Pathological findings in stranded harbour seals (Phoca vitulina) and grey seals (Halichoerus grypus) in the Netherlands between 2009 and 2014.

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Abstract

In this study necropsy has been performed on 206 wild seals from the Wadden Sea, of which 158 harbour seals (Phoca vitulina), 22 grey seals (Halichoerus grypus) and 26 of which the species could not be determined, to determine what caused the animals to strand. The seals stranded on the Dutch coastlines between 2009 and 2014. They were further divided depending on the fact if the animal was found dead or died soon after stranding or if the animal was euthanized. The presence of morphologic abnormalities were the highest in the alimentary tract (203 abnormalities were scored in this category. In most cases the cause of death remained unknown after necropsy (50,5%). The hypothesis of the study was, that there were no differences in causes of death between the species, between the animals that were found dead or that were euthanized and that there were no differences between this studies results when comparing the to earlier studies. The cause of death that was established the most in the seals that were examined, was parasitic bronchopneumonia (13,5%), followed by septicaemia (12,6%). The grey seals significantly died more often due to emaciation (P=0.023) or inferred bycatch (P=0.040), in comparison with the harbour seals. Euthanized harbour seals, in comparison with harbour seals that were found dead or died soon after stranding (<24 hours), significantly died more often due to parasitic bronchopneumonia (P=0.004) and significantly more often the cause of death could be determined in this group (P=0.012).
In a previous study that examined seals between 1996 and 2005(1), significantly more harbour seals died from parasitic bronchopneumonia (P=0.007), perinatal death (P=0.000) and ecto- and endoparasitism (P=0.012). The other way around, more harbour seals along the Dutch coastline died due to flipper abscesses (P=0.029), pup starvation (P=0.009), foreign bodies (P=0.001) and causes Comparing the findings in seals in the Wadden Sea with earlier periods, significantly more harbour seals died due to pup starvation (P=0.012) and inferred bycatch (P=0.000) between 1979 and 2008. On the other hand, between 2009 and 2014 significantly more harbour seals died due to parasitic bronchopneumonia (P=0.000), septicaemia (P=0.000), emaciation of unknown origin (P=0.000) and physical trauma (P=0.026).

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1. Introduction

Harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) are the most common seal species in the Wadden Sea in the Netherlands. These seals often strand on the Dutch coastlines(2). In most cases the animals are found dead or die soon after stranding. If an animal is in relatively good condition, the animal will be taken to a seal rehabilitation shelter to recover.

The Dutch population of seals has decreased severely two times caused by phocine distemper virus (PDV) epizootics, the first in 1988 and the second in 2002(1,4,5). This could have been caused partly by human activities in and around the Wadden Sea, since chemical pollutants decrease the effectiveness of the immune response(1). An experimental study in 1994 tested if there is was a significant difference in the effectiveness of the seals immune system after feeding them fish out of polluted waters for two years, comparing to seals that were fed with relatively unpolluted fish(6). A significant difference was shown in the functionality of natural killer-cells and lymphocyte proliferation, indicating a negative influence of pollution of the seals habitat towards the immune system of these animals, especially in female seals(6). The seals can get more vulnerable for bacterial infections due to higher levels of granulocytes(6). All together the less active immune system could cause the seals to be more vulnerable for viruses, bacteria and parasites(6).
Fishing activities in the natural habitat of the seal can also be a great health risk for the animals. The ingestion of fishing hooks can cause trauma in the animals gastro-intestinal tract and ingestion of fishing sinkers can cause acute lead toxicosis(7). Seals can also get caught in fishing-gear, when the animals cannot get themselves out of the nets they drown.
The impact human activity has on the population makes it very important to keep investigating the health status of the seal population(1). The health status in seals is also important for public health, because of the seals niche on top of the food chain it gives an indication on the levels of toxins in humans, that eat the same polluted fish as the seals do(3).
The seal population is recently counted, In August 2013, 26.788 harbour seals(8) were counted by the Trilateral Seal Expert Group (TSEG) and in the winter (2012-2013) 2.785 grey seals (9).

The Dutch Wildlife Health Centre (DWHC)[1] is studying the stranding patterns and pathology among the harbour seals and grey seals stranded on the island of Texel and the northern parts of North Holland. The aim of the overall study is to investigate occurrence of changes in pathological findings, causes of death and stranding patterns over time. With this knowledge it will be possible to link these differences to the human activities in the area, such as pollution, disturbance of the seals in their habitat and fishing.

The aims of this study were to investigate occurrence and frequency of pathological findings in stranded seals, and to assess causes of death in stranded seals. The hypothesis tested in this report is that there will be no significant difference in the frequency of the most common causes of death between this study and the earlier studies in the same areas.

This report gives an overview of the pathological findings and causes of death that were determined in the stranded harbour seals and grey seals brought to the DWHC between 2009-2014. The results were compared with earlier studies in the same areas(1,2).

[1] Dutch Wildlife Health Center (DWHC), Faculty Veterinary Medicine, Utrecht University, Yalelaan 1, Utrecht

2. Materials and methods

In this study, necropsy has been performed on 206 wild seals from the Wadden Sea. Among these seals were 158 harbour seals (*Phoca vitulina*) of which 70 males and 88 females. There were 22 stranded grey seals (*Halichoerus grypus*) examined of which 14 males and 8 females. There were also seals where could not be determined what species it was, this group of undetermined species consisted of 26 seals. Within this group sex and age was undeterminable as well, because of this, the frequency of males and females is unknown in this group.

The stranded animals were collected by Ecomare[2] and the Institute for Marine Resources (Imares)[3] between 2009 and 2014. At the pathobiology department of the Faculty of Veterinary Medicine at Utrecht University, pathologists performed necropsy on the stranded animals to determine pathological lesions and causes of death. They were supported by students of the Faculty of Veterinary Medicine in Utrecht, that perform research internships at this department. Pathologists that performed necropsy were: L. Begeman, G. C. M. Grinwis, A. Gröne, J. IJzer, R. Keesler and M. J. L. Kik. Histology was performed by A. Gröne, J. IJzer and R. Keesler. Collecting the seals started in 2009 until January 2014, the last carcasses were examined in March 2014. Not all seals that were examined between 2009 and 2014 were included in the study. The seals that had been in a rehab centre for over more than twenty-four hours were excluded, because this study only focusses on the wild animals. The rehab could have had influence on the animals condition, health and cause of death, because of forced feeding and medication. The findings at necropsy would have said something about the cause of death and nutritional state of the animal, but it could not have been used to draw conclusions towards the health status of the wild population of seals in the Wadden Sea, which we want to monitor. After exclusion of these rehab animals, there were 206 wild seals left within the study.

Due to logistical limitations carcasses were stored in freezers at minus 20 degrees Celsius until necropsy was performed. A few days before necropsy the seals were collected and stored in a refrigerator, that way the carcasses would slowly defrost. This was important, because if the carcasses would defrost very quickly, they would have been too putrefied for necropsy.

2.1 Necropsy

At necropsy, the seals were given a carcass number that consisted of the abbreviation of the species: PV for *Phoca* vitulina, HG for *Halichoerus grypus,* and PV/HG for seals where species could not be determined and a number. These numbers increased, so the first harbour seal that was examined was named PV 1 and the seventh grey seals that was examined, was named HG 7.
The stranding data were collected, these included stranding date, location of stranding and who found and transported the animal. The seals were identified if they had a tag of chip.

The seals of each species were divided in two groups: one group consisted of animals that were found death or died within 24 hours of stranding and the other group consisted of animals that were euthanized within 24 hours of stranding. Animals that were taken into rehabilitation centres, Ecomare for example, were euthanized when their survival chances decreased.

The group of harbour seals contained 15 seals that had been euthanized within 24 hours of stranding and 143 seals that were found dead or died within 24 hours of stranding. The group of grey seals contained two seals that had been euthanized and 20 that were found dead or died within 24 hours of stranding. Within the group of undetermined seals species, all of the seals were found dead or died within 24 hours of stranding.

[2] Ecomare, Ruijslaan 92, de Koog, Texel
[3] Institute for Marine Resources (Imares), Landsdiep 4, 't Horntje, Texel

The seals were divided in four age categories: neonate, only when the umbilical cord was still present, juvenile, subadult and adult. For division of the last three groups a method was followed described by McLaren (1993) using the standard length (SL): straight line distance from snout to tip of tail(2,10). See table 1.

species	sex	category	age (years)	body length (cm)
Phoca vitulina	male	neonate		umbilical cord present
		juvenile	≤ 1	≤ 107
		subadult	1 ≤ 4.7	107 ≤ 142
		adult	> 4.7	> 142
	female	neonate		umbilical cord present
		juvenile	≤ 1	≤ 103
		subadult	1 ≤ 3.7	103 ≤ 129
		adult	> 3.7	> 129
Halichoerus grypus	male	neonate		neonate haircoat present
		juvenile	< 1	≤ 134
		subadult	1 ≤ 4.9	134 ≤ 174
		adult	> 4.9	> 174
	female	neonate		neonate haircoat present
		juvenile	< 1	≤ 126
		subadult	1 ≤ 4.0	126 ≤ 158
		adult	> 4.0	> 158

Table 1 Age determination table for *Phoca vitulina* and *Halichoerus grypus*, according to McLaren (1993) using the standard length (SL): straight line distance from snout to tip of the tail(2,10).

At necropsy the stage of decomposition of the carcasses (DCC) was scored according to the determination table(11) in table 2. The DCC was scored from 1 (very fresh), to 5 (very putrefied). When the carcass was fresh, many the organs were examined and flaws were described following a detailed necropsy protocol added as addendum 1. When decomposition was scored as very putrefied or the carcass consisted of remains only there was little possibility of identifying histo-pathological lesions and very few samples were taken, see sampling guidelines in the necropsy protocol (Addendum 1).

