

## Increase in parasite burden and associated pathology in harbour porpoises (*Phocoena phocoena*) in West Greenland

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**Abstract** Knowledge about parasitism in harbour porpoises and their health status around Greenland is scarce. This study provides knowledge about the poorly studied cetacean in its rapidly changing environment. Parasites and pathological findings in 20 harbour porpoises (*Phocoena phocoena*) hunted in waters around West Greenland are presented. Carcasses were dissected, and parasitological, histological and bacteriological investigations were carried out. Protozoa (*Sarcocystis* sp.), Nematoda (*Halocercus invaginatus*, *Stenurus minor*, *Anisakis simplex* sensu lato (s.l.), *Crassicauda* sp.), Trematoda (*Campula oblonga*) and Cestoda (*Phyllobothrium delphini*, *Monorygma grimaldii*) were found. Parasitic infection of the peribullar cavity and lung with pseudaliid nematodes was found in most animals. Sixty per cent of the porpoises were infected with stomach

worms, and trematodes were present in liver and pancreas of 90 and 30 % of the porpoises, respectively. *Crassicauda* sp. was isolated from perimuscular fascia in 45 % of the animals. This is the first record of tetraphyllidean merocercoids in harbour porpoises. *M. grimaldii* and *P. delphini* were found in blubber layer of 15 % and abdominal cavity of 50 % of the porpoises. Bronchopneumonia, gastritis, cholangitis, pancreatitis and panniculitis were almost exclusively associated with parasitic infection and usually mild. Compared with a previous study of Greenlandic porpoises from 1995, a significant increase in severity of parasitic infections and the emergence of new parasite species were observed, most likely associated with changes in diet, influenced by increasing sea temperatures and receding ice cover.

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## Introduction

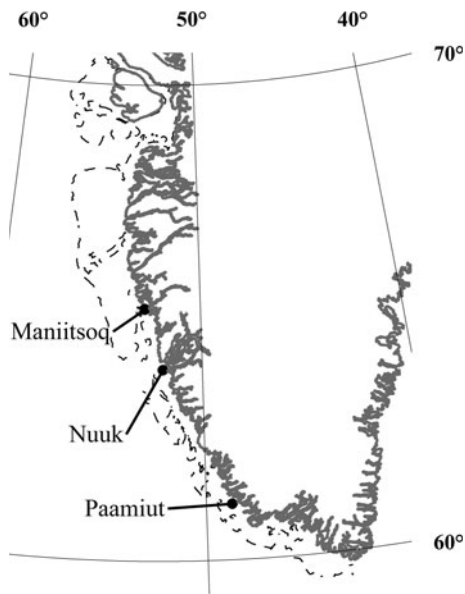
The harbour porpoise (*Phocoena phocoena*) is a cetacean species native in the coastal waters of the North Atlantic (Hammond et al. 2002). Genetic investigations revealed a fine-scaled genetic population structure (Tolley et al. 2001; Andersen 2003) in which restricted exchange occurs between areas of distribution (Rosel et al. 1999). Investigations on the health status of harbour porpoises in European waters have been performed for many years (Clausen and Andersen 1988; Baker and Martin 1992; Siebert et al. 2001; Wünschmann et al. 2001; Jauniaux et al. 2002; Jepson et al. 2005; Lehnert et al. 2005), and they have demonstrated the importance of parasitic infections and associated lesions for the health status of porpoises. Previous investigations showed that animals from the North and Baltic Seas suffered from parasitic and bacterial infections, particularly those of the respiratory tract (Jepson et al. 2000; Siebert et al. 2001; Jauniaux et al. 2002; Lehnert et al. 2005; Siebert et al. 2009). Harbour porpoises from the Baltic Sea showed a significantly higher prevalence of severe bacterial infections than those from less polluted waters around Greenland (Wünschmann et al. 2001). Polychlorinated biphenyl (PCB) concentrations in adult harbour porpoises were ten times lower in the Arctic than in the Baltic and North Seas (Kleivane et al. 1995; Bruhn et al. 1999). In harbour porpoises from Icelandic waters, concentrations of PCBs and polybrominated diphenyl ethers (PBDEs) were on average five times lower than in those from Norwegian and German waters; as for toxaphene concentrations, however, arctic porpoises showed higher burdens (Thron et al. 2004) than porpoises from the North and Baltic Sea.

