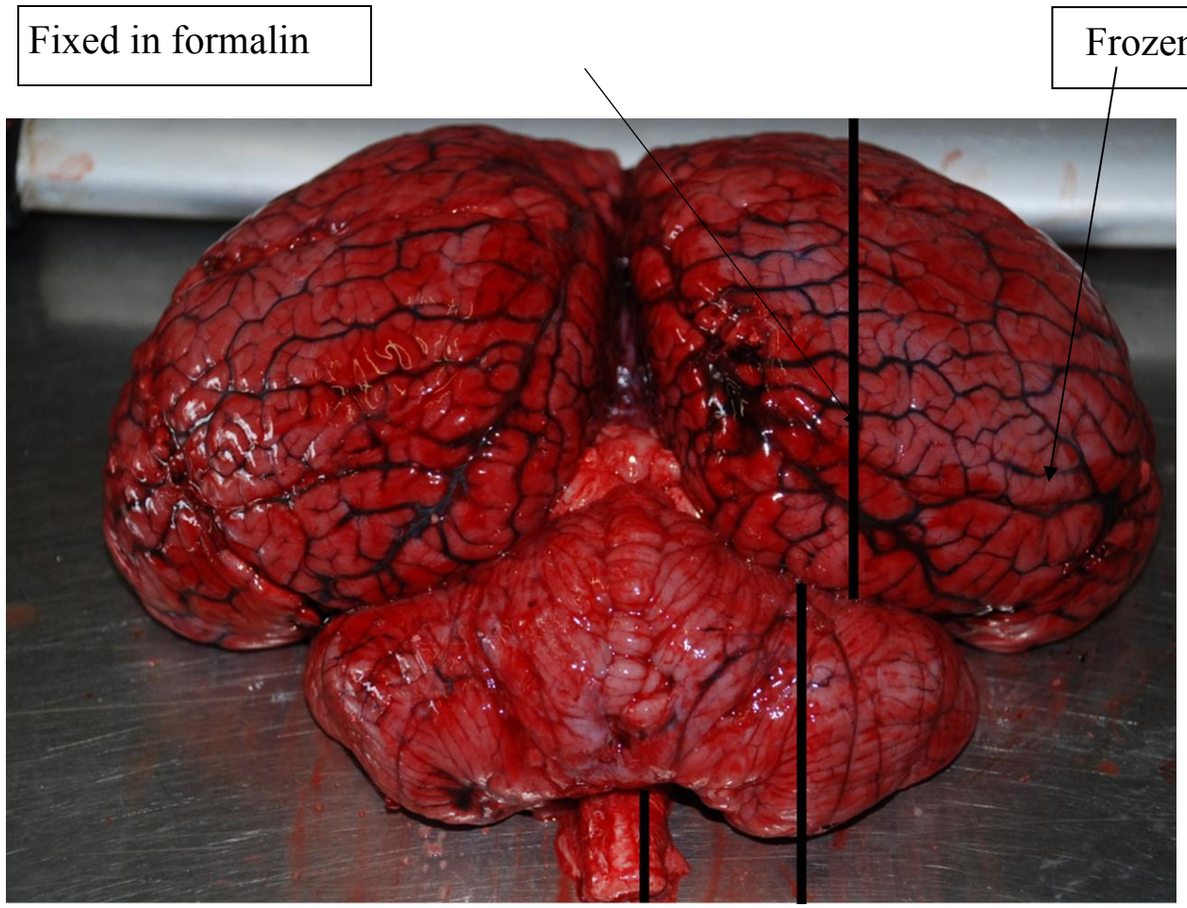

Fig. X: Sagittal paramedian cut of the brain. The largest portion fixed in 10% buffered formalin for histopathological examination and the smallest portion stocked frozen for microbiological, ecotoxicological and virological investigations. Image credit: C.Re.Di.Ma.

Keywords

- Post-mortem investigation vs Necropsy
- No short-cuts but guidance
- Cause of death vs mechanism of death
- Veterinarian vs biologists

Glossary

DISSECTION/PROSECTION: Medical and/or biological procedure to dismember the body of a deceased animal according to specific protocols in order to study its anatomical structure and/or to evaluate and sample specific organs and tissues.

NECROPSY/AUTOPSY/POST-MORTEM/POST MORTEM EXAMINATION Synonyms for a specialised medical procedure comprising of a thorough examination of a carcass by dissection to determine the cause, the mechanism and manner of death through the collection of evidence. In the case of wild animals this requires the involvement of a veterinary pathologist or a veterinarian with specific training in animal pathology, diseases and assessment of health.

POST MORTEM INVESTIGATIONS: All studies and investigations carried out on an animal's carcass and/or samples taken after death, including those aimed to determine the cause of death.

HEALTH STATUS: Subjective assessment of diseases, conditions, or injuries that not only contributed to the proximal cause of death but which characterize the ante-mortem health status of the individual and the possible health status of cohort animals.

CAUSE OF DEATH/STRANDING: The disease, injury or abnormality that alone or in combination with other factors (environmental, other concurrent diseases, age, etc.) is responsible for initiating the sequence of functional disturbances that resulted in live stranding and death. In the case of an aquatic animal stranded on shore, the post mortem investigation is aimed to determine the cause of stranding. During this procedure the following may be further defined:

- Immediate cause of death: final disease or condition resulting in death;

- Underlying cause of death: the disease or injury that initiated the chain of morbid events that led directly and inevitably to death;

- Contributing factors: other significant diseases, conditions, or injuries/impacts/influences that may have contributed to death but which did not constitute an underlying cause of death.

Protocol for basic post-mortem examination: multi-level approach

TIER 1 - external examination and stranding data collection

- Who: Wide range of personnel who have basic training. .

- To be assessed: External examination only, aiming to collect

. basic morphometric data,

. assessment of decomposition condition,

. sex and age class determination,

. photographs of external features

- **DO NOT** permit any reliable assessment of health status nor allow conclusions to be drawn as to the cause of death.

Protocol for basic post-mortem examination: multi-level approach

<u>TIER 2</u> - Dissection with sampling for postmortem investigations

- *Assessment level: trained responders with skills and experience.*

-*To be assessed: thorough post-mortem investigation, involving the visualization and gross inspection of all organ systems and a detailed description of findings.*

- *Samples should be collected to allow assessment of health status but not the cause of death (i.e. diet, life history, contaminant)*

- *Findings should be considered informative, but not conclusive on the cause of death.*

- ***Marine litter presence/ingestion and interaction with fisheries could be assessed at this level***

Protocol for basic post-mortem examination: multi-level approach

<u>TIER 3:</u> necropsy (dissection with diagnostic aim)

Assessment level: **by professional** (e.g. an experienced veterinary or biologists), and always including a veterinary pathologists.