Decomposition Condition Code (DCC)	Criteria
DCC 1	Very fresh, less than 48 hours dead, may show signs of rigor mortis (< 24 h), blood still separates serum (24-48 h), rigidity of eyes is diminished but not very flaccid, cornea is not cloudy
DCC 2	Fresh, first signs of decomposition visible, eyes and surface quality of the skin reveal decomposition, otherwise good state, organs look intact, blood does not separate the serum, no smell of decomposition.
DCC 3	Skin peeling, moderate but clear signs of decomposition (changes in colour and consistency [flaccid]) of skin and organs, not suitable for bacteriology because of overgrowth, moderate smell of decomposition.
DCC 4	Advanced decomposition, skin and organs clearly altered, the loss of consistency changes the organ's shapes (liver!), clear smell of decomposition, not suitable for any tissue analysis, even gross pathology is very unclear and can hardly be interpreted at all.
DCC 5	Completely useless for pathological examination, organs are beyond clear recognition or absent, may be mummified, etc.

Table 2 Determination table for decomposition condition code. According to Kuiken et al (1993)(11).

Each seal was given a nutritional condition code (NCC) according to table 3(12), which gives an indication about the nutritional state of the animal. When there was a DCC established for the carcass from 4 or 5, the NCC was not determined, because when de carcass was putrefied, the blubber could have been vanished because of the rotting. In this case the NCC would say nothing about the nutritional state of the seal in these cases.

Nutritional Condition Code (NCC)	Nutritive condition	Blubber thickness (mm)	External factors and subcutaneous fat
NCC 1	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.
NCC 2	Well fed		A good nutritive condition, well nourished, abundant blubber, some subcutaneous fat, longissimus dorsi and neck are straight or slightly convex.
NCC 3	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.
NCC 4	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.
NCC 5	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
NCC 6	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 Determination table for the nutritional condition code (NCC) (12).

To determine the probability of bycatch, we used external observations of the carcass, such as specific trauma or the presence of fishing lines and internal observations such as health state, contact with fishing gear, lack of oxygen, damage during release of the net and other relevant characteristics, see guidelines in the necropsy protocol for criteria for the diagnosis of bycatch (Addendum 1).

All macroscopic abnormalities, external and internal, were described. Photos were taken of the whole carcass and the found abnormalities. Abnormalities include, but are not limited to, missing of fur and/or skin, missing flippers, fishing gear, visible fractures, haemorrhages or lumps, or internal abnormalities such as rupture of orangs, severe inflammation of organs, pooling of blood and parasites and other visible damages. Depending on the decomposition state of the carcass samples were taken from the organs. Which samples were taken at each DCC, the collection/DCC correlation, is shown with color-codes in part 6 (sample collection) of the necropsy protocol (addendum I). Samples for histology were taken from skin, muscle, genital split, mammary gland/penis, gonad, reproductive tract, reproductive tract lymfnode, placenta, umbilical cord, urinary bladder, ileocecal lymfnode, mesenteric lymfnode, prescapular lymfnode, pancreas, spleen, liver, kidney, adrenal, lung, pulmonary lymfnode, heart, thymus, thyroid, eye, cerebellum, cerebrum, and intestine.

To be able to identify microscopic abnormalities, histology was performed. The samples were fixed in 10% neutral buffered formalin, routinely paraffin bedded, cut in 4-6 µm slides and stained with haematoxylin and eosin (HE). When necessary, additional stainings were performed: PAS (Periodic Acid Schiff) and ZN (Ziehl Neelsen).

Parasites that were found were fixed in 70% ethanol. Determination was macroscopically performed by students that performed internships at the pathobiology department, according to determination criteria provided by parasitologist Herman Cremers.

Per animal, a final report was issued containing all collected data regarding identification, stranding, biometrical data, macroscopic and microscopic pathological lesions and most likely cause of death. The data were collected and categorized for the grey seals, harbour seals and the carcasses of which determination of species was impossible (addendum 2). All pathological findings were collected in spreadsheets, categorized for each species. Subsequently, all reported macroscopic and microscopic pathological lesions, and determined causes of death were scored by Jooske IJzer, according to the scoring system used by Osinga et al(2). The determination table that was used to determine the cause of death is presented in table 4.

The ten most occurring pathological findings in the different species are presented in table 5. In table 6 all causes of death established in the examined animals are presented. Statistical tests showed whether there was a significant difference between the harbour seals and the grey seals, and between the seals that were found dead or died within 24 hours of stranding compared to the seals that had been euthanized within 24 hours of stranding. The results of these tests are shown in table 7 and 8.

The results of this study were statistically compared to earlier similar studies of Osinga et al(2) and Siebert et al(1) to evaluate potential difference in causes of death in the seals of the Wadden Sea in different periods of time.
The study of Siebert contained only harbour seals. In order to be able to compare with this study, only the harbour seals out of this report were compared to it.
The study of Osinga contained both harbour seals and grey seals, but only seals with a scored DCC of 1, 2 or 3. To compare this report with Osinga et al(2), the seals with DCC 4 and 5 were excluded before comparing the results between the studies. The results of these statistical tests are shown in table 9 and 10.

2.2 Statistical analysis

To determine whether there was a statistical difference between these groups, the Fisher's exact test was used. This test was chosen, because there were two independent groups to be compared. The grey seals versus the harbour seals and the group seals that died themselves versus the group that had been euthanized. The seals that were examined for this investigation were also compared to earlier studies that also investigated the health status of seals in the Wadden Sea, between 1996 and 2005(1) and between 1979-2008(2). Because of the small n value there was a relatively low expected value in some groups, for example the grey seals that were euthanized (n=2). When the expected value becomes less than 5 in more than 20% of the cells, the Fisher's exact test is recommended(13,14).

Table 4 Determination table causes of death(2).

Causes of death	The criteria that had been used to categorize the seals into the correct category causes of death.
Parasitic bronchopneumonia	Severe inflammation of the lungs inflicted by parasites, detected macroscopically and/ or histologically(2).
Pneumonia of undetermined cause/ unknown etiology	Severe inflammation of the lungs but the etiology was not detected/ not proven(2).
Flipper abscess	Encapsulated purulent process in the flipper.
Septicaemia	Systemic presence of bacteria indicated by histological changes and/or culture. Seals were also included when hepatitis, abscessation, generalized lymphadenopathy or polyarthritis were detected.
Gastro-enteritis	Severe inflammation of stomach and/or intestinal tract.
Pup starvation	The seal was estimated less than 6 months of age (June- December for harbour seals, October-April for grey seals), blubber thickness was < 1 cm, there was no food in the stomach, and other significant lesions were absent(2).
Perinatal death	Death of mother and/or pup around moment of birth, related to pupping or in absence of other significant lesions.
Emaciation of undetermined cause	Emaciated animal, nutritive condition code 6 (Table 3), no cause for the emaciation could be detected.
Physical trauma	Severe physical trauma including bone fractures and/or organ ruptures associated with haemorrhages(2).
Dystocia	Abnormal parturition(2).
Bycatch, confirmed	Cases found dead in a fishing net(2).
Bycatch, inferred	Cases found dead on the coast or in the water. Although the signs fulfilled the criteria of bycatch, these cases were not found in a net, neither did they have external evidence of contact with fishing gear. Seals were categorized as inferred bycatch only if they fulfilled all of the following criteria: 1. Fish (remains) in stomach or intestines, 2. Good nutritional condition, 3. No other significant pathological findings and 4. Presence of lung oedema(2).
Foreign body	Death caused by the presence of a foreign body in the trachea or other abnormal locations(2).
Fatal haemorrhage, non-traumatic	Presence of large amount of blood in body cavity related to vascular rupture, absence of traumatic lesions in skin and musculoskeletal system.
Intestinal torsion	Dislocation or twisting of (part of) the intestinal tract with necrosis of intestinal wall.
Other	Cause of death was determined, but was not included in the abovementioned categories.
Unknown	The cause of death was not determined.

3. Results

3.1 Morphological findings

Overall the presence of morphologic abnormalities was the highest in the alimentary tract, there were 203 abnormalities scored in this category. The second highest amount of abnormalities was scored in the respiratory tract with 156 abnormalities in this category. The third highest amount of abnormalities was seen in the category haematopoietic and endocrine system, with 136 scored abnormalities. There can be more abnormalities scored in one category for one seal. For example in seal HG 1 both nematodirasis in the bronchial tree and bronchopneumonia were scored in the category respiratory tract.

The ten most occurring morphological findings per seal species are presented in table 5. Within the group of undetermined seal species very little specific abnormalities were found due to rotting.

In the group of the undetermined seal species, there are only three categories of morphological findings presented in the table. This is due to the fact that there were only three frequent found morphological findings, nine other findings only were found in one seal each. These findings included nematodiriasis, liquothorax, ascites, intestinal parasites, hepatitis, fractured skull or skeletal bones, haemorrhages in the muscles, splenic hyperplasia and lymphadenitis. Because only one seal was scored in each category of the 26 seals in this group, the percentage seals in these categories was 3,8%. This made it impossible to make a reliable top 10 out of all morphological findings in this group of seals, so only the three categories of morphological findings that occurred more than once were presented.

Shortlist of morphological findings per seal species		
Harbour seal (n=158)	**Number**	**%**
Nematodiriasis bronchial tree	56	35,4
Gastric parasites	55	34,8
Bronchopneumonia	51	32,3
Hyperplasia lymphnodes	44	27,8
Intestinal parasites	28	17,7
Lymphadenitis	28	17,7
Hepatitis	24	15,2
Bleeding/ haematoma of the skin	22	13,9
Enteritis	22	13,9
Nematodiriasis in cardio vascular system	18	11,4
Grey seal (n=22)	**Number**	**%**
Gastric parasites	11	50,0
Bleeding/ haematoma of the skin	8	36,4
Nematodiriasis bronchial tree	6	27,3
Hyperplasia lymphnodes	5	22,7
Bronchopneumonia	4	18,2
Wounds of the skin	3	13,6
Gastritis	3	13,6
Enteritis	3	13,6
Lymphadenitis	3	13,6
Foreign body	2	9,1
Undetermined seal species (n=26)	**Number**	**%**
Bleeding/ haematoma of the skin	4	15,4
Nematodiriasis bronchial tree	3	11,5
Bronchopneumonia	2	7,7

Table 5 Ten most occurring morphological findings in each seal species.

In the harbour seals the abnormality that was scored most often, was nematodiriasis in the bronchial tree. In the grey seals the presences of gastric parasites was the most frequent found abnormality. In the undetermined seal species the most frequent found abnormality was bleeding or haematoma of the skin.

