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Health assessment of harbour porpoises (*PHOCOENA PHOCOENA*) from Baltic area of Denmark, Germany, Poland and Latvia

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ABSTRACT

Harbour porpoise (*Phocoena phocoena*), the only resident cetacean species of the Baltic Sea is formed of two subpopulations populations, occurring in the western Baltic, Belt Seas and Kattegat and the Baltic Proper, respectively. Harbour porpoises throughout these areas are exposed to a large number of human activities causing direct and indirect effects on individuals, that might also harm this species on a population level. From Latvia, Poland, Germany and Denmark 385 out of 1769 collected dead harbour porpoises were suitable for extensive necropsy. The animals were collected between 1990 and 2015 and were either by-caught or found dead on the coastline. Following necropsies, histopathological, microbiological, virological and parasitological investigations were conducted. Females and males were equally distributed among the 385 animals. Most animals from the different countries were juveniles between 3 months and 3 years old (varying between 46.5 and 100% of 385 animals per country). The respiratory tract had the highest number of morphological lesions, including lungworms in 25 to 58% and pneumonia in 21 to 58% of the investigated animals. Of those with pneumonia 8 to 33% were moderate or severe. The alimentary, hearing, and haematopoietic systems had inflammatory lesions and parasitic infections with limited health impact. 45.5 to 100% of the animals from the different countries were known by-caught individuals, of which 20 to 100% varying between countries had netmarks. Inflammatory lesions, especially in the respiratory tract were found in higher numbers when compared to control populations in areas with less human activities such as arctic waters. The high number of morphological changes in the respiratory tract and of bycatches especially among immature animals before reaching sexual maturity is of serious concern, as well as the low number of adult animals among the material. Data on health status and the causes of death are valuable for management. A next step in this regard will combine data from health and genetic investigations in order to detect differences between the two populations of the Baltic.

1. Introduction

The harbour porpoise (*Phocoena phocoena*; HP) is the only reproducing cetacean species in the Baltic Sea as defined under HELCOM and ASCOBANS (Hammond et al., 2002, 2013; Siebert et al., 2006; Scheidat et al., 2008; HELCOM, ASCOBANS Jastarnia Plan). Two HP populations are distinguished in Baltic waters, one in the western Baltic and inner Danish waters called the Belt Sea population and one in the Baltic Proper divided around 13.5°E (Wiemann et al., 2010; Sveegaard et al., 2015; Galatius et al., 2012; Lah et al., 2016). The Baltic Proper population has been severely reduced during the last century (Koschinski, 2002; Skora and Kuklik, 2003) and is currently estimated

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Table 1
Sex, age and by-catch/stranding distribution of harbour porpoises from the Baltic and adjacent waters (1990–2015).

	Germany (Schleswig-Holstein) animals	Germany (Mecklenburg-Prepommerania) animals	Denmark animals	Poland animals	Latvia animals
Total (all states of preservation)	1.222	460	65	20	2
Number of animals suitable for necropsies	231	84	58	12	2
Sex	**number (%)**	**number (%)**	**number (%)**	**number (%)**	**number (%)**
Female	107 (46.3)	33 (39.3)	32 (55.0)	6 (50.0)	1 (50.0)
Male	121 (52.4)	39 (46.4)	26 (45.0)	6 (50.0)	1 (50.0)
Unknown	3 (1.3)	12 (14.3)	0 (0)	0 (0)	0 (0)
Age distribution	**number (%)**	**number (%)**	**number (%)**	**number (%)**	**number (%)**
≤3 months (incl. foetuses)	52 (22.5)	8 (9.5)	11 (19.0)	1 (8.3)	0 (0)
> 3 months ≤ 3 years	137 (59.3)	39 (46.4)	35 (60.3)	7 (58.3)	2 (100)
> 3 years	41 (17.7)	25 (29.8)	12 (20.7)	4 (33.3)	0 (0)
Unknown	6 (0.4)	12 (14.3)	0 (0)	0 (0)	0 (0)
Stranded or by-catch	**number (%)**	**number (%)**	**number (%)**	**number (%)**	**number (%)**
By-catch	112 (48.9)	38 (45.5)	49 (84.5)	11 (91.7)	2 (100)
Stranding	119 (51.5)	46 (54.7)	9 (5.5)	8 (8.3)	0 (0)

at less than 600 remaining animals (Carlén et al., 2018). The western subpopulation is living in the area from Skagerak to the Darss and Limhamn ridge. In 2012, an international survey estimated the abundance of HPs in the Kattegat, Belt Sea and the Western Baltic (GAP-area) at 40,475 animals (95% Confidence interval (CI): 25,614–65,041. Coefficient of Variation (CV) = 0.235) with an associated density of 0.79 animals km^{-2} (95% CI: 0.50–1.24. CV = 0.24) and an average group size of 1.49 animals (Viquerat et al., 2014). Similar densities were observed during the SCANS surveys in 1994, 2005 and 2016 (Hammond et al., 2002, 2013, 2017; Viquerat et al., 2014).

Anthropogenic activities such as fisheries, ship traffic, oil exploration, industrial chemical, marine litter, offshore constructions and noise pollution are causing continuous pressure on the HP populations (Siebert et al. (1999, 2012); Richardson et al. (1995); Kock and Benke (1996); Skora and Kuklik (2003); Beineke et al. (2005ab); Das et al. (2006); Wright et al. (2013); Jepson et al. (2016); Unger et al. (2017)). In addition, several studies on the acoustic impact on hearing, behaviour and foraging of harbour porpoises have shown the high potential of anthropogenic activities such as ramming for the construction of offshore windfarms (Lucke et al., 2009; Tougaard et al., 2009; Schaffeld et al., 2020), shipping (Wisniewska et al., 2018) as well as military activities (Jepson et al., 2003; Wright et al., 2013; Siebert et al., 2013) to disrupt the natural behaviour of the animals, cause stress and negatively impact the health status.

Several agreements and management steps have been undertaken to enhance the conservation of the HP to protect the species in accordance with different agreements for the Baltic Area as ASCOBANS (Agreement on the conservation of Small Cetaceans of the Baltic and North Sea), HELCOM (Helsinki Commission) and MSFD (Marine Strategy Frame Directive), but still, the knowledge on numbers of by-catches and effects of chemical and noise pollution, habitat loss as well as cumulative effects of anthropogenic activities is scarce (Siebert et al., 2012). In order to detect and quantify various environmental treats and changes that lead to population decrease and reduced health and reproduction of this species, knowledge of diseases and mortality factors based on necropsy findings is strongly required.

Investigations of diseases and causes of HP mortality are vital for the understanding and management of the populations in relation to anthropogenic activities. The knowledge of HPs health and reproduction in European waters has increased over the last two decades revealing a need to further examine anthropogenic effects on the marine environment (Siebert et al., 2001; Wünschmann et al., 2001; Jauniaux et al., 2002; Jepson et al., 2005; Siebert et al., 2006, 2009; Kesselring et al., 2017).