To be assessed: **cause of death**.

This involves additional or detailed analysis of the data and samples collected during post-mortem investigation (tier two), aiming to understand also wider parameters of ecological health.

This tier often requires specialized laboratories and can be carried out in collaboration with other stranding investigation groups.

POST-MORTEM INVESTIGATIONS vs NECROPSY

“Dissection with diagnostic aims”.

Main goals
a. establish the cause of death (N)
b. confirm a clinical diagnosis (N)
c. detect diseases (N&P)
d. collect data for management and conservation (P)
e. increase biological and medical knowledge (P)

It is a simple and cheap medical analysis:
- to detect and manage infectious diseases (epidemiology)
- assess the role of anthropic stressors (conservation)
- assess possible responsibility (forensic)
- evaluate existing management problem
- health and welfare assessment

A NEGATIVE DATA IS AN INFORMATION!!!

Necropsy limits

- ✓ Clinical course and type of pathological changes
- ✓ Carcass decomposition
- ✓ Diagnostics tools and analyses
- ✓ Skills and expertise

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2. Basic field equipment

The minimum material necessary to perform a necropsy of a stranded animal should be the following:

- Latex gloves (sanitary conditions, not plastic ones)
- Data sheets
- Waterproof markers
- Measuring equipment
- Knives, scissors, scalpel, plastic knives, string
- Sample containers
- Aluminium foil and new plastic bags and sacs
- Kitchen paper roles
- Roman balance or dinamometres
- Camping cooler box with cold accumulators
- Preservatives (70% ethanol, 10% formalin, others)
- First-Aid kit
- Photographic camera and film

DECOMPOSITION CONDITION CODE - DCC

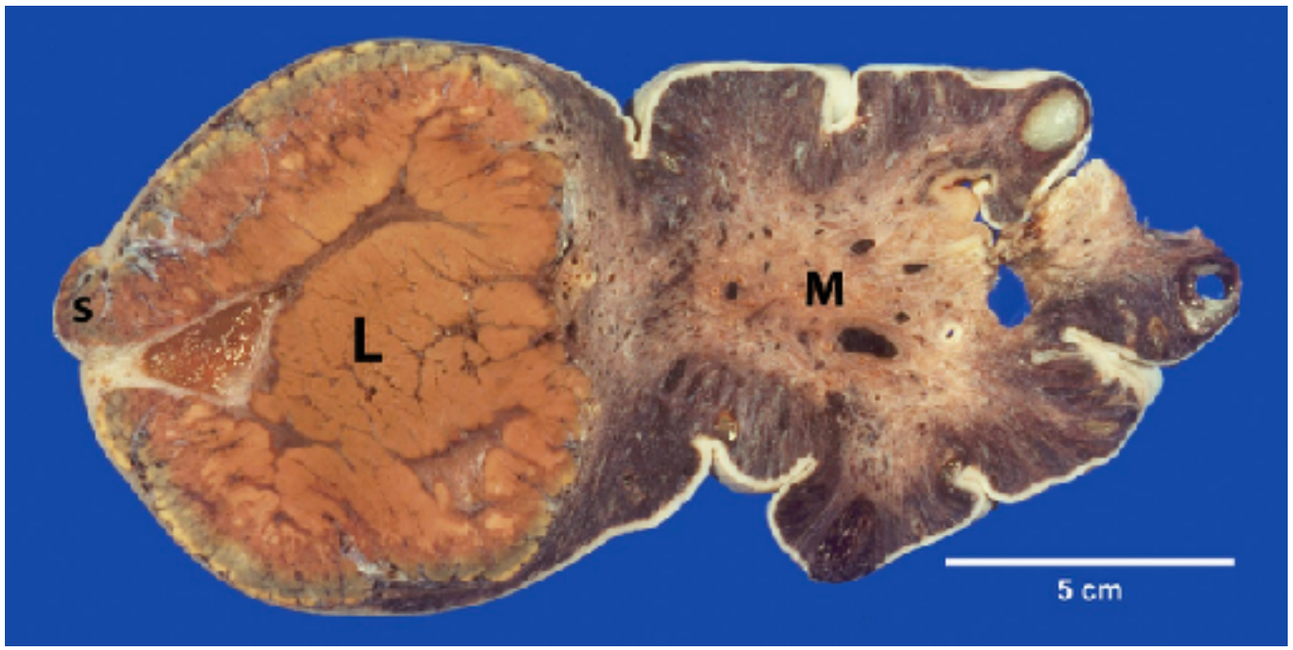
CONDITION CODE	DESCRIPTION
1	**Extremely fresh carcass, just dead**. Usually live stranded and died on the beach or stranded right after death, and exhibiting no post mortem changes (e.g. no bloating or sloughing of skin); fresh smell; clear, glassy eyes; blubber firm and white; muscles firm, dark red, well-defined; viscera intact and well-defined; gut contains no to little gas; brain firm with no discoloration, surface features distinct, easily removed intact.
2	**Fresh carcass** Normal appearance, fresh smell, minimal drying and wrinkling of skin, eyes and mucous membranes; carcass not bloated, tongue and penis not protruded; blubber firm and white, occasionally tinged with blood
3	**Moderate decomposition**. Bloating evident (possible with tongue and penis); skin cracked and started sloughing; characteristic (mild) odor can be expected; mucous membranes dry, eyes sunken. Blubber blood-tinged and oily; muscles are softer and poorly defined. Organs are basically intact, still well recognizable and can be easily removed and assessed, although colour is more uniformly throughout thoracic and abdominal cavity and organ consistency affected by decomposition (softer, friable). Gut segments are gas holding; brain with lost consistency.
4	**Advanced decomposition**. Carcass may be intact, but collapsed, skin sloughing, often severe scavenger damage, strong odor, blubber or muscle easily torn or falling off bones, liquefied internal organs
5	**Mummified or skeletal remains**. Characteristics: Skin may be draped over skeletal remains; any remaining tissues are desiccated. Organs partially or totally disappeared, or if present not completely identifiable.