Figure 1 shows the difference in percentage of found abnormalities between the harbour seals and grey seals. The grey seals seems to suffer more often from gastric parasites and bleeding or haematoma of the skin compared to the harbour seals that were examined. Within the harbour seals nematodiriases in bronchial tree and bronchopneumonia were found more often compared to the grey seals that were examined.

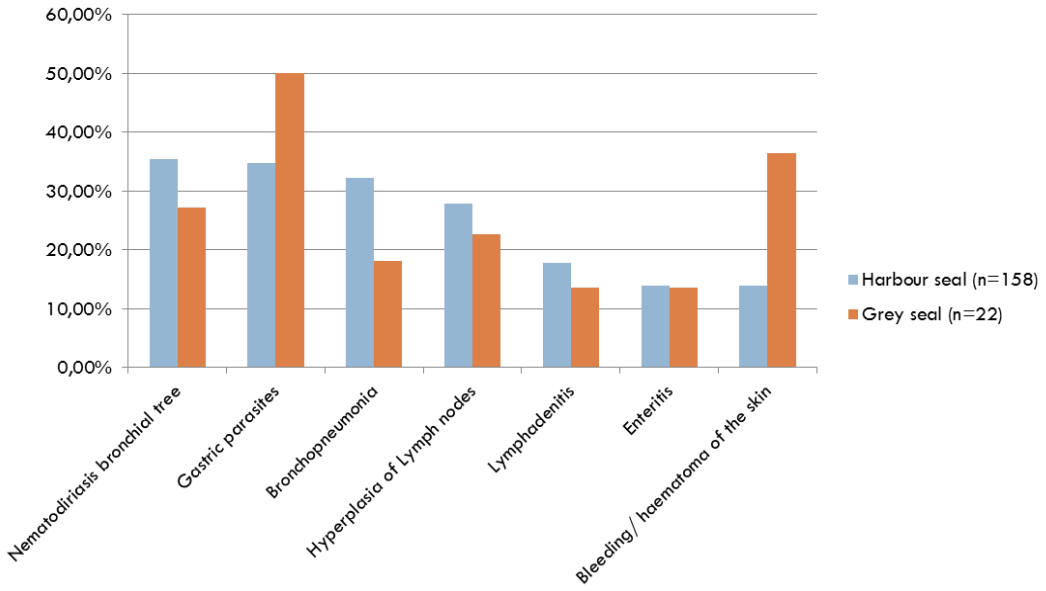

Figure 1 The difference in percentage of found abnormalities in harbour seals comparing to the grey seals.

3.2 Causes of death

All determined causes of death per seal species are presented in table 6 on the next page. In 50,5 percent (n=104) of the cases that were examined in this study the actual cause of death cannot be determined, and is scored as unknown. With 13,6 percent of all animals parasitic bronchopneumonia is considered to be the most common cause of death. The second important cause of death, established in 12,6 percent of all the examined animals, turned out to be septicaemia. Other important causes of death were emaciation (7,3 %), physical trauma (5,8 %) and death caused by a foreign body (3,4 %).

Each cause of death will be discussed separately. The criteria that were used to determine whether an animal died from each cause are presented in table 4 but will be mentioned at each specific cause of death as well. Most of these criteria were cited from the criteria that were used by Osinga (2012) to be able to compare the results of the study to that report.
The amount of seals in each seal species group that died from a certain cause of death will be described.
The results of the Fisher's exact test that compared the frequency in causes of death found in the harbour and grey seals are reported. Also the comparison made between the seals that were euthanized within 24 hours of stranding or that were found death/died within 24 hours of stranding using the Fisher's exact test will be described at each cause of death. The results of these tests are shown in table 7 and 8 (see page 13 and 14).

	Harbour seal						Grey seal						Undetermined species						Total					
	Found dead		Euthanized		Subtotal		Found dead		Euthanized		Subtotal		Found dead		Euthanized		Subtotal		Found dead		Euthanized		Subtotal	
	n=143		n=15		n=158		n=20		N=2		n=22		n=26		n=0		n=26		n=189		n=17		n=206	
Determined cause of death	**Number**	**%**	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Parasitic bronchopneumonia	19	13,3	7	46,7	26	16,5	1	5,0	0	0,0	1	4,5	1	3,8	0	0,0	1	3,8	21	11,1	7	41,2	28	13,6
Pneumonia of undetermined cause/ unknown etiology	2	1,4	0	0,0	2	1,3	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	2	1,1	0	0,0	2	1,0
Flipper abcess	2	1,4	1	6,7	3	1,9	0	0,0	1	50,0	1	4,5	0	0,0	0	0,0	0	0,0	2	1,1	2	11,8	4	1,9
Septicaemia	18	12,6	4	26,7	22	13,9	2	10,0	1	50,0	3	13,6	1	3,8	0	0,0	1	3,8	21	11,1	5	29,4	26	12,6
Gastro-enteritis	1	0,7	0	0,0	1	0,6	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	1	0,5	0	0,0	1	0,5
Pup starvation	4	2,8	0	0,0	4	2,5	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	4	2,1	0	0,0	4	1,9
Perinatal death	2	1,4	0	0,0	2	1,3	0	0,0	0	0,0	0	0,0	1	3,8	0	0,0	1	3,8	3	1,6	0	0,0	3	1,5
Cachexia/ emaciation of undetermined cause	8	5,6	2	13,3	10	6,3	5	25,0	0	0,0	5	22,7	0	0,0	0	0,0	0	0,0	13	6,9	2	11,8	15	7,3
Physical trauma	10	7,0	0	0,0	10	6,3	2	10,0	0	0,0	2	9,1	0	0,0	0	0,0	0	0,0	12	6,3	0	0,0	12	5,8
Dystocia	2	1,4	0	0,0	2	1,3	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	2	1,1	0	0,0	2	1,0
Bycatch, confirmed	0	0,0	0	0,0	0	0,0	1	5,0	0	0,0	1	4,5	0	0,0	0	0,0	0	0,0	1	0,5	0	0,0	1	0,5
Bycatch, inferred	1	0,7	0	0,0	1	0,6	2	10,0	0	0,0	2	9,1	0	0,0	0	0,0	0	0,0	3	1,6	0	0,0	3	1,5
Foreign body	5	3,5	1	6,7	6	3,8	1	5,0	0	0,0	1	4,5	0	0,0	0	0,0	0	0,0	6	3,2	1	5,9	7	3,4
Intestinal torsion	4	2,8	0	0,0	4	2,5	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	4	2,1	0	0,0	4	1,9
Other	1	0,7	1	6,7	2	1,3	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	0	0,0	1	0,5	1	5,9	2	1,0
Unknown	70	49,0	2	13,3	72	45,6	8	40,0	1	50,0	9	40,9	23	88,5	0	0,0	23	88,5	101	53,4	3	17,6	104	50,5

Table 6 All causes of death in all wild seals examined between 2009 and 2014, devided by species. A devision has been made between seals that were found dead and seals that had been euthanized within 24 hours of stranding. The group of seals that were found dead also contained seals that died themselves within 24 hours of stranding.

	Harbour seal		Grey seal		Significancy
	Subtotal		Subtotal		
	n=158		n=22		
Cause of death	Number	%	Number	%	Pvalue
Parasitic bronchopneumonia	26	16,5	1	4,5	0,206
Pneumonia of undetermined cause	2	1,3	0	0,0	1,000
Flipper abcess	3	1,9	1	4,5	0,409
Septicaemia	22	13,9	3	13,6	1,000
Gastro-enteritis	1	0,6	0	0,0	1,000
Pup starvation	4	2,5	0	0,0	1,000
Perinatal death	2	1,3	0	0,0	1,000
Emaciation of undetermined cause	10	6,3	5	22,7	0,023
Physical trauma	10	6,3	2	9,1	0,644
Dystocia	2	1,3	0	0,0	1,000
Bycatch, confirmed	0	0,0	1	4,5	0,122
Bycatch, inferred	1	0,6	2	9,1	0,040
Foreign body	6	3,8	1	4,5	1,000
Intestinal torsion	4	2,5	0	0,0	1,000
Other	2	1,3	0	0,0	1,000
Unknown	72	45,6	9	40,9	0,820

Table 7 All causes of death and statistical tested difference (Fisher's exact test) between the harbour seal and the grey seal.

	Harbour seal					Grey seal				
	Found dead or died within 24 hours of stranding		Euthanized within 24 hours of stranding		Statistics	Found dead or died within 24 hours of stranding		Euthanized within 24 hours of stranding		Statistics
	n=143		n=15			n=20		n=2		
Cause of death	Number	%	Number	%	P value	Number	%	Number	%	P value
Parasitic bronchopneumonia	19	13,3	7	46,7	0,004	1	5,0	0	0,0	1,000
Pneumonia of undetermined cause	2	1,4	0	0,0	1,000	0	0,0	0	0,0	-
Flipper abcess	2	1,4	1	6,7	0,260	0	0,0	1	50,0	0,091
Septicaemia	18	12,6	4	26,7	0,229	2	10,0	1	50,0	0,260
Gastro-enteritis	1	0,7	0	0,0	1,000	0	0,0	0	0,0	-
Pup starvation	4	2,8	0	0,0	1,000	0	0,0	0	0,0	-
Perinatal death	2	1,4	0	0,0	1,000	0	0,0	0	0,0	-
Emaciation of undetermined cause	8	5,6	2	13,3	0,242	5	25,0	0	0,0	1,000
Physical trauma	10	7,0	0	0,0	0,600	2	10,0	0	0,0	1,000
Dystocia	2	1,4	0	0,0	1,000	0	0,0	0	0,0	-
Bycatch, confirmed	0	0,0	0	0,0	-	1	5,0	0	0,0	1,000
Bycatch, inferred	1	0,7	0	0,0	1,000	2	10,0	0	0,0	1,000
Foreign body	5	3,5	1	6,7	0,456	1	5,0	0	0,0	1,000
Intestinal torsion	4	2,8	0	0,0	1,000	0	0,0	0	0,0	-
Other	1	0,7	1	6,7	0,181	0	0,0	0	0,0	-
Unknown	70	49,0	2	13.3	0,012	8	40,0	1	50,0	1,000

Table 8 The difference between seals that were found dead or died within 24 hours of stranding and seals that have been euthanized within 24 hours of stranding. These findings were statistically tested using the Fisher's exact test.

