



EVIDENCE-BASED DIAGNOSTIC ASSESSMENT FRAMEWORKS FOR CETACEAN NECROPSIES ON SPECIFIC ISSUES/THREATS

Sandro Mazzariol & Cinzia Centelleghè
Department of Comparative Biomedicine and Food Science - University of Padova

Interpreting post-mortem findings and evidences collected during a thorough necropsy, not limited to gross examination, needs specific skills and expertise. More in detail, these data should be elaborated by skilled professionals to properly hypothesize the possible cause, mechanism and manner of death.

A necropsy is a specialized medical procedure comprising of a thorough examination of a carcass by dissection. Sampling and testing should be complete and not be driven by any previous hypothesis or speculation; interpretation of evidences should be based on the best existing literature and protocols already published and/or used, ruling out any possible causes of death without bias. Even if it depends on the specific country's legal framework, post-mortem investigations with diagnostic aims should be performed by a veterinarian trained in animal pathology with an experience in marine mammal diseases.

In the present document, best practices and criteria associated with diagnoses of the most relevant threats for marine mammals (i.e. bycatch, marine litter ingestion, underwater noise) found during cetacean post-mortem examinations are resumed along with the most recent pertinent literature. These set of findings constitute an evidence-based diagnostic assessment framework and could support the interpretation of data and observations collected during a thorough and complete necropsy by a veterinary pathologist and/or a governmental veterinarian.

It should be stressed that the following frameworks are not shortcuts that justifies rapid diagnoses from inexperienced personnel; rather they are a support tool for trained and authorized professionals to harmonize interpretation and evaluation. Total or partial presence or absence of the reported evidences obtained through the reported best practices should be considered along with the other results from the entire necropsy in order to gain the final diagnosis, and evidences should be interpreted by the experienced veterinarian or biologist involved after a complete necropsy. Without a complete post-mortem investigation, carried out according to a standardized procedure by expert and trained personnel, final diagnoses are not supported and have no value.

The following issues are herein resumed:

- a. [bycatch](#)
- b. [entanglement](#)
- c. [marine litter ingestion](#)
- d. [underwater noise](#)
- e. [ship strikes](#)
- f. [infectious diseases](#)

All the most relevant findings and diagnostic criteria for each single issue and reported in the most relevant literature will be summarized in tables including the type of examination, the tiers at which it could be detected according the European Cetaceans post-mortem investigations best-practices and some notes. It is not necessary that all the listed evidences are contemporary present, but they should be interpreted with the results of the complete necropsy and all the other possible causes of death should be ruled out. Since this information is included in the most recent literature, it is highly recommended a periodic update.

a. Bycatch

The challenge of identifying the cause of death in bycaught cetaceans arises from the nonspecific nature of the lesions of drowning/asphyxiation, lack of previous history of the dead animal and the varied nature of fishing gear, with no pathognomonic changes recognized for acute underwater entrapment. Several publications recognize signs of acute external entanglement, bulging or reddened eyes, recently ingested gastric contents, pulmonary changes, and decompression-associated gas bubbles as most commonly reported changes, but these findings cannot be surely related to acute bycatch and many others could support the interpretation and final diagnosis.

All these findings should be collected during a standardized, thorough necropsy performed by skilled personnel. The necropsy could allow to interpret all the reported findings, to exclude any other cause of death and to advance a final diagnosis.

