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Analysis of stranding data and pathological findings in stranded harbor seals and grey seals on Texel and the North-west coast of the Netherlands between 2009 and 2012



Master Research Project Veterinary Medicine
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Contents

Summary

Stranding data and pathological findings were analyzed for harbor seals and grey seals that stranded on Texel and the North-West coast of the Netherlands between 2009 and 2012. Stranding data were analyzed for 170 stranded seals (137 harbor seals, 16 grey seals, 17 unknown) and consisted of the date of stranding, stranding location, age category, sex and species. Pathological findings were evaluated for 40 seals (38 harbor seals and 2 grey seals) that died or were euthanized due to severe illness in rehabilitation centre Ecomare during the winter of 2011 to 2012. Weight, sex, lengths, decomposition condition code, nutritive condition code and macroscopical observations of organs and lesions were analyzed. Samples of organs were collected for histological, virological (morbillivirus, phocine herpesvirus, influenzavirus), bacteriological and parasitological examination. Stranded seals were mainly in the age category of juvenile. Strandings were most frequent in June and July (neonate harbor seals) which corresponds with the pupping season of this species. The highest stranding rate was during the winter months (December and January), which indicates that juveniles in particular may have difficulties with surviving winter due to bad weather conditions and a not completely developed immune system. Most frequently found observations in rehabilitation seals were a poor nutritive condition (52,5%), skin ulceration (28,9%), subcutaneous hemorrhage (18,4%), lungworm infection (70%), interstitial edema (62,5%) and hyperemia (47,5%) (acute pneumonia), lung atelectasis (26,3%), multifocal necrosis in the liver (10,5%) and parasite infections of the gastro-intestinal tract (72,5%). Acute pneumonia was in most cases associated with the presence of lungworms (*Otostrongylus circumlitus* and *Parafilaroides gymnurus*). Bacterial infections were suggested to be secondary to verminous pneumonia. Poor nutritive condition in stranded seals was probably due to disease and a lack of feeding because of separation from the mother. Lungworm infections were in most cases mild, but because of the frequent findings of acute pneumonia and the relatively high number of unexpected deaths of seals after an apparently successful completed treatment, it is suggested that that the provided treatment in rehabilitation was not sufficiently effective in overcoming the lungworm infestation and associated inflammation.

Introduction

Harbor seals and grey seals

Since seals populating the Dutch Wadden Sea are accessible for a wider audience, they are an important topic of wildlife in the Netherlands. The expansion of recreation along the Dutch coast, information provided by the media and the attention of environmental organizations and seal rehabilitation centers have ensured that more interest has arisen in the Dutch seal population.
The Wadden Sea is mainly populated by harbor seals (*Phoca vitulina*) and fewer grey seals (*Halichoerus grypus*). Among the harbor seals, a distinction is made between four subspecies: *P. vitulina stejnegeri* (West-Pacific Ocean), *P. vitulina richardsi* (East-Pacific Ocean), *P. vitulina concolor* (West-Atlantic Ocean), and *P. vitulina vitulina* (East-Atlantic Ocean). The latter subspecies is found in the waters around Iceland and in Europe around the British Islands, Ireland, the North-Sea coasts of Denmark, Bretagne (France) and the Netherlands, Skaggerak and Kattegat, along the West coast of Norway and the South-West part of the Baltic Sea. Sandy beaches and sand bars along coasts are used by seals as haul-out sites, which are used for resting, thermoregulation, access to foraging sites and pupping, nursing and moulting (Murray et al., 2008). Harbor seals are considered as non-migrating, but the distance of travelling varies within a population. Most seals stay in a range of 5 to 25 kilometres close to their primary haul-out site, but others may travel more than 100 kilometres for foraging and mating (Peterson et al., 2012, Sharples et al., 2012). Female harbor seals have a gestation time of 8-8,5 months (Siebert et al., 1990) and their pupping season is from May to July. Juveniles are weaned at 3 to 6 weeks. Compared to grey seals, harbor seals have a round head and a relatively distinct forehead when viewed in profile (*Photo 1a*). Grey seals have a much flattened forehead and nose (*Photo 1b*). In Europe they are mainly found in the waters around Great-Britain, Ireland and Bretagne (France), along the West coast of Norway, in the Wadden Sea and in the Baltic Sea, forming the subspecies *H. grypus balticus*. They forage close to their primary haul-out site, but may travel more than 100 kilometres for foraging (Breed et al., 2006). Female grey seals have a gestation time of 11 months (Yunker et al., 2006) and their pupping season is from September to December. For seals in the Wadden Sea, the pupping season may be prolonged up to January. Pups are weaned at 17-18 days and are recognizable by the typical white, long fur which they lose in two weeks after birth.

The Wadden Sea seal population

Seal population counting in the Wadden Sea is carried out by the Trilateral Seal Expert Group (TSEG). Seals are counted when hauling out on sandbanks in the Wadden Sea of Denmark, Germany and the Netherlands. A number of 6,529 harbor seals was counted in the Dutch Wadden Sea in August 2012, which is a decline of 12% compared to the counting in 2011. However, the total number of seals in the Wadden Sea including Germany and Denmark increased with 11% compared to 2011 to a total of 26,220 seals (TSEG, 2012). By contrast, in 1977 only 430 harbor seals were observed in the Dutch Wadden Sea (van Haaften et al., 1978). A total of 3,059 grey seals were counted in the Dutch Wadden Sea in the winter of 2012, which is an increase of 28% compared to 2011 (TSEG, 2012). Mortality among the population changes from year to year and depends on diseases, from which an important example is the outbreak of morbillivirus which caused mass mortality in 1988 and 2002 (Harris et al., 2008, Lonergan et al., 2010), weather conditions (TSEG, 2002), or food availability (TSEG, 2012), but also on bycatch (Roger et al., 2012) and other human-related activity (Osinga et al., 2012, TSEG, 2002).

The effect of human activity on the seal population

Despite a record number of seals in the Wadden Sea since the start of counting it is important to remember that human activity may have a significant effect on the health as well as the size of the seal population (Siebert et al., 2007, Osinga et al., 2012). Until the seventies, the population in the Wadden Sea decreased due to intensive hunting and pollution (Osinga et al., 2012, Siebert et al., 2007). When it was recognized that the conservation of the environment and marine wildlife was a concern, chemical pollutants (PCBs) and hunting were banned in the mid- and late

seventies (Kaley et al., 2006). Even though improvement has been made, the environment of the Wadden Sea today is still influenced by factors of human activity such as fishing and recreation. Chemical pollutants are still present in the aquatic environment, seen their properties of chemical stability and resistance to metabolic breakdown (Kaley et al., 2006). Since seals are on the top of the aquatic food chain, they occupy a position of great risk for accumulation of persistent pollutants, which can lead to toxic responses and an impairment of the immune response in seals (Frouin et al., 2010, De Swart et al., 1995). It is suggested that exposure to environmental factors such as PCBs and heavy metals makes marine mammals more susceptible for infectious diseases by inducing an impairment of the immune system (Beineke et al., 2007, De Swart et al., 1995). Inhibition of cellular immune function has been described in harbor seals that were experimentally fed with PCB contaminated fish (De Swart et al., 1995). On the other hand it may be possible that pathogens itself cause an impairment of the immune system. In this study I aim to investigate whether there is an immune impairment in stranded seals and if this is associated with lungworm infection. The pros and cons of the currently used method for examining the immune system in this study are also discussed.

Seal rehabilitation

Weak, ill and dead-stranded seals that strand along the Dutch coast are reported and collected by the Institute for Marine Resources and Ecosystem Studies (IMARES, Wageningen UR), Seal Rehabilitation Centre Ecomare. These organizations cover the coast of North Holland and Texel. Strandings on the rest of the Dutch coast are covered by the Seal Rehabilitation and Research Centre (SRRC). Weak and ill seals that strand alive are brought to Ecomare (Texel) or the SRRC (Pieterburen), where a rehabilitation process is started with medication, force feeding if necessary and strict monitoring. Ecomare reports that in the winter of 2011 to 2012, the condition of seals in rehabilitation deteriorated without a clear cause, followed by death or euthanasia due to severe complications. Compared to earlier years, this happened to relatively many seals, despite an apparently successful completed treatment. The symptoms that the seals showed on the first day of entry at the centre were mainly dyspnoea, tightened, shallow and abdominal breathing, coughing (in some cases coughing up blood, mixed with parasites), squeaking while breathing, emaciation, diarrhea and small wounds around the beak and on the flippers. A majority of the juvenile rehabilitation seals showing respiratory complications were suffering from lungworm infection. The lungworms that are most frequently found in harbour seals and grey seals are *Parafilaroides gymnurus* and *Otostrongylus circumlitus* (Vercruysse et al., 2003). Juvenile seals are most susceptible for the impact of lungworms, soon after weaning, while lungworm infestation is rarely found in adult seals (Vercruysse et al., 2003, Rijks et al., 2008). In this study I investigate the pathological findings in rehabilitation seals from Ecomare that stranded in the winter of 2011 to 2012. I aim to get more knowledge on frequent complications in the seal population by examining a selected group, but also aim to provide this information for the preparation of a sufficient treatment protocol and a better view on the predictability of prognosis in the future. I also pay attention to the question to which extends the rehabilitation of wildlife marine mammals is appropriate, which is currently frequently discussed by wildlife organisations, rehabilitation centers and animal welfare organisations.

Evaluation of stranding data and pathological findings

Osinga et al. (2012) reported an overview of the stranding data and pathological findings of harbor seals and grey seals that stranded on the Dutch coast between 1979 and 2008. The analysis of seasonality and geographical distribution of stranded seals was discussed and pathological examination was performed in order to provide knowledge on the most common causes of death. The results showed that the total stranding rates per month peaked in June and July for harbor seals and in January for grey seals, which corresponds with the pupping seasons of these seal species. The most common causes of death were by-catch, pup starvation, intestinal volvulus, parasitic bronchopneumonia (harbor seals) and by-catch, pup starvation and trauma (grey seals). Siebert U. et al (2007) published a simultaneous study which reports the

pathological findings of harbor seals that stranded on the coast of Schleswig-Holstein, Germany between 1995 and 2006. The results showed that the mostly affected organs of stranded seals were the respiratory and alimentary tract. The most common cause of death was bronchopneumonia caused by parasitic and/or bacterial infection of the lungs.
In this study I aim to make a comparison between the results in this study and the results of Osinga et al. (2012) and Siebert et al. (2007). I attempt to discover similarities or differences in the findings of stranding data and pathological results between the different locations (Texel and the North-West coast of the Netherlands in this study, the rest of the Dutch coast (Osinga et al., 2012) and Schleswig-Holstein (Siebert et al., 2007)), in the course of time. I am also interested in the differences between pathological findings in the studied group of rehabilitation seals and the pathological findings in dead-stranded (free-ranging) seals between 2009 and 2012, which were studied earlier in connection with this research project.

Photo 1a. Juvenile harbor seal, Department of Veterinary Pathobiology, Utrecht University, 2012

Photo 1b. Subadult grey seal, Department of Veterinary Pathobiology, Utrecht University, 2012

Materials and Methods

Stranding data

A total of 170 seals were used for the analysis of stranding data, which include the date of finding, stranding location, age class and sex. The age class and sex were determined at the Department of Veterinary Pathobiology, Utrecht University by macroscopical examination and measuring as will be described later. A distinction was made between species (PV: *Phoca vitulina* and HG: *Halichoerus grypus*). This study included 137 harbor seals and 16 grey seals. Of 17 seals the species was unknown due to the state of the carcass. The investigated group consisted of seals that were either found dead stranded, died in rehabilitation centre Ecomare or were euthanized in Ecomare between February 2009 and September 2012 on Texel and the North-West coast of the Netherlands. One seal included in this study stranded in 2007.

Statistics

The significance of an even or uneven sex ratio in the group of harbor seals and in the group of grey seals was determined by statistic calculation with the use of a two-proportion z-test. H_0: $p_1 = p_2$ was used, stating that the proportion of females is equal to the proportion of males, where p_1 is the proportion of female seals and p_2 is the proportion of male seals. The One-way ANOVA; LSD Post Hoc test (SPSS statistics version 18) was used for statistical analysis of significant differences between groups (mild lungworm infection, moderate lungworm infection, severe lungworm infection). The dependent factor used in this calculation is the number of days that seals with lungworm infection survived in rehabilitation, while the independent factor is the severity of lungworm infection in those seals. H_0: the means of days of survival are equal for each grade of severity of lungworm infection (mild, moderate and severe).