Parasitic bronchopneumonia

The cause of death of seals was considered to be parasitic bronchopneumonia, when macroscopic and microscopic findings showed severe inflammation of the lungs caused by parasites (see table 4). There are two lungworms often shown in seals: the *Otostrongylus circumlitus*, with a thickness of 1-2 mm and the *Parafilaroides gymnurus,* with a thickness of less than 5 mm.(15) Both of these parasites were found in the seals autopsied in this study. The parasites were found in the lung, bronchia and/or trachea.

Of the 158 harbour seals, 26 died from parasitic bronchopneumonia (16,5%) , only one of the twenty-two grey seals (4,5%) and only one of the twenty-six seals of undetermined species(3,8%). Lungworms were found in 31,6% of all the examined seals.

Figure 2 Photographed lungworms in one of the examined seals in this study. Photo gained from photo

The harbour seals that were euthanized had significantly more animals that died from parasitic bronchopneumonia (46,7%), comparing to the harbour seals that were found dead or died within twenty-four hours of stranding (13,3%) (P=0.004).

There was no significant difference between these two groups in the grey seals (P=1.000), because there was only one grey (from the twenty-two) that died from this disease. Comparing the species, there was no statistical difference in parasitic bronchopneumonia between the harbour seals and the grey seals examined in this study (P=0.206).

Septicaemia

The conclusion that a seal died from septicaemia as primary cause, was drawn when bacteria were detected in multiple organs, for example polyarthritis (see table 4). This indicates that bacteria entered the bloodstream and could spread the infection to other organs. Septicaemia was also scored as primary cause of death if bacteria were found in multiple enlarged lymph nodes, or the liver.

Bacteria were found if there was a positive culture or if bacteria were seen in histological changes of the organ tissue.

Twenty-two of the 158 harbour seals died primarily because of septicaemia (13,9%), three of the 22 grey seals(13,6%) and one of the 26 undetermined species (3,8%). There was no significant difference between the harbour seals and the grey seals in frequency of septicaemia as primary cause of death . There were 143 harbour seals that were found dead or died soon after stranding, in 19 of them septicaemia was scored as the primary cause of death (13,3%). Fifteen harbour seals had been euthanized, of which four died of septicaemia (26,7%). There was no significant difference between these two groups of harbour seals (P=0.229). Because only one of the grey seals died from this disease, one that was found dead, the grey seals also did not show significantly differences whether the seals where euthanized or not (P=0.260).

Emaciation

When seals had a very poor nutritional state they were categorized as nutritional condition code 6 (see table 3 for criteria). When no other possible causes of death were detected at necropsy, and no cause for the poor nutritional state was detected, the animals cause of death was scored as emaciation of unknown origin.

Ten of the 158 harbour seals (6,3%) died due to emaciation of undetermined cause, five of the 22 grey seals (22,7%) and none of the undetermined species (n=26). Statistically grey seals died more often due to emaciation comparing to the harbour seals (P=0.023).

Two of the 15 euthanized harbour seals died due to emaciation (11,8%) and eight of the 143 seals that were found dead (5,6%). There was no significant difference between these two groups of harbour seals (P=0.242). The five grey seals that died due to their poor nutritional condition all belonged to the group of grey seals that were found dead (n=20). None of the euthanized grey seals died from emaciation. There was no significant difference between these groups of grey seals(P=1.000).

Physical trauma

Physical trauma was primary cause of death when severe trauma, such as broken bones, or ruptures of multiple organs, that were associated with haemorrhage (see table 4). Ten of the 158 harbour seals (6,3%) in this study died due to severe physical trauma, two of the 22 grey seals (9,1%). There was no significant difference between the harbour seals and the grey seals (P=0.644). All of the ten harbour seals were found dead, there was no significant difference with the euthanized group (P=0,600). Two of the 20 grey seals that were found dead died due to severe trauma (10,0%), there was no significant difference between this group and the grey seals that were found dead of which none died due to trauma (P=1.000)

Foreign body

Death caused by foreign body was scored, when foreign bodies were found in irregular places (see table 4). For example in this study fish hooks and fishing lines were found in some seals stomach or oesophagus, also fish were found stuck in the trachea of some seals. In six of 158 harbour seals (3,8%) a foreign body was established as the cause of death and only one of the 22 grey seals (4,5%) died due to a foreign body. None of the undetermined species died because of a foreign body. The was no significant difference between the group of harbour seals and the group of grey seals. Foreign body was the cause of death in five of the 143 harbour seals that were found dead (3,5%) and one of 15 harbour seals that were euthanized (6,7%), there was no significant difference between these two groups (P=0.456). Neither did the grey seals showed a significant difference between these groups (P=1.000), there was only one seal that died from a foreign body.

Inferred bycatch

Only in one of the 158 harbour seals (0,6%), the cause of death was determined as inferred bycatch. When comparing this result to the grey seals (n=22) of which two died due to inferred bycatch, there were significantly more grey seals that died due to inferred bycatch (P=0.040).

Unknown causes of death

In 50,5 % of the examined animals, the cause of death could not be determined (n=104). These animals were scored unknown in this category. From the 158 harbour seals, there were 72 in which the cause of death was scored unknown. In total 15 harbour seals had been euthanized, two of them were scored under unknown. (13,3%). There were 143 harbour seals that were found dead or died within 24 hours of stranding, in 70 of them the cause of death could not be determined (49,0%) **T**here were significantly more unknowns in the group of harbour seals that were found dead (P=0.012). Between these groups among the grey seals, there was no statistical difference detected.

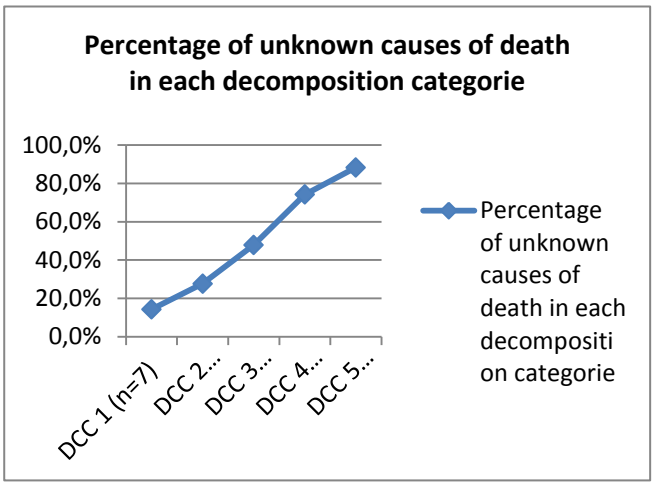

Figure 3 The relation between the scored decomposition code and the percentage of seals where cause of death could not be determined, the percentage of unknowns.

The percentage of unknown causes of death was comparable to the stage of decomposition of the carcass. Figure 3 shows an increasing percentage of unknown causes of death as the DCC (decomposition code) was scored higher. In animals that were relatively fresh there were more causes of death determined with certainty.

3.3 Comparing pathological findings found in harbour seals to harbour seals examined between 1996-2005(1)

Looking at the percentages of the most frequent pathological findings in the seals within this study, there are a few differences with the seals examined between 1996 and 2005. Between 2009 and 2014 nematodirasis in bronchial tree was found in 56 harbour seals (35%), between 1996 and 2005 there were 147 harbour seal (41%) suffering from these parasites, which indicates a decreasing of the frequency of these parasites.

The nematodiriasis in pulmonary vessels also decreased from 13% of the harbour seals between 1996 and 2005 to 4% between 2009 and 2014. The frequency of the presence of bronchopneumonia among the seals had decreased from 42% to 32% of the examined harbour seals.

In contradiction the nematodes in the cardiovascular system increased during the years. Within the seals examined between 1996 and 2005(1) 6% of the seals suffered from these parasites and between 2009 and 2014 the parasites were established in 11% of the examined harbour seals. The presence of hepatitis seems to be increased from 7%, between 1996 and 2005(1), to 15% between 2009 and 2014. The largest difference was found in the lymphatic system. Lymphadenitis and hyperplasia of lymph nodes were found in respectively 5% and 3% of the examined harbour seals (n=355) between 1996 and 2005(1), and between 2009 and 2014 they both increased respectively to 18% and 28% of the examined harbour seals (n=158) that period.

Due to time limitations of this study these differences were not statistically tested.

3.4 Comparing causes of death established in harbour seals with harbour seals examined between 1996 and 2005(1)

The study between 1996 and 2005 contained only harbour seals. In order to be able to compare with this study, only the harbour seals out of this report were compared to it. The results of these statistical tests are shown in table 9. When comparing the causes of death in the harbour seals examined in this study (n=158) to the results Siebert et al(1) presented from seal necropsies between 1996 and 2005 (n=355), there were significant differences.

Cause of death	Utrecht Harbour seals		Siebert		Statistics
	Harbour seal		Harbour seal		
	n=158		n=355		
	Number	%	Number	%	P value
Parasitic bronchopneumonia	26	16,5	98	27,6	0,007
Pneumonia of undetermined cause	2	1,3	0	0,0	0,094
Flipper abcess	3	1,9	0	0,0	0,029
Septicaemia	22	13,9	67	18,9	0,207
Gastro-enteritis	1	0,6	11	3,1	0,116
Pup starvation	4	2,5	0	0,0	0,009
Perinatal death	2	1,3	32	9,0	0,000
Emaciation of undetermined cause	10	6,3	36	10,1	0,183
Physical trauma	10	6,3	13	3,7	0,246
Dystocia	2	1,3	5	1,4	1,000
Bycatch, confirmed	0	0,0	0	0,0	-
Bycatch, inferred	1	0,6	0	0,0	0,308
Foreign body	6	3,8	0	0,0	0,001
Intestinal torsion	4	2,5	16	4,5	0,334
Other	2	1,3	9	2,5	0,516
Unknown	72	45,6	41	11,5	0,000
Ecto- endoparasitism	0	0,0	13	3,7	0,012
Keratitis corneal rupture	0	0,0	7	2,0	0,106
Dermatitis	0	0,0	6	1,7	0,184

Table 9 The differences between the harbour seals examined between 2009 and 2014 compared with the harbour seals examined between 1996 and 2005(1). It was tested whether the differences were significant, using the Fisher's exact test.