Previous investigations have shown that HPs from the North and Baltic Seas suffer from parasitic and bacterial infections, particularly in the respiratory tract (Jepson et al., 2000; Siebert et al., 2001; Jauniaux et al., 2002; Lehnert et al., 2005). HPs from the Baltic Sea had a significantly higher incidence of severe bacterial infections than those from less polluted waters around Greenland, Iceland and Norway (Wünschmann et al., 2001; Siebert et al., 2006, 2009). Polychlorinated biphenyl (PCB) concentrations in adult HPs from Greenland were 10 times lower than in HPs from the Baltic and North Seas (Kleivane et al., 1995; Bruhn et al., 1999) where high PCB levels potentially causing negative health effects were found (Falandysz et al., 1994; Jepson et al., 2016).

The present study gives an overview of the lesions ascertained in HPs from the Baltic Sea and adjacent waters over the past 25 years. It represents the largest data set on health of harbour porpoises from the Baltic Sea and summarizes necropsy findings in order to further assess effects from anthropogenic activities in the future. The results of this study have the potential to serve as the basis for the MSFD and HELCOM obligations in the future. Political demands for the protection and support for Baltic harbour porpoises can be warranted based on these findings.

2. Material and methods

Between 1990 and 2015 a total of 1,769 harbour porpoises (HP) were collected through the stranding networks of the Baltic Sea. The animals were by-caught (delivered directly by fishermen) or found dead on the beach during the survey period. The animals originated from the German-Schleswig-Holstein (SH; n = 1,222), German-Mecklenburg-Prepommeranian (MP; n = 460), Danish (DK; n = 65), Polish (PL; n = 20) and Latvian (LV; n = 2) waters and coastline of the Baltic as defined under HELCOM (Table 1). It was decided that reference of locations was made to national borders or states as it allowed the different stranding networks and governments to see findings from their responsible areas in a first common publication. Furthermore, without genetic information harbour porpoises found especially on the German coast could not be related with certainty to the different subpopulations.

Of the 1,769 HPs, 385 individuals were suitable for a full necropsy examination as described by Siebert et al. (2001, 2009) and IJesseldijk et al. (2019) with the majority of animals (n = 231) originating from the waters of Schleswig-Holstein (Table 1). HPs were either examined without freezing or if immediate necropsy was not possible, the carcasses were stored at −20 °C until examination.

The animals were weighed and measured, and 6 lower incisors (I_1-I_3, left and right) were removed for age determination by counting the annual growth layers (Lockyer, 1995). Animals were grouped into different classes according to length and age determination (Siebert et al., 2001). The nutritional state was based on the weight (kg) of the total blubber and muscle (along the dorsal column) tissue as well as by evaluating blubber thickness and muscle mass. The blubber thickness (mm) was measured in four different locations (sternal, caudodorsal, caudolateral, and caudoventral to the dorsal fin) (Siebert et al., 2001, 2006).

The carcasses were examined for external lesions, in particular netmarks, and all organ systems were examined macroscopically. Samples for histopathology were collected as described in Siebert et al. (2001, 2006) including lesions and different organ systems: lungs, trachea, stomach (1st, 2nd and 4th compartments), intestine, oesophagus, liver, pancreas, thyroid gland, adrenal gland, kidney, urinary bladder, testis, uterus, ovary, spleen, lymph nodes (pulmonary, intestinal and retropharyngeal), heart, aorta, rete mirabilis, skeletal muscles, diaphragm, intercoastal musculature, tongue, skin, blubber, eye, acoustic fat and other tissue, brain, spinal cord. Parasites were fixed in 70% ethanol and identified as described by Lehnert et al. (2005). Lung, liver, kidney, spleen, intestine, mesenteric lymph nodes and tissues with lesions underwent microbiological investigation as described by Siebert et al. (2009).

Lungs were examined immunohistochemically for the presence of morbillivirus antigen by means of the avidin–biotin complex (ABC)-technique using a polyclonal antibody against canine distemper virus (Örvell and Norrby, 1980) that is known to cross-react with porpoise morbillivirus (Müller et al., 2000). The procedure of the immunohistological analysis was performed as described by Baumgärtner et al. (1995).

3. Results

3.1. Sources, sex and age distribution

Fishermen submitted all animals from Latvia and 91.7% of the HPs from Poland, 84.5% from Denmark, 48.9% from SH and 45.5% from MP as by-catches (Table 1). The age distribution is shown in Table 1. Males and females were almost equally distributed among the HPs found on beaches or delivered as by-catch by fishermen (Table 1). The highest percentage of neonates was found among HPs from SH (20.3%) while none of the animals from Latvia were neonatal (≤3 months). In all areas, the majority of the animals belonged to the age group between 3 months and 3 years. None of the individuals from Latvia were older than three years, while 33.3% of the animals from Poland were older than 3 years.

3.2. Nutritional condition

More than half of the investigated animals were in good nutritional state, including both animals submitted from Latvia (Table 2). Of the animals 18% from SH, 12% from MP, 21% from DK and 50% from PL were moderately undernourished while 13% from SH and MP, 12% from DK and 17% from PL were emaciated.

3.3. Pathological findings

Pathological findings are divided by organ system. Results are presented in Table 2 and not all results are repeated in the text. The figures in the text are rounded up or down which has no impact on the quality of the data.

Respiratory tract. Depending on the region, pulmonary lesions included oedema (32 to 100%), congestion (17 to 100%), emphysema (2 to 29%) and haemorrhage in (1 to 5%) of the porpoises. Lungworms (*Pseudalius inflexus*, *Torynurus convolutus* and *Halocoercus invaginatus*) were found in 25 to 58% (Tables 2). Both animals from Latvia did not have parasites. Parasites were usually present in the bronchial tree and venous and arterial vessels. Pneumonia of varying intensity and type occurred in 21 to 58% of HPs originating from SH, MP, DK and PL. Both animals from Latvia were devoid of inflammatory lung lesions. Eight to 33% of the HPs had moderate or severe inflammatory lesions of the lung. Animals from MP had the lowest frequency of moderate to severe pneumonia while animals from PL had the highest prevalence of moderate and severe pneumonia. Inflammatory changes consisted in most cases of granulomatous or suppurative lesions (Figs. 1, 2) or were characterized by necrosis or formation of abscesses. Other pathological findings of the respiratory tract were alveolar histiocytosis, calcifications of lung tissue, atelectasis, pulmonary fibrosis, fibrinous, suppurative-fibrinous or serous pleuritis, lymphcytic or mucopurulent bronchitis and lymphocytic-plasmacellular tracheitis. Intraalveolar keratin squames and fetal atelectasis were findings in neonatal animals. There was no histological evidence of viral infections in the investigated organs and immuno-histochemistry on CeMV was negative in all tested animals. The lung was the organ with the highest burden and variety of isolated bacteria (data is not shown).

