





ACCOBAMS training on necropsies

Part I - Online, 28 - 29 June 2021



Necropsy: a tool for the identification of the causes of death and threats

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:

- Belgian coastline: 2013: 180 stranded marine mammals Highest density in the world (//km coastline)

2020 different unusual stranding (Belgique/France):

- Jan. : 1 Sowerby beaked whale (Mesoplodon bidens)

- Sept.: 1 fin whale, 1 Bryde's whale and 1 tropical whale (B. edeni): fis stranding in Europe

- Oct.: 3 fin whales

- Dec.: : 1 Sowerby beaked whale

2020 Belgian coastline: 94 marine mammals dead stranded





Different "options" for stranded cetaceans (lead by local priorities)

Calais (France): Nov. 2015: 10 pilot whales (*Globicephala melas*): immediate necropsy



Yoff- Dakar (Senegal): May 2008: 34 pilot whales: 32 « dissappeared as « marine bushmeat »; 2 were necropsied





Collision between a ship and a whale (ship strike): reason of post-mortem investigations?











Sperm whales Unusual Mortality Event: links with human activities?

SPERM WHALE STRANDINGS 2016





Narwhal: Arctic species One individual stranded in Belgium (2016): link with global changes?



Narwhal: 11 strandings recorded in the North Sea between 1588 and 1949!!!



To understand cetaceans' strandings, causes of death, main threats:

OONE TOOL:

NECROPSY (gross lesions and histopathology) and complementary investigations (microbiology, parasitology...), toxicology,....



Introduction: the North Sea situation

Where is the North Sea?

What marine mammal species are present?

Why and how to study marine mammals?

How many strandings?

What are the main causes of death?

The continental coastline of the southern North Sea:

Causes of death of small cetaceans: the harbour porpoise

Causes of death of large cetaceans



« ... cet animal appartenait à l'embranchement des vertébrés, à la classe des mammifères, au groupe des pisciformes, et finalement à l'ordre des cétacés. Quant à la famille dans laquelle il prenait rang, baleine, cachalot ou dauphin, quant au genre dont il faisait partie, quant à l'espèce dans laquelle il convenait de le ranger, c'était une question à élucider ultérieurement.
 Pour la résoudre, il fallait disséquer ce monstre inconnu,... »

To solve the enigma, it is necessary to dissect such unknown monster

Vingt mille lieues sous les mers

Jules Vernes,





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The continental coastline of the southern North Sea: Causes of death of small cetaceans: the harbour porpoise

Causes of death of large cetaceans: sperm whale and fin whale



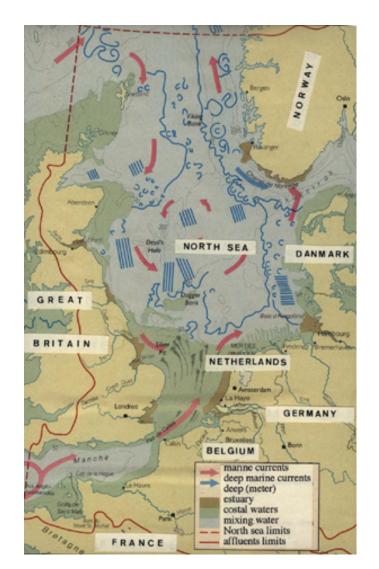
Different agreements for the conservation and protection of marine mammal species

- International: Convention on International Trade in Endangered Species (CITES), Convention on Migratory Species
- European: Habitat directives
- Regional: (1) Agreement on the Conservation of Small Cetaceans of the Baltic and North Sea (ASCOBANS),
- (2) Agreement on the Conservation of Cetaceans in the Black Sea, Mediterranean Sea and contiguous Atlantic area (ACCOBAM), OSPAR
- national: federal laws

PROTECTED SPECIES



Where is the North Sea?









What marine mammal species are present?

Cetaceans in the southern North Sea

Odontoceti Harbour porpoise

White-beaked dolphin

Atlantic white-sided dolphin

Striped dolphin

Common dolphin

Bottlenose dolphin

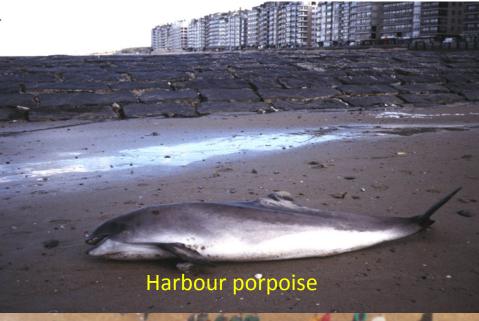
Sperm whale

Mysticeti Minke whale

Fin whale

Humpback whale



















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Why to study marine mammals?

