Pathological findings in 132 Dutch harbour seals (*Phoca vitulina*)

(source: http://en.wikipedia.org/wiki/Harbor_seal)

Veterinary Medicine 12 week research project by Stijn Tegenbosch,
(3260062), (L.A.Tegenbosch@students.uu.nl).
at Utrecht University, division Pathobiology.

Supervised by Jooske IJzer (J.Ijzer@uu.nl).

11-07-2014

Table of contents

Summary	4
Introduction	5
Materials and Methods	6
Results	9
Discussion	20
References	23
Addendum I	27
Addendum II	30

Summary

Between 2009 and 2013 the carcasses of 132 harbour seals (*Phoca vitulina*), originating from the Dutch coast, were investigated for pathological changes and cause of death at the University of Utrecht, division pathobiology. This provided information on the causes of mortality and disease in the population, which can be used to monitor the health status of the population. This is the first study done by the University of Utrecht and the intention is to repeat this study in the future with standardized protocols. So that long term changes and patterns in the populations health status can be recognized.

The seals were either found dead or euthanized in rehabilitation within 24 hours without being given any prior medication. Necropsy was performed in each case. Depending on macroscopical findings and decomposition of the carcass, histopathological, microbiological and parasitological examinations were performed. In this study, the respiratory and alimentary tracts were the organ systems most consistently affected by pathological change, specifically parasitic infections. The most common cause of death was parasitic bronchopneumonia (21%), followed by septicaemia (12%) and physical trauma (5%). Also frequently identified changes included: hyperplasia of lymph nodes (29%), hepatitis (17%), cardiovascular nematodiriasis (16%), bleeding/ haematoma of the skin (15%), alopecia (11%) and lymphadenitis (11%).

Introduction

There are approximately 8000 Harbour seals (*Phoca vitulina*) and 3000 Grey seals (*Halichoerus grypus*) in the Dutch waters.

The harbour seal population in the Wadden Sea declined until the 1970s, due to the impact of hunting (Reijnders, 1983; Heide-Jorgensen and Härkönen, 1988), chemical pollution (Koeman *et al*., 1973; Van Haaften, 1974, 1978; Reijnders, 1980, 1986; Brouwer *et al*., 1989; Siebert *et al*., 1999; Beineke *et al*., 2005; Das *et al*., 2007; 't Hart, 2007), and habitat disturbance (Reijnders, 1983; Thiel *et al*., 1992). After this the management and conservation of the harbour seal was secured by the establishment of the Trilateral Wadden Sea Agreement.

"In the Netherlands, human activity may have an effect on the harbour seal (*Phoca vitulina*) population, because of the densely populated coastline and the Dutch waters are heavily used. Many industrial pollutants from the discharge of several large rivers draining the European hinterland flow into Dutch coastal waters. These factors make it important to investigate seals stranded dead on the Dutch coast for human activity related and other causes of death" (Osinga *et al*., 2012).

Seals that strand on the Dutch coast are mainly harbour seals and grey seals. In this study only the harbour seals are discussed.

Since 1979 post-mortem examinations of seals stranded on the Dutch coast (except the Texel region) were performed by the Seal Rehabilitation and Research Centre (SRRC) (Osinga *et al*., 2012). In 1988 and 2002 phocine distemper virus caused mass mortality among common seals. Studies of stranding and mortality of harbour seals in the Netherlands, apart from the phocine distemper outbreaks, have been done by Osinga *et al*. (2012) until 2008. Outside the Netherlands studies of stranding and mortality of seals in the Wadden Sea have been published in Germany (Schumacher *et al*., 1990; Siebert *et al*., 2007). Outstanding findings in these studies were the increase of parasitic bronchopneumonia and the shift to seals being infected by parasites at a younger age.

Since 2009 necropsies of stranded seals from the Texel region were performed at the University of Utrecht, division pathobiology. In this study, the results of pathological examination of 132 harbour seals examined between 2009 and 2013 are presented. Thus hoping to gain more knowledge of the health status of the harbour seal in the Netherlands. Protocols of post-mortem examination and storage of the results have been standardized by the Utrecht University. So that it should be possible to repeat this study in the future and compare the results to see trends and changes over a longer period which cannot be seen with this study alone.

The purpose of this study is to set a starting point, from where further seal research by the Utrecht University can be related to. We also want to compare our data with the above mentioned earlier studies. We expect to find juvenile animals with parasitic bronchopneumonia.

Materials and Methods

A total of 132 harbour seal carcasses, collected between 2009 and 2013, were examined in this study. The collection and transport of the carcasses was a collaboration of Ecomare (address: Ruijslaan 92, 1796 AZ De Koog), IMARES (address: Landsdiep 4, 1797 SZ Den Hoorn) and the Utrecht University. The carcasses were either found dead along the Dutch coast around the island Texel, or euthanized in within 24 hours of their arrival in rehabilitation due to severe illness and were given no medication prior to euthanasia. The carcasses were then frozen at -20 °C (7 animals were not frozen but examined directly) and transported to the University of Utrecht, division pathobiology, their strandingsdata can be found in Addendum I.

Necropsy examinations were done by pairs of research students (Erik Groeneveld, Eveline Mus, Ivanna Nijenhuis, Lucy v Eldik, Monique Folkerts, Sanne Roozen, Stephanie Wigman), each pair examining about 25 carcasses, under supervision of a pathologist (Jooske IJzer, Rebecca Keesler). All following the same necropsy protocol, see Addendum 2. The carcasses were first cleaned with water to remove excess sand, and then weighed and measured from nose to the tip of the tail. Animals were classified in 4 age classes: neonate, juvenile, sub adult, adult (see criteria in Table 1 (Osinga *et al*., 2012)). The carcasses were examined for external lesions and all organs were examined macroscopically. The nutritional condition code (NCC, see criteria in Table 2) was determined on blubber thickness on the neck and breast. After opening the body cavities and macroscopically examining the state of decomposition of the organs, the decomposition code (DCC, see criteria in Table 3) was classified. Macroscopic evaluation was done by pathologists: Andrea Gröne, Rebecca Keesler, Marja Kik, Guy Grinwis and Jooske IJzer.

Whether or not samples for histological examination were taken depended on the DCC classification. Histological samples were only taken from carcasses with a DCC1 or DCC2. They were collected from the skin, gonad and reproductive tract, urinary bladder, lymph nodes (ileocecale, mesenteric, pre scapular, pulmonary and reproductive tract), spleen, liver, kidney, adrenal, lung, heart, thymus, thyroid, eye, cerebellum, cerebrum and intestine of DCC1 and DCC2 carcasses. Muscle, genital split, mammary gland/penis, placenta, umbilical cord and pancreas were also collected from DCC1 carcasses. Microscopic evaluation was done by pathologists: Andrea Gröne, Rebecca Keesler and Jooske IJzer.

The samples were fixed in 10% formalin and embedded in paraffin wax. Tissue sections were cut (3µm) and stained with haematoxylin and eosin (HE). When appropriate sections were stained by periodic acid-Schiff (PAS) or by Ziehl-Neelsen.

Parasites were fixed in 70% ethanol, and identified by light and binocular microscopy. This was done by parasitologist Herman Cremers.

Incidentally samples were taken for bacteriological examination. These samples were examined by the Veterinary Microbiological Diagnostic Centre (VMDC) (address: Yalelaan 1, 3584 CL Utrecht) of the Utrecht University.

After all examinations a pathological report with the results was created per animal. In these reports were the stranding data, all collected samples and all pathological findings. At the end of each report was a conclusion and the probable cause of death of the animal. Pathological results and causes of death were classified by pathologist Jooske IJzer as showed in Table 7 and the top ten findings were summarized, see Table 8.

Table 1
Age determination criteria (Osinga *et al.*, 2012)

species	sex	category	age (years)	body length (cm)
Phoca vitulina	male	neonate		umbilical cord present
		juvenile	≤ 1	≤ 107
		subadult	$1 \leq 4.7$	$107 \leq 142$
		adult	> 4.7	> 142
	female	neonate		umbilical cord present
		juvenile	≤ 1	≤ 103
		subadult	$1 \leq 3.7$	$103 \leq 129$
		adult	> 3.7	> 129

Table 2
NCC criteria (Jauniaux *et al.*, 2005)

Nutritive condition	Blubber thickness (mm)	External factors and subcutaneous fat
Very well fed		Very good nutritive condition, very well nourished, abundant blubber, significant other subcutaneous fat present in the dorsal neck and sometimes on the lateral thorax, longissimus dorsi and neck are convex. The whole animal makes a "round, barrel-like" body shape.
Well fed		A good nutritive condition, well nourished, abundant blubber, some subcutaneous fat, longissimus dorsi and neck are straight or slightly convex
Normal	> 15	A normal nutritive condition, the blubber thickness is normal, no subcutaneous fat present, neck and longuisimus dorsi are straight, on movement of the animal sometimes slightly convex
Poor	11-15	A bad nutritive condition, the blubber thickness is on the thin side, skin thickness can be increased, neck and longuisimus dorsi are visibly concave
Very poor	< 11	A very bad nutritive condition, the blubber thickness is thin, skin thickness most often increased, longuisimus dorsi and neck are clearly concave.
Emaciated		An extremely bad nutritive condition, severely emaciated, the blubber thickness is very thin, neck an dlonguisimus dorsi are severely concave, the contour of the scapula (especially the spina scapulae) may be visible.

Table 3
DCC criteria (Kuiken *et al*., 1991)

	DCC
1	Very fresh
2	Fresh
3	Putrefied
4	Very putrefied
5	Remains

Results

Sex and age distribution

132 harbour seals were examined between 2009 and 2013 included 55 males and 75 females. The sex of 2 animals could not be determined. 83 seals were juvenile, 12 sub adult, 28 adult, 6 neonate and the age of 3 seals could not be determined (see Table 4).

Table 4
Sex and age distribution

	neonate	juvenile	sub-adult	adult	unknown	total
male	1	38	6	9	1	55
female	5	45	6	19	0	75
unknown	0	0	0	0	2	2
total	6	83	12	28	3	132

General findings

The NCC distribution was: NCC1: 14, NCC2: 16, NCC3: 31, NCC4: 33, NCC5: 17. The nutritional status of 14 animals was undetermined (see Table 5).

