

Post-mortem Findings in Cetacean Stranded along Italian Adriatic Sea coastline (2000-2006)

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Abstract

Stranded cetaceans offer a unique opportunity for collecting data on the health status of dolphin and whale free-living populations. Between 2000 and 2006, the National Stranding Networks described 163 stranded marine mammals along the Italian coastline of the Adriatic Sea. A complete post-mortem examination was possible on 13 entire and well preserved animals. Pneumonia was one of the most commonly encountered lesion, especially *Halocercus lagenorhynchi*-associated bronchopneumonia. Additional findings were nephritis, hepatitis and *Pholeter gastrophilus*-associated gastritis. Parasites were often found in most of the examined organs and tissues and in many cases, Granulomatous meningoencephalitis by *Cladosporium* spp. was observed in a female bottlenose dolphin, while papilloma-like lesions were observed on the rostrum and in the oral cavity of other two female bottlenose dolphins. Other peculiar findings were heterotopic renal tissue in the lung parenchyma of the common dolphin. In a few cases, putative zoonotic agents, such as *Vibrio alginolyticus*, were isolated from these stranded dolphins. In agreement with other authors, parasites were the most frequently encountered pathogens but, despite noticeable tissue damage, they were not life-threatening organisms. Pneumonia was a very common disease condition also in the cetaceans included in this study.

Adriatic Sea: cetaceans population and strandings data.

The Adriatic Sea is an arm of the Mediterranean Sea lying between Italy and the Balkan Peninsula. It extends northwest from 40° to 45° 45' N., with an extreme length of about 770 km, with a total surface area of about 160,000 km² with a mean depth of the sea is estimated at 240 m. The Italian coasts border the basin to the North and the West and is relatively straight and continuous, having no islands, with long and flat sandy shores.

In the Adriatic Sea, common bottlenose dolphin (*Tursiops truncatus*) is the most regularly encountered cetacean, while striped dolphin (*Stenella coeruleoalba*) is diffuse mainly in the central and southern parts. Risso's dolphins (*Grampus griseus*), long-finned pilot whales (*Globicephala melas*), Cuvier's beaked whales (*Ziphius cavirostris*), sperm whales (*Physeter macrocephalus*) and fin whales (*Balaenoptera physalus*) are occasionally encountered in the southern waters, in particular close to the border with the Ionian Sea. Short-beaked common dolphin (*Delphinus delphis*) population is drastically declined over the past decades, in particular in the northern Adriatic Sea, becoming rarer or disappearing (Notarbartolo di Sciara G. and Demma M., 1997).

In the seven years, between 2000 and 2006, the Museum of Natural History of Milan (MNHM), in collaboration with the National Stranding Network (Centro Studi Cetacei Onlus, CSC), reported 166 cetacean strandings along Italian Adriatic coastline. Strandings data are analyzed in detailed in table 1.

<i>Species/year</i>	2000	2001	2002	2003	2004	2005	2006	TOT
Common bottlenose dolphins (<i>Tursiops truncatus</i>)	28	15	7	9	8	8	9	84
Striped dolphins (<i>Stenella coeruleoalba</i>)	4	2	3	2	-	-	4	15
Risso's dolphin (<i>Grampus griseus</i>)	2	-	-	1	2	2	-	7
Long-finned pilot whales (<i>Globicephala melas</i>)	-	1	-	-	-	-	-	1
Cuvier's beaked whales (<i>Ziphius cavirostris</i>)	-	1	-	-	1	1	-	3
Sperm whales (<i>Physeter macrocephalus</i>)	-	-	-	-	-	1	-	1
Short-beaked common dolphin (<i>Delphinus delphis</i>)	1	-	-	-	-	-	-	1
Fin whales (<i>Balaenoptera physalus</i>)	-	-	-	-	1	-	-	1
Undetermined	11	6	4	8	10	10	4	53
TOT	46	25	14	20	22	22	17	166

Table 1: Strandings of the Adriatic Sea (2000-2006; data provided by the Museum of Natural History of Milan)

Main pathological findings

Stranded animals offer a unique opportunity for collecting data on the health status of free-living cetaceans. In collaboration with the National Stranding Network, the MNHM, the Mediterranean Marine Mammals Tissue Bank (MMTB) and other institutions, it was possible to perform detailed postmortem examination on 14 (8,4%) of the above mentioned marine mammals in the cited period. Six bottlenose dolphins, three striped dolphins, three Risso's dolphins and one common dolphin were investigated. Data on examined animals are listed in table 2.