Parasitic bronchopneumonia as cause of death in 26 of all of the harbour seals in this study(n=158) were compared to the harbour seals examined between 1996 and 2005(1) that also died from parasitic bronchopneumonia (98 of 355 seals). Between 2009 and 2014 16,5% of the harbour seals died of this disease, between 1996 and 2005 27,6%. This difference was significant(P=0.007), which indicates that in the earlier period more harbour seals died of this parasitic disease.
The findings of septicaemia in harbour seals (13,3%, n=158) where compared to the outcomes of necropsies between 1996 and 2005(1). In the period from 1996-2005, there were 67 of the 355 harbour seals (18,9%) that died due to septicaemia(1). There is no significant difference between these two periods of time (P=0.207). When comparing the ten of 158 harbour seals (6,3%) to the 36 of 355 harbour seals (10,1%) that have died from emaciation of unknown origin between 1996 and 2005(1), no statistical difference between the groups was found(P=0.183). Comparing the harbour seals that died due to physical trauma from this study (6,3%) with the harbour seals that died from this between 1996 and 2005(1), 13 of 355 seals (3,7%) showed no significant differences (P=0.246).
Between 1996 and 2005, none of the 355 harbour seals in the study died due to a foreign body(1). A significant difference was found when this group of animals was compared with this study's harbour seals (n=158) of which six died due to a foreign body(3,8%). There were significantly more seals between 2009 and 2014 that had foreign bodies that caused the seals to die (P=0.001).
When comparing the harbour seals where the cause of death could not be established out of this

study (45,6%) to the harbour seals stranded between 1996 and 2005(1) (11,5%) there were significantly more seals of which the cause of death was unknown after necropsy in this study (P=0.000). Between 1997 and 2005(1), significantly more harbour seals died from perinatal death (P=0.000) and ecto- and endoparasitism (P=0.012). The other way around, more harbour seals between 2009 and 2014 died due to flipper abscesses (P=0.029), pup starvation (P=0.009).

3.5 Comparing causes of death found in harbour seals and grey seals with harbour seals and grey seals examined between 1979 and 2008(2)

In the article of Osinga et al (2), all seals with a decomposition code of 4 or 5 where excluded from the study. After excluding the seals with these DCCs from this study the results could be compared to the study in which Osinga et al (2)reported causes of death in seals examined between 1979 and 2008. The results of these statistical tests are shown in table 10.

Cause of death	Utrecht University		Osinga			Utrecht University		Osinga		
	Harbour seals		Harbour seals			Grey seals		Grey seals		
	n=110		n=286			n=16		n=93		
	Number	%	Number	%	P value	Number	%	Number	%	P value
Parasitic bronchopneumonia	26	23,6	17	5,9	0,000	1	6,3	1	1,1	0,273
Pneumonia of undetermined cause	2	1,8	13	4,5	0,253	0	0,0	1	1,1	1,000
Flipper abcess	3	2,7	4	1,4	0,403	0	0,0	0	0,0	-
Septicaemia	20	18,2	14	4,9	0,000	3	18,8	3	3,2	0,040
Gastro-enteritis	1	0,9	0	0,0	0,278	0	0,0	0	0,0	-
Pup starvation	1	0,9	20	7,0	0,012	0	0,0	10	10,8	0,352
Perinatal death	0	0,0	9	3,1	0,068	0	0,0	3	3,2	1,000
Emaciation of undetermined cause	9	8,2	0	0,0	0,000	5	31,3	0	0,0	0,000
Physical trauma	9	8,2	8	2,8	0,026	1	6,3	5	5,4	1,000
Dystocia	1	0,9	1	0,3	0,479	0	0,0	1	1,1	1,000
Bycatch, confirmed	0	0,0	4	1,4	0,579	1	6,3	5	5,4	1,000
Bycatch, inferred	0	0,0	50	17,5	0,000	1	6,3	9	9,7	1,000
Foreign body	4	3,6	10	3,5	1,000	1	6,3	4	4,3	0,555
Fatal hemorrhage (non traumatic)	0	0,0	4	1,4	0,579	0	0,0	3	3,2	1,000
Intestinal torsion	4	3,6	20	7,0	0,248	0	0,0	2	2,2	1,000
Other	1	0,9	6	2,1	0,679	0	0,0	0	0,0	-
Unknown	38	34,5	106	37,1	0,727	5	31,3	46	49,5	0,278

Table 10 The differences in frequency of causes of death in seals examined between 2009-2014 and seals examined between 1979-2008(2).

First the harbour seals of each study were statistically compared to each other. The current study contained 110 harbour seals and the study Osinga et al described(2) contained 286 harbour seals. Significant differences were detected in six causes of death. Significantly more harbour seals died due to pup starvation (P=0.012) and inferred bycatch (P=0.000) between 1979 and 2008(2). On the other hand, between 2009 and 2014 significantly more harbour seals died due to parasitic bronchopneumonia (P=0.000), septicaemia (P=0.000), emaciation of unknown origin (P=0.000) and physical trauma (P=0.026).

The grey seals of each study were compared as well. Between 1979 and 2008 there had been 93 stranded grey seals that were examined. Between 2009 and 2014 there were 16 grey seals that had a decomposition code of 1, 2 or 3. Comparing these groups showed that there were statistically more grey seals between 2009 and 2014 that died due to septicaemia (P=0.040) and emaciation of unknown origin (P=0.000).

After exclusion of the seals with DCC 4 and 5 there were 110 harbour seals left of which 20 died due to septicaemia (18,2%) and 16 grey seals of which three seals had septicaemia as cause of death (18,8%). These new values were compared to the findings between 1979 and 2008(2). In the period 1979-2008 there were 14 of 286 harbour seals (4,9%) with septicaemia and three of 93 grey seals (3,2%). Between these periods there was a significant difference in occurrence of septicaemia as cause of death in harbour seals (P=0.000) and in grey seals (P=0.040).

After exclusion of the seals with DCC 4 and 5 there were nine harbour seals that had died due to their poor nutritional condition (8,2%). When comparing to the harbour seals examined between 1979 and 2008(2), where none of the 286 harbour seals died from this abnormality, there is a significant difference (P=0.000). There were 16 grey seals that had DCC 1-3, of which 5 died because of malnutrition (31,3%). These were compared to the grey seals (n=93) examined between 1979 and 2008(2) of which not one grey seal died from emaciation. This difference was significant (P=0.000).

Between 2009 and 2014 nine of 110 harbour seals that died because of trauma (8,2%) and one of 16 grey seals (6,3%). From the seals examined between 1979 and 2008 there were eight of 286 harbour seals (2,8%), in which death was caused by severe trauma and five of 93 grey seals (5,4%) (2). Comparing the harbour seals and grey seals from the current study with the seals examined between 1979 and 2008, (2) showed no significant difference in grey seals (P=1.000) but in the harbour seals dying from severe trauma turned out to be significantly more present in the current study.

Between 2009 and 2014 four of the stranded harbour seals (3,6%) and four of the stranded grey seals died due to foreign bodies (4,3%). There were no significant differences found when the harbour seals were compared to the harbour seals examined between 1979 and 2008(2) (P=1.000) neither when the grey seals were compared to the grey seals examined between 1979 and 2008 (2) (P=0.555).

4. Discussion

4.1 Morphological findings

Comparing the morphological findings between the seal species in the study, grey seals proved to suffer more often from gastric parasites and bleeding or haematoma of the skin, compared to the harbour seals. In harbour seals nematodiriasis in the bronchial tree and bronchopneumonia were found more often. These findings could not be statistically tested, because of time limitations of the study.

4.2 Causes of death

Comparing the causes of death between the seal species, it was shown that grey seals died significantly more often due to emaciation (P=0.023) and inferred bycatch (P=0.040), than harbour seals. To explain this, the causes of death has to be compared to the stranding data that were collected during this study. The grey seals that died from emaciation, mostly died during the winter months, this has to be statistically tested, but that was not in this study's scope. One possible interpretation of this finding is as follows. The grey seals pupping season is in the winter, from October to November(16,17), the seals might have higher energy needs in this season, because they need to feed their pups. Because the most important food source of the grey seal, herring, is mostly caught in October, November and December(18) (See figure 4), there might develop a shortage of food for the grey seals. Seals could get emaciated because of these circumstances. The harbour seals pupping season is in the summer months(19), so they would not have as much problems with the herring being cached mostly in the winter. This could explain why the grey seals were scored as inferred bycatch as well, they might had to take more risks to find enough food. However there were only three seals of the entire study that died due to inferred bycatch(n=206), of which one was a harbour seal and two were grey seals. Because these experimental groups are very small, it is questionable whether they can be used to couple these findings to the wild population of seals in the Wadden Sea. This explanation is speculative, further research is needed to be able to draw conclusions regarding these findings.

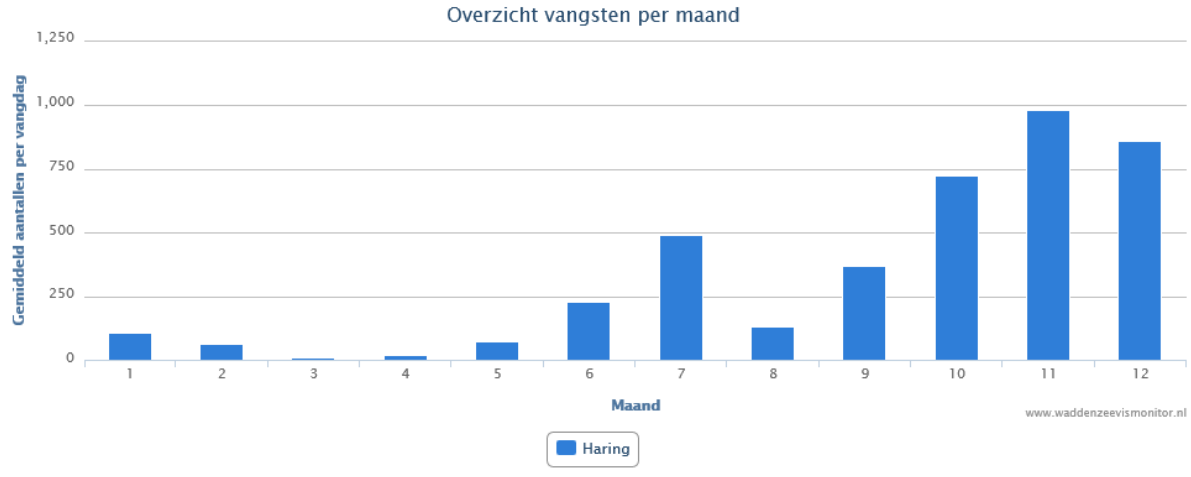

Figure 4 Herring is mostly caught in the months October, November and December(18).

