Aquatic Mammals 2016, *42*(3), 292-299, DOI 10.1578/AM.42.3.2016.292

Short Note

Necropsy Findings of 11 White-Beaked Dolphins (*Lagenorhynchus albirostris*) Stranded in Denmark During 2008-2014

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The white-beaked dolphin (*Lagenorhynchus albirostris*) was recognized as a separate species by the British cetologist John Eward Gray in 1846, based on a specimen collected from the English North Sea coast near Great Yarmouth in 1845. The same year, the first Danish specimen was collected and described by D. F. Eschricht (1846) as *Delphinus ibsenii* named after the donor of the specimen Ib Pedersen Ibsen (Kinze, 2008). Today, the white-beaked dolphin is considered the second most common cetacean species in Danish waters and a native inhabitant of the North Sea (Kinze, 2007). Although, the white-beaked dolphin is listed as LC (least concern) in the International Union for Conservation of Nature's *Red List of Threatened Species*, the species is in need of dedicated studies and management (Hammond et al., 2015). The only current knowledge for Danish waters as well as the adjacent Baltic and North Sea waters, therefore, relies on rather heterogeneously compiled sets of data. Potential threats to the species in these waters include incidental catches, pollution, disturbances, and, on a larger time scale, climate changes. Since 1845, several hundred specimens have been documented by Danish natural history museums with the main focus on describing the distribution, abundancy, and anatomy of the species. Stomach parasites were also retrieved from a specimen collected as early as 1860 (Krabbe, 1878). Until the onset of a systematic national stranding scheme in 1981 (Kinze, 2007), the information on this species remained very scattered; but in subsequent years, the collected data also included body measurements, age determination, reproductive status, body condition, and, more recently, health status. Tissues from some of the white-beaked dolphins (e.g., liver, kidney, and blubber) were sampled and stored for later analyses of heavy metals and other contaminants. Herein, we present necropsy findings of 11 white-beaked dolphins stranded in Denmark from 2008 through 2014.

In Denmark, a national contingency plan for marine mammals (Jensen et al., 2012) provides effective monitoring of the health of marine mammals and ensures that Denmark complies with international obligations to protect white-beaked dolphins, among other endangered whale species. As part of this contingency plan, reports on dead and live stranded marine mammals are registered by Regional Nature Agency units, which receive the information from the public or through active regional surveillance. This information is further disseminated to the National Nature Agency and the Fisheries and Maritime Museum for collection of cadavers and for their destruction after measuring and sexing; or for necropsy, mainly at the National Veterinary Institute, where material for diagnostic and research purposes are sampled. Between 2008 and 2014, 39 white-beaked dolphin strandings were registered in Denmark (Figure 1). Necropsy was performed on 11 animals (Table 1). The others were either too decomposed to be necropsied or were stranded in areas inaccessible for collection either because they were in the tide water or because a vehicle could not get to the stranding place in due time. Eight of the necropsied dolphins were found dead on the beach, while two individuals were euthanized (C347 and C348) and one died shortly after stranding (C342). The distribution of sex among the necropsied white-beaked dolphins was not statistically significantly

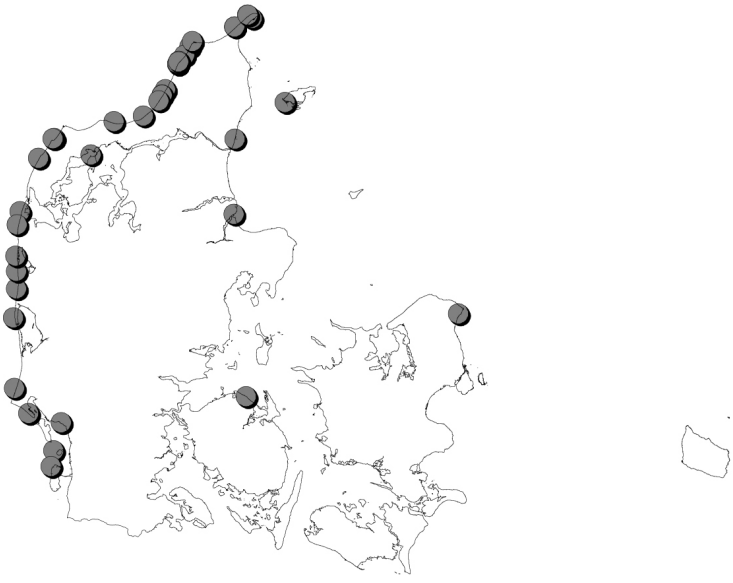

Figure 1. Map of the 39 white-beaked dolphin (*Lagenorhynchus albirostris*) strandings between 2008 and 2014 in Denmark

