

Grey seals (*Halichoerus grypus*) in the Dutch North sea: population ecology and effects of wind farms

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Contents

Executive Summary	6
1 Assignment	9
1.1 WE@SEA	9
1.2 Aim of this study.....	9
2 General introduction.....	11
2.1 Grey Seals in Dutch Waters	11
2.2 Effect of human activity on seals: wind farms	12
2.2.1 Disturbance of marine mammals	12
2.2.2 Expected effects.....	13
2.2.3 Studied effects	14
2.3 Layout of this study.....	14
3 Population Study.....	17
3.1 Introduction.....	17
3.2 Material and methods	17
3.3 Results.....	18
3.3.1 Population Dynamics.....	18
3.3.2 Distribution	22
3.4 Discussion	30
3.4.1 Population dynamics	30
3.4.2 Distribution	31
4 Diet of grey seals in the Netherlands	33
4.1 Introduction.....	33
4.2 Material and methods	33
4.2.1 Analysis of hardparts.....	34
4.2.2 Statistical analyses.....	35
4.3 Results.....	36
4.3.1 Samples.....	36
4.4 Discussion.....	42
5 Spatial distribution of individual seals.....	43
5.1 Introduction.....	43
5.2 Material and Methods.....	44
5.2.1 Maps on environmental conditions.....	45
5.2.2 Tagging of individual seals: Grey seals studied in the Netherlands	46
5.2.3 Animal tracking filtering procedure for ARGOS data	47
5.2.4 Analysis of trips.....	48
5.2.5 Spatial Modelling: Defining the habitat preference function .	48
5.2.6 Accounting for unequal habitat availability.....	49

5.2.7	Likelihood function and parameter estimation	50
5.2.8	Spatial prediction of usage and preference	50
5.3	Results.....	52
5.3.1	Tracking results; filtered data.....	52
5.3.2	Dive data.....	54
5.3.3	Behavioural data.....	54
5.3.4	Habitat modelling.....	57
5.4	Discussion.....	62
6	Quality Assurance.....	63
	References.....	65

Executive Summary

There is increasing, however circumstantial evidence that anthropogenic activity such as wind farming, could influence marine mammals. Construction and operational activity, including traffic in relation to maintenance, augment the human influence already present in a heavily exploited Southern North Sea.

This study was setup to gain an understanding of the possible effects of large-scale development of wind farms in Dutch waters on grey seals (*Halichoerus grypus*). This should be considered a first step in doing so as up until now relatively little was known about the species in Dutch waters. The study was carried out in the framework of WE@SEA a foundation aimed at acquiring knowledge in the field of offshore wind energy.

Defining the effects of human activities such as wind farming on grey seals requires an observed change or absence of change in the seals population, be it in numbers, distribution, diet or habitat use. In the case of grey seals in Dutch waters, identifying a cause and effect relationship between the wind farm (construction and operation) and the well-being of the seals was held back, due to insufficient information collected on this relatively new species. In lack of such detailed references on this species, the prerequisite of this study was to include basic data on the species. Data on population development, diet and habitat use is presented here.

Chapter 3 *Population Study*, shows that grey seal numbers have impressively grown in the past 30 years from an occasional individual observed, to a maximum count in the Dutch Wadden Sea of over 2000 animals during the moult, when most are seen. In addition, growing numbers of grey seals are seen in the Dutch Delta area, sometimes exceeding the number of harbour seals (Strucker et al., 2007). Presumably, most animals originated from the British coasts where the largest grey seal population in the world resides. Migration might still play a role in the observed annual population changes.

Other strongholds of the grey seals on the continental coasts are found in Germany, but numbers remain below several hundreds. It is clear that the Netherlands house the most individuals of this species on the European continent. This heightens the responsibility to protect them in the frame work of the Habitat Directive. Currently the species is protected under Annex II and Annex V of the European Community's Habitats Directive. In the future, yearly monitoring of the population development will show when the population stabilizes both in size as in the use of the haul-outs.

In chapter 4 *Diet of grey seals in the Netherlands*, we show using scat analysis, that grey seals along the Dutch coast mainly feed on a variety of predominantly benthic prey species. Most numerous are sole in spring and flounder in autumn. This is comparable to the diets of grey seals from the east coasts of the UK though there, more sandeel is eaten. On average prey is seldom larger than 20cm, only slightly larger compared to the harbour seals' diet.

As scat analysis (like all methods for dietary research of cryptic animals) does create a bias, additional information was collected for fatty acid analysis. However, the results of the analysis are not yet satisfactory. In the near future, we expect to use this method in parallel to scat analysis to understand more fully the dietary preference of the species.

We have concluded in chapter 5 *Spatial distribution of individual seals* that the Dutch North Sea zone plays an important role for grey seals both in terms of migration and foraging. Although most seals spend the majority of their time close to their central place (haul-outs), the model predicts that areas further offshore such as the Frisian front and the Dogger Bank provide suitable foraging areas. McConnell et al (1999) found that grey seals from UK populations travel to, and feed off the Dogger Bank. Comparably, large distance migrations along the continental coasts and to the UK are observed. This suggests that the Dutch grey seal population is indeed open. Consequently, increase in human activity along these migration routes has the potential to disturb the seals in their movement. In our small sample size, a relatively large number of seals are found to make these journeys which suggests that it is common, rather than rare, practise for the seals to travel such long distances. In terms of preference to particular areas, our model indicates that the grey seals prefer sandy areas and relatively shallow waters. This lends support to previous studies in which similar results were found (e.g. Aarts et al. 2008, and in the case of harbour seals, Brasseur et al. 2009). These findings allow the prediction of spatial distribution, even in areas with little telemetry data.

When attempting to calculate the effect of pile driving activity at the wind farm the numbers of seals tagged at that moment was relatively low. Furthermore, many seals were too far from that area to perceive any activity. Though circumstantial, the seals seem to move more towards the wind farm area after the pile driving stopped this is shown by the tracks of the seals that were tagged during the pile driving activity

1 Assignment

1.1 WE@SEA

The Dutch government has set a target for the development of 6,000 MW of wind power in the Dutch part of the North Sea by 2020. In order to meet this target, knowledge and technical expertise are required to build and operate wind farms in a sustainable way. The provision of a subsidy to gain expertise and knowledge for this goal was the driving force in the formation of the consortium We@Sea.

The consortium We@Sea consists of two foundations: the foundation We@Sea and the foundation We@Sea/ BSIK. The foundation We@Sea is an organization aimed at acquiring knowledge in the field of offshore wind energy. The foundation has acquired a grant (BSIK) for a research and development programme with 7 outlines.

0. Integration & scenarios
1. Offshore wind power generation
2. Spatial planning & environmental aspects
3. Energy transport & Distribution
4. Energy market & Finance
5. Installation, operations and maintenance
6. Training, education & dissemination of knowledge
7. Programme management

The effects of wind farms on the grey seals in the Netherlands, is one of the projects subsidised under 2: Spatial planning & Environmental aspects.

The foundation We@Sea/ BSIK is the channel for this funding, from SenterNovem to the project. Both foundations have the same administration. The foundation We@Sea is the secretary of the consortium and the management was financed under BSIK conditions: 50% is subsidised and the other 50% allocated proportionally among all projects. This project was co-financed through other studies on grey seals including population studies and diet studies under the ministry of LNV, and the studies of the effects of the OWEZ wind farm on seals.

1.2 Aim of this study

This study was carried out in the framework of WE@SEA. It was setup to gain an understanding of the possible effects of large-scale development of wind farms at sea on grey seals (*Halichoerus grypus*) in Dutch waters. However, when this study commenced in 2005 relatively little was known about the species in the Netherlands, certainly in comparison with the harbour seal (*Phoca vitulina*). Grey seals recolonised the area in the late 20th century, breeding since the late 1980's. This study thus aimed primarily at acquiring general knowledge on the species. Only then is it possible to estimate the effects mentioned below, of wind farming on the seals and the possible actions to be taken to minimise these effects.

It is generally expected that the effects of wind farming on grey seals may include disturbance because of underwater noise generated during the building and/or operational phase. As the seals' habitat is altered, the animals may also be affected directly. As phocid seals in general can hear low frequency sounds relatively well (Kastak et al., 1998), grey seals are expected to be aware of underwater noise expected during such human activities at large distances well beyond 10km. The seals are even expected to hear operating windmills at <5 km (pers. comm. O.

Hendriksen). Other effects could be expected when the wind farms are being serviced or decommissioned.

2 General introduction

2.1 Grey Seals in Dutch Waters

The grey seal (*Halichoerus grypus*) belongs to the family phocidae and is protected under several conventions and treaties in the Netherlands and in Europe as a whole. It is listed as an Appendix III species under the Bern Convention, and the subpopulations in the Baltic and Wadden Seas are listed as an Appendix II species under the Bonn Convention. The species is also listed as a protected species under Annex II and Annex V of the European Community's Habitats Directive, and several important sites for the grey seal have been proposed in EC member countries as Special Areas of Conservation.

The grey seal is a medium-sized seal found on both sides of the North Atlantic Ocean of the Northern hemisphere. Three main populations are recognized: the Northwest Atlantic population, the Baltic Sea population and the Northeast Atlantic population. The Northwest Atlantic population ranges from Labrador in the north to Nantucket in the south. The Baltic population includes the Gulfs of Bothnia, Finland and Riga and south to the Gulf of Danzig, extending as far as the border between Poland and Germany, and on the Swedish coast as far as Malmö. The Northeast Atlantic population is found along the coast of Iceland, around the British Isles, along the Atlantic and North Sea coasts, and down to Brittany in France (de Jong et al., 1997). The three populations are not considered to be sub-species (Anderson, 1990; Bonner, 1989; De Jong et al., 1979). Along the European continent strongholds for breeding are the Dutch Wadden Sea, Amrum in the German Wadden Sea and Helgoland (Härkönen, 2007).

Historical data indicates that grey seals were common in the entire Wadden Sea until the 6th century (Reijnders, 1995) but the species, decreased substantially thereafter. It is postulated that the virtual disappearance of grey seals, was due to the increase in human settlement and to intensified hunting pressure (Reijnders, 1995). As grey seal pups remain ashore during the suckling period and even some time after weaning, they are a relatively easy catch compared to harbour seals that have the ability to swim almost directly after parturition. This could explain the absence of the species until the hunting ban in the late 20th century, whilst harbour seals remained present.

In the 1960s, a colony began to form around the German Wadden Island of Amrum (Caudron, 1997; Quedens, 1988). A short while thereafter (1980) grey seals were observed, in increasing numbers, in Dutch waters. This study will elaborate on the recovery of the species in the Netherlands and the habitat use of this "new" species, including modern aspects such as the effect of wind farming at sea.

Two wind farms have already been built: OWEZ (Offshore Windfarm Egmond aan Zee), which has 36 windmills with a hub height of 70 meters above mean sea level (NAP), each producing 3 MW. It is located 8-18 km offshore with an approximately

area of 40 km². Construction began in April 2006 with all the turbines standing by August 2006 (pile driving period). The official opening of the wind farm was in April 2007. Slightly west of OWEZ wind farm Prinses Amalia Windpark is located, 23 kilometres off the coast of IJmuiden. The total area of this wind farm is 14 km². There are 60 2MW turbines with a hub height of 59 meters. Pile driving for this wind farm began in October 2006 and ceased in April 2007. Further construction continued until April 2008 with the official opening in June 2008.

The Dutch North Sea coastal zone is known to play an important role as a foraging area for grey seals, but also as a migration route between the Wadden Sea and the Delta area, and vice versa . Existing wind farms such as OWEZ and Prinses Amalia Windpark are located in this area. New wind farms are planned in the same general area though somewhat further away from shore (Figure 1).

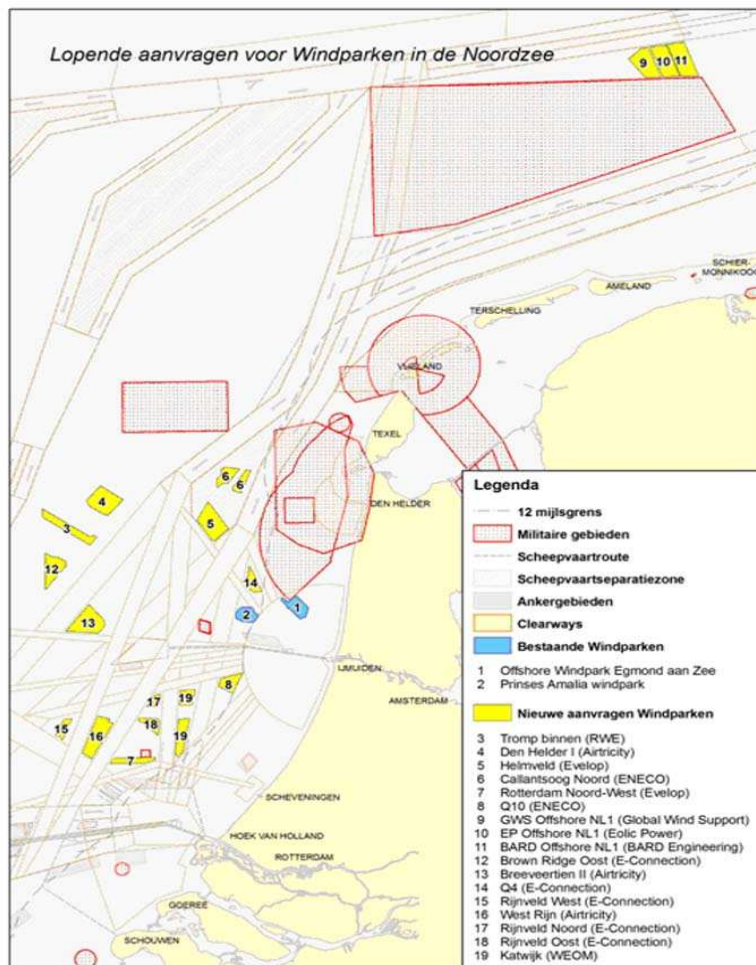


Figure 1. Planned locations for wind farms in the Netherlands (yellow) and existing wind farms (blue). (Source:http://www.rijkswaterstaat.nl/actueel/nieuws_en_persberichten/2009/maart2009/huizingabehan_delt17aanvragenwindturbineparkopzee.aspx#)

2.2 Effect of human activity on seals: wind farms

2.2.1 Disturbance of marine mammals

Extensive research has shown that human activity, even the sheer presence, has a real potential to negatively affect wildlife. The response of wildlife depends on a

number of factors, both stimulus related and inherent to the animal, and the effects vary from short term e.g. fleeing an area, through to long-term e.g. permanent physical damage, even death. In the case of marine mammals, only a few cases of direct deaths are recorded (Jepson et al., 2003). Whilst many examples can be found on humans affecting the behaviour of animals through disturbance (noise or other). For example, a study on bottlenose dolphins (*Tursiops truncatus*) found that an increase in the number of tourist boats resulted in a decrease in resting behaviour (Constantine et al., 2004). In seals, comparable results were found in an experiment manipulating the extent of human disturbance (Brasseur et al., 2001; Reijnders et al., 2000). Despite these studies, and a large number of others (Hayward et al., 2005; McMahon et al., 2005; Nordstrom, 2002; van Polanen Petel et al., 2006), disturbance is seldom quantified such that effects on the population could be measured or estimated. Moreover, for obvious reasons, disturbance of seals on land is understood much better than disturbance under water. When at sea where most wind farms are located, seals and marine mammals in general, have the potential to be affected by underwater noise, as well as underwater structures and the presence of ships, while the animals are much more difficult to observe.