Investigation	Evidences	Tier	Notes	Literature
External examination	Nutritional condition: very good to sub-optimal	1	difficult to detect in case of interaction with gillnets and trawling	1. Bernaldo de Quiros et al., 2018 2. Moore et al., 2013 3. Kuiken et al., 1994
	In vivo evidence of entanglement: . contact with fishing gear (superficial: impressions, depigmentation etc.) . presence of fishing gear . physical injuries (amputation, laceration, fracture etc.) . haemorrhagic findings			
	Bulging/red eyes			
Pathological examination (gross and/or microscopic)	Evidence of undigested gastro-oesophageal contents	1	nonspecific and/or pathognomonic	1. Bernaldo de Quiros et al., 2018 2. Moore et al., 2013 3. Kuiken et al., 1994 4. Bernaldo de Quiros et al., 2016
	Multi-organ congestion	2		
	Multi-organ gas bubbles with high score in coronary, renal, iliac, subcutaneous vessels and perirenal tissues	2	requires training	
	Pulmonary oedema	2	nonspecific finding associated to many other pathological conditions	
Chemical analyses of gas bubbles	Gas bubbles are not consistent with post-mortem gases.	3	sampling requires training and very few laboratories are skilled in this type of analyses	5. Bernaldo de Quiros et al., 2013 6. Bernaldo de Quiros et al., 2011
Microscopic and immunohistochemical examination	Muscle changes consistent with stress	3	sampling requires training and very few laboratories are skilled in this type of analyses	7. Sierra et al., 2017.
Pathological and microbiological examinations	Absence of infectious agents impairing animal health	3	results from microbiology should be compared to microscopic examination	2. Moore et al., 2013 3. Kuiken et al., 1994
Diatoms research technique	Diatoms in the long bones	3	not pathognomonic; may support diagnosis	8. Rubini et al., 2018

1. Bernaldo de Quirós Y, Hartwick M, Rotstein DS, Garner MM, Bogomolni A, Greer W, Niemeyer ME, Early G, Wenzel F, Moore M. **Discrimination between bycatch and other causes of cetacean and pinniped stranding.** *Dis Aquat Organ.* 2018 Jan 31;127(2):83-95.
2. Moore MJ, der Hoop Jv, Barco SG, Costidis AM, Gulland FM, Jepson PD, Moore KT, Raverty S, McLellan WA. **Criteria and case definitions for serious injury and death of pinnipeds and cetaceans caused by anthropogenic trauma.** *Dis Aquat Organ.* 2013 Apr 11;103(3):229-64. Kuiken T, Simpson VR, Allchin CR, Bennett PM, Codd GA, Harris EA, Howes GJ, Kennedy S, Kirkwood JK, Law RJ, et al. **Mass mortality of common dolphins (*Delphinus delphis*) in south west England due to incidental capture in fishing gear.** *Vet Rec.* 1994 Jan 22;134(4):81-9
3. Bernaldo de Quirós Y, Saavedra P, Møllerløkken A, Brubakk AO, Jørgensen A, González-Díaz O, Martín-Barrasa JL, Fernández A. **Differentiation at necropsy between in vivo gas embolism and putrefaction using a gas score.** *Res Vet Sci.* 2016 Jun;106:48-55.
4. Bernaldo de Quirós Y, Seewald JS, Sylva SP, Greer B, Niemeyer M, Bogomolni AL, Moore MJ. **Compositional discrimination of decompression and decomposition gas bubbles in bycaught seals and dolphins.** *PLoS One.* 2013 Dec 19;8(12):e83994.
5. Bernaldo de Quirós Y, González-Díaz O, Saavedra P, Arbelo M, Sierra E, Sacchini S, Jepson PD, Mazzariol S, Di Guardo G, Fernández A. **Methodology for in situ gas sampling, transport and laboratory analysis of gases from stranded cetaceans.** *Sci Rep.* 2011;1:193
6. Sierra E, Espinosa de Los Monteros A, Fernández A, Díaz-Delgado J, Suárez-Santana C, Arbelo M, Sierra MA, Herráez P. **Muscle Pathology in Free-Ranging Stranded Cetaceans.** *Vet Pathol.* 2017 Mar;54(2):298-311.
7. Rubini S, Frisoni P, Russotto C, Pedriali N, Mignone W, Grattarola C, Giorda F, Pautasso A, Barbieri S, Cozzi B, Mazzariol S, Gaudio RM. **The diatoms test in veterinary medicine: A pilot study on cetaceans and sea turtles.** *Forensic Sci Int.* 2018 Sep;290:e19-e23

b. Entanglement

Entanglement refers to the wrapping of materials of anthropogenic origin like lines, ropes or nets around the body of an animal and differs from bycatch, which refers to the unintentional capture of species such as small cetaceans in fishing nets.