The DCC distribution was: DCC1: 7, DCC2: 52, DCC3: 33, DCC4: 36, DCC5: 4 (see Table 6).

Table 5
NCC distribution

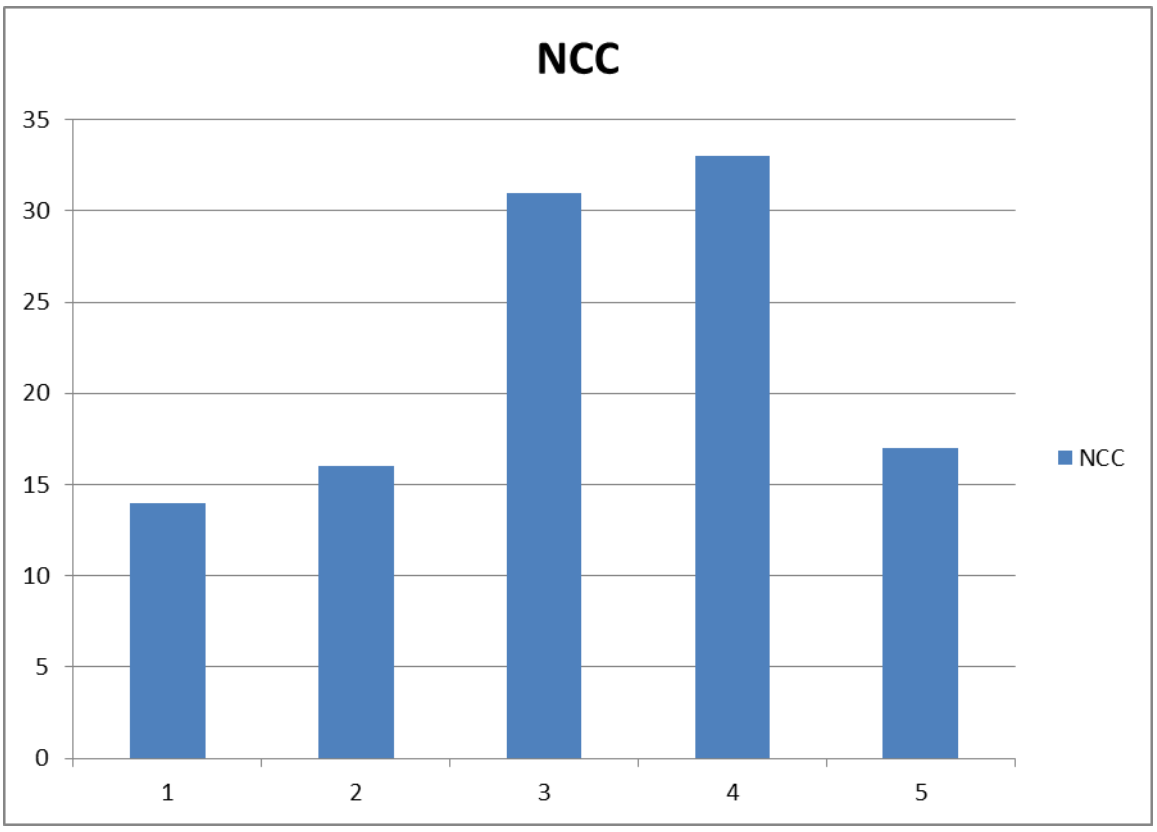
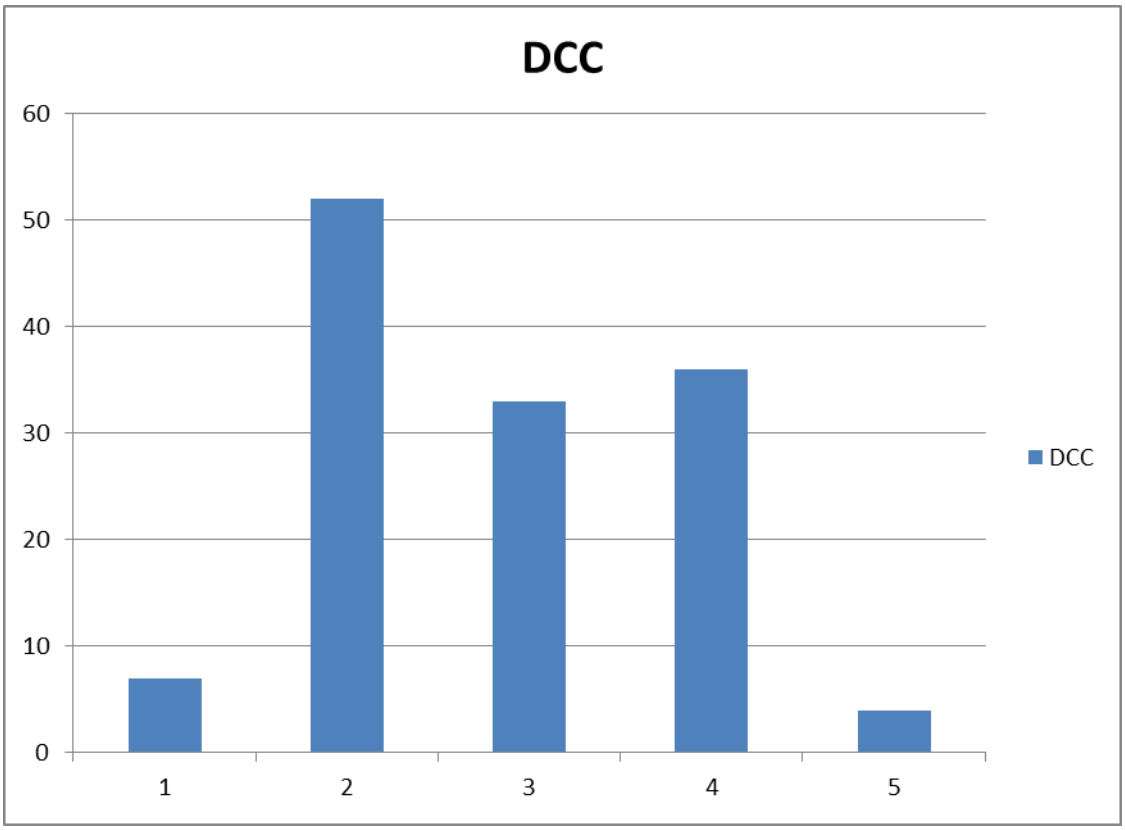


Table 6
DCC distribution



Macroscopical and histopathological findings
Macroscopical and histopathological findings are classified and summarized in Table 7.

Respiratory system
Parasitic infection (*Otostrongylus circumlitis, Parafilaroides gymnurus*) of the bronchial tree was recorded in 51 of 132 seals (39 percent). Parasites in the trachea were found in 13 animals (10 percent) (see figure 1). Parasites had infected the pulmonary blood vessels in 3 animals (2 percent). The majority of parasitized animals was juvenile (90%).
A bronchopneumonia was found in 49 animals (37 percent). Bronchopneumonia was most often found in juvenile animals and was associated with parasitic infection.
Pulmonary haemorrhage, pulmonary rupture and foreign body/food aspiration were also found in small numbers, see Table 7.

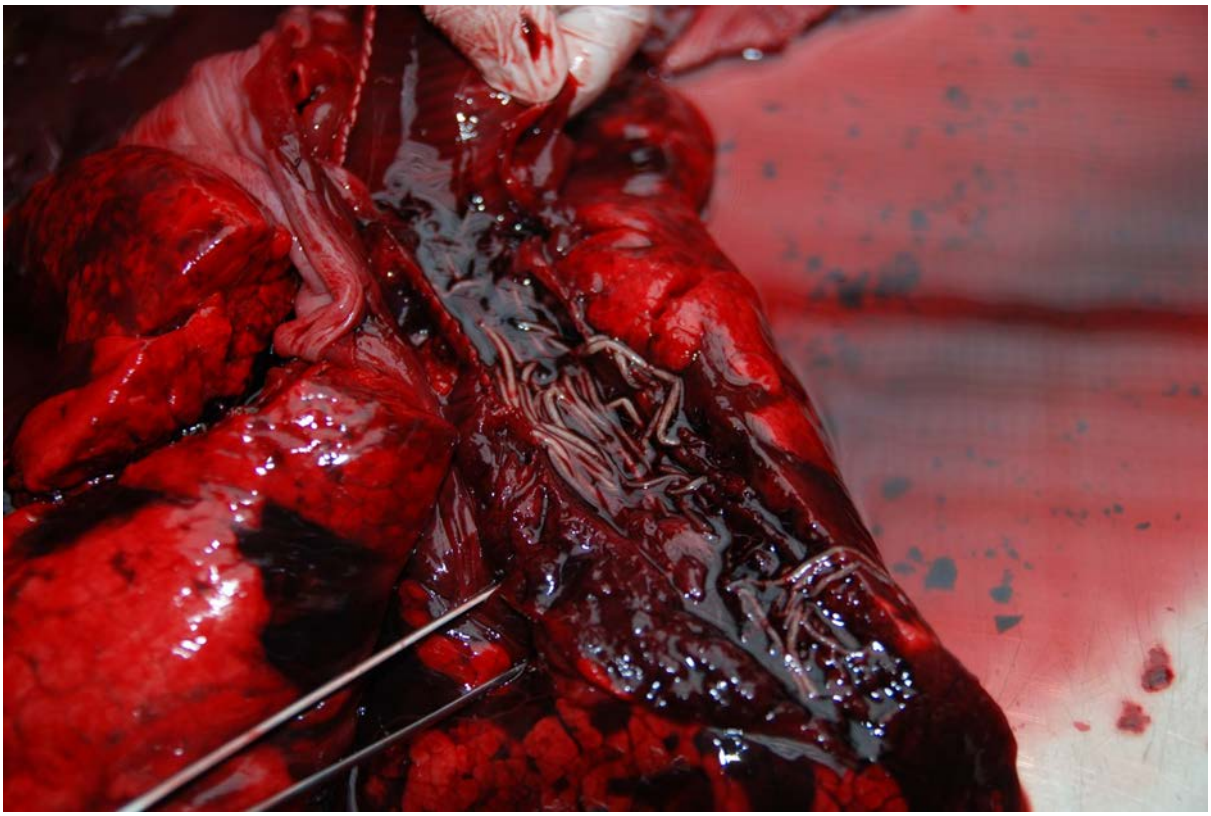


Figure 1. Parasites in trachea of a juvenile male harbour seal.