Macroscopical evaluation

The Department of Veterinary Pathobiology, Utrecht University, performs pathological examination on dead-stranded seals as well as on rehabilitation seals. Dead-stranded seals were often found more putrefied, but could still be used for research. As far as possible, the nutritive condition and the probability of bycatch can be estimated and a macroscopical conclusion can be drawn by external and internal observations. There is a close co-operation between the Department of Veterinary Pathobiology and IMARES, which performs several research projects associated with the ecology of marine wildlife in Dutch waters. For this reason data and samples are shared with the centre in order to get a view on the Dutch seal population from different perspectives. This only applies to seals that were free-ranging and have not been in rehabilitation. For the research projects by IMARES, samples of the muscle, blubber, kidney and liver were collected for toxicological research. By examining the accumulation of toxic substances, more clarity can be obtained about pollution in the Wadden Sea and the risks for marine mammals. The mandibles were collected to determine the age more precisely by examinating the teeth. The stomachs were collected for research on the stomach contents to get a clear view on the food that free-ranging seals mainly ingest. Until transport to IMARES, all samples were stored in a freezer at - 20°C. A piece of skin was collected from free-ranging as well as from rehabilitation seals for DNA tissue banking at the Department of Veterinary Pathobiology, which can be used for later research.

For this study, a total of 40 seals that had been in rehabilitation were used for the analysis of pathological findings. These included 38 harbor seals and 2 grey seals which died a natural cause or were euthanized in rehabilitation centre Ecomare during the winter of 2011 to 2012.
Necropsies were performed at the Department of Veterinary Pathobiology, Utrecht University. At the start of the necropsy the seals were weighed (kg) and measured from nose to hind flippers (*total length, TL*), from nose to tail (*standard length, SL*), from axilla to tail (*reduced length, RL*) and at the level of the axilla (perimeter) (*axillary girth, AG*). The age category was determined as neonate-juvenile, juvenile, subadult or adult, based on the state of development of the reproductive tract and the standard length as described by McLaren et al. (1993). Characterizations that led to the determination of neonate-juvenile were the presence of the umbilical cord, a puppy coat (grey seals) and milk in the stomach. The sex was determined by external observation or by internal observation of the gonads during macroscopical examination. For the macroscopical evaluation of the carcasses a standard protocol was used which is based on the descriptions of Kuiken et al. (1991). Only carcasses with a decomposition condition (DCC) (Kuiken et al., 1991) of 1 to 3 were included in this study (*Table 1*). The nutritive condition code (NCC) was based on the thickness of the blubber and subcutaneous fat, state of the musculature and body weight as described by Siebert et al (2001) (*Table 1*).

Table 1. The nutritive condition (NCC) code and decomposition condition (DCC) code

	NCC	*DCC*
1	Very well fed	Very fresh
2	Well fed	Fresh
3	Normal	Putrefied
4	Poor	Very putrefied
5	Very poor	Remains
6	Emaciated	
	Unknown	

The carcasses were fully intact and were delivered from Ecomare directly after death (*n=4*) or had been stored in a freezer (*n=36*) at -20°C until necropsy. Blubber thickness was measured at the level of the neck and at the level of the breast, cranially from the front flipper. Photographs were taken according to a standard protocol and include the entire body, the head, middle part of the body and hind flippers (lateral view), the front flippers (dorsal view), the external abdomen (ventral view) and the lungs of all fresh carcasses with the trachea and bronchi cut open to the caudal lobes in order to systematically survey the lung damage in stranded seals. Extra photographs of external and internal lesions were added when observed. Internal observations and lesions were defined for the head and neck region, the thorax and the abdomen. The examined internal organs are described in *Table 2*.
Lesions in external or internal organs were defined by composing a morphological diagnosis. This includes the severity, the time, the distribution, and the anatomic site of the lesion. When parasites were present, the location was noted and the amount was scored as none (0), mild (1), moderate (2) or severe (3).

Table 2. Internal observation for macroscopical evaluation: examined organs

Region	*Examined organs*
Head and neck	Larynx, thyroid, oral cavity, nostrils, eyes, teeth, auditory system, skull and brain.
Thorax	Trachea, lungs, pulmonary lymph nodes, heart, esophagus and thymus.
Abdomen	Urinary bladder, mesenteric lymph nodes, intestines, stomach, spleen, pancreas, liver, adrenals, kidneys, genital tract and gonads.

Sampling

For this study, samples were collected for histological, virological, bacteriological and parasitological examination. Standard samples were collected and samples of a specific organ were added if specific lesions were observed. Histological samples (*Table 3*) were collected from seals that were scored as DCC 1 and DCC 2 if lesions were observed. The tissues were cut in blocks of 10 x 10 x 4 mm and fixed in 4% formaldehyde. 6 histological samples (cranial lobe, middle part and caudal lobe of each lung) were collected as described by Piché et al. (2010) from all seals that were scored as DCC 1 and DCC 2, in order to systematically survey the lung damage in stranded seals. The thymus, spleen and lymph nodes (prescapular, mesenteric, ileocecal and pulmonary) were sampled in order to examine the immune status of the animal.

Table 3. Organs sampled for histological, virological and bacteriological examination

Field of study	*Sampled organs*
Histology	Gonad and reproductive tract, reproductive tract lymph node, placenta and umbilical cord, urinary bladder, ileocecal lymph node, mesenteric lymph node, prescapular lymph node, pancreas, spleen, liver, kidney, adrenal, lung, pulmonary lymph nodes, heart, thymus, thyroid, cerebrum, cerebellum and intestine.
Virology	Gonad and reproductive tract, placenta and umbilical cord, prescapular lymph node, stomach, spleen, liver, kidney, lung, pulmonary lymph node, heart, cerebrum, cerebellum and intestine.
Bacteriology	Swab of the genital split, gonad and reproductive tract, reproductive tract lymph node, spleen, liver, kidney, lung, lungworms, pulmonary lymph node, caecum

After fixation, the tissues were dehydrated by using an alcohol series of 70%, 80%, 96% and 100% and xylene, followed by impregnation with paraffin. The tissues were embedded in paraffin, cut in coupes of 6 μm and stained with haematoxylin and eosin (HE).

Virological samples (*Table 3*) were collected from 18 rehabilitation seals by cutting the tissues in blocks of approximately 20 x 20 x 20 mm and were stored in sterile plastic containers at -80˚ C, as described by Kuiken et al. (1991). Samples were collected if lesions with a suspected virological etiology were observed. The samples were sent to the Erasmus University in Rotterdam to test for morbillivirus, influenzavirus and phocine herpesvirus. Polymerase chain reaction (PCR) was used for the detection of morbillivirus and phocine herpesvirus. Carré staining (Avidin-Biotin Immunohistochemistry method) was performed only on tissues from which histological coupes were available and therefore only from fresh carcasses (DCC1 and 2). The Matrix Taqman RT-PCR method was used to detect the influenzavirus.

Bacteriological samples (*Table 3)* were collected from 10 rehabilitation seals by cutting the tissues in blocks of approximately 40 x 40 x 40 mm in which a zone of juxtaposition of normal tissue and the lesion was included, as described by Kuiken et al. (1991). Bacterial culture examination was performed on samples of lesions with signs of inflammation with purulent exudate or any lesion with a suspected bacteriological etiology. Standard samples were collected from fresh carcasses (DCC1 and 2). One sample was tested for *Mycobacterium* spp. by Ziehl-Neelsen staining and one was tested for gram-positive cocci by Gram staining. The genital split was sampled with a swab and sterile tube. The samples were stored in plastic sealed bags at -20º C and sent to the Veterinary Microbiologic Diagnostic Centre (VMDC) of Utrecht University, or the laboratory of the Department of Veterinary Pathobiology for staining or were kept stored for eventual later research on *Brucella ceti* or *Brucella pinnipedi*. Blood agar plates (aerobe and anaerobe), McConkey agar plates and chocolate agar plates were used for growing bacterial culture.
Parasites were fixed in a solution of 70 % ethanol and determined microscopically.

Results

The stranding data were analyzed for 170 seals, which include 137 harbor seals, 16 grey seals and 17 seals of unknown species. Pathological findings, including macroscopy, histology, virology, bacteriology and parasitology were analyzed for 40 rehabilitation seals (38 harbor seals and 2 grey seals).

Distribution of age classes and sex

Of both harbor seals and grey seals most stranded seals were in the age class of juvenile (*n=99* harbor seals, 72,3% and *n=12* grey seals, 75%). Of the remaining stranded harbor seals, 7 were neonates (5,1%), 12 were sub adults (8,8%) and 19 were adults (13,8%). Of the remaining grey seals, 3 were sub adults (18,8%) and 1 was an adult (6,2%).
The sex ratio in the group of harbor seals as well as in the group of grey seals was even. The group of stranded harbor seals included 75 females and 62 males and the group of stranded grey seals included 6 females and 10 males. There differences between males and females within both groups were not significant (harbor seals: Two-proportion z-test= 1,03, P= 0,30, grey seals: Two-proportion z-test= 0,75, P= 0,45). Of one seal the sex was unknown due to the state of the carcass. In *Table 4* an overview is presented of the sex ratio within the different age classes. Stranded neonate harbor seals were female in 71% of the cases and adult harbor seals were female in 74% of the cases. The sex distribution among stranded juvenile and subadult harbor seals was equal. The sex distribution in grey seals shows that juveniles were male in 67% of the cases, sub adults were male in 67% of the cases and adults were female in 100% of the cases.

Table 4. The distribution of age classes by sex in harbor seals (a) and grey seals (b).

a.	*Neonate*	*Juvenile*	*Subadult*	*Adult*	*Total*
Female	5	50	6	14	75
	71%	*51%*	*50%*	*74%*	
Male	2	49	6	5	62
	29%	*49%*	*50%*	*26%*	
Total	7	99	12	19	137

b.	*Neonate*	*Juvenile*	*Subadult*	*Adult*	*Total*
Female	0	4	1	1	6
	0%	*33%*	*33%*	*100%*	
Male	0	8	2	0	10
	0%	*67%*	*67%*	*0%*	
Total	0	12	3	1	16

Stranding data

The analysis of stranding data shows a seasonal distribution in strandings. In *Fig. 1* the seasonal distribution of strandings of harbor seals and grey seals are presented per age class. There is an increase in harbor seal stranding rate in the summer, which is caused by the strandings of neonates in the months June (3) and July (4) (*Fig. 1a*). The most notable peak in harbor seal stranding rate however, is during the winter months (*Fig. 1a*). 38% of all harbor seals stranded in December and January during the period of 2009 – 2012. This was caused by the high stranding rate of juveniles is these months (43,4% of all juveniles stranded in December and January). Adult harbor seals also mostly stranded in winter (Dec-Jan) (42,1%) and in spring (Apr-May) (26,3%). A peak in stranding rate of grey seals is observed in December: 31,3% of all grey seals stranded in December during the period of 2009 – 2012 (*Fig 1b*). Only juvenile grey seals stranded in December, which was 41,7% of all juveniles.

Stranding locations

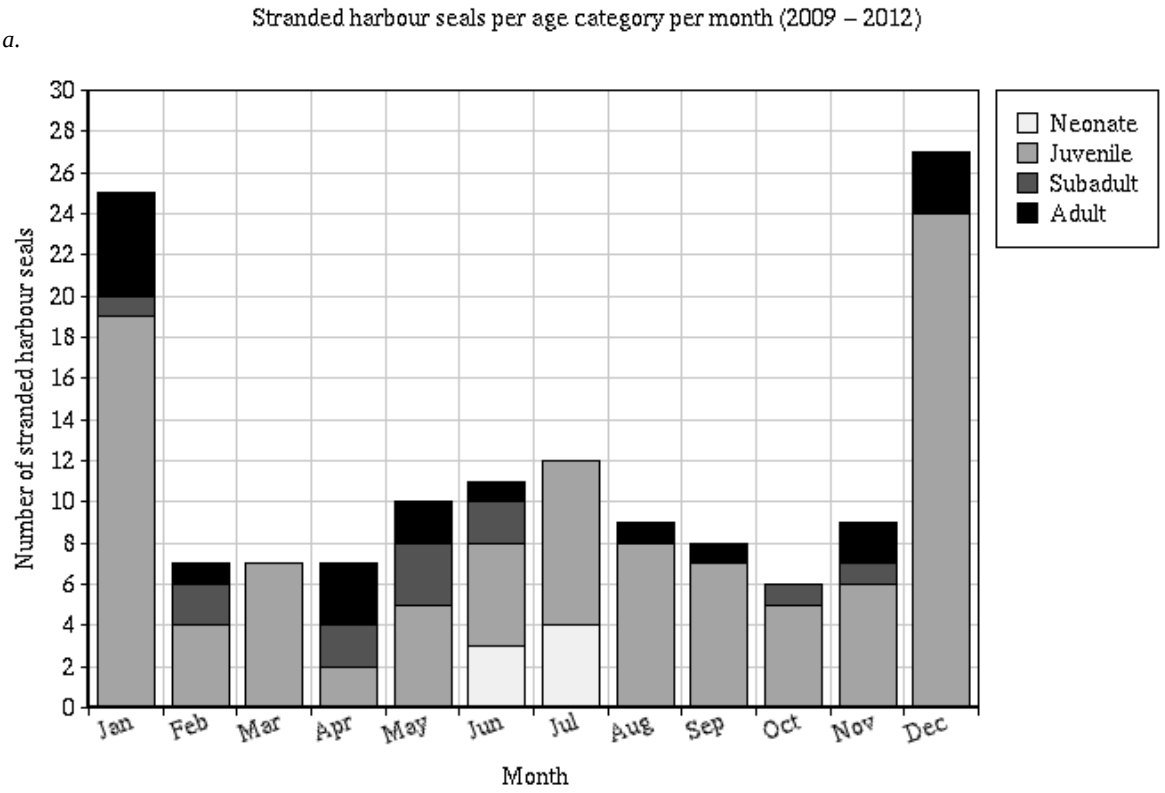
The locations of stranded seals along the Dutch coast between 2009 and 2012 are shown in *Table 5* and the geographical distribution is set out in *Fig. 2*. Strandings were most frequent on Texel (harbor seals: 61,3% and grey seals: 52,9%). Most strandings on Texel were reported from the west-coast of the island. Note that strandings along the coast of North-Holland and Texel are covered by Ecomare and IMARES which co-operate with the Department of Veterinary Pathobiology, while the rest of the Dutch coast is covered by the SRRC, Pieterburen. Two seals stranded at inland locations (one harbor seal at the IJsselmeer and one grey seal at the Amstelmeer). These were in both cases adults. Of 7 seals the stranding location was unknown.