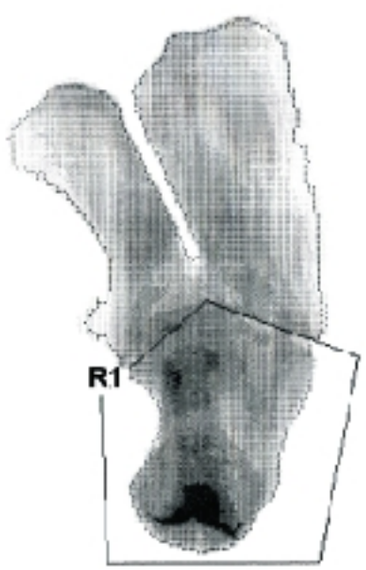
Limits? Decomposition code of the carcass (DCC)

Analytical procedure	DCC 1	DCC 2	DCC 3	DCC 4	DCC 5	Comments/recommendations
Genetics	✓	✓	✓	✓	✓	For DCC4 or 5: paleopathological procedures may be required on account of degraded DNA (eg extracting DNA from bone medulla)
Diet and marine debris	✓	✓	✓	✓	(✓)	If GIT is not intact, eg from post mortem scavenger damage, results are compromised
Age determination	✓	✓	✓	✓	(✓)	
Fatty acids and stable isotopes	✓	✓	✓	✓	(✓)	Depending on analysis planned
Parasitology	✓	✓	✓	✓	(✓)	Depending on analysis planned
Morphometrics	✓	✓	✓	(✓)	(✓)	Girth measurements can be disrupted by bloating due to autolysis in DCC4-5
Gross pathology	✓	✓	✓	(✓)	(✓)	Recommended for DCC4-5 in cases of forensic investigation
Reproductive studies	✓	✓	✓	(✓)	✘	
Toxicology	✓	✓	✓	(✓)	✘	Depending on pollutants. DCC1-2 for biomarker investigation.
Ear investigation	✓	✓	✓	✘	✘	Inner ear analysis specifically: DCC1, histopathology of fixed ears possible up to DCC3
Microbiology	✓	✓	(✓)	(✓)	✘	Depending on analysis planned. For DCC3-4 microbiology can still be worthwhile for detection of certain bacteria and fungi using specific culture methods. Should a septicaemia be suspected in DCC3-4 animals, then microbiological investigations should be undertaken on the kidney, as this is resilient to microbial post mortem invasion using specific culture methods.
Histopathology	✓	✓	(✓)	(✓)	✘	Recommended for DCC4-5 in cases of forensic investigation
Virology	✓	✓	(✓)	✘	✘	Depending on analyses planned.
Biotoxins	✓	✓	(✓)	✘	✘	
Gas bubble analysis	✓	✓	✘	✘	✘	If this procedure is conducted: it should be done first, before undertaking further assessments and dissections, particularly prior opening any part of the vascular system or removing the head.
Serology	✓	(✓)	(✓)	✘	✘	Advisable both on blood serum and on cerebro-spinal fluid, the latter of which should be collected as soon as possible. In heavily autolyzed specimens, alternatives are “juice” obtained from skeletal muscle or lung, vitreous humour or pericardial fluid

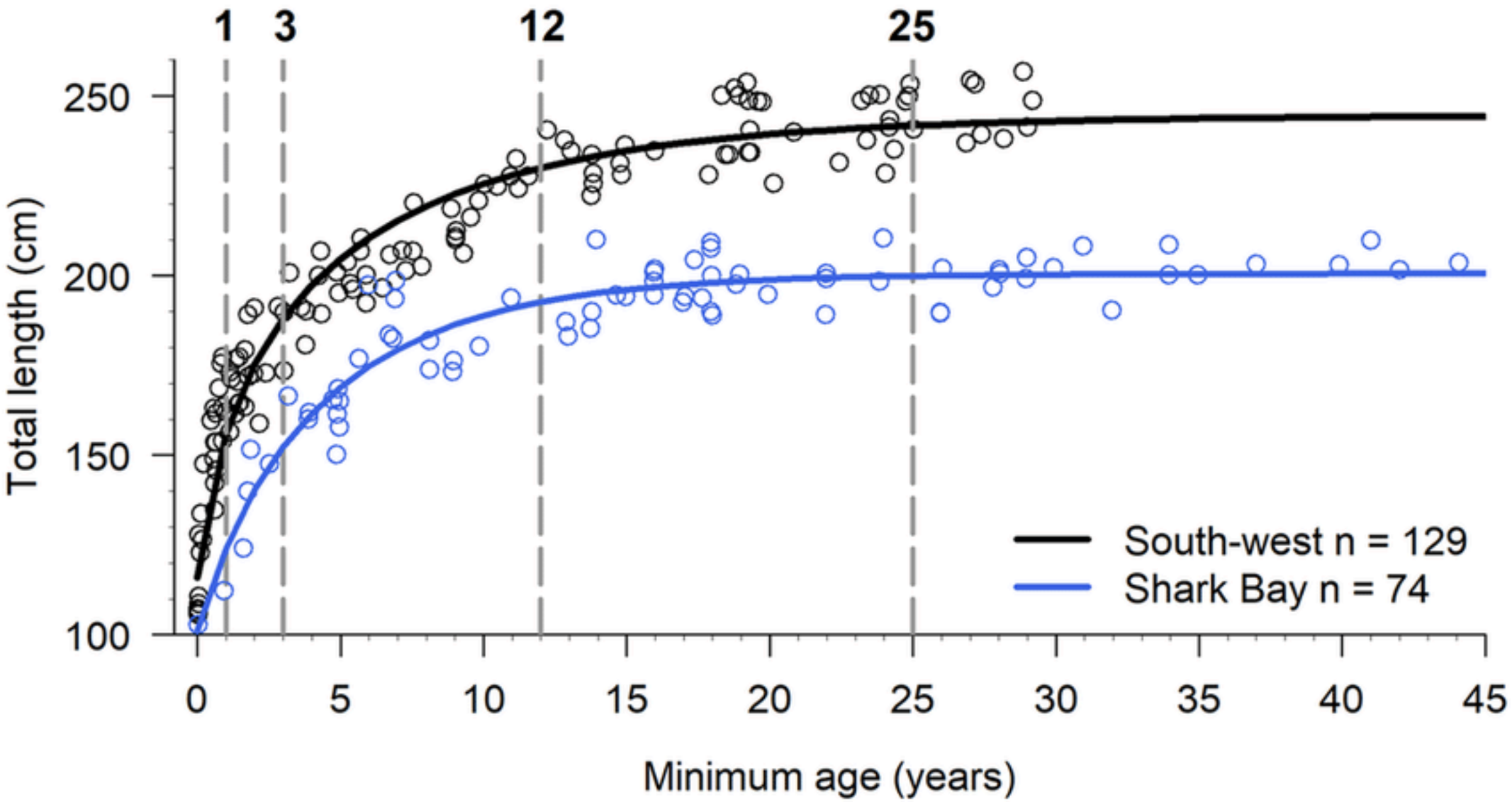
AGE AND SEX

- ✓ Morphology and growth curves.
- ✓ Gonads and renal glomeruli
- ✓ Bones (X-ray, DEXA)
- ✓ Teeth
- ✓ DNA methylation

