Comparative biology of common and grey seals along the Dutch coast : stranding, disease, rehabilitation and conservation

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Synthesis

A multidisciplinary approach has been applied in this thesis, to learn more about the different aspects of the biology and pathology of seals. This has allowed a comprehensive view of their status in Dutch waters. The two seal species, the common seal and the grey seal differ in physiology, behaviour and the use of the Wadden Sea and North Sea ecosystems. These two species were compared with regard to stranding patterns, prevalence of diseases and genetics. The degree to which these species are impacted by human presence was also studied.

The key results from the chapters and publications are given in the synthesis, followed by a discussion of these findings. Finally, overall conclusions of the thesis are given, followed by recommendations for research and conservation.

Key results

The following key results were formulated based on the chapters and publications of this thesis.

Section A. Patterns and trends in strending

- Combining analyses of live-stranded and dead-stranded seals results in four main causes of disease and mortality of seals: orphanage, parasitic bronchopneumonia (common seals only), phocine distemper (common seals only) and by-catch. Orphaned seals and seals with parasitic pneumonia were animals under four weeks of age and under one year of age, respectively. Phocine distemper and by-catch occurred in seals of all ages.
- The number of live-stranded common seals with parasitic bronchopneumonia has increased steeply in recent years: from 0-30 per year for 1971-1997, 30-160 per year for 1998-2009 to 300-500 per year for 2009-2013. Seals also suffer from parasitic bronchopneumonia at a much younger age now compared to previous decades.

Section B. Genetic variation

- The level of genetic variation in common seals was lower than in grey seals. However, in neither species was there any cause for concern in terms of potential genetic vulnerability to disease.
- Grey seals recolonised Dutch waters in the 1980s. The observed high incidence of melanism in Dutch grey seals may be a genetic consequence of the recolonisation.

Section C. Parasitic infections

- The lungworms *Parafilaroides gymnurus* from common seals of the Wadden Sea have the longest body lengths that have been described for this species. We suggest that both the immunosuppression and the young ages of exposure to larvae in common seals of the Wadden Sea provide an optimal environment for *P. gymnurus* and enable them to reach the long lengths.

- First-stage *P. gymnurus* larvae were detected in fish species that are part of the weaned common seal's diet: hooknose, juvenile dab, and in juvenile plaice. The finding of first-stage larvae suggests that these fish species may be part of the lifecycle of this lungworm. However, experimental infections are required to further test this.
- In 2012, a young common seal pup (1.5-3 months of age) died mid-August and had an infection with mature *Otostrongylus circumlitus* lungworms. We also found unidentified nematode larvae in placenta material. These findings suggest that vertical transmission of larvae – that is from mother to pup – may play a role in the life cycle of seal lungworms.

Section C. Breeding biology

- Seals in the Dollard were frequently disturbed by pedestrians on the dyke area. Disturbance by agricultural activities and by boats occurred less frequently. The observations showed that human disturbance resulted in seals being harassed into the water on average once every seven hours and seals being alerted on average once every three hours.
- The pupping season of common seals in the Wadden Sea area has advanced progressively by 26 days over a period of 34 years. Such a shift in phenology could reflect an adaptive response of the animals to altered local circumstances, which in turn may be induced by larger scale phenomena such as climate change. There were no indications of any negative impact on the weight of the pups. This suggests that the most likely explanation for the change in phenology is a corresponding change in the timing of cessation of the period of delayed implantation rather than a shortening of the period of active gestation.

Discussion

The main findings of the thesis have been explored in the discussion sections of the publications and chapters. Here, an overall discussion on the status of seals in the Dutch waters is provided. First, the methodology of analysing stranding data and aerial survey data is discussed (2.1). Then, the population trends are discussed and estimates of recruitment are given (2.2). Thereafter, the different human impacts on seals are listed (2.3), followed by a comparison between the effects of human impacts on common seals and grey seals (2.4). Finally, several important facets of seal rehabilitation in the Netherlands are discussed (2.5).

2.1 Methodology

Most of the findings in this thesis have been the result of information gained from either live-stranded or dead-stranded seals. Combining these data with estimates of population size and trends can reveal increases in disease relative to population size, such as found for parasitic pneumonia in common seals (see **Chapter 1** and **Chapter 2**). However, both

types of data have potential biases that should be considered when interpreting results retrieved from these data.

Analyses of stranding data

Stranding records, life history data, veterinary diagnoses and samples collected from stranded animals are an important source of information on the health status of a population. Stranding data provide a minimum estimate of the level of mortality as well as an inventory of causes of disease and mortality. The following points should be considered when using data of stranded seals.

The Netherlands has an accessible coastline and therefore a high detection rate for stranded marine mammals. After its establishment in 1971, the SRRC stranding network expanded during the initial years, but remained stable over the past 30 years. In the latter period, it may be assumed that reporting effort of live-stranded seals and collection effort of dead-stranded seals has been more or less constant.

The number of stranded seals is an underestimation of total mortality in the population. This is because not all seals that die at sea wash up on the coast, not all stranded seals are collected for post-mortem examination, and usually only relatively fresh carcasses are included. Therefore, the true scale of mortality in the population cannot be determined from stranding data. This is a limitation with all stranding investigation programmes (Eguchi 2002).

When using stranding data as a proxy for its source population, it should be realised that biases in the occurrence and discovery of stranded marine mammals may occur. It is important to consider whether a species has a (seasonal) near shore distribution as this is an important parameter determining the chance of stranding (Byrd *et al.* 2014). Winds and currents are also important factors determining stranding rates (Peltier *et al.* 2012). Furthermore, chances of discovery, and perhaps collection effort, of stranded carcasses may vary spatially and seasonally.

Causes of stranding found in dead-stranded seals differ from those in live-stranded seals. Generally, seals suffering from chronic disease are more likely to strand alive than seals with an acute disease. The majority of orphaned pups and seals with parasitic pneumonia strand while still alive (**Chapter 1** and **Chapter 2**). On the contrary, during the PDV epidemics, the majority of seals stranded dead (2154 out of a total of 2284 strandings in the 2002 epidemic; Rijks *et al.* 2005). Seals that are caught in operational fishing nets drown and strand dead, whereas those entangled in lost fishing nets usually strand alive. The chances that seals are found while they are still alive will vary between countries and types of coastlines. For example, in a review study of pathological findings in common seals stranded on the German coast (1996–2005), bronchopneumonia caused by parasitic and/or bacterial infection of the lung was the most common cause of death (Siebert *et al.* 2007). On the contrary, in our review study bacterial and/or parasitic pneumonia constituted only 8% of causes of mortality (**Chapter 2**). This difference is probably caused by the quick rescue response to live-stranded seals in the Netherlands.

There is also a difference in age categories among dead-stranded and live-stranded seals. The dataset of dead-stranded seals consists of seals of all ages (**Chapter 2**), while live-stranded seals are typically seals under one year of age (**Chapter 1**). Possibly, subadult and adult seals suffer less often from chronic diseases that causes them to strand alive. The chances of stranding alive may also be reduced as they spend more time offshore. Finally, it may also be a function of behaviour; i.e. sick subadult and adult seals may avoid coming ashore in populated areas.

For the interpretation of the stranding rates, it should be taken into account that for the Dutch coast strandings of live seals are two to three times more frequent than strandings of dead seals. By-catch was recorded as the main cause of mortality in dead-stranded seals (**Chapter 2**). However, this should be put into perspective as the total number of established by-catch cases was never higher than five and three cases per annum for common seals and grey seals, respectively. This is a very low number compared to the hundreds of live-stranded seals that were orphaned or suffered from a parasitic infestation. At the same time, it should be realised that the presented mortality figures are an underestimation and that the true scale of mortality in the population is higher.