Human activity in Dutch waters is obvious and evident. Shipping traffic is heavy, in some areas as many as 70,000 shipping hours/year (MARIN Wageningen), with a number of large international ports along the Dutch coast. Commercial fishers utilise the area for a variety of species and recreational boating is popular. The coast itself is popular for recreation and activities such as beach nourishment occur almost constantly. There are also two operational wind farms 8-18 km and 24-30 km off shore respectively. In the near future, human activity in Dutch waters will increase substantially, with the construction and operation of a large number of new wind farms and large scale sand mining activities. Additionally, the harbour of Rotterdam is being enlarged. All of these activities have the potential to negatively affect both the quality of the marine ecosystem and the wildlife that inhabits it.

2.2.2 Expected effects

The construction and operation of wind farms at sea has the potential to affect the marine life in and around the area. The construction phase often includes profiling, shipping, pile-driving of heavy steel jackets into the seabed, trenching and dredging (Nedwell et al., 2004). All of these activities generate noise of varying intensity, duration and frequency, with pile-driving producing powerful shock waves. The operational phase generates mechanical noise transmission from moving parts and blade beat frequencies, next to boating activity related mainly to maintenance. The physical presence of the turbines also has the potential to affect marine life. In general it is agreed that in the construction of a wind farm pile-driving, is the activity most likely to affect marine mammals (Koschinski et al., 2003; Madsen et al., 2006; Thomsen et al., 2006). Although of relatively short duration, this generates intense impulses that are likely to induce hearing impairment at close range, and to disturb the behaviour of marine mammals at ranges of many kilometres. As harbour seals are relatively sensitive at low frequency, and sounds at low frequency travel relatively well in seawater, it is to be expected that these animals may be aware of such an intensive sound at very large distances. In Madsen et al. (2006) the modelled ranges indicate that pile-driving sounds should be audible to the marine mammals at very long ranges of more than 100 km.

Operating wind turbines commonly generate low sound levels, unlikely to impair hearing in marine mammals. Despite this, it is still possible that wind turbines affect animals, by causing changes in foraging behaviour for example. More importantly in this particular case, it could influence the movement pattern of the seals and thereby limiting the number of animals migrating south towards the Dutch Delta area, or travelling westwards to the UK coast and islands, or East towards the German Wadden Sea.

Another effect that is often readily stated is the possible amelioration of feeding possibilities for the marine mammals as the piles could serve as substrate for growth, thereby attracting fish. The parks themselves are forbidden for fisheries. In the Dutch case where seal populations are growing at exponential rates (chapter 3), unlimited by food availability. It is therefore unlikely that more fish would have measurable effects.

2.2.3 Studied effects

Research on the effects of wind farm construction and operation on marine mammals is limited, with much of the literature in the form of reports (Edrén et al., 2004; Tougaard et al., 2006). For example, a study on harbour seals, hauled out 10 km from the Nysted wind farm, at Rødsand seal sanctuary using remote video monitoring, showed that there was no change in disturbance rates during the construction period (thought to be due to boat regulations), but that during ramming periods the number of seals on land decreased significantly (between 31 and 61%) (Edrén et al., 2004). Fewer seals were observed in the wind farm and in the immediate surroundings during the construction period which was attributed to the high levels of underwater noise generated by pile driving operations (Edrén et al., 2004). Similarly, a study by Teilmann *et al.* (2006) found indications of disturbance to seals (both harbour and grey seals) from pile driving at two Danish wind farms (i.e. reduced numbers observed on land during pile driving). In their study, Teilmann *et al.* (2006) do state that no changes in abundances were observed during construction at either site (Horns Rev) and that no effects were documented from the operation of the wind farms. In a different study, (but on one of the same wind farms (Horns rev) and on the same species), Tougaard *et al.* (2006) state that they were unable to determine whether there were any effects of the wind farm on harbour seals. This was attributed to the limitations of the methods used.

It is obvious from the above studies that seals reacted to wind farm related activity, albeit up till now only a short-term response in the case of pile driving was observed. However, it is unclear as to whether these have negative effects and to the level of impact, i.e. individual animal or at the population level. Moreover, cumulative effects and long term consequences in terms of populations and even region species is unknown.

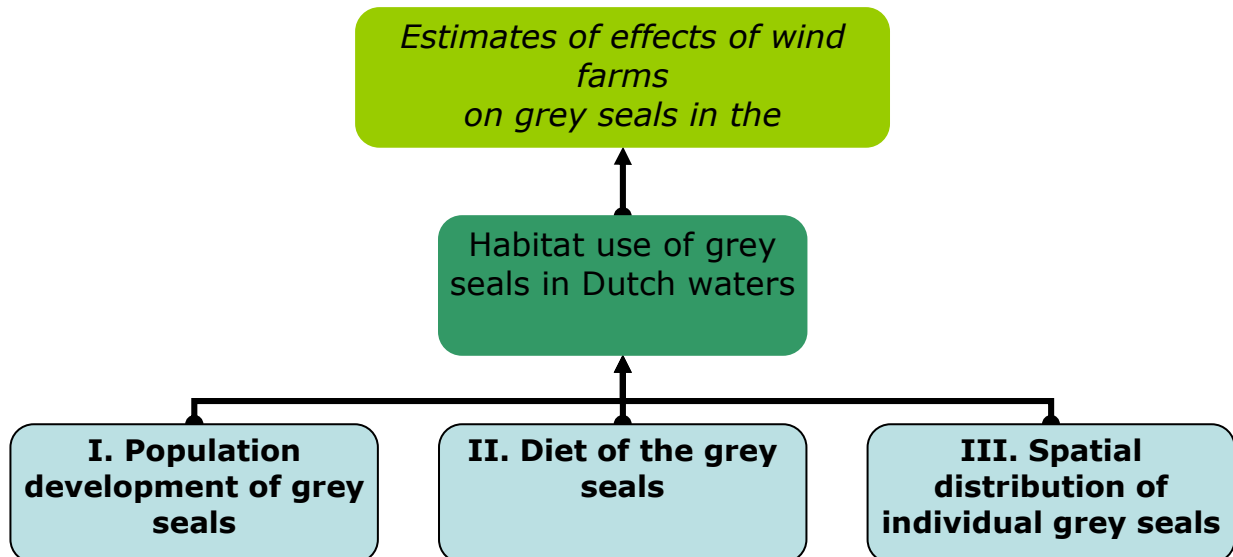
2.3 Layout of this study

In this project three main topics are examined:

1. Population development of grey seals along the Dutch coast;

2. Diet of grey seals in Dutch waters;
3. Spatial distribution and diving behaviour of individual grey seals in the North Sea.

The scheme below shows how the collected information can and will be used to study the effects of windfarms.



3 Population Study

3.1 Introduction

Grey seals are sometimes considered to be a new species in the Netherlands, however, sub fossil remains, of which some were dated up to 10,000 BC (Joensen et al., 1976, Bree van et al., 1992) show that grey seals were present in all Wadden Sea regions and along the Dutch North Sea coast during the Neolithic and early Bronze Age (Joensen et al., 1976; Reijnders, 1978a; Requate, 1956). Most of the seal remains found in Dutch deposits dated from between 2,000 BC and 1,000 AD, and originated from grey seals (Clason, 1988; Griffen, 1913), which is comparable to finds in the other parts of the Wadden Sea (Reijnders et al., 1995). Historical data indicates that grey seals were common in the Wadden Sea until the 6th century (Reijnders et al., 1995), but the species decreased substantially thereafter. The ratio of grey seals to harbour seals seems to have gradually changed and by 1,000 AD finds of the two species occurred in about the same quantities. This shift coincides well with the growing numbers of human settlements in this area. As dykes were built, more humans settled, and more grey seals could be hunted. By the end of the Middle Ages (1400-1500 AD) grey seals virtually disappeared from the area (Reijnders et al., 1995).

Thus, following a long-term decline since the Neolithic, grey seals became extinct in the Wadden Sea and the Dutch North Sea coast by about 1500 AD (Reijnders et al., 1995). Up until the mid 20th century, only straggling animals were reported on the Dutch, German, and Danish North Sea coasts (Haaften, 1974; Mohr, 1952). In the 1960s, a colony began to form around the German Wadden Island of Amrum (Caudron, 1997; Quedens, 1988). Since 1980, grey seals are observed, in increasing numbers, in Dutch waters, most probably originating from the closest grey seal populations in the Farne Islands (east coast of UK). More (young) animals were believed to be migrating to the Dutch Wadden Sea where the first colony formed between the islands of Vlieland and Terschelling in 1980 (Reijnders et al., 1995). Since 1985 pups have been born in this colony (pers. obs. Reijnders), with the number of grey seals surpassing 1000 in 2003 (Härkönen, 2007; Reijnders, 2003). Post 2000, the seals were found to be spreading to other haul-out sites, namely, around the Island of Texel (Noorderhaaks, Vliehors).

The aim of this section of the study is to investigate and describe the presence, number and distribution of grey seals in the Netherlands.

3.2 Material and methods

Grey seals are generally counted during aerial surveys at low tide, when the maximum number of haul-out sites are available. The dedicated grey seal counts in the Wadden Sea are carried out during the pupping period and the moult (December-

February and March-April respectively). During other periods of the year when flights were undertaken to survey harbour seals, the grey seals were also counted, albeit opportunistically. Dedicated grey seal counts were initiated in 2000 by the authors (IMARES), contracted by the Ministry of Agriculture, Nature and Food Quality. Multiple counts (5-8 counts a year) in this period provide the necessary accuracy for long term monitoring and population studies (Meesters et al., 2007; Reijnders, 1978b; Reijnders et al., 1997). The data also provides information on the spatial distribution of the seals and their pups whilst hauled out

(http://www.zeezoogdieren.alterra.wur.nl/p1a1_zeehondentelling.htm). Before this, from 1985 onwards yearly counts during the pupping and moult of the grey seals were conducted by boat (Ministry of Agriculture, Nature and Food Quality). A large part of this data set has been discussed in (Reijnders, 1995). In the southern Netherlands (Delta area) seals are counted during a monthly count (Biologisch Monitoring Programma Zoute Rijkswateren van het RIKZ, Rijksinstituut voor Kust en Zee, now Waterdienst).

Statistical analysis was conducted using R software (R Development Core Team, 2008). All data were tested for normality and homogeneity of variance using exploratory analysis and residual plots. Transformations were used where necessary.

3.3 Results

Prior to discussing the results it is important to mention a caveat of the study. It is believed, as mentioned above, that grey seals in the Netherlands originate from populations in the UK. The latter is estimated to have reached a size of 182,000 (C.I 96,200 to 346,000) animals (Duck, 2008). The population in the UK is estimated on pup counts, where approximately 20% of the pups are born along the east coast of Brittan and another 40% in the Orkney Islands. From these areas it is known that grey seals exchange with grey seals along the Dutch coast.

This together with the fact that grey seals are known to swim between the UK and the Netherlands insinuates that the Dutch population is not a closed population. Moreover, it is most likely that the population is not a true population, but a colony. This could have two consequences; first, growth in numbers is not only attributed to the Dutch colony, and secondly, the seals in the Netherlands could be quite dependant on the possible crossing of the North Sea. Thus changes in the North Sea, as a result of large building activities, could have consequences for the seals.

3.3.1 Population Dynamics

During the last 24 years, the grey seal population in the Dutch Wadden Sea has grown exponentially to 2108 individuals (moult count 2009; **Error! Reference source not found.**Figure 2). It is important to note that this figure does not include all individuals as there are always individuals who are either at sea or underwater during the surveys. Even when a maximum number of seals is ashore and can be counted, a large percentage could be swimming and are thus not surveyed. For grey seals in the Netherlands the percentage is still to be determined but for harbour

seals, it has been estimated that at low tide, during the pupping period, only 68% of seals are hauled out (Ries et al., 1998).