Table 1. Necropsy results for 11 white-beaked dolphins (*Lagenorhynchus albirostris*)

ID*	Year	Month	Sex	Reproductive status	Length (cm)	Weight (kg)	Pathological examinations
C354	2008	January	F	Mature	270	230	Macroscopic, histology, laboratory
C274	2009	February	M	Mature	250	176	Macroscopic, histology
C314	2009	November	F	--	260	--	Macroscopic, histology, laboratory
C325	2011	May	M	--	193	--	Macroscopic
C326	2011	May	M	Mature	280	--	Macroscopic, histology, laboratory
C340	2013	March	F	Mature	239	--	Macroscopic, histology
C342	2013	June	M	Immature	218	--	Macroscopic, histology
C347	2013	December	M	Mature	260	236	Macroscopic, histology, laboratory
C348	2013	December	F	Mature, lactating	242	224	Macroscopic, histology, laboratory
C349	2014	January	F	Mature, pregnant	262	--	Macroscopic, laboratory
MCE1643	2014	January	F	Mature, lactating	254	280	Macroscopic, laboratory

*ID numbers are generally assigned sequentially; however, in some cases (C354), the stranded dolphin is sent directly by the Danish Nature Agency to the Veterinary Institute for autopsy without notifying the Fisheries and Maritime Museum who assign ID numbers. In such a case, the ID number will only be assigned when analysed tissue samples arrive at the museum for storage. *Weight:* It often was not possible to determine weight under field conditions.

different from a 1:1 distribution (G test, $p = 0.76$). Most of the necropsied dolphins stranded during winter and spring, which probably indicates that the low water and air temperatures at that time prevented rapid decomposition, allowing standardized necropsies to be carried out. Based on total body lengths, the majority of necropsied dolphins were estimated to be adults or young adults

(Galatius et al., 2013). The blubber thickness of the necropsied dolphins was relatively thin (front flipper: 1.6 ± 0.4 cm; max. abdomen width: 1.6 ± 0.2 cm).

Necropsies took place between 1 and 6 d after stranding. In cases of postponed necropsies, as for C347, C348, and C349, the bodies were either kept in a freezer or cooled with ice to prevent further decomposition. Prior to necropsy, external measurements were taken according to an unpublished internal protocol used by the Fisheries and Maritime Museum in Esbjerg, Denmark. Necropsies were performed by a multidisciplinary team of biologists and veterinarians. The procedure followed the guideline given by the marine mammal necropsy guide (Pugliares et al., 2007). For histology, tissue samples were collected and fixed in 10% neutral buffered formalin for ten of the dolphins. No tissue samples were collected from MCE1643. The samples were submitted for routine histologic processing and stained with hematoxylin and eosin. Aerobic and anaerobic cultures for bacteria were done on indication for four of the dolphins by use of blood agar supplemented with 5% calf blood and incubated at 37° C for 24 to 48 h. McMaster and/or Baermann was used for parasitological examinations on six dolphins. Parasites from heart and stomach chambers were sampled and stored in 70% ethyl alcohol until further examination. Species identification of parasites (*Pseudoterranova decipiens* and so on) was carried out by morphology using comparison with published accounts (Olson et al., 1983). Tissues from four dolphins were tested for morbillivirus by RT-PCR as described by Barrett et al. (1993). One white-beaked dolphin was tested serologically and bacteriologically for brucellosis. All laboratory examinations were carried out at the National Veterinary Institute, Danish Technical University, by routine diagnostic methods. Major findings for each of the necropsied dolphins are presented in Table 2.