Lungworms were found in 31,6% of all seals. Infestation of parasites in the lung have negative effect on the ability of breathing and diving of the animal, which makes it harder to catch fish(15). Seals that suffer from parasites in the lung therefore are at risk to develop emaciation. Comparing to the amount of seals in which the cause of death was determined as parasitic bronchopneumonia (13,6 % of all seals), this shows parasites in the lung do not necessarily kill them.

The causes of death in seals that were found dead or died within 24 hours of stranding, were compared to seals that had been euthanized within 24 hours of stranding. There were significantly more euthanized seals that died from parasitic bronchopneumonia (P=0.004) compared to the seals that were found dead.
Seals with parasitic bronchopneumonia do not necessarily die from this disease, but when they were coughing and had trouble breathing, this could have been a reason for the rehabilitation shelter to euthanize the seal. However, the exact reasons to euthanize these seals were not recorded.
In seals that were found dead on the coastlines, pathologists were significantly less frequent able to determine the cause of death (P=0.012) in comparison to the euthanized harbour seals. This could have been cause due to the fact that they probably were less fresh than the euthanized seals. The seals that were euthanized, mostly were frozen immediately after the animal was euthanized, the seals that were found on the beach had to be found by someone at first and then had to be transported to a freezer and in this time the carcass already starts rotting. The cause of death is difficult to determine when a carcass is rotten.

Of the 26 harbour seals that died from parasitic bronchopneumonia, there was only one subadult, all of the others were juvenile. This indicates that juvenile seals are more vulnerable for parasitic infections, compared to subadult and adult seals. Within this research there were no clues why the juvenile seals were more vulnerable but other studies suggest it could have something to do with the effectiveness of the immune system in juvenile seals could have been decreased by PCB's(20). The level of PCBs is extremely high in juvenile seals, because when the mother mobilises lipids for the milk out of the blubber layer, the PCBs in the blubber layer also are being mobilised(21). The mother transfers the PCBs she stored in the blubber to the pup(21). The PCB level rises till the moment the pup becomes independent from the mother's milk, it accumulates in the body when the pup ages and only starts decreasing when the seal gets pups(21). This indicates that juvenile seals have higher risk of inadequate immune system due to PCBs. The PCB levels were not measured in this research, but in following studies they might be interesting to investigate.

4.3 Comparing causes of death found in harbour seals with harbour seals examined between 1996-2005(1)

The cause of death significantly more often remained unknown in seals that stranded between 2009 and 2014, comparing to seals that were examined between 1996 and 2005(1) (P=0.000). This can be explained, because between 1996 and 2005 a lot of ill seals that were severely weakened had been killed by shootings, 161 animals from a total of 280 seals in the study(1). These seals were examined and included in the study(1). Because so many seals were killed, comparing to only 17 euthanized seals in this study which contains 206 seals, a very high percentage of seals was examined soon after it died. Between 2009 and 2014 the larger part of the seals was found on the beach and could have started rotting before they were collected and frozen. This could have had negative influence on the ability to determine the cause of death in these animals.

Between 2009 and 2014 significant more seals died from pup starvation (P=0.009). In the seals examined between 1996 and 2005 there was not a single seal of all 355(1), in which this cause of death was determined. This suggests that the two studies used different criteria to categorize the seals in this group. Comparing the seals examined between 2009 and 2014 to the seals examined between 1996 and 2005(1) also shows, that more harbour seals died due to flipper abscesses (P=0.029) and foreign bodies (P=0.001). These differences could not be declared.

Between 1996 and 2005(1) significantly more seals died due to perinatal death (P=0.000) and ecto- and endoparasitism(P=0.012), comparing with this recent study. Significantly more seals also died due to parasitic bronchopneumonia(P=0.007).
This could be related to the phocine distemper virus epidemic in 2002, which is suspected to have had big influence on the immune system of the seals(4,5). The fact that less seals died from these causes between 2009 and 2014, indicates that the populations immune system has recovered from this outbreak in 2002.

4.4 Comparing causes of death found in harbour seals and grey seals with harbour seals and grey seals examined between 1979-2008(2)

When comparing the harbour seals examined between 2009 and 2014 to the harbour seals examined between 1979 and 2008(2), there are significantly more seals with parasitic bronchopneumonia the recent period described in this report (P=0.000). This seems to be in contradiction with the earlier found significant difference, that showed that more seals died due to parasitic bronchopneumonia between 1996 and 2005(1).
This shift is the effect of removing all seals with DCC 4 and 5 out of this study, which had to be done because these seals were excluded from the report describing the seals examined between 1979 and 2008(2) as well. When these seals with DCC 4 and 5 had not been removed before comparing the results from this study to the results in the report of the seals examined between 1979 and 2008(2), comparing would not be possible because of the different criteria used for the two groups. Because of the fact that these two groups with high decomposition code (DCC 4 and 5) both had high percentages of unknown causes of death, respectively 74,2% and 88,2%, removing the seals with the DCCs 4 and 5 (total of n=79), resulted in a shift from unknown cause of death to other causes including parasitic bronchopneumonia.

The occurrence of septicaemia as cause of death in both harbour seals and grey seals was significantly higher between 2009 and 2014 comparing with the results between 1979 and 2008(2). This could have been caused by a broader interpretation of septicaemia.
The amount of both harbour seals and grey seals that died due to emaciation of unknown origin has increased from zero between 1979 and 2008(2), to 15 of 206 examined seals between 2009 and 2014. Because none of the seals that were examined between 1979 and 2008 died from emaciation(2), there might have been a different formulation that was used in both studies for emaciation of unknown origin, that resulted in these differences.
Harbour seals that stranded between 2009 and 2014 died more often due to trauma comparing with harbour seals that stranded between 1979 and 2008(2). In grey seals there was no significant difference. This difference could not be declared.
Of both species there were significantly more that died due to septicaemia between 2009-2014 comparing to 1979-2008(2).

4.5 Discussion points of the study

The study contained 158 harbour seals and 22 grey seals. Because of the relatively small group of grey seals, one grey seal with a specific cause of death had much more impact on the percentage of seals that died from it than one harbour seal.
In August, 26.788 harbour seals(8) were counted by the Trilateral Seal Expert Group (TSEG) and 2.785 grey seals were counted(9). Because one grey seal has such a big influence in percentages of causes of death, and the experimental group is so small comparing to the total population of seals in the Wadden Sea, it is hard to extract these findings to the wild population.
There also was a large difference in n-value between the euthanized seals and the seals that were found dead or died soon after stranding. This division between seals has been made to be able to investigate the difference in cause of death in euthanized seals and seals that died themselves. Because of the small group of euthanized seals the distribution of causes of death between these groups is unstable. More research is required in larger groups, with large n-values in both euthanized seals and seals that died themselves, to investigate if there are significant differences between these groups of seals.

In several carcasses there were multiple important abnormalities found that could have caused the animal's death. In these cases there were more causes of death scored for one animal. In the cases of harbour seals PV 14, PV 24, PV 79, PV 103, PV 104, PV 143 and PV 144 the animals suffered from bronchopneumonia as well as septicaemia, the animals could have died from both these diseases. In harbour seal PV 199 pneumonia of undetermined cause and septicaemia were found. Both of these findings were scored as cause of death for this animal, since the animal could have died due to both of these abnormalities. Of the grey seals, there were three that had multiple causes of death. HG 6, where there was a foreign body found and septicaemia, HG 17, which suffered from parasitic bronchopneumonia and septicaemia, and HG 24, where both the flipper abscess and the septicaemia could have been the trigger for the decision to euthanize the seal.
The fact that we have two causes of death for 11 animals has to be taken in account when interpreting the percentages given in table 6.

Of all examined seals between 2009 and 2014, the cause of death could not be determined in 50,5%. This high percentage can be accounted for, because a lot of them were putrefied at necropsy. There were a lot of carcasses that were scored as putrefied (DCC 4) or very putrefied (DCC 5), of these seals there was a high percentage in which the cause of death could not be determined and were scored as unknown (see Figure 3). When these seals are excluded the percentage unknown causes of death decreased and relatively more causes of death were determined. It is recommended to use only relatively fresh seals, with DCC up to 3, as it is shown this increases the chance that the macroscopic abnormalities are found and there are better possibilities to determine the cause of death. In these seals it was still possible to describe the macroscopic abnormalities and to perform histology on the samples that were taken. Because the organs were still intact and not rotten, the cause of death can be determined more often.

4.6 Recommendations further research

Because of time limitations of this research project, it was not possible to statistical analyse all found abnormalities and compare them to earlier studies. This would have been interesting, because then it would be possible to compare very specific findings, to see if there are significant differences in, for example, the presence of heart- or lungworms between these periods of time. This cannot be concluded when only the causes of death are being compared, because not every seal dies due to these parasites.

It would have been very interesting to see if there was a relation between the stranding locations and the causes of death, but due to time limitations of the study this was not possible. This could have given information about the prevalence of interaction with fishing material that could be higher in specific areas in the Wadden Sea or if seals got sicker in specific area's possibly due to pollution.

Despite the fact the found carcasses were stored frozen, some carcasses that lay on the beach long time before they were collected or even died before they stranded, were putrefied at necropsy. In animals that were rotten or only consisted of remains the cause of death could not be determined. Because of so many cases in which the cause of death was unknown, it is not possible to draw conclusions of it.
In future researches it will be very important to examine the possibility to perform necropsy on as many fresh animals as possible.
Hopefully there will be time and money enough, so that the seals do not need be frozen at first. In frozen animals the interpretation of lung oedema becomes impossible. Because of the freezing the lungs show oedema, but it is still possible to determine whether there also was lung oedema before the carcass was frozen. There cannot be concluded whether the seal had drowned or not. Since drowning/lung oedema is a very important criteria for bycatch, it will be very interesting if we can be certain the seal has drowned in future studies. The chance to actually score an animal as bycatch would improve, and this would decrease the percentage of seals in which the cause of death could not be determined.