Parasitic infections in harbour porpoises were found in the respiratory and alimentary tract, heart, liver, pancreas, auditory sinuses and skin (Raga 1994; Siebert et al. 2001; Lehnert et al. 2007). Infections with lung worms are commonly associated with secondary bacterial infections (Siebert et al. 2001; Lehnert et al. 2005) and are known to negatively affect the health of marine mammals. In general, a lower prevalence of infectious diseases has been reported in harbour porpoises from northern regions (Norway, Iceland) in comparison with animals from German waters (Siebert et al. 2006, 2009). Assessments of the health status of porpoises in Greenlandic waters were undertaken in 1995 (Wünschmann et al. 2001) and showed lower intensities of parasitic infection and less severe lesions than observed in porpoises from German waters (Wünschmann et al. 2001). Immune suppression caused by accumulation

of contaminants may increase the susceptibility of harbour porpoises towards infectious disease such as from parasites and bacteria (Aguilar and Borrell 1995; Jepson et al. 1999; Siebert et al. 1999). The diet of porpoises from coastal shelf waters in the North and Baltic Sea likely differs from animals feeding in deeper and pelagic waters around Greenland (benthic vs. pelagic prey) (Gilles 2009; Heide-Jørgensen et al. 2011). Diet composition will influence porpoise parasite fauna as fish species occurring in temperate and arctic waters play different roles as intermediate hosts (Klimpel et al. 2004; Lehnert et al. 2010). Knowledge about parasitism in harbour porpoises and their health status around Greenland is scarce. We aim to provide knowledge about the poorly studied cetacean in this rapidly changing environment (Heide-Jørgensen et al. 2011). Special attention was paid to parasite species diversity and prevalence, associated lesions and their impact on the harbour porpoises' health status. Changes in the prevalence and level of parasitic infections in Greenlandic animals since 1995 and with changing environmental conditions are discussed.

## Materials and methods

Harbour porpoises are hunted regularly in West Greenland for subsistence purposes (Teilmann and Dietz 1998). The 20 examined harbour porpoises were shot in West Greenlandic waters by Greenlandic hunters and landed in Maniitsoq in September 2009 (see Heide-Jørgensen et al. 2011) (Fig. 1), and the samples were purchased under permission provided by the Greenlandic Home Rule Government. The fresh carcasses were frozen prior to dissection. Nutritional status was determined by blubber thickness and state of muscle (Siebert et al. 2001). The reproductive status was judged by the activity of the gonads and mammary glands as well as gestation. Necropsies were performed at the Greenland Institute of Natural Resources in Nuuk, Greenland, according to international guidelines (Pugliares et al. 2007); all organs were examined macroscopically and histologically (Siebert et al. 2001). Furthermore, microbiological investigations were carried out on the porpoises (Siebert et al. 2009). Parasites were collected during necropsies, preserved in 70 % ethanol and identified microscopically after preparation in lactophenol (Lehnert et al. 2005). Voucher specimens were deposited in the Senckenberg Institute, Forschungsinstitut und Naturmuseum Frankfurt, Frankfurt, Germany (Accession Nos. SMF 15186-15189; SMF 16976-16979). The level of parasitic infection was determined (during necropsy) semiquantitatively as severe, moderate or mild, as described by Siebert et al. (2001). For merocercoids and *Crassicauda* sp., prevalence was recorded. Porpoises were divided into age



**Fig. 1** Map of West Greenland showing sampling area of the two studies in 1995 and 2009

classes according to Siebert et al. (2001):  $\leq 0.5$  year (neonate), 0.5–4 year (juvenile) and  $>4$  year (adult).

All statistical analyses were performed with the open source software R (R Development core team 2012, version 2.15.0). Prevalence (presence/absence) and level of infection were compared between 1995 and 2009, and differences between sexes and age classes were tested as main, fixed effects using the original data (Wünschmann et al. 2001). Minimal adequate models were determined by single-factor deletion and selected based on Akaike's Information Criterion (AIC). To estimate overall parasite diversity, the number of parasite species per porpoise based on presence/absence data was tested in a generalized linear model (GLM) with the quasi-Poisson link function to account for overdispersion (Crawley 2012). The semi-quantitative level of infection was defined as ordered factor, and cumulative link models were fitted (CLM, package ordinal, Christensen 2012). GLMs were fitted for the prevalence data with binomial errors. Emerging parasite species and species with low prevalence (1–3 infected animals) were included only in statistical analyses for parasite diversity.

For *Campula oblonga*, in addition to the overall study questions (comparison years, age and sex), an additional main effect “infected organ” was included into the models for the prevalence and intensity of infection. It was tested whether liver or pancreas was more severely affected. For the parasite species *Halocercus invaginatus* and *Stenurus minor*, which belong to the same family Pseudaliidae, it was tested whether the prevalence and level of infection differed between species.

## Results

The harbour porpoise sample consisted of seven male and 13 female animals. Their nutritional status was good. The age classes represented were seven juveniles (4 females and 3 males) and 13 adults (9 females and 4 males); no neonates were examined.