Alimentary system. Parasitic infection was found in the oesophagus, stomach compartments and the intestine of 2 to 12% of the animals from SH, MP and DK (*Anisakis simplex*, *Contracaecum osculatum*, *Stenurus minor*, *Diphyllobothrium* sp., *Hysterothylacium aduncum*, *Pholeter gastrophilus*) (Table 2). None of the Polish or Latvian HPs had parasitic infections. Ulcerative and eosinophilic oesophagitis and gastritis were found in some cases associated with parasitic infections. Seventeen percent of the Polish HPs had a catarrhal oesophagitis without parasitic infection. Overall, the health impact of parasitic infections in the alimentary system was mainly mild or moderate. Granulomatous, eosinophilic, ulcerative, suppurative, necrotising, lymphocytic or catarrhal enteritis was found in 9% of the animals from SH and in 22% from DK in some cases associated with macroscopic parasitic burdens in other cases without. None of the Polish or Latvian animals had enteritis.

Adult trematodes and their eggs (*Campula oblonga*) were found in the liver of 16% of the SH, 5% of the MP, 22% of Danish HPs and 8% of the Polish HPs. Trematode infection was associated with cholangitis, pericholangitis (Fig. 3), hepatitis, duct epithelial hyperplasia and periductal fibrosis. In Polish HPs, similar lesions were also found without signs of trematode infection. Diffuse hepatocellular lipidosis was only found in 3–14% of the animals from MP, SH and DK but not in HPs from Poland and Latvia. *Campula oblonga* in the pancreas was only found in 3% of the HPs from SH and not in animals from other areas. Granulomatous, eosinophilic or interstitial pancreatitis occurred in 17% of the Polish, 3% of the Danish and 2% in the SH HPs without macroscopic or histological indications of *Campula oblonga*. In animals from MP and Latvia no histological changes referring to pancreatitis where found.

Urinary system. The urinary system only had few lesions. Congestion of the kidneys was the most common finding and seen in 8.3 to 25.9% of HPs from SH, MP, DK and PL. Inflammatory lesions such nephritis, pyelonephritis, and lipidosis of renal tubular cells were found in 1 to 8% of all HPs.

Genital system. Few lesions were noted in the genital system. Inflammatory lesions such as vaginitis, endometritis, mastitis, and balanitis were found in 1 to 8% of all HPs.

Skin and subcutis. Most lesions in the skin and subcutis were associated with by-catch of the HPs (Figs. 4, 5). Netmarks were the most characteristic lesion in by-caught animals and found in 20–100% of the by-caught handed over by fishermen and stranded animals (SH: 42%, MP: 20%, DK: 55%, PL: 58%, LV: 100%). In addition, subcutaneous haemorrhages (Fig. 6) and haematomas mainly on the lower jaw, scars, dermatitis, panniculitis, and wounds were found (Table 2).

Central nervous system, eye and ear. Nematodes *(Stenurus minor*) were found in the Eustachian tube and the aural peribullar cavity in 14% to 47% of the HPs from SH, MP, DK and PL (Table 2). Both HPs from Latvia were not infected by parasites in the ear. In addition, single cases of haemorrhage in the eye chambers (SH: 1%), haemorrhage in CNS (SH: 4%, DK: 5%), congestion in the CNS (SH: 8%) and suppurative-necrotizing encephalitis/leptomeningitis caused by beta-haemolytic streptococci septicaemia (SH: 4%, MP: 2%, DK: 3%) were found (Fig. 7).

Haematopoetic and endocrine systems. Lymphadenitis was found in

Table 2
Selected pathological findings in harbour porpoises from the Baltic and adjacent waters (17.09.17).