5. Conclusion

The first hypothesis of the study was, that there were no differences in causes of death between the species, but statistical analysis showed that there was. The grey seals significantly died more often due to emaciation (P=0.023) or inferred bycatch(P=0.040), in comparison with the harbour seals. The null hypothesis is rejected in this case.

The second hypothesis of the study was, that there were no significant differences in causes of death between the seals that were found dead or died within 24 hours of stranding compared to the seals that were euthanized. In the grey seals there was no difference between these groups, the null hypothesis was retained in this case. Statistical analysis showed that there was a significant difference between these groups in the harbour seals. Harbour seals that had been euthanized significantly died more often due to parasitic bronchopneumonia (P=0.004). In harbour seals that were found dead or died within 24 hours of stranding died the cause of death significantly more often remained unknown.

The last hypothesis of the study was, that there were no differences between this studies results when comparing to earlier studies(1,2)**.**
The harbour seals of this study were compared to harbour seals examined between 1996 and 2005(1). Significantly more harbour seals died from parasitic bronchopneumonia (P=0.007), perinatal death (P=0.000) and ecto- and endoparasitism (P=0.012) between 1996 and 2005. The other way around, more harbour seals examined between 2009 and 2014 died due to flipper abscesses (P=0.029), pup starvation (P=0.009), foreign bodies (P=0.001) and unknown causes (P=0.000).
Seals were also compared with seals that were examined between 1979 and 2008(2). Significantly more harbour seals died due to pup starvation (P=0.012) and inferred bycatch (P=0.000) between 1979 and 2008. On the other hand, between 2009 and 2014 significantly more harbour seals died due to parasitic bronchopneumonia (P=0.000), septicaemia (P=0.000), emaciation of unknown origin (P=0.000) and physical trauma (P=0.026).

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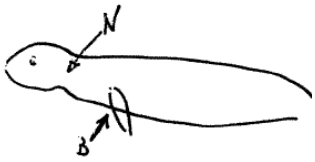
Addendum 1: Seal necropsy protocol

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

skin)

Diagram 1 – blubber

(including

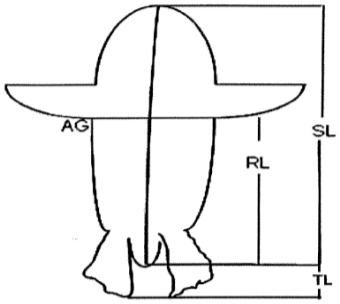


Diagram 2 - morphometry

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)	

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

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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 Necropsy form - 3	Number	.....	GLIMS	.....
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				

Texel *UU* *CVI*

	Cass. Nr. formaline	*4 hoekig buisje*	*zakje*	*Schroefdop Alc. 70%*	*Bruin epje halfvol*	*Melk buisje*	*zakje*	*zakje*	*Epje Alc. 70%*
	HP	**-80**	**-20**	**Parasites**	**Vit. A (- 20)**	**Brucella CVI (-20)**	**TX Alu**	**TX PL**	**Life History**
Skin		Lesions	Lesions						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!!!

Addendum 2: Database of all seals included in this study with determined cause of death.

Serie	Carcass	Age	Sex	DCC	NCC	SL (cm)	Macro conclusion	Cause of death
PV	1	S	F	2	1	120	Infectious	Unknown
PV	2	S	M	2	3	107,5	Infectious	Septicaemia
PV	4	J	F	2	4	94	Unknown	Septicaemia + trauma
PV	5	J	M	3	2	94	Trauma	Physical trauma
PV	9	A	F	1	3,5	146	Tijdens zenderen dood	Unknown
PV	10	J	M	4	6	73	Starvation	Unknown
PV	13	J	F	4	U	78,5	Unknown	Unknown
PV	14	J	F	1	4	84	Infectious	Parasitic bronchopneumonia + sepsis
PV	15	J	M	3	5	84	Emaciation of unknown origin	Cachexia/ emaciation of undetermined cause
PV	16	A	F	2	3	138	Infectious	sepsis (from endometritis: perinatal death?)
PV	18	J	M	2	3	89	Pneumonia	Parasitic bronchopneumonia
PV	19	J	F	3	3	90,5	Unknown	Unknown
PV	20	J	F	2	3	91	Pneumonia	Parasitic bronchopneumonia
PV	21	J	M	3	4	84,5	Starvation	Pup starvation
PV	23	J	M	3	4	83	Emaciation of unknown origin	Parasitic bronchopneumonia
PV	24	J	F	2	3	90	Pneumonia	Parasitic bronchopneumonia + sepsis
PV	25	J	F	2	4	85	Emaciation of unknown origin	Cachexia/ emaciation of undetermined cause
PV	27	J	M	2	4	80,5	Unknown	Unknown
PV	28	J	M	2	4	79	Infectious	Euthanasia; Flipper/ skin abcess
PV	30	J	F	2	3	91,5	Infectious	Septicaemia incl multiparasitic infestation
PV	32	J	F	2	3	82	Trauma?	Unknown
PV	33	J	F	2	3	94	Pneumonia	Parasitic bronchopneumonia
PV	34	A	F	2	2	134	Unknown	Unknown
PV	35	A	F	2	2	138	Unknown	Septicaemia incl multiparasitic infestation
PV	36	A	F	3	2	140	Liver rupture?	Unknown/ Fatal hemorrhage (non traumatic)
PV	37	J	M	3	4	89	Infectious	Septicaemia incl multiparasitic infestation
PV	38	J	M	2	4	106	Infectious	Flipper/ skin abcess
PV	39	N	F	4	3	70	Unknown	Unknown
PV	41	A	M	3	4	144	Possible bycatch	Unknown
PV	44	J	F	3	6	85	Blunt Trauma	Physical trauma
PV	45	S	F	2	5	105	Unknown	Parasitic bronchopneumonia

PV	47	J	M	2	4	102	Infectious	Parasitic bronchopneumonia
PV	49	S	M	3	5	136,5	Trauma	Physical trauma
PV	51	J	F	3	1	103	Unknown	Unknown
PV	52	A	F	3	3	152	Unknown	Unknown
PV	54	J	M	3	4	88	Emacation of unknown origin	Unknown
PV	56	J	M	3	3	97	pneumonia	Parasitic bronchopneumonia
PV	60	J	F	3	5	80	T61 in rehab	Euthanasia; Parasitic bronchopneumonia
PV	62	J	F	3	3	95	Unknown	Unknown
PV	66	J	M	2	4	96	T61 in rehab	Euthanasia; Parasitic bronchopneumonia
PV	68	J	F	2	5	86	Drowning	Parasitic bronchopneumonia
PV	69	J	F	3	4	90	Unknown	Cachexia/ emaciation of undetermined cause
PV	71	J	M	3	3	96	Died in rehab	Euthanasia; Unknown, possibly trauma
PV	72	J	M	3	3		Unknown	Unknown
PV	73	A	F	3	3	139	Unknown	Unknown
PV	75	A	F	3	6	135,5	T61 in rehab / unknown.	Foreign body (food aspiration)
PV	76	J	M	4	4	94	Unknown	Unknown
PV	78	U	F	1	1		Unknown	Septicaemia incl multiparasitic infestation
PV	79	J	M	2	5	95	T61 in rehab	euthanasia (paras br pneum + sepsis omphalophlebitis)
PV	81	A	F	2	4	138	Unknown	Unknown
PV	82	A	F	3	1	140	Trauma	Physical trauma
PV	85	A	M	2	2	147	Torsio of the jejunum trough an opening in the mesenterium	Intestinal torsion
PV	86	A	F	3	2	134	Unknown	Unknown
PV	87	J	F	2	5	90,5	Parasitic infestation and damage to the lungtissue	Parasitic bronchopneumonia
PV	88	J	M	2	3	96	Pneumonia, lungworm	Septicaemia incl multiparasitic infestation
PV	92	A	F	3	1	150	Blunt trauma	Physical trauma
PV	94	A	M	3	4	152	Sepsis because of old wounds?	Unknown (possibly sepis from skin wound)
PV	96	S	F	3	3	114	Blunt Trauma	Unknown (suspected trauma)
PV	99	J	F	2	4	64	T61 in rehab	euthanasia (paras br pneum)
PV	100	J	F	2	4	97	Extensive necropurulent peritracheitis	Septicaemia incl multiparasitic infestation
PV	103	J	F	1	3	85	T61 in rehab	euthanasia (paras br pneum + septicaemia)
PV	104	J	F	1	3	90	T61 in rehab	euthanasia (paras br pneum + septicaemia)
PV	109	A	F	4	2	135	Unknown	Unknown

PV	110	J	F	2	5	93	Parasite infestation, multiple organs	Unknown
PV	111	S	F	4	4	118	Unknown	Unknown
PV	112	A	F	4	3	134	Unknown	Unknown
PV	114	S	F	2	2	104	Sepsis	Unknown
PV	115	J	M	3	2	102	Pneumonia	Pneumonia of undetermined cause/ unknown etiology
PV	117	N	M	5	6	77	Unknown	Unknown
PV	118	J	M	4	2	106	Unknown	Unknown
PV	119	J	M	2	2	102	Possibly pneumonia	Pneumonia of undetermined cause/ unknown etiology + septicaemia
PV	121	J	M	4	5	84	Unknown	Unknown
PV	123	N	F	4	3	79	Unknown	Unknown
PV	124	J	F	4	1	96	Unknown	Unknown
PV	126	J	F	3	5	89	Pancreatitis	Gastro-enteritis
PV	128	J	M	2	4	81	Sepsis due to polyarthritis	Septicaemia incl multiparasitic infestation
PV	132	J	F	2	6	94	Lungworm infestation with emaciation	Parasitic bronchopneumonia
PV	133	J	F	2	1	103	Liver disease	Unknown (possibly trauma)
PV	136	A	M	4	3	145	Severely enlarged right kidney	Septicaemia incl multiparasitic infestation
PV	137	S	F	2	2	127	Trauma	Unknown
PV	138	J	F	4	-	76	Gastric ulcers	Unknown
PV	141	U	M	4	-	-	Unknown	Unknown (possibly foreign body: fish hook in stomach)
PV	142	J	M	4	-	97,5	Unknown	Unknown
PV	143	J	F	1	2	96	Lungworms, chronic pneumonia right with pyothorax and metastatic hepatitis.	Parasitic bronchopneumonia + sepsis
PV	144	J	F	2	3	83	Pneumonia, lungworm	Parasitic bronchopneumonia + sepsis
PV	148	N	F	4	4	73	Unknown	Unknown
PV	149	N	F	2	6	72	Unknown	Cachexia/ emaciation of undetermined cause
PV	151	J	F	4	-	89	Possibly trauma	Unknown (possibly trauma)
PV	153	J	M	4	6	82	Unknown	Septicaemia incl multiparasitic infestation
PV	154	S	M	4	4	136	Unknown	Unknown
PV	155	S	M	4	4	110	Trauma	Physical trauma
PV	160	S	M	2	2	133	Infectious	Intestinal torsion
PV	161	J	M	2,5	3	92	Infectious?	Unknown
PV	162	S	F	4	-	125	Unknown	Unknown
PV	163	J	F	2,5	1	101	Possible bycatch	Foreign body (food aspiration)
PV	165	J	F	3	3,5	88,5	Possible bycatch	Unknown
PV	167	J	M	2,5	4	85	Infectious due to severe parasitic infestation of the	Unknown