All adult female porpoises were pregnant and six of them lactating. Seven parasite species belonging to Nematoda, Cestoda and Trematoda were found: *H. invaginatus* in the lung; *Anisakis simplex* sensu lato (s.l.) in the oesophagus and the first (forestomach), second (main stomach) and fourth (pyloric stomach) stomach compartments; *C. oblonga* in the liver, bile ducts, pancreas and its ductular system; and *S. minor* in the auditory sinus (Table 1). Nematodes of the *Crassicauda* sp. were located between the subcutaneous fat layer and the skeletal musculature across the flank and around the mammary gland. Two types of cestode merocercoids were isolated: *Monoorygma grimaldii* from the peritoneum of the abdominal cavity and *Phyllobothrium delphini* encysted in the subcutaneous blubber.

Parasite diversity per porpoise as tested in a generalized linear model (quasi-Poisson link function) increased significantly in porpoises from Greenlandic waters from 1995 (Wünschmann et al. 2001) to 2009 (this study) ( $p < 0.001$ ). Moreover, adult porpoises had accumulated more parasites in both studies ( $p = 0.008$ ).

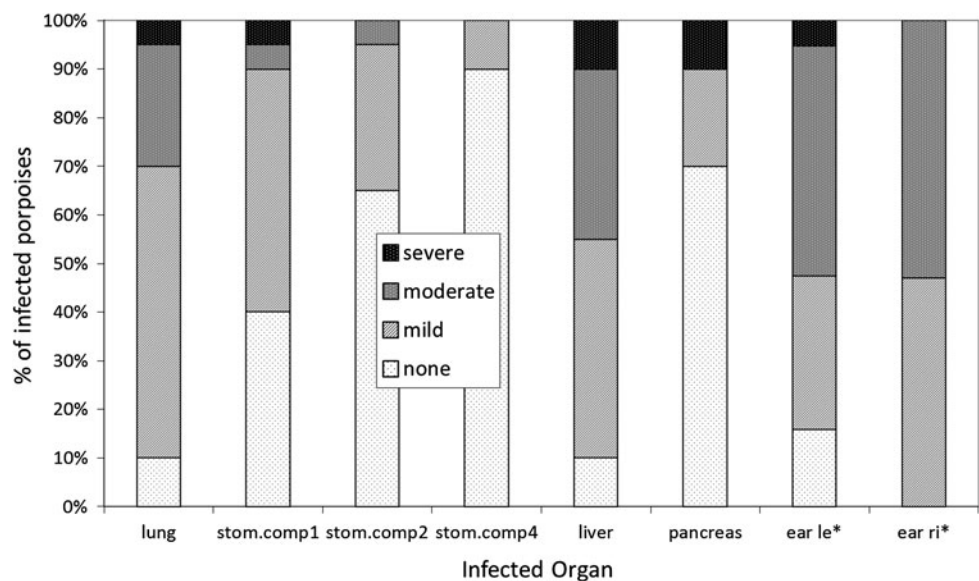
One juvenile female harbour porpoise was not infected by grossly visible parasites, and its stomach contained milk constituent parts. Presumably, it was primarily still nursing and had not been feeding on prey items acting as parasite intermediate hosts. Nineteen (95 %) porpoises were infected with *H. invaginatus* in the lung. Most (13) infections were mild, five were moderate, and one adult female was severely infected (Fig. 2). Pulmonary infections were characterized by adult nematode worms in bronchioles and pulmonary blood vessels. Lungworms were found in the pulmonary parenchyma and alveoli and formed multiple nodules that were often calcified. In bronchioles, a mild to moderate diffuse lymphohistiocytic inflammation associated with bronchiectasia was observed (Fig. 3).

Affected blood vessels showed mild muscular hypertrophy and a generally mild to moderate perivascular infiltration, dominated by lymphocytes. Infection with lung nematodes was in most cases associated with a moderate to severe granulomatous pneumonia ( $n = 10$ ) (Table 2). In some animals, a mild to moderate interstitial ( $n = 9$ ) or catarrhal to suppurative pneumonia ( $n = 5$ ) was found. Severity of parasitic infections with the pseudaliid nematodes *H. invaginatus* and *S. minor* had increased in comparison with 1995 (Wünschmann et al. 2001) (cumulative link model (CLM),  $p = 0.001$ ), and a marginally significant

**Table 1** Parasite species, infection site and prevalence found in harbour porpoises from Greenlandic waters in 1995 and 2009