Observation	Germany (Schleswig-Holstein) Animals (n = 231)	Germany (Mecklenburg-Prepommerania) Animals (n = 84)	Denmark Animals (n = 58)	Poland Animals (n = 12)	Latvia Animals (n = 2)
Nutritional state	**number (%)**	**number (%)**	**number (%)**	**number (%)**	**number (%)**
Good	127 (55.0)	31 (36.9)	35 (60.3)	3 (25.0)	2 (100)
Moderate	41 (17.8)	10 (11.9)	12 (20.7)	6 (50.0)	0 (0)
Emaciated	29 (12.6)	11 (13.1)	7 (12.1)	2 (16.7)	0 (0)
Not determined	34 (14.7)	32 (38.1)	4 (6.9)	1 (8.3)	0 (0)
	Germany (Schleswig-Holstein) number (%)	**Germany (Mecklenburg-Prepommerania) number (%)**	**Denmark number (%)**	**Poland number (%)**	**Latvia number (%)**
Respiratory system					
Pulmonary oedema	181 (78.4)	27 (32.1)	49 (84.5)	9 (75.0)	2 (100)
Pulmonary congestion	83 (35.9)	15 (17.9)	25 (43.1)	4 (33.3)	2 (100)
Pulmonary emphysema	52 (22.5)	2 (2.4)	17 (29.3)	1 (8.3)	0 (0)
Pulmonary haemorrhage	4 (1.7)	0 (0)	3 (5.2)	0 (0)	0 (0)
Pulmonary nematodiasis	105 (45.5)	21 (25.0)	33 (56.9)	7 (58.3)	0 (0)
Pneumonia	97 (42.0)	18 (21.4)	32 (55.2)	7 (58.3)	0 (0)
None	134 (58.0)	66 (78.6)	26 (44.8)	5 (41.7)	2 (100)
Mild	23 (10.0)	3 (3.6)	6 (10.3)	0 (0)	0 (0)
Moderate	46 (19.9)	7 (8.3)	15 (25.9)	3 (25.0)	0 (0)
Severe	28 (12.1)	8 (9.5)	11 (19.0)	4 (33.3)	0 (0)
Alveolar histiocytosis	21 (9.1)	1 (1.2)	8 (13.8)	0 (0)	0 (0)
Atelectasis	6 (2.6)	2 (2.4)	2 (3.4)	0 (0)	0 (0)
Calcification	19 (8.2)	6 (7.1)	5 (8.6)	3 (25.0)	0 (0)
Fibrosis	4 (1.7)	3 (3.6)	3 (5.2)	0 (0)	0 (0)
Pleuritis	3 (1.3)	3 (3.6)	0 (0)	0 (0)	0 (0)
Bronchitis	5 (2.2)	5 (6.0)	2 (3.4)	0 (0)	0 (0)
Tracheitis	4 (1.7)	0 (0)	3 (5.2)	0 (0)	0 (0)
Keratin flakes	5 (2.2)	1 (1.2)	0 (0)	1 (8.3)	0 (0)
Cardiovascular system					
Parasites in the heart	9 (3.9)	0 (0)	2 (3.4)	0 (0)	0 (0)
Thrombosis	7 (3.0)	1 (1.2)	1 (1.7)	0 (0)	0 (0)
Pericardial effusion	0 (0)	3 (3.6)	0 (0)	0 (0)	0 (0)
Myocarditis	5 (2.2)	1 (1.2)	0 (0)	0 (0)	0 (0)
Alimentary system					
Oesophageal parasites	6 (2.6)	0 (0)	1 (1.7)	0 (0)	0 (0)
Gastric parasites	27 (11.7)	6 (7.1)	6 (10.3)	0 (0)	0 (0)
Enteric parasites	5 (2.2)	0 (0)	1 (1.7)	0 (0)	0 (0)
Stomatitis/Glossitis/Gingivitis	3 (1.3)	0 (0)	2 (3.4)	0 (0)	0 (0)
Oesophagitis	5 (2.2)	0 (0)	0 (0)	2 (16.7)	0 (0)
Gastritis	30 (13.0)	7 (8.3)	7 (12.1)	0 (0)	0 (0)
Enteritis	21 (9.1)	2 (2.4)	13 (22.4)	0 (0)	0 (0)
Hepatic trematodiasis	36 (15.6)	4 (4.8)	13 (22.4)	1 (8.3)	0 (0)
Congestion liver	49 (21.2)	11 (13.1)	16 (27.6)	3 (25.0)	0 (0)
Hepatitis	14 (6.0)	6 (7.1)	7 (12.1)	3 (25.0)	0 (0)
Hyperplasia of bile ducts	31 (13.4)	5 (6.0)	5 (8.6)	2 (16.7)	0 (0)
Fibrosis	33 (14.3)	7 (8.3)	6 (10.3)	1 (8.3)	0 (0)
Cholangitis/pericholangitis	41 (17.7)	6 (7.1)	16 (27.6)	1 (8.3)	0 (0)
Hepatocellular lipidosis	24 (10.4)	3 (3.6)	8 (13.8)	0 (0)	0 (0)
Pancreatic trematodiasis	7 (3.0)	0 (0)	0 (0)	0 (0)	0 (0)
Pancreatitis	4 (1.7)	0 (0)	2 (3.4)	2 (16.7)	0 (0)
Urinary and genital system					
Renal congestion	41 (17.7)	13 (15.5)	15 (25.9)	1 (8.3)	0 (0)
Nephritis	4 (1.7)	1 (1.2)	0 (0)	1 (8.3)	0 (0)
Renal lipidosis	5 (2.2)	2 (2.4)	1 (1.7)	0 (0)	0 (0)
Pyelonephritis	3 (1.3)	2 (2.4)	0 (0)	0 (0)	0 (0)
Genital system					
Vaginitis/Endometritis/Mastitis	2 (0.9)	2 (2.4)	6.8	0 (0)	0 (0)
Balanitis	0 (0)	0 (0)	0 (0)	1 (8.3)	0 (0)
Skin and subcutis					
Netmarks	98 (42.4)	17 (20.2)	32 (55.2)	7 (58.3)	2 (100)
Subcutaneous haemorrhage	16 (6.9)	2 (2.4)	6 (10.3)	1 (8.3)	2 (100)
Haematoma	13 (5.6)	2 (2.4)	6 (10.3)	0 (0)	0 (0)
Scars of the skin	11 (4.8)	0 (0)	0 (0)	0 (0)	0 (0)
Dermatitis	12 (5.2)	1 (1.2)	1 (1.7)	1 (8.3)	0 (0)
Panniculitis	10 (6.5)	1 (1.2)	1 (1.7)	1 (8.3)	0 (0)
Skin wounds	14 (6.1)	2 (2.4)	4 (6.9)	1 (8.3)	0 (0)
Central nervous system and ears					
Nematodiasis of the Eustachian tube and peribullar cavity	75 (32.5)	12 (14.3)	27 (46.6)	4 (33.3)	0 (0)
Haemorrhage in CNS	8 (3.5)	0 (0)	3 (5.2)	0 (0)	0 (0)
Enzephalitis/Leptomeningitis	9 (3.9)	2 (2.4)	2 (3.4)	0 (0)	0 (0)
Hyperaemia/congestion of meninges	19 (8.2)	0 (0)	0 (0)	0 (0)	0 (0)
Hyphaema of eye	3 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)

(*continued on next page*)

Table 2 (*continued*)

Observation	Germany (Schleswig-Holstein) Animals (n = 231)	Germany (Mecklenburg-Prepommerania) Animals (n = 84)	Denmark Animals (n = 58)	Poland Animals (n = 12)	Latvia Animals (n = 2)
Haematopoetic and endocrine systems					
Hyperplasia of spleen	15 (6.5)	2 (2.4)	4 (6.9)	0 (0)	0 (0)
Haematopoesis of spleen	8 (3.5)	0 (0)	1 (1.7)	0 (0)	0 (0)
Lymphadenitis	60 (25.1)	7 (8.3)	18 (31.0)	0 (0)	0 (0)
Fibrosis of thymus	1 (0.4)	0 (0)	2 (3.4)	0 (0)	0 (0)
Lymphocytic depletion of lymph nodes	6 (2.6)	0 (0)	2 (3.4)	0 (0)	0 (0)
Hyperplasia of lymph nodes	65 (28.1)	1 (1.2)	25 (43.1)	0 (0)	0 (0)
Hyperplasia of tonsils	6 (2.6)	0 (0)	2 (3.4)	0 (0)	0 (0)
Hyperplasia of Peyeŕs patches	9 (3.9)	1 (1.2)	1 (1.7)	0 (0)	0 (0)
Thymus depletion	9 (3.9)	0 (0)	5 (8.6)	2 (16.7)	0 (0)
Adrenal hyperplasia	7 (3.0)	1 (1.2)	15 (25.7)	0 (0)	0 (0)

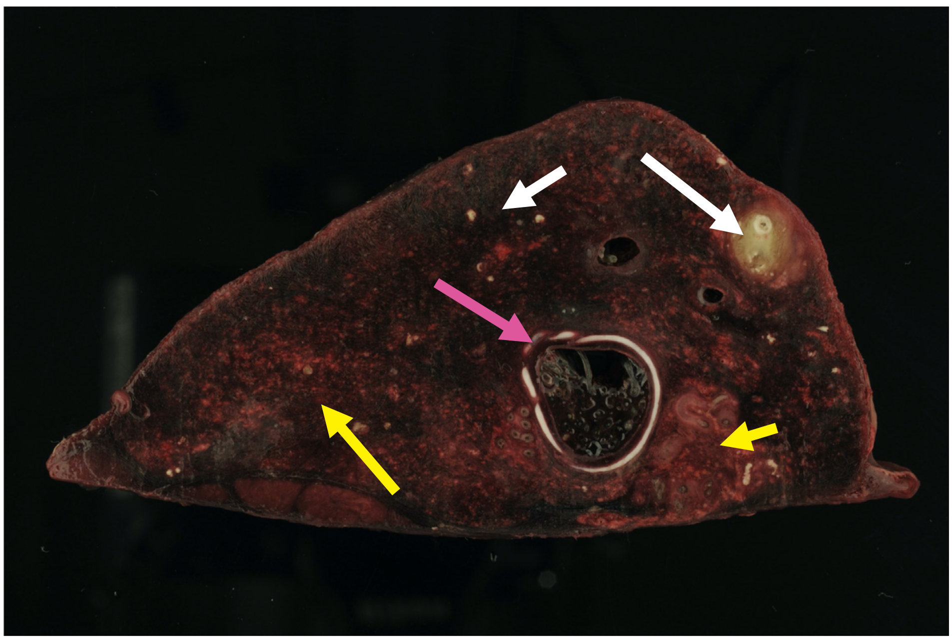

Fig. 1. Lung of a harbour porpoise with an intrabronchial nematodes (pink arrow), bronchopneumonia (yellow arrows) and calcification (white arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