Cardiovascular system
Parasitic infection (*Acanthocheilonema spirocauda, Otostrongylus circumlitis*) was found in 21 animals (16 percent). Pathological findings in the heart other than nematodiriasis were rare and included the following findings: Myocarditis/epicarditis occurred in 3 seals. Perforation of the heart was found 1 time and was associated with blunt trauma as the animal showed bone fractures and haemorrhages (see figure 2). Myocardial fibrosis was found once. Also one congenital defect was found (persistent foramen ovale).

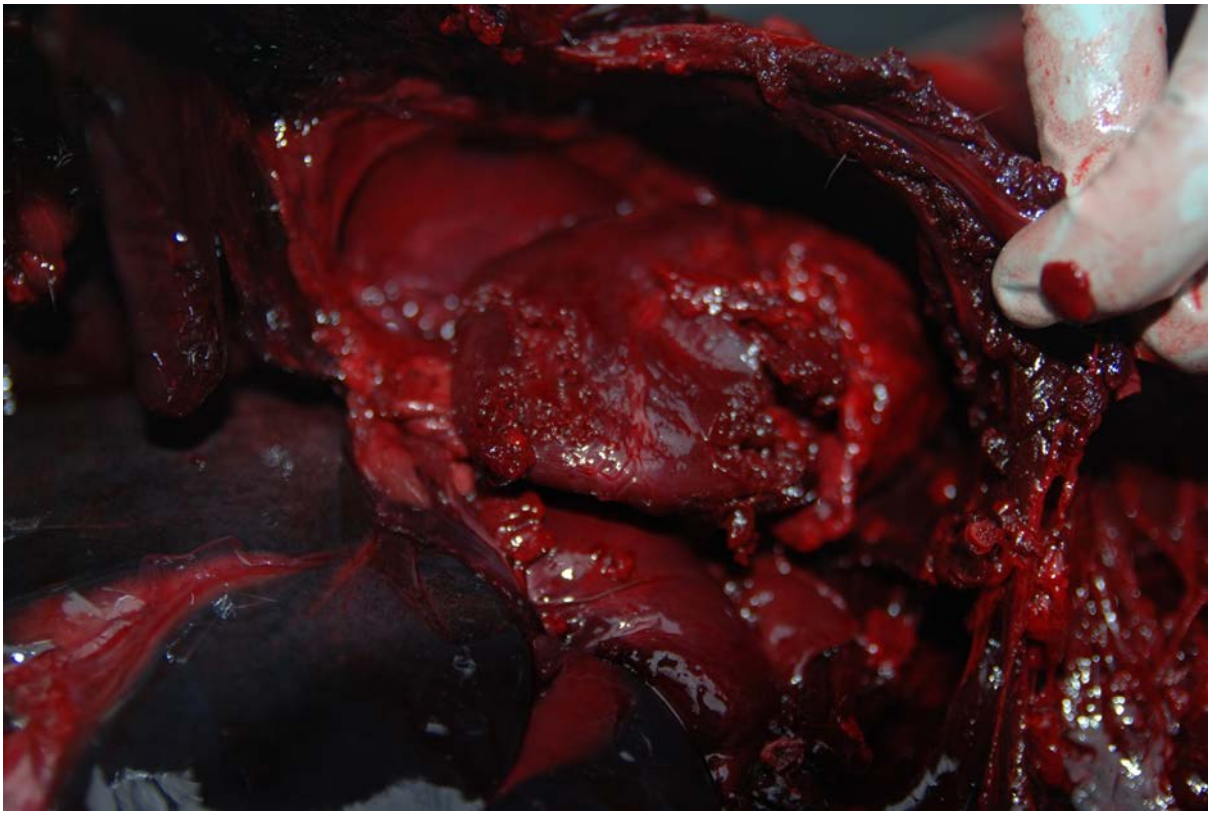


Figure 2. Ruptured heart due to blunt trauma in a juvenile female harbour seal.

Thoracic cavity
Mediastinal emphysema was most often found in the thoracic cavity, 6 seals (5 percent). Haemothorax/liquothorax was found 3 times, but it was sometimes hard to make the difference between primary pathologic fluid or artefact because the carcasses were defrosted, because body fluids leak from the blood vessels and cells after defrosting, due to freezing damage to the cells. Pneumothorax/perforation was found one time and was associated with blunt trauma as this animal showed fractured bones, haemorrhages and a perforated heart.
Mediastinal haemorrhage and pyothorax were also found once.

Abdominal cavity
Haemoabdomen was found 3 times (2 percent). Ascites 3 times and peritonitis once. But just as with the haemothorax/liquothorax these findings are not easy to distinguish from freezing artefacts. Peritonitis was found once.

Alimentary system
Parasitic infection (*Pseudoterranova decipiens, Contracaecum osculatum*) of the stomach was found in animals 52 (39 percent). The presence of parasites was incidentally associated with mild gastritis. Intestinal parasites (*Corynosoma strumosum, Corynosoma semerme*) were found in 32 animals (24 percent). Oesophageal parasites were found in 8 animals (6 percent).

Gastritis was found in 7 animals (5 percent) and was mostly associated with parasites and eosinophilic inflammation.
Enteritis was found in 11 animals (8 percent) and was associated with eosinophilic inflammation and microscopically findings of parasitic larvae, but not with macroscopically finding any parasites.
Hepatitis was found in 22 animals (17 percent), eosinophilic hepatitis was found 10 times and was associated with migrating parasites, which were once identified as microfilariae of *Dipetalonema spirocauda*. One seal had a bacterial hepatitis (*Streptococcus* spp. group G).
Hepatic parasites occurred in 7 animals (5 percent) and was mostly associated with eosinophilic hepatitis. Foreign body was found in 5 animals (4 percent) they were 3 times hooks and line in the stomach (see figure 3), once net/line in the stomach and once sand in the colon.

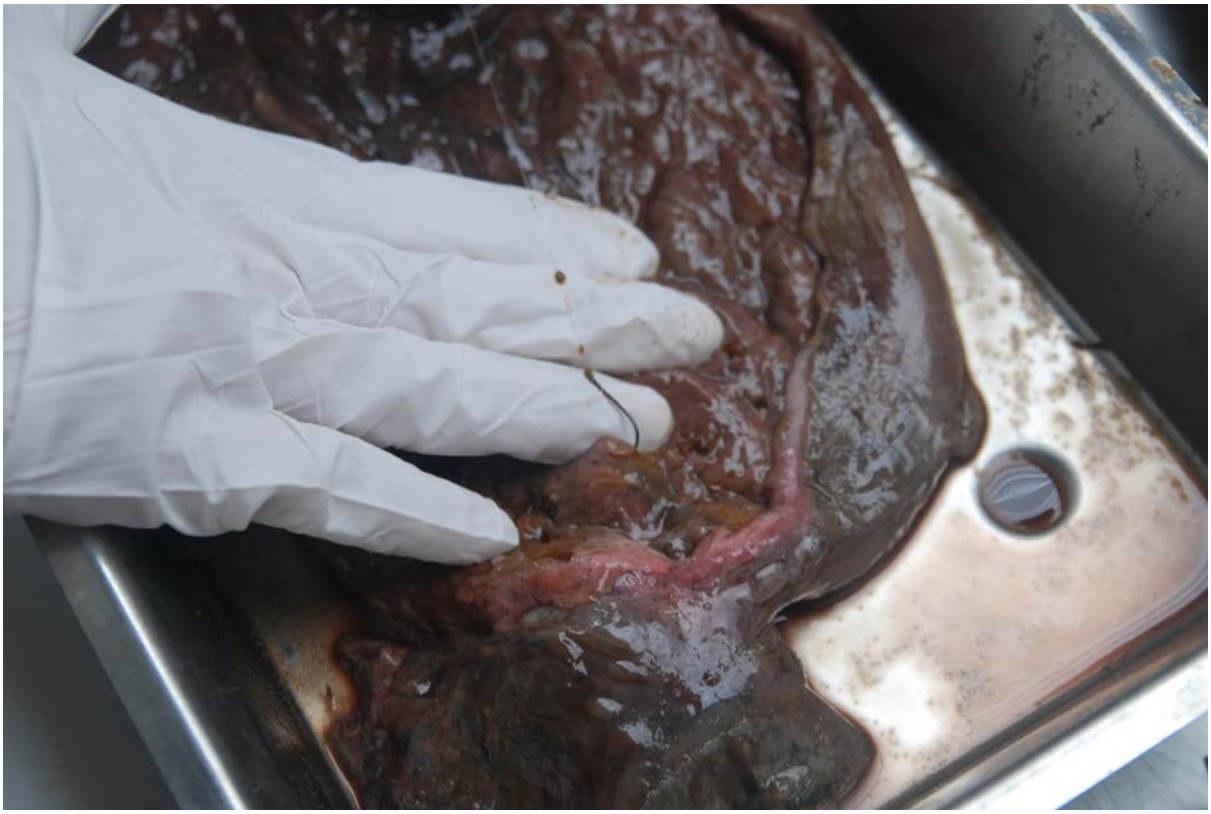


Figure 3. Fish hook in the stomach of a juvenile female harbour seal.

Urinary tract
Nephritis was found 4 times (3 percent) and was mostly neutrophilic.
Haemorrhages in kidney and perirenal haemorrhage was found 2 times and associated with blunt trauma due to the fact that these animals also had fractures and haemorrhages.
Renal tubular necrosis, renal calcification and urinary bladder calculi were all found once.

Genital tract
7 Females were pregnant (5 percent) which was 37% of all stranded adult females.
Endometritis was found in 2 animals, balanophtitis and uterine parasites were found once.