All dolphins, especially C347, C348, and C349, were emaciated with scant subcutaneous and visceral fat deposits (Table 1). Externally, they had superficial cutaneous excoriations (C274), scars (C314), and ulcerations (C354, C274, C340, C342, and C349), most likely related to physical trauma associated with stranding. Eyes (C274 and C340) or part of tails and flippers (C274) were missing in two dolphins. Since there were no apparent tissue reactions, all these external findings may have arisen *postmortem*. In a single dolphin (C325), the presence of a nylon rope embedded in the muscles of the tail indicated that the individual had been bycaught (Figure 2).

Major pathological findings were related to the lungs and the heart. A single dolphin had lung

Figure 2. In a single dolphin (C325), the presence of a nylon rope embedded in the muscles of the tail indicated that the individual had been bycaught.

Table 2. Major necropsy findings in stranded white-beaked dolphins between 2008 and 2014 in Denmark.

	Major pathological examinations and findings			Cause of death/ reason for euthanasia
ID	Macroscopic	Histology	Laboratory	
C354	Lungs: Stasis; enlarged bronchial lymph nodes Oedematous stomach containing nematodes and fish bones Bleedings and oedema in intestines, spleen, and intestinal lymph nodes Swollen liver with bleedings Uterus wall and ovarian bursa thickened and dark	Lungs: Atelectasis, massive stasis, and alveolar oedema Bacteria Bronchial lymph nodes: Bleeding, fibrosis, and stasis Chronic gastritis Intestines: Eosinophil and mononuclear cells in mucosa and muscle Liver: Stasis and lipid-containing liver cells Stasis in spleen, kidneys, adrenal glands, and uterus Oedema in uterus	*Clostidium perfringens* + *Edwardsiella tarda* lymph nodes + uterus Brucella antibodies detected Negative tests: *Brucella* spp. (lymph nodes), oocysts (McMaster), and Distemper virus	Sepsis
C274	Eyes, part of tailfin, and front flippers missing Two holes in right side and in the jaw; no tissue reactions Part of the lung stasis Stomach: Few nematodes; cod otholit Several ulcers penetrating mucosa Intestinal tract missing Liver: Hemorrhagic; enlarged Spleen: Hemorrhagic; decomposed	Lungs: Massive stasis, mild infiltration of neutrophils, and mononuclear cells Gastritis nonsuppurative with ulcers, massive chronic multifocal infiltration of mononuclear cells, eosinophils, and parasites Liver: Massive stasis Kidney: Decomposed Spleen: Decomposed stasis; reactive lymphoid tissues Lymph node: Stasis; fibrosis	Not performed Nematode larva Negative tests: Camphylobacter and oocysts (McMaster)	Emaciated Focal ligation of lung Parasitic gastritis and penetrating ulcers Reactive splenomegaly
C314	Several scars in cutis Right side of thoracic cavity: Cyst Stomachs: Nematodes (> 1,000); bleedings	No inflammation of cyst wall Stomach: Mucosal bleedings and infiltration of mononuclear cells	No pathogenic bacteria identified in cyst	Not known
C325	Very decomposed Nylon rope in muscles and bones of tail (from fishing tackle?)	Not performed	Not performed	Bycatch?
C326	Lungs: Bleedings Lymph nodes enlarged Blood (1 L) in abdomen Intestinal lymph nodes enlarged	Mild, nonsuppurative interstitial pneumonia, oedema, and infiltration of macrophages in alveoli Intestinal lymph node with microabscess, fibrosis, and suppurative vasculitis Decomposed liver with infiltration of inflammatory cells and fibrosis in portal areas	Decomposed Saprophytic (?) bacterial growth in all organs	Not known Abdominal infection/ inflammation (enteritis/ peritonitis)

Table 2. (cont.)