lymph nodes associated with parasitic infection or inflammation of the respiratory and alimentary system in 25% of SH, 8% of the MP, and 31% of the DK HPs (Fig. 8). Hyperplasia of lymph nodes was diagnosed in 28% of HPs from SH, 43% of the DK and 1% of MP HPs. Thymic depletion was found in 4% of the SH animals, 9% of the DK and 17% of PL HPs. The adrenal glands had nodular proliferative hyperplasia of the cortex in 26% of the DK porpoises, 3% in SH and 1% in MP HPs (Table 2).

4. Discussion

Investigations of the health status were conducted on 385 HPs collected among 1,769 individuals from the southern HELCOM area including Danish inner, German and Polish waters and in addition two individuals from Latvian waters. The respiratory tract was the organ system with the highest prevalence of pathological findings in animals from all areas. Some of the findings such as pulmonary oedema and congestion are considered agonal findings commonly found in by-caught animals and no indication for an association with a disease was found (Kuiken et al., 1994; Siebert et al., 2001).

Main pathological respiratory findings in the studied HPs are pneumonia and pulmonary nematodiasis. These findings did also occur in by-caught animals and individuals being in relatively good nutritional status indicating that the nutritional status does not necessarily reflect the health status of a harbour porpoise. Nematodes in the lungs of HPs are mainly associated with consumption of fish as intermediate hosts (Lehnert et al., 2010; Andreasen et al., 2017) and occur in the bronchial tree and the pulmonary blood vessels where they can be associated with thrombosis and sclerosis (Jepson et al., 2000; Siebert et al., 2001). Several studies have indicated that pseudaliid lungworms have heteroxenous lifecycles (Houde et al., 2003; Measures 2001) using fish intermediate hosts and although some species may use (also) direct transmission (Reckendorf et al., 2018), infection patterns in harbor porpoises indicate that they get infected when starting to ingest prey species after weaning.

Pneumonia similar to that described in the present investigation has previously been described for HPs in the North Sea (Clausen and Andersen, 1988; Jepson et al., 2000; Siebert et al., 2001; Jauniaux et al., 2002). In HPs from the North and Baltic Seas, granulomatous, suppurative or necrotizing pneumonia were the most common types occasionally with abscesses caused by primary or secondary bacterial infection (Jepson et al., 2000; Siebert et al., 2001; Jauniaux et al., 2002). In HPs from the Baltic and North Seas, bacteria most commonly isolated from the respiratory tract include *Brucella* spp., *Erysipelothrix rhusiopathiae*, β-haemolytic streptococci and *Staphylococcus aureus* (Jepson et al., 2000; Siebert et al., 2002, 2009).

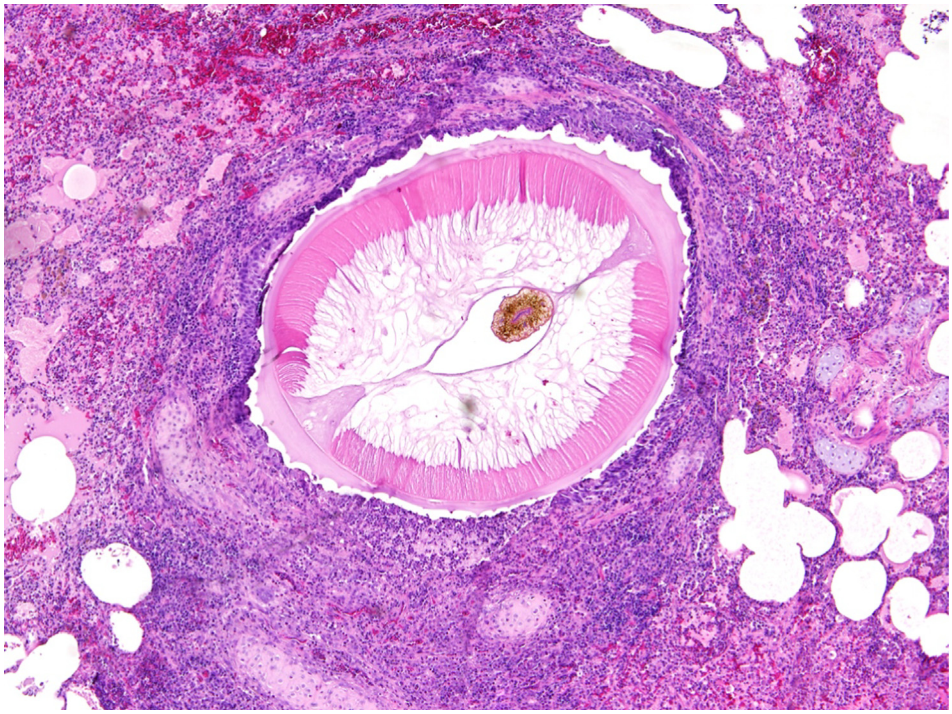

Fig. 2. Lung of a harbour porpoise with an intrabronchial nematode and chronic bronchitis, haematoxylin and eosin staining, magnification 40×.

The health impact of parasitic infections in the alimentary system was mainly mild or moderate and comparable to previous investigations from the North and Baltic Seas (Baker and Martin, 1992; Siebert et al., 2001; Wünschmann et al., 2001; Jauniaux et al., 2002). This is in contrast to HPs from Greenland, which had a higher incidence of lesions in the alimentary system compared to animals from the North and Baltic Seas which mainly had pulmonary lesions (Siebert et al., 2006; Lehnert et al., 2014).