Species	Sex/Age	Year	Site of Stranding	Stranding conditions
Common bottlenose dolphins (<i>Tursiops truncatus</i>)	F, adult	2000	Jesolo (VE) Northern Adriatic Sea	Found dead on the beach
Short-beaked common dolphin (<i>Delphinus delphis</i>)	F, 15 yo	2000	Lido degli Estensi (RA) Northern Adriatic Sea	Alive, dead after 14 days in rehabilitation
Striped dolphins (<i>Stenella coeruleoalba</i>)	M, adult	2001	Atri Pineto (AN) Central Adriatic Sea	Alive, it died close to the shore
Striped dolphins (<i>Stenella coeruleoalba</i>)	M, adult	2001	Chioggia (VE) Northern Adriatic Sea	Found dead close to the shore
Risso's dolphin (<i>Grampus griseus</i>)	M, juvenile	2001	Lignano (UD) Northern Adriatic Sea	Alive, it died close to the shore
Striped dolphins (<i>Stenella coeruleoalba</i>)	F, juvenile	2002	Malamocco (VE) Northern Adriatic Sea	Found dead on the beach
Risso's dolphin (<i>Grampus griseus</i>)	F, newborn	2003	Rimini (RN) Northern Adriatic Sea	Alive, it died close to the shore
Common bottlenose dolphins (<i>Tursiops truncatus</i>)	F, adult	2004	Civitanova Marche (AN) Central Adriatic Sea	Alive, dead after 1 day in rehabilitation
Risso's dolphin (<i>Grampus griseus</i>)	F, 18 yo	2005	Ancona (AN) Central Adriatic Sea	Alive, dead after 3 days in rehabilitation
Common bottlenose dolphins (<i>Tursiops truncatus</i>)	F, adult	2005	Marina di Montemarignano (AN) Central Adriatic Sea	Alive, it died close to the shore
Common bottlenose dolphins (<i>Tursiops truncatus</i>)	F, adult, pregnant	2006	Cavallino-Treporti (VE) Northern Adriatic Sea	Found dead on the beach
Common bottlenose dolphins (<i>Tursiops truncatus</i>)	M, adult	2006	Riccione (RN) Northern Adriatic Sea	Alive, it died close to the shore
Common bottlenose dolphins (<i>Tursiops truncatus</i>)	M, newborn	2006	Pescara (PE) Central Adriatic Sea	Found dead on the beach
Common bottlenose dolphins (<i>Tursiops truncatus</i>)	M, juvenile	2006	Porto Tolle (RO) Northern Adriatic Sea	Found dead on the beach

Table 2: Animal submitted to postmortem examination

During *post-mortem* examination, tissues were sampled and formalin-fixed for microscopic examination. Microbiological and toxicological exams were done when suggested by

pathological changes observed during necropsy. More in detail, a retrospective study was performed to assess the presence of any lesion related to “gas and fat embolic syndrome” or to anthropogenic factors, with detailed immunohistochemical investigations against specific *Morbillivirus* antigens being also carried out in 7 number of animals (Fernandez A. *et al.*, 2005; Jepson P.D. *et al.*, 2005).

Table 3 summarizes the main lesions found in the 14 investigated animals, along with the number of cetaceans showing changes in any organ or tissue and indicating the number and the type of findings observed.