Entangled animals do not die immediately after wrapping but the materials around the cetacean's body could injure it and impair its swimming, diving and feeding, inducing a chronic condition. In these conditions, death could be due to progressive starvation due to a reduction in food intake and an increase of energetic cost. Possible secondary infections could infect wounds associated with entanglement or affect the animal due to an impairment of the immune system. The following table resumes the main finding that could be reported during post-mortem examinations on entangled cetaceans.

Investigation	Evidences	Tier	Notes	Literature
External examination	Nutritional condition: poor to cachectic	1		1. Moore et al., 2006 2. Moore et al., 2013
	In vivo entanglement evidence: . contact with anthropogenic materials around the body of the animal (superficial changes) . presence of anthropogenic materials around the body of the animal . chronic physical injuries (laceration, scars, etc.)			
Gross examination	Muscular atrophy	2	possible findings that may be detected singularly or associated with muscular atrophy	1. Moore et al., 2006 2. Moore et al., 2013
	Absence of food remains in the stomach	2		
	Pale discoloration of muscle and tissues	2		
	Severe parasitic infestation	2		
	Gelatinous atrophy of the subcutaneous tissues	3		
	Haemorrhagic changes to subcutaneous and serosal surfaces (petechiae, bruises, etc.)	3		
	Opportunistic infections	3		
Microscopic examination	Muscular atrophy with scattered fiber necrosis	3	described in terrestrial mammals; only in single case reports in cetaceans	3. Sierra et al., 2017.
	Liver steatosis and/or hemosiderotic pigment in Kupffer cells	3		4. Gerdin et al., 2016
	Splenic hemosiderophages	3		
	Opportunistic infections	3		2. Moore et al., 2013
Microbiological investigations	Possible infectious diseases	3		2. Moore et al., 2013

1. Moore MJ, der Hoop Jv, Barco SG, Costidis AM, Gulland FM, Jepson PD, Moore KT, Raverty S, McLellan WA. **Criteria and case definitions for serious injury and death of pinnipeds and cetaceans caused by anthropogenic trauma.** *Dis Aquat Organ.* 2013 Apr 11;103(3):229-64.

2. Kuiken T, Simpson VR, Allchin CR, Bennett PM, Codd GA, Harris EA, Howes GJ, Kennedy S, Kirkwood JK, Law RJ, et al. **Mass mortality of common dolphins (*Delphinus delphis*) in south west England due to incidental capture in fishing gear.** *Vet Rec.* 1994 Jan 22;134(4):81-9
3. Moore MJ, Bogomolni AL, Bowman R, Hamilton PK. **Fatally entangled whales can die extremely slowly.** *Ocean'06 MTS/IEEE, Boston, MA:* 2006.
4. Sierra E, Espinosa de Los Monteros A, Fernández A, Díaz-Delgado J, Suárez-Santana C, Arbelo M, Sierra MA, Herráez P. **Muscle Pathology in Free-Ranging Stranded Cetaceans.** *Vet Pathol.* 2017 Mar;54(2):298-311.
5. Gerdin JA, McDonough SP, Reisman R, Scarlett J. **Circumstances, Descriptive Characteristics, and Pathologic Findings in Dogs Suspected of Starving.** *Vet Pathol.* 2016 Sep;53(5):1087-94.

c. Marine litter

The ingestion of marine litter can occur in many cetacean species and the number of reports of foreign bodies found in the stomachs of stranded marine mammals is increasing. Despite these numbers, it should be noted that findings of plastic debris are not often deemed to be the main cause of stranding and are poorly reported in pathology literature. Recent papers published in the Canary Islands (Díaz Delgado et al., 2018; Puig-Lozano et al., 2018) underline that only a few species seem to be lethally affected by plastic ingestion, with deep divers such as sperm whales and beaked whales more affected than others; young age and poor nutritional condition seems to be another relevant factor. With regards to the nutritional condition, it is not yet clear if it is a predisposing factor for the ingestion of marine litter, or a consequence thereof.

While, during necropsy, it is easy to state the possible ingestion of marine debris, it is more difficult to assess the impact it has on the animal's health. The findings summarized in the above table could be observed, alone or associated, and they can support the interpretation of the pathologist in the assessment of the cause of death during the complete necropsy.