Parasites	Infection site	Prevalence % (n)	
		Greenland 1995 n = 14	Greenland 2009 n = 20
<b>Nematoda</b>			
Pseudaliidae; Metastrongyloidea			
<i>Halocercus invaginatus</i>	Bronchioles, lung, pulmonary blood vessels	86 (12)	95 (19)
<i>Stenurus minor</i>	Eustachian tube, peribullar cavity	86 (12)	95 (19)
Anisakidae; Ascaridoidea			
<i>Anisakis simplex s.l.</i>	Oesophagus, 1st, 2nd and 4th stomach compartments	0	60 (12)
Crassicaudidae			
<i>Crassicauda</i> sp.	Perimuscular fascia, subcutaneous fat	36 (5)	45 (9)
<b>Digenea</b>			
Brachycladiidae; Allocreadioidea			
<i>Campula oblonga</i>	Liver, pancreas	71 (10)	90 (18)
<b>Cestoda</b>			
Phyllobothriidae; Tetraphyllidea			
<i>Monorygma grimaldii</i>	(Merocercoid) peritoneum, abdominal cavity	0	15 (3)
<i>Phyllobothrium delphini</i>	(Merocercoid) subcutaneous blubber	0	50 (10)
<b>Protozoa</b>			
<i>Sarcocystis</i> sp.	Skeletal musculature	14 (2)	5 (1)

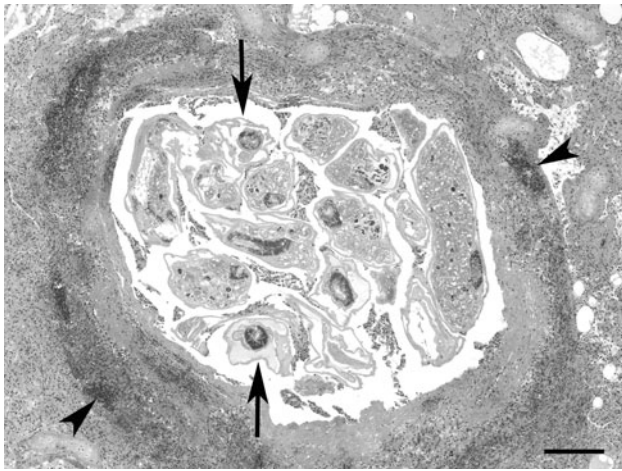
**Fig. 2** Prevalence and qualitative level of parasitic infections in different organs of 20 porpoises from West Greenland (asterisks include peribullar sinus, Eustachian tube and cranial sinuses)



increase in the severity of lesions in the respiratory tract compared with the 1995 study ( $p = 0.057$ ) was observed.

Nearly all porpoises ( $n = 19$ ; 95 %) were infected with *S. minor* in one or both peribullar cavities and cranial sinuses. Two adult females and one adult male had no infection in the left but moderate or mild infections in the right peribullar cavities. In two adult porpoises, the right

peribullar cavity was not examined due to trauma (shot through the head), and in one juvenile animal, both peribullar cavities were not examined. No gross lesions due to *S. minor* were observed. *S. minor* shows higher levels of infection than *H. invaginatus* (CLM,  $p < 0.001$ ) in both studies. No significant difference in the prevalence of the two pseudaliid nematodes between both studies was found.



**Fig. 3** Lung with severe intravascular infection of nematodes (arrows) with perifocal chronic granulomatous perivasculitis; HE, bar = 200  $\mu$ m

Twelve individuals (60 %) were infected with *A. simplex* (s.l.) in the stomach, seven animals in the first stomach compartment, three of those additionally in the second and/or fourth compartments and four porpoises in the second compartment only and once additionally in the fourth compartment. In one juvenile animal, four anisakid larvae were found in the oesophagus. Four adult and four juvenile porpoises had no stomach nematodes. One severely infected adult female had two non-penetrating chronic ulcerations in the first compartment, one containing anisakid larvae. One adult female with a mild parasitic infection in the first and second compartments showed a chronic non-penetrating ulceration in the first compartment, and another adult female had a mild and moderate infection in the first and second compartments that was associated with multifocal acute and subacute ulcerations (Fig. 4). In 1995, none of the individuals was infected with *A. simplex*.

Eighteen porpoises (90 %) were infected with *C. oblonga* in the liver and bile ducts. Nine animals were infected mildly, seven showed a moderate infection, and two adult males had a severe infection (Fig. 2). Two juvenile animals were not infected. Severity and prevalence of *C. oblonga* infections increased with age (CLM,  $p = 0.014$ ; and GLM (binomial),  $p = 0.013$ , respectively). The liver was more affected in both severity of infections and prevalence than the pancreas ( $p < 0.001$ ). No differences between the studies were found.

Six porpoises (30 %) were infected with *C. oblonga* in the pancreas. Three adults and one juvenile showed a mild infection, and two adults were severely infected. Fourteen porpoises (70 %) had no parasites in the pancreas. Parasitic lesions in liver, bile ducts, pancreas and pancreatic ducts were similar. Ducts were dilated with accumulated cellular debris and inflammatory cells. In addition, mural

fibrosis with a generally severe mononuclear and eosinophilic inflammation often associated with trematode eggs (Fig. 5) or trematodes (Fig. 6) was found. Bile and pancreatic ducts showed multifocal proliferation with adjacent fibrosis.