Skin and subcutis
Bleeding/haematoma was found in 20 animals (15 percent) and was found more in male than female seals. Wounds of the skin, single or multiple and of various sizes and shapes were found times 10 (8 percent) and were usually found on the extremities and more in male than female seals(see figure 4).
Dermatitis including focal/multifocal, suppurative, necrotizing, ulcerative, granulomatous or eosinophilic occurred 12 times (9 percent). Alopecia was found 15 times (11 percent) and was found

more in male than female seals. Ectoparasites (*echinophtihirius horridus*, *Demodex*) were found on 5 animals (4 percent).
Scarring was found 3 times and panniculitis 2 times.

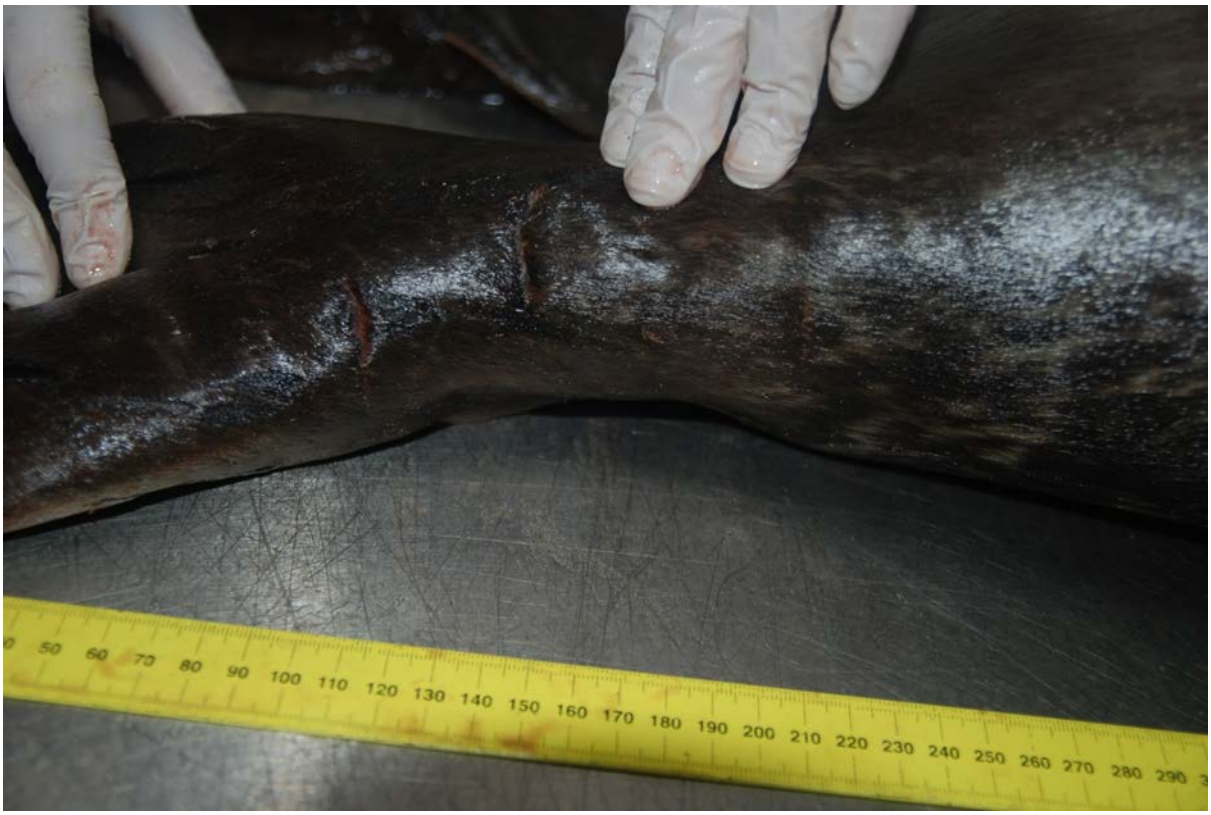


Figure 4. Wounds of the skin on the right tail fin of an adult male harbour seal.

Locomotor system
Fractured skull or skeletal bones were most often found in the locomotor system (8 times, 6 percent) and had often haemorrhages around them. Ostitis/ osteomyelitis, arthritis/ polyarthritis, myositis/ abces and haemorrhages in the muscle all occurred 3 times (2 percent). An altered bone metabolism was found 2 times .

Central nervous system, eye and ear
Meningeal + spinal haemorrhages were found 2 times (2 percent). Haemorrhage in the inner ear was also found 2 times. Meningitis, conjunctivitis and intraocular haemorrhage were found once.

Haematopoietic and endocrine system
Hyperplasia of lymph nodes was by far the most prominent finding (38 times, 29 percent) and was associated with bronchopneumonia, enteritis hepatitis and dermatitis. Lymphadenitis was found 15 times (11 percent) and was associated with the same conditions as hyperplasia of the lymph nodes. Parasites were found in lymph nodes 9 times (7 percent) and were almost always found in the mesenterial lymph node.
Splenic hyperplasia was found in 5 animals (4 percent), splenic haemosiderosis in 3 and splenic congestion in 2.
Adrenalitis occurred 2 times, lipidosis of the adrenal gland and hyperplasia of the adrenal gland once.

Table 7
Pathological findings in 132 harbour seals.

	n =	132	
		number	%
Morphological findings			
Respiratory system			
	Nematodiriasis pulmonary vessels	3	2
	Nematodiriasis trachea	13	10
	Bronchopneumonia	49	37
	Pulmonary rupture	1	1
	Pulmonary hemorrhage	3	2
	Foreign body/ food aspiration	3	2
Cardiovascular system			
	Nematodiriasis	21	16
	Myocarditis + epicarditis	3	2
	Perforation of the heart	1	1
	Myocardial fibrosis	1	1
	Congenital defect	1	1
Thoracic cavity			
	Pneumothorax/ perforation	1	1
	Haemothorax/ liquothorax	3	2
	Mediastinal hemorrhage	1	1
	Mediastinal emfysema	6	5
	Pyothorax	1	1
Abdominal cavity			
	Peritonitis	1	1
	Haemoperitoneum/ haemoabdomen	3	2
	Ascites	3	2

Table 7 (continued)

Alimentary system			
	Broken/ fractured teeth	2	2
	Stomatitis/ glossitis	3	2
	Oesophageal parasites	8	6
	Gastric parasites	52	39
	Gastritis	7	5
	foreign body	5	4
	Intestinal parasites	32	24
	Enteritis	11	8
	Intestinal torsion	2	2
	Hemorrhages/ hemorrhagic infarct	4	3
	Hepatic parasites	7	5
	Hepatitis	22	17
	Periportal fibrosis	1	1
	Proliferation of bile ducts	2	2
	Hepatocellular lipidosis	1	1
	Hepatocellular hemosiderosis	1	1
	Liver rupture	1	1
	Liver congestion	4	3
Urinary tract			
	Nephritis	4	3
	Renal tubular necrosis	1	1
	Renal calcification	1	1
	Hemorrhages in kidney + perirenal	2	2
	Urinary bladder calculi	1	1
Genital tract			
	Uterine parasites	1	1
	Endometritis	2	2
	Pregnancy	7	5
	Balanophtitis	1	1
Skin and subcutis			
	Ectoparasites	5	4
	Dermatitis incl ulcerations	12	9
	Pannuculitis	2	2
	Alopecia	15	11
	Scarring	3	2
	Wounds of the skin	10	8
	Bleeding/ haematoma of the skin	20	15

Table 7 (continued)

Locomotor system			
	Ostitis/ osteomyelitis	3	2
	Arthritis/ polyarthritis	3	2
	Fractured skull or skeletal bones	8	6
	Altered bone metabolism	2	2
	Myositis/ abcess	3	2
	Haemorrhages in the muscles	3	2
Central nervous system, eye, ear			
	Meningeal + spinal haemorrhages	2	2
	Meningitis	1	1
	Conjunctivitis	1	1
	Intraocular haemorrhage	1	1
	Haemorrhage in inner ear	2	2
Haemopoetic and endocrine system			
	Splenic haemosiderosis	3	2
	Splenic hyperplasia	5	4
	Splenic congestion	2	2
	Extramedullary haematopoesis	6	5
	Parasites in Lymphnodes	9	7
	Lymphadenitis	15	11
	Hyperplasia of Lymph nodes	38	29
	Hyperplasia of adrenal gland	1	1
	Lipidosis of adrenal gland	1	1
	Adrenalitis	2	2

Table 8
Top 10 pathological findings in 132 harbour seals.

	Top 10 pathological findings		
		n=132	
		number	%
1	Gastric parasites	52	39
2	Bronchopneumonia	49	37
3	Hyperplasia of lymph nodes	38	29
4	Intestinal parasites	32	24
5	Hepatitis	22	17
6	Cardiovascular nematodiriasis	21	16
7	Bleeding/ haematoma of the skin	20	15
8	Alopecia	15	11
8	Lymphadenitis	15	11
10	Nematodiriasi trachea	13	10

Causes of death

The causes of death are summarized in Table 9. Please note that some animals had more than one cause of death.
Parasitic bronchopneumonia was the most common cause of death (28 cases, 21 percent) and almost all seals (90%) that died from parasitic bronchopneumonia were juvenile.
Septicaemia, including hepatitis, abcessation, generalised lymphadenopathy and polyarthritis, was the second major cause of death for the seals (16 cases, 12 percent). It appeared most often secondary to bronchopneumonia, enteritis, polyarthritis or skin wounds. Due to the state of the carcasses, findings of bacteria were rare.
7 seals (5 percent) were killed by physical trauma as evidenced by the presence of multiple fractures and/or ruptured internal organs and body cavity haemorrhage. Cachexia/emaciation of undermined cause was cause of death for 6 animals (5 percent). Foreign body killed 5 animals (4 percent). Unfortunately it was not possible to determine the cause of death in 65 cases (49 percent).

Table 9
Causes of death in 132 harbour seals.