Bioindicators

Long life span
Mammals (homeothermic)
Contaminated environment
Top predator

Accumulation of lipophilic contaminants in marine food web Bioaccumulation: concentration ratio between predator and prey

Marine mammals will indicate early alteration associated with contaminant exposure

<u>High concentration of contaminant in tissues of stranded marine mammals: BUT</u> High concentration responsible for diseases/lesions?

or

Diseases/lesions responsible for contaminant increasing in tissues

NEED: multidisciplinary approach of the cause of marine mammals death, from the individual to evaluate the population health status

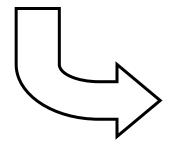


How to study marine mammals?

Stranding network: MARIN aims:

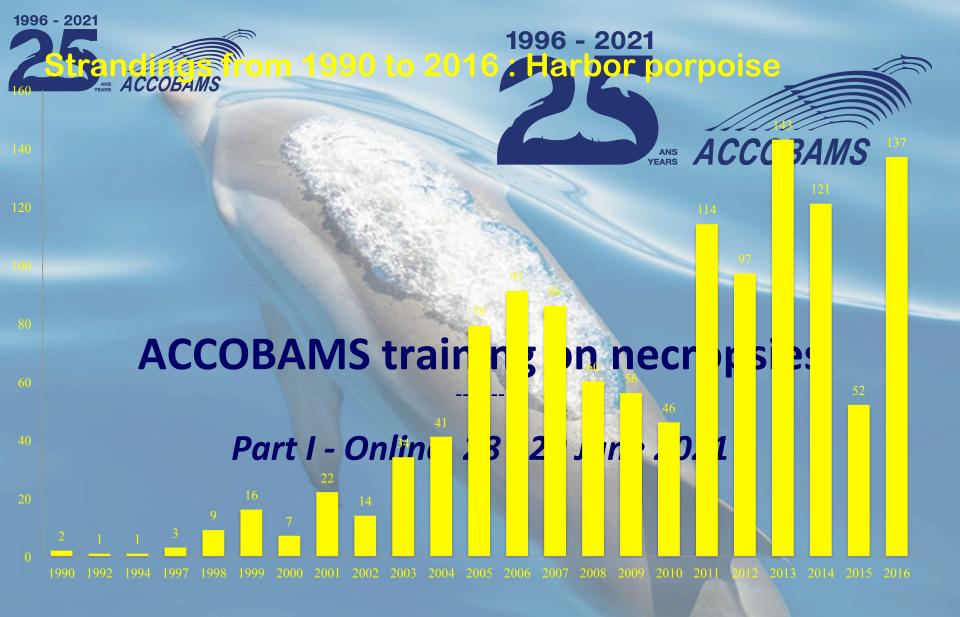
Identification of lesions and causes of death of cetaceans and pinnipeds- stranded on the continental coastline of the southern North Sea by:

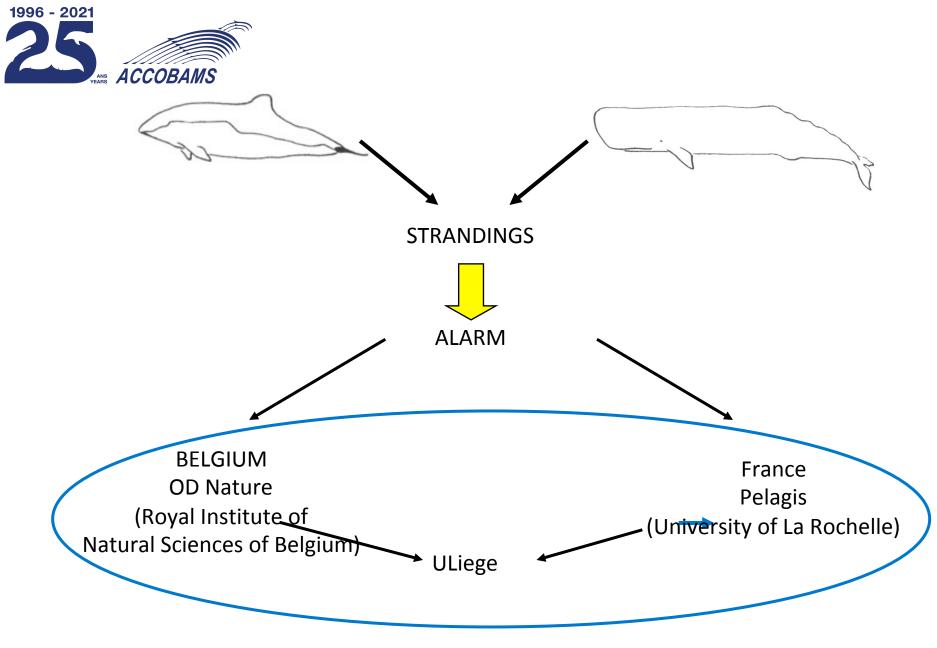
- Systematic necropsy using specific protocol;
- Complete samplings and tissues banking;
- Database with all results;
- Detailed description of lesions and their origin;
- Highlighting main mortality processes.