Macroscopical and histological findings

All rehabilitation seals (*n=40*) that were investigated for pathological evaluation were juveniles. These seals were either euthanized or died a natural cause in Ecomare during the winter of 2011 - 2012. The decomposition condition codes of the carcasses were in most cases scored as ‘fresh’ (DCC2). 4 seals were scored as DCC1, 21 as DCC2 and 15 as DCC3. The nutritive condition codes were in most cases scored as ‘normal’ (NCC3) or ‘poor’ (NCC4). 2 seals were scored as NCC2, 16

Fig. 1 a and b. Seasonal distribution of strandings of harbor seals (a) and grey seals (b) per age class.

a.



b.

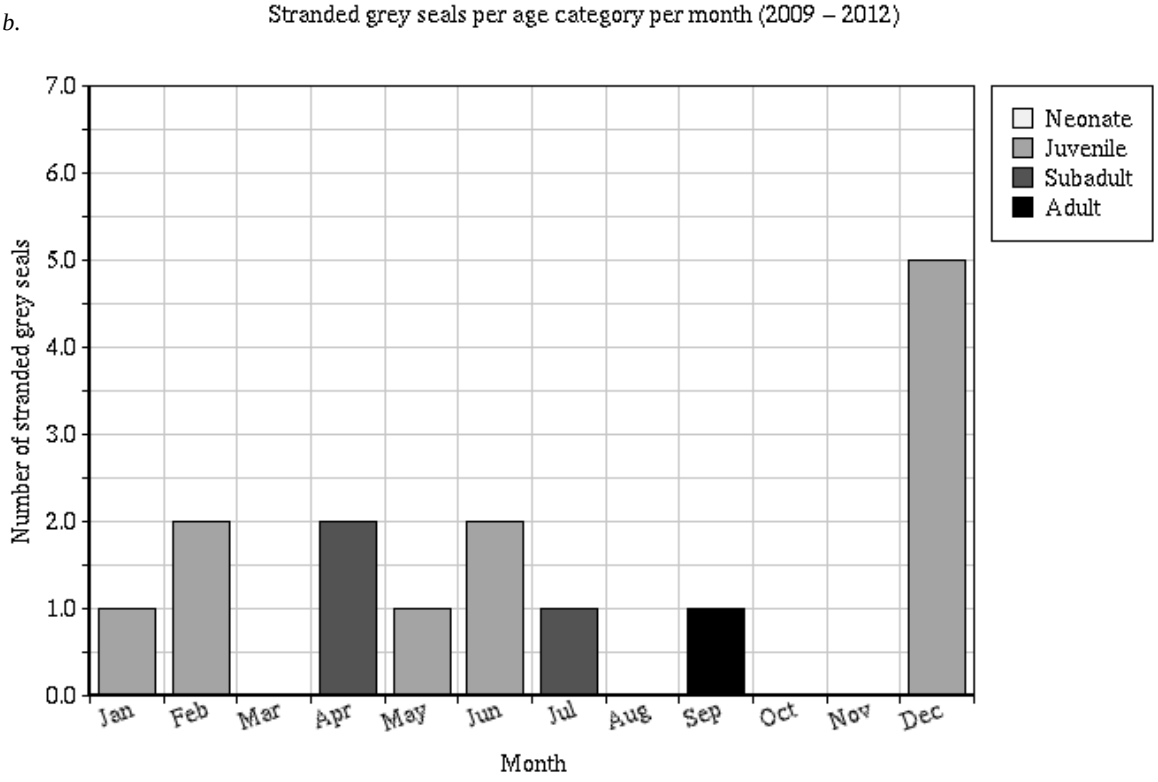
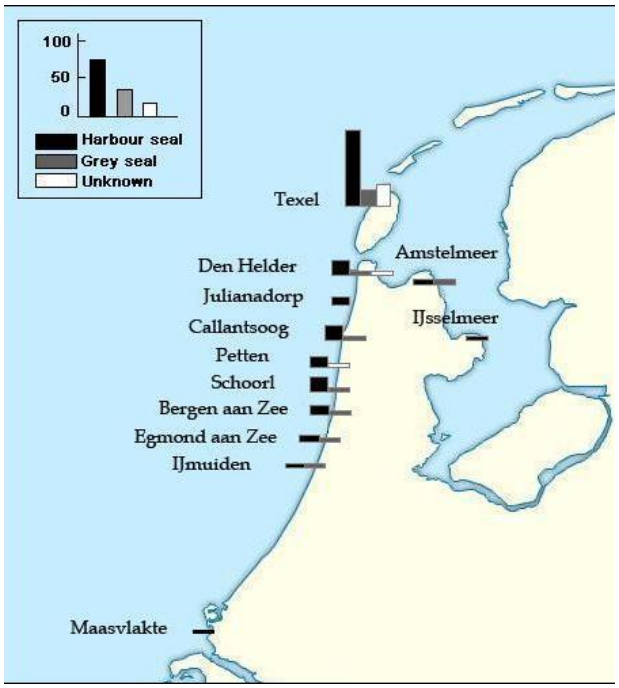


Table 5 and Fig. 2. Distribution of stranding locations of harbor seals, grey seals and seals of unknown species PV = Phoca vitulina, HG = Halichoerus grypus, Un = Unknown species.

Stranding location	*Species*		
	PV	*HG*	*Un*
Texel	84	8	15
Den Helder	9	1	1
IJsselmeer	1		
Amstelmeer	1	1	
Julianadorp	4		
Callantsoog	8	1	
Petten	5		1
Schoorl	9	1	
Bergen aan Zee	5	1	
Egmond aan zee	2	1	
IJmuiden	1	1	
Maasvlakte		1	
Location unknown	7		
Total	*137*	*16*	*17*



as NCC3, 14 as NCC4, 7 as NCC5 and 3 as NCC6, which means that 52,5% had a poor nutritive condition (*Photo 2a and b*).

The pathological findings in the investigated group of rehabilitation seals are presented in *Table 8*. The most commonly found lesions and conditions were skin ulceration, subcutaneous hemorrhage, lungworm infection, acute interstitial pneumonia, acute bronchopneumonia, lung atelectasis, hepatitis and parasite infections of the gastro-intestinal tract.

Skin ulceration was found in 11 harbor seals (28,9%) and 2 grey seals (100%) and presented itself by one or more ulcerations with well-defined edges of approximately 10 by 5 mm that extended up to the dermis. Skin ulceration was often found on the flippers, from which mainly the hind flippers (*Photo 3*).

One harbor seal had hyperemic sclera and gingiva and in one harbor seal conjunctivitis was found, in combination with hyperemic edges of the tongue and mucopurulent exudate around the eyes and the beak. In one grey seal parasitic mites were found in the oral cavity. Scarring was found in 2 harbor seals from which in one case on the cheek and in the other case on the ventral abdominal wall. Lesions of the palatum durum (2 harbor seals) were characterized by erosions of the mucosa of approximately 5 mm in diameter. In one seal the umbilical cord was inflamed.

Subcutaneous hemorrhage was found in 7 harbor seals (18,4%). In 4 of these seals subcutaneous hemorrhage was located at the level of the ribs and scapulae (*Photo 4*). In some cases this was associated with muscular hemorrhage (2) and muscular emphysema (1). In 2 seals the subcutaneous hemorrhage was found between the mandibles, in one seal at the level of the right axilla and in one seal at the level of the knee, which was associated with skin ulceration at the same location.

Cases of acute interstitial pneumonia and acute bronchopneumonia were characterized by the presence of interstitial edema (*n=24* harbor seals, 63,2% and one grey seal, 50%) and moderate to severe lung hyperemia (*n=18* harbor seals, 47,4% and one grey seal, 50%) (*Photo 5a and b*). Gross observations in these cases were similar to observation of interstitial lung edema described by McGavin et al (2012): 'the failure of the lungs to collapse when the thoracic cavity is opened, the occasional presence of rib impressions on the lung's pleural surface, indicating poor deflation and the lack of visible exudates in the airways unless complicated with secondary bacterial pneumonia'. The color of lungs with interstitial edema varied from diffusely red in acute cases to diffusely pale gray to mottled red in chronic cases (McGavin et al., 2012). Froth in the trachea was found in 13 harbor seals (34,2%) and 2 grey seals (100%), which was associated with interstitial edema of the lungs and originates from the mixing of edema fluid and air (McGavin et al., 2012). Lung atelectasis was found in 10 harbor seals (26,3%) and was associated with obstruction by parasites and/or bronchopneumonia

(obstruction by inflammatory exudates in bronchi, bronchioles and alveoli). Atelectasis was found in parts of the lungs or one lobus (multifocal or focal), while the distribution of interstitial edema was diffuse and involved all pulmonary lobes. The distribution of hyperemia was focal extensive or diffuse. The presence of lung parasites was in all cases associated with interstitial edema and hyperemia.
Parasite infection (*n=27* harbor seals, 71,1% and 1 grey seal, 50%) was mild in most cases (*n=12* harbor seals, 31,6%); in 8 cases the infestation was moderate (21,1%) and in 7 cases the infestation was severe (18,4%) (*Photo 5a and b*). In *Table 6* the duration of survival in rehabilitation of seals with lungworm infections is set out to the severity of their infection.

Table 6. The number of days in rehabilitation (days of survival) compared to the severity of the lungworm infection

Parasite score	*Days in rehabilitation*						
Mild	7	3	5	18	18	1	
Moderate	5	2	6	9	2	3	23
Severe	2	2	2	5	1	1	

Seals with mild lungworm infections averagely survived 8,7 days in rehabilitation, while seals with severe infections averagely survived 2,2 days in rehabilitation. Statistics show that there were no significant differences in means of days of survival between the group of seals with 'mild' and 'moderate' infections (P= 0,667), between 'moderate' and 'severe' infections (P= 0,171) and between 'mild' and 'severe' infections (P= 0.09). However, the Post Hoc LSD test showed a P-value of 0,09 between the group of mild infection and severe infection, which is close to a significant difference.
In 16 harbor seals (42,1%) the pulmonary lymph node was enlarged, which was in all cases associated with acute pneumonia and the presence of parasites. Purulent bronchopneumonia (*n=3* harbor seals) presented itself by the presence of pus in the bronchi, thickened walls of the bronchi, hyperemia and a firm consistency of the lung parenchyma. Chronic bronchopneumonia (*n=2* harbor seals) presented itself by a pale grey color of the lungs and obstructive atelectasis. Parasitic infestation of the heart and the arteria pulmonalis was found in 6 harbor seals (15,8%). Epicardial emphysema was found in 5 harbor seals (13,2%).
In 4 harbor seals (10,5%) multifocal necrosis of the liver was observed. The pattern of these white foci (1 mm in diameter) was random; there was no predictable location within a lobule. 5 seals showed paleness of the liver and in 5 seals the livers were congested, which presented itself by bleeding of the tissue on cut surface. In one harbor seal an abscess in the liver was observed and was characterized by a not well demarcated yellow focus, extending on cut surface of approximately 1 cm in diameter.
Parasite infections of the alimentary tract were in most cases mild. Gastric ulcers and gastric hyperemia were associated with the presence of parasites in the stomach (*n=15* harbor seals, 39,5% and *n=2* grey seals, 100%), although not all seals with gastric parasites showed these lesions (gastric ulcers: *n=3* harbor seals, 7.9%, gastric hyperemia: *n=4* harbor seals, 10,5%). Parasitic infections of the intestines were in most cases mild.
Lesions of the urogenital tract were pale kidneys (*n=4* harbor seals, 10,5%), urolithes (1), kidney abscesses (1) and a pink to red colored urine (1).
In 12 seals (31,6%) and 2 grey seals (100%) the thymus was present. In the remaining harbor seals the thymus was absent due to atrophy or was not determined. Other findings of the hematopoietic system were lymphadenopathy (*n=1*), splenic congestion (*n=2*), splenic hyperplasia (*n=1*) and spleen megaly (*n=3*).