Investigation	Evidences	Tier	Notes	Literature
External examination	Nutritional condition: normal to poor	1		
Gross examination	Plastic debris	1		1. Puig-Lozano et al., 2018 2. Díaz-Delgado et al., 2018
	Gastric perforation	2	may lead to acute death	
	Ulcerative gastritis	2		
	Gastric impaction/obstruction	2		
	Muscular atrophy	2	only when a poor nutritional condition has been determined	
	Severe parasitic infestation	2		
	Opportunistic infections	3		
Microscopic examination	Muscular atrophy	3		3. Sierra et al., 2017.
	Opportunistic infections	3		
Microbiological investigations	Possible infectious diseases	3		

1. Puig-Lozano R, Bernaldo de Quirós Y, Díaz-Delgado J, García-Álvarez N, Sierra E, De la Fuente J, Sacchini S, Suárez-Santana CM, Zucca D, Câmara N, Saavedra P, Almunia J, Rivero MA, Fernández A, Arbelo M. **Retrospective study of foreign body-associated pathology in stranded cetaceans, Canary Islands (2000-2015)**. *Environ Pollut.* 2018 Dec;243(Pt A):519-527.
2. Díaz-Delgado J, Fernández A, Sierra E, Sacchini S, Andrada M, Vela AI, Quesada-Canales Ó, Paz Y, Zucca D, Groch K, Arbelo M. **Pathologic findings and causes of death of stranded cetaceans in the Canary Islands (2006-2012)**. *PLoS One.* 2018 Oct 5;13(10):e0204444.
3. Sierra E, Espinosa de Los Monteros A, Fernández A, Díaz-Delgado J, Suárez-Santana C, Arbelo M, Sierra MA, Herráez P. **Muscle Pathology in Free-Ranging Stranded Cetaceans**. *Vet Pathol.* 2017 Mar;54(2):298-311.

d. Underwater impulsive noise-related strandings

The diagnostic assessment framework for the investigation of underwater impulsive noise as a possible cause of strandings is not as complete as for other causes due to lack of knowledge. In fact, only a spatial and temporal association of middle and low frequency military sonar to a gas and fat embolic syndrome developed in beaked whales has been reported, while for any other species and/or sound sources there is not yet enough literature to draw possible diagnostic criteria. Investigations performed on the inner ear conducted according to a specific protocol could support the diagnosis of cochlear damage.

Due to these limitations, to date, it is only possible to exclude any other possible cause through a complete and detailed necropsy. The stranding pattern (active vs. passive, location of strandings, marine currents etc.), the number of animals involved (individual or multiple animals in good nutritional condition stranded within hours or a few days of a military exercise), the spatial and temporal association with a functioning impulsive noise source are fundamental to support the diagnostic hypothesis. From a pathological point of view, the post-mortem findings included in the following table may be observed.

Investigation	Evidences	Tier	Notes	Literature
External examination	Bleeding from main orifices	1		1. Fernandez et al., 2005 2. Bernaldo de Quiros et al., 2019
	Good nutritional status	1		
Gross examination	food remnants in the first gastric compartment ranging from undigested food to squid beaks	2		
	abundant gas bubbles widely distributed in veins (subcutaneous, mesenteric, portal, coronary, subarachnoid veins, etc.)	2	requires training	
	gross subarachnoid and/or acoustic fat hemorrhages;	2		
	absence of other relevant diseases	2		
Microscopic examination	microscopic multi-organ gas and fat emboli associated with bronchopulmonary shock	3		
	diffuse, mild to moderate, acute, monophasic myonecrosis (hyaline degeneration) in fresh and well-preserved carcasses	3		
	multi-organ microscopic hemorrhage of varying severity in lipid-rich tissues such as the central nervous system, spinal cord, and the coronary and kidney fat (when present)	3		
	Hemorrhage in the inner ear visible with HE-stain after decalcifying tympano-periotic complex	3	decalcification process may alter microscopic findings	
	absence of other relevant diseases	3		
Chemical analyses of gas bubbles	mainly N2	3	requires training to collect bubbles from veins and perform chemical analyses	4. Bernaldo de Quiros et al., 2011
Electron microscopy	scars and damage to the cochlear hair cells of the inner ear	3	requires training to collect and preserve inner ear; possible until 30 hours after death	5. Morell et al., 2017