For the interpretation of pathological diagnoses, it should be realised that there may be differences between pathologists in their diagnoses of causes of death. By-catch is a diagnosis that is particularly difficult to establish in the absence of external evidence of contact with fishing gear. Pathologists use different sets of criteria and may be more or less conservative in their diagnoses. For example, by-catch was not reported for any of the 141 dead-stranded common seals examined in Germany, 1996-2005 (Siebert *et al.* 2007). On the contrary, by-catch was the most frequent diagnosis among post-mortem investigated dead-stranded seals in our analysis (**Chapter 2**). Probably this difference is not caused by differences in fisheries between the Dutch and German coast, but rather by a more conservative approach of attributing pathological findings to by-catch in the German study.

Methodology of aerial surveys

In the Dutch Wadden Sea, aerial surveys are conducted both by the SRRC and the Trilateral Seal Expert Group (TSEG). In the Southwest Delta, aerial surveys are conducted by the Rijkswaterstaat (executive body of the Dutch ministry of infrastructure and the environment). The numbers of seals are counted in the pupping season and in the subsequent moulting season during which the maximum number of seals haul out on the sand banks. Aerial surveys with consistent methodology provide reliable data on trends over the years. However, there are difficulties in the estimation of the true number of seals present in the area. These difficulties are in survey methodology and in the extrapolation of counted numbers.

Difficult aspects of the survey methodology are orientation and prevention of disturbance to seals. Especially in an area with many similar looking sandbanks and few orientation points such as the Wadden Sea, it is easy to lose track of which sandbanks were

counted or which still need to be counted. Sandbanks with seals can be missed or they can be counted twice. Therefore, combining seal counting data with a GPS position for each data registration point is essential. Another important factor to take into account is disturbance. Reactive movement of seals in response to low flying airplanes were observed in the Dollard (**Chapter 9**). Combining simultaneous land-based counting (**Chapter 9**) with aerial counting is recommended, as this provides a valuable check on results obtained by both methods.

For the conversion of counted numbers to estimates of population size, an extrapolation factor is often established based upon the percentage of seals hauling out on land in a particular season. Such a percentage is usually determined by equipping seals with transmitters (Thompson *et al.* 1997; Ries *et al.* 1998). But there are several major potential sources of error. First, there are concerns regarding the sample that is tagged. Sample sizes are often small, for example only 15 seals were tagged by Ries *et al.* (1998). Also, samples of marked animals are often not representative for age and sex of the population, implying skewed haul-out patterns (Härkönen & Harding 2001). Second, haul-out behaviour will depend on the type of coastline; e.g. haul-out locations can be permanently exposed or only during low tide. Results obtained on the percentage of time seals spent on haul-out locations (such as Huber *et al.* 2001; Boveng *et al.* 2003) are area-specific and thus cannot be applied to other areas. Third, externally attached devices influence the seal's hydrodynamics and therefore these animals may not exhibit natural behaviour, i.e. they may spend longer foraging in the water to compensate for the greater energy expenditure (Hazekamp *et al.* 2010).

Although current aerial surveys provide a good index of population trends, there is a need to improve the extrapolation of counted numbers to the true number of seals present in the Wadden Sea. Therefore, in this thesis (**Chapter 2** and **Synthesis paragraph 2.2**), we used non-extrapolated survey data.

2.2 Population and recruitment

Here the most important factors determining population dynamics are discussed, such as reproduction, recruitment, disease rate and mass mortalities. To determine recruitment levels, data on stranded seals (**Chapter 1** and **Chapter 2**) were combined with data from aerial surveys. Finally, population trends were compared with those of neighbouring populations.

Reproduction

In the 1980s, common seal pup production in the Netherlands was too low to sustain the local population. This was ascribed to impairment of reproduction in females with high organochlorine pollutant levels (Reijnders 1986). Levels of reproduction have recovered in the 1990s (Reijnders *et al.* 1997) and currently, good pup production levels are reported for both common seals and grey seals in the Wadden Sea (Härkönen *et al.* 2007; Reijnders *et al.* 2010).

Recruitment

First year mortality is an important factor determining population growth in seals. First year mortality for Dutch waters was estimated, based on a combination of different datasets (Table 1). The number of pups counted during the pupping seasons for both seal species was taken from aerial survey data of the Wadden Sea (TSEG 2013) and Southwest Delta (Strucker *et al.* 2011, 2012, 2013). Stranding data were based on data from **Chapter 1** (with updated data in **Appendix 1**) and **Chapter 2**. Also, rehabilitation data from the other rehabilitation centre, Ecomare at Texel, was added (Ecomare 2010, 2011, 2012). It should be taken into account that the number of strandings is an underestimate of the true mortality. Similarly, the number of pups counted during surveys is an underestimate of the true number of pups born. Furthermore, severe weather conditions were reported to have affected the counting of grey seals for the aerial surveys of 2011/2012 and 2012/2013.

For common seals, the percentage of yearlings that stranded varied between 35.9% and 58.1% for the studied period (Table 1). The comparable figures for grey seals varied between 28.2% and 67.0%. The percentage of stranded pups appears to be similar for both seal species; roughly one third to two third of counted pups strand during the first year. Although the percentages appear to be similar for both species, the reasons of stranding differed. Along with orphanage, parasitic pneumonia was the main reason for stranding in common seals, while almost all grey seals stranded due to orphanage or shortly thereafter as weaned seals (**Chapter 1, Appendix 1**).

The calculated stranding rates for the Netherlands should be considered as potential mortality rates. This follows because live-stranded seals are admitted to rehabilitation and an average of 92% of common seals and 95% of the grey seals survive and are released (**Chapter 1**).

Earlier estimates of first year mortality of common seals of the Dutch or German Wadden Sea are: 25% (Borchardt, 1995), 35% (Abt 2002), 43% (Ries, 1999). No published first year mortality rates are available for grey seals and pup mortality rates were used here. Earlier estimates of pup mortality of grey seals from the United Kingdom are: 7.5-12.0% (Baker 1988), 15% (Baker 1984), 14-25% (Boyd & Campbell 1971) and 26%-42% (Anderson *et al.* 1975). For the Norwegian coast, a very low level of pup mortality of 1.1% was reported. For both seal species, there were no earlier reports of mortality rates over 50% as found in our analysis. It appears that for the Dutch coast, the first year stranding rate (roughly 30% to 70%) is higher than reported previously (roughly 10% to 40%).

Disease rate

The number of live-stranded common seals with parasitic bronchopneumonia has increased steeply in recent years (see data presented in **Appendix 1**). However, the number of common seals has also increased in recent decades (CBS *et al.* 2013). Combining stranding data with number of counted seals, shows that the number of stranded common seals with a parasitic pneumonia has increased relative to population size (Figure 1). Such an increase relative to population growth was not found for grey seals. The sample sizes of

Table 1. Number of yearlings stranded per seal year, number of pups counted in the Wadden Sea and percentages of stranded yearlings of the total number of pups counted.

		Live strandings seals < 1 year	Live strandings seals < 1 year, Ecomare [*1]	Dead strandings seals < 1 year	Number of pups counted Wadden Sea	Number of pups counted, South west Delta [*2]	Total percentage of pups that stranded	Percentage of pups that stranded alive	Percentage of pups that stranded dead
Common seal	2010-2011	457	59	16	1451	32	35.9	34.8	1.1
	2011-2012	771	58	29	1445	32	58.1	56.1	2.0
	2012-2013	467	57	23	1473	32	36.3	34.8	1.5
Grey seal	2010-2011	66	15	11	322	4	28.2	24.8	3.4
	2011-2012	171	19	11	288	12	67.0	63.3	3.7
	2012-2013	83	23	3	355	7	30.1	29.3	0.8

[*1] Data for Ecomare is per calendar year; data for 2010 is used for seal year 2010-2011 etc.
[*2] Common seal aerial survey data of 2012 was not available yet, therefore data for 2011 was used for 2012.