C340	Eyes missing and multiple (> 100) small ulcerations in cutis Chronic pericarditis Stomachs: Nematodes, otholits, fish bones, and blood Enlarged intestinal lymph nodes; calcification Enlarged liver	Bronchial (cardiac) lymph node with neutrophil granulocytes Eosinophilic granules in liver cells	Not performed	Not known
C342	Small ulcerations on flippers Blood in trachea, lungs, and bronchi Stomach: Ematodes + otholits	Lungs: Chronic, nonsuppurative bronchointerstitial pneumonia; suppurative bronchopneumonia Liver: Ecrosis; infiltration of neutrophil granulocytes Stasis of liver and spleen	Not performed	Sepsis following severe pneumonia (stranded alive)
C347	Cachectic; teeth worn down Heart: Nematodes Stomach: Nematodes (> 1,000)	No lesions observed	Heart: *Acanthocheilonema spirocauda* Stomach: *Ascaridia* spp. and *Pseudoterranova decipiens* Negative test: Distemper virus	Euthanasia
C348	Cachectic Chronic pericarditis Stomach with nematodes	No lesions observed	Stomach with *P. decipiens* Negative test: Distemper virus	Euthanasia
C349	Cachectic; small ulcerations in abdominal cutis Stomach: Nematodes and fish bones Adherences between diaphragm and intestines	Not performed	Stomach: *Ascaridia* spp. Negative test: Distemper virus	Not known
MCE 1643	No lesions observed	Not performed	Stomach with light infestation of nematodes	Not known

fibrosis and pulmonary congestion (C274), while three others exhibited pulmonary hyperemia (C354) or pulmonary hemorrhage (C326 and C342). Enlarged bronchial lymph nodes (C354 and C326) also were observed. Histological examinations of the lungs showed diffuse hyperemia (C354 and C274), atelectasis (C354), and edema (C354) in two of the dolphins, as well as mild bronchointerstitial (C342) or interstitial pneumonia (C326) (see Figure 3). One other dolphin had hemorrhage and congestion in the enlarged bronchial lymph nodes (C354) from which *Clostridium perfringens* and *Edwardsiella tarda* could be recovered. No parasites were observed in any of the lungs, but several nematodes (*Acanthocheilonema spirocauda*) were found in the heart of a single dolphin (C347)—a new host record for this species—without signs of heart or lung disease. In two dolphins (C340 and C348), chronic pericarditis was observed without other heart pathology. Unfortunately, bacteriology was not performed. In a single dolphin (C314), a cyst measuring approximately 7 × 12 cm (see Figure 4) was found in the right side of the inlet

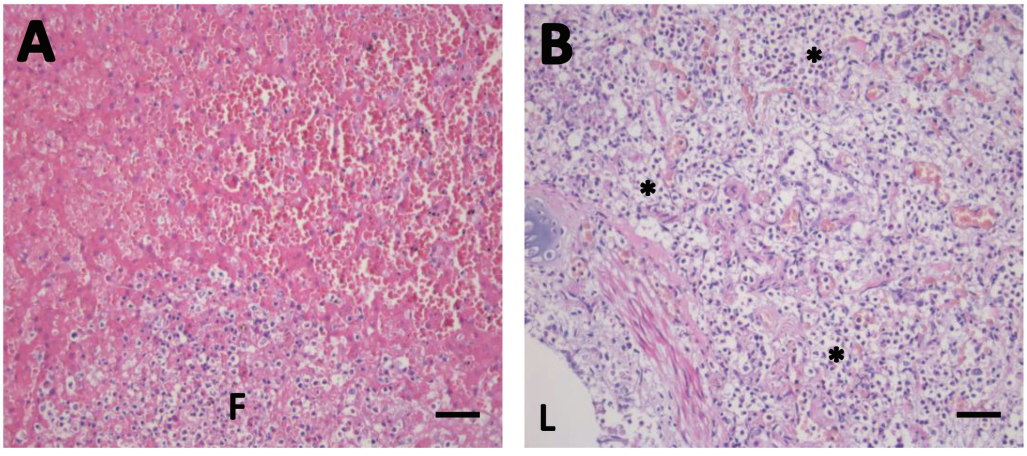

Figure 3. Histologic sections of organs from C342: hematoxylin and eosin. Scale bars = 100 µm. (A) Liver with congestion and focal infiltration of neutrophilic granulocytes with degeneration and necrosis of hepatocytes (F), and (B) section of lung with bronchopneumonia. * = neutrophilic granulocytes in alveoli; L = lumen of small bronchus.

to the thoracic cavity (apertura thoracis cranialis), but histological examination did not show any signs of inflammation, and no pathogenic bacteria were identified in the cyst fluid. Lung congestion in one of the dolphins (C274) was so massive that it may seriously have affected the lung function. The other thoracic findings probably had minor effects on the dolphins.