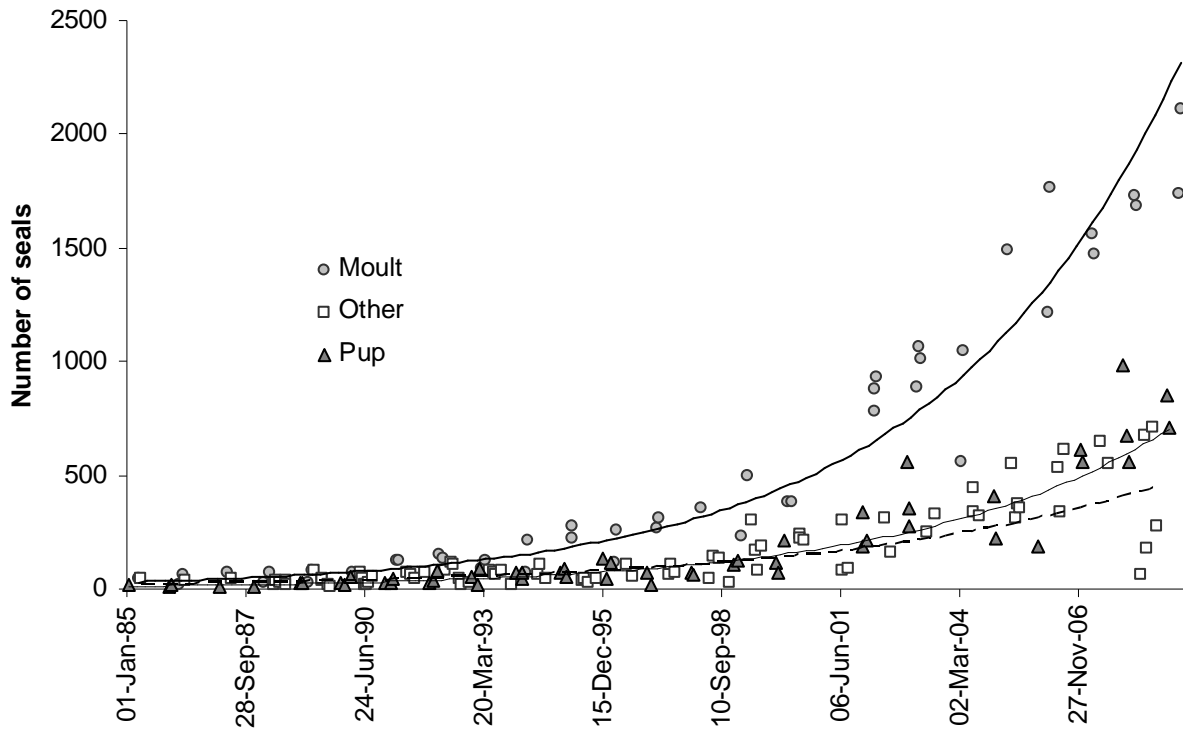


Figure 2. Exponential growth of grey seals in the Dutch Wadden Sea during the moult (March/April -18.74%), the pupping period (December – February -19.76%) and during the remaining time 'other' - 14.65%.

Table 1. Regression coefficients of a regression of $\log(\text{counts}) \sim I + \text{year.dec} * \text{Type}$, with $\log(\text{total})$, the total number counted logarithmically transformed; year.dec, the time as year with decimals; Type, as the three data collections Moulting, Other, and Pupping. The * denotes that the model also contains an interaction which allows for a different regression coefficient for each Type.

Coefficients:	Estimate	Std. Error	t-value	Prob.(> t)	
(Intercept)	-3.544e+02	2.100e+01	-16.878	< 2e-16	***
year.dec	1.803e - 01	1.051e -02	17.147	< 2e-16	***
TypeOther	8.604e+01	2.715e+01	3.170	0.00180	**
TypePup	1.602e+01	2.948e+01	0.543	0.58761	
year.dec:TypeOther	-4.360e - 02	1.359e -02	-3.208	0.00159	**
year.dec:TypePup	-8.544e - 03	1.476e -02	-0.579	0.56345	

Table 2. Results from 999 bootstraps of the model in Table 1. Estimated median values and 95% confidence limits are also given.

Type	Estimate	95% confidence limits	Rate of increase % (Conf. limits)
Pupping period	0.172	0.155, 0.188	18.74 (16.8,20.7)
Moult	0.180	0.166, 0.197	19.76 (18.1,21.8)
Other	0.136	0.113, 0.158	14.65 (12,17.1)

Three data types are recognised because of the phenology of the grey seal and the nature of the behaviour of grey seals in Dutch waters. Grey seals haul-out to rest, for parturition and to moult. Grey seals in the Netherlands pup during December-February and moult during March/April (this is in contrast to other grey seal colonies that pup during autumn and moult in winter in western UK waters (Pomeroy et al., 1999)). The numbers of seals that haul-out can obviously vary between periods, for example, mothers must haul-out to care for their young, while males do not, while during the moult, all seals haul-out (this is the period where maximum numbers are counted). Within a time period, numbers can also vary, for example, during the pupping period, stormy weather can result in pups washing off the sandbank, which will result in lower total counts.

A linear regression of counts versus time and type (i.e. pup, moult and 'other'), (counts logarithmically transformed) is highly significant (F-statistic: 199.1 on 5 and 174 DF, p-value: < 2.2e-16) and explains 85% of the variation (Table 1). The interaction is also very significant (p=0.0026, F=6.16, df=2). Table 1 reads as follows; for data collected during the moult, the first two lines are to be considered: there is an intercept of -354.4 at year.dec=0, however given that the start date is 1985, we must add 1985 * 0.1803, which results in 3.49. Back-transforming the estimate results in 33 individuals. For the data from the pupping period it is necessary to add 16.02 to the intercept and -0.008544 from the slope of 0.1803. The probabilities (Prob.) of the coefficients suggest that the extra effect on the slope for the pupping data is not significantly different from the slope of the data from the moult period (p=0.56), while the other data are significantly different (p=0.00159). Bootstrap estimates for the coefficients were obtained using 999 bootstraps. These resulted in coefficient estimates shown in Table 2. The different slopes can also be converted into yearly rates of increase. Thus, growth rates are 18.74%, 19.76% and 14.65% for pupping data, moult data, and 'other' data respectively. The confidence limits indicate that 'other data' has a significantly lower rate of increase than the other two groups of data. There is no significant difference between the rates of increase in the data collected during the moult and during the pupping period.

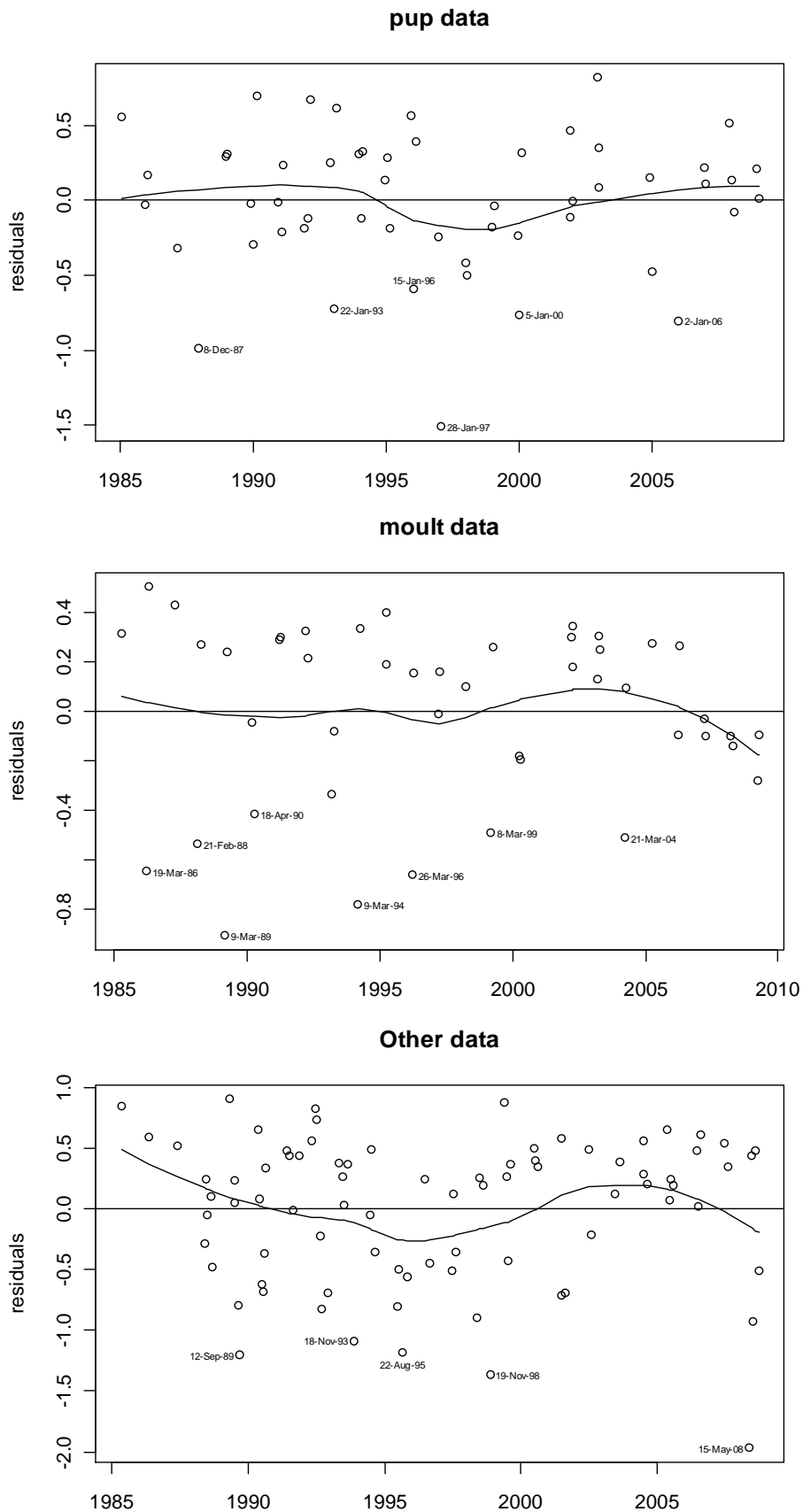


Figure 3. Residuals of pup (above) moult (middle) and other (bottom) counts of grey seals in the Dutch Wadden Sea. Residuals should be randomly distributed around zero with no obvious pattern discernible. A smoothing line for pattern detection has been added to each plot. Absolutely larger residuals are denoted by their date of collection.

Although the above graph capture the general trends, there remain irregularities in the data. This can be investigated by looking at the residuals over time. The three graphs below (Figure 3) show the residuals for the moult, 'other' and pupping period. The residuals are the differences between the fitted values and the actual values. Evident from the graphs is that the larger residuals are mainly negative residuals. These data are thus generally lower than would be expected based on the regression. This means that the regressions are not completely valid. However, this means, that the results are conservative in the sense that if all the data that are too low would be removed, the growth estimates of the grey seal population would be even higher. Also evident from the residual plots is that residuals of the data from the moult period are decreasing through time. In the 'other data', low counts in the last years apparently pull the regression down and 2001 to 2006 shows mainly positive residuals, indicating that the regression in this period is not correct.

In terms of actual pup numbers, the data indicates that the number of pups is also increasing exponentially with a growth rate of 21.3%. A linear regression of counts versus time (counts logarithmically transformed) is highly significant (F-statistic: 76.31 on 1 and 15 DF, p-value: 2.868e-07) and explains 83% of variation. Bootstrap estimates for the coefficients were obtained using 999 bootstraps. The bootstrapped 95% slope intervals were 15.9-25.9. Of interest is that the maximum pups counted per month within the pupping period tends to be December with the exception of in 2006 and 2008 (Figure 4). In these years the greatest numbers of pups (of the season) were recorded in January.

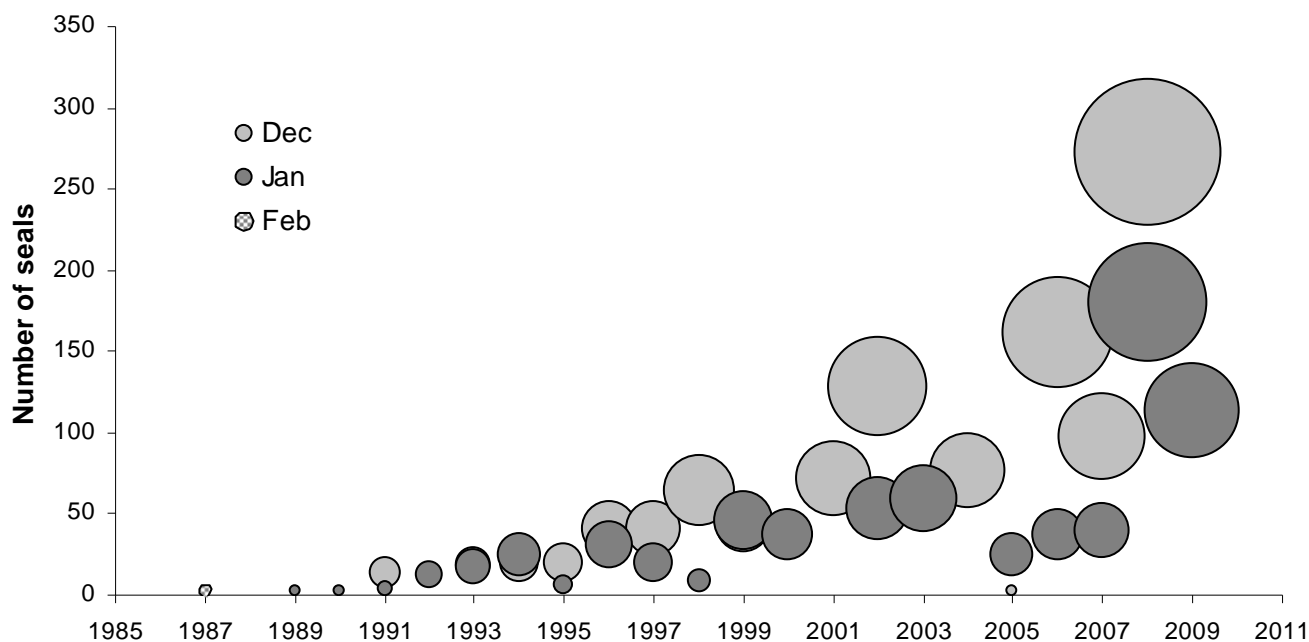


Figure 4. Monthly maximum of the number of pups counted in the Dutch Wadden Sea (Dec-Feb).