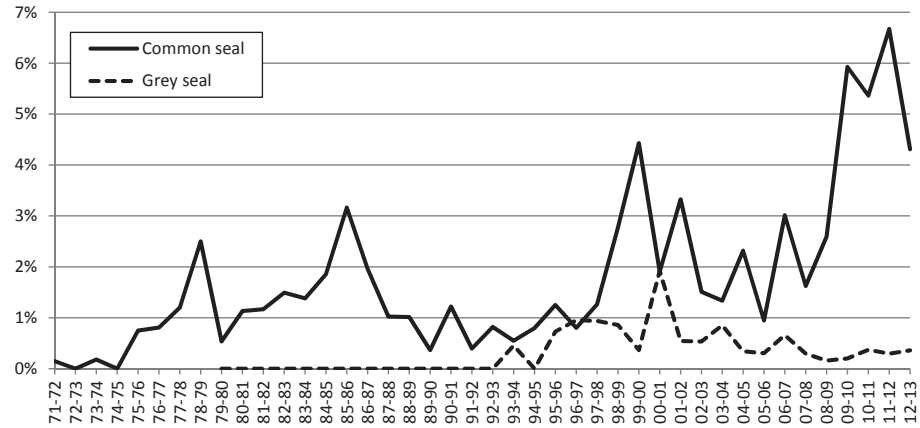

Figure 1. Number of admitted common and grey seals diagnosed with parasitic pneumonia as a percentage of the number of seals counted in Dutch waters (Delta area and Wadden Sea combined; CBS *et al.* 2013).

data on disease are much smaller for the dead-stranded seals and no significant changes over time in the occurrence of disease could be detected in this dataset, except for a decrease in the occurrence of by-catch in grey seals (Table 4, **Chapter 2**).

Mass mortalities

Phocine distemper was the main cause of mortality for the common seal population in two major epidemics (1988 and 2002) (**Chapter 2**), whereas it caused no mortality among grey seals. Two major outbreaks of phocine distemper virus (PDV), in 1988 and in 2002, had large effects on the common seal population (Osterhaus & Vedder 1988; Jensen *et al.* 2002). The estimated cumulative mortality in 2002 (54%) was similar to that in 1988 (53%) (Rijks *et al.* 2005). For both epidemics, the first cases of mortality were noted on Anholt Island, Denmark with a subsequent spread of the disease east to Germany and Denmark, and west to Belgium, France, the United Kingdom, and Ireland (Rijks *et al.* 2005). Phocine distemper is likely to reoccur in epidemic form and substantially impact common seal populations in the North Sea. Recently, the percentage of common seals in the Wadden Sea that have protective immunity against PDV was estimated to be of the order of only 11% (Bodewes *et al.* 2013a). This proportion of immune individuals is not sufficient to provide effective herd immunity against PDV should it be introduced again into the population. In the above study, it was concluded that PDV re-introduction in this area therefore may cause a major epizootic with infection of >80% and mass-mortality of >50% of the population.

Other populations

At present, common seal populations are reported to be declining in several areas of Europe, including some that are strongholds for this species, such as Scotland and Norway. In Scotland, strong declines have been seen since 2000; Shetland 30%, Orkney

75% and the Firth of Tay 85% (SCOS 2013). The number of seals declined In Norway by 10% between surveys carried out in the period 1996-1999 and in 2003-2005 (Nilssen *et al.* 2010). Other areas have been surveyed less well, but available data indicate concerns for conservation, e.g. for the Murman coast of Russia (Zyryanov & Egorov 2010), the Baltic (Härkönen & Isakson 2010), Iceland (Hauksson & Einarsson 2010) and Greenland (Rosing-Asvid 2010). In recent decades, there has been an increase in the number of common seals in the Wadden Sea and Southwest Delta (CBS *et al.* 2013). A similar increasing trend was found for common seals in Denmark and Southern Sweden (Regions Skagerrak, Kattegat, Limfjord and western Baltic; Olsen *et al.* 2010). There has been a different trend in England, in comparison to Scotland. Counts in the Wash and eastern England did not demonstrate any recovery from the 2002 epidemic until 2009, but numbers have increased in the past three years (SCOS 2013).

Grey seal populations in Europe are increasing in many regions, including the mainland European coast, United Kingdom (Duck & Thompson 2007), Norway (Nilssen & Haug 2007), and the Baltic (Harding *et al.* 2007). A decline was reported for Iceland (Hauksson 2007). No recent data were available for the Murmam coast of Russia (Ziryanov & Mishin 2007) and the Faroe islands (Mikkelsen 2007).

Generally, a reduction in recruitment seems to play a role in the areas with a declining abundance of common seals (Bowen *et al.* 2003; Thompson *et al.* 2010). Declines were also observed in North American common seal populations. For Sable Island, Canada, food shortages arising from competition with grey seals were suggested to play a role, along with shark-inflicted mortality (Bowen *et al.* 2003). The decline in the western Gulf of Alaska was thought to be related to overfishing of shrimp and capelin which are important prey species for young common seals in this area (Hansen 1996). Several possible causes were suggested for the decline in Scotland, namely competition with grey seals through dietary and foraging area overlap, disease, and in particular the ingestion of toxins from harmful algae and physical trauma (SCOS 2013). For Norway, increased hunting, high by-catch levels and the PDV epidemic were considered to be the main reasons for the decrease (Nilssen *et al.* 2010).

2.3 Human impacts

Seals that are dependent on coastal waters are vulnerable to the impacts of human activities. The latter can cause direct mortality, such as fisheries leading to by-catch of seals (**Chapter 2**). They can also be indirect, such as disturbance, pollution and food availability. These may affect the occurrence and severity of orphanage and parasitic pneumonia (**Chapter 1**, **Chapter 2, Chapter 9**). It is difficult to distinguish between natural and human-caused mortality and these two cannot be easily teased apart. For example, mother-pup separations and parasites may be natural causes of morbidity or mortality, however human influences can exacerbate or contribute. In this paragraph, all human activities are listed which are expected to have an effect on seal survival. Finally, the cumulative impact is discussed.

Direct human-induced mortality

Causes of death in seals that are directly linked to human activities include by-catch, human-induced trauma and ingestion of human-produced foreign bodies. Orphanage can also be a consequence of direct human impact, when disturbance causes separation of mothers and pups (Doornbos 1980; **Chapter 9**). However, in the case of pups it is impossible to determine the cause of separation, i.e. these could have occurred due to disturbance (**Chapter 9**), extreme weather conditions (Bonness *et al.* 1992) or other reasons.

For common seals, 24% of post mortem investigated animals died from direct human-induced mortality (confirmed by-catch: n=4, inferred by-catch: n=50, human produced foreign body: n=6, physical trauma: N=8, total number of necropsied seals 1979-2008: n=286; **Chapter 2**). Orphanage was not included in this analysis as the percentage caused by disturbance cannot be determined. This calculation therefore needs to be regarded as a minimum estimate. For grey seals, also 24% of post mortem investigated animals died from direct human-induced mortality (confirmed by-catch: n=5, inferred by-catch: n=9, human produced foreign body ingestion n=3, physical trauma: n=5, total number of necropsied seals 1979-2008: n=93; **Chapter 2**)**.** For some of the seals in the dataset, no cause of death could be established at post-mortem examination. Assuming there is no difference in detection for certain causes of death, these seals can be omitted from the current analyses (remaining number of necropsied common seals n=186 and grey seals n=50). Then, the percentage of direct human-induced mortality amounts to a total of 37% for common seals and a total of 44% for grey seals for all post-mortem investigated seals in which a cause of death could be established. It should be realised that the numbers presented above are an underestimation of the true mortality in the population. This is because not all seals that die at sea wash up on the coast, not all stranded seals are collected for post-mortem examination, and for this study only relatively fresh carcasses were included.

By-catch, foreign body ingestion and trauma most often cause acute death rather than chronic disease (**Chapter 2**). Therefore, direct human impact constitutes a relatively small part of all live strandings. Live seals that strand from direct human impact are those that are entangled in lost fishing nets or other marine debris (Van Liere *et al.* 2012a) or those that have ingested fishing hooks but are still alive (Osinga & 't Hart 2006). Currently less than ten entangled live common and grey seals strand per year (unpublished data SRRC) and the number of live stranded seals with an ingested fishhooks was not higher than one case per year (Osinga & 't Hart 2006).