Histology

Histology was performed on 9 rehabilitation seals at the moment of writing. Ulcerations of the flipper showed an irregularly and thickened epidermis due to focal orthokeratotic hyperkeratose, moderate hypergranulation and acanthose of the associated epithelium. Histological findings of the lung parenchyma were multiple foci of necrosis (*n=3*), infiltrates of neutrophils (*n=7*), eosinophils (*n=3*) or macrophages (*n=1*), interstitial edema (*n=1*), emphysema (*n=2*), hyperplasia of the bronchial epithelium (*n=3*), the presence of fibrin (*n=2*) and intralesional parasites (*n=6*) in combination with inflammatory infiltrates at the site of attachment to the lung tissue. Lungs containing a lot of inflammation infiltrate histologically appeared atelectatic. In one case, the activation of bronchus associated lymphoid tissue (BALT) was observed in association with confluent active pulmonary lymph follicles in the cortex of the pulmonary lymph node. Histological findings of the spleen were active lymph follicles in the white pulp (*n=3*) and extra medullary hematopoiesis (*n=2*). Moderate chronic gastritis was observed in 3 stomachs and was in all cases associated with intralesional parasitic remnants. This histologically presented itself by multifocal lymphoplasmacellular foci in the lamina propria and submucosa. In one of these cases different types of inflammatory cells, including

multinucleated giant cells were observed. The mesenteric lymph nodes of 5 seals histologically appeared active. In one mesenteric lymph node, one ileocecal lymph node and in one sample of intestinal mucosa, the observed reaction was defined as the formation of intensely eosinophilic material in radiate configurations ('Splendore-Hoeppli phenomenon') (Hussein et al., 2008) and may indicate bacterial sepsis. Histological findings of one liver with fibrin on its surface showed moderate lymphoplasmacellular infiltration of the portal areas and a milliar distribution of multiple acute hemorrhages throughout the parenchyma. This was often associated with necrotic foci, also surrounded by lymphoplasmacellular infiltration. Lymphoid organs mainly showed reaction of the lymph nodes and depletion and regression of the thymus. In other organs no significant microscopical lesions were found or the lesions were not interpretable due to severe autolysis and freeze artifacts.

Virology

The virological samples of 18 rehabilitation harbor seals that were tested for *morbilli* virus, influenza virus and *phocine herpes* virus were all tested negative.

Bacteriology

Of the 10 rehabilitation seals from which samples were collected for bacteriological examination, no bacteria were isolated from the tested samples in 2 cases. From one lung showing gross lesions of chronic pneumonia, *Staphylococcus* spp, *Streptococcus* spp. and a mixed culture were isolated. From one lung that showed signs of purulent bronchopneumonia, *Escherichia coli* was isolated. From 5 lungs showing acute or sub-acute interstitial pneumonia and bronchopneumonia, *Escherichia coli*, *Proteus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., and coliforms were isolated. In 6 seals, samples of the lung showed mixed cultures of bacteria. From liver samples of one seal a mixed culture including coliformes en ß-haemolytic streptococci were isolated. *Klebsiella* spp. was isolated from kidney samples of the same seal. Organs showing the Splendore-Hoeppli phenomenon were tested negative for bacterial infection.

Parasitology

Results of parasitology are presented in *Table 7*. Lungworm infection was caused by *Otostrongylus circumlitus* (*n=23* harbor seals, 60,5% and one grey seal, 50%) and *Parafilaroides gymnurus* (*n=5* harbor seals, 13,2%). *O. circumlitus* was found in the large bronchi and at the bifurcation of the lungs, while *P. gymnurus* was found in the bronchioles and in the lung parenchyma. Intralesional small lungworms were found during histological examination (*n=5*) and were presumptive *P. gymnurus*, but the parasite could not be determined. Infection of the heart and arteria pulmonalis was caused by *Dipetalonema spirocauda* (*n=5*) and *Acanthocheilonema spirocauda* (*n=1*). In one case of a severe lungworm infection *O. circumlitus* was found in the arteria pulmonalis. *Corynosoma strumosum* was found in the intestines of 18 harbor seals (47,3%) and 2 grey seals (100%). Other intestinal parasites were *Cryptocotyle lingua* (*n=6*), *Ascocotyle septentrionalis* (*n=6*) and *Diphyllobothrium spp.* (*n=1*).
Parasites that were found in the stomach were *Contracaecum osculatum* (*n=6*), *Anisakis spp.* (*n=4*) and *Pseudoterranova decipiens* (*n=2*).
In one grey seal the mite *Halarachne halichoeri* was found in the oral cavity.

Table 7. Parasites observed in rehabilitation harbor seals and grey seals

	Otostrongylus circumlitus	Parafilaroides gymnurus	Dipetalonema spirocauda	Acanthocheilonema spirocauda	Corynosoma strumosum	Contracaecum osculatum	Anisakis spp.	Cryptocotyle lingua	Ascocotyle septentrionalis	Pseudoterranova decipiens	Diphyllobothrium spp.	Halarachne halichoeri	Not determined
Harbor seals	23	5	5	1	18	6	4	6	6	2	1		10
Grey seals	1				2	1						1	

Photo 2a. Juvenile harbor seal with a very good nutritive condition (NCC 1). Department of Veterinary Pathobiology, Utrecht University, 2012

Photo 2b. Juvenile harbor seal with a very poor nutritive condition (NCC 6). Department of Veterinary Pathobiology, Utrecht University, 2012

Table 8. The pathological findings in rehabilitation harbor seals (n=38) and grey seals (n=2) that were euthanized or died a natural cause in Ecomare between November 2011 and March 2012.

	Harbor seals		*Grey seals*		*Total*
	n	*%*	*n*	*%*	*n*
Poor nutritive condition	19	50	2	100	21
Skin and subcutis					
Scarring	2	5,3			2
Skin ulceration	11	28,9	2	100	13
Hyperkeratosis	1	2,6			1
Subcutaneous interstitial edema	3	7,9			3
Subcutaneous emphysema	2	5,3			2
Subcutaneous hemorrhage	7	18,4			7
Subcutaneous noduli			1	50	1
Inflammation of the umbilical cord	1	2,6			1
Mites			1	50	1
Eyes					
Hyperemic sclera	1	2,6			1
Conjunctivitis	1	2,6			1
Purulent exudate	1	2,6			1
Nasal cavity					
Purulent nasal discharge	2	5,3			2
Oral cavity					
Lesions on palatum durum	1	2,6	1	50	2
Hyperemia of the gingiva	1	2,6			1
Cuts on tongue	1	2,6			1
Parasites	3	7,9			3
Musculoskeletal system					
Muscular hemorrhage	2	5,3			2
Muscular emphysema	1	2,6			1
Thoracic cavity					
Fluid in thorax	1	2,6			1
Respiratory system					
Hyperemia of the larynx	2	5,3			2
Froth in the trachea	13	34,2	2	100	15
Parasites in the trachea	9	23,7			9
Fluid in the trachea	1	2,6			1
Hyperemia of the trachea	2	5,3			2
Lung atelectasis	10	26,3			10
Lung interstitial edema	24	63,2	1	50	25
Lung hyperemia	18	47,4	1	50	19
Lung emphysema	7	18,4			7
Lung hemorrhage	5	13,2			5
Purulent bronchopneumonia	3	7,9			3
Chronic bronchopneumonia	2	5,3			2
Parasites in the lungs	27	71,1	1	50	28
Mild	12	31,6			12

	n	*%*	*n*	*%*	*n*
Moderate	8	21,1	1	50	9
Severe	7	18,4			6
Reactive pulmonary lymph node	16	42,1			16
Cardiovascular system					
Parasites in heart or a. pulmonalis	6	15,8			6
Epicardial emphysema	5	13,2			5
Thoracic cavity					
Mediastinal emphysema	5	13,2			5
Abdominal cavity					
Fluid in abdomen	2	5,3			2
Alimentary system					
Hyperemia of the oesophagus	2	5,3			2
Abces liver	1	2,6			1
Liver megaly	1	2,6			1
Multifocal white foci / necrosis	4	10,5			4
Pale liver	5	13,2			5
Liver congestion	5	13,2			5
Fibrin on liver surface	1	2,6			1
Pancreas megaly	1	2,6			1
Gastric hyperemia	4	10,5			4
Gastric ulcers	3	7,9			3
Gastrointestinal parasites	27	71,1	2	100	29
Oesophagus	3	7,9	2	100	5
Stomach	15	39,5	2	100	17
Intestines	20	52,6	2	100	22
Hemorrhagic contents intestines	1	2,6			1
Corpora aliena in intestines	1	2,6			1
Reactive mesenteric lymph node	7	18,4			7
Urinary system					
Pink to red colored urine	1	2,6			1
Pale kidneys	4	10,5			4
Abces kidney	1	2,6			1
Urolithes	1	2,6			1
Hematopoietic system					
Lymphadenopathy	1	2,6			1
Splenic congestion	2	5,3	1	50	3
Splenic hyperplasia	1	2,6			1
Spleen megaly	3	7,9			3
Endocrinological system					
Thymus present	12	31,6	2	100	14

Photo 3. Skin ulceration on the right front flipper of a juvenile harbor seal, euthanized in rehabilitation. Department of Veterinary Pathobiology, Utrecht University, 2012

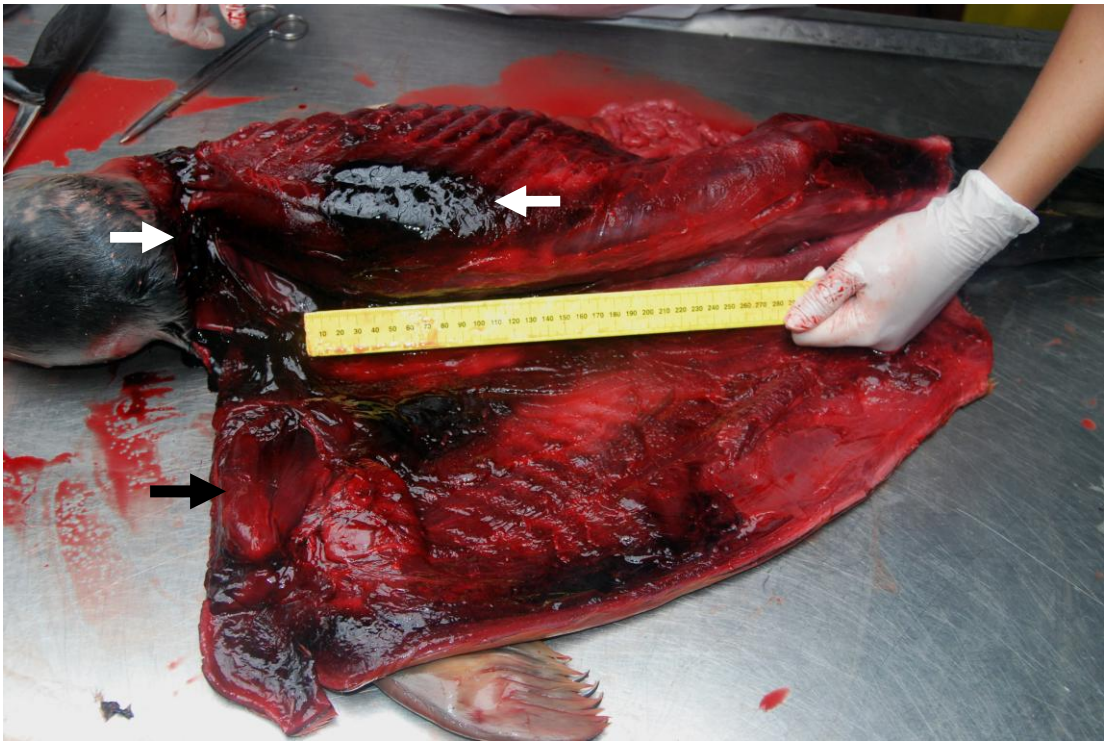


Photo 4. Subcutaneous hemorrhage (white arrows) at the level of the scapula (black arrow), neck and ribs in a juvenile harbor seal. On the left is the head, the skin and subcutis are stripped. Department of Veterinary Pathobiology, Utrecht University, 2012