3.3.2 Distribution

Grey seals in Dutch waters are currently found in the North sea, in the Dutch Delta area and in the Wadden sea. The stronghold of the seals in continental Europe is in

the Dutch Wadden sea (Härkönen, 2007), of which haul-out sites between Vlieland and Terschelling, namely the Richel and the Engelsehoek are of key importance (Figure 5). These two sites are also the main pupping sites for this species. In recent years, the seals have been observed more frequently in the eastern sections of the Wadden Sea, with 38 seals counted during monitoring flights in 2008 as far east as the Sparregat and the Eems-Dollard.

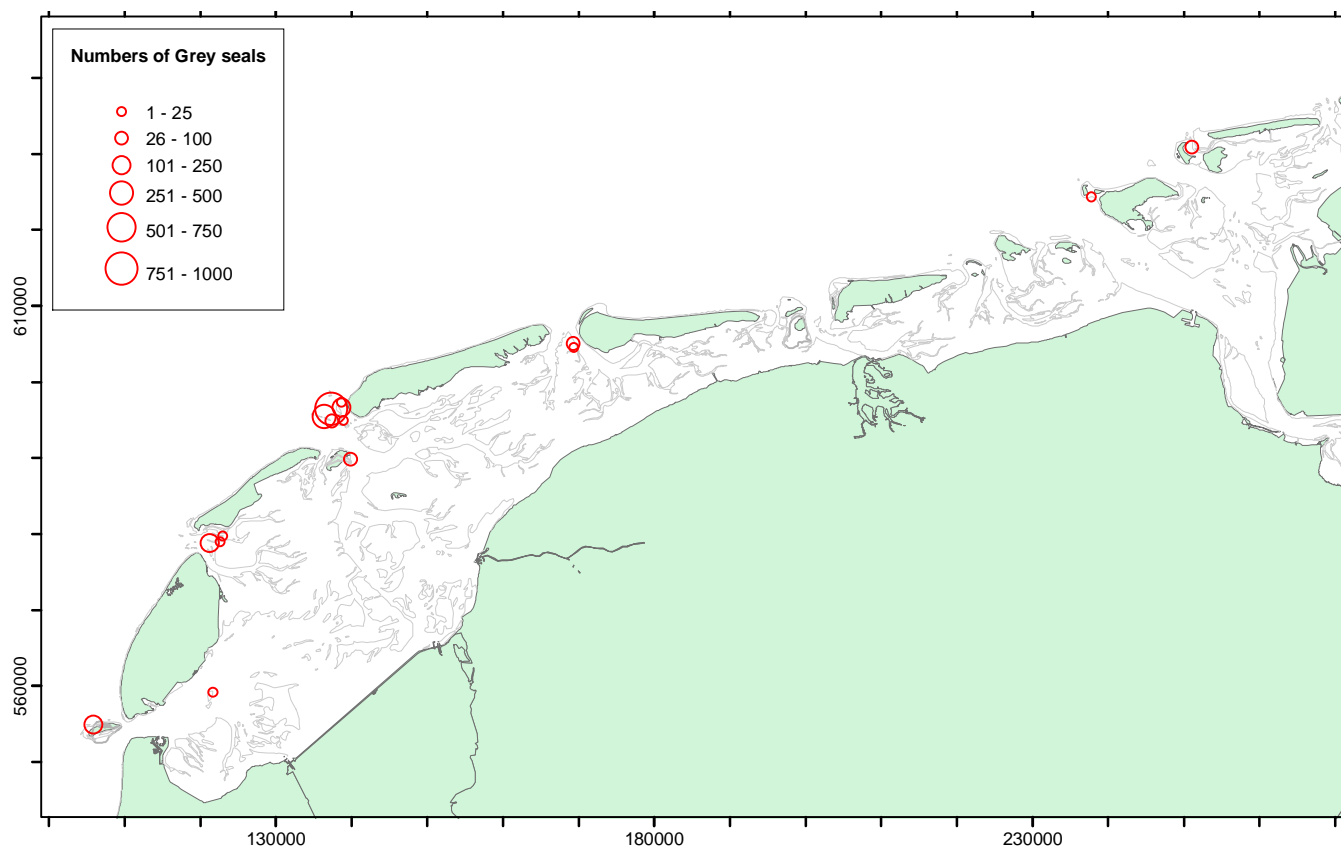


Figure 5 The distribution of grey seals in the Dutch Wadden Sea during the moult (April) 2009 when the greatest number of hauled out seals are observed.

Figures 5-7 show the preferred haul-out locations during the pupping period and the maximum number of seals (range) hauled out. Here too it is obvious that the population is increasing. Of interest from these figures is the expanding of the population to new locations, both in terms of general haul-out sites and in terms of pupping sites. From 2001 onwards surveys were conducted in a larger area, as shown in figure 6, which resulted in the observation of grey seals at multiple haul-out sites. Not all haul-out sites are pupping sites as seen in Figures 6 & 7. It is also evident from the graphs that some haul-out sites are always pupping sites. The figures also indicate that some haul-out sites are used in one year and not in another year. Despite this, there seems to be a growing interest in western sandbanks (2007 & 2008).

The majority of seals haul-out on sandbanks between Vlieland and Terschelling. The number of haul-out locations during the moult in the Wadden Sea have also increase over the years as seen in Figures 8-10. Here too is a growing interest in locations to the west of the key haul-out sites (2008 & 2009).

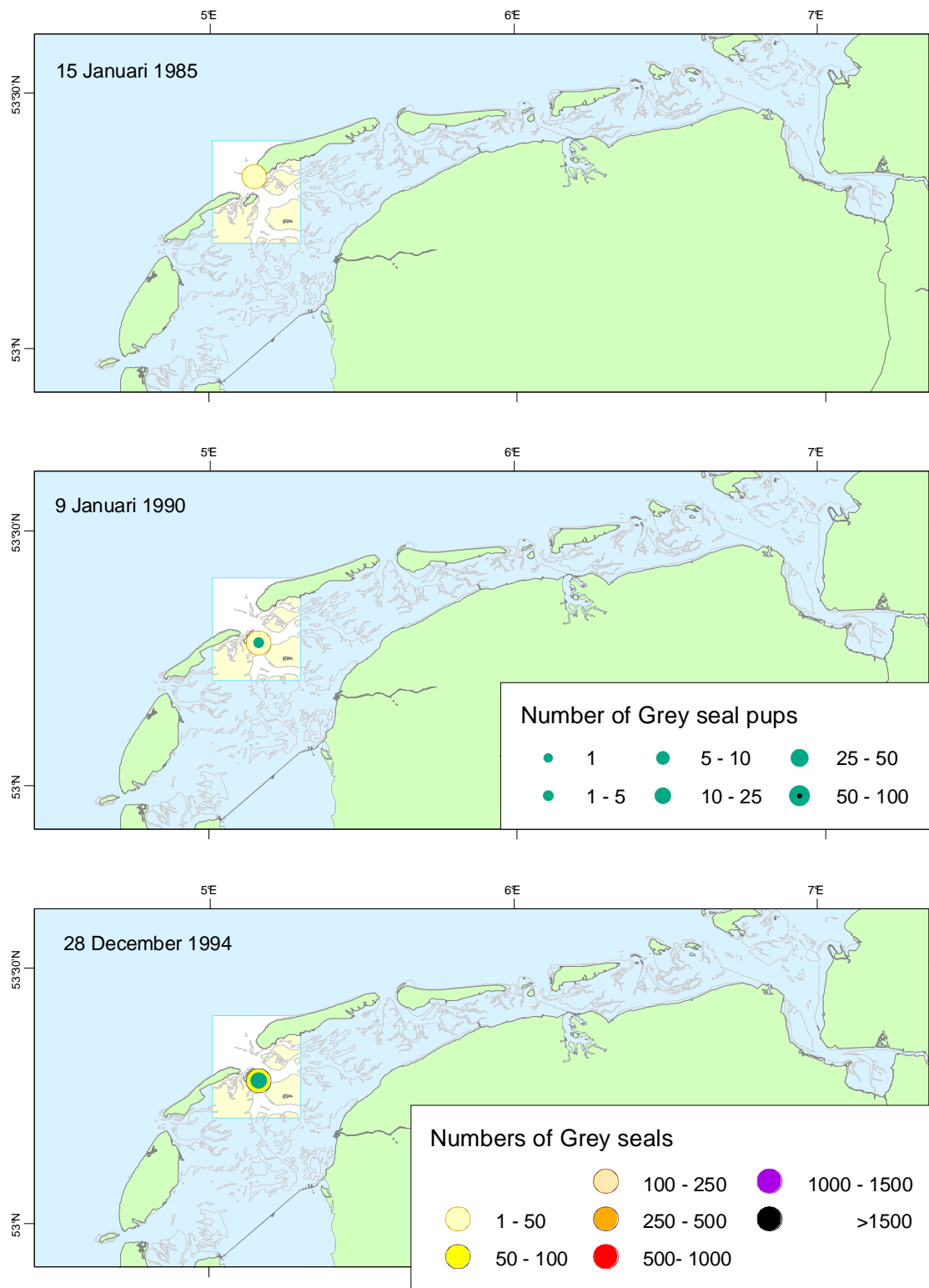


Figure 6 The haul-out location of grey seals in the Dutch Wadden Sea during the pupping season (Dec-Feb) 1984/85-1994/95, showing the maximum number of pups counted during the season (green dots) as well as the maximum number of all grey seals (coloured circles). The white area shows the survey area.

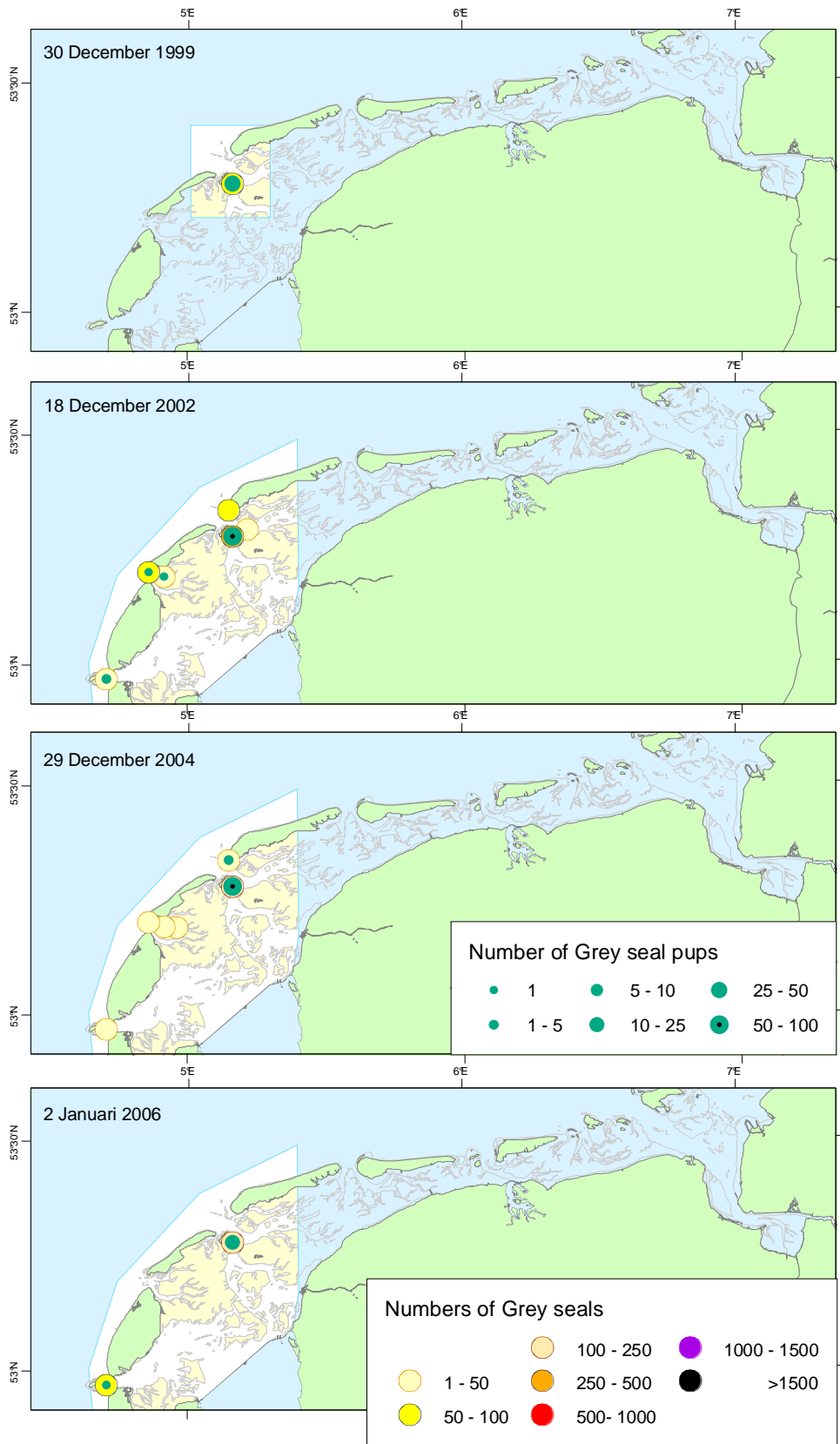


Figure 7 The haul-out location of grey seals in the Dutch Wadden Sea during the pupping seasons from 1999/00 - 2005/06, showing the maximum number of pups counted, as well as the maximum number of all grey seals. The colour indicates the actual number of seals and the white square shows the survey area