*Phyllobothrium delphini* merocercoids were found in the blubber layer of two adult females (15 %) and one juvenile male. No inflammatory changes due to encysted cestode larvae were observed grossly or histologically.

Within the mesenteries of the abdominal cavity, often around the testes and uterus, encapsulated merocercoids of *M. grimaldii* were isolated from ten porpoises (50 %). Seven adult females, two adult males and one juvenile female were infected. Cestode larvae were surrounded by a demarcating collagen fibre-rich connective tissue with focal inflammatory infiltration.

*Crassicauda* sp. was found in nine porpoises (45 %), two male adults, five female adults and two male juveniles. They were located along the dorsolateral flank of the animals between the subcutaneous fat and the perimuscular fascia (Figs. 7, 8), reaching the mammary glands in their female hosts. Histologically, fibrosis, calcifications and chronic granulomatous panniculitis were associated with *Crassicauda* sp. infections. In one female porpoise, *Crassicauda* sp. was found within the mammary gland. The prevalence of *Crassicauda* sp. showed no statistical differences between age classes, sexes or the two studies.

In one adult male porpoise, unidentified parasitic (cestode) structures were observed histologically in the perimuscular connective tissue, but not observed grossly.

A thin-walled cyst containing numerous bradyzoites consistent morphologically with *Sarcocystis* sp. without inflammatory changes was found in the musculature of one adult female (Fig. 9).

Several ( $n = 13$ ) species of bacteria were isolated from selected organs of 16 porpoises (Table 3). The bacterial growth was mild in most cases and only moderate in three cases. Most bacteria were considered to be unspecific and were regarded as post-mortem invaders or contaminants due to the lack of inflammatory changes. Potential pathogenic bacteria were  $\beta$ -haemolytic streptococci and *Escherichia coli*.

## Discussion

This is the second comprehensive study of the health status of harbour porpoises in Greenland where complete post-mortem investigations and continuative analyses have been conducted. The first study involved examination of samples from 14 harbour porpoises landed in Maniitsoq in August and September of 1995 (Wünschmann et al. 2001). There is a temporal change in the diversity of parasite fauna and an

**Table 2** Predominant pathological findings in 20 harbour porpoises from West Greenland

Organ	Findings	Number of affected animals
Respiratory system		
Trachea	Tracheitis	1
Lungs	Intraluminal bronchial and pulmonary nematode infection	19
	Bronchopneumonia	15
	Granulomatous with intralesional parasites	10
	Interstitial	9
	Catarrhal–suppurative	5
	Bronchitis	3
	Fibrosis	3
Digestive tract		
Stomach	Gastritis without intralesional/luminal nematodes	4
	Gastritis with intralesional/luminal nematodes	5
	Intraluminal nematode infection without inflammatory changes	8
Intestine	Enteritis without intralesional parasites	2
Abdominal cavity and peritoneum	Serositis with parasitic cyst formation	10
Pancreas	Pancreatitis with intraluminal trematodes	6
	Inflammation of ductus pancreaticus	3
	Fibrosis	2
Liver	Cholangitis and pericholangitis with intraluminal trematodes	18
	Proliferation of bile ducts	12
	Fibrosis	11
	Hepatitis	6
Lung-associated lymphatic tissue	Hyperplasia	7
Thymus	Thymic cysts	2
Central nervous system and ears		
Ear sinus	Otitis media with nematode infection in the Eustachian tube and the tympanic cavity	19
Locomotor system		
Skeletal muscles	Perimuscular fibrous tissue with focal parasitic cyst formation	7
	Myositis	3
	Sarcocystosis	1
Skin and subcutis		
Subcutis	Panniculitis with focal parasitic cyst formation	8

increase in the prevalence and severity of infections between the two studies (Tables 1, 2).

Severity of parasitic infections with the pseudaliid lung nematodes *H. invaginatus* and *S. minor* and of lesions in the respiratory tract (e.g. pneumonia) has increased in comparison with 1995 (Wünschmann et al. 2001). Lungworms are often associated with secondary bacterial infections and bronchopneumonia (Jepson et al. 2000; Lehnert et al. 2005), which can seriously impair the health status and are a common cause of death in porpoises (Siebert et al. 2001).

The pseudaliid nematodes *Pseudalius inflexus* and *Torrynurus convolutus*, common lungworms in porpoises, were not found in porpoises from Greenlandic waters in

this or the previous study from 1995 by Wünschmann et al. (2001). Flatfish have been reported to be potential intermediate hosts for these metastrongyloid lungworms of marine mammals (Houde et al. 2003; Lehnert et al. 2010). Analyses of prey items from the animals examined here for parasites (Heide-Jørgensen et al. 2011) demonstrated that comparatively few flatfish were contained in the diet.