Human disturbance

Common seals give birth on sandbanks in the Wadden Sea. In this thesis a study is included on common seals in the Dollard, one of the core breeding areas of the Wadden Sea (**Chapter 9**). In this area, seals haul-out close to the mainland where recreational activities are the primary cause of disturbance to seals. The causes of disturbance in other breeding areas of the Wadden Sea have not recently been investigated. However, studies in

the 1980s have shown that boats (both those used for professional or recreational purposes) are important (Reijnders 1981, Doornbos 1980, Van Wieren 1981). More research on the disturbing effects of boats is recommended, especially because the number of ships and boats that use the Wadden Sea has nearly doubled since 1982 (Convenant vaarrecreatie Waddenzee 2007). There are no studies on disturbance of seals in the Southwest Delta area, but a comparable level of human activities as found for the Wadden Sea is expected here. Another source of disturbance comes from building activities in harbours. For example, in 2009/2010 the harbour Eemshaven (eastern Wadden Sea) was enlarged. Observations of seals on a nearby sandbank 'De Hond' revealed that during the pupping season particular building activities were associated with a decrease in the number of seals (Van Liere *et al.* 2012b).

Grey seals give birth further away from the coast, on the three permanently exposed sandbanks in the tidal outlets between islands. They also give birth in winter when recreational pressure is low. However, disturbance is affecting grey seals, but only in those years when these sandbanks are submerged due to severe weather conditions. They then relocate to beaches of the Wadden Sea islands, which are used year-round for recreational purposes. Just as for common seals, disturbance was observed to lead to separations of grey seal mothers and pups (SRRC unpublished data). This phenomenon is also reflected in the grey seal stranding data; where a high stranding rate of orphaned and weaned grey seal pups was found in particular years (**Chapter 1**, **Appendix 1**).

Alongside separation, repeated human disturbances will have negative energetic consequences and could be detrimental for the condition of the pups. The survival chance of pups depends largely on an undisturbed nursing period, and the nutritional status at weaning (Drescher 1979). During low tide when sandbanks emerge, there is only a limited time available for suckling and resting of pups on land. Drescher (1979) found that because of frequent disturbance, mother and pup spend much of the low tide period in the water. A lower body condition for common seals in autumn has been linked to first year survival (Harding *et al.* 2005). For grey seals on tourist beaches, it was observed that mothers stayed at the waterline and postponed suckling until more quiet times of the day (SRRC unpublished data). Repeated disturbance can thus negatively affect the body condition of weaned seals. Chronic stress can also have a negative effect on an animal's immunological and physiological functioning (Kight & Swaddle 2011). To conclude, repeated disturbance can thus potentially reduce weaning weights and/or negatively affect immune system functioning and therefore disturbance may eventually play a role in the occurrence of disease after weaning.

Fisheries and food availability

Fisheries reduce fish stocks and therefore lower the prey availability for seals. Daily catches of a fyke net near Texel have been monitored by the NIOZ since 1965 (Royal Netherlands Institute for Sea Research) (Van der Heij & Streefkerk 2014). These authors have concluded that both fish abundance and the number of species in the Wadden Sea have declined

dramatically since 1965. In 1965, catches of a fykenet which was emptied every 24 hours weighed an average 50 kg, whereas they currently only weigh about 5 kg. Van der Veer *et al.* (2011) combined data from the same fyke net program with additional flatfish surveys in the western Wadden Sea and found a decline of juvenile flatfish over the period 1975-2007. Since the 1980s, they found a decrease of plaice (*Pleuronectus platessa*) and flounder (*Pleuronectus flesus*) in the Western Wadden Sea and a disappearance of I- or II -group plaice. Another fish-monitoring program is the Dutch Demersal Fish Survey (DFS) that has been carried out since 1970. They have found that total fish densities, expressed both in numbers and biomass, have decreased strongly since the mid-1980s for the Wadden Sea (Tulp *et al.* 2008). The trend for the Westerschelde (Southwest Delta) was comparable except that after 2000 the decreasing trend reversed and an increase in fish densities was found (Tulp *et al.* 2008). For the Wadden Sea, an overall increase was shown for smelt, flounder, herring and sprat, but an overall decrease was found for eelpout, plaice, sole, dab, cod and whiting (Jager *et al.* 2009). These surveys have also revealed that since the 1990s, young flatfish and shrimp of the Wadden Sea have undergone a distribution shift from coastal to more offshore waters, and also to northerly waters (Jager *et al.* 2009). The distribution shifts of juvenile flatfish are thought to be due to changed conditions in the Wadden Sea nursery, which may have become less favourable due to higher water temperatures during summer.

The weight of seals in their first months of life is a combination of the weaning weight and the energy ingestion after weaning. Recently weaned seals prefer species of fish that are easy to catch and have a high lipid content (Hansen 1996). Harding *et al.* (2005) studied first year survival of common seals, and found that for weaned seals it is important to reach at least 26 kg in the autumn, since the survival rate declines steeply for smaller seals. For four months old common seals, the probability of surviving to an age of 1 year is only 0.63 for the smallest pups at 17 kg, whereas pups of 32 kg have a survival probability of 0.96 (Harding *et al.* 2005). Small seals suffer from heat loss which must be compensated by increased food intake. Obviously, there must be an upper limit to the amount of food that can be caught and metabolized during a day. Harding *et al.* (2005) therefore concluded that common seal pups are sensitive to food availability and that annual fluctuations in food supply can affect survival of entire cohorts.

Negative associations between host body condition and intensity of nematode infections were found in several wildlife studies, e.g. for baboons (Eley *et al.* 1989), geese (Shutler *et al.* 2012), and spoonbills (Sepulveda *et al.* 1994). It often remains unclear whether such associations are caused by the depressing effect of parasites on the host's body condition or alternatively that an initial poorer nutritional state made these hosts more susceptible to infection, see e.g. Irvine *et al.* (2006).

Food shortages can also trigger a change in diet. This may increase parasite infestation levels if the change means an increased consumption of infected intermediate host species. Unfortunately, monitoring efforts of non-commercial coastal fish species are limited and also diet studies of seals in the Wadden Sea and Southwest Delta are lacking.

Overfishing may have played a role in the spread of the phocine distemper virus to Wadden Sea common seals. The original source of the epidemics is still unknown as well as where the virus remained between epidemics (Rijks 2008). However, it is often suggested that the origin of the 1988 PDV epidemic is possibly linked to the invasion of harp seals which took place on a large scale in 1987 and 1988 (Harder 1997). This invasion is thought to have occurred because the seals were searching for food (Haug *et al.* 1991; Haug & Nilssen 1995; Bogstad *et al.* 2000). If the shortage of food was caused by overfishing, then the introduction of PDV to the common seals can be attributed indirectly to human activities.

Pollution

Seals are particularly exposed to pollution because they are top predators, characterized by relatively long life spans and large deposits of fat tissue reserves. Contaminants can impair physiological functions in the organism, including the immune system. Several studies have indicated that there is an association between environmental pollution and infectious disease mortality in marine mammals found in the North Sea (Jepson *et al.* 1999; Siebert *et al.* 1999; Jepson *et al.* 2005). The most detailed study is that of Ross (1995) and De Swart (1995) who conducted a two year study feeding fish from the polluted Baltic Sea to captive common seals. Significant differences in adaptive and innate immunity were found between experimental and control groups, with significantly reduced immune function observed in those animals fed contaminated fish. This study demonstrated that ambient levels of environmental contaminants were immunotoxic to captive common seals.

In a more recent study, Rijks (2008) investigated the level of contaminants in juvenile common seals that stranded along the Dutch coast during the 2002 epidemic and the preceding decade. The results of this study showed that the levels of major organohalogen contaminants, including PCBs and DDT, had not declined in tissues of common seals, and still exceeded the levels at which immunosuppressive effects have been measured.