Another frequent finding was nematodiasis in the ear, peribullar sinuses and Eustachian tube due to *Stenurus minor* infection (Siebert et al., 2001; Lehnert et al., 2005; Morell et al., 2017; Wohlsein et al., 2019). The importance of parasitic infection in the ear complex still remains unclear. Generally, as harbour porpoises from the Baltic Sea show higher parasitic infections when compared to animals from Norway, Iceland and Greenland it cannot be ruled out that environmental factors have some influence on the nematodiasis in the ear complex (Wünschmann et al., 2001; Beineke et al., 2005a,b; Siebert et al., 2006; Lehnert et al., 2014). Recent investigations of the ear complex indicated that harbour porpoises from the Baltic Sea had traumatic and inflammatory lesions at surprisingly high rates (Siebert

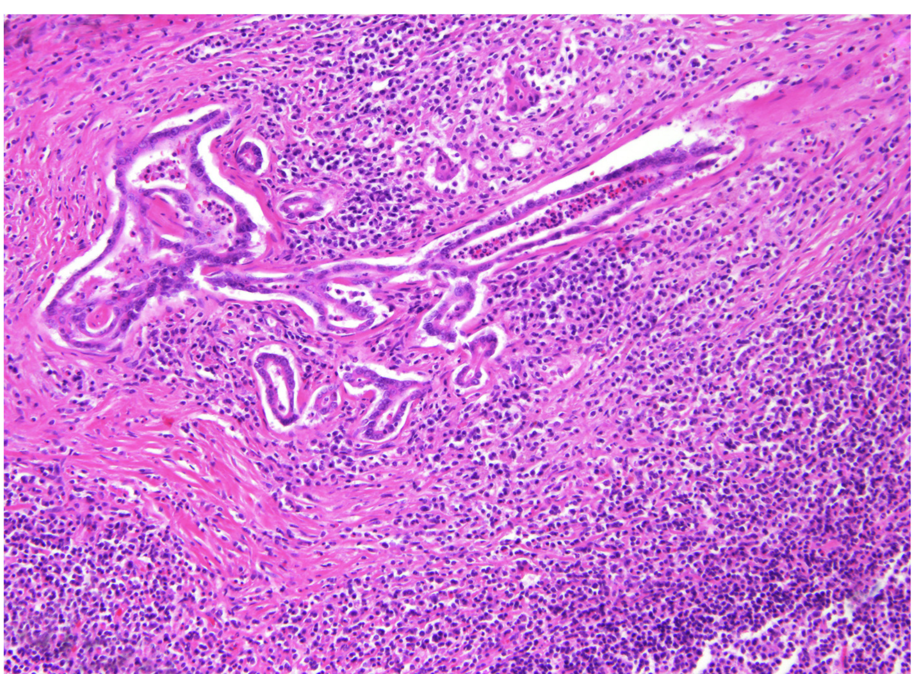

Fig. 3. Liver of a harbour porpoise with chronic proliferative cholangitis and pericholangitis, haematoxylin and eosin staining, magnification 100×.

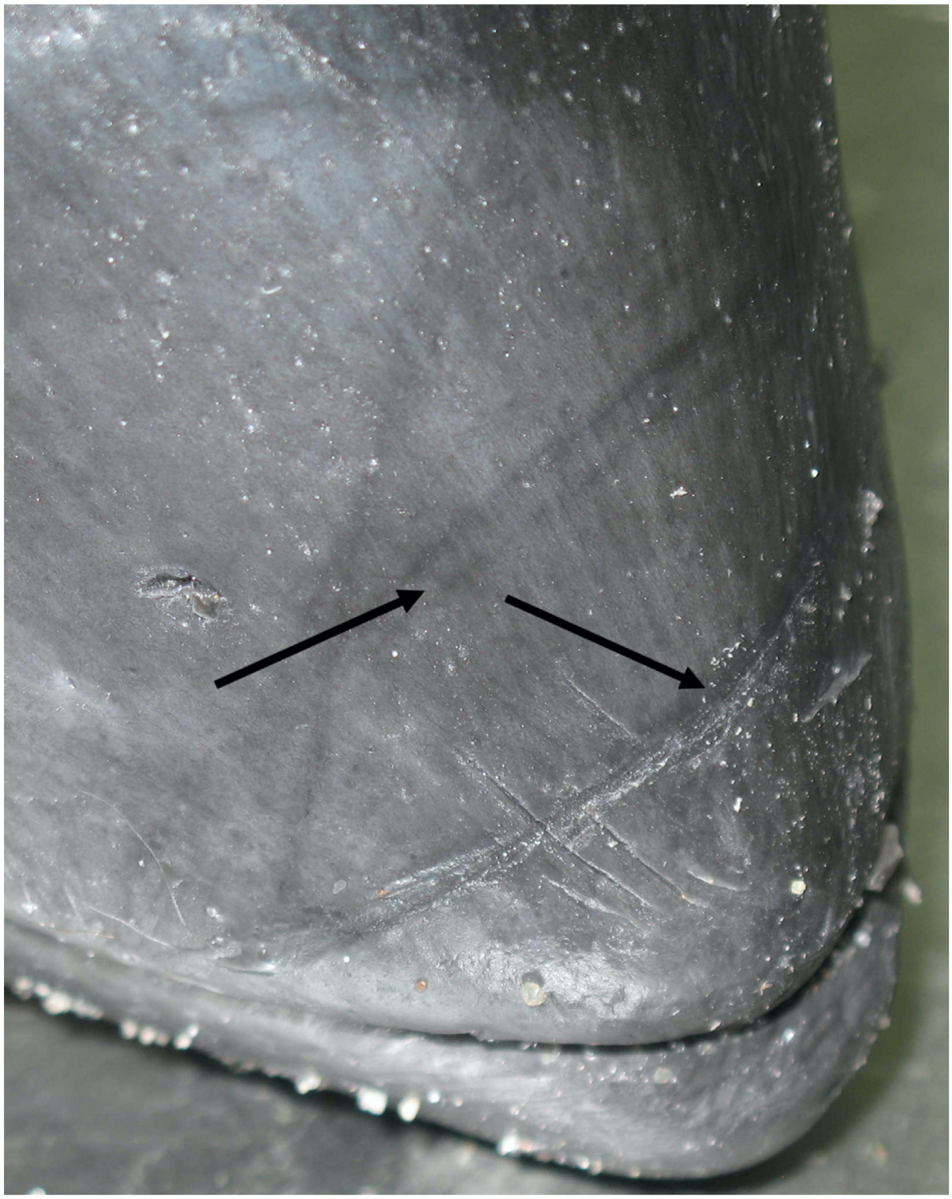

Fig. 4. Harbour porpoises with netmarks on the head (arrows).

et al., 2019; Wohlsein et al., 2019).

Most of the encountered parasite species rely on indirect trophic transmission therefore differences in parasite fauna and intensity of infections of course also reflect (apart from potentially immune suppression caused by pollutants in animals more susceptible for infectious disease) oceanographic differences in geographic regions. Oceanic vs. shelf waters with different prey species distribution and abundance as well as environmental conditions influencing intermediate host and life cycle viability, as oxygen and salinity, may reflect this.

Lymphadenitis and hyperplasia of lymph nodes and spleen were most often associated with inflammatory processes in the respiratory or alimentary tract as described by other studies from the North Sea (Jepson et al., 2000; Siebert et al., 2001, 2006; Jauniaux et al., 2002). Lesions of the urinary and genital systems were rare and mostly characterized by inflammation and infection, as opposed to leiomyoma, stenosis and occlusions of the reproductive tract described for grey and ringed seals from the Baltic Sea during the 1970s and 1980s (Bergman, 1999, 2007).