1. Fernández A, Edwards JF, Rodríguez F, Espinosa de los Monteros A, Herráez P, Castro P, Jaber JR, Martín V, Arbelo M. **"Gas and fat embolic syndrome" involving a mass stranding of beaked whales (family Ziphiidae) exposed to anthropogenic sonar signals.** *Vet Pathol.* 2005 Jul;42(4):446-57
2. Bernaldo de Quirós Y, Fernandez A, Baird RW, Brownell RL Jr, Aguilar de Soto N, Allen D, Arbelo M, Arregui M, Costidis A, Fahlman A, Frantzis A, Gulland FMD, Iñíguez M, Johnson M, Komnenou A, Koopman H, Pabst DA, Roe WD, Sierra E, Tejedor M, Schorr G. **Advances in research on the impacts of anti-submarine sonar on beaked whales.** *Proc Biol Sci.* 2019 Jan 30;286(1895):20182533
3. Jepson PD, Deaville R, Acevedo-Whitehouse K, Barnett J, Brownlow A, Brownell RL Jr, Clare FC, Davison N, Law RJ, Loveridge J, Macgregor SK, Morris S, Murphy S, Penrose R, Perkins MW, Pinn E, Seibel H, Siebert U, Sierra E, Simpson V, Tasker ML, Tregenza N, Cunningham AA, Fernández A. **What caused the UK's largest common dolphin (*Delphinus delphis*) mass stranding event?** *PLoS One.* 2013 Apr 30;8(4):e60953. doi: 10.1371/journal.pone.0060953.
4. Bernaldo de Quirós Y, González-Díaz O, Saavedra P, Arbelo M, Sierra E, Sacchini S, Jepson PD, Mazzariol S, Di Guardo G, Fernández A. **Methodology for in situ gas sampling, transport and laboratory analysis of gases from stranded cetaceans.** *Sci Rep.* 2011;1:193
5. Morell M, Brownlow A, McGovern B, Raverty SA, Shadwick RE, André M. **Implementation of a method to visualize noise-induced hearing loss in mass stranded cetaceans.** *Sci Rep.* 2017 Feb 6;7:41848. doi: 10.1038/srep41848

e. Ship strikes

In the last decades, collisions between vessels and cetaceans have significantly increased worldwide and they are deemed to be a major threat for large cetaceans living in the ACCOBAMS area. In case of collisions, external features may be pathognomonic with extensive subcutaneous, muscular and visceral hemorrhage and hematomas, indicating unequivocal *ante-mortem* trauma. However, when carcasses are highly autolyzed, it is challenging to distinguish whether the trauma occurred *ante-* or *post-mortem*. The presence of fat emboli within the lung microvasculature is used to determine a severe “*in vivo*” trauma in other species, and they can be used also in these cases. These aspects are summarized in the following tables.

Investigation	Evidences	Tier	Notes	Literature
External examination	Sharp traumas with one or more linear to curvilinear laminar incising wounds that cause damage to axial muscles, skull and vertebral column	1	mainly on the back and sides	1. Moore et al., 2013 2. Campbell-Malone et al., 2008
Gross examination	Blunt traumas with hemorrhage and edema in the blubber, subcutaneous tissue, and skeletal muscle	2		
	fractures and luxations	2		
Microscopic examination	Muscular hemorrhages and edema	3		3. Sierra et al., 2014.
	flocculent, granular or/and hyalinised segmentary degeneration; contraction band necrosis; discoid degeneration or fragmentation of myofibres	3		
	Fat emboli in the lung tissue	3	not relevant if death is immediate after trauma	4. Arregui et al., 2019