Causes of death		
	n=132	
	number	%
Parasitic bronchopneumonia	28	21
Pneumonia of undetermined cause/ unknown etiology	2	2
Flipper abcess	2	2
Septicaemia (hepatitis, abcessation, generalised lymphadenopathy, polyarthritis)	16	12
Gastro-enteritis	1	1
Pup starvation	2	2
Cachexia/ emaciation of undetermined cause	6	5
Physical trauma	7	5
Bycatch, confirmed	1	1
Foreign body	5	4
Intestinal torsion	2	2
Other	1	1
Unknown	65	49
NB some animals >1 cause of death		

Discussion

Pathological research on stranded harbour seals gives useful information about the causes of death and presence of diseases in the Dutch seals. There are however some limitations in this research.

In this research only wild animals were used. Wild being defined as stranded dead, or stranded alive and euthanized in rehabilitation within 24 hours, without being given medication. So pathological findings are not influenced by human interference. Also the group of seal carcasses which was examined is not a random sample of the Dutch harbour seal population since they only came from the Texel region. Not all dead seals will be found on the beach and carcasses are not always collected if they aren't fresh enough anymore. This is a problem for all stranding programmes (Eguchi, 2002).

The carcasses were frozen after their finding. This made pathological examination and interpretation very difficult, especially microscopic examination because of cellular destruction. Also while being frozen and defrosted, parts of the carcass were not frozen and autolysis occurred. This makes that at the moment of examination the carcasses are more putrefied than at the moment of finding of the carcass. This gives a worse DCC and makes examination and interpretation harder. This is a reason why findings in other studies, which use fresh carcasses, are not or less found in this study.

The pathological examinations were done by multiple different research students (n=8) under the supervision of multiple pathologists (n=6). However strict protocols were followed, it is possible that this inter-observer variation has had an influence on the results.

Results of post-mortem examinations of rehabilitation seals are not included, results in this group differ from the ones in this research. This has to be taken into account when interpreting these results. For example, seals suffering from chronic disease are more likely to strand alive and to be admitted for rehabilitation than seals with an acute disease (Osinga *et al*., 2012).

The most found cause of death was parasitic bronchopneumonia. The majority of parasitized animals was juvenile. "This may be related to the short lactation period of 4-6 weeks in this species (Ross *et al*., 1994) and a higher pressure on the immune system during the first months after weaning" (Siebert *et al*., 2007). "The seals become infected in with parasites (*Otostrongylus circumlitus* and *Parafilaroides gymnurus*) after weaning when they start to feed on fish that contain larvae of these parasites" (Osinga *et al*., 2012). However in contrast to results from harbour porpoises (Siebert *et al*., 2001; Jepson *et al*., 2005; Lehnert *et al*., 2005) harbour seals appear to suffer more from severe parasitic infections of the lung and associated lesions in the respiratory tract between 1 and 18 months of age (Claussen *et al*., 1991). But this does not correspond with the findings in this study and Osinga and 't Hart (2010), where the majority of parasitized animals was juvenile and less than one year old. "It appears that seals suffer from parasitic bronchopneumonia at a much younger age now compared to previous decades" (Osinga *et al*., 2012). However the age categories in this study and Osinga and 't Hart (2010) are based on standard length, so it is possible that animals aren't always categorized in the right category. "Infections with *P. gymnurus* are considered to be more pathogenic than those with *O. circumlitus* (Vercruysse *et al*., 2003)" (Osinga *et al*., 2012). Possibly parasitic bronchopneumonia is not a cause of death in older animals because they develop active immunity (Claussen *et al*., 1991). Osinga and 't Hart (2010) found a significant increase in parasitic bronchopneumonia in live stranded harbour seals. In 1971-1997 they found 0-30 cases per year to 400 cases of parasitic bronchopneumonia in 2009-2010. The reason for this is not clear but might be related to environmental pollution, which affects the immune system of the seal (Ross *et al*., 1996). The increase of parasitic bronchopneumonia corresponds with the finding in our study, where it was found most frequently.

The second most diagnosed cause of death was septicaemia, including hepatitis, abcessation, generalized lymphadenopathy and polyarthritis. It appeared most often secondary to bronchopneumonia, enteritis, polyarthritis or skin wounds. Findings of bacteria were rare because the carcasses were putrefied, therefore the carcasses were usually contaminated with environment

bacteria. In literature septicaemia in seals is associated with infection by *streptococci*, E. *coli* and C. *perfringens* infections (Siebert *et al*., 2007). Molecular characterization of streptococci found in harbour seals identified the organism as *Streptococcus phocae* (Vossen *et al*., 2004). *Streptococci*, E. *coli* and C. *perfringens* are also reported as pathogenic bacteria from other pinniped species and cetaceans (Dunn *et al*., 2001; Siebert *et al*., 2001; Siebert *et al*.; 2007). Zoonotic bacteria as *Brucella spp.* and *Erysipelothrix rhusiopathiae* are sometimes isolated (Siebert *et al*., 2007). This means pathological examination of harbour seals should always be done as hygienic as possible to prevent zoonotic infection.

Death caused by physical trauma was regularly found (5%). Injuries included fractures, ruptured organs and body cavity haemorrhage. The causes of the injuries were not identified. In literature a number of possibilities are mentioned. A potential cause at the Dutch coast is the dashing of young animals against basalt blocks or dykes (Osinga *et al*., 2012). Other causes are storms and high seas dashing the animals against rocks, collisions with vessels and deliberate killing by man (Baker *et al*., 1998).

Cachexia/ emaciation of undetermined cause was found as a cause of death in five percent of the seals. It is possible that other contributing factors to the death of the animal have been missed due to the state of the carcass. But fish populations are declining in the Dutch waters and fisheries may reduce fish stocks (Nillsen *et al*., 1998) and make it harder for seals to catch enough fish.

Confirmed bycatch was only found once (PV-nr: 152, see Addendum I). This does not correspond with earlier reports in which bycatch was found more than three times a year (Osinga *et al*., 2012; van Haaften, 1982). It does correspond with Siebert *et al*. (2007) who found no confirmed bycatch in 141 examined harbour seals. The true scale of death due to bycatch in the population cannot be determined since dead seal strandings represent an unknown proportion of the total number of seals that die at sea (Osinga *et al*.,2012). The studies with low or none confirmed bycatch used more recent data, so it might be that modern fishing equipment is safer for harbour seals, but this has to be investigated further. However Osinga *et al*. (2012) mention the fact that in their study, none of the confirmed bycatch cases had external evidence of contact with fishing gear. “This is in contrast witch the situation in small cetaceans, in which by-caught animals may show cuts at the edge of mouth, in the skin or tail, and encircling lesions around an extremity (Kuiken, 1996). Possibly this is because seals have tougher skin than cetaceans and their fur masks any subtle lesions” (Osinga *et al*., 2012). Therefore it is possible that very subtle clues are missed.

Another cause of death, linked to human activity, is ingestion of a foreign body. Which was found in 4 percent of the seals. 75% of the foreign bodies were fishing gear. Unfortunately it’s difficult to get fishermen to clean up all their gear and prevent seals from ingesting it.

Causes of death can also be linked indirectly to human activities. For example, “environmental pollution, which affects the immune system of the seal (Ross *et al*., 1996). Human disturbance in pupping areas, which causes separation of pups from their mothers (Doornbos, 1980; Osinga *et al*., 2012). Fossil fuel combustion resulting in climate change, which is associated with advancement of pupping dates (Osinga *et al*., 2011) and the combination of several human activities, which has caused decline and fragmentation of seal populations in north west Europe and rendered them susceptible to epidemics of acute viral disease such as phocine distemper (Rijks, 2008)” (Osinga *et al*., 2012) .

However no “lesions indicating increased exposure to chemical pollutants such as stenosis of occlusion of the uterus, osteoporosis, colonic ulceration and lymphoid depletion of the thymus were found in this study (Bergman and Olsson, 1985; Reijnders, 1986; Schumacher *et al*., 1990; Bäcklin *et al*., 2003). However analogous to harbour porpoises in which higher pollutant burden was associated with higher incidence of infectious diseases (Siebert *et al*., 1999; Das *et al*., 2004; Jepson *et al*., 2005) it remains possible that the high level of parasitic infections in the seals may be related to the effects of chemical pollutants” (Siebert *et al*., 2007). Changes in the immune and endocrine system were described for marine mammals origination from the Wadden Sea (Brouwer *et al*., 1989; Schumacher *et al*., 1993; De Swart *et al*., 1996; Ross *et al*., 1996; Beineke *et al*., 2005; Kakuschke *et al*., 2005; Das *et al*., 2007). More investigation and monitoring are needed to elucidate the impact of chemical

pollutants on seals in the Wadden Sea. During the necropsies, tissue samples have been collected and have been sent to another research facility (IMARES), so the impact of chemical pollutants can be investigated further.

Pup starvation was only found twice, which is in contrast with Osinga *et al*. (2012) who found pup starvation to be the main cause of death in juveniles. But the present results do correspond with Siebert *et al*. (2007) who had no case of pup starvation in 141 examined seals. "Causes of pup starvation include separation from the mother due to human disturbance (Osinga *et al*., 2012) or severe weather conditions (Van Wieren, 1981) and failure to find food in the post-weaning period (Osinga *et al*., 2012). Seal pups suffering starvation are more often found stranded alive than dead" (Osinga *et al*., 2012). This may explain the difference in results, because seals in rehab aren't included in this study.

The most frequently detected pathological finding was gastric parasites. They occurred in 39 percent of the seals. This is less than the 67% reported by Schumacher *et al*. (1990). But that in that study the necropsies were done on seals that had died from Phocine Distemper Virus (PDV). So the data might be influenced by the PDV infection and cannot be compared easily.

Hyperplasia of the lymph nodes was found in 29 percent of the examined seals in this study, and usually occurred in seals with parasitic infection. This does not correspond with Siebert *et al*. (2007), who found lymph node hyperplasia in only 3 percent of 141 examined harbour seals. The criteria for lymph node hyperplasia were not publicized in this study, but it is likely that their criteria were different from the ones in our study, and that it caused the difference in results.

Scarring, alopecia and wounds of the skin were regularly found and more in male than in female seals and mostly on the extremities, possibly caused by fighting. Skin wounds are reported in other literature (Zimmerman and Nebel, 1975, Siebert *et al*., 2007) but were not described by Schumacher *et al*. (1990).