Organ/tissue	N.	Pathological process (associated to parasites)				
		Cong.	Traum.	Inflam.	Deg./met	Neop.
Integument (skin/blubber/mammary gland)	7	-	3	4 (3)	2	-
Skeletal system (bones/articulations)	-	-	-	-	-	-
Muscles	5	-	-	5 (5)	-	-
Serous cavities (thoracic, abdominal, pericardium)	4	-	-	3 (3)	1	-
Oral cavity (mouth/teeth/tongue)	3	-	-	2 (-)	-	1
Gastric concamerations	7	-	-	7 (3)	-	-
Intestine	5	-	-	4 (1)	-	-
Pancreas	3	-	-	2 (1)	1	-
Liver	9	-	-	6 (-)	6	-
Upper air tracts (sinuses/larynx/trachea/bronchi)	3	-	-	3 (2)	-	-
Lungs	14	-	-	14 (12)	-	-
Kidneys	7	-	-	5 (-)	8	-
Urinary tract (ureter/bladder/urethra)	2	1	-	1 (-)	-	-
Gonads	2	-	-	2 (-)	1	-
Uterus/prostate	3	-	-	1 (-)	1	1
Vagina/penis	2	-	-	2 (-)	-	-
Adrenal glands	3	-	-	1 (-)	2	-
Thyroids	-	-	-	-	-	-
Spleen	3	-	-	-	3	-
Lymph nodes	2	-	-	2 (1)	-	-
Bone marrow/thymus	-	-	-	-	-	-
Heart	4	-	-	-	4	-
Vessels (arteries/veins/lymphatic)	3	-	-	-	3	-
Nervous System (Central Nervous System, CNS/Peripheral Nervous System, PNS)	6	-	-	4 (-)	5	-
Melon/sensory organs (eyes, ears, echolocation system)	1	-	-	1 (-)	-	-

Table 3: Pathological findings distribution in organs/tissues and number of lesions (both macroscopic and microscopic) found in the 14 investigated animals. N.: number of subjects presenting lesions in correspondent tissue/organs. Cong.: congenital lesion; Traum.:

traumatic lesion; Infl.: inflammation; Deg/met.: degenerative/metabolic lesion; Neop.: neoplasia; (n) lesions associate to parasites.

Pneumonia was one of the most commonly encountered lesion, especially associated to lungs parasitosis. 12 animals (85,7%) show a chronic bronchopneumonia, most of the time associated to parasites-induced lesions as bronchiolar calcifications (5 animals) and sub-pleural granulomas (6 cases). Parasitological examination was performed in 6 cetaceans and *Halocercus* sp. was recognized. The same nematode was seen also in the upper respiratory tract of a striped dolphin, while, *Crassicauda grampicola* caused a moderate catarrhal chronic sinusitis in an adult female Risso's dolphin. Rarely a respiratory lesion was not associate to nematodes: in a common bottlenose dolphin an uremic pneumonia was described while, in a newborn *Grampus griseus*, inflammatory changes were no related to any etiological agent. An extremely peculiar and serendipitous finding was an heterotopic kidney tissue "island" in the lung parenchyma of the common dolphin investigated herein (Di Guardo *et al.*, 2005).

Parasites are often involved in other organs or tissues. Tetraphyllidea cystic larvae were encountered during necropsies in the thickness of the body walls. In particular, *Phyllobothrium delphini* was found in the dorsal muscles and in the blubber of the dorso-caudal regions of 9 animals, while *Monorygma grimaldii* was observed in retro-peritoneal tissues of 3 animals. Parasites were less frequently involved in inflammation in other sites as in stomach (*Pholeter gastrophylus* granulomas were seen in the submucosa of the second and third gastric concamerations in 3 cetaceans), intestine (adult nematodes were found in the intestinal lumen of an adult Risso's dolphin), pancreas (trematodes, possibly *Campula* sp., were microscopically recognized in the pancreatic ducts of a striped dolphin) and lymph nodes (ova were observed in subcapsular and medullar sinuses of meseraic lymph nodes of the previous striped dolphin, in association with an eosinophilic lymphadenitis).