							lungs?	
PV	168	N	F	4	4	68,5	Starvation	Pup starvation
PV	169	J	M	2	5	84	Euthanesia, due to slow reactions	Euthanasia (multiple parasites, possibly sepsis)
PV	170	J	M	2	4	91	Unknown	Unknown
PV	171	S	F	2	2	108,5	Suffocation	Foreign body (food aspiration)
PV	172	A	M	2	1	147	Suffocation	Unknown
PV	173	J	F	3	5	84	Emaciation	Cachexia/ emaciation of undetermined cause
PV	174	J	M	2	5	95	Unknown, probably lungworms	Parasitic bronchopneumonia
PV	175	J	M	2	4	77	Euthanesia, lungworm	euthanasia (paras bronchopneumonia)
PV	177	J	F	3	5	82	Lung + heartworm, emaciation	Parasitic bronchopneumonia
PV	178	J	M	2	2	97	Lungworm	Parasitic bronchopneumonia
PV	179	A	F	4	1	158	Unknown	Unknown (perinatal death?)
PV	180	A	F	4	1	143	Unknown	Unknown (perinatal death?)
PV	181	A	F	4	1	151	Unknown	Unknown
PV	182	A	F	4	1	134	Unknown	Unknown
PV	183	J	F	4	-	93	Unknown	Unknown
PV	184	A	M	5	-	149	Bycatch	foreign body (fish hooks)
PV	185	A	M	4	-	143	Unknown	Unknown
PV	186	J	F	4	3	66	Emaciation	Cachexia/ emaciation of undetermined cause
PV	188	J	M	2	4	98,5	Euthanasia	euthanasia (other causes)
PV	189	J	M	4	-	78	Unknown	Unknown
PV	190	J	M	2	4	85	Heartworm + stomatitis	Septicaemia incl multiparasitic infestation
PV	192	S	F	5	-	106	Bycatch	foreign body (fish hooks stomach)
PV	193	J	F	4	-	73	Unknown	Unknown
PV	194	J	M	2	5	57,5	Likely lungworm infection	Parasitic bronchopneumonia
PV	196	J	M	2	5	92	Euthanasia	Euthanasia (cachexia/ emaciation of undetermined cause)
PV	197	J	M	4	unknown	99	Unknown	Unknown
PV	198	J	F	2	6	84	Emaciation	Cachexia/ emaciation of undetermined cause
PV	201	J	M	2	6	85	Euthanasia	Euthanasia; Cachexia/ emaciation of undetermined cause
PV	202	J	M	2	4	85	Euthanasia	Euthanasia (flipper/ skin abcess)
PV	203	S	M	2	2	114	Hepatitis?	Unknown
PV	205	S	F	2	6	123	Emaciation	Cachexia/ emaciation of undetermined cause

PV	206	A	M	4	unknown	154	Unknown	Unknown
pv	207	A	F	3	2	149	Unknown	Unknown
PV	208	S	F	3	3	129	Suffocated in fish	Foreign body (food aspiration)
PV	209	J	M	1	6	91	Bite trauma in head, with penetrations in skull	Physical trauma (bite wounds)
PV	210	S	F	3	2	127	Unknown	Unknown
PV	212	A	M	4	unknown	152	Bycatch highly probable	Bycatch, inferred
PV	213	S	M	4	unknown	140	Unknown	Unknown
PV	214	A	F	3	2	160	External trauma, broken neck	Physical trauma
PV	215	A	F	4	unknown	144	Dystocia?	Dystocia
PV	218	J	F	2	2	83	Euthanasia	Euthanasia (Unknown)
PV	219	S	F	3	-	114	Unknown	Unkown
PV	220	J	F	2	2	96	Unknown	Unknown
PV	221	A	F	2	2	143	Torsio Coli	Intestinal torsion
PV	225	U	F	4	3	98	Trauma	Unknown
PV	226	N	M	4	unknown	73	unknown	Pup starvation
PV	227	J	F	2	2	98	unknown	Unknown
PV	228	A	M	4	unknown	147	Unknown	Unknown
PV	229	N	M	4	unknown	75	Unknown	Pup starvation
PV	231	J	M	2	3	95	Unknown	Unknown
PV	232	J	F	4	unknown	100	Unknown	Unknown
PV	233	N	M	5	unknown	81	Unknown	Unknown
Pv	234	J	M	2	3	88	Sulfocation?	Parasitic bronchopneumonia
PV	236	N	F	4	unknown	80	Possibly abortion	Unknown
PV	238	J	F	2	2	95	Unknown	Unknown
PV	243	A	M	4	unknown	136	Unknown	Other (peritonitis of undetermined origin)
PV	246	S	F	4	unknown	99	Unknown	Unknown
PV	247	S	M	3	unknown	110	Trauma	Trauma
PV	248	S	M	2	2	109	Unknown	Unknown
PV	215F	N	F	4	unknown	unknown	Dystocia mother	Intestinal torsion (of mother)
PV	221F	J	M	2	2	76	Intra-Uterine death due to torsio coli mother	Dystocia (of mother)

Serie	Carcass	Age	Sex	DCC	NCC	SL (cm)	Macro conclusion	Cause of death
HG	1	J	F	4	4	88,5	No evidence	Cachexia/ emaciation of undetermined cause
HG	2	J	M	2	6	100,5	No evidence	Cachexia/ emaciation of undetermined cause
HG	3	J	M	2	5	93	No evidence	Unknown, possibly sepsis (adrenalitis)
HG	4	S	M	3	3	153	Unknown	Unknown, possibly sepsis (stomatitis)
HG	5	J	M	2	2	127	Certain	Bycatch, confirmed
HG	6	S	F	2	4	158	No evidence	Foreign body (fish in trachea) + Septicaemia incl multiparasitic infestation
HG	7	J	M	2	3	112	No evidence	Unknown (possibly trauma)
HG	9	J	F	3	2	118	Possible	Physical trauma
HG	10	J	M	4	4	134	Unknown	Physical trauma
HG	11	J	F	2	2	-	Possible	Unknown
HG	14	J	F	2	4	90	No evidence	Cachexia/ emaciation of undetermined cause
HG	17	J	M	2	5	109	No evidence	Parasitic bronchopneumonia + Septicaemia incl multiparasitic infestation
HG	19	A	F	3	2	171	Probable	Bycatch, inferred
HG	24	J	F	2	4	88	No evidence	Euthanasia; flipper/ skin abcess + Septicaemia incl multiparasitic infestation
HG	25	N	M	2	5	95	Possible	Pup starvation? Or Cachexia/ emaciation of undetermined cause
HG	26	N	F	2	1	100	No evidence	Unknown
HG	27	J	M	4	Unknown	132	No evidence	Bycatch, inferred
HG	28	A	M	3	4	208	No evidence	Euthanasia; Unknown
HG	29	J	M	4	Unknown	84	Unknown	Unknown
HG	30	S	M	2	6	143	No evidence	Cachexia/ emaciation of undetermined cause
HG	32	A	M	4	Unknown	205	No evidence	Unknown
HG	131	S	M	4	4	145,5	No evidence	Unknown

Serie	Carcass	Age	Sex	DCC	NCC	SL (cm)	Macro conclusion	Cause of death
PV/HG	11	S	U	5	1	128	Unknown	Unknown
PV/HG	12	N	U	5	4	81	Unknown	Unknown
PV/HG	15	N	M	3	5	84	Emaciation of unknown origin	Unknown
PV/HG	16	N	F	2	3	138	Infectious	Septicaemia incl multiparasitic infestation
PV/HG	31	J	F	5	3	104	Unknown	Unknown
PV/HG	48	A	M	4	3	150	Unknown	Unknown
PV/HG	50	N	M	4	6	87	Unknown	Unknown
PV/HG	80	J	F	5	unknown	94	Unknown	Unknown
PV/HG	83	U	M	3	3	unknown	Chronic pneumonia	Parasitic bronchopneumonia
PV/HG	105	J	F	5	unknown	81	Unknown/possible bycatch?	Unknown
PV/HG	120	N	M	4	5	69	Unknown	Unknown
PV/HG	122	N	F	4	3	77	Still birth	Perinatal death
PV/HG	135	S	M	4	3	134	Unknown	Unknown
PV/HG	139	N	M	4	unknown	74	Unknown	Unknown
PV/HG	145	U	U	5	unknown	unknown	Unknown	Unknown
PV/HG	147	N	F	5	unknown	58	Unknown	Unknown
PV/HG	150	J	F	4	4	93	Unknown	Unknown
PV/HG	176	U	U	5	unknown	unknown	Unknown	Unknown
PV/HG	216	J	F	4	unknown	88	Unknown	Unknown
PV/HG	224	U	F	4	unknown	unknown	Unknown	Unknown
PV/HG	230	N	F	5	unknown	80	Unknown	Unknown
PV/HG	235	U	M	5	unknown	unknown	Unknown	Unknown
PV/HG	237	N	U	5	unknown	unknown	Unknown	Unknown
PV/HG	239	U	M	5	unknown	unknown	Unknown	Unknown
PV/HG	240	U	U	5	unknown	unknown	Unknown	Unknown
PV/HG	241	N	M	4	unknown	71	Unknown	Unknown