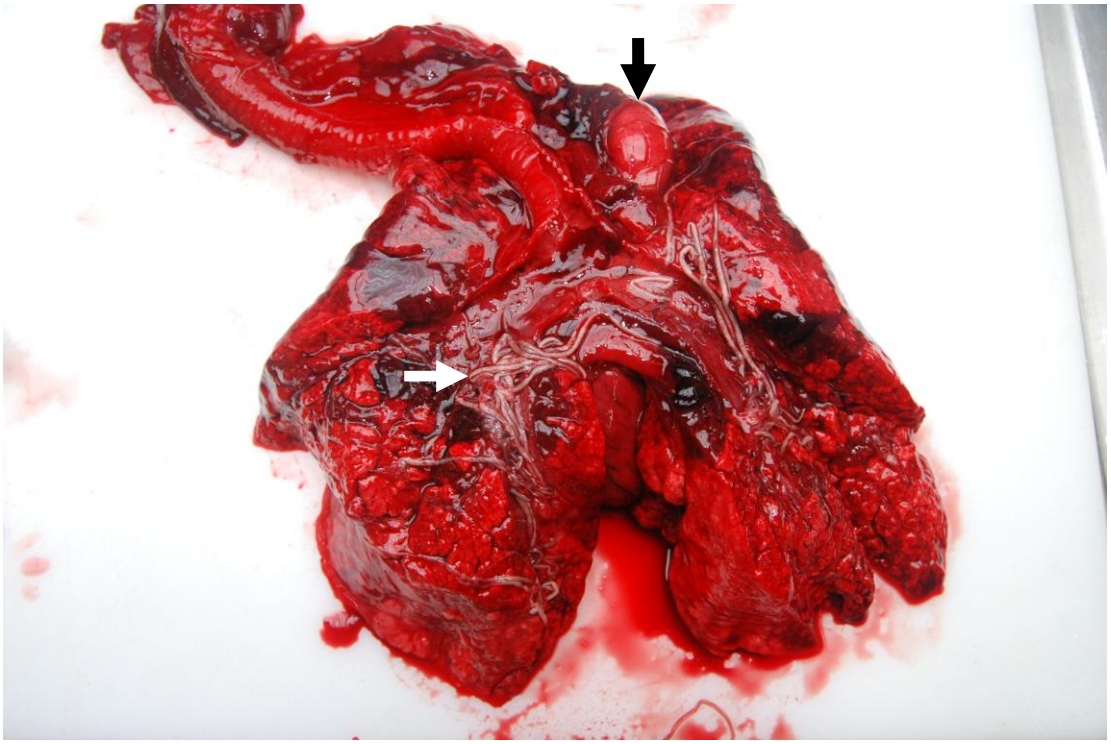


Photo 5a. Severe parasite infestation by Otostrongylus circumlitus in the large bronchi (white arrow) and Parafilaroides gymnurus (not visible with the naked eye) in the lungs of a juvenile harbor seal, euthanized in rehabilitation. Note the enlarged pulmonary lymph node (black arrow). Department of Veterinary Pathobiology, Utrecht University. 2012

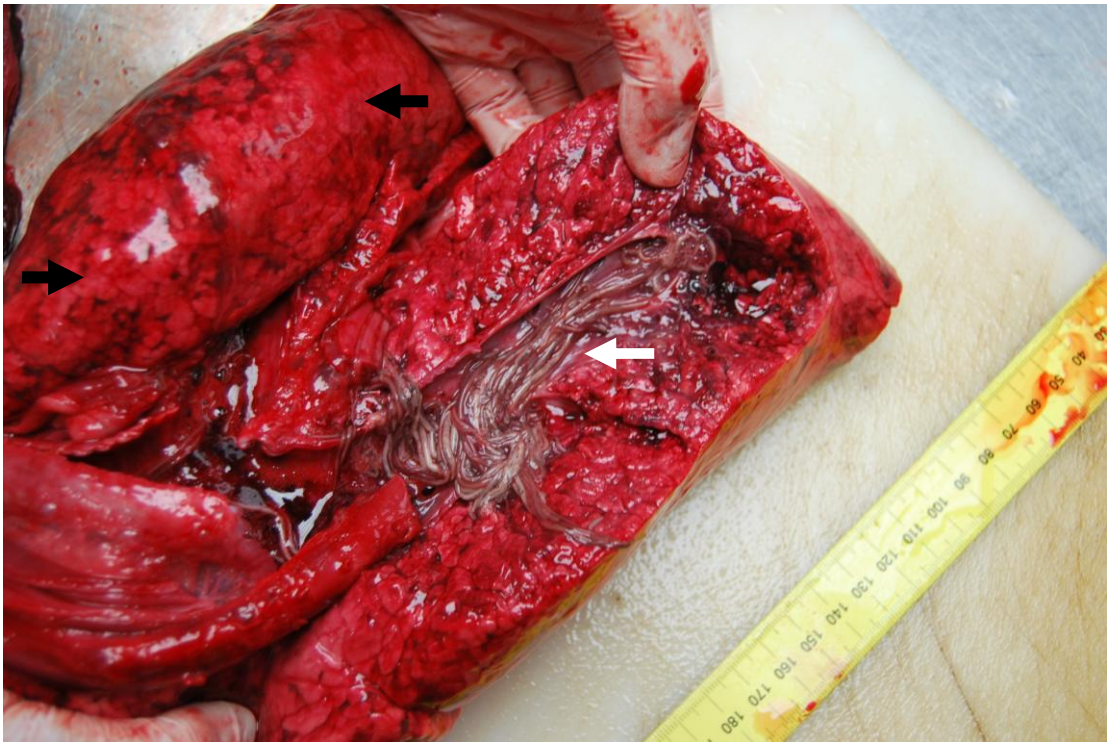


Photo 5b. Severe parasite infestation by Otostongylus circumlitus in the large bronchi (white arrow) in the lungs of a juvenile harbor seal, died after one day in rehabilitation. Note that the septa of the lobi are broadened and that the lung is not well collapsed (interstitial edema) (black arrows). Department of Veterinary Pathobiology, Utrecht University. 2012

Discussion

Stranding data were evaluated for 170 harbor seals and grey seals that stranded on Texel and the North-West coast of the Netherlands between 2009 and 2012. The results showed that stranded seals were mainly juveniles (72,3% of the harbor seals en 75% of the grey seals). This corresponds with the study of van Haaften (1982) who also found a large proportion of stranded juvenile harbor seals (55%) in an analysis of the strandings of dead-stranded seals between 1960 and 1981. Juvenile harbor seals stranded mostly in December and January and grey seals stranded mostly in December. This suggests that juveniles in particular may have difficulties with surviving winter, in which the weather conditions may play an important role. It is known that neonate-juvenile harbor seals are more likely to get lost from their mother during bad weather, for example, in storms (TSEG, 2002). Because of the separation the juvenile is not fed, which leads to a decrease in energy reserves and weakness because of emaciation. This weakness, together with the fact that the immune system of juvenile animals during the first months after weaning is not yet completely developed, may lead to an increased susceptibility to disease; juveniles are more likely to develop serious complications after exposure to pathogens, in contrast to adult seals. Neonate harbor seals stranded in June and July, which corresponds with the pupping season of harbor seals (May – July). Because there were few grey seals included in this study, peaks in stranding rate are not very clear.

The sex ratio for harbor seals was even (P= 0,30). Osinga et al. (2012) also reported an even sex ratio in stranded female and male harbor seals. Statistics show that the sex ratio between male and female grey seals in this study is also even (P= 0,45). This is in contrast with the results of Osinga et al. (2012), which reports that significantly more male than female grey seals stranded between 1978 and 2008. However, there are few grey seals included in this study, while it is difficult to find significant results in a small sample. Therefore is not responsible to confirm or refute the findings in the study of Osinga et al.

Analysis of the sex ratio within the different age classes shows that stranded adult harbor seals were female in 74% of the cases and male in 26% of the cases. Van Haaften et al. (1982) also reported a distribution of 75% female harbor seals and 25% male harbor seals among stranded seals between 1960 and 1981. It is unclear why the majority of stranded adult harbor seals were female, but it is noted that halve of these stranded females had complications that may have been related to gestation (pregnant of a fetus (*n=5*), uterus involution (*n=1*), endometritis (*n=1*).

Strandings were mostly reported from Texel. This is probably due to the fact that Ecomare and IMARES are located at Texel and therefore the strandings at this location are dealt with more accurately, but also because the rest of the Dutch coast, including the North-West coast is covered by the SRRC in Pieterburen. Stranded seals are picked up from these locations by the SRRC and are submitted for necropsy at the SRRC itself. In the future it may be useful to cooperate with the SRRC, but also other seal research centers in the Wadden Sea (Seehundstations Norden-Norddeich and Friedrichskoog in Germany and Fiskeri-og Søfartsmuseet Esjberg in Denmark) in order to have a greater number of seals that can be submitted for research and to prevent different approachings to pathological findings by the separate research centers.

Pathological findings were evaluated for 40 rehabilitation seals which were either euthanized or died a natural cause in rehabilitation centre Ecomare during the winter of 2011 to 2012. Most carcasses were frozen before necropsy which negatively influenced the interpretation of the tissues and the results of histological, virological and bacteriological examination (Kuiken et al., 1991). Histological changes that are known to be caused by the freezing process are an eosinophilic, homogenous, extracellular fluid accumulation, cellular shrinkage, loss of staining, fractures, hemolysis, and hematin formation (Baraibar et al., 1985). In the lungs specifically it may lead to bronchial transudate, loss of cilia, alveolar transudate, alveolar wall changes, hemolysis, loss of vascular endothelium and pleural and intralobular connective tissue changes (Baraibar et al., 1985), which mimics lung edema and acute pneumonia. Fractures in particular may be mistaken by interstitial edema and should therefore be diagnosed with some caution in the future. In general it would be useful to only use carcasses that were submitted for necropsy directly after death of the animal in order to prevent artifacts in the tissues.

The most common findings in the investigated rehabilitation seals were a poor nutritive condition (52,5%), skin ulceration (32,5%), subcutaneous hemorrhage (17,5%), necrotic foci of the liver (10%), reactive pulmonary lymph nodes (40%), lung interstitial edema (62,5%), lung hyperemia (47,5%), lungworms (70%) and gastrointestinal parasites (72,5%).

Over half of the rehabilitation seals had a poor nutritive condition. In an earlier study, 41% of dead-stranded and killed seals between 1996 and 2002 were emaciated and they were mostly between 0 and 6 months of age of (Siebert et al., 2007). Poor nutritive condition in juveniles may be caused by the lack of feeding when separated from the mother or by disease. For example, animals with lungworm infections in rehabilitation often showed a loss of appetite, which was probably due to malaise and the unwillingness to swallow because of dyspnoea.

Many seals showed ulceration of the skin on the flippers. In other studies, juvenile harbor seals and grey seals in rehabilitation developed the same lesions in combination with spheric dermal elevations, and verrucose, rounded nodules in the oral cavity approximately 1–2 cm in diameter, which healed spontaneously in a period of about 3 weeks to 10 months (Hicks et al., 1987, Müller et al., 2003). These lesions were associated with the presence of the *Parapoxvirus* and were not observed in adult seals at the centre (Müller et al., 2003). Based on macroscopical evaluation it is presumable that the ulcerative skin lesions found in the seals of this study are associated with the *Parapoxvirus*. If there is interest for this to be diagnosed in the future, samples of these lesions can be tested for *Parapoxvirus* by polymerase chain reaction (PCR) and in situ hybridization (ISH). Virological studies were performed on swabs and biopsy specimens obtained from pox-like lesions in living animals (Müller et al., 2003, Nollens et al., 2006, Ohno et al., 2011).

Subcutaneous hemorrhage was common in the investigated group of seals and was in most cases located at the level of the scapulae and ribs. Again it is noted that the freezing process caused changes in the tissues and therefore may have led to discoloration of the subcutis and muscles, which may be mistaken by hemorrhage. It is also speculated that the hemorrhage is related to handling at the rehabilitation centre, because the seal caretakers position the animal firmly between the knees to avoid escaping of the animal and bite accidents during force feeding. This may also have contributed to the hematomas at this location, because it corresponds with the areas of pressure.

Multifocal white foci were found in the livers of 4 harbor seals and are presumptive lesions of hepatitis. However, this cannot be diagnosed without histological, virological, bacteriological and parasitological examination. At the moment of writing, histological examination has not yet been performed on 31 rehabilitation seals that are discussed in this study. If it is presumed that these seals had hepatitis based on macroscopical evaluation, this leads to a percentage of 10,5% of the rehabilitation harbor seals showing hepatitis. Siebert et al. (2007) found that 7% of the examined harbor seals had hepatitis; these include seals found dead or killed due to severe illness. Hepatitis in these seals was associated with migrating parasites or umbilical infection or septicaemia due to *E. coli* and α/β-haemolytic streptococci (Siebert et al., 2007). On one liver in this study with presumptive hepatitis, bacteriological examination has been performed and resulted in the isolation of a mixed culture including coliformes en ß-haemolytic streptococci, corresponding with the results of Siebert et al. (2007).