Inflammatory reactions were frequently described in the kidneys and in the liver. A nonspecific chronic reactive hepatitis was often observed (6 animals) with a severe and diffuse congestion. Degeneration (hepatic, midzonal and centrolobular lipidosis in 2 dolphins; mild peri-portal hemosiderosis in 2 animals in poor body conditions) or mild biliary stasis were sometimes observed. In 3 cases liver degeneration was seen without any inflammatory reaction. The most peculiar finding was a diffuse glycogenosis with a severe hepatomegaly in a Risso's dolphin newborn which was associated to moderate ascites and bilateral, retro-peritoneal, air-filled, multi-cystic formations (12 cm long) developed around ureters (likely congenital lesions).

Mild, multifocal interstitial chronic nephritis was described in 5 cases and in 4 animals calcifications were noticed disseminated in the medulla. In one bottlenose dolphin, a severe membranoproliferative glomerulonephritis and a multifocal, moderate chronic tubulo-interstitial nephritis determined a condition of renal failure. Medullary nephrocalcinosis was observed in other 4 cetaceans with no inflammatory changes. In the only common dolphin investigated, a large, 6.5 x 3.5 x 4 cm white calculus was found in the vagina with a mild chronic inflammation. Inflammatory findings in the genital apparatus were seen in other 3 dolphins (1 oophoritis, 1 vaginitis and 1 endometritis with mild mucometra).

Heart and vessels showed mainly degenerations (valvular endocardiosis and arteriosclerosis). Exceptions were diffuse medial and intimal calcifications occurred in a systemic uremic syndrome, seen in the subject with renal failure and uremic pneumonia, and a massive myocardial fibrosis in the left ventricle of the heart of another adult female bottlenose dolphin, as a consequence of an infarct.

Lipofuscinosis and spheroids were observed in the central nervous system of 5 animals. These nonspecific changes are considered age-related, but in two of the investigated cetacean, the age determination by the teeth analysis suggested a different pathogenesis (the common dolphin and a Risso's dolphin were respectively about 15 and 18 years old). Lipofuscinosis is

mostly related to old age but it seems to be age-independent in diseases which enhance autophagy, increase oxidative stress, augment the amounts of redox-active iron, or reduce the capacity of intralysosomal degradation (Terman A. and Brunk U.T., 2004). There are strong indications that progressive lipofuscin accumulation promotes the development of age-related pathologies, as neurodegenerative diseases, heart failure. In both cases, a mild perivascular, non suppurative encephalitis with gemistocytic astrogliosis and edema were seen. More in detail, in the *Grampus griseus* a peculiar enlargement of the adrenocortical nerve sheaths compressing the contiguous *zona fasciculata* was observed associated with central findings. This animal stranded alive with a female juvenile subject and it was lactating: for all these reasons and for high POPs concentration in the milk, a chronic toxicity was supposed.

In a pregnant female, a 3 cm diameter abscess in the left occipital region was found as the most significant finding, possibly determining a central blindness. A severe, chronic granulomatous meningoencephalitis by *Cladosporium spp.* was observed in a female bottlenose dolphin, determining a moderate hydrocephalus: some *Cladosporium* genus members, which show a specific neurotropism, have been reported as causative agents of meningoencephalitis in humans, dogs and cats (Guillot J. *et al.*, 2004; David T.P. *et al.*, 2004). Lymphoreticular system did not show any significant change: hyperplastic reactive lymphadenopathy and lymphocytic depletion are frequently found. In two animals, in the muscular layer of the medium size arteries yellow-greenish pigment aggregates (hematoidin) were described.

Photobacterium damsela from mesenteric lymph nodes of a striped dolphin, *Vibrio alginolyticus* from the intestine of a bottlenose dolphin, *Aeromonas hydrophyla* from several organs of a bottlenose dolphin were putative zoonotic agents isolated by inflammatory lesions. Immunohistochemistry did not revealed any morbilliviral infection in the investigated animals.

During postmortem examination, no by-catch lesions were noted: traumatic findings were mainly blunt trauma or excoriations due to social interactions or related to stranding. No animals showed any change related to sonar (resembling “gas and fat embolic syndrome”) or caused by collision with vessels, even if further investigations are in progress using histochemical fat emboli detection techniques (4, 5). Only two neoplastic findings were observed, both benign: an oral fibropapilloma and a genital fibroma in two different adult female *Tursiops truncatus*.