1. Moore MJ, der Hoop J, Barco SG, Costidis AM, Gulland FM, Jepson PD, et al. **Criteria and case definitions for serious injury and death of pinnipeds and cetaceans caused by anthropogenic trauma.** *Dis Aquat Organ.* 2013; 103 (3): 229–264
2. Campbell-Malone R, Barco SG, Daoust PY, Knowlton AR, McLellan WA, Rotstein DS, et al. **Gross and histologic evidence of sharp and blunt trauma in North Atlantic right whales (*Eubalaena glacialis*) killed by vessels.** *J Zoo Wildl Med.* 2008; 39 (1): 37–55.
3. Sierra E, Fernández A, Espinosa de los Monteros A, Arbelo M, Díaz-Delgado J, Andrada M, et al. **Histopathological muscle findings may be essential for a definitive diagnosis of suspected sharp trauma associated with ship strikes in stranded cetaceans.** *PLoS One.* 2014
4. Arregui M, Bernaldo de Quirós Y, Saavedra P, Sierra E, Suárez-Santana CM, Arbelo M, Díaz-Delgado J, Puig-Lozano R, Andrada M and Fernández A (2019) **Fat Embolism and Sperm Whale Ship Strikes.** *Front. Mar. Sci.* 6:379

f. Infectious diseases

Cetaceans can be affected by many infectious agents that can cause diseases and death. Among these pathogens, Cetacean Morbillivirus (CeMV), Brucella spp. and Toxoplasma gondii are the most relevant ones.

As in terrestrial mammals, the diagnosis of a disease is supported by the contemporary evidence of pathological changes, immunohistochemical and microbiological findings. If all three are not present at the same time, the diagnosis is weak, and it should be interpreted in accordance with other findings. In the following table, main findings for CeMV diseases are reported in order to aid pathologists in their diagnosis for this virus considered as the more dangerous for the cetaceans' conservation in the ACCOBAMS waters. Other pathogens are often reported as single case reports.

Investigation	Evidences	Tier	Notes	Literature
Gross examination	Meningeal congestion	2	not always present at the same time	1. Van Bresse et al., 2014
	Lymph node enlargement	2		
	Bronchopneumonia	2		
	Secondary infections and parasitic infestation	2		
Microscopic examination	Chronic meningoencephalitis with astrogliosis and possible demyelination	3		
	Interstitial bronchopneumonia	3		
	Lymphoid depletion with multinucleated giant cells	3		
	Secondary infections and parasitic infestation	3		
Immunohistochemistry	Positive using anti-CDV antibodies	3		
Molecular analyses	Positive target organs (brain, lymph nodes, spleen, thymus, lungs)	3	highly specific but limited by conservation code	
		3	time-consuming but highly sensitive for large cetaceans and conservation codes 3-4	3. Centelleghé et al., 2016
		3	all CeMV strains	4. Rubio-Guerri et al., 2013

1. Van Bresse MF, Duignan PJ, Banyard A, Barbieri M, Colegrove KM, De Guise S, Di Guardo G, Dobson A, Domingo M, Fauquier D, Fernandez A, Goldstein T, Grenfell B, Groch KR, Gulland F, Jensen BA, Jepson PD, Hall A, Kuiken T, Mazzariol S, Morris SE, Nielsen O, Raga JA, Rowles TK, Saliki J, Sierra E, Stephens N, Stone B, Tomo I, Wang J, Waltzek T, Wellehan JF. *Cetacean morbillivirus: current knowledge and future directions*. *Viruses*. 2014 Dec 22;6(12):5145-81.
2. Verna F, Giorda F, Miceli I, Rizzo G, Pautasso A, Romano A, Iulini B, Pintore MD, Mignone W, Grattarola C, Bozzetta E, Varello K, Dondo A, Casalone C, Gorla M. *Detection of morbillivirus infection by RT-PCR RFLP analysis in cetaceans and carnivores*. *J Virol Methods*. 2017 Sep;247:22-27.
3. Centelleghé C, Beffagna G, Zanetti R, Zappulli V, Di Guardo G, Mazzariol S. *Molecular analysis of dolphin morbillivirus: A new sensitive detection method based on nested RT-PCR*. *J Virol Methods*. 2016 Sep;235:85-91.
4. Rubio-Guerri, C. et al. *Simultaneous diagnosis of Cetacean morbillivirus infection in dolphins stranded in the Spanish Mediterranean Sea in 2011 using a novel Universal Probe Library (UPL) RT-PCR assay*. *Vet Microbiol* 165, 109–114 (2013).