Pulmonary lymph nodes were reactive in 42,1% of the rehabilitation seals and were associated with acute pneumonia. Reaction was determined by the observation of enlargement of the lymph node and histological observation of a broadening of the cortex.

Interstitial edema was macroscopically observed in well over half of the rehabilitation seals, which was in most cases associated with moderate to severe hyperemia. The lung parenchyma histologically showed bronchitis and alveolitis by infiltrates of neutrophils, eosinophils and macrophages, thickened bronchial walls and inflammatory infiltrates at the site of attachment of parasites to the lung tissue, which corresponds with the histological findings in an earlier study on harbor seals naturally infected with *O. circumlitus (*Piché et al., 2010).

In the seals that were submitted for virological examination, observed lesions were highly probable not caused by the morbillivirus, influenzavirus and phocine herpesvirus, because these viruses were not detected in the samples. However, other viruses which have not been tested may still play a role. Bacterial infections were found in 7 lungs showing signs of acute, purulent or chronic bronchopneumonia, which were probably secondary to parasitic infection.

Parasite infection was associated with signs of acute pneumonia: interstitial edema, hyperemia,

froth in the trachea, lung hemorrhage and inflammatory cell infiltrates in the bronchi. This suggests that acute pneumonia was primarily caused by parasite infestation, which was found in 70% of the rehabilitation seals. The study of Siebert et al. reports that lungworms were observed in the bronchial tree in 37% of the seals that were shot due to severe illness between 1996 and 2002 and 57% of the seals that were shot between 2002 and 2005. Compared to the 70% in this study, these results suggest that the number of seals in the Dutch Wadden Sea infected with lungworms is increasing. However, seals of all age classes were included in the study of Siebert et al., while this study only included juveniles. Since lungworm infection in older seals (from the age of 2 years) is rare (Vercruysse et al., 2003, Rijks et al., 2008), this may explain part of the difference between the results of Siebert et al. and this study.

Otostrongylus circumlitus was found in 23 harbor seals (60,5%) and one grey seal, while *Parafilaroides gymnurus* was found in only 5 harbor seals (13,2%). By contrast, in other studies percentages were found of 70,8% (van den Broek and Wensvoort, 1959), 87,8% (van de Broek, 1963), 23% (Bus and Verplanke, 1988) and 26,9% (Claussen et al., 1991) for *P. gymnurus.* It may be possible that infection with *P. gymnurus* in the seal population has decreased, or that infestation has been tackled by treatment in rehabilitation. On the other hand it is possible that the method in this study for the detection of this parasite is not sufficient. In some seals intralesional parasites were observed during histological examination of the lung parenchyma, while *O. circumlitus* was not observed during parasitological evaluation in these seals. The small parasites captured in the histological samples were therefore highly probable *P. gymnurus* or larvae of *O. circumlitus*. *P. gymnurus* resides in the small bronchioles and alveoli of the lung parenchyma (Borgsteede et al., 1991, Vercruysse et al, 2003, Piché et al., 2010), which is also where the samples were taken. In contrast to *O. circumlitus*, *P. gymnurus* is not clearly visible with the naked eye and was therefore in most cases sampled unconsciously in a clump of large lungworms and mucus. It is suggested that seals with acute pneumonia, that are lacking the presence of large lungworms, may have an infection with *P. gymnurus* but it is not detected. Vedder (1998) also states that mainly in young seals up to 3 to 4 months small lungworms can be found without the presence of large lungworms. This indicates that parasitological examination must be performed more accurately in the future.

Parasites found in the gastro-intestinal tract were in most cases mild. *Halarachne halichoeri* was found in one grey seal and was earlier described in grey seals showing associated upper respiratory infections, which was not observed in the seal in this study (Alonso-Farré et al., 2012).

As mentioned earlier, the Department of Veterinary Pathobiology also performs necropsies on dead-stranded (thus, free-ranging) seals. The most commonly found lesions in dead-stranded seals between 2009 and 2012 were a poor nutritive condition (mainly in juveniles), skin ulceration, from which the lesions were similar to those described earlier in rehabilitation seals, lungworms, lung interstitial edema, lung hyperemia, subcutaneous hemorrhage, hepatitis with necrotic foci and gastrointestinal parasites. These are the same findings as those that were most commonly found in the investigated group of rehabilitation seals. Other common findings in dead-stranded seals that were less commonly or not found in rehabilitation seals were fractures of the flippers, conjunctivitis, fragile livers, lesions on the palatum durum, purulent bronchopneumonia and pregnancy in adult females. Also, all dead-stranded seals were scored for probability of bycatch. 17 dead-stranded seals were assigned as ‘possibly bycatch’, 4 seals as ‘probable’ or ‘highly probable bycatch’ and one seal as ‘certainly bycatch’. This leads to a total of 17,2% of the dead-stranded seals that were possible bycatch and 0,8% that were confirmed bycatch. The remaining seals were scored as ‘no evidence’ or ‘unknown’. In all rehabilitation seals there was no evidence for bycatch, since internal lesions that may have been caused by drowning, in particular those in the lungs, are not reliable due to the rehabilitation process. The results in this study correspond with the results of Osinga et al. (2012), who reports that bycatch was one of the most common causes of death among dead-stranded harbor seals and grey seals (17,5% inferred and 1,4% confirmed for harbor seals and 9,7% inferred and 5,4% confirmed for grey seals).

Parasitic bronchopneumonia was also one of the most common causes of death among stranded seals in the study of Osinga et al. (2012) (5,9%) and Siebert et al. (2007) (27,6%). Parasitic bronchopneumonia was not mentioned by Osinga et al. (2012) as common cause of death in grey seals which also applies to the results in this study: in the group of dead-stranded seals, lungworms were relatively much less found in grey seals compared to harbor seals. Both studies also report intestinal volvulus as common cause of death (7%, Osinga et

al. (2012), 4,5%, Siebert et al. (2007)), while in this study this was only observed in one dead-stranded seal.

The rehabilitation seals that were evaluated all showed signs of respiratory disease at the first day of entry to Ecomare. These signs included dyspnoea, tightened, shallow and abdominal breathing, squeaking while breathing and coughing. The medication that was standard provided on the first day were an injection of 0,3-0,5 mL Ivomec® (ivermectin) as anthelmintic treatment, 2 mg/mL Dexadreson® (dexamethasone) to reduce inflammatory reaction and 2-5 mL Engemycin® (oxytetracyclin), because a common complication of verminous pneumonia is bacterial infection (Vercruysse et al., 2003). Vercruysse et al. (2003) performed a study on the effectiveness of a treatment with either 0,2 mg/kg ivermectin orally or 0,2 mg/kg moxidectin subcutaneously against lungworm and the results showed that 'a single treatment with either ivermectin or moxidectin was highly efficacious in treating infections with *P. gymnurus* or *O. circumlitus* in harbor seals' (Vercruysse et al., 2003). It was noted that the treatment was more effective against *O. circumlitus* than *P. gymnurus*, which is supported by Borgsteede et al. (1991). This was stated since *O. circumlitus* was no longer found after 20 days of anthelmintic treatment, while *P. gymnurus* was still present in the small bronchi, bronchioles and alveoli. Dierauf et al. (1990) states that an oral treatment with ivermectin is more effective than a parental treatment, because of the thick layer of blubber. A course with 100 mg Ronaxan® (doxycyclin) was set in 2 dd with a maximum of 10 days on the second day in rehabilitation. When it was noted that Ronaxan was not effective enough in some seals, in addition a course of 50 mg Enrofloxoral® or Baytril® (enrofloxacin) 1 dd for 7 days was set in after one to two weeks of rehabilitation. However, the condition of 10 seals deteriorated after 12 to 24 days with signs of lethargia, sopor, dyspnoea, coughing and a fast and shallow breathing, followed by euthanasia. An equal number of seals was euthanized or died a natural cause due to severe respiratory disease between one and 5 days in rehabilitation, showing the same respiratory signs. When comparing the means of days that seals with different grades of severity of lungworm infection survived in rehabilitation, it is notable that seals with mild infections averagely survived longer in rehabilitation than seals with severe lungworm infections. However, no significant differences were found between the groups of seals with mild, moderate or severe lungworm infections. In the group of seals with moderate lungworm infections, there was one seal that survived for 23 days in rehabilitation. This leads to a large distribution of dependent variables and the absence of a stable mean in this group. There were also too few animals in these groups, while it is difficult to find significant results in a small sample. The comparison of means of days of survival between seals with mild infections and seals with severe infections resulted in a P-value of 0,09. This result is worth mentioning, because it is close to the level of significance. It is presumable that the use of larger group samples for this comparison may lead to more reliable results, since in that case it is more likely to find a significant difference. Also, in this evaluation the data are missing of seals that received the same treatment, completely recovered and were released back into the wild. The impression is that these are relatively few, but these data are indispensable for analyzing the sufficiency of treatment.
Ecomare is particularly questioning about those seals that seem to recover by treatment and survive for one week and longer, but still deteriorate unexpectedly and die or must be euthanized due to severe complications. Concerning the seals with mild infections it is suggested that the medication provided in rehabilitation did quell the infestation or that the seals already had mild infestations when they entered the centre and therefore treatment was more successful than in seals with severe lungworm infestations. Because of the frequent findings of acute pneumonia and lungworms during necropsy, in can be concluded that the provided treatments were not sufficiently effective in overcoming the lungworm infestation and associated inflammation. At the moment, the protocol of Ecomare is to euthanize seals that show severe respiratory complications. I confirm this course of action, since the results in this study show that these seals suffered from severe lungworm infestations and damage to the lung tissue, which is a poor prognosis for the juvenile animal. In view of the well-being of the animal, it is therefore appropriate to euthanize rather than starting with therapy that is not sufficiently effective in severe cases.

Environmental factors may induce an impairment of the immune system in marine mammals and an increased susceptibility for infectious diseases. Several reports have demonstrated that environmental contaminants are suspected of exhibiting immunotoxic effects in marine mammals

and therefore have an adverse effect on their health status (Luebke et al., 1991, De Swart et al., 1995, Ross et al., 1996, Siebert et al., 1999, Beineke et al., 2007). It may also be possible that pathogens itself cause an impairment of the immune system. In order to investigate whether there is an immune impairment in the investigated group of rehabilitation seals, histological, virological and bacteriological examinations were performed on lymphoid tissues (thymus, spleen and prescapular, mesenteric, ileocecal and pulmonary lymph nodes). The most observed changes in the investigated lymphoid tissues were reaction of the lymph nodes (broadening of the cortex) and depletion and regression of the thymus.

However, discrimination has to be made between pathological changes that indicate an impairment of the immune response and physiological changes that occur in lymphoid organs during the lifetime of marine mammals, for example, age-related lymphoid depletion and lymphocyte loss of the thymus after puberty (Beineke et al., 2007). In this study, the thymus was in regression or was not determined in some seals, which suggests that this may not be the most efficient organ to investigate.

In several studies the effects of chemical pollutants on seal lymphoid tissues were investigated and they report changes in lymphoid cell proliferation after the exposure to toxic substances (Ross et al., 1996, Kakuschke et al., 2009, Neale et al., 2002). These studies support the hypothesis that chemical pollutants lead to an impaired immune status in marine mammals. However, these results are based on clinical trials and in vitro methods. By contrast, with the used material in this study only retrospective cohort studies can be performed because pathological findings were examined only in seals that were dead-stranded or died in rehabilitation. Therefore the history of the animal is not known and a difference between exposed and non-exposed animals cannot be made. The disadvantage of this type of study is that changes caused by the effect of chemical contaminants cannot be discriminated from other factors such as malnutrition, emaciation or infections, which also may have contributed to the observed alterations in lymphoid tissues (Beineke et al., 2007).

The Netherlands is one of the few countries in the world where there is regularly a debate on to which extends rehabilitating wildlife marine mammals is appropriate. At this moment the seal population is doing well, so it is discussed whether it is necessary to help seals in need. Justifications from rehabilitation centers include animal welfare, management of beach use conflict, research, conservation, and public education (Moore et al., 2007). The SRRC, Pieterburen states that environmental factors related to human activity are the major cause of strandings of ill seals from the Wadden Sea. It is suggested that it is important to take responsibility to save weak and stranded animals, because humans have ensured that these animals are in trouble. Every animal that is saved is an asset to the population, because the genetic variation among harbor seals in the Wadden Sea is low, which is a risk in view of the outbreaks of diseases (Kappe et al., 1997). Justifications from opponent organizations are ignorance of recipient population ecology, poor understanding of long-term survival, introduction of novel or antibiotic-resistant pathogens, harm to human health, costs, the high public profile and labor efforts involved in rehabilitation programs with unclear aims (Moore et al., 2007). From the Scientific Platform for seals in the Wadden Sea it was stated that "in order to preserve the seal population, it is not necessary to remove, care for and return sick, weakened or deserted seals to the Wadden Sea". (Wetenschappelijk Platform Zeehonden Waddenzee, 2002). Uncertainties concerning marine mammal wildlife rehabilitation are the lack of information about how best to release an animal, ignorance about the reproductive potential of released animals, risk of abnormal behavior in the wild resulting from human interactions during captivity, capacity to forage successfully once released and ignorance about long-term survival (Moore et al., 2007). Ecomare minds these justifications, but still continues rehabilitating stranded seals (event though in far fewer numbers than the SRRC), in view of animal protection and the relatively large percentages of seals that do well after being released. Also they state to be huge contributors in spreading knowledge about environmental problems of the Wadden sea by providing education to a wide audience. Still much research is needed in order to be able to make substantiated decisions for the seal as an individual as well as for the seal population.