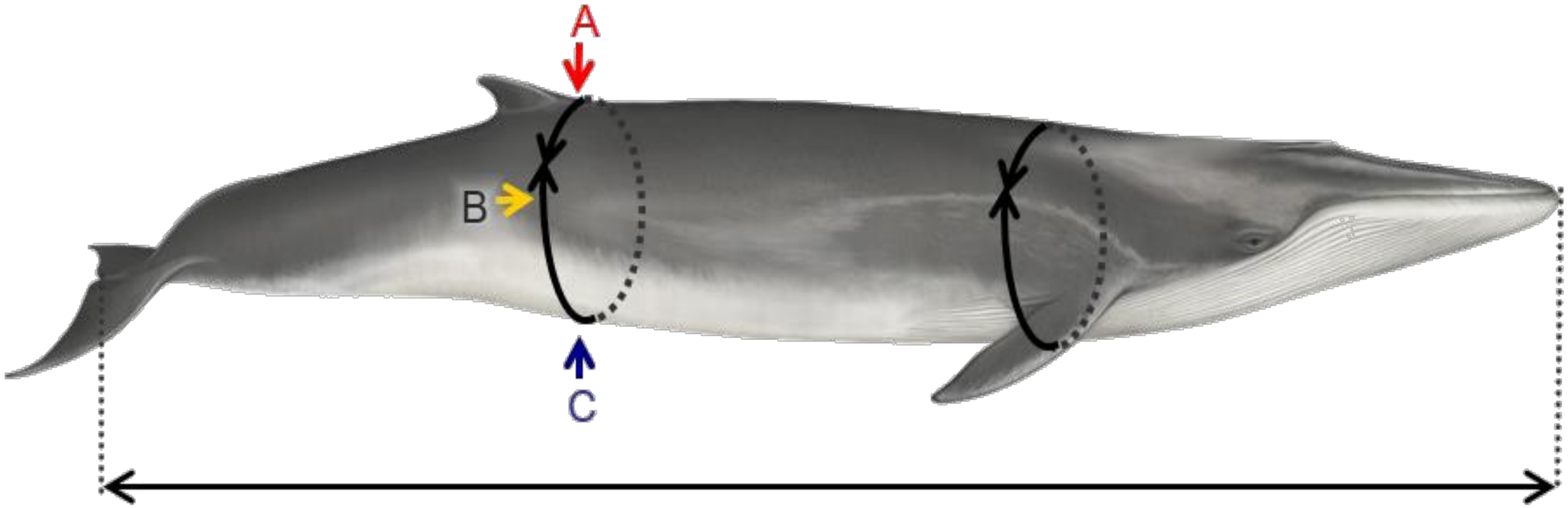


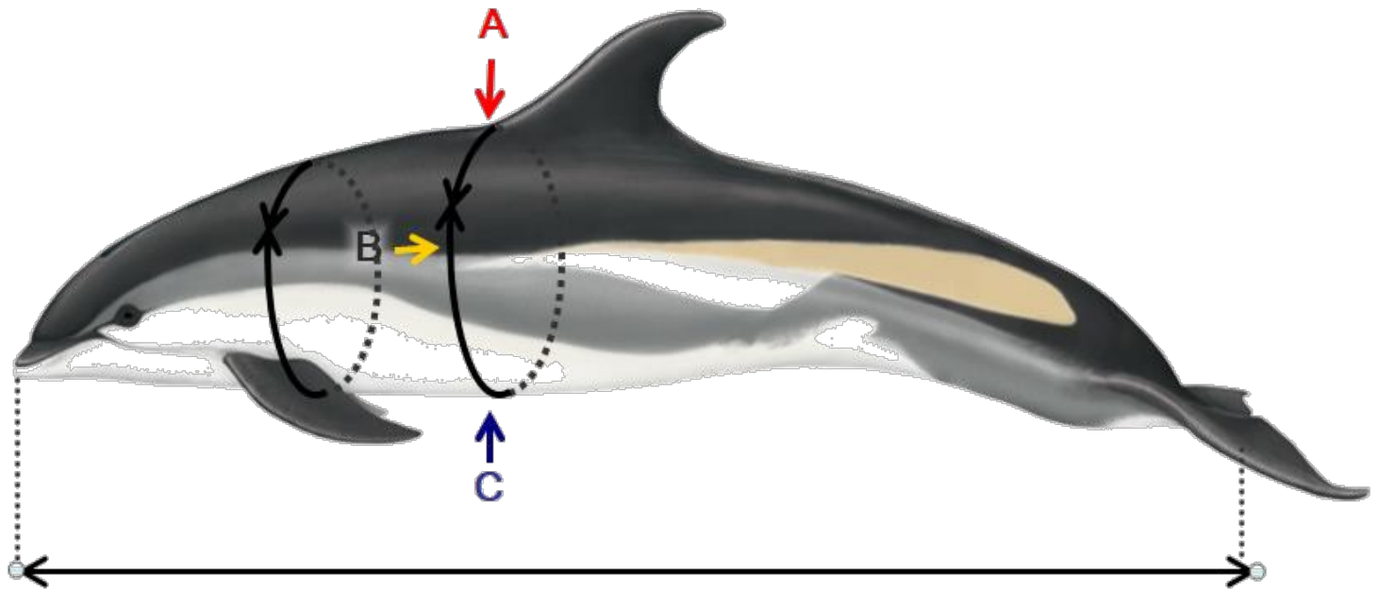


Dimension - age estimation



MEASUREMENTS





MEASUREMENTS

Family	Sex	a	b
Myticetes	M	-7.347	2.329
	F	-7.503	2.347
Odontocetes	M	-8.702	2.382
	F	-9.003	2.432

$$\log_e M_{media}\ 0\ a + b \log_e L_{max}$$

WHALESCALE

EXTERNAL EXAMINATION

SEX DETERMINATION

✓ To determinate the sex of a small cetacean, examine the ventral midline of the animal. Both male and female cetaceans possess a genital silt between the umbilicus and anus