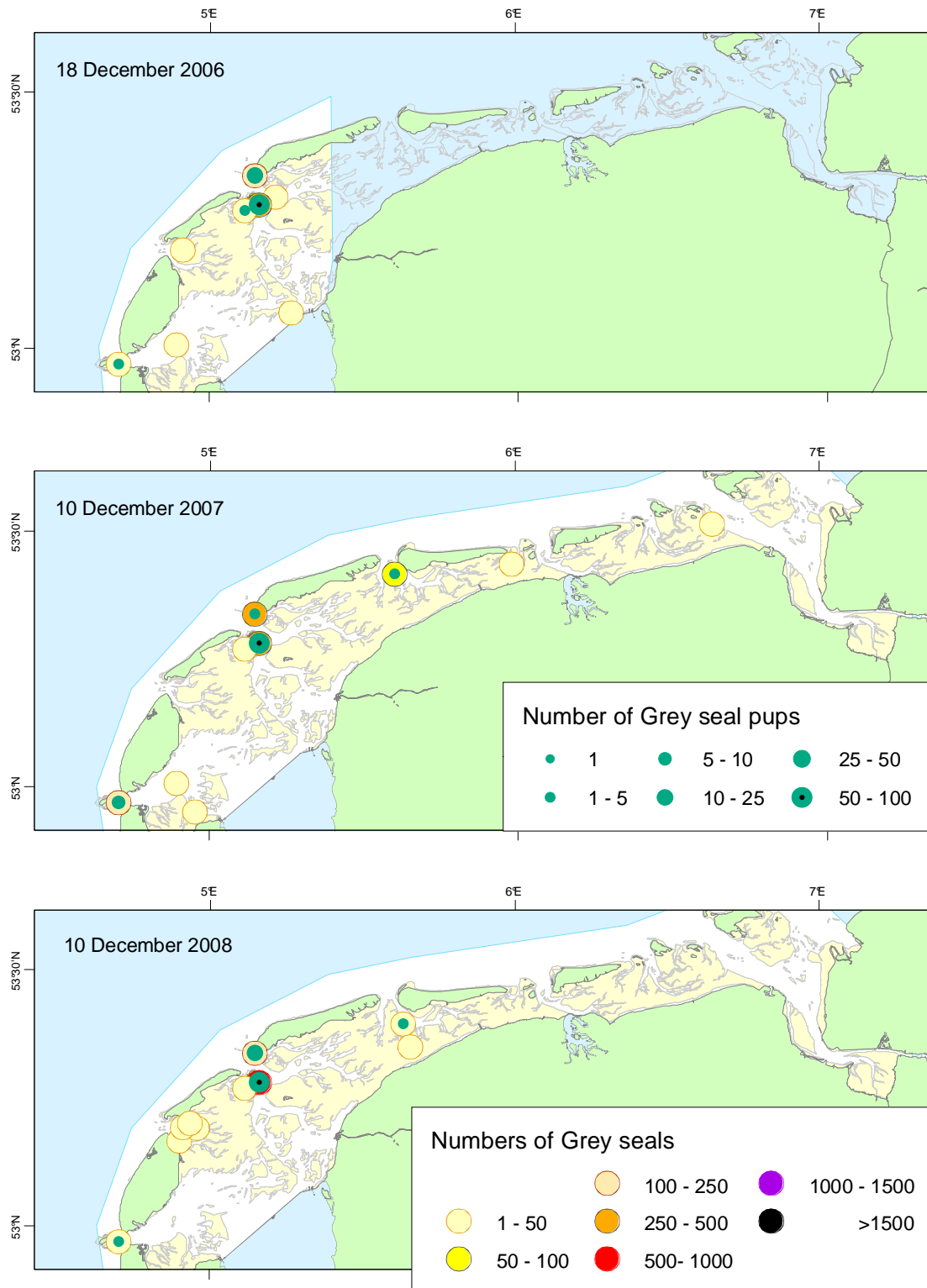


Figure 8 The haul-out location of grey seals in the Dutch Wadden Sea during the pupping season of 2006/07 - 2008/09 showing the maximum number of pups counted as well as the maximum number of all grey seals. The colour indicates the actual number of seals and the white square shows the survey area.

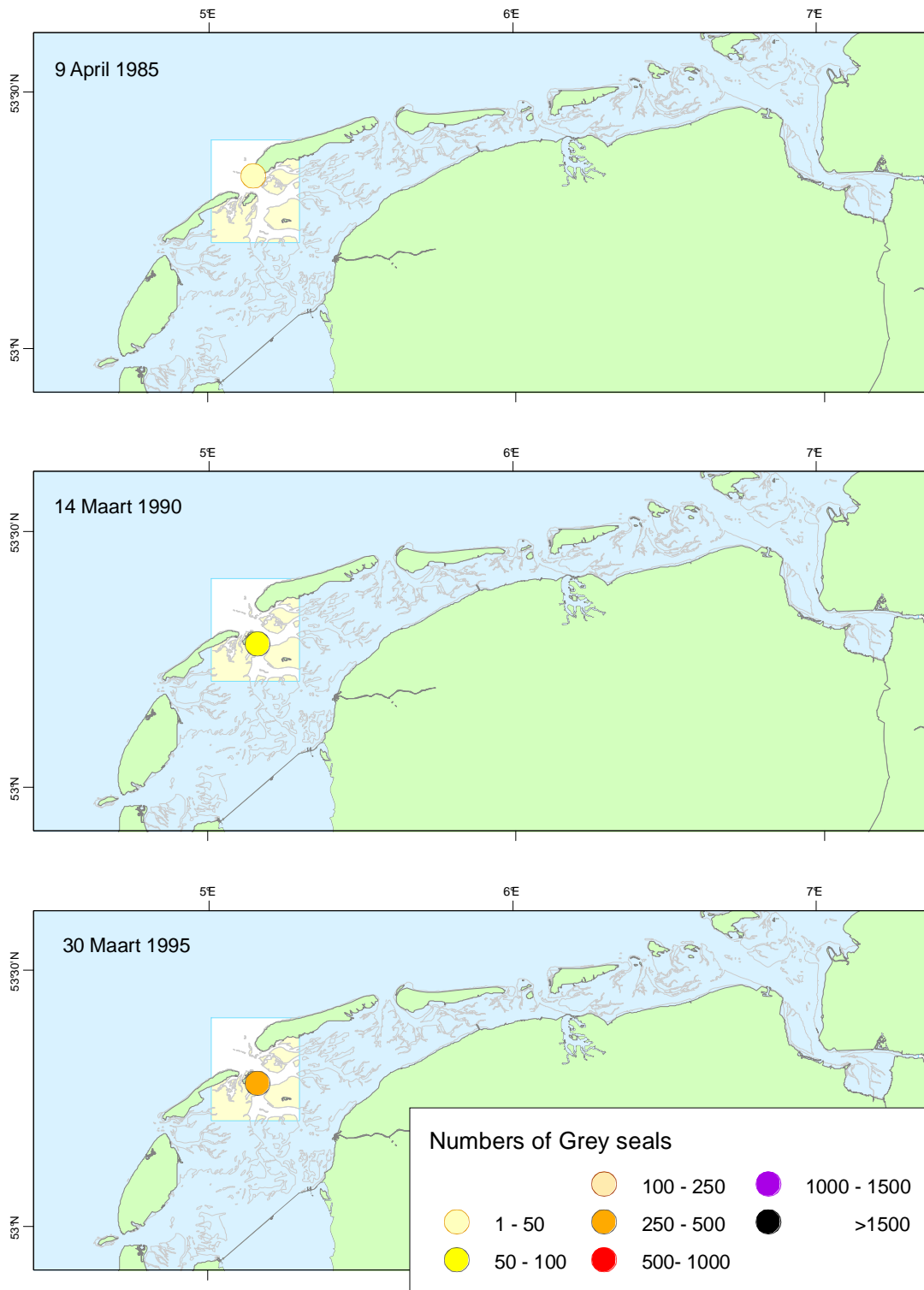


Figure 9 The maximum number of grey seals counted during the moult (March/April) between 1985 – 1995, showing the location of haul-out sites in the Dutch Wadden sea. The colour indicates the total number and the white areas indicates surveyed area.

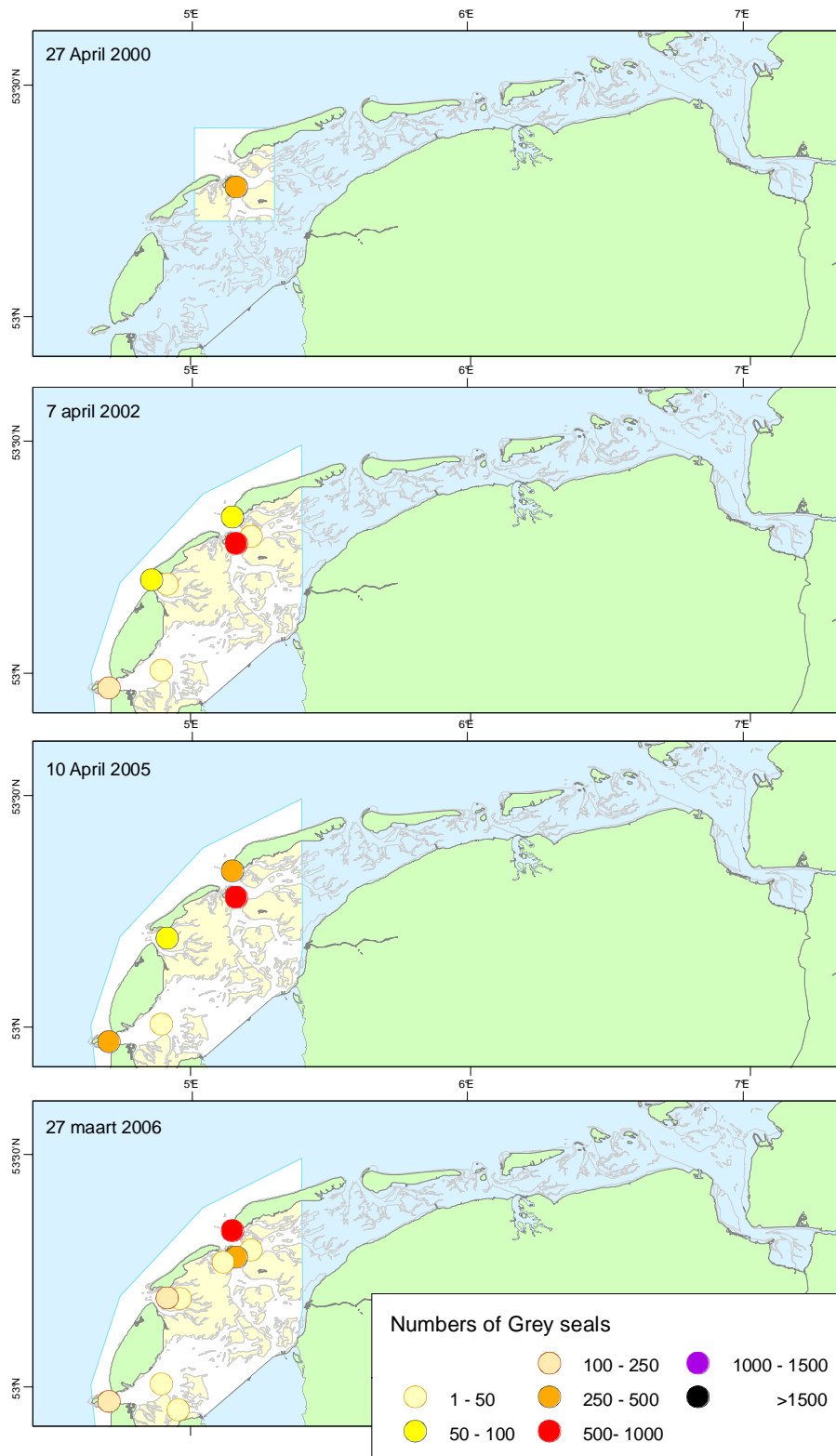


Figure 10 The maximum number of grey seals counted during the moult (March/April) between 2000 – 2006, showing the location of haul-out sites in the Dutch Wadden sea. The colour indicates the total number and the white areas indicates surveyed area.