- 1. Creation of specific theories for stranding;
- 2. Evolution of the causes of death;
- 3. Impacts of human activities;
- 4. From the individuals to the population

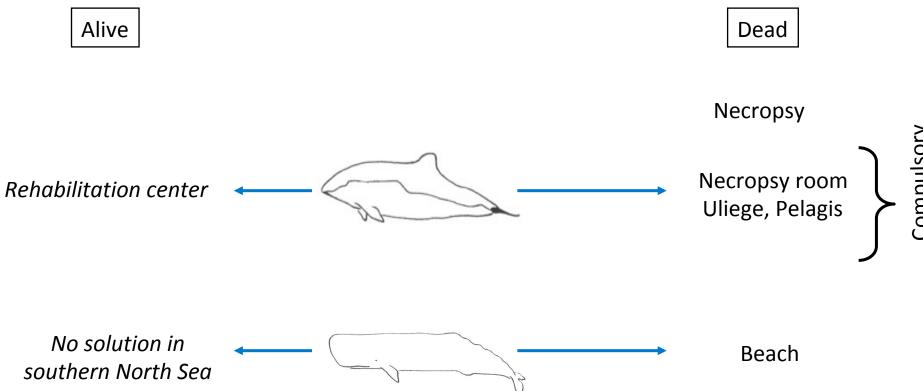
One health, multidisciplinary, holistic approach





INTERVENTION









"A pod of almost 48 Sperm Whales have stranded two days ago on a sand bar at Perkins Island near Smithton, on Tasmania's north-west coast. During these two days Australian rescuers have tried to save 12 of them that were still alive, but the sperm whales are simply too big and heavy to be moved and when the sun's out the animals just heat up incredibly quickly, the blubber, which is a real asset to them in the deep cold waters, just really makes them heat up quickly, on top of that the site of the stranding can only be reached with boats and during the high tide making everything more difficult"

http://seawayblog.blogspot.be/2009/01/slow-death-of-sperm-whales-pod.html



	Repro	HP.	Viro.	Bact.	Parasi	ML	POPs	Alim	Genet
Peau									alcool
Glande mammaire G/D						lait	lait	lait	
Dents						3			5:-20°C
Graisse									
Muscle									
Foie					parasite				
Surrénale									
Ganglion mésentérique									
Rate									
Gonade									
Tractus reproducteur complet									
Prostate G/D									
Estomac					parasite				
Restes alimentaires									
Intestin									
Rein									alcool
Vessie									
Pancreas									
Poumon					parasite				
Ganglion bronchique									
Coeur G/D				sang					
Aorte									
Oesophage				+ulcère					
Thymus									
Thyroide									
Système nerveux central									
Hypophyse									
Sang									
Placenta, cordon, membrane									
Conduit auditif									
Oeil									
Squelette/einture pelvienne									
Lésions									



Histopathology: 10% buffered formol

Virology: samples stored -80°C ou -20°C (no formol no ethanol)

Bacteriology: samples stored 4°C (no formol no ethanol)

Parasitology: parasites stored in 70 % ethanol with 5% glycerin

Toxicology:

Heavy metals (>50 g.): samples stored in plastic bags -20°C Persistant Organic Pollutants (>50 g.): : samples stored in aluminium -20°C

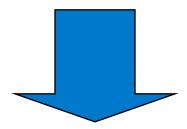
Stomach content (prey investigation): samples stored -20°C

Genetic: samples stored in 70 % ethanol



Necropsy of marine mammals

- 1. Report of all observed lesions
- 2. Collection of samples for complementary investigations (histopathology, toxicology, bacteriology virology, parasitology, mycology,...)
 - 3. Complementary investigations analyses and results



One health, multidisciplinary, holistic approach of the causes of death and identification of major threats



Marine mammal necropsies:



scientific, political, mediatic and sanitary reasons

Team composed of veterinarian (vet. Pathologist), with assistants (or volunteers such as veterinary and biologist students)

Specific procedures and protocoles (Join ASCOBANS-ACCOBANS protocol)

Equipment

Necropsy of all stranded animals whatever the size (0,8 m to 20 m) or the conservation code (1 very fresh to 5 severe putrefaction)



Cetaceans necropsy in the context of ACCOBAMS (also ASCOBANS and other agreements)

- Identification of lesions and causes of death, categorization of main threats
- Tool for elaboration of strategies for conservation and protection concepts



Major threats for marine mammals in the North Atlantic

Net capture

Viral infection

Parasites infestation

Bacterial infection

Contaminants/Pollutants

Miscellaneous





Main threats and causes of death of marine mammals in the southern North Sea

1. Net entraptment (by catch):

- First cause of porpoises death
- Removal rate > population growth rate (2 4%)
- Porpoise population estimated: 350.000 (2%: 7.000)

1.1. Evaluation by observers on fishing vessels (number of porpoises)

Country	Fishing area	Period	Annual rate	Reference
Denmark	North Sea	1993-1994	7000 🕺 🤻	Vinther, 1996
Great-Britain	Irish Sea	1993-1994	2200 🙎	Tregenza <i>et al.,</i> 1997
Denmark	Baltic and North Seas	1994-1998	6785 🙎 🕱	Vinther, 1999