Intestinal volvulus in harbour seals has been reported regularly (Siebert *et al*., 2007; Ulloa *et al*., 2002), but was only found twice in this study, the reason for this is not clear.

This study is beneficial because we now know the most important causes of death and disease in Dutch harbour seals. And it is possible to monitor their health status for further research and environmental management.

Parasitic bronchopneumonia is the most found cause of death. "The underlying causes of the increasing frequency of this disease and the apparent shift to younger age of infection need to be investigated" (Osinga *et al*., 2012). "Also human activities as recreation and offshore construction may still be a threat to the marine ecosystem of the harbour seal (Reijnders *et al*., 2005.). Therefore, monitoring of the health status of seals should continue" (Siebert *et al*., 2007).

In this study no statistical analysis was performed on the data. This is because there is no earlier data which has been collected and stored in the same way, with the same protocols as in this study. And also because the amount of time of this project was to short. If, in the future, more harbour seals have been pathologically examined with these standardised protocols, it is possible to perform statistical analysis of those data to the ones in this study. This will help to find patterns of findings over time and this will better help protecting the harbour seal population in the Dutch and other European waters.

Parasitic bronchopneumonia was the most frequently found cause of death in this study, there seems to be a shift in the age of infected animals, because most animals infected now are juveniles. The reason for this is not clear and should be investigated further. Possibly when this study is repeated in the future.

References

B. **Bäcklin**, M.L. Eriksson, M. Olovsson, Histology of uterine leiomyoma and occurrence in relation to reproductive activity in the Baltic Gray Seal (Halichoerus grypus), *Veterinary Pathology*, 40 **(2003)**, pp. 175–180

J.R. **Baker**, P.D. Jepson, V.R. Simpson, T. Kuiken, Causes of mortality and non-fatal conditions among grey seals (Halichoerus grypus) found dead on the coasts of England, Wales and the Isle of Man, *Veterinary Record*, 142 **(1998)**, pp. 595–601

A. **Beineke**, U. Siebert, M. MacLachlan, R. Bruhn, K. Thron, K. Failing, G. Müller, W. Baumgärtner, Investigations upon the potential influence of environmental contaminants on thymus and spleen of harbor porpoises (Phocoena phocoena), *Environmental Science and Technology*, 39 **(2005)**, pp. 3933–3938

A. **Bergman**, and Olsson, M.. Pathology of Baltic grey seal and ringed seal females with special reference to adrenocortical hyperplasia: is environmental pollution the cause of a widely distributed disease syndrome? *Finnish Game Research*, 44 **(1985)**, pp. 47–62

A. **Brouwer**, P.J.H. Reijnders, J.H. Koeman, Polychlorinated biphenyl (PCB) contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal (Phoca vitulina), *Aquatic Toxicology*, 15 **(1989)**, pp. 99–106

D. **Claussen**, V. Strauss, S. Ising, M. Jäger, T. Schnieder, M. Stoye, The helminth fauna of the common seal (Phoca vitulina vitulina, Linne, 1758) of the Wadden Sea in lower Saxony, *Journal of Veterinary Medicine*, 38 **(1991)**, pp. 649–656

K. **Das**, U. Siebert, M. Fontaine, T. Jauniaux, L. Holsbeek, J.M. Bouquegneau, Ecological and pathological factors related to trace metal concentrations in harbour porpoises (Phocoena phocoena) from the North Sea and adjacent areas, *Marine Ecology Progress Series*, 281 **(2004)**, pp. 283–295

K. **Das**, Vossen, A., Tolley, K., Vikingsson, G., Thron, K., Müller, G., Baumgärtner, W. and Siebert, U. Interfollicular fibrosis in the thyroid gland of the harbour porpoise: an endocrine disruption? *Archive of Toxicology and Environmental Contamination*, **(2007)** in press.

R.L. **De Swart**, P.S. Ross, J.G. Vos, A.D.M.E. Osterhaus, Impaired immunity in harbour seals (Phoca vitulina) exposed to bioaccumulated environmental contaminants: review of a long-term feeding study, *Environmental Health Perspectives*, 104 (Suppl. 4) **(1996)**, pp. 823–828

G. **Doornbos**, Gedrag van zeehonden (Phoca vitulina L.) in het stroomgebied van de oude Lauwers (oostelijke Waddenzee) in 1978, *Rijksinstituut voor Natuurbeheer*, Texel **(1980)** p. 24

J.L. **Dunn**, Buck, J.D. and Robeck, T.R.. Bacterial diseases of cetaceans and pinnipeds. *In: Handbook of Marine Mammal Medicine*, L.A. Dierauf and F.M.D. Gulland, Eds, CRC Press, Florida, **(2001)** pp. 309–335.

T. **Eguchi,** A method for calculating the effect of a die-off from stranding data, *Marine Mammal Science*, 18 **(2002)**, pp. 698–709

M.P. **Heide-Jørgensen**, T.J. Härkönen, Rebuilding seal stocks in the Kattegat-Skagerrak, *Marine Mammal Science*, 4 **(1988)**, pp. 79–111

T. **Jauniaux**, Beans, C., Dabin, W. Stranding, necropsy and sampling: collection data, sampling level and techniques. *Student European Cetacean Society workshop*, **(2005)**.

P.D. **Jepson**, P.M. Bennett, R. Deaville, C.R. Allchin, J.R. Baker, R.J. Law, Relationships between polychlorinated biphenyls and health status in harbor porpoises (Phocoena phocoena) stranded in the United Kingdom, *Environmental Toxicology and Chemistry*, 24 **(2005)**, pp. 238–248

A. **Kakuschke**, E. Valentine-Thon, S. Griesel, S. Fonfara, U. Siebert, A. Prange, Immunological impact of metals in harbor seals (Phoca vitulina) of the North Sea, *Environmental Science and Technology*, 39 **(2005)**, pp. 7568–7575

J.H. **Koeman**, W.H.M. Peeters, C.H.M. Koudstaal-Hol, P.S. Tjioe, J.J.M. De Goeij, Mercury–selenium correlations in marine mammals, *Nature*, 245 **(1973)**, pp. 385–386

T. **Kuiken**, Review of the criteria for the diagnosis of by-catch in cetaceans, *European Cetacean Society Newsletter*, 26 **(1996)**, pp. 38–43

K. **Lehnert**, J.A. Raga, U. Siebert, Macroparasites in stranded and bycaught harbour porpoises (Phocoena phocoena) from German and Norwegian waters, *Diseases of Aquatic Organisms*, 64 **(2005)**, pp. 265–269

K.T. **Nilssen**, T. Haug, T. Øritsland, L. Lindblom, S.A. Kjellqwist, Invasions of harp seals Phoca groenlandica Erxleben to coastal waters of Norway in 1995: ecological and demographic implications, *Sarsia*, 83 **(1998)**, pp. 337–345

N. **Osinga**, I. Pen, H.A. Udo de Haes, P.M. Brakefield, Evidence for a progressively earlier pupping season of the common seal (Phoca vitulina) in the Wadden Sea, *Journal of the Marine Biological Association of the United Kingdom* **(2011)**

N. **Osinga**, M.M. Shahi Ferdous, D. Morick, M. García Hartmann, J.A. Ulloa, L. Vedder, H.A. Udo de Haes, P.M. Brakefield, A.D.M.E. Osterhaus, T. Kuiken, Patterns of Stranding and Mortality in Common Seals (Phoca vitulina) and Grey Seals (Halichoerus grypus) in The Netherlands between 1979 and 2008, *Journal of Comparative Pathology*, 147 **(2012)**, pp. 550-565

N. **Osinga**, P. 't Hart, Harbour seals (Phoca vitulina) and rehabilitation, *NAMMCO Scientific Publications*, 8 **(2010)**, pp. 355–372

P.J.H. **Reijnders**, Organochlorine and heavy metal residues in harbour seals from the Wadden Sea and their possible effects on reproduction, *Netherlands Journal of Sea Research*, 14 **(1980)**, pp. 30–65

P.J.H. **Reijnders**, The effect of hunting on the further existence of a harbour seal population in the Dutch Wadden Sea, *Zeitschrift für Säugetierkunde*, 48 **(1983),** pp. 50–54

P.J.H. **Reijnders**, Reproductive failure in common seals feeding on fish from polluted coastal waters, *Nature*, 324 **(1986)**, pp. 456–457

R.J.H. **Reijnders,** Abt, K.F., Brasseur, S.M.J.M., Camphuysen, K.C.J., Reineking, B., Scheidat, M., Siebert, U., Stede, M., Tougaard, J. and Tougaard, S. Marine Mammals. In: Wadden Sea Quality Status Report 2004. Wadden Sea Ecosystem No. 19. K. Essink, C. Dettmann, H. Farke, K. Laursen, G. Lürßen, H. Marencic and W. Wiersinga, Eds, *Trilateral Monitoring and Assessment Group, Common Wadden Sea Secretariat, Wilhelmshaven, Germany*, **(2005)**, pp. 305–318.

J.M. **Rijks**, Phocine Distemper Revisited: Multidisciplinary Analysis of the 2002 Phocine Distemper Virus Epidemic in the Netherlands, *PhD thesis, Erasmus Medical Centre, Rotterdam*, **(2008)** pp. 192.