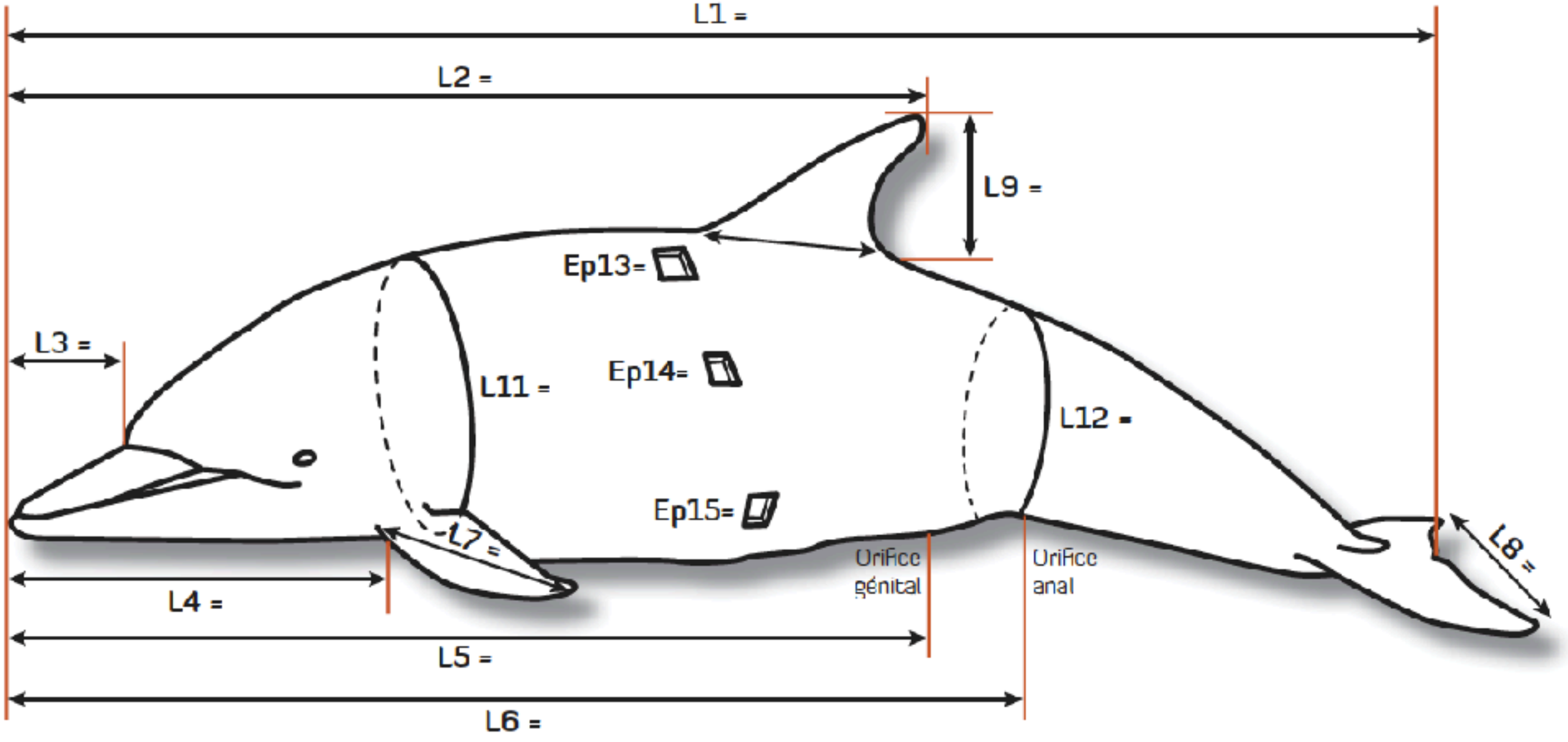
✓ For **female** cetaceans, there should generally be less than 10 cm distance between the centers of the anal opening and the genital slit. Whereas with a male, the distance between the anus and genital slit is much greater

NUTRITIONAL CONDITION CODE - NCC

- **Very good**: the animal's outlining on a cranial perspective is convex; round appearance caudal to the skull and lateral to the dorsal fin visible; subcutaneous-, pleural and other visceral fat present; blubber layers are thick..
- **Good**: the animal's outlining on a cranial perspective is convex; no hollow appearance caudal to the skull and lateral to the dorsal fin visible; possible some subcutaneous-, pleural and other visceral fat present.
- **Suboptimal:** the animal's outline on a cranial perspective is not fully round; a slight hollow appearance caudal to the skull and lateral to the dorsal fin is visible (slightly hollow or almost flat); no internal fat is observed.
- **Poor:** the animal's outline on a cranial perspective shows moderate concavity, and outline of lateral aspects of the vertebrae; a hollow appearance caudal to the skull and lateral to the dorsal fin is visible; scapula's can be observed sticking out.
- **Emaciated:** the animal's outlining on a cranial perspective is very concave and the lateral aspects of the vertebrae are easily palpable; an extremely hollow appearance caudal to the skull and lateral to the dorsal fin is visible; scapulas can be observed sticking out; blubber layers are minimal (in small odontocetes <1 cm).