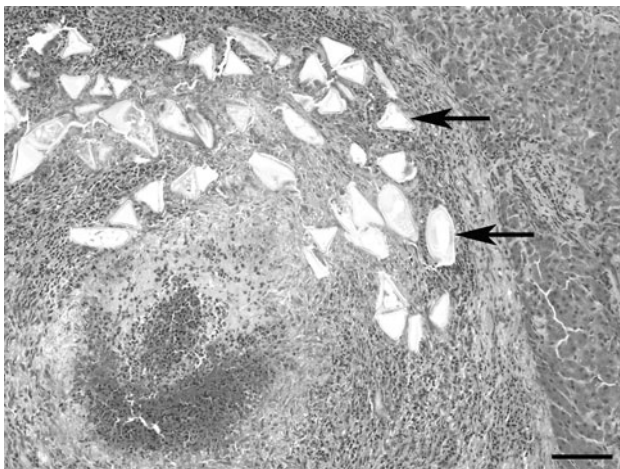
In the German North and Baltic Sea, all three pseudaliid lungworms are commonly found in high prevalence (Lehnert et al. 2005; Siebert et al. 2006), and the diet consists in large parts of benthic fish (Gilles 2009). In British waters, Gibson et al. (1998) and Jepson et al. (2000) found *P. inflexus*, *T. convolutus* and *H. invaginatus* in the respiratory tract of stranded and by-caught harbour porpoises,



**Fig. 4** Proventricular stomach with nematode larva attached to the mucosa (arrow), bar = 350 μm



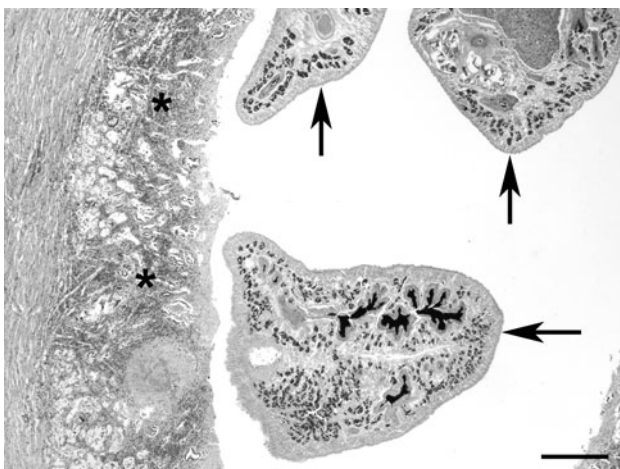
**Fig. 7** Musculature, harbour porpoise, focal parasitic cystic cavity (black asterisk) with thick fibrous wall (white asterisk) and focal chronic non-suppurative inflammation (arrow); HE, bar = 300 μm



**Fig. 5** Liver with eosinophilic and granulomatous inflammation and numerous trematode eggs (arrows); HE, bar = 80 μm



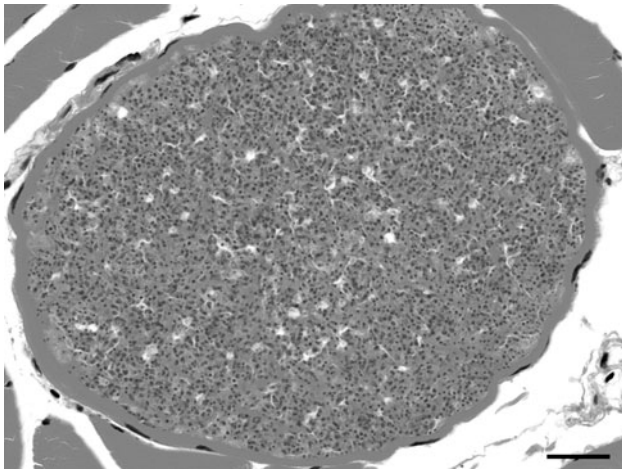
**Fig. 8** Blubber, harbour porpoise, cystic cavities (asterisks) with mineralizations surrounded by fibrous connective tissue suggestive of *Crassicauda* sp.; HE, bar = 300 μm



**Fig. 6** Dilated bile duct with trematodes (arrows) and proliferation of the mucosa (asterisks); HE, bar = 350 μm

although the prevalence of *H. invaginatus* in those animals was very low (2 %) compared with the findings of other studies (Wünschmann et al. 2001; Siebert et al. 2006). The absence of *P. inflexus* and *T. convolutus* from the Greenlandic porpoises is striking, as they belong to the same family (Pseudaliidae) as *H. invaginatus* and *S. minor*, which are the parasites with the highest prevalence found in this study.