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Appendix - Stranding data of stranded seals between 2009 and 2012
(Database of the Department of Veterinary Pathobiology, 2012

2009

Serie	Carcass	GLIMS	EHBZ/Imares	Dd	Mm	Yy	Stranding location	Age	Sex	DCC	Frozen	State of carcass	Scavenging	NCC	Macro conclusion
PV	1	3090514051		22	2	2009	Texel paal 23	S	F	2	Y	Fully intact	None	1	Infectious
PV	2	3090514052		20	2	2009	Texel paal 20	S	M	2	Y	Fully intact	None	3	Infectious
PV	4	3090630003		22	5	2009	Texel paal 21	J	F	2	Y	Fully intact	None	4	Unknown
PV	5	3090630004		16	4	2009	Ijmuiden	S	M	3	Y	Scavenged	Moderate	2	Trauma
PV	8	3090703044		2	7	2009		N	M	1	N	Fully intact	None	5	T61
PV	10	3090901024		2	7	2009	Texel paal 23	J	M	4	Y	Scavenged	Severe	6	Starvation
HG	5	3090619034	D-SH 383 groen	17	6	2009	Maasvlakte	J	M	2	N	Fully intact	None	2	Certain bycatch
HG	6	3090702001		15	4	2009	Texel paal 8	S	F	2	Y	Fully intact	Moderate	4	Asphyxiation
HG	7	3090703001		27	5	2009	Bergen aan Zee, km 31,5	J	M	3	Y	Scavenged	Moderate	3	Unknown

2010

Serie	Carcass	GLIMS	EHBZ/Imares	Dd	Mm	Yy	Stranding location	Age	Sex	DCC	Frozen	State of carcass	Scavenging	NCC	Macro conclusion
HG	1	3100616042		21	12	2009	Egmond aan Zee	J	F	2	Y	Fullyintact	Mild	4	Emaciation of unknown origin
HG	2	3100616041		20	12	2009	Callantsoog	J	M	2	Y	Scavenged	Mild	6	Emaciation of unknown origin
HG	3	3100616043		30	12	2009	Texel paal 18	J	M	2	Y	Scavenged	Mild	5	Emaciation of unknown origin
HG	4	3100616044		5	4	2010	Togen, Texel	S	M	4	Y	Scavenged	Moderate	3	Unknown
PV/HG	11	3100531001	203	23	4	2010	Texel paal 28	S	U	5	Y	Incomplete	Severe	1	Unknown
PV/HG	12	3100817035		2	7	2009	Dijk Zeeburg, Texel	N	U	5	Y	Incomplete	Severe	4	Unknown
PV	13	3100819049		24	6	2009	Oudeschild, Texel	J	F	4	Y	Scavenged	Moderate	U	Unknown
PV	14	3101228024	238	29	12	2010	Texel paal 7	J	F	1	N	Fullyintact	None	4	Infectious
PV	16	3100616045		18	5	2010	Schoorl aan zee	A	F	2	Y	Fullyintact	None	3	Infectious
PV	17	3100616046	302	10	5	2010		S	M	3	Y	Scavenged	Mild	3	Possibe bycatch
PV	18	3100616047		4	12	2009	De Hors, Texel	J	M	2	Y	Scavenged	Mild	3	Pneumonia
PV	19	3100616048		31	10	2009	Noord Slufter, Texel	J	F	3	Y	Scavenged	Mild	3	Unknown
PV	20	3100616049	946 DLD	11	4	2010	Texel paal 6	J	F	2	Y	Scavenged	mild	3	Pneumonia
PV	21	3100616050		10	4	2010	Texel paal 7	J	M	3	Y	Scavenged	Mild	4	Starvation
PV	22	3100616051		13	9	2009		J	M	2	Y	Fullyintact	None	6	Pneumonia
PV	23	3100616052		26	10	2009	Texel paal 15,2	J	M	3	Y	Scavenged	Moderate	4	Emaciation of unknown origin
PV	24	3100616053		24	12	2009	Texel paal 8	J	F	2	Y	Scavenged	Mild	3	Pneumonia
PV	25	3100616054	914 geel	19	3	2010		J	F	2	Y	Scavenged	None	4	Emaciation of unknown origin
PV	26	3100616055		2	11	2009	Egmond aan Zee	J	F	2	Y	Fullyintact	Mild	4	Infectious
PV	27	3100616056		7	8	2009	Texel paal 17	J	M	2	Y	Scavenged	None	4	Unknown
PV	28	3100616057		31	8	2009	Texel paal 25	J	M	2	Y	Fullyintact	None	4	Infectious
PV	29	3100616058	991 geel	8	5	2010	Egmond aan Zee	J	F	2	Y	Fullyintact	None	3	Unknown
PV	30	3100616059		13	2	2009	Hondsbosche zeewering	J	F	2	Y	Scavenged	Mild	3	Infectious
PV/HG	31	3100616006		3	6	2009	Texel, pl 24,5	J	F	5	Y	Incomplete	Moderate	3	Unknown
PV	32	3100616061		9	10	2009	Texel, pl 26,5	J	F	2	Y	Scavenged	Mild	3	Trauma?
PV	33	3100616062		16	5	2010	Groote Keeten km 11	J	F	2	Y	Fullyintact	None	3	Pneumonia
PV	34	3100616063		2	1	2010	Texel, pl 28	A	F	2	Y	Fullyintact	None	2	Unknown
PV	35	3100616064		11	1	2010	Texel, pl 33	A	F	2	Y	Scavenged	Mild	2	Unknown
PV	36	3100616065		12	4	2010	Ijzerenkaap, Texel	A	F	3	Y	Scavenged	Mild	2	Liver rupture?
PV	36	fetus							M						

2011

Serie	**Carcass**	**GLIMS**	**EHBZ/Imares**	**Dd**	**Mm**	**Yy**	**Stranding location**	**Age**	**Sex**	**DCC**	**Frozen**	**State of carcass**	**Scavenging**	**NCC**	**Macro conclusion**
HG	8							A	M	2,5	Y	Fully intact	None	3	Unknown
HG	9	3110429032	232	17	12	2010	Texel paal 17	J	F	3	Y	Scavenged	Mild	2	Unknown
HG	10	3110429033	210	12	6	2010	Vuurtorenstrand, Texel	J	M	4	Y	Scavenged	Mild	4	Blunt Trauma
HG	11	3110601039	234	17	12	2010	Texel paal 26,4	J	F	2	Y	Incomplete	None	2	Unknown
HG	12	3110601040	250	26	2	2011	Vuurtorenstrand, Texel	J	M	2	Y	Fully intact	Mild	4	T61 in rehab
HG	13	3110604041	239	26	12	2010	Texel paal 18	J	M	3	Y	Fully intact	None	5	T61 in rehab
HG	14	3120126051		19	1	2012	Hargen aan zee, km 27	J	F	2	Y	Peck or bite wound	Mild	4	Trauma or hypothermia
PV	15	3110601042	243	11	1	2011	Texel paal 31	J	M	3	Y	Fully intact	Mild	5	Emaciation of unknown origin
PV	37	3110314039	219	3	9	2010	Julianadorp paal 13	J	M	3	Y	Fully intact	None	4	Infectious
PV	38	3110314043	213	26	7	2010	Texel paal 30	J	M	2	Y	Fully intact	Mild	4	Infectious
PV	39	3110314038	212	17	6	2010	Ceres, Texel	N	F	4	Y	Scavenged	Moderate	3	Unknown
PV	40		215	11	8	2010	Groote Keeten	J	M	2	Y	Fully intact	None	4	T61 in rehab
PV	41		217	31	8	2010	Cocksdorp, Texel	A	M	3	Y	Fully intact	None	4	Possible bycatch
PV	42	3110614042	218	1	9	2010	Texel paal 17	J	F	3	Y	Fully intact	None	4	Blunt Trauma
PV	44	3110429034		18	6	2010	Den Helder	J	F	3	Y	Fully intact	Mild	6	Blunt Trauma
PV	45	3110429035	209	11	6	2010	Texel paal 20	S	F	2	Y	Fully intact	None	5	Unknown
PV	46	3110429036	228	10	12	2010	Texel paal 11	J	M	2	Y	Fully intact	None	3	T61 in rehab
PV	47	3110429037	205	21	5	2010	Texel paal 12	J	M	2	Y	Fully intact	None	4	Infectious
PV/HG	48	3110429038	206	22	5	2010	Texel paal 20	A	M	4	Y	Incomplete	Severe	3	Unknown
PV	49	3110429039	208	5	6	2010	Oudeschild, Texel	S	M	3	Y	Fully intact	Mild	5	Trauma
PV/HG	50	3110429040	211	14	6	2010	Vuurtorenstrand, Texel	N	M	4	Y	Fully intact	Mild	6	Unknown
PV	51	3110429041	231	17	12	2010	Texel paal 22	J	F	3	Y	Incomplete	Severe	1	Unknown
PV	52	3110601043	244	18	1	2011	Vuurtorenstrand, Texel	A	F	3	Y	Fully intact	None	3	Unknown
PV	53	3110601044	225	8	11	2010	Julianadorp	J	F	3	Y	Fully intact	None	4	T61 in rehab
PV	54	3110601045		12	10	2010	Den Helder paal 5	J	M	3	Y	Fully intact	None	4	Emacation of unknown origin
PV	55	3110601046	226	5	12	2010	Vuurtorenstrand, Texel	J	M	3	Y	Fully intact	None	5	Emaciation of unkown origin

PV	56	3110601047	233	17	12	2010	Texel paal 16	J	M	3	Y	Fully intact	None	3	pneumonia
PV	57	3110601048	245	15	1	2011	Sint Maartenzee km 18	J	M	3	Y	Fully intact	None	5	T61 in rehab
PV	58	3110601049	229	11	12	2010	Schoorl paal 15	J	F	3	Y	Fully intact	None	6	T61 in rehab
PV	59	3110601050	253	26	2	2011	Texel paal 21	J	F	3	Y	Fully intact	None	3	T61 in rehab
PV	60	3110601051	227	5	12	2010	Texel paal 23,4	J	F	3	Y	Fully intact	Mild	5	T61 in rehab
PV	61	3110601052	236	22	12	2010	Bergen aan Zee	J	M	3	Y	Fully intact	None	4	T61 in rehab
PV	62	3110601053		12	12	2010	Den Helder paal 3	J	F	3	Y	Scavenged	Mild	3	Unknown
PV	63	3110601054	256	13	3	2011	Texel paal 12	J	F	3	Y	Fully intact	None	4	T61 in rehab
PV	64	3110601055	254	7	3	2011	Texel de Hors	J	M	3	Y	Fully intact	None	4	T61 in rehab
PV	65	3110601056	291	25	2	2011	Hargen paal 27,250	J	F	3	Y	Fully intact	None	3	T61 in rehab
PV	66	3110601057	222	1	11	2010	Texel paal 23	J	M	2	Y	Fully intact	None	4	T61 in rehab
PV	67	3110601058	237	22	12	2010	Den Helder	J	M	3	Y	Fully intact	None	4	T61 in rehab
PV	68	3110601059		3	1	2011	Zwanenwater Noord-Hollan	J	F	2	Y	Fully intact	Mild	5	Drowning
PV	69	3110601060	255	23	3	2011	Texel paal 17	J	F	3	Y	Scavenged	Moderate	4	Unknown
PV	70	3110601061	247	2	1	2011	Petten km 20	J	F	3	Y	Fully intact	None	4	T61 in rehab
PV	71	3110601062	242	8	1	2011	Texel Mokbaai strand	J	M	3	Y	Fully intact	None	3	Pneumonia? Trauma?
PV	72	3110601063		1	11	2010	Groote Keeten, km 10	J	M	3	Y	Fully intact	None	3	Unknown
PV	73	3110601064	240	4	1	2011	Texel paal 9	A	F	3	Y	Scavenged	Mild	3	Unknown
PV	74	3110621036	223	17	11	2010	Texel paal 29	J	M	3	Y	Fully intact	None	6	Pneumonia
PV	75	3110621037	220	13	9	2010	Vuurtorenstrand, Texel	A	F	3	Y	Fully intact	None	6	T61 in rehab / unknown.
PV	76	3110621038		24	10	2010	Camperduin, km 26	J	M	4	Y	Scavenged	Mild	4	Unknown
PV	77	3111123001		20	9	2011		J	M	4	Y	Fully intact	None	3	Possibly bycatch
PV	78	3111216004		14	12	2011	Texel, Paal 18	A	F	1	N	Fully intact	None	1	Unknown