1.2. Evaluation by necropsy on stranded porpoises

... most of by-caught animals being thrown back into water after capture

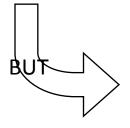
Location	Porpoise	Period	Reference
British islands	24%	1989-1991	Baker & Martin, 1992
British islands	34%	1990-1996	Jepson, 2000
German coasts	51%	1991-1993	Benke, 1998
German coasts	47%	1991-1996	Siebert <i>et al.,</i> 2001

The Netherlands, Belgium (2008): 50%)



Post-mortem diagnosis based on:

- evidence of capture : skin lacerations, hematomas
- evidence of release from net: flipper amputation, eventration
- good health: absence of disease, good nutritional status
- evidence of hypoxia: lung congestion and edema, petechia



lesions not always present and non-pathognomonic, and no diagnostic test



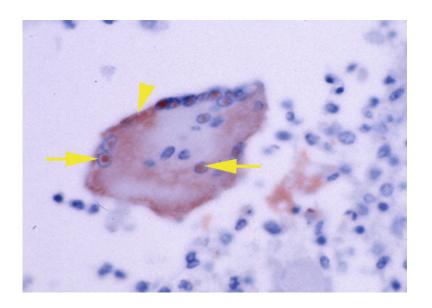


Estimation of by-caught only by necropsy underestimated the impact on population



2. Viral disease: Morbillivirus (similarity with Distemper, measles, rinderpest)

See Sandro's presentation



Multinucleate cell, Immunohistochemistry straining Morbillivirus +, lymph node, Balaenoptera physalus

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3. Parasites infestation

Respiratory nematodiasis with secondary bacterial/fungal infection: first infectious cause of death

Harbour porpoise: high prevalence of nematodes infestations in lung (tract, pulmonary arteries), heart, stomach, middle ear.

Also trematodes infestation in biliary ducts, stomach.

Also cestodes infestation in intestine

Nematodes: suspected to play a rule for transmission of Brucella or morbillivirus

4. Bacterial infection

Most opportunistic germ secondary parasites or viral infection leading to pneumonia, septicaemia

Risk of zoonosis:

Erysipelothrix rhusiopathiae: skin lesion, septicemia, endocarditis,

Brucella sp: 4 human cases reported of neurobrucellosis and osteomyelitis (B.ceti)

5. Fungal infection

Opportunistic, secondary to parasites: Aspergillus fumigatus, Candida sp.

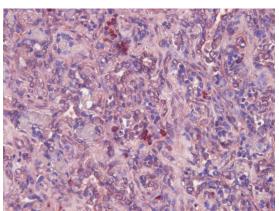




 For cetaceans: Brucella ceti:
 High zoonotic risk (4 cases reported)
 1 case of profesional exposition low consequences: fever, tiredness



3 other cases: unknown origin (no link with marine mammals)
BUT raw fish and crustacean consumers (sushi eater)
Severe consequence: encephalitis



For seals: Brucella pinnipedialis
 Zoonotic risk :? (no case reported)
 Seal meat consumption by nordic people



6. Contaminants

<u>Immunosuppression</u>: suspicion in morbillivirus diseases emergence but epizootics of rinderpest and measle before presence of such contaminants



<u>Alteration of reproduction and endocrine disruptor</u>: uterine stenosis, reduction of reproduction

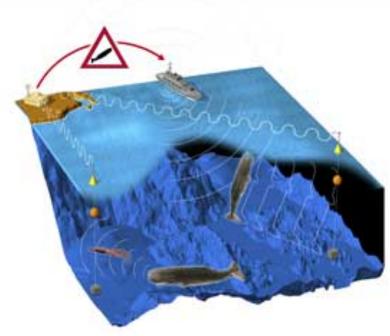
Tumor induction:

St-Laurent beluga whale, the highest prevalence of tumor compared with other species linked with high concentration of B(a)P



Collision or ship strike: area with high density of traffic or with high speed:

- Tankers: no specific location
- Jet sky
- Fast ferry: between islands (Canaries) or between continent and island (Corsica, Baleares) specific location: anti-collision system



http://rolexawards.com/en/the-laureates/michelandre-home.jsp



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The continental coastline of the southern North Sea:

Causes of death of small cetaceans: the harbour porpoise

Causes of death of large cetaceans: sperm whale and fin whale

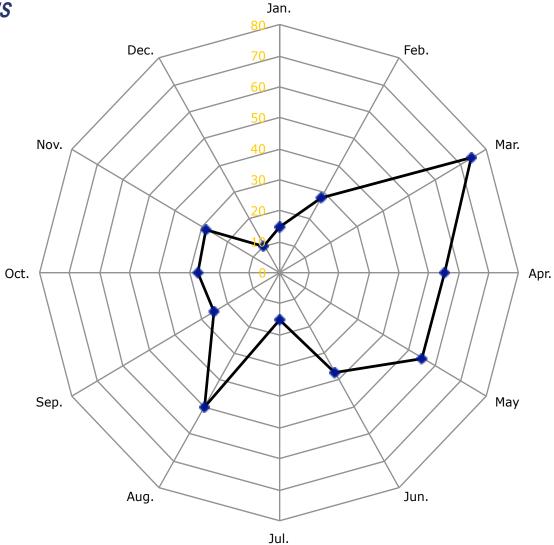




- 1. >3000 porpoises stranded between 1990 and 2017
- 2. >1000 necropsied and sampled
- 3. Identification of relevant lesions and causes of death
- 4. Samples for histology, microbiology, toxicology, age determination, preys identification, stable isotopes, ...





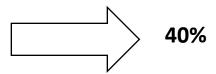


End of winter and August peaks



Causes of death (when the cause of death is determined!) net capture

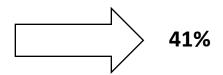
- -External observations : 30% skin lacerations, net marks, amputation
- Other observations :
 subcutaneous hemorrhages
 lung edema and congestion
 recent feeding
 good nutritional status



!!! BUT (severe) diseases also on by-caught porpoises (then not a "control" population de facto without necropsy

infectious diseases and emaciation

emaciation
parasitosis
acute pneumonia
severe gastritis
no evidence of recent feeding





Captures linked with recreational fishing activities -beach set gillnet;

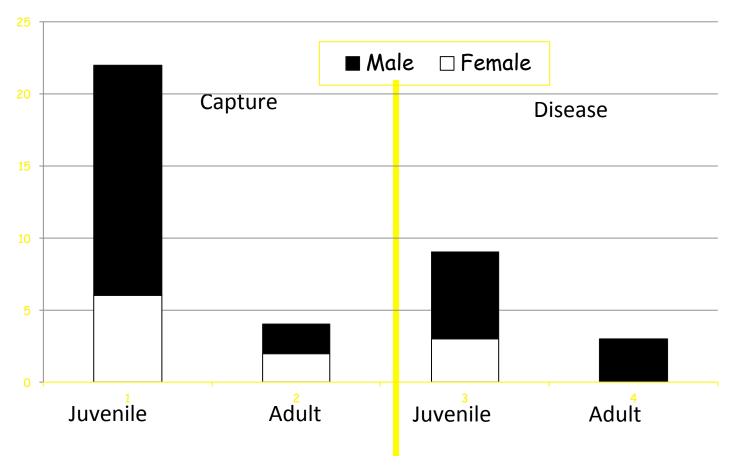
Frequently other lesions on by-caught porpoises, some being potentially fatal (severe emaciation, acute pneumonia)

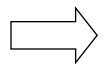
Lesions related to net-entrapment: not always present (skin marks,..) and not specific: underestimation of net capture

To evaluate health status of stranded animals: Needs of complete necropsy by pathologist of all animals including animals with clear evidence of net-entrapment



Sex and age





- ✓ More males than females
- ✓ More juveniles
- ✓ Predisposition for capture amongst juveniles

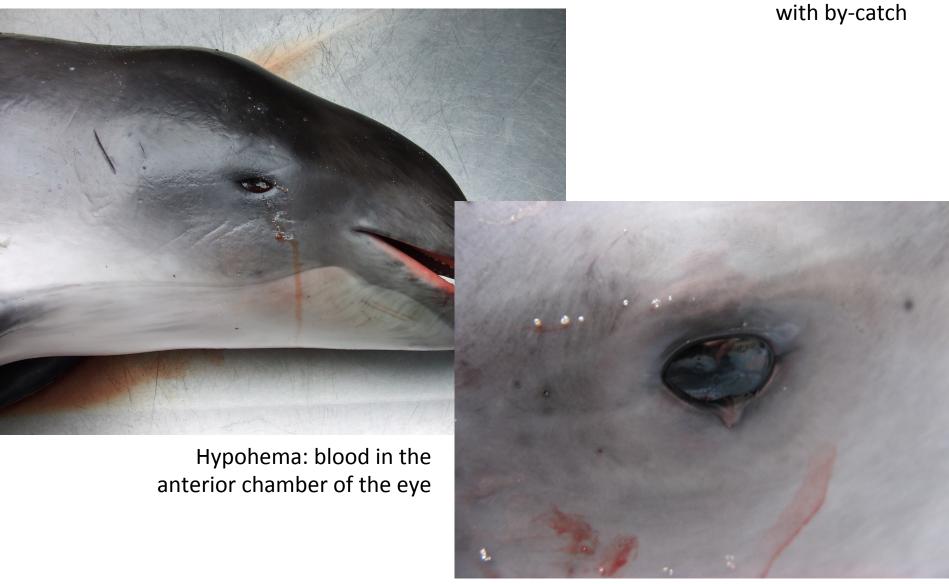








Most common external observations frequently associated with by-catch





France: Bay of Biscay situation: Every winter, hundreds of dolphins stranded with evidences of capture