A


B


L1 =
L2 =
L9 =
Ep13=
L3 =
L11 =
Ep14=
L12 =
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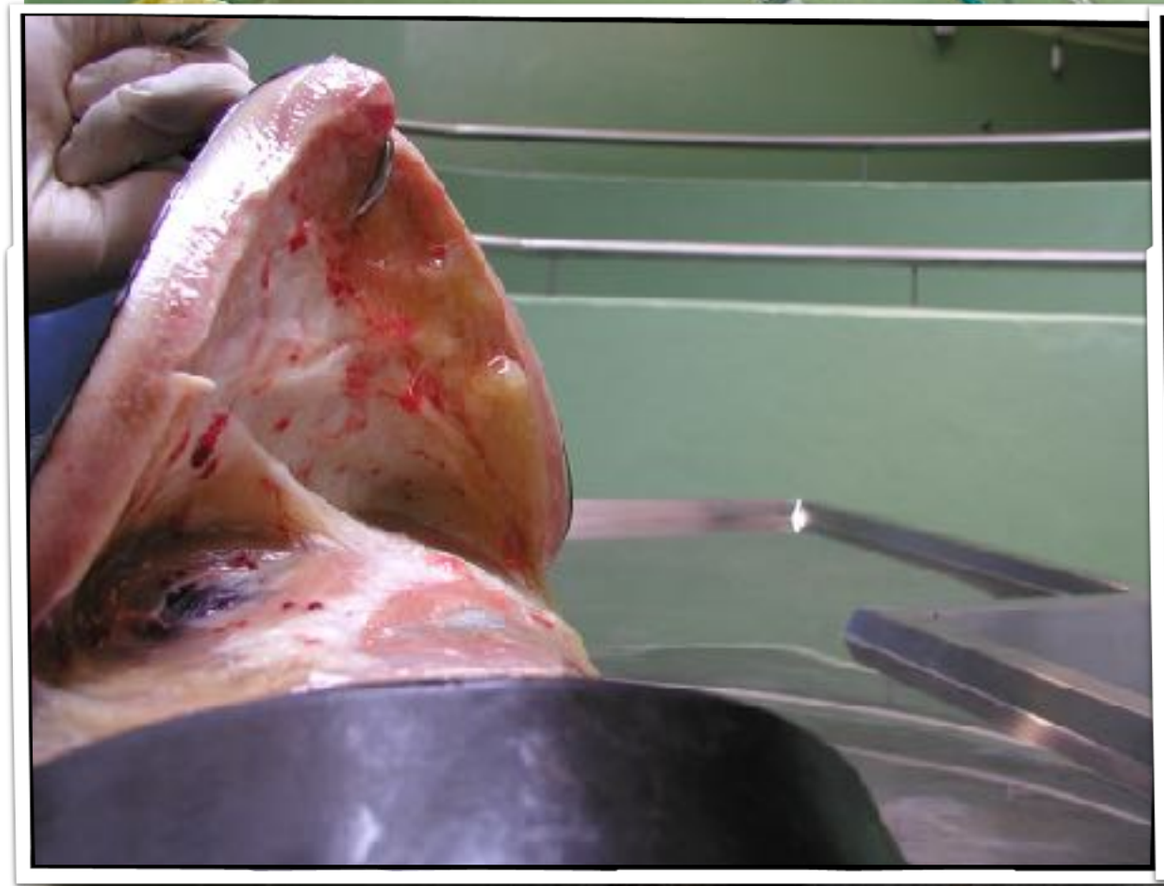
L7 =
L8 =
Orifice génital
Orifice anal
L4 =
L5 =
L6 =

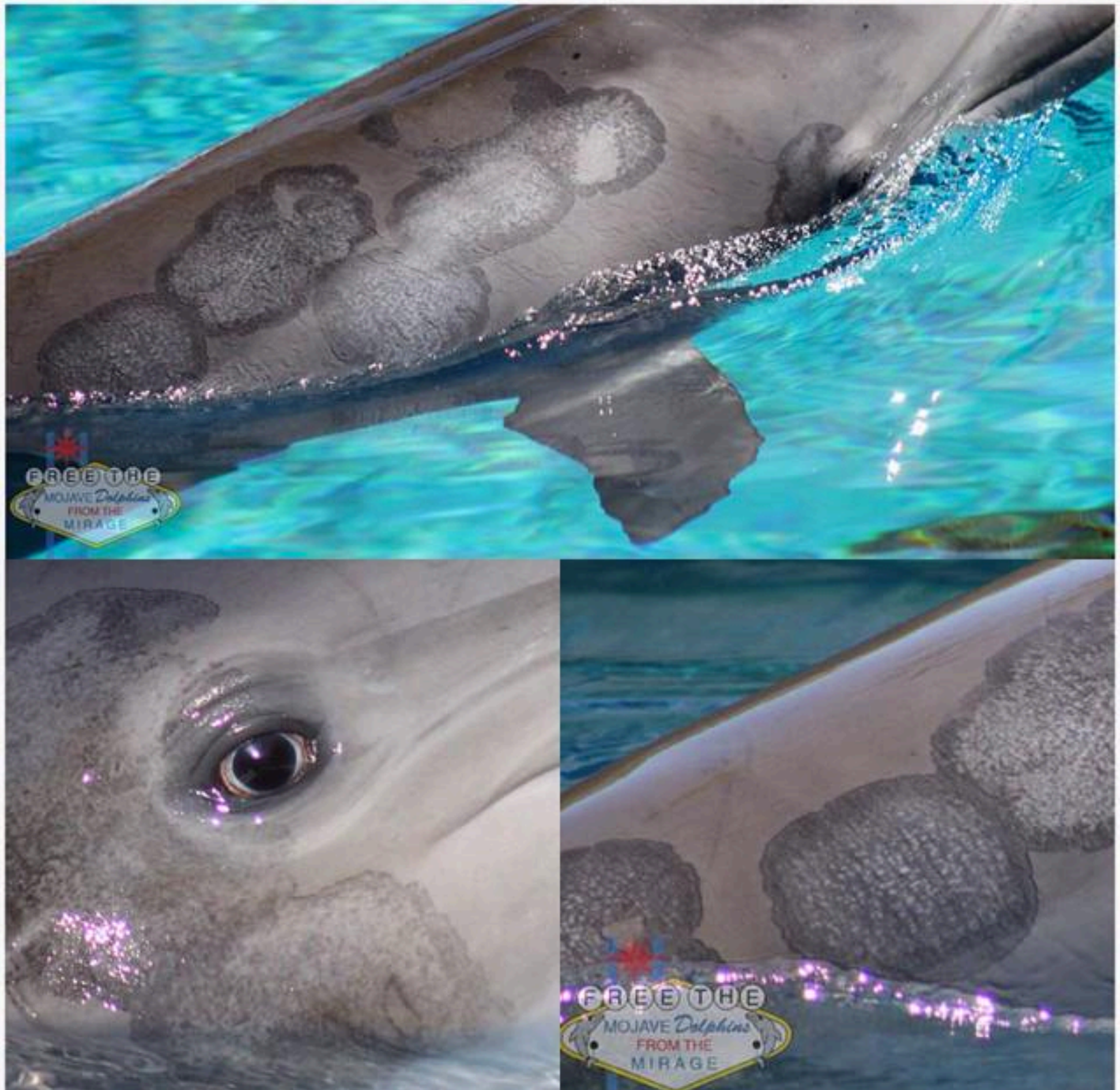


EXTERNAL EXAMINATION

SKIN AND BLUBBER

- ✓ Examine and document any scars, abscesses, ulcerations, erosions, wounds and parasites on the skin
- ✓ Make note of the size (length x width x depth/height), shape, color, texture, location and distribution of all abnormalities

Dolphin pox virus



External parasites: *Pennella* spp.