Net marks have previously been reported in by-caught and suspected by-caught animals on the head, flipper, dorsal fin and flukes (Kuiken et al., 1994; Wünschmann et al., 2001). By-caught individuals directly handed over by fishermen were generally in a good state of preservation, and therefore all included in the presented study. On the other hand, individuals suspected as by-caught among the stranded individuals are an indication that the number of by-catches among the more decomposed animals is higher than possible to diagnose. If the number of by-caught harbour porpoises is reduced by e.g. alerting devices it can also be expected that the total number of strandings will decrease. Although the necropsy results do not allow to give total by-catch numbers for different areas, they are important indications for a by-catch problem which needs to be further examined and managed, especially as by-catch is suspected to be the most important direct threat for harbour porpoises in the Baltic Sea (Kock and Benke, 1996; Koschinski 2002; ICES, 2017). The presented results demonstrate that by-caught HPs in the Baltic also suffer from infectious diseases especially in the respiratory tract. Therefore, excluding unhealthy stranded animals from being by-caught individuals underestimates the by-catch numbers. Bulging eyes or gas bubbles in different organs as described in by-caught cetaceans and pinnipeds from the East coast of the United States (de Quirós et al., 2018) were not observed in a single by-caught

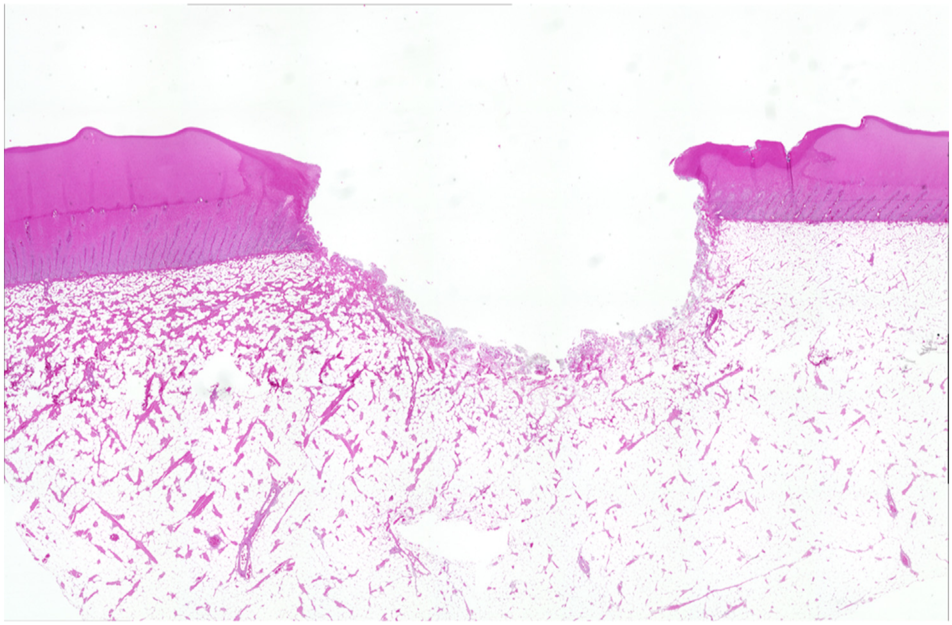

Fig. 5. Skin of a harbour porpoises with histological lesions of a netmark, haematoxylin and eosin staining, magnification 40×.

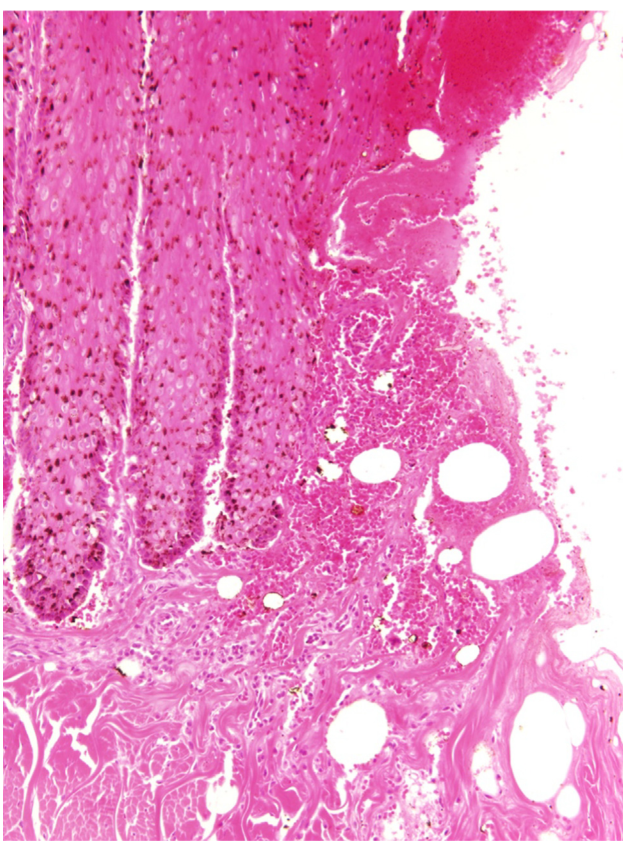

Fig. 6. Skin of a harbour porpoises with histological lesions of a netmark including bleedings in the cutis and subcutis, haematoxylin and eosin staining, magnification 100×.

harbour porpoise from the Baltic Sea.

As a next step biological parameters and pathological findings of harbour porpoises from the Baltic Sea will be combined with results from genetic analyses to distinguish between individuals from the subpopulation of the Baltic proper and Western Baltic as it has been shown that the location of dead stranded animals alone is too uncertain to be able to distinguish between individuals from the two subpopulations (Lah et al., 2016).

5. Conclusions

Summarizing the above findings, the lungs are the organ displaying most lesions impacting health status. The severity of lesions in the respiratory tract was not reflected by changes in the nutritional status as lesions of the respiratory tract were also found in by-caught animals. Therefore, for a health monitoring if restrictions apply and targeted examination of the carcasses are needed the respiratory tract should be the organ system to focus on for HPs from the Baltic Sea. Lung lesions caused by parasites and bacteria do not only affect the respiratory tract itself, but may also lead to septicaemia and spread to other vital organs including heart and brain (Siebert et al., 2009). Additionally, birth distress, particularly hypoxia, can be evaluated in the lung by fetal pulmonary atelectasis with numerous desquamated cells and keratinized lamellae as indication of aspiration of amniotic fluid (Siebert et al., 2001). The respiratory tract is also one of the target organs for viral diseases such as morbillivirus and influenza infections (Kennedy, 1998; Wohlsein et al., 2007; Bodewes et al., 2015).

Lesions in the respiratory tract have been associated with chemical pollutant burdens and other human impacts most likely due to an impaired immune and endocrine system (Siebert et al., 1999, 2006; Bruhn et al., 1999; Jepson et al., 2000; Beineke et al., 2005ab; Das et al., 2006). The respiratory tract can therefore be recommended as the indicator organ for HPs from the Baltic Sea if one organ has to be chosen for future monitoring of the health status. Hence determination will be possible, whether the condition of the target organ is changing during time and meets the Good Environmental Status according to MSFD and HELCOM as it can be found in HPs from Norwegian and Icelandic waters.