Annually, end of winter around 800-1,000 stranded dolphins on French coastline of bay of Biscay

Model estimation: 5-8 % offshore capture

Cause of death: mostly capture

Final evaluation: 10.000 à 16.000 by-caught dolphins

⇒ Significant local treath

BUT post-mortem investigations done by non-veterinarians and post-mortem conclusions invalidate by advocates

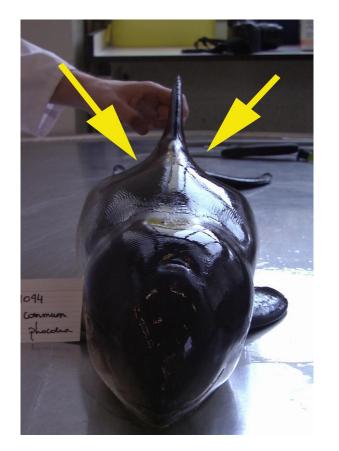
Strong need of veterinary expoerts to validate post-mortem investigations



Diseases & emaciation



Good nutritional status



Poor nutritional status (severe emaciation)



Parasitism (presence of parasites) vs parasitosis (disease associated with parasites)



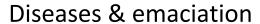
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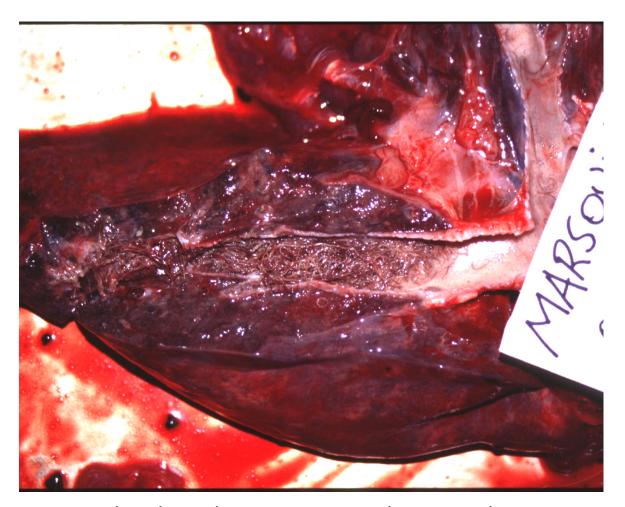
Gastric nematodes

Airways nematodes

Peritympanic nematodes



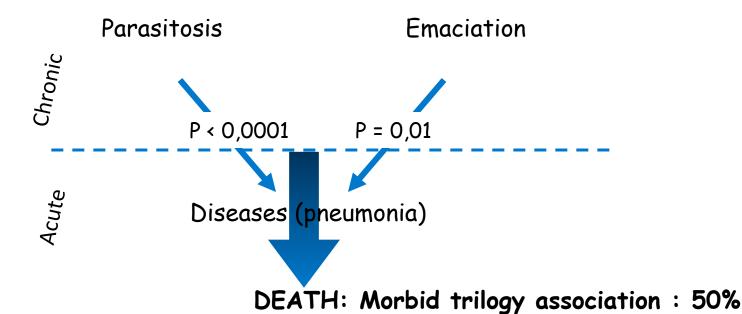




Acute purulent bronchopneumonia with nematodes in airways

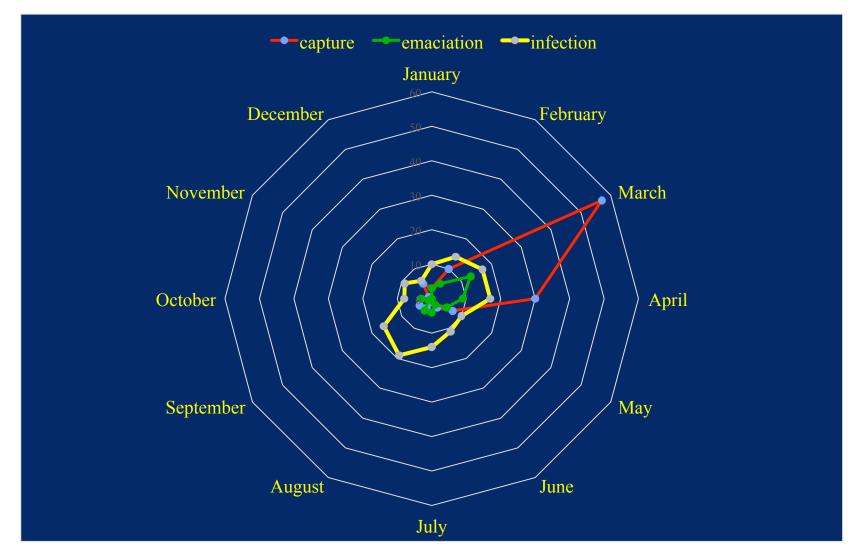


Causes of death



Human activities: accidental net capture 40%







Why such porpoises strandings increase on the continental coastline of the southern North Sea