External evidences of Human interaction

Injures due to direct interaction

- ✓ lacking of extremities
- ✓ fins, head and rostral injures
- ✓ lacerations and nets marks (features could suggest the type of gear)
- ✓ incisions and deep wounds due to sharp objects
- ✓ penetration wounds
- ✓ tail abrasions

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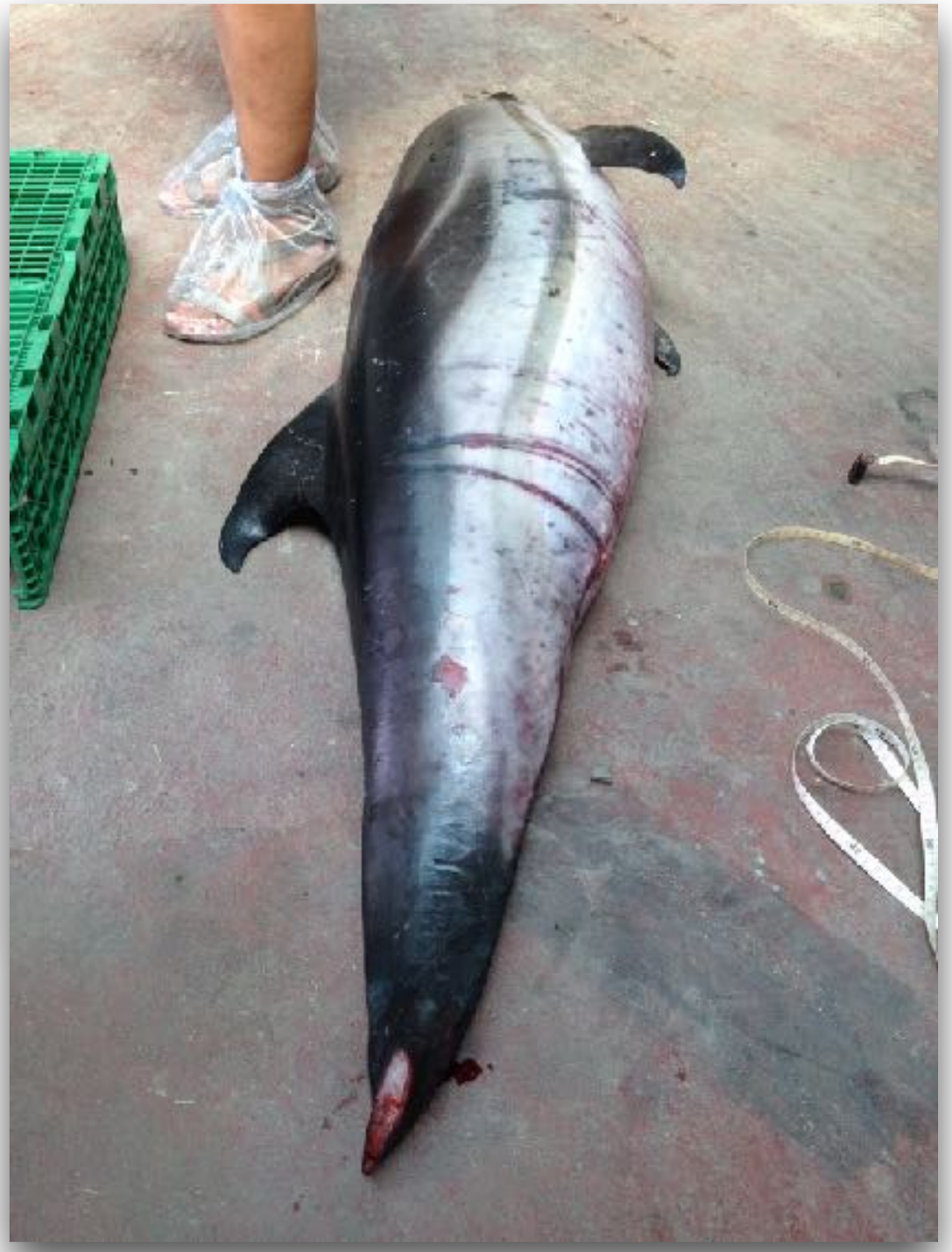
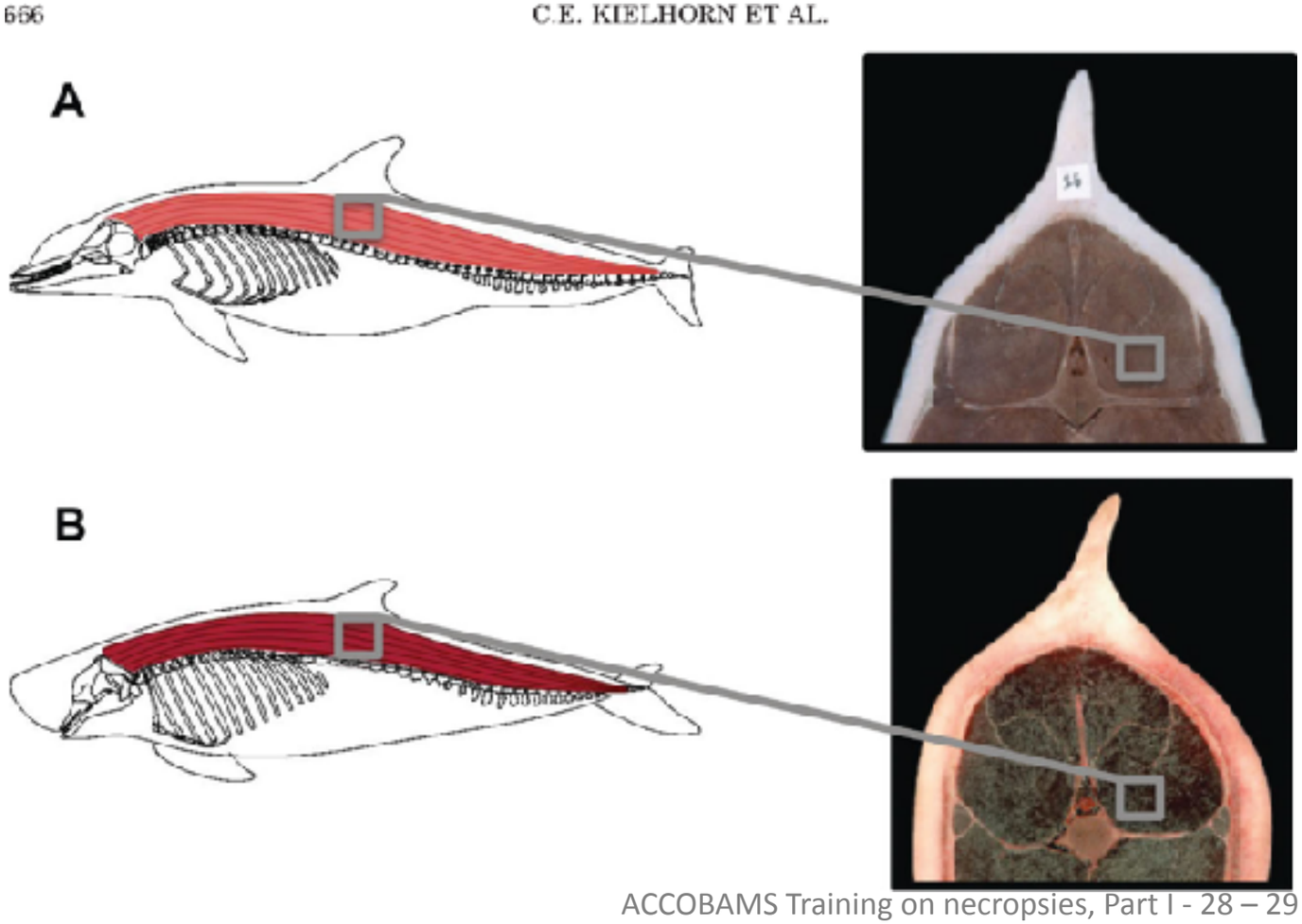