Persistent organic pollutants are transferred from mother to pup, both via placental transfer and via lactation (Debier *et al.* 2003). Lactating female grey seals appear to excrete about 15% of the PCB body burden during the nursing period (Addison & Brodie 1977). These pollutants may have a negative effect on the growth, development and the immune system functioning of young seals. A mark–recapture survival study of young grey seals by Hall *et al.* (2009) revealed that although condition at weaning and sex were the most important predictors of survival, evidence was also found that higher blubber contaminants decrease first-year survival probability.

Experimental studies that relate pollution to parasitic infections are scarce due to the complicated study design required (Sures 2008). Gendron *et al.* (2003) conducted an experimental study with leopard frogs which showed that exposure to a pesticide mixture increased the infection rate with lungworms. Furthermore, for glaucous gulls, a correlation was found between pollution levels and intensity of parasite infections (Sagerup *et al.* 2000). Similarly, Rohde (1984) reported on higher levels of ectoparasites in fish from

polluted waters compared to fish from non-polluted waters. A direct causal relationship between pollution and intensity of parasite infections in seals is lacking. However, there is ample evidence of the immunosuppressive effect of pollution (see above). Clearly, good immune system functioning is key to controlling parasite infections (Wakelin 1996). Therefore, it can be concluded that pollution may play a role in the susceptibility of seals to parasitic infection. However, the scale of the effect of pollution and its importance relative to other factors remains unclear.

There may also be an effect of pollution involved in the scale of the outbreaks of phocine distemper. It is believed that massive mortality may have partly been exacerbated by PCB exposure (Thompson & Hall 1993). Also, according to Ross (1995) and De Swart (1995), exposure to immunotoxic chemicals acted as a co-factor in the mortality. They conclude this may have facilitated the emergence of the epizootic by aggravating the severity and extent of the infection, leading to increased numbers of affected animals and case fatality rates.

Climate change

Climate change could also have an indirect effect on seals. Based on the advanced pupping dates, we suggested that common seals of the Wadden Sea have changed their phenology in response to an earlier peak in food abundance (**Chapter 10**). It is interesting to note that in the analysis, the mean stranding date of seals in the Dutch Wadden Sea appears not to have advanced any further since 2000, perhaps indicating that the maximal forward timing of females has been reached. If there is a limited flexibility in timing, then a mismatch with prey availability can occur, such as found for migratory birds (Saino *et al.* 2011). Such a mismatch could lead to overall lower prey availability, or a switch to less preferred prey species. Eventually this could affect the body condition of weaned pups.

Cumulative human impact

Cumulatively, direct and indirect human impact on seal mortality is substantial in Dutch waters (**Chapter 2**). This is also found for other mammal species. For instance, in a meta-study of 27 land mammal species in North America, human causes of mortality were found to be more frequent than natural causes (Collins & Kays 2011). These anthropogenic mortalities may represent strong selective forces for animal populations and it would be interesting to investigate how human impacts alter selective forces.

New types of human impact may also arise and new diseases may emerge. For example, a new development that requires more research is the building of windmill parks at sea and associated increased ship activity. In the United Kingdom, there has been an unusual mortality of seals with corkscrew injuries since 2008. The injuries were consistent with the animals being drawn through the ducted propellers of marine vessels. The first recorded cases in the UK appeared to coincide with the recent expansion of the offshore renewable energy industry and the associated increase in work boat activity in littoral waters. Also for the Dutch coast, there has been an increase in stranded seals with similar injuries (SRRC unpublished data). For instance, at the island of Vlieland, 13 seals with traumatic

injuries stranded from mid-2012 until December 2013. This is 18% of the total of 73 seals that stranded dead on this island during this period. These traumatic injuries were found both for common seals and grey seals. However, the source of these injuries in Dutch seals is yet to be identified.

2.4 Common seals in comparison to grey seals

Generally, the population of grey seals in Dutch waters appears to be flourishing. In contrast, there are concerns about the high level of disease in common seals and about declines in neighbouring populations. The main diseases causing morbidity and mortality in common seals, but not in grey seals, are phocine distemper and lungworm infections (**Chapter 1** and **Chapter 2**).

With regard to PDV, the species immunological defence to the virus is important. Grey seals which had been infected with the phocine distemper virus remain asymptomatic (Hammond *et al.* 2005; Pomeroy *et al.* 2005). It was also found that the antibody response of grey seals was more competent than that of common seals with respect to morbillivirus antigens (Duignan *et al.* 1997).

Possible explanations for the increased occurrence of parasitic pneumonia in common seals are discussed below.

Genetic variability

Although we found evidence that the genetic variation of common seals was lower than for grey seals, there were no indications for concern in terms of potential genetic vulnerability to disease (**Chapter 4**). Future studies of immune response genes, such as those of the major histocompatibility complex (MHC) are needed. Their overall variation or the occurrence of specific MHC alleles may provide more insight in to the common seal's vulnerability to disease.

Host-parasite compatibility and environment within the host

Common seals may be a more suitable host for the seal lungworms *Otostrongylus circumlitus* and *Parafilaroides gymnurus* than grey seals are (**Chapter 6**). Host-parasite compatibility is an important factor determining infection rates of parasites (Johnson & Hartson 2009; Lagrue *et al.* 2011). While parasites infect a wide variety of hosts, they often reach maturity in only a sub-set of hosts. Interspecific differences in infection levels may be related to morphological and/or physiological compatibility, affecting parasite growth and fecundity (Lagrue *et al.* 2011). Interesting in this regard are the long body lengths of the lungworm *P. gymnurus* in Wadden Sea common seals (**Chapter 6**). Host-parasite compatibility, the young ages of exposure to larvae (**Chapter 8**) and immunosuppression in this population (De Swart *et al.* 1996) may provide an optimal environment for these lungworms and enable them to reach the long lengths.

The balance of the relationship between common seals and these lungworms appear to be disturbed. In parasite-host evolution there is generally a tendency towards mutual adjustment between the two species; the parasite reducing its pathogenicity and

immunogenicity to elicit weaker host responses, the host reducing its responsiveness so that parasite control is achieved without concomitant pathological change (Wakelin 1996). Such long-term adaptation can be affected by extrinsic factors, including changes in the environment. Further study of host-parasite dynamics is therefore interesting as such dynamics can be considered as indicators of environmental stress.

Diet and foraging ecology

Overall, the diet of common seals and grey seal adults is rather similar (Brown *et al.* 2012). The diet of recently weaned pups may, however, differ between the species because of seasonality and location of foraging. This may result in differences in dietary intake of lungworms (**Chapter 7**). There may be differences in diet with regard to the occurrence of fish species that serve as parasite intermediate hosts. In addition to diet, the foraging area may be important for dietary intake. Even though both species of seals haul out in the Wadden Sea and the Southwest Delta, there is only a partial overlap in foraging areas. Grey seal adults make more extensive movements than common seal adults. Research in the Moray Firth in Scotland, showed that common seals forage within 60 km of their haul-out sites whereas grey seals forage within 145 km of their haul-out sites and they occasionally moved to other haul-out sites 125-365 km away (Thompson *et al.* 1996). The foraging area of weaned pups is expected to differ between common and grey seals. Common seals are born on sandbanks in the Wadden Sea and also in estuaries whereas grey seal pups are born on sandbanks on the border of the Wadden Sea and the North Sea. It is probable that fish in the Wadden Sea carry more larvae than fish from offshore waters due to the higher density of defecating seals. This may be especially true for the estuary of the Dollard, which is one of the main pupping areas of common seals in the Dutch Wadden Sea (**Chapter 9**).

Food availability

Since a much smaller range of foraging habitats is available for common seals, this makes them more vulnerable to local changes in food availability. Fish stocks of the coastal waters are especially important for the foraging of young common seals after weaning. It is therefore worrying that there are major concerns about fish stock declines in the Wadden Sea (Tulp *et al.* 2008; Van der Veer *et al.* 2011; Van der Heij & Streefkerk 2014). Furthermore, there is no recent fish monitoring data available for the Dollard estuary. Moreover there are concerns about the increased levels of mud and water turbidity in this estuary (Spiteri *et al.* 2011). Grey seals were found to avoid muddy waters as a foraging area (McConnell *et al.* 1999; Aarts *et al.* 2008). Muddy sea bottoms are thought to be unfavourable because of the seal's sand digging foraging technique (Bowen *et al.* 2002) and because of the lower distribution of sediment-burrowing prey such as sandeels (Wright *et al.* 2000). Therefore, the increase in muddiness in the Dollard could be disadvantageous for the body condition of weaned common seals.