P.S. **Ross**, R.L. de Swart, I.K.G. Visser, L.J. Vedder, W. Murk, W.D. Bowen, A.D.M.E. Osterhaus, Relative immunocompetence of the newborn harbour seal, Phoca vitulina, *Veterinary Immunology and Immunopathology,* 42 **(1994)**, pp. 331–348

P.S. **Ross**, R.L. De Swart, H.H. Timmerman, P.J.H. Reijnders, J.G. Vos et al., Suppression of natural killer cell activity in harbour seals (Phoca vitulina) fed Baltic Sea herring, *Aquatic Toxicology*, 34 **(1996)**, pp. 71–84

U. **Schumacher**, H.P. Horny, G. Heidemann, W. Schultz, U. Welsch, Histopathological findings in harbour seals (Phoca vitulina) found dead on the German North Sea coast, *Journal of Comparative Pathology*, 102 **(1990)**, pp. 299–309

U. **Schumacher**, S. Zahler, H.P. Horney, G. Heidemann, K. Skrinisson, U. Welsch, Histological investigation on the thyroid glands of marine mammals (Phoca vitulina, Phocoena phocoena) and the possible implication of marine pollution, *Journal of Wildlife Diseases*, 29 **(1993)**, pp. 103–108

U. **Siebert**, C. Joiris, L. Holsbeek, H. Benke, K. Failing, K. Frese, E. Petzinger, Potential relationship between mercury concentrations and necropsy findings in cetaceans from German waters of the North and Baltic Seas, *Marine Pollution Bulletin*, 38 **(1999)**, pp. 285–295

U. **Siebert**, A. Wünschmann, R. Weiss, H. Frank, H. Benke, K. Frese, Post-mortem findings in harbour porpoises (Phocoena phocoena) from the German North and Baltic Seas, *Journal of Comparative Pathology*, 124 **(2001)**, pp. 102–114

U. **Siebert**, P. Wohlsein, K. Lehnert, W. Baumgärtner, Pathological Findings in Harbour Seals (Phoca vitulina): 1996–2005, *Journal of Comparative Pathology*, 137 **(2007)**, pp. 47-58

P. 't Hart, Zeehondenjacht in Nederland 1591–1962, *PhD thesis, Vrije universiteit Amsterdam*, **(2007)** pp. 351

M. **Thiel**, G. Nehls, S. Bräger, J. Meissner, The impact of boating on the distribution of seals and moulting ducks in the Wadden Sea of Schleswig-Holstein, *Netherlands Institute of Sea Research Publication Series*, 20 **(1992)**, pp. 221–233

A. **Ulloa**, J. Van der Kamp, L. Vedder, T. Kuiken, Epidemiology of intestinal volvulus in harbour and grey seals, *16th European Cetacean Society Conference 'Marine Mammals Health: from Individuals to Populations'*, **(2002)** Liege, Belgium

J.L. **Van Haaften**, Zeehonden langs de Nederlandse kust, *Wetenschappelijke Mededelingen KNNV*, 101 **(1974)**, pp. 1–35

J.L. **Van Haaften**, De Waddenzee-zeehonden in 1977, *Waddenbulletin*, 13 **(1978)**, pp. 98–499

J.L. **Van Haaften**, Sectiebevindingen bij de in de natuur gestorven zeehonden, *Tijdschrift voor Diergeneeskunde*, 107 **(1982)**, pp. 379–383

S.E. **Van Wieren**, Broedbiologie van de gewone zeehond, Phoca vitulina, in het Nederlandse Waddengebied, *Rijksinstituut voor Natuurbeheer*, Texel **(1981)**

J. **Vercruysse**, A. Salomez, A. Ulloa, M. Alvinerie, A. Osterhaus et al., Efficacy of ivermectin and moxidectin against Otostrongylus circumlitus and Parafilaroides gymnurus in harbour seals (Phoca vitulina), *Veterinary Record*, 152 **(2003)**, pp. 130–134

A. **Vossen**, A. Abdulmawjood, C. Lämmler, R. Weiß, U. Siebert, Identification and molecular characterization of beta-hemolytic streptococci isolated from harbor seals (Phoca vitulina) and grey seals (Halichoerus grypus) of the North and Baltic seas, *Journal of Clinical Microbiology*, 42 **(2004)**, pp. 469–473

T. **Zimmermann**, W. Nebel, Über Erkrankungen von Seehunden aus dem Gebiet der nordfriesischen Küste, *Deutsche Tierärztliche Wochenschrift*, 82 **(1975)**, pp. 221–260

Addendum I
Strandingsdata of 132 used harbour seals.

PV nr	Glims nr	sex	age	stranded	location
1	3090514051	f	s	22-2-2009	Texel paal 23
2	3090514052	m	s	20-2-2009	Texel paal 20
4	3090630003	f	j	22-5-2009	Texel paal 21
5	3090630004	m	s	16-4-2009	Helling haringhaven Ijmuiden
13	3100819049	f	j	24-6-2009	Texel, Oudeschild
14	3101228024	f	j	unknown	unknown
15	3110601042	m	j	11-1-2011	Texel paal 31
16	3100616045	f	a	18-5-2010	Schoorl aan zee
17	3100616046	m	s	10-5-2010	unknown
18	3100616047	m	j	4-12-2009	Texel, De Hors
19	3100616048	f	j	31-10-2009	Texel, Noord-slufter
20	3100616049	f	j	11-4-2010	Texel paal 6
21	3100616050	m	j	10-4-2010	Texel paal 7
23	3100616052	m	j	26-10-2009	Texel paal 15.2
24	3100616053	f	j	24-12-2009	Texel paal 8
25	3100616054	f	j	19-3-2010	Norddeig
26	3100616055	f	j	2-11-2009	Egmond aan zee
27	3100616056	m	j	7-8-2009	Texel paal 17
28	3100616057	m	j	31-8-2009	Texel paal 25.4
30	3100616059	f	j	13-2-2009	Schoorl,Hondsbosche zeewering, KM 24
32	3100616061	f	j	9-10-2009	Texel paal 26.5
33	3100616062	f	j	16-5-2010	Groote Keeten km 11
34	3100616063	f	a	2-1-2010	Texel paal 28
35	3100616064	f	a	11-1-2010	Texel paal 33
36	3100616065	f	a	12-4-2010	Texel, Ijzeren kaap
37	3110314039	m	j	3-9-2010	Julianadorp paal 13
38	3110314043	m	j	26-7-2010	Texel paal 30
39	3110314038	f	n	17-6-2010	unknown
40	3110314040	m	j	11-8-2010	Groote Keeten
41	3120516028	m	a	31-8-2010	Texel, Cocksdorp
42	3110614042	f	j	unknown	unknown
44	3110429034	f	j	18-6-2010	Den Helder
45	3110429035	f	s	11-6-2010	Texel paal 20
46	3110429036	m	j	10-12-2010	Texel paal 11
47	3110429037	m	j	21-5-2010	Texel paal 12
49	3110429039	m	s	5-6-2010	Texel, Haven oudeschild
51	3110429041	f	j	17-12-2010	Texel paal 22
52	3110601043	f	a	18-1-2011	Texel, Vuurtorenstrand
54	3110601045	m	j	12-10-2010	Den Helder paal 5
56	3110601047	m	j	17-12-2010	Texel paal 16
58	3110601049	f	j	11-12-2011	Schoorl paal 15
59	3110601050	f	j	26-2-2011	Texel paal 21

60	3110601051	f	j	5-12-2010	Texel paal 23.4
61	3110601052	m	j	22-12-2010	Bergen aan zee
62	3110601053	f	j	12-12-2010	Den Helder paal 3
63	3110601054	f	j	13-3-2011	Texel paal 12
64	3110601055	m	j	7-3-2011	Texel, De hors
65	3110601056	f	j	25-2-2011	Hargen paal 27.250
66	3110601057	m	j	1-11-2010	Texel paal 33
67	3110601058	m	j	22-12-2010	Den Helder
68	3110601059	f	j	3-1-2011	Zwanenwater
69	3110601060	f	j	23-3-2011	Texel paal 17
70	3110601061	f	j	2-1-2011	Petten km 20
71	3110601062	m	j	8-1-2011	Texel, Mokbaai
72	3110601063	m	j	1-11-2010	Groote Keeten km 10
73	3110601064	f	a	4-1-2011	Texel paal 9
75	3110621037	f	a	13-9-2010	Texel, Vuurtorenstrand
76	3110621038	m	j	24-10-2010	Camperduin km 26
77	3111123001	m	j	20-9-2011	Den Helder
78	3111216004	f	a	14-12-2011	Texel paal 18
81	3120105057	f	a	18-11-2011	Texel, Havenkantoor
82	3120105058	f	a	16-11-2011	Den Oever, Zuidermeerhaven
85	3120112004	m	a	17-12-2011	Texel paal 31
86	3120112006	f	a	21-21-2011	Huisduinen
87	3120112009	f	j	17-12-2011	Texel, De Slufter
88	3120112011	m	j	26-12-2011	Petten paal 19
92	3120126048	f	a	17-1-2012	Texel, Ijzeren kaap
94	3120126050	m	a	20-1-2012	Bergen aan zee
96	3120126053	f	s	19-1-2012	Callantsoog km 13
99	3120126056	f	j	19-1-2012	Texel paal 34
103	3120131045	f	j	30-1-2012	Texel paal 28/29
109	3120427031	f	a	22-4-2012	Ijsselmeer bij Andijk
110	3120525033	f	j	3-7-2011	Zwanenwater km 14
111	3120525036	f	s	4-5-2011	Texel, Dijkmanshuizen
112	3120525040	f	a	2-5-2011	Texel paal 34
114	3120601043	f	s	4-5-2011	Texel, Volharding
115	3120525042	m	j	14-7-2011	Texel, Cocksdorp
116	3120525043	m	a	unknown	Texel paal 13
117	3120525044	m	n	13-7-2011	Texel paal 20
118	3120601042	m	j	16-7-2011	Texel, Cocksdorp
119	3120601045	m	j	31-7-2011	Coog, km 12.250
121	3120601048	m	j	25-6-2011	Texel paal 28
123	3120601050	f	n	5-6-2011	Texel paal 26
124	3120601051	f	j	21-7-2011	Den Helder, Marinehaven
126	3120608052	f	j	29-3-2012	Texel paal 12
132	3120608062	f	j	4-2-2012	Slufter paal 26.400
133	3120608063	f	j	22-9-2011	Texel paal 29