It is very alarming that among 385 investigated harbour porpoises none of the individuals from Latvia and only 17.7% from SH, 20.7% from DK, 29.8% from MP and 33.3% from Poland were older than 3 years. Harbour porpoises can reach > 20 years (Lockyer, 2003). The lack of older animals among the bycaught and stranded porpoises from all study areas may be a signal that Baltic harbour porpoises may be impacted from cumulative human activities as also previously suggested (Kesselring et al., 2017). Therefore, protective measures and reduction of human impacts are urgently needed.

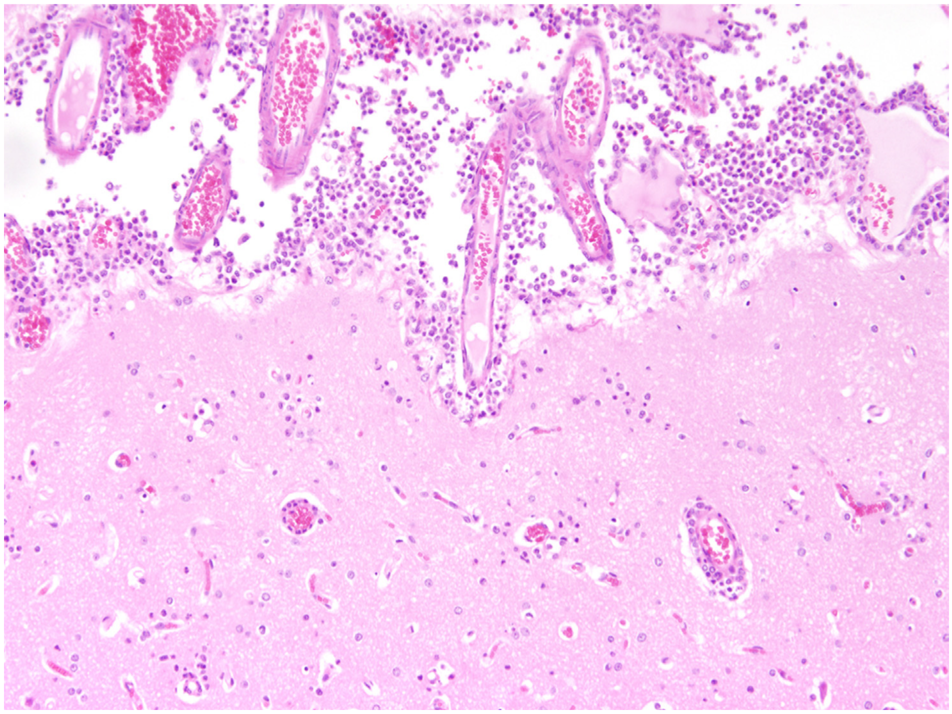

Fig. 7. Brain of a harbour porpoise showing suppurative meningoencephalitis due to beta-haemolytic streptococci septicaemia, haematoxylin and eosin staining, magnification 200×.

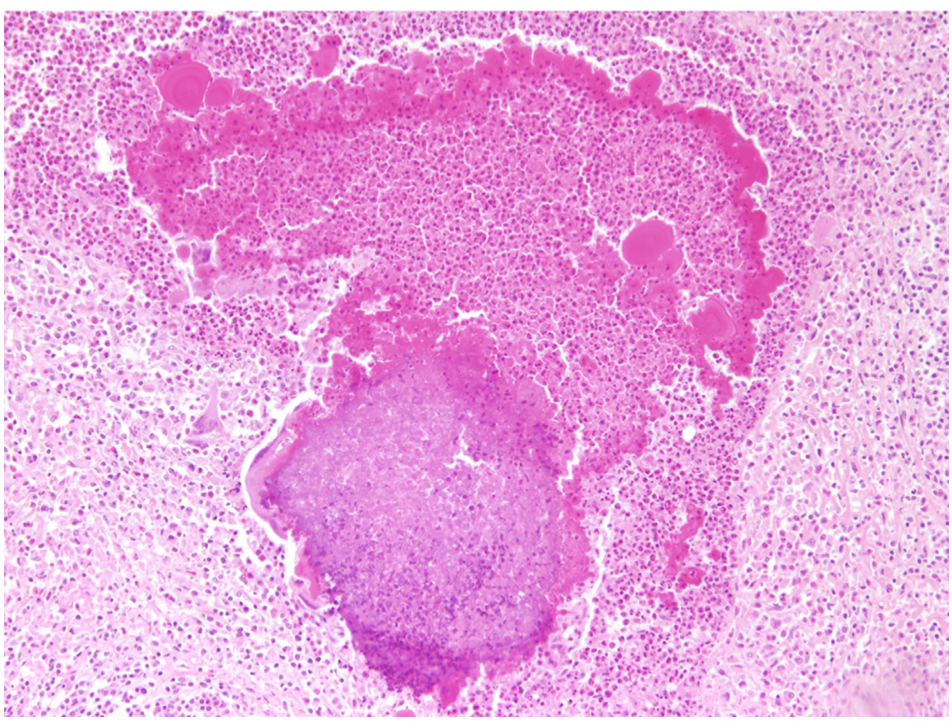

Fig. 8. Mesenteric lymph node of a harbour porpoise with granulomatous-necrotising lymphadenitis due to a bacterial and parasitic infection, haematoxylin and eosin staining, magnification 100×.

Stranded HPs suspected as by-catched were often very decomposed and not suited for extensive necropsy. This indicates that the number of by-catches might be higher than is possible to diagnose. Assuming that diseased stranded animals were not by-caught will therefore inherently result in underestimated by-catch numbers.

CRediT authorship contribution statement

U. Siebert: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **I. Pawliczka:** Funding acquisition, Investigation, Methodology, Writing - review & editing. **H. Benke:**

Investigation, Methodology, Writing - review & editing. **V. von Vietinghoff:** Investigation, Methodology, Writing - review & editing. **P. Wolf:** Visualization, Investigation, Methodology, Writing - original draft, Writing - review & editing. **V. Pilāts:** Funding acquisition, Investigation, Methodology, Writing - review & editing. **T. Kesselring:** Investigation, Methodology, Writing - original draft, Writing - review & editing. **K. Lehnert:** Investigation, Methodology, Writing - original draft, Writing - review & editing. **E. Prenger-Berninghoff:** Methodology, Writing - review & editing. **A. Galatius:** Investigation, Methodology, Writing - review & editing. **L. Anker Kyhn:** Investigation, Methodology, Writing - review & editing. **J. Teilmann:** Investigation, Methodology, Writing - review & editing. **M. S. Hansen:** Investigation, Methodology, Writing - review & editing. **C. Sonne:** Funding acquisition, Writing - original draft, Writing - review & editing. **P. Wohlsein:** Investigation, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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