Food shortages can also trigger a prey switching event. Host diet is a principle factor determining helminth infection patterns (Lagrue *et al.* 2011). For instance, the occurrence

of parasite species in Norwegian common and grey seals depended on whether the seals diet consisted of benthic or pelagic fish species (Bjørge 1984). A change in diet, driven by food shortages, could thus have played a role in the increase of parasite infections of common seals in Dutch waters (**Chapter 8**).

Level of immunosuppressive toxins and/or susceptibility to immunosuppressive effects of toxins

Good immune system functioning is crucial for controlling parasite infections (Wakelin 1996). Infection with lungworm larvae could result in parasitic pneumonia when the immunological system is compromised. Pollution may play a role in the susceptibility of seals to parasitic infection. Since current pollution levels were reported to remain sufficiently high to exceed the levels at which immunosuppressive effects have been measured for seals (Ross 1995; Rijks 2008). Common seals forage closer to the coast than grey seals and it could be hypothesised that this results in common seals being exposed to a higher level of pollution.

Interestingly, immunological factors also play a role in susceptibility to immunosuppressive toxins. Common seals and grey seals were found to differ in their susceptibility to the immunosuppressive effects of toxins (Hammond *et al.* 2005b). In the study of Hammond *et al.* it was demonstrated that exposure to PCBs significantly reduced immune functions of common seal leucocytes in vitro, but had no effect on the same cells in grey seals from sympatric wild populations. The mechanisms by which such differences arise are still unclear, although it was suggested that a species difference in the way PCBs are able to bind to cellular receptors may play a role. The authors conclude that the detrimental effect that these contaminants could have on the innate immune functions of common seals could influence their overall survivorship.

Only for common seals lungworm infections were found to occur at very young age (**Chapter 8**). This is an important finding because at this age their immune systems are not fully developed yet (Ross *et al.* 1993; Ross *et al.* 1994). More research is needed into the immune response of both common seals and grey seals to lungworms. Such research should include a study of how immunity to parasties develops with increasing age.

Conclusion

The higher disease rate for certain pathogens in common seals compared to grey seals (**Chapter 1** and **Chapter 2**) is most likely to reflect a combination of species immunology and dietary factors. In addition, the fact that common seals are found in a more coastal environment provides an increased exposure to anthropogenic influences such as a reduction of fish stocks and pollution.

The sudden and strong increase since 2009 of numbers of stranded seals with parasitic pneumonia, suggests that changes have occurred in the Wadden Sea ecosystem. As was suggested by Measures (2001), during times of environmental stress, lungworm infections might predispose healthy seals to respiratory disease. Further research is needed to find out which changes have occurred in the Wadden Sea ecosystem five years ago. More

research is also needed to better understand why the immune systems of young common seals are not able to defend against lungworms.

2.5 Seal rehabilitation

Rehabilitation of stranded marine mammals elicits polarized attitudes. Rehabilitation is primarily done for animal welfare reasons, but also has other benefits such as in research, human impact reduction, conservation, and public education. However, opponents point to potential disadvantages of rehabilitation such as poor understanding of long-term survival, support of the genetically not-so-fit, introduction of novel or antibiotic-resistant pathogens, and costs. A publication on the rehabilitation of common seals in the Netherlands is included in this thesis (**Chapter 1**). Several other review articles on rehabilitation have been published (St. Aubin *et al.* 1996; Measures 2004; Moore *et al.* 2007). Here I elaborate on several facets of the discussion such as animal welfare, population effects, genetics and the risk of introducing pathogens. I also stress the importance of vigilant and professional care for seals in rehabilitation. Science needs to be a fundamental component of rehabilitation. Not only because of the huge scientific spin-off from the collected data and materials, but also for the responsible and professional care for seals.

Animal welfare

Human activities may cause conservation concerns when animal populations or ecosystems are harmed and animal welfare concerns when individuals are harmed. Wildlife conservation and animal welfare share the common goal of preventing harm to wildlife, but the differences between these areas of concern have been a focus of much discussion (see e.g. Dubois & Fraser 2013). From the beginning, conservation science has set out to protect the integrity and continuity of natural processes, populations and ecological systems (Soulé 1985), whereas animal welfare science focuses on the quality of life of individuals (Fraser 2008). When population numbers are robust, some biologists consider care for individuals unnecessary or even a potential hazard to the population. Those biologists overcome their emotional identification with individual victims. For example, they see an abandoned, sick or wounded animal as part of the process of natural selection and they are not deceived that rescuing individuals is serving the species or the cause of conservation (Soelé 1985). Contrary, welfare-oriented people focus on the quality of life of individuals, a value which applies to all sentient beings (Fraser 2008).

In practice, the majority of the public, especially those who are confronted with an orphaned, ill or injured animal, care about the individual and expect a professional and humane response. The welfare of individual seals is the primary reason for responding to stranded seals in the Netherlands. When people encounter seals in distress, the first thing that usually comes to mind is that they want to help them. It is with this basic animal welfare principle in mind that many people set out to rehabilitate seals and other wildlife species. People often find that it is our duty as sentient human beings to relieve the suffering of animals, preferably by rescuing the animal or if needed by euthanizing it.

Rehabilitation is indicative of the way society treats nature and wild animals in a broader sense. Providing care for animals in need of help inspires people to develop compassion and an understanding of our wildlife and the environment. Rehabilitation of seals has become an activity that is anchored in the present day society of many countries. Common and grey seals are rehabilitated in most countries along the seal's range; for instance in Belgium, Canada, France, Germany, Ireland, Poland, Russia, Spain, Sweden, the Netherlands, United Kingdom, and the United States. There are also rehabilitation centres for many other wildlife species in the Netherlands, such as for birds, hedgehogs and roe deer. The knowledge on wildlife medicine has increased over the last decades and several handbooks are now available on the treatment of marine mammels (Dierauf & Gulland 2001; Geraci & Lounsbury 2005) and other wildlife species (e.g. Stocker 2005).

Several law and legislation in The Netherlands are applicable to stranded seals. First, both species of seals are listed as protected species (Flora and Faunawet 1998). In the Netherlands animals are recognised as sentient beings (Verdrag van Amsterdam 1997), which aplies to both captive and wild animals (RDA 2012). Also the intrinsic value of individual animals is recognized by the Dutch government (Nota Rijksoverheid en dierenbescherming 1981; Wet Dieren 2015). The recognition of animals as sentient beings and the recognition of the intrinsic values of animals, implies that humans have a moral responsibility with regard to the welfare of animals, regardless of species or context (RDA 2012). The responsibility to care for animals in need of help is laid down in the Gezondheids en Welzijnswet voor Dieren and its successor the Wet Dieren (2014).

Population effects

Rehabilitation of seals of the Wadden Sea and Southwest Delta has been very important for the recoveries of the populations since the 1970s. Currently, rehabilitation provides a considerable quantitative contribution to the population (see Synthesis Table 1. paragraph population and recruitment). Although seals are rehabilitated all over the world, the Dutch coastline is characterized by accessible sandy beaches close to densely populated areas which, together with a dedicated stranding network, maximizes the chances of detecting stranded seals, and increases numbers admitted for rehabilitation.

It needs to be considered that the effect of seal rehabilitation in the Netherlands is so substantial that it could have prevented a potential declining trend of the populations. It is interesting that Thompson *et al.* (2010) remark that since the 2002 epidemic the disparity between the failure of the UK coast population to recover and the continued rapid growth of the Wadden Sea population has become more pronounced. Although seal rehabilitation occurs in the UK, the rehabilitation centres are smaller. Also seals are less likely to be found in large parts of the UK, where the coastlines are difficult to access. Therefore differences in rehabilitation effort may well explain the increasing trend for common seals that is observed here, compared to the declining trends in parts of the United Kingdom.