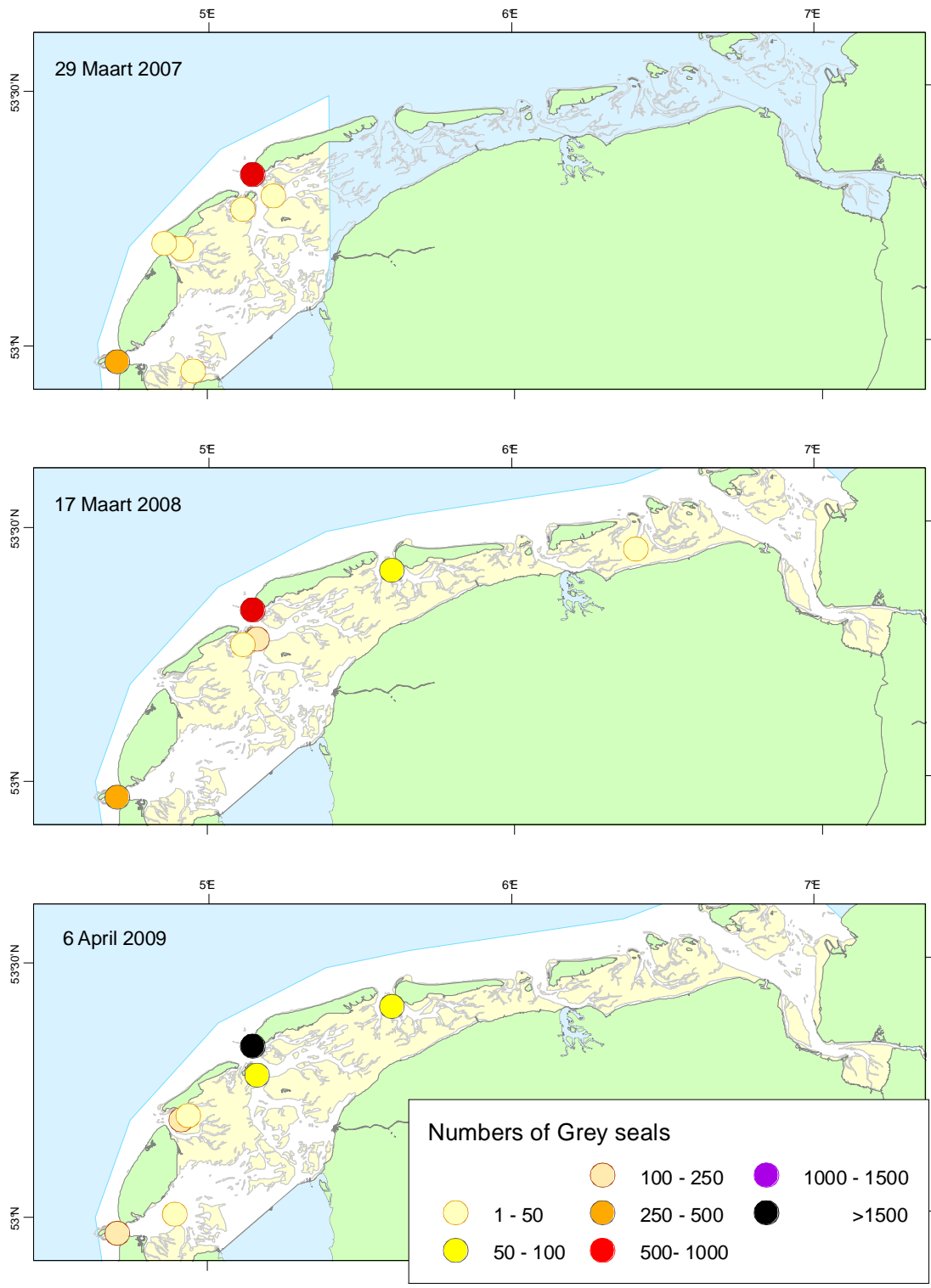


Figure 11 The maximum number of grey seals counted during the moult (March/April) between 2007 – 2009, showing the location of haul-out sites in the Dutch Wadden sea. The colour indicates the total number and the white areas indicates surveyed area.

3.4 Discussion

The results show that the grey seal population in the Dutch Wadden Sea is growing exponentially with an expanding distribution. On average, counts during the moult augment with 19.7% yearly, this is much higher than the maximum growth capacity of harbour seals (in lack of data on grey seals), which is estimated at 12% (Harkonen et al., 2002). Bowen *et al.*, (2003) report that grey seal numbers on Sable Island in Canada have been having sustained exponential pup growth at 12.8%. During the moult in 2009, 2108 seals were counted. During the pupping period, considerably less seals are counted (855 during the breeding season of 2008/2009). This includes 272 pups. The continuous exponential growth could imply that at present the population has not reached its carrying capacity, which in turn means that resources, such as food and haul-out sites are not limiting the population.

3.4.1 Population dynamics

Given the fact that the Dutch grey seal population is most probably not a closed population, it is possible that the high growth rates, calculated for the pupping and moult periods, are influenced by an influx of seals from neighbouring populations. The magnitude of this influx, from elsewhere, is not clear, however this would explain the extremely high growth rates. Seasonal changes in the influx would be one explanation for the variable growth rates in the different periods. Alternatively, the seasonal changes in growth rate could be related to the phenology, and thus behaviour of the seals. That is, the necessity to haul-out for long periods of time outside of the moult and pupping period is reduced. Thereby reducing the chances of observing seals on the sandbank during a survey flight outside the moult and pupping periods.

The high rate of increase in pup numbers (yearly) may be inflated due to poor counts in the years between 1985 and 2001. These counts were made by boat, during mid winter and are thus highly dependant on the weather. For example, the boat could not always sail close to the sandbanks due to ice (pers. obs Reijnders). Also wind periodically pushing the seals away from the boating lane made it difficult to count the seals. Moreover, during the winter, storms can result in high seas, which have the real potential of washing the non-swimming pups off the sandbanks. Wind conditions, i.e. north and north-easterly, can also result in high seas, again increasing the chance that pups are washed off the sandbank. Thus, it is highly likely that these counts often underestimated the number of pups, especially in the early years. Despite this, the rate of increase is still extremely high. Again pointing to an influx of seals from elsewhere who then pup in the Dutch Wadden Sea.

Despite there being no significant difference in growth rates from the pupping period and the moult, estimating the population size in the Netherlands is best done using the moult counts. Not only is the total number of seals observed higher, but more importantly, there is less variation in counts during the moult (fig 2). The relatively low variation between counts during the moult is most probably related to better weather conditions experienced in spring, the increased sample size and the behaviour of the seals, i.e. aggregating during the moult.

3.4.2 Distribution

It is clear from the maps (figures Figure 7Figure 11) that multiple haul-out sites exist for grey seals in the Wadden Sea. Although the counts prior to 2001 were within a small area, i.e. around the two known grey seal pupping and moulting sites, it is still obvious that the population increased rapidly. Between 1985 and 1990 it is also apparent that the seals shifted haul-out sites with the later site (Richel) remaining the most important grey seal haul-out site. After the winter of 2000/2001 when flights were initiated, new haul-out sites were located by the observers. It is unknown as to which year the seals first starting using these sites. It is therefore possible that the counts in those early years were underestimates. However, if continuous growth can be expected, only the numbers in the season of 1999/2000 would be underestimated.

It is interesting to note that there were four pupping sites in 2001/02 while in 2004/05 and 2005/2006 there were only two. This increased to four again in 2006/7 and remained that way. Although the reasons for this are unknown, it is likely to have been related to the changing sand banks, making them variably suitable as pupping sites. The actual location of the pupping sites was shown to vary, with the exception of the Engelsehoek and the Richel. These two sites are the two preferred haul-out sites for both pupping and moulting.

In terms of general numbers, thus using moult counts, the change in the seals haul-out distribution in the Dutch Wadden Sea is more involved. Here too, the Engelsehoek and the Richel are the preferred haul-out sites, even though the importance of the one site over the other changed between 2005 and 2006, with a shift to the Engelsehoek from the Richel. It appears that some of the seals using the Richel ventured out to discover new sites as seen in Figure 7 by the decrease in numbers from 2005 through to 2007. It is also interesting to note that the greatest number of haul-out sites occurred in 2006 despite the survey area still be restricted by today's standard. However four of these sites had less than 50 individuals. It might be due to weather conditions, i.e. water regularly washed over the sandbank, and was therefore not used again. An alternative explanation, and one that may be more likely, is that of the locations in which there were only a few individuals, the seals observed at these sites, were not hauled out to moult but instead were only briefly visiting the sandbank to rest.

What is evidently clear from the two years in which the whole Dutch Wadden Sea was surveyed (2008 & 2009), is that the seals are venturing out to new areas to the west. Although the reason is unclear as to why this is the case, we can say with certainty that it is not related to the carrying capacity at other haul-out sites (Figure 10Figure 11). What this may indicate is that the decrease in numbers at haul-out sites in the east may be related to disturbance. For example, the number of seals hauled out on the Noordehaaks (south-west of Texel) is variable and appears to be decreasing, which may be related to an increase in disturbance e.g. boating and recreation on the sandbank.

4 Diet of grey seals in the Netherlands

4.1 Introduction

Relation presence of species/ food

It might be clear that the grey seals numbers have increased at a very high rates since the late 1980's, and that there are no indications that the seals have reached, or are reaching, their carrying capacity. Studying diet in this respect serves a dual purpose: firstly, understanding if and in what this species might compete for resources with other predators, mainly the harbour seal. Secondly whether this information helps to estimate the habitat use of the seals, as to a great extent, movement is driven by necessity to feed and most movements are to and from foraging areas.

Individual variation is also evident in the diets of grey seals, with feeding distribution varying by time of day and foraging strategy (Austin et al., 2006). Research suggests that individuals can learn to specialise, and that a population consists of specialists and generalists (Grellier et al., 2006; Hammond et al., 1994b). Temporal variation in the species composition of grey seal diets is well documented (Benoit et al., 1990; Bowen et al., 1993; Hammond et al., 1990). Similarly, diet composition is dependent on geographical location, with dominant or important prey species varying between locations (e.g. there are many studies from all parts of the species distribution, describing the diet of grey seals, see for example (Bowen et al., 1994; Haug et al., 2007; Rae, 1973). Despite these differences, grey seals almost invariably feed at or near the sea bed and have been described both as actively searching for their prey but also as being a sit and wait predator (McConnell et al., 1999; Thompson et al., 1993; Thompson et al., 1991). The daily intake of grey seals is estimated at approximately an average of 5500 kcal per day (Fedak et al., 1985 ; Greenstreet et al., 1996; Hammond et al., 1994a; Sparling et al., 2003) , though this can depend sometimes strongly on season or the size of the animal. Research suggests that this equates to approximately 5 kg per day depending on the prey species, although it is thought that grey seals do not eat every day (Anderson, 1992). Both males and females fast during the breeding season and during the moult (Iverson et al., 1993; Lidgard et al., 2005; Walton et al., 2000), when they resume they may feed at an increased rate in an attempt to regain lost fat stores (Murie et al., 1992).

Here a description of the seals' diet is given both in regards to the species composition and the size frequency. Also, a first comparison is made between the diet of grey seals and harbour seals in the Netherlands.

4.2 Material and methods

Seal faeces were conveniently collected from haul-out sites when seals had already been disturbed for example during a tagging expedition. Many haul-out sites are simultaneously used by both grey and harbour seals. As it was impossible to discern the origin of the procured scat, it was necessary to develop a method to determine the seal species from the scat itself. This was done in close cooperation with the

molecular laboratory of the NIOZ. A diagnostic method, based on the differences in genetic code, was developed by successfully designing species specific primers for the two seal species. However, the details of this study is clearly beyond the scope of this report. We therefore refer to van Bleijswijk, Brasseur *et al* in prep.

4.2.1 Analysis of hardparts

Faeces were placed into plastic bags and stored at -20°C until processing. Each scat was considered to come from one individual. Hard parts were extracted from the faeces as follows. After removing a small sample for DNA (see above), the individual scat was placed in a 300-µm mesh bag closed by folding the opening twice and secured by wrapping a rubber band around it. This bag was then placed inside a 120-µm mesh bag closed in the same way. The outer bag prevented small parts (mostly sand) from being lost and also protected the inner bag from wear.

Samples were placed in a commercial clothes washing machine. Each load was run through a normal cycle with prewashing, washing and rinsing at a temperature of 70°C. For the prewashing 50g of detergent with enzymes (Biotex blue, Sara Lee H&HB Nederland B.V.) were added and for the main washing cycle we used 50g of common laundry detergent (Dreft). After washing, the bags were rinsed and dried (Brasseur & Jansen, unpublished data).

All fish otoliths were identified to species using a reference collection and identification guides (Härkönen 1986, Leopold et al. 2001). Length and width of each otolith were measured to the nearest 0,01 mm with digital callipers and a projection microscope (Projectina). Occasionally otoliths were found broken and only one of the measures could be taken. For each otolith, a wear class was also estimated (Leopold et al. 2001) and according to this wear class a correction factor was used to calculate the size of the undigested otolith (Van Damme & Leopold unpublished data). Fish length and fish weight were estimated from otolith size using relationships described in Leopold et al. (2001).

Other hard parts such as vertebrae, premaxilla, urohyals, were also identified using a reference collection of fish bones and an identification guide (Watt et al. 1997), but in lack of knowledge on the size in relation to the fish, they were not measured. The minimum number of individual prey (MNI) of each prey species was estimated by finding the pairs of otoliths, and in addition, the unique or paired bones structures (urohyal, premaxilla, preoperculae, atlas vertebrae). Non-unique fish remains (teeth, denticles, vertebrae) were not used to enumerate the fish but only to determine their presence.

We estimated the percentage of each species in the diet by weight (Prime and Hammond, 1987). In several samples, some species were identified using only bones. As the bones were not measured, the weight of the fish was estimated to be equivalent to the average of the weight of the species in all the samples.

Occasionally, remains of shrimp (*Crangon crangon*), worms (*Nereis sp.*) or other small organisms were found in the samples. As these were often found in conjunction with fish that prey on these species, we considered them to be secondary prey, that is eaten by the seals' prey. Moreover, the number of unique identifiable remains never reached a level that could be significant to our results (i.e. <5 individual shrimps). Therefore, they were not included in the analyses. This is justified by the fact that

several studies show that these animals are the main food of fish species found in faecal samples (Braber and De Groot 1973, Hostens and Mees 1999, Pedersen 1999).