1. Southern shift of the porpoises population

porpoise population estimated: 350.000

SCANS 1: 1994: northern North Sea SCANS 2: 2005: southern North Sea

2. Increase of capture

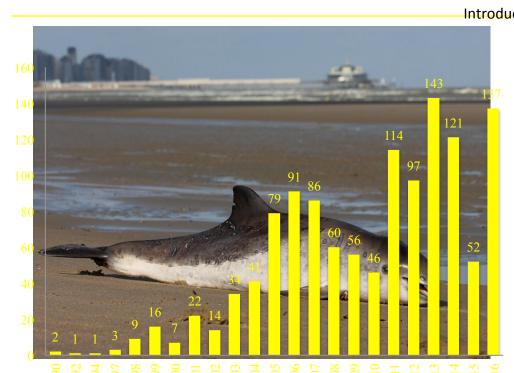
1990-2000: 20%

2001-2013: >40%

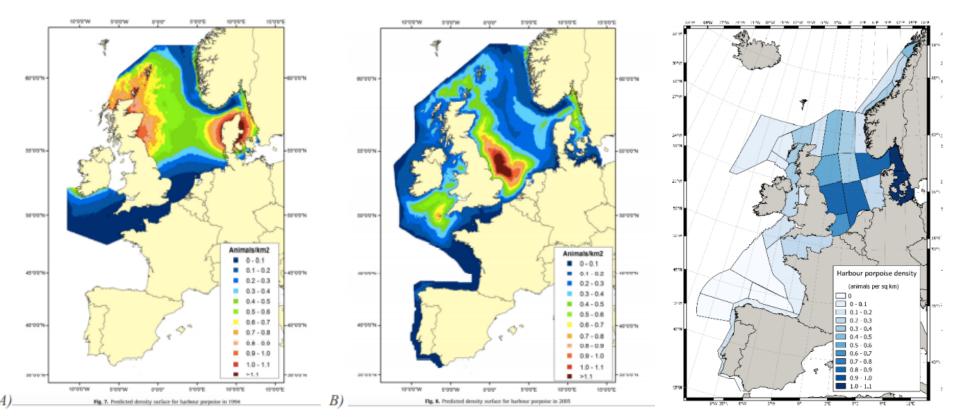
recreational fishing activities

(beach set gillnet)

3. Oceanographic (currents) Predisposition for Belgium







Distribution of harbour porpoises A) SCANS-I (1994) B) SCANS-II (2005) C) SCANS-III (2016) (Source : Hammond et al. 2013 et Hammond et al. 2017)





Since 2011, porpoises with severe skin lacerations









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- 1. Hemorragic lesions: ante-mortem
- Edges of lesion: regular but finely serratedBUT not done by a knife
- 3. Parallel edge AND punctifurm lesion
 - = Bite: marine predator

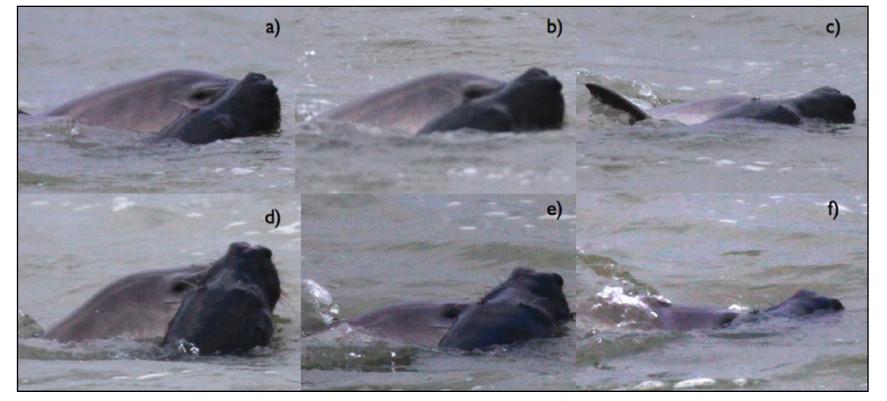
4. Space between edges of lesion: 6 cm

Candidates: Common seal and grey seal

Space between inter-canine teeth: Common seal (n= 52): 1,4 à 4,8 cm

Grey seal (n= 87): 1,9 à 7, 1 cm







The final proof: demonstration of seal's DNA in porpoise's lesion: the forensic pathology method













Jauniaux T., Garigliany M.-M., Loos P., Bourgain J.-L., Bouveroux T., Coignoul F., Haelters J., Karpouzopoulos J., Pezeril S., Desmecht D., Bite Injuries of Grey Seals (*Halichoerus grypus*) on Harbour Porpoises (*Phocoena phocoena*), PLoS ONE 9(12): e108993. doi: 10.1371/journal.pone.0108993