The main findings of the gastrointestinal system were related to the stomachs. In general, the stomachs were empty or nearly empty, but several contained nematodes (C354, C274, C314, C340, C342, C347, C348, and C349) in the stomachs. Parasitological examination of nematodes revealed dual infection in one dolphin (C347), and one each were infected by *Ascarida* spp. (C349) and *P. decipiens* (C348). In a single case (C314), more than 1,000 nematodes were present, and histology of the stomach wall showed mononuclear cell infiltrations and hemorrhage into the gastric lumen. Another dolphin had fewer nematodes (C274) but several ulcers in the gastric wall. Histopathology revealed a chronic mononuclear eosinophilic gastritis. The intestinal lymph nodes were enlarged (C340), fibrotic with neutrophils and suppurative vasculitis (C326), or hemorrhage (C354); the latter dolphin also had intestinal hemorrhage. In general, the parasitic gastritis and penetrating ulcers may have had major effects on two dolphins (C314 and C274), while the other findings probably only have had minor effects.

Swollen livers were found in three dolphins (C354, C274, and C340). Two dolphins had congestion and hemorrhage (C354 and C274); and for the third (C340), eosinophilic granules of unknown composition could be demonstrated in the liver cells by histology. In a single dolphin (C326) with blood in the abdomen, but without macroscopic liver pathology, histological examinations showed fibrosis and infiltration of inflammatory cells around the bile ducts and in the connective tissue. Both liver necrosis and congestion was seen in a single dolphin (C342) (see Figure 3). In two dolphins (C354 and C274), the spleens were congested and hemorrhagic, and one of them (C274) had reactive lymphoid tissue. Congestion of the spleen also was seen in another dolphin (C242). In one dolphin (C354), congestion was observed in kidneys, adrenal glands, and the uterus; the uterus was edematous, and *E. tarda* could be recovered. Two of the largest females (C314 and C349) were pregnant. Reactive splenomegaly may have affected another dolphin (C274), and congestion of several abdominal organs indicated sepsis in two others (C354 and C342). Adherences between the diaphragm and the intestines indicated chronic peritonitis in a single dolphin (C349).

In summary, sepsis, and probably gastritis, enteritis, peritonitis, and bycatch were causing stranding and death for most of the dolphins, while no causes were found in the rest. As already mentioned, potential threats to the dolphins in Danish waters include incidental catches, pollution, disturbances, and climate changes. However, we only found a cause of death for a few of the dolphins. Necropsy and laboratory tests (see Table 2) indicated sepsis as the cause of stranding/death in two of the 11 necropsied dolphins. None of the four dolphins that were tested for morbillivirus

were positive. Morbilliviruses are known to cause epidemics in dolphins (Domingo et al., 1990; Raga et al., 2008); therefore, the majority of dolphins that had stranded after 2011 were tested due to an ongoing epidemic in Denmark among terrestrial wildlife (Trebbien et al., 2014). Although white-beaked dolphins are not believed to develop symptoms after morbillivirus infections (van Elk et al., 2014), the virus might contribute to weakness and increased susceptibility to other infections. However, the standard laboratory tests used were not able to detect the agents responsible for sepsis. Several dolphins had gastric ulcers, which are not believed to be the cause of death, but in striped dolphins (*Stenella coeruleoalba*), white sided dolphins (*L. acutus*), and short beaked common dolphins (*Delphinus delphis*), these ulcers have been found to cause weakness when associated with helicobacter infection (Davison et al., 2014).

Bacteriology or histochemistry for identification of helicobacter was not performed on samples from the gastric ulcers; thus, it is not known if helicobacter was present, but the ulcers could be a contributing factor to the emaciation found in C314, for example. Dolphin C342 had severe pneumonia, which, based on the histopathological findings, probably caused sepsis, but, unfortunately, no bacteriological examinations were performed. Infection with *E. tarda* was believed to cause sepsis and subsequent death in C354, which also has been reported from a sperm whale (*Physeter macrocephalus*) (Cools et al., 2013).