It has been suggested that some pseudaliid nematodes (e.g. *P. inflexus*) in cetaceans have a life cycle using flatfish intermediate hosts (Houde et al. 2003; Lehnert et al. 2010), but the data collected here indicate that *H. invaginatus* (and *S. minor*) may use a different mechanism. They could be transmitted directly or prenatally, as suggested by Dailey et al. (1991) in *Halocercus lagenorhynchi* of bottlenose



**Fig. 9** Protozoan cyst in the skeletal musculature. Note no inflammatory changes were identified, and cyst is surrounded by a thin wall containing numerous bradyzoites suggestive of *Sarcocystis* sp.; HE, bar = 40  $\mu$ m

dolphins (*Tursiops truncatus*). If an intermediate host is involved, it may be pelagic fish or cephalopods.

In the present study, *A. simplex* s.l. was found in more than half of the examined porpoises. *A. simplex* was not found in animals examined in 1995 by Wünschmann et al. (2001). The occurrence of the gastric nematode *A. simplex* s.l. in this study indicates a major shift in the diet of the porpoises and/or in fish distribution around Greenland since 1995. This is supported by Heide-Jørgensen et al. (2011), whose diet analyses on the same animals revealed a considerable change in prey composition with a major

increase in cod (Gadidae), snailfish (Liparidae) and squid (Cephalopoda), with Gadidae being an important intermediate host for anisakid larvae (Aspholm 1995).

The prevalence of *C. oblonga* in 2009 is similar to the observations from other geographical areas (Siebert et al. 2001; Lehnert et al. 2005). The trematode *C. oblonga* is regularly found in the liver of porpoises from the North and Baltic Seas, although an infection of pancreatic tissue is rarely observed in those waters (6 % prevalence ( $n = 133$ ) in Siebert et al. 2001). Gibson et al. (1998) found *C. oblonga* in the pancreas of 16 % of the examined 173 porpoises around Britain. Infections with *C. oblonga* in pancreatic tissue have been found more frequently in Norwegian (18 %;  $n = 22$ ) and Icelandic (25 %;  $n = 12$ ) porpoises (Lehnert et al. 2005; Siebert et al. 2006). This may be due to a higher prevalence of infected prey acting as intermediate host in northern areas. The trematode *Orthosplanchnus mironovi* was not isolated from Greenlandic porpoises in this study, in contrast to the study in 1995 (Wünschmann et al. 2001).

The deep waters around Greenland show different habitat characteristics than the coastal shelf waters in the German North and Baltic Sea. Cold arctic waters have a different species composition compared with temperate waters, and we assume that the pelagic lifestyle of Greenlandic porpoises results in a different prey composition (cephalopods, etc.); thus, the parasite fauna differ between European and Greenlandic porpoises.

The prevalence of *Crassicauda* sp. has increased in Greenlandic porpoises from 1995 to 2010. This large nematode, which can rarely be dissected in one piece from a cetacean carcass, has not been found in harbour porpoises

**Table 3** Bacteria isolated from harbour porpoises from Greenlandic waters

Bacteria	Greenland 1995 ( $n = 11$ ) (Wünschmann et al. 2001)	Greenland 2009 ( $n = 16$ )
Considered pathogenic		
$\beta$ -haem streptococci	0	1 (INLY)
<i>E. coli</i>	6 (LU, LI, KI, SP, IN)	1 (KI, IN)
Potentially pathogenic		
$\alpha$ -haem streptococci	1 (LU)	2 (LU, LI, KI, SP, IN, LULY, INLY)
<i>Aeromonas</i> spp.	0	1 (KI, IN)
<i>Pseudomonas aeruginosa</i> /spp.	4 (LU, KI, IN)	5 (LU, LI, KI, SP, IN, INLY)
<i>Corynebacterium</i> spp.	6 (LU, LI, KI, SP, IN)	4 (LI, SP, IN, LYLY, INLY)
<i>Lactobacillus</i> spp.	2 (LI, KI)	0
<i>Actinobacillus</i> spp.	1 (LI)	0
<i>Acinetobacter</i> spp.	0	1 (KI, LU)
Aerobic bacilli	0	3 (KI, LULY, INLY)
<i>Pantoea</i> spp.	0	1 (IN)
<i>Staphylococcus epidermidis</i>	0	6 (LU, LI, KI, SP, IN, LULY, INLY)
<i>Enterococcus</i> spp.	0	1 (LI)
<i>Micrococcus</i> spp.	0	2 (KI, LU, IN)
Mould fungi	0	1 (LU, LI, KI, SP, LULY)

$N = 16$  (numbers of cases and organs from which bacteria were isolated are given)

LU lungs, LI liver, KI kidney, SP spleen, IN intestine, LULY lung lymph node, INLY intestinal lymph node



from the German North and Baltic Sea (Siebert et al. 2001; Lehnert et al. 2005) but was recorded in porpoises from waters around the United Kingdom (Gibson et al. 1998). Its prevalence could be associated with certain fish species serving as intermediate hosts that may not occur in coastal shelf waters. Surprisingly, no *Crassicauda* sp. were found by Siebert et al. (2006) when investigating harbour porpoises from Norwegian and Icelandic waters. In diet analyses performed by Heide-Jørgensen et al. (2011), a nearly threefold increase in cephalopod beaks in harbour porpoise stomachs was found compared with diet analysis from the 1995 study (Lockyer et al. 2003), indicating that cephalopods have constituted a larger fraction of the diet in recent years and thus could potentially serve as an intermediate host to *Crassicauda* sp.