Table 4 reports causes of death or main pathological findings which are involved in determining stranding events or peculiar changes. It was possible to determine a certain cause of death in 8 animals out of 14 (57,1%).

Species	Cause of death/strandings	Findings predisponent to stranding
Common bottlenose dolphin (<i>Tursiops truncatus</i>)		- Melon: multiple abscesses. - Oral fibropapilloma
Short-beaked common dolphin (<i>Delphinus delphis</i>)		- Heterotopic renal tissue in the lung. - Vaginal calculus. - Chronic oophoritis and ovary calcifications. - Ovarian arteriosclerosis.
Striped dolphin (<i>Stenella coeruleoalba</i>)	Severe acute necrotizing pancreatitis and severe multifocal adrenal gland necrosis	

Striped dolphin (<i>Stenella coeruleoalba</i>)		
Risso's dolphin (<i>Grampus griseus</i>)		<ul style="list-style-type: none"> - Moderate splenic haemocytoatheresis - Mild liver hemosiderosis - Hematic pigment in renal tubuli.
Striped dolphin (<i>Stenella coeruleoalba</i>)	Infection by <i>Photobacterium damsela</i> and severe parasitosis	
Risso's dolphin (<i>Grampus griseus</i>)		<ul style="list-style-type: none"> - Retroperitoneal periurethral multiple air-filled cysts - Severe liver glycogenosis - Ascites
Common bottlenose dolphin (<i>Tursiops truncatus</i>)	Severe hydrocephalus secondary to chronic granulomatous multifocal mycotic meningoencephalitis due to <i>Cladosporium</i> sp.	<ul style="list-style-type: none"> - Severe parasitosis
Risso's dolphin (<i>Grampus griseus</i>)		<ul style="list-style-type: none"> - Severe lipofuscinosis with severe astrogliosis and adrenocortical nerves sheaths enlargement - Severe pulmonary edema - Severe cerebral edema.
Common bottlenose dolphin (<i>Tursiops truncatus</i>)	Heart failure due to chronic myocardial infarct	
Common bottlenose dolphin (<i>Tursiops truncatus</i>)	Cerebral abscess on the left occipital region	
Common bottlenose dolphin (<i>Tursiops truncatus</i>)	Uremic syndrome due to renal failure.	
Common bottlenose dolphin (<i>Tursiops truncatus</i>)	Respiratory failure secondary to severe parasitic bronchopneumonia due to <i>Halocercus lagenorhynchi</i> .	<ul style="list-style-type: none"> - Accessory spleens (<i>extra-lien</i>) within gastro-lienal ligaments.
Common bottlenose dolphin (<i>Tursiops truncatus</i>)	Septicemia due to <i>Aeromonas hydrophyla</i>	

Table 4: Causes of death and main pathological findings presumably related to stranding

In spite of the fact of the limited investigated number, death diagnosis percentage is quite high. Many of the studied animals stranded alive and postmortem examination could be carry

out in a short time after death. In other cases, stranding site was not too far from our facilities and this allowed a very rapid action.

On the basis of the data obtained in the present study and in agreement with previous reports (Di Guardo G. *et al.*, 1995; Cornaglia E. *et al.*, 2000), pneumonia was the most frequently observed pathological finding in these 14 Odontocete cetaceans stranded on the Adriatic coastline. All findings were related to single spontaneous disease conditions and no stranding was directly related to human activities (by-catch, military sonar, collisions) or to epidemic episodes, as in the well-known example of *Morbillivirus* infections. Most of the time it was associated with respiratory or extra-pulmonary parasitosis but only in one case, severity and extension of the infestation suggest parasitic bronchopneumonia as the likely cause of death. In other cases, a severe inflammatory lesion or its consequences led to an organ failure and then to death or stranding. Even if *Vibrio* spp., *Aeromonas hydrophila* and *Photobacterium* spp. are opportunistic bacteria, their isolation from cetaceans emphasizes necessity of using precautions during marine mammals necropsies.

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