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Large cetaceans necropsy: ALWAYS MAJOR CHALLENGE

Sperm whales

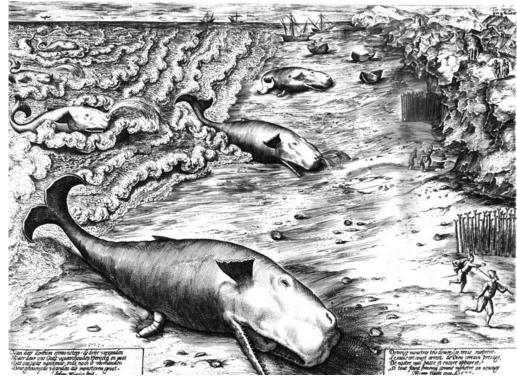
Mass stranding since 1577 (25 % in the North Sea)

Common findings : animals : adult males

period: winter

area: 4 mains areas with complex coastal topography

BUT virtual absence of data on the pathology of stranded sperm whales





Sperm whales stranding: « navigation error »

Death: cardiovascular failure secondary to the stranding

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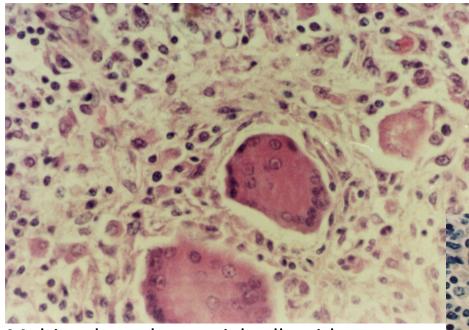
Fin whales (Balaenoptera physalus)



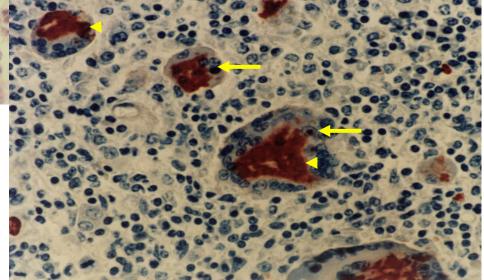
Gross findings: emaciation, severe parasitosis



First cases of morbilliviral infection in fin whales (sse Sandro's cases)



Multinucleated syncytial cells with eosinophilic intranuclear inclusion body (arrow). Lymph node



Intranuclear (arrowhead) and intracytoplasmic (arrow) using MoAb against PDV. Lymph node



Humpback whale



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Minke whale (Balaenoptera acutorostrata)



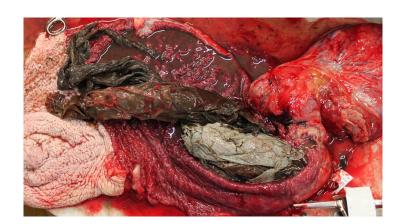
Severe emaciation



Generalized edema, hypoproteinemia



Generalized edema, hypoproteinemia



Plastic impaction in stomach

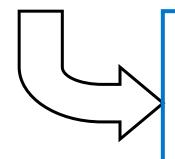


Summary

Stranding network aims with veterinarian support:

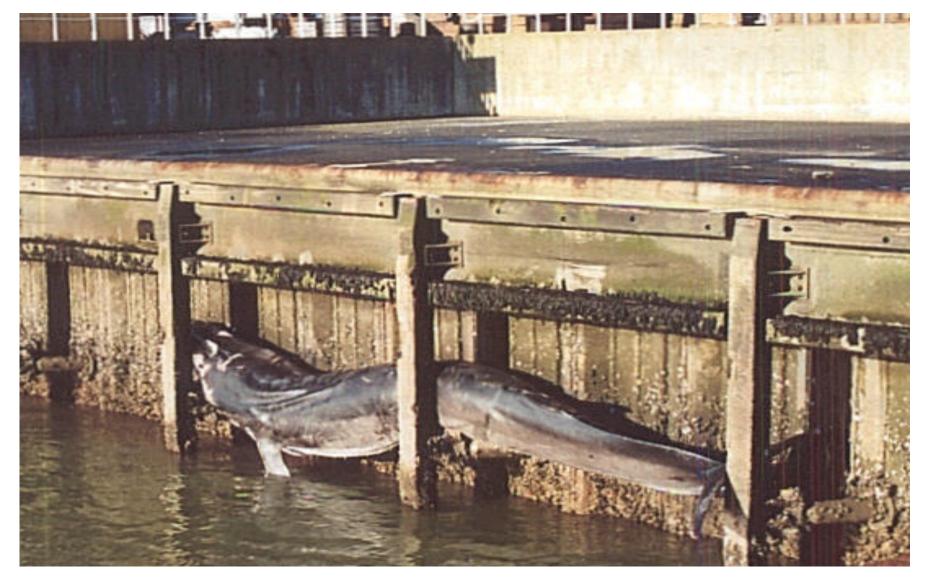
Identification of lesions and causes of death of cetaceans:

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