In this study, tetraphyllidean merocercoids were recorded for the first time in Greenlandic porpoises. To our knowledge, this is the first host record of tetraphyllidean merocercoids (*P. delphini* and *M. grimaldii*) in harbour porpoises. These cestodean larvae are described in studies from a large range of cetaceans but usually not found in harbour porpoises (Gibson et al. 1998; Siebert et al. 2001, 2006; Lehnert et al. 2005). Sharks are believed to be the final hosts, and it is assumed that the fish serving as intermediate host may be pelagic and too large to be ingested by porpoises (Gibson et al. 1998; Aznar et al. 2007). The two species isolated belong to the same group but are found in different locations within their (intermediate) mammalian host (Agusti et al. 2005): *P. delphini* parasitizes in the subcutaneous blubber and *M. grimaldii* in the serosa of the abdominal cavity. Siebert et al. (2006) did not record any merocercoids from Norwegian and Icelandic porpoises. As Greenlandic waters are inhabited by the Greenland shark (*Somniosus microcephalus*), an apex predator, known consumer of marine mammal carcasses (MacNeil et al. 2012) and a presumable final host for these cestodes, the prevalence of larval tetraphyllidean specimens in the ecosystem is consequential.

Histopathological lesions were mostly associated with parasites. While lesions in the digestive tract, liver and muscles were similar to those described for animals in 1995 (Wünschmann et al. 2001), there was an increase in the severity of lesions in the respiratory tract compared with the 1995 study. Thymic cysts were also found in 1995 (Wünschmann et al. 1999).

As the respiratory system is one of the most important organs responsible for good health status (Jepson et al. 2000; Siebert et al. 2001), continued monitoring of the immune and health status of harbour porpoises in Greenland is relevant. Pollution in Greenlandic waters increased over the last decades, as has been shown for different compounds in polar bears (*Ursus maritimus*) and ringed seals (*Phoca hispida*) (Dietz et al. 2008, 2011; Aubail and Ridoux 2010) as well as marine fish and blue mussels (Christensen et al. 2002).

Contaminants may be influencing the immune system and susceptibility towards infectious diseases in harbour porpoises. However, porpoises from Greenland are known to have relatively low levels of OCs (HCB, DDTs and PCB) compared with other arctic marine mammals and with porpoises from the North Atlantic (Borrell et al. 2004). However, toxaphene concentrations, as reported by Thron et al. (2004), were higher in porpoises from arctic regions.

Impairment of the immune and endocrine systems was found in harbour porpoises from the Baltic and North Seas (Beineke et al. 2005, 2007; Das et al. 2006), which show more infectious diseases associated with the respiratory system and have more chronic diseases than porpoises from Norwegian or Icelandic waters (Lehnert et al. 2005; Siebert et al. 2006).

Microbial fauna has changed to some degree, but bacterial load remains low when compared to harbour porpoises from the North and Baltic Seas (Siebert et al. 2009). Most of the bacteria found are considered to be unspecific; only  $\beta$ -haemolytic streptococci and *E. coli* were potentially pathogenic. Neither abscessation and septicaemia nor zoonotic bacteria such as *Brucella pinnipedialis*, *Brucella ceti* or *Erysipelothrix rhusiopathiae* (Siebert et al. 2009) were found, similar to microbiological findings of harbour porpoises dissected in 1995 (Wünschmann et al. 2001).

Overall, an increase in the severity of parasitic infection has occurred over the past 15 years in all organ systems. Parasite species that previously did not appear in porpoises from Greenland are now emerging (*A. simplex* s.l.). Tetraphyllidean merocercoids are appearing for the first time, as this is the first record of *P. delphini* and *M. grimaldii* in harbour porpoises. This emergence of new species and increase in severity of infection with certain species may be due to the rapidly changing marine environment in West Greenland (Heide-Jørgensen et al. 2011) that has caused a shift in availability of prey species or a new susceptibility of the animals to parasitic infection. We suggest that the absence of *P. inflexus* and *T. convolutus* in this study may indicate that the pseudaliid lungworms *H. invaginatus* and *S. minor* use other fish intermediate hosts or the transmission of these species occurs directly (vertically/horizontally).

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