4.2.2 Statistical analyses

Statistical multivariate analyses were performed to describe the grey seals' diet, define variation in time and delineate whether there were differences between the diet of grey and harbour seals.

All otoliths and other remains found in each scat were analysed to the highest possible species level. For otoliths, the average fish length and weight were also determined. A matrix was generated, holding for each scat, species and number of prey.

Table 3. Overview of scat samples of grey seals (top) and harbour seals (below) included in this study.

year	month	Noorder- haaks	Richel	Simonzand	Steenplaat	Texel	Zeeland	Total
GREY SEALS								
2002	Jun.					1		1
	Nov.					1		1
2002 Grey seal total						2		2
2004	Apr.		32					32
	Jun.	9						9
2004 Grey seal total		9	32					41
2005	Mar.	25	10					35
	Apr.	6						6
	Sep.			4				4
	Nov.				5			5
2005 Grey seal total		31	10	4	5			50
2007	Mar.				5			5
	Sep.				2		1	3
2007 Grey seal total					7		1	8
Grey seal total		40	42	4	12	2	1	101

HARBOUR SEALS								
2002	Nov.					1		1
2002 Harbour seal total						1		1
2004	Apr.		4					4
2004 Harbour seal total			4					4
2005	Mar.	8						8
	Apr.	2						2
	Aug.	7						7
	Sep.			14				14
	Oct.						1	1
	Nov.					1		1
2005 Harbour seal total		17		14	1		1	33
2007	Mar.				23			23
	Aug.						6	6
	Sep.				23		6	29
2007 Harbour seal total					46		12	58
Harbour seal total		17	4	14	47	1	13	96

The analysis was done using the program PRIMER version 6 (Clarke et al., 2006). The data consisted of a matrix describing scat number and the number of individuals of encountered prey species. As mentioned before shrimp and Nereis species were excluded as well as prey species that only occurred once. Shrimp and Nereis spp. were considered secondary prey. Prey species that only occurred once in a sample do not really contribute to a comparison between scats. Because the large variation in prey numbers data were fourth root transformed, a common practice to prevent the domination of single dominant species in subsequent analyses. Next Bray-Curtis similarity was calculated between each pair of scats. The resulting triangular matrix was then analysed by non-metric Multi-Dimensional Scaling (Shepard 1962, Kruskal 1964) further called MDS. MDS constructs a configuration in 2 or 3 dimensional space such that the rank order of the samples in the matrix is mimicked as best as possible. Samples that are more similar to each other are more close in space than samples that are less similar.

Another multivariate analysis that was used was the so-called ANOSIM procedure (Analysis Of SIMilarities) which applies a non-parametric permutation procedure to the (rank) similarity matrix to test for a difference between groups (Clarke and Green, 1988)

4.3 Results

4.3.1 Samples

The amount of scat samples collected varied strongly as a result of the size and species composition of the group of seals on the sandbank and the height of the tide and the sandbank. Grey seals generally aggregate in large groups on relatively high (tidal) flats. A total of 548 scats were collected from the sandbanks between 1999 and 2008. To this date, 4382 prey remains have been found in 197 scats, which were successfully diagnosed as belonging to one of the seal species (Table 3). The remaining scats still need to be analysed.

The grey seals' diet determined by scat samples shows great diversity and large variation in size (Table 4).

Table 4. Results of grey seal' scat analysis of samples collected in Dutch waters between 2002-2008. Including were possible species, number of individual fish found and size (average, median maximum and minimum length)

Species or group	Latin name	Number of fish identified (MNI)	Average length (cm)	Median length (cm)	Maximum length (cm)	Minimum length (cm)
Cod	<i>Gadus morhua</i>	6	25.0	22.8	34.6	20.0
Whiting	<i>Merlangius merlangus</i>	54	18.4	18.3	31.1	8.5
	<i>Trisopterus minutus</i>	6	15.3	15.6	19.2	9.9
Pollack	<i>Pollachius pollachius</i>	2	20.6	20.6	21.3	19.9
Five-Bearded Rockling	<i>Ciliata mustela</i>	2				
Four-Bearded Rockling	<i>Enchelyopus cimbrius</i>	1				
Three-	<i>Gaidropsarus</i>	1				

Bearded Rockling	<i>vulgaris</i>					
Rockling	<i>Unidentified</i>	1				
Gadidae	<i>Unidentified</i>	6				
<hr/>						
	<i>Pleuronectes</i>					
Plaice	<i>platessa</i>	21	20.0	17.0	44.4	12.2
Flounder	<i>Platichthys flesus</i>	77	17.0	15.6	39.4	6.0
Dab	<i>Limanda limanda</i>	40	12.8	10.6	23.9	5.0
Long Rough Dab	<i>Hippoglossoides platessoides</i>	7	10.5	9.3	16.8	7.6
Turbot	<i>Scophthalmus maximus</i>	15	9.1	9.3	13.7	5.4
Brill	<i>Scophthalmus rombus</i>	1	18.8		18.8	18.8
Sole	<i>Solea solea</i>	275	22.0	22.7	46.9	4.5
Solenette	<i>Buglossidium luteum</i>	18	8.3	7.7	12.3	5.7
Soleidae	<i>Unidentified</i>	1				
Flatfish	<i>Unidentified</i>	18	23.9		30.4	17.2
<hr/>						
Goby	<i>Pomatoschistus sp., Gobius sp.</i>	12	5.7	5.7	8.0	3.9
Sandeel	<i>Ammodytes sp.</i>	567	16.6	16.6	24.8	6.9
Greater Sandeel	<i>Hyperoplus lanceolatus</i>	29	20.5	20.6	30.5	12.6
<hr/>						
Sprat	<i>Sprattus sprattus</i>	2	8.8	8.8	13.5	4.1
Herring	<i>Clupea harengus</i>	13	14.5	13.9	23.8	8.3
Clupeidae	<i>Unidentified</i>	3				
<hr/>						
Dragonet	<i>Callionymus lyra</i>	28	17.3	16.7	23.4	11.3
Garfish	<i>Belone belone</i>	6				
River Lamprey	<i>Lampetra fluviatilis</i>	5				
Scad	<i>Trachurus trachurus</i>	1				
Scaldfish	<i>Arnoglossium lanterna</i>	3				
Sea Lamprey	<i>Lampetra fluviatilis</i>	1				
Viviparous Blenny	<i>Zoarces viviparus</i>	2				
Fish	<i>Unidentified</i>	135				
Grand Total or Average		1359	17.8	14.8	46.9	13.9

The difference in scat content through the years was investigated by MDS analysis and is shown in Figure 12. The stress value shown in the graph is 0.17 which is considered to be just appropriate to present the similarity matrix in the number of dimensions used. The differences between years was tested with the ANOSIM test and shows a clear significant difference between years ($R=0.1$, $p<0.003$). Possible R values lie between 0 and 1 and indicate how much the average similarity between groups equals the average within groups. At zero there is no difference and at 1 all replicates within groups are more similar to each other than any replicates from different groups. An R value of 0.1 indicates that there is considerable overlap between similarities within and between years. The R values for the pair-wise comparisons are more informative and results are shown in the next table. The clearest differences are mainly between 2004 and 2007 and to a lesser degree between 2005 and 2007. Between 2004 and 2005 there is no statistical difference. The other comparisons allow only a very limited number of permutations, making the output of the tests not very informative.

Table 5. ANOSIM pairwise tests

Pairwise Tests					
Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
2002, 2004	0.256	12.2	41	41	5
2002, 2005	0.032	40.8	49	49	20
2002, 2007	0.438	22.2	9	9	2
2004, 2005	0.012	21.2	Very large	99999	21236
2004, 2007	0.546	0.007	Very large	99999	6
2005, 2007	0.167	3.9	Very large	99999	3852

The main difference between the years 2004 and 2007 was that Raitt's sandeel was not found in 2004, while it was present in 7 of the 8 samples in 2007. In 2004 sole and sandeel were eaten more than in 2007. The limited amount of samples in 2007 however, makes a clear and robust interpretation difficult. Moreover, the very low R value for the comparison between 2004 and 2005 indicate that the difference between 2 years with reasonably large numbers of scats was not significant, lending more evidence to assume that there was generally no real difference between years.

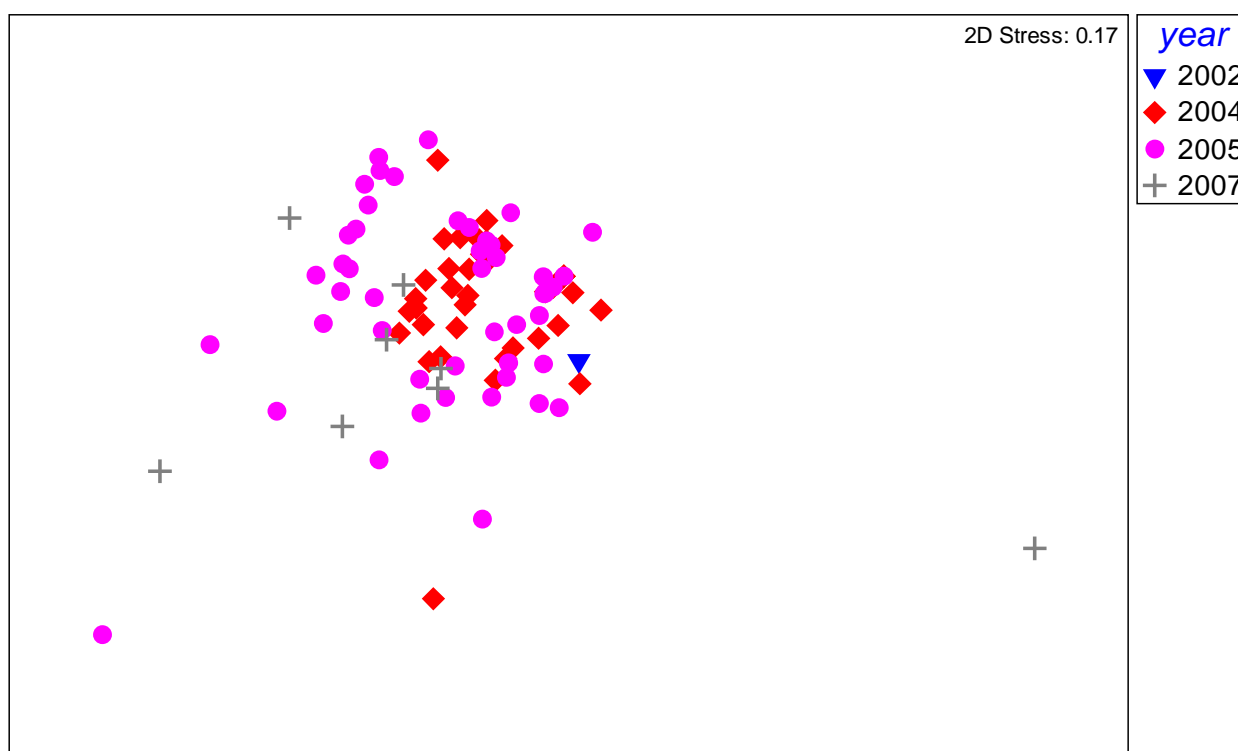


Figure 12. MDS-plot showing the differences between scat contents of grey seals collected in different years. (Fourth root transformed; Resemblance: S17 Bray Curtis similarity)

In the same way, possible seasonal effects were tested: spring (n=88) vs. autumn (n=13). The ANOSIM test indicates a highly significant result (R=0.35, Significance level: 0.00008, 99999 permutations). The two groups showed an average dissimilarity of 81.3 (Table 6). Striking within the species contributing most to the overall dissimilarity is the abundance of sole in spring and flounder in autumn.

Table 6. Result of a simpler analysis for the dissimilarity between the contents of grey seal scats in two different seasons. Only the 10 most important species are shown. Av.Diss, is the average dissimilarity between samples of the 2 groups. Diss/SD is a measure of the constancy of the difference between the samples. The higher the more samples share the same characteristic. Contrib% is the percentage contributed to the total dissimilarity.

Species	Spring Av.Abund	Autumn Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Sandeel	0.69	0.93	13.14	0.99	16.17	16.17
Sole	1.05	0.42	11.87	1.2	14.6	30.77
Flounder	0.27	0.61	7.23	0.92	8.9	39.67
Raitt's_Sandeel	0.12	0.4	5.42	0.5	6.67	46.34
Turbot	0.04	0.43	4.56	0.69	5.61	51.95
Plaice	0.1	0.32	4	0.63	4.92	56.87
Dab	0.31	0.11	3.8	0.66	4.68	61.55
Viviparous_Blenny	0	0.17	2.72	0.41	3.35	64.89
Whiting	0.22	0	2.46	0.43	3.03	67.92
Dragonet	0.22	0	1.98	0.48	2.44	70.36
Cod	0.05	0.08	1.98	0.34	2.43	72.79
Solenette	0.17	0	1.86	0.42	2.28	75.08
Sandeel_Sp	0	0.17	1.83	0.29	2.26	77.34
Herring	0.13	0	1.59	0.37	1.95	79.29
Crab	0	0.08	1.55	0.28	1.9	81.19
Lernaecocera_Branchialis	0.08	0.08	1.53	0.41	1.88	83.07
River_Lamprey	0.05	0.08	1.48	0.35	1.82	84.89
Flatfish	0.1	0	1.27	0.3	1.56	86.45
Poor_Cod	0.02	0.08	1.22	0.31	1.5	87.95
Pagurus_Sp.	0	0.08	1.15	0.29	1.41	89.36
Scad	0	0.08	1.02	0.29	1.26	90.62

Finally, a comparison between grey and harbour seal scats was made (Figure 13). Despite the fact that the stress value is a little too high to ensure correct display of samples in 2 dimensions, the main centroids of the two species appear not in the same place. The ANOSIM test indicates a significant difference ($R=0.14$, Significance level: 0.00001, 99999 permutations).