For some endangered populations each released individual is an important contribution to the survival chanches of the population. For this reason both the SRRC and the Marine

Mammal Center in Sausalito (USA) have set up rehabilitation centres in other parts of the world where endangered seal species occur. The SRRC has established rehabilitation centres for the help of Mediterranean monk seals *(Monachus monachus*; in Mauretania, Greece and Turkey), Caspian seals (*Pusa caspica*; in Iran) and Ladoga ringed seals (*Pusa hispida ladogenesis*; in Rusia). Similarly, the Marine Mammal Center has established a rehabilitation centre for Hawaiian monk seals (*Monachus schauinslandi*; Hawaii). Besides the direct support for the population in numbers, rehabilitation together with education can act as a catalyst for the protection of the endangered population.

Genetics

Seals are primarily rehabilitated for animal welfare reasons. No negative effects of rehabilitation on the populations have yet been documented. It is, however, sometimes argued that release of rehabilitated animals may eventually have negative consequences for the population where there are individuals with genetic weaknesses among the admitted seals. Orphanage (both seals species) and parasitic pneumonia (common seals only) are the main causes of seals being admitted to rehabilitation (**Chapter 1**). For orphans, disturbance and extreme weather conditions are considered to be the most probable causes of separations between mothers and their pups. These two factors are expected to affect the seals in a random manner. A number of marine mammal studies postulate a relationship between disease and heterozygosity, which is the level of genetic variation within an individual animal selected at random from the population (Acevedo-Whitehouse *et al.* 2003; Valsecchi *et al.* 2004; Rijks *et al.* 2008). Disease can also be related to a specific locus rather than overall genetic variability (Bowen *et al.* 2005; Acevedo-Whitehouse *et al.* 2009). Rijks *et al.* (2008) reported on what they described as a signal in the direction of reduced heterozygosity correlating with lungworm burden in seals. They suggest a genome-wide effect since they found no indications of a relationship with a specific locus. For phocine distemper, the other important disease of common seals, no association was found between disease susceptibility and genes coding for immunity to viruses (McCarthy *et al.* 2011).

With regard to the debate on the genetics of rehabilitated seals, I would like to add the following points to the discussion that have not been addressed before.

First, there is much scientific debate on heterozygosity-fitness correlations. Such associations were found to be typically weak and also the design of such studies has been criticized, for example because of small sample sizes, a small number of markers, and publication bias towards publishing significant results (David 1998; Coltman & Slate 2003; Balloux et al. 2004). Caution is therefore warranted in using detected average heterozygosity levels among individuals as a direct index of fitness.

Although specific alleles will be passed on the next generation, heterozygosity is in principle not heritable. In each new generation alleles of both parents are combined, thus a parent with low heterozygosity can have offspring with higher levels of heterozygosity when opposite alleles are inherited from the second parent. This was demonstrated in a

recent study by Forcada *et al.* (2014), in which they found that heterozygote advantage in Antarctic fur seals was not transmitted from one generation to the next.

If seals that stranded because of disease have a lower fitness, this would be expected to be reflected in the survival rates after release. However, in contrast to the expectation, a preliminary analysis of tagged seals showed that released seals that initially stranded with a parasitic bronchopneumonia did not have a higher chance of being reported back after release than was found for orphaned pups (**Chapter 1**). The anticipated next PDV mass mortality could provide a good opportunity to study survival and reproduction success of rehabilitated seals.

Stranding chances are determined by many different factors, or a combination of factors. Pathogens (viruses, bacteria and parasites) have an important role in regulating natural populations or in contributing to health through immunity or natural selection. However, selection processes in populations are not solely determined by survival of the genetically fittest individuals, but many more factors - both environmental and human-related - play a role. To which degree natural, environmental or human-related factors determine survival in a population will differ between relatively pristine areas and densely populated areas such as the Dutch coast. Currently for both seal species in Dutch waters, one-third to two-third of the pups strand within the first year of life (paragraph 2.2). It is unlikely that all these seals have an underlying genetic weakness. The sudden and strong increase since 2009 in the numbers of seals that stranded with parasitic pneumonia, suggests an ecological cause rather than an increased genetic vulnerability to parasites in the population. This is in line with Measures (2001) who suggested that during times of environmental stress, lungworm infections might predispose healthy seals to respiratory disease.

Introduction of novel pathogens and resistant bacteria

Infectious diseases in the wild and their management during rehabilitation is an important issue. Certain diseases can potentially cross the species barrier from human to seal, such as influenza (Osterhaus *et al.* 2000; Bodewes *et al.* 2013b), pox (Osterhaus *et al.* 1990; Osterhaus *et al.* 1994) and brucellosis (Jepson *et al.* 1997; Foster *et al.* 2002). Therefore strict protocols have to be in place at all rehabilitation centres to prevent contamination of infectious diseases from human to seal and vice versa. Disease transmission has been studied extensively for seal rehabilitation in the Netherlands over the past thirty years. Although possibly overlooked, no serious cases of disease transmission were found. Furthermore, contact between seals and humans not only occurs at rehabilitation centres, as seals in Dutch waters occur close to the inhabited coast and for instance haul out on tourist beaches. Nonetheless, rehabilitators should be vigilant and work professionally. One could also argue that without central rehabilitation facilities being available, private initiatives will take place anyway, but in such a situation quality assurances would not exist.

Treatment of marine mammals in rehabilitation often involves the use of antimicrobial drugs (Gulland *et al.* 2001). There is concern that rehabilitated seals may disseminate

antimicrobial resistant bacteria once released into back into the wild. Antimicrobial resistance of *Escherichia coli* was studied for northern elephant seals (*Mirounga angustirostris*) in rehabilitation (Stoddard *et al.* 2009). They found that at release from rehabilitation 77.8% of the elephant seals had antimicrobial resistant *E. coli* compared to 38.4% of the elephant seals at admission. Conversely, no such increase during rehabilitation was found in a study of rehabilitated common, grey and harp seals (*Phoca groendlandica*) of the Northwest Atlantic (Wallace *et al.* 2013). Although microbial resistance may increase during rehabilitation, there appears to be a high environmental load of resistant *E. coli*. Stoddard *et al.* (2009) conclude that the environmental load of resistant *E. coli* that is contributed by the faeces of released seals is likely small compared to the entire *E. coli* population present in marine ecosystems due to run-off from human sewage, wildlife, domestic animals, farms and surface water. Antiparasitic drugs are another type of medicines that are often used in rehabilitation centres. No published records of resistance to these drugs are currently available. Clearly, it is important that rehabilitators closely monitor for potential resistance of seals to these drugs.

The Dutch government called for a reduction of veterinary use of antimicrobials (LNV 2010). Although no official guidelines exist, a reduction of antibiotics usage is also wanted for wildlife. Preferably culture and sensitivity testing should be performed before selecting the appropriate antibiotic. However, frequently treatment must be implemented before culture results are available. It is therefore crucial that clinicians build up an extensive knowledge of bacterial pathogens in the seal population and their antimicrobial susceptibility. The immunosuppressive effects of antibiotics are another reason to avoid the use of antibiotics when possible (Banck & Forsgren 1979). In addition to appropriate use of medication, high levels of hygiene are essential in the rehabilitation of wild animals.

Professional care

In addition to strict protocols for animals under care, expertise is also required for decisions on intake and release of seals. For decision making on live-stranded seals, rehabilitators and stranding network members should have sufficient knowledge of the biology of the species concerned and also know the area of stranding. To exemplify this, the response to lone common seal pups should depend on whether they are found in or outside breeding areas and therefore the possibility of mothers being present (**Chapter 9**, **Appendix 9**).

The success of seal release can be monitored by studying data on seals that are reported again after release. At the SRRC all seals are released with a tag in their hind flippers and since 1995 a microchip transponder has been implanted in each animal. Analyses of these tags have shown long-term survival of the released seals (**Chapter 1**). Also rehabilitated seals were re-reported during the epidemics (**Chapter 1**) and the post-mortem examined animals were found to be in a good body condition.