2012

Serie	Carcass	GLIMS	EHBZ/Imares	Dd	Mm	Yy	Stranding location	Age	Sex	DCC	Frozen	State of carcas	Scavenging	NCC	Macro conclusion
PV/HG	15	3120601044		15	7	2011	Texel, Paal 20,8	N	M	4	Y	Scavenged	Mild	6	Unknown
PV/HG	16	3120601052		22	10	2011	Texel, Paal 19 - 20	N	F	4	Y	Incomplete	Moderate	6	Severe parasite infestation heart
HG	17	3120608067		2	2	2012	Wijk aan Zee	J	M	2	Y	Fully intact	None	5	Severe parasite infestation lungs and stomach
HG	19	3120925038		24	9	2012	Breezand, Ijsselmeer	A	F	3	N	Scavenged	Mild	2	Probably bycatch
PV	79	3120102034	620	12	12	2011	Vuurtorenstrand, Texel	J	M	2	Y	Fully intact	None	5	T61 in rehab
PV/HG	80		616	24	12	2011	Schorren, Texel	J	F	5	Y	Skeletel parts	Severe	-	Unknown
PV	81	3120105057	615	18	11	2011	Havenkantoor, dijk Texel	A	F	2	Y	Fully intact	None	4	Unknown
PV	82	3120105058		16	11	2011	Zuidermeerhaven, Den Helder	A	F	3	Y	Fully intact	None	1	Trauma
PV/HG	83	3120105059		28	11	2011	Lange Jaap, Den Helder	J	M	3	Y	Incomplete	Severe	3	Chronic pneumonia
PV	84	3120111059	612	12	12	2011	Cocksdorp, Texel	J	F	2	Y	Fully intact	None	3	T61 in rehab
PV	85	3120112004	624	17	12	2011	Paal 31, Texel	A	M	2	Y	Fully intact	None	2	Torsio of the jejunum through an opening in the mesenterium
PV	86	3120112006		21	12	2011	Huisduinen	A	F	3	Y	Fully intact	None	2	Unknown
PV	87	3120112009		17	12	2011	De slufter, Texel	J	F	2	Y	Fully intact	None	5	Parasitic infestation and damage to the lungtissue
PV	88	3120112011		26	12	2011	Petten, paal 19	J	M	2	Y	Fully intact	None	3	Pneumonia, lungworm
PV	89	3120112014	626	5	12	2011	Paal 27,5, Texel	J	F	2	Y	Fully intact	None	3	T61 in rehab
PV	90	3120112016	627	17	12	2011	Paal 31, Texel	J	M	2	Y	Fully intact	None	3	T61 in rehab
PV	91	3120112018		27	12	2011	Callantsoog	J	F	2	Y	Fully intact	None	3	T61 in rehab
PV	92	3120126048	635	6	2	2012	Ijzeren Kaap, Texel	A	F	3	Y	Fully intact	None	1	Blunt trauma
PV	93	3120126049	634	9	1	2012	Julianadorp	J	F	2	Y	Fully intact	None	4	T61 in rehab
PV	94	3120126050	641	20	1	2012	Bergen aan Zee, Castricum	A	M	3	Y	Fully intact	None	4	Sepsis because of old wounds?
PV	95	3120126052	636	5	1	2012	Paal 10, Texel	J	M	3	Y	Fully intact	None	3	Pneumonia, lungworm
PV	96	3120126053		19	1	2012	Callantsoog, km 13	S	F	3	Y	Fully intact	None	3	Blunt Trauma
PV	97	3120126054	637	18	12	2011	Den Helder	J	M	2	Y	Fully intact	None	3	T61 in rehab
PV	98	3120126055	640	18	1	2012	Paal 28, Texel	J	F	2	Y	Fully intact	None	4	T61 in rehab
PV	99	3120126056	638	19	1	2012	Paal 34, Texel	J	F	2	Y	Fully intact	None	4	T61 in rehab
PV	100	3120126057	639	19	1	2012	Texel, Slufter	J	F	2	Y	Fully intact	None	4	Extensive necropurulent peritracheitis
PV	101	3120131043	644	14	1	2012	Petten	J	M	1	N	Fully intact	None	3	T61 in rehab
PV	102	3120131044	643	18	1	2012	St. Maarten zee (17.500)	J	M	1	N	Fully intact	None	2	Died in rehab
PV	103	3120131045	645	30	1	2012	Texel, Paal 28	J	F	1	N	Fully intact	None	3	T61 in rehab
PV	104	3120131046	646	30	1	2012	Ten noorden van de badweg	J	F	1	N	Fully intact	None	3	T61 in rehab
PV/HG	105	3120314045		12	8	2011	Hondsbosche Zeewering, km2	J	F	5	Y	Scavenged	Severe	-	Unknown / possible bycatch?
PV	106	3120314046	642	12	1	2012	Noord Hollandse kust	J	M	2	Y	Fully intact	None	3	Died in rehab on 29-1-2012
PV	107	3120314047	170	12	1	2012	Noord Hollandse kust	J	M	2	Y	Fully intact	None	4	T61 in rehab on 28-1-2012
PV	108	3120314048	282	7	9	2011	Hargen aan Zee	J	M	2	Y	Fully intact	None	6	Died in rehab, emaciation of unknown origin
PV	109	3120427031		22	4	2012	Andijk, Ijsselmeer	A	F	4	Y	Scavenged	Severe	2	Unknown
PV	110	3120525033		3	7	2011	Zwanenwater km 14	J	F	2	Y	Scavenged	Mild	5	Parasite infestation, multiple organs
PV	111	3120525036	263	4	5	2011	Dijksman Huizen, Texel	S	F	4	Y	Scavenged	Moderate	4	Unknown
PV	112	3120525040	262	2	5	2011	Paal 34, Texel	A	F	4	Y	Incomplete	Moderate	3	Unknown
PV	113	3120525041	269	30	6	2011	Paal 30, Texel	N	F	2	Y	Fully intact	None	6	T61 in rehab
PV	114	3120601043	264	4	5	2011	van de Harding rechts, Texel	S	F	2	Y	Scavenged	Mild	2	Sepsis
PV	115	3120525042	271	14	7	2011	Cocksdorp, Texel	J	M	3	Y	Fully intact	None	2	Pneumonia
PV	116	3120525043	296	1	6	2012	Paal 13, Texel	A	M	4	Y	Scavenged	Mild	5	Unknown
PV	117	3120525044	270	13	7	2011	Paal 20, Texel	N	M	5	Y	Scavenged	Moderate	6	Unknown
PV	118	3120601042	272	16	7	2011	Cocksdorp, Texel	J	M	4	Y	Scavenged	Mild	2	Unknown
PV	119	3120601045		31	7	2011	Texel, km 12-50	J	M	2	Y	Fully intact	None	2	Possibly pneumonia
PV/HG	120	3120601047	261	30	4	2011	Cocksdorp, Texel	N	M	4	Y	Scavenged	Mild	5	Unknown
PV	121	3120601048	280	25	6	2011	Paal 28, Texel	J	M	4	Y	Scavenged	Mild	5	Unknown
PV/HG	122	3120601049	265	5	5	2011	Schans, Texel	N	F	4	Y	Scavenged	Mild	3	Still birth
PV	123	3120601050	266	5	6	2011	Cocksdorp, Texel	N	F	4	Y	Scavenged	Moderate	3	Unknown
PV	124	3120601051		21	7	2011	Marinehaven, Den Helder	J	F	4	Y	Scavenged	Mild	1	Unknown
PV	125	3120608051	648	25	1	2012	Paal 28, Texel	J	F	2	Y	Fully intact	None	3	T61 in rehab
PV	126	3120608052	711	29	3	2012	Paal 12, Texel	J	F	3	Y	Scavenged	Mild	5	Pancreatitis
PV	127	3120608054	649	22	1	2012	Hondsbosche Zeewering, km2	J	M	2	Y	Fully intact	None	3	T61 in rehab
PV	128	3120608055	275	20	8	2011	de Schans, Texel	J	M	2	Y	Fully intact	None	4	Sepsis due to polyarthritis
PV	129	3120608057	629	28	12	2011	Schoorl	J	F	2	Y	Fully intact	None	3	T61 in rehab
PV	130	3120608058	628	16	12	2011	Julianadorp	J	M	2	Y	Fully intact	None	4	T61 in rehab

HG	131	3120731047	green 78	30	7	2012	Fort Erfprins, Den Helder	S	M	4	N	Scavenged	Mild	4	Infectious
PV	132	3120608062	651	4	2	2012	Slufter Paal 26 - 400	J	F	2	Y	Scavenged	Mild	6	Lungworm infestation with emaciation
PV	133	3120608063	284	22	9	2011	Paal 29, Texel	J	F	2	Y	Fully intact	None	1	Liver disease
PV	134	3120608066		28	12	2011	Schoorl	J	F	2	Y	Fully intact	None	3	Liquothorax
PV/HG	135	3120608068	702	19	3	2012	Paal 19,5, Texel	S	M	4	Y	Scavenged	Severe	3	Unknown
PV	136	3120608069	662	8	4	2012	Zeeburg, Texel	A	M	4	Y	Scavenged	Mild	3	Severely enlarged right kidney
PV	137	3120914043	709	1	4	2012	Paal 18, Texel	S	F	2	Y	Fully intact	None	2	Trauma
PV	138	3120914048	664	24	5	2012	Vuurtorenstrand, Texel	J	F	4	Y	Scavenged	Mild	-	Gastric ulcers
PV/HG	139	3120914050	665	24	5	2012	Paal 33, Texel	N	M	4	Y	Scavenged	Mild	-	Unknown
PV	140	3120914051	657	29	1	2012	Vuurtorenstrand, Texel	J	F	2	Y	Fully intact	None	3	T61 in rehab
PV	141	3120914052	673	26	6	2012	Paal 20,5, Texel	U	M	4	Y	Incomplete	Mild	-	Unknown
PV	142	3120914053	669	5	6	2012	Paal 28, Texel	J	M	4	Y	Incomplete	Moderate	-	Unknown
PV	143	3120920001		18	9	2012	Camperduin km 26	J	F	1	N	Fully intact	None	2	Chronic pneumonia right, pyothorax, hepatitis
PV	144	3120924056	703	19	3	2012	Paal 9,6, Texel	J	F	2	Y	Fully intact	Mild	3	Pneumonia, lungworm
PV/HG	145	3120924057	679	14	7	2012	Paal 24, Texel	J	-	5	Y	Incomplete	Severe	-	Unknown
PV	146	3120924058		8	3	2012	Ecomare	J	F	2	Y	Fully intact	None	2	T61 in rehab
PV/HG	147	3120924059	671	8	5	2012	Haven N/102, Texel	N	F	5	Y	Scavenged	Moderate	-	Unknown
PV	148	3120924060	670	4	7	2012	IJzeren Kaap, Texel	N	F	4	Y	Scavenged	Moderate	4	Unknown
PV	149	3120924061	676	4	7	2012	Julianadorp	N	F	2	Y	Fully intact	None	6	Unknown
PV/HG	150	3120924063		15	5	2012	Mokbaai, Texel	J	F	4	Y	Scavenged	Moderate	4	Unknown
PV	151	3120924062		9	7	2012	Wieringen	J	F	4	Y	Scavenged	Moderate	-	Possibly trauma
PV	152	3120927046		7	11	2007	NIOZ, Texel	J	F	2	Y	Fully intact	None	1	Bycatch probable
PV	154	3121005022	224	23	10	2012	Vlieland Boulevard, Texel	S	M	4	Y	Scavenged	Moderate	4	Unknown
PV	155	3121005024		4	11	2010	Groote Keeten, km 9	S	M	4	Y	Scavenged	Mild	4	Trauma
PV	156	3121005026	689	3	8	2012	Bergen aan Zee, Paal 33	J	M	2	Y	Fully intact	None	6	T61 in rehab
PV	157	3121005028	694	14	8	2012	Paal 15, Texel	J	M	3	Y	Fully intact	None	4	T61 in rehab
PV	158	3121005029	688	4	8	2012	Schoorl	J	M	2	Y	Fully intact	None	6	T61 in rehab
PV	159	3121005030	693	20	8	2012	Paal 16, Texel	J	M	2	Y	Fully intact	None	6	T61 in rehab

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