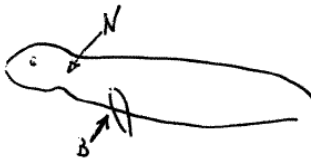
136	3120608069	m	a	8-4-2012	Texel, Zeeburg
137	3120914043	f	s	1-4-2012	Texel paal 18
138	3120914048	f	j	24-5-2012	Texel, Vuurtorenstrand
141	3120914052	m	unknown	26-6-2012	Texel paal 20.5
142	3120914053	m	j	5-6-2012	Texel paal 28
143	3120920001	f	j	18-9-2012	Camperduin km 26
144	3120924056	f	j	19-3-2012	Texel paal 9.6
148	3120924060	f	n	4-7-2012	Texel, Ijzeren kaap
149	3120924061	f	n	4-7-2012	Julianadorp
151	3120924062	f	j	9-7-2012	Wieringen
152	3120927046	f	j	7-11-2007	Texel, Nioz fuik
154	3121005022	m	s	23-10-2010	Texel, Vlieland boulevard
155	3121005024	m	s	4-11-2010	Groote Keeten km 9
160	3130204036	m	j	24-1-2013	Schoorl km 29
161	3130312015	m	j	24-10-2012	Groote Keeten km 10
162	3130312017	f	a	27-10-2012	Vlieland, Nioz Texel
163	3130312019	f	j	8-11-2012	Texel paal 14
164	3130312020	unkr	unknown	29-11-2012	Texel paal 9.7
165	3130312021	f	j	20-12-2012	Schoorl km 30
166	3130312022	unkr	unknown	28-10-2012	Texel paal 28
167	3130312023	m	j	5-11-2012	Den Helder
168	3130801037	f	n	unknown	Serooskerke (Schelphoek)
169	3131014008	m	j	19-032013	Slufter naar Krim
170	3131014010	m	j	18-2-2013	Waddenstrand
171	3131014013	f	a	25-4-2013	Julianadorp km 8
172	3131014015	m	a	31-3-2013	Schorren paal 22.2
173	3131014017	f	j	3-5-2013	Texel, Oudeschild, dijk
174	3131014019	m	j	16-4-2013	Keele km 10
175	3131014020	m	j	25-3-2013	unknown
177	3131018021	f	j	13-12-3012	Texel paal 17
178	3131018024	m	j	30-1-2013	Texel paal 21
179	3131018025	f	a	17-122013	De Hors, De Mok
180	3131018026	f	a	13-1-2013	Texel, Nioz haven
181	3131018027	f	a	13--01-2013	Texel, Krassekeet
182	3131018028	f	a	14-1-2013	Texel, Oudeschild
183	3131028007	f	j	15-8-2013	Ijmuiden, Middensluis
184	3131028008	m	a	11-9-2013	Den Helder paal 0
185	3131028009	m	a	7-9-2013	Texel, Nioz haven
186	3131028010	f	j	1-7-2013	Walsoorden
188	3131028012	m	j	14-7-2013	Razende Bol
189	3131028013	m	j	15-7-2013	Texel, Oudeschild, jachthaven
190	3131028014	m	j	2-9-2013	Texel paal 125.4
192	3131028016	f	j	1-9-2013	Texel paal 28
193	3131028017	f	j	10-7-2013	Texel, Volharding
194	3131120027	m	j	7-11-2012	Texel paal 28

Addendum II

Seal necropsy protocol

Record forms SEAL Necropsies

Part 1 Identification	Number	.....	GLIMS	.....
	Stranding date:			
	Autopsy date:			
	Autopsied by:			
Chip check[1]:				
□ yes / □ no negative / positive	True location:	.....	NSO	.....
	Provided by:	□ EHBZ □ EcoMare □ Other		



thickness

Diagram 1 – blubber (including skin)

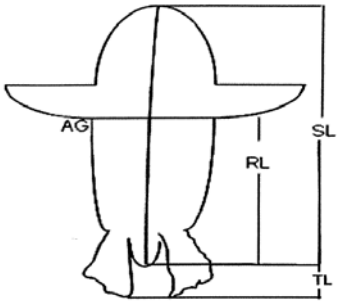


Diagram 2 - morphometry

Part 2 Biometrics	Morphometry (see diagrams above)	Blubber thickness **neck** (N)..... mm Blubber thickness **breast** (B)..... mm	**TL**.....cm **SL**.....cm **RL**.....cm **AG** (axillary girth).....cm
Sex:	□ ♂ □ ♀ (certain / uncertain) □ sex unknown	♂ large anogenital distance ♀ vulva located just ventral to anus	
Body mass:	.....kg	yes/ almost / no	
Nutritive condition code:	□NCC**1** □NCC**2** □NCC**3** □NCC**4** □NCC**5** □ NCC**6** □ unknown		
Storage:	□ Direct delivery □ Cooled (ca. ……hrs) □ Frozen		
Expected age:	□ Neonate □ Juvenile □ Adult □ Unknown		
Decomposition DCC:	□ Very fresh DCC**1** □ Fresh DCC**2** □ Putrefied DCC**3** □ Very putrefied DCC**4** □ Remains DCC**5**		

State of carcass:	□ fully intact □ peck or bite wounds □ incomplete □ skeletal parts, namely: .....
Bycatch: (based on external observation only)	□ certain □ highly probable □ probable □ possible □ no evidence □ unknown **Only wildlife**
Part 2 Photography	
Entire body	
Head only	
Snout	
Eyes	
Teeth	
Urogenital region	
External Observations (Specify lesion and location)	
Internal observations (Specify organ)	

Only in Wildlife!

Estimated significance of the presence/absence of criteria for the diagnosis of bycatch			
Criteria	**Presence**	**Absence**	**Observed**
1. Health state			**yes ? no**
A. Exclusion of other causes of death	**+**	**--**	□ □ □
B. Good nutritional condition	**+**	**-**	□ □ □
C. Evidence of recent feeding	**+**	**0**	□ □ □
2. Contact with fishing gear			
A. Superficial skin lesions			**yes ? no**
1. cuts in edge of mouth, fin or tail	**++**	**0**	□ □ □
2. encircling lesions around extremity	**++**	**0**	□ □ □
B. Bruises	**+**	**0**	□ □ □
C. Skull fractures	**+**	**0**	□ □ □
3. Lack of oxygen (hypoxia)			**yes ? no**
A. Oedematous lungs	**+**	**-**	□ □ □
B. Persistent froth in the airways	**+**	**-**	□ □ □
C. Bullous emphysema in the lungs	**+**	**0**	□ □ □
D. Epicardial and pleural petechiae	**+**	**0**	□ □ □
4. Damage during release of the net			**yes ? no**
A. Amputated fin, fluke or tail	**++**	**0**	□ □ □
B. Penetrating incision into body cavity	**++**	**0**	□ □ □
C. Rope around tail stock	**++**	**0**	□ □ □
D. Gaff mark	**++**	**0**	□ □ □
5. Other relevant characteristics			**yes ? no**
A. Sharp edged cuts or blubber defects on body	**++**	**0**	□ □ □
B. Sharp edged cuts or blubber defects on mandible	**++**	**0**	□ □ □
			□ □ □

++ consistent with bycatch + bycatch possible 0 no significance for diagnosis - bycatch less likely -- bycatch unlikely

[1]Kuiken T. 1994. Review of the criteria for the diagnosis of by-catch in cetaceans. *In:* Kuiken T. (ed.) Diagnosis of By-Catch in Cetaceans. Proc. 2nd. ECS workshop on cetacean pathology, Montpellier, France, 2 March 1994. European Cetacean Society Newsletter 26: 38-43

Part 3 Pathology	Number	.....	GLIMS	.....
Necropsy form – 1				
External observations & lesions				
□ Scavenging	□ Severe □ Moderate □ Mild □ None			
Subcutaneous observations & lesions				
□ Sub cut.fat	□ Absent □ Present, approximate thickness: ..... □ Unknown			

Part 3 Pathology	Number	.....	GLIMS	.....
Necropsy form - 2				

Internal observations & lesions

Abdomen (tick if normal, describe if abnormal) □ Urinary bladder □ Mesenteric LN □ Intestine □ Stomach □ Spleen □ Pancreas □ Liver □ Adrenal □ Kidney □ Genital tract □ Gonads	Sex □ ♂ □ ♀ □ ND Age □ Neonatal □ Juvenile □ Adult □ Undetermined

Thorax (tick if normal, describe if abnormal) □ Trachea □ Lungs □ Bronchial LN □ Heart □ Oesophagus □ Thymus (present/absent)	

Part 3 Pathology	Number	.....	GLIMS	.....
Necropsy form - 3				

Head and Neck (tick if normal, describe if abnormal) □ Larynx □ Thyroid □ Oral cavity □ Nostrils □ Eyes □ Teeth □ Auditory system □ Skull □ Brain	
Conclusions	

Probable cause of death	

Part 6 Sample Collection	Number	.....	GLIMS	.....
Sample list				

	UU					*CVI*	*Texel*			
	Cass. Nr. formaline	*4 hoekig buisje*	*zakje*	*Schroefdop Alc. 70%*	*Bruin epje halfvol*	*Melk buisje*	*zakje*	*zakje*	*zakje*	*Epje Alc. 70%*
	HP	**-80**	**-20**	**Parasites**	**Vit. A (- 20)**	**Brucella CVI (-20)**	**TX Alu**	**TX PL**	**Life History**	**Life History**
Skin		Lesions	Lesions						Whisker	Skin&Hair
Blubber					Inner + outer		3x TX	2xTX		
Muscle	Dcc1						TX	2xTX		
Genital split	Dcc1		Dcc1 Swab							
Mam.gland/penis	Dcc1									
Gonad & reproductive tract										
Reproductive tract LN										
Placenta, umbilical cord	Dcc1									
Urinary bladder										
Ileocecale LN										
Mesenteric LN										
Pre scapular LN										
Stomach				Parasites				SB		
Pancreas	Dcc1									
Spleen										
Liver				Parasites			3x TX	2xTX		
Kidney							3x TX	2xTX		
Adrenal										
Lung			Parasites	Parasites						
Pulmonary LN										
Heart										
Blood & / Serum										
Thymus										
Thyroid										
Eye										
Teeth										2x Mandible
Cerebellum										
Cerebrum										
Intestine			Caecum - WL							
Intestinal contents										
lungworm										

Collection/ DCC correlation	DCC 1		DCC 2				DCC 3	DCC 4 and 5		

BD: bijzondere dieren

WL: Wildlife

Caecum – WL – alleen bij niet gevroren dieren!!!