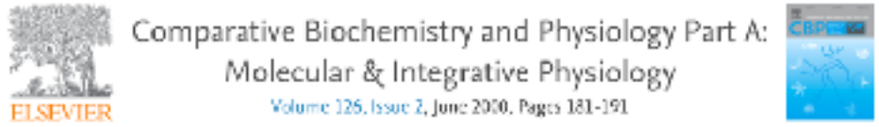
Tissue or Organ	Diagnostic investigation	max DCC	Aseptic fresh tissue	Frozen -20'C	Frozen -80'C	Ethanol	10% Buffered formalin	RNA Later	Quantity	Comment
Blubber	Contaminants	3		1					>10g, wrapped in aluminium foil	
	Stable isotopes and fatty acids	4		1					2 cm3 of aseptic sample	Freeze, -70/80°C
Skin	Biomarkers	1			1			1	2 cm3 of aseptic sample	
	Contaminants	3		1					>10g, wrapped in aluminium foil	
	Genetics	4		1		1		1	2 cm3 of aseptic sample	
	Histopathology	4					1			
	Stable isotopes and fatty acids	4		1					2 cm3 of aseptic sample	Freeze, -70/80°C

INTERNAL EXAMINATION

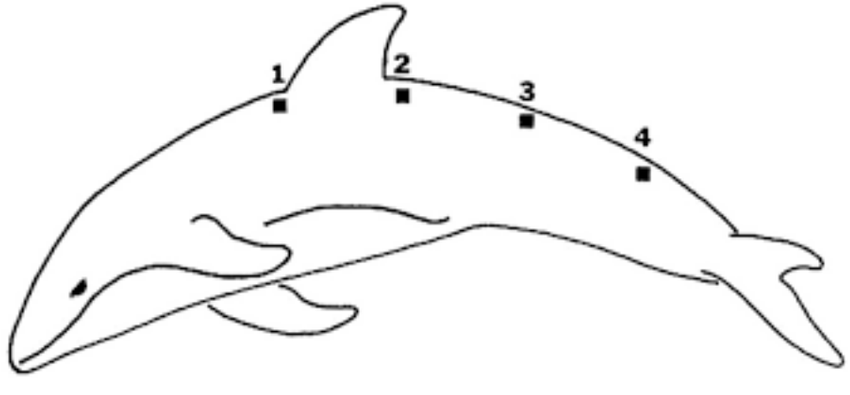
SKELETAL MUSCLE

- ✓ Examine the quality of the fascia and muscle on the body before removing it
- ✓ Note the color, texture, thickness and abnormalities
- ✓ Look for hemorrhage, post mortem pooling of blood in vessels (hypostasis or post mortem lividity) and bruising (hematoma)





Body size and skeletal muscle myoglobin of cetaceans: adaptations for maximizing dive duration



Tissue or Organ	Diagnostic investigation	max DCC	Aseptic fresh tissue	Frozen -20'C	Frozen -80'C	Ethanol	10% Buffered formalin	RNA Later	Quantity	Comment
Liver	Biomarkers	**2**			**1**			**1**		
	Contaminants	**3**		**1**					>10g, wrapped in aluminium foil	
	Histopathology	**3**					**1**		Sections of 1cm thickness over normal/abnormal border	
	Microbiology	**3**	**1**						Aseptic sample or swab	Refrigerated, +1°C prior to culture
	Virology	**3**			**1**				2 cm3 of aseptic sample	Freeze, -70/80°C
	Parasitology	**4**		**1**		**1**			Parasitology samples collected whole, dissect out head attachments of parasitic worms	Freeze, -20°C
	Stable isotopes and fatty acids	**4**		**1**						

DESCRIPTIVE PATHOLOGY

Distribution and location: note the anatomical region, organ and/or tissue involved. Report if the abnormality is bilateral or unilateral, diffuse, focal, multifocal or multiple, patchy;

Size: measure and scale any finding and/or compare with commonly known objects if a ruler is not available. In order to evaluate if any organ or body part dimension is increased or decreased compared to normal, the assessing person should be experienced in this species.

Shape: bi-dimensional or tri-dimensional description of the lesion(s) (circular, oblong, spheroid, ovoid, target-like, wedge-shaped, irregular, papillary, pedunculated, sessile, villous);

Margins: note the edges of lesions (indistinct, infiltrative, papillary, pedunculated, serpiginous, serrated, sessile, villous, well-demarcated);

Surface: describe the surface of the organ or lesion (bulging, cobblestoned, corrugated, crusted, eroded, granular, pitted, rough, smooth, striated, ulcerated, umbilicated, verrucous);

Colour: note the colour of any change. Usual colours in a carcass could be: black, brown, grey-green, mahogany, red, tan, white, yellow;

Consistency: note any changes compared to normal features of the tissue and/or organ of interest. Consistency cannot be evaluated by simply observing the organ/tissue, but should be done by palpating and comparing with known materials.

REGULATION (EC) No 1069/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 21 October 2009

laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation

THANK YOU FOR YOUR ATTENTION!

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