The release weight is crucial for survival after release. Harding *et al.* (2005) provided data on weight and survival rates of young common seals. Based on these data, a release

weight of at least 26 kg is required for yearling common seals. It may be desirable to increase the release weight by several kilograms, as the seals may need some time to adapt after release. Therefore, a minimum release weight of 30 kg is advised for common seals. No such studies exist for grey seals, but their optimal release weight would be expected to exceed 30 kg, since they are larger animals than common seals. Analyses of release weights showed that common seals released with a weight below 30 kg were more frequently reported back sick or dead (8/91=8.8%) than those released with a weight above 30 kg (90/1261=7.1%) (data of seals released 2011-2013; SRRC unpublished data). However this difference was more pronounced when comparing seals released with a weight below 35 kg (48/525=9.1%) to those released with a weight above 35 kg (50/831=6.0%) (Chi-square $p<0.05$). This suggests that a release weight of 35 kg increases the survival chances of released seals.

Science

Marine mammals are important sentinels for the monitoring of ocean and human health (Reif 2011). Reif gives several reasons why marine mammal species are important sentinels. First, marine mammals have relatively long lifespans that permit the expression of chronic diseases, abnormalities in growth and development, and reproductive failure. Second, as apex predators, marine mammals feed at or near the top of the food chain. As the result of biomagnification, the levels of anthropogenic contaminants found in marine mammal tissues are typically high, often higher than those found in humans. Further, the subdermal blubber layer provides a repository for lipophilic contaminants. Finally, the application of clinical examination procedures, combined with pathological examination, has led to the development of health assessment methods at the individual and population levels.

There are many advantages to using animals under human care as sentinels (Dierauf & Gulland 2001). Free-living marine mammals usually die at sea and their carcasses are rarely discovered when tissues are fresh enough for productive pathological examination (Gulland 1995). However, clinical data and samples, such as tissues and blood, can be regularly and systematically collected while animals are in rehabilitation. Seals in a captive environment also enable the design of studies like those which tested the effect of seals eating polluted fish (Ross 1995; de Swart 1995).

The Dutch stranding network has been set up in 1971 by the SRRC for the rescue of live seals (**Chapter 1**). The main motivation for the volunteers of the stranding network is the rescue of live seals that are in need of help. Having a strandingnetwork of trained volunteers also allowed the collection of carcasses of dead-stranded seals (since 1979, see **Chapter 2**). The stranding data, veterinary diagnoses and samples that have been collected over the past decades provide a unique research opportunity. These data have been the impetus of the current thesis and for many earlier studies (see e.g. Ross 1995; de Swart 1995; Harder 1997; Kappe 1998; Martina 2003; Rijks 2008). Rehabilitators often collaborate with scientists from various disciplines of science, but also with experts in

human health, which allows a multidisciplinary approach to the study of the health of seals and their ecosystem.

It is important to note that funds which are available for rehabilitation would generally not be available for research since the general public specifically supports the help for individual seals. Rehabilitation therefore has a positive effect on the total research effort, either through research executed at rehabilitation centres or via increased awareness of the importance of seal research.

Summarizing conclusions

After discussing the findings of the chapters and publications of this thesis, the following overall conclusions can be drawn.

1. For both seal species, one third to two third of counted pups strand during the first year. These first year stranding rates appear to be higher than found in any other region or in previous Dutch studies. The majority of these seals strand whilst still alive and are admitted for rehabilitation.
2. The causes of live stranding differed between the two seal species. Alongside orphanage, parasitic pneumonia was the main reason for stranding in common seals, whereas almost all grey seals had stranded as orphans or shortly thereafter as weaned seals.
3. In dead-stranded seals, mortality caused by direct human impact constitutes a substantial part of the total mortality. Estimations of direct human-induced mortality varied between 24% and 37% for common seals and between 24% and 44% for grey seals. In addition, human disturbance, overfishing, pollution and possibly climate change will have an indirect impact on seals. Therefore, cumulatively human impact on seals in Dutch waters is substantial.
4. Common seals are affected by infectious disease more frequently than grey seals, however this difference could not be explained by a lower genetic variation at neutral markers.
5. For the higher occurrence of parasitic pneumonia in common seals compared to grey seals, a combination of species immunology and dietary factors may play a role. In addition, the fact that common seals are a more coastal species exposes them to more anthropogenic influences such as a reduction of fish stocks and pollution.

Recommendations

Based on the findings in this thesis, the following recommendations are given for further research and conservation.

Further research

1. In order to reduce orphanage in both seal species, the sources of disturbance in breeding areas of both seal species need to be better determined. Particularly more research is needed on the disturbing effects of boats, because the number of ships and

boats that use the Wadden Sea has nearly doubled since 1982 (Convenant vaarrecreatie Waddenzee 2007).

2. For by-catch, there is a need to know which fisheries are involved and to take measures to reduce mortality associated with their activities.
3. The relationship between genetic variation and fitness is not well understood and needs further investigation. Future studies of immune response genes, such as those of the major histocompatibility complex (MHC) are needed. Their overall variation or specific MHC alleles may provide more insight in to the common seals vulnerability to parasite infections.
4. More research is needed on the causes of the increased incidence of parasitic bronchopneumonia in common seals and the apparent shift to infection at younger ages. To this end, the following research is recommended: 1. study of the weaned seal's diet combined with a study of food availability, 2. testing whether hooknose is indeed a *Parafilaroides gymnurus* intermediate host species by conducting experimental infections, 3. searching for the intermediate hosts of *Otostrongylus circumlitus*, 4. testing the possibility of vertical transmission of lungworms, 5. study of the immune responses of both seal species to lungworms, including a study on how immunity to parasties develops with increasing age.
5. Research into the use of host-parasite dynamics as indicators of environmental stress would be most interesting. Such a study should compare parasite infestation levels in host populations that occur in ecosystems with different levels of environmental pollution.
6. It is recommended that fish monitoring efforts should be increased in Dutch coastal waters as well as to extend survey areas and include the estuaries. More study is also needed to determine if climate-induced distribution shifts of juvenile flatfish from the Wadden Sea to the North Sea result in a lower availability of prey for young common seals. Furthermore, the effect of increased mud levels and turbidity in the Dollard on the available prey for seals needs further investigation.
7. Rehabilitators have the responsibility to continuously investigate admitted seals. This is not only because of the scientific spin-off from the collected data and materials, but also for the welfare of the seals in rehabilitation. Research topics should include: understanding when animals are in need of help; avoiding the introduction of pathogens and resistant bacteria; and optimisation of release criteria to increase survival rates. The fact that as yet no general relationship has been established in animals in nature between genetic variation and individual fitness does not rule out the possibility that rehabilitation of seals and their subsequent release could influence the population, whether positively or negatively. More research would be needed to come to a rigorous conclusion on this issue. However, my results show that minimising the ecological and human-related challenges to the seal populations of the Wadden Sea should be the present priority for conservation policy.

Conservation

1. Population models for common seals should take into consideration that another Phocine distemper epidemic is likely to reoccur soon, which will substantially impact the population.
2. Pollution still appears to have a substantial indirect impact on seals. Monitoring of the level of pollutants in the seas and in marine life remains essential, in particular for a timely reaction to increased levels or new pollutants.
3. Protected areas for pupping sites of both seal species are important for preventing human disturbance and therefore prevent separation of mothers and pups. Preventing disturbance is also important to reduce the energetic costs of pups responding to repeated disturbances.
4. Healthy fish stocks in coastal waters are vital for flourishing populations of marine mammals and piscivorous birds. Conservation efforts should therefore not solely focus on mammals and birds, but should aim at protecting all parts of the coastal ecosystem.
5. This thesis underpins that having a comprehensive stranding network linked to rehabilitation of live-stranded seals and post-mortem examination of dead seals provides a wealth of information on the health status of seals, which is vital for research and for the conservation of the seal populations.

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