As mentioned earlier, another common cause of death in dolphins is bycatch (Mannocci et al., 2012). Except for the nylon rope in a single animal (C325) and the residue of fishing net in the stomach of another dolphin (C354), no scars or wounds indicating an interaction with fishing gear was found. However, we cannot rule out bycatch as a potential cause of death of other dolphins (e.g., C326 with lung edema) since animals may have drowned in fishing gear without evidence of external trauma (Kuiken, 1994; Mannocci et al., 2012). Therefore, future studies should have a greater focus on signs of bycatch as a potential cause of death. As we unexpectedly found *A. spirocauda* in the heart of a single dolphin, it also

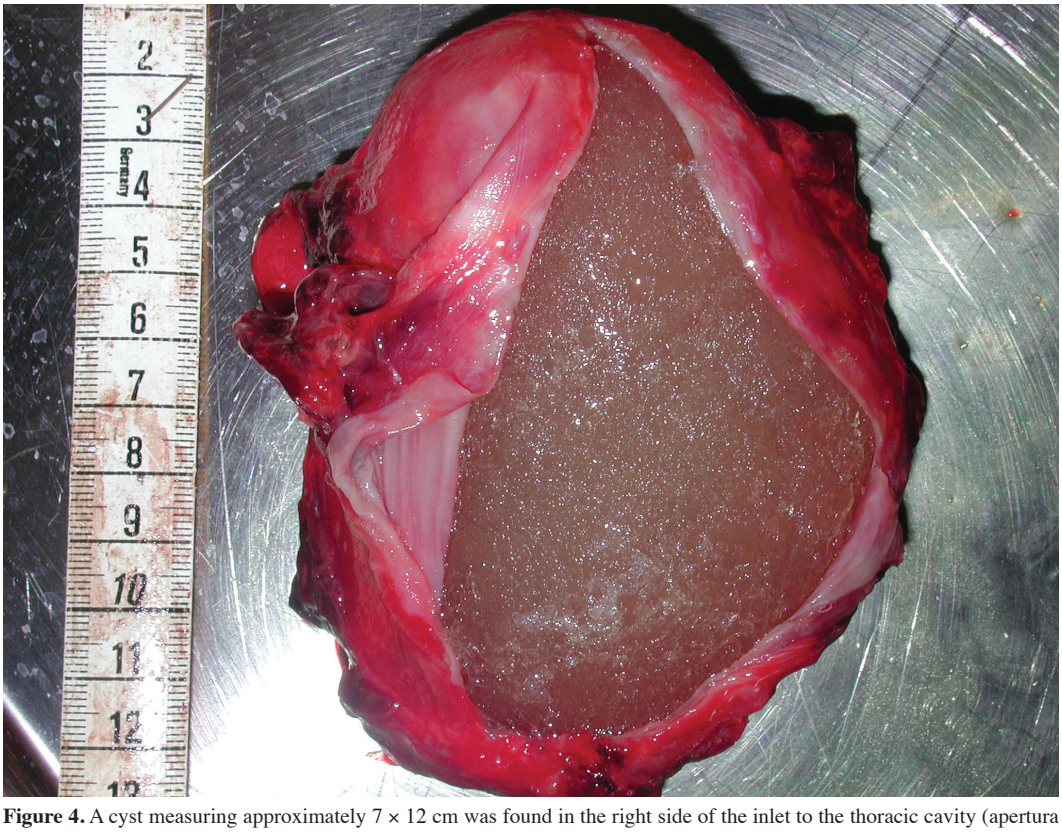

Figure 4. A cyst measuring approximately 7 × 12 cm was found in the right side of the inlet to the thoracic cavity (apertura thoracis cranialis) in a single dolphin (C314).

would be of interest to focus more on this in future studies: Was it merely an incidental finding or does it also exist in other individuals? Also, does it have any clinical significance? This heartworm is well-known from the fast-growing population of harbour seals (*Phoca vitulina*) in Scandinavia (Leidenberger & Boström, 2008).

Our study indicates that a higher number of systematic necropsies would be required in order to elucidate the causes for dolphin strandings quantitatively. Furthermore, it could be relevant to include more laboratory diagnostic tests so the causes of the sepsis could be detected. From a preservative perspective, it would be relevant to know whether sepsis is caused by a specific agent or whether it occurs in dolphins that are already weakened. *A. spirocauda* were found in the heart of a single dolphin (C347) without signs of heart or lung disease, for example.

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