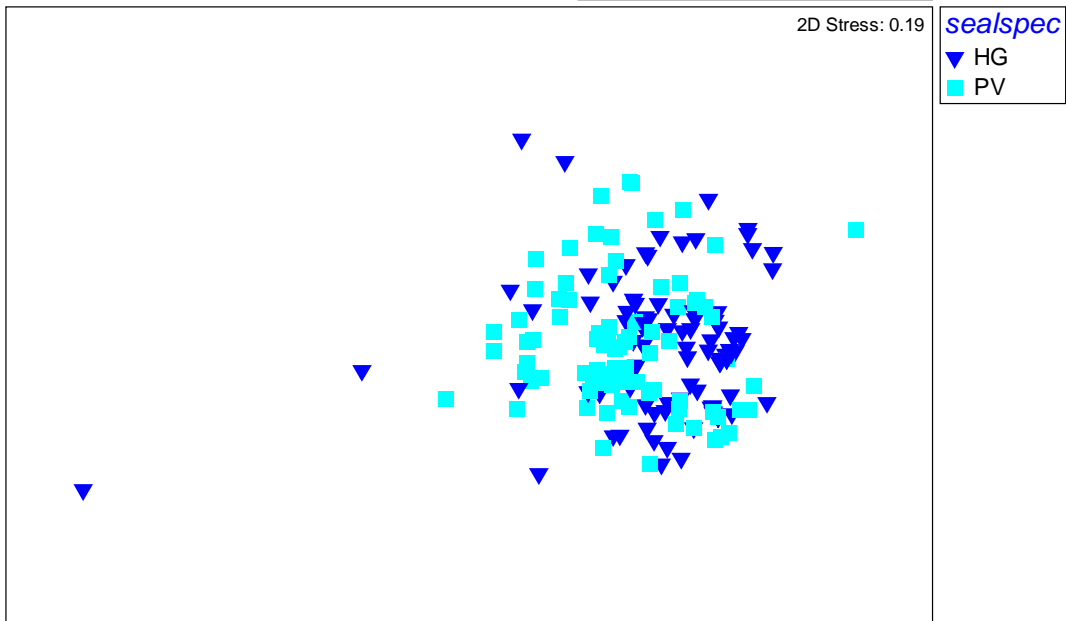


Figure 13 MDS-plot showing the differences between grey and harbour seal prey remains derived from the scats. The stress value indicates that 2 dimensions is not enough to distinguish the species well enough. (Fourth root transformed; Resemblance: S17 Bray Curtis similarity)

Table 7. Result of a SIMPER analysis for the dissimilarity between the contents of harbour and grey seal scats. Av.Diss, is the average dissimilarity between samples of the 2 groups. Diss/SD is a measure of the constancy of the difference between the samples. The higher the value the more samples share the same characteristic. Contrib% is the percentage contributed to the total dissimilarity.

Prey	grey seal Av.Abund	harbour seal Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Sandeel	0.73	0.14	8.99	0.81	11.69	11.69
Sole	0.99	0.74	8.33	0.98	10.83	22.52
Flounder	0.31	0.67	7.91	0.93	10.28	32.8
Dragonet	0.19	0.46	5.9	0.72	7.66	40.47
Dab	0.28	0.32	4.75	0.75	6.18	46.64
Plaice	0.13	0.38	4.54	0.71	5.9	52.55
Solenette	0.15	0.25	3.59	0.63	4.67	57.21
Whiting	0.2	0.12	3.03	0.49	3.94	61.15
Turbot	0.09	0.19	2.65	0.49	3.44	64.59
Raitt's_Sandeel	0.16	0.12	2.51	0.4	3.27	67.86
Flatfish	0.09	0.1	1.95	0.38	2.54	70.4
Sand_Goby	0.02	0.17	1.84	0.4	2.4	72.79
Sandeel_Sp	0.02	0.12	1.71	0.31	2.23	75.02
Herring	0.12	0.03	1.63	0.37	2.12	77.14
Ectoparasite	0	0.14	1.52	0.38	1.98	79.12
Gadidae	0.03	0.08	1.29	0.32	1.67	80.79
Goby	0.06	0.07	1.24	0.32	1.62	82.41
Greater_Sandeel	0.07	0.06	1.18	0.31	1.53	83.94
Long_Rough_Dab	0.06	0.07	1.07	0.33	1.39	85.33
Lernaeocera_Branchialis	0.08	0.02	0.99	0.32	1.29	86.62
Five-Bearded_Rockling	0.02	0.08	0.93	0.26	1.21	87.83
Common_Goby	0.01	0.09	0.92	0.26	1.2	89.03
River_Lamprey	0.05	0.03	0.78	0.28	1.01	90.04

A further SIMPER-analysis was used to define the basis of the difference between the two seal species. It is clear that grey seal scats contain more sole and sandeel than the harbour seal remains. On the other hand, the presence of flounder and dragonet and plaice would indicate remains of harbour seals (Table 7).

The length of prey found in the scats differed only slightly between seal species. Only few species overlapped enough to allow for comparison (Figure 14).

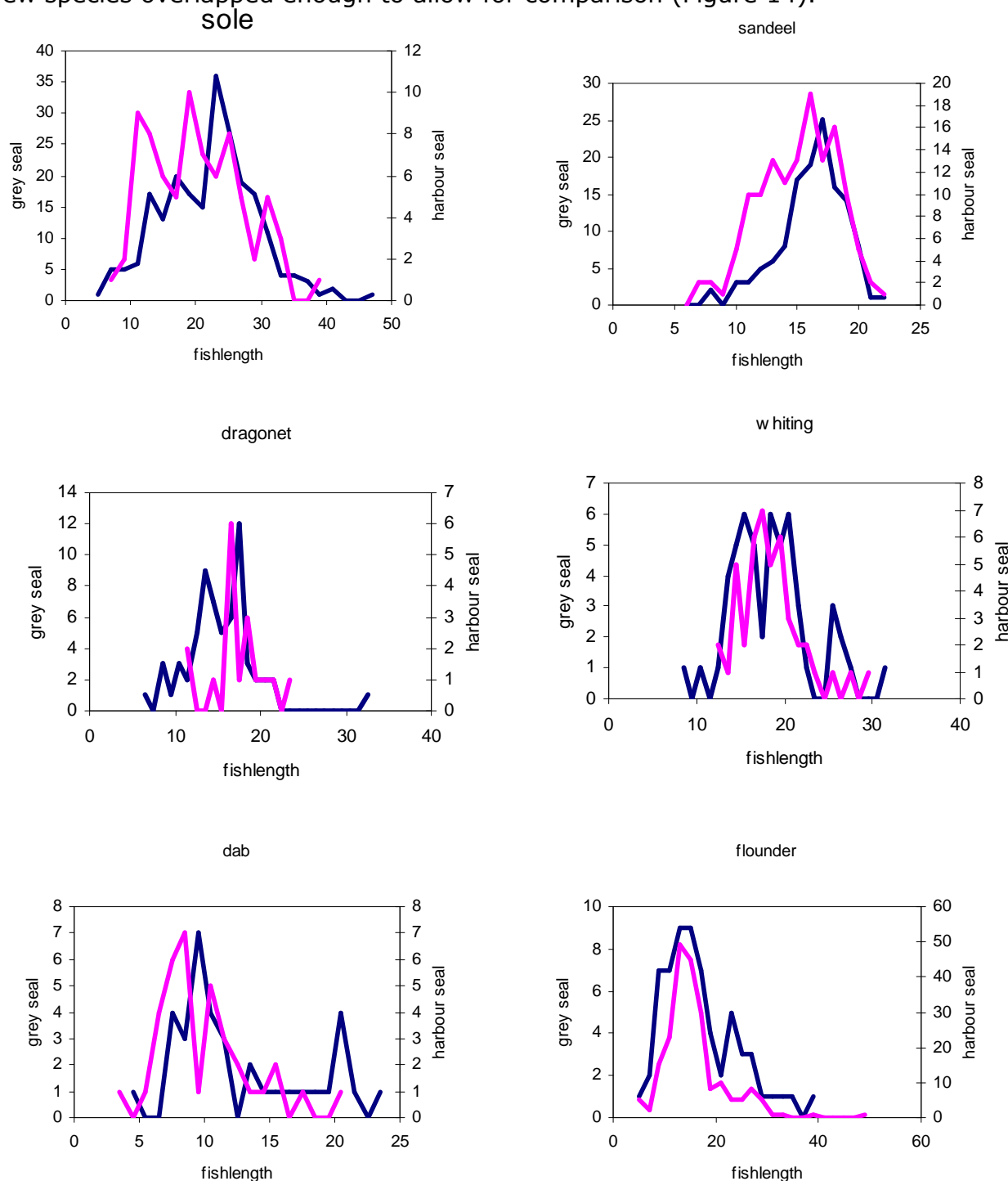


Figure 14. Length-frequency plots of fish species eaten by both harbour and grey seals in the Netherlands. Bleu :grey seal; Pink: harbour seal

4.4 Discussion

The diet study presented here is the first overview for the grey seals' diet on the European continent. It is commonplace to state that the scat analysis may have serious limitations (Pierce et al., 1991; Trites et al., 2005) to the method used as scats do not represent all prey eaten by the seals. For example, it is improbable that much remains come from prey eaten far away from the haul-out sites. So this majorly shows local diet. In addition, some prey contains more robust hard parts than others, enlarging the possibility of finding these. The behaviour of the animals themselves would also cause a bias; grey seals tend to choose areas that are flooded less often than harbour seals. Consequently, chances of finding grey seal scat are higher. There might also be differences in behaviour with regard to the age class the animals are in.

The method does however give insight in the scope of prey species and sizes taken and results are comparable to other scat analysis. As this method does create a bias, additional information was collected for fatty acid analysis. However, as of now, the results of the analysis are not yet satisfactory as large uncertainties remain with regard to the interpretation. The progress of the method is ongoing and results are expected soon.

The development of a method to discern grey seals from harbour seals based on DNA extracted from the scats has ameliorated our insight in the diet of the two species. The large sexual size dimorphism in grey seals can only lead to adult male and female to differ in dietary niche breadth and foraging behaviours. In Canadian waters the male and female grey seals were found to have different strategies of energy accumulation, with females having a more consistent strategy (Austin et al., 2006). Females typically showed smaller foraging ranges and tend to consume a higher percentage of pelagic and semi pelagic prey species of higher energy density (Austin et al., 2006; Beck et al., 2002). Males spent more time feeding and tend to have longer feeding events (Beck et al., 2003a). Despite this, females' foraging effort was found to be greater than males'; however, the levels of effort exhibited by the sexes are most similar just prior to the breeding season (Beck, 1994; Beck et al., 2003a; Beck et al., 2003c). Logically, a second development in the future should include the recognition of the sex of the animal that produced the scat.

Here we see that grey seals along the Dutch coast mainly feed on a variety of benthic prey species, most numerous is sole in spring and flounder in autumn. This is comparable to the diets found on the east coasts of the UK though there, even more sandeel is eaten. In average prey seldom is larger than 20 cm, not much bigger than the harbour seals' diet. In the future competition between the species is possible though the differences in phenology cause the animals to concentrate feeding efforts in other periods.

Understanding the dynamics behaviour and distribution of the prey species will, in time, help explain the habitat necessity of the seals. Most studies of these species is now concentrated around stock assessment for human consumption.

5 Spatial distribution of individual seals

5.1 Introduction

The spatial distribution of grey seals' may be defined by both abiotic and biotic factors. Abiotic factors can include sediment type, depth and distance to haul-out, while biotic factors can include food resources and the level of human activity in the area. For example, (Aarts, 2008) showed that grey seals in UK waters have a preference for coarse sediment type, which may be related to the fact that this is also the preferred burrowing habitat of sand eels (Wright et al., 2000). The influence of both biotic and abiotic factors on grey seals, both on an individual level and on a population level, is extremely difficult to quantify as they are very variable in both space and time. Not only do the seals live in a 3-dimensional environment, where accurately tracking an animal is difficult, but establishing cause and effect relationships, particularly with respect to human activity, is both problematic and challenging.

Grey seals spend the majority of their time (80%) in the water (McConnell et al., 1999). They haul-out on sandbanks, rocky shores or ice (depending on their geographical location), to rest, moult and give birth to a single white-coated pup. Time of parturition is dependent on geographic location, with grey seals in Dutch waters giving birth between November and January (see also Population Study). The moult, in Dutch waters, occurs between March and April. Adult grey seals show strong fidelity to haul-out sites, however, during the foraging season they may alternate between several haul-out sites (Bjorge et al., 2002; McConnell et al., 1992).

Telemetry studies have shown that adult grey seals may repeatedly travel hundreds of kilometres from one haul-out site to another (McConnell et al. 1992; Thompson et al. 1991; Hammond et al. 1992; Thompson et al. 1996a). Grey seal movements in the North Sea have been broadly categorized by McConnell et al., (1999) into two movement types: long and distant travel (up to 2100 km), and local, repeated trips to discrete offshore areas. Data from satellite tags have revealed that individual seals often, but not always return to the same foraging areas, making many repeated movement patterns (Harris, 2007). Despite this, grey seals are considered to be central-place foragers (McConnell et al., 1992; McConnell et al., 1999; Sjoberg, 2000). They concentrate their activities within ca 50 km of one or several haul-out sites from which they perform a series of return trips to sea (Harvey et al., 2008). In a study on grey seals, McConnell et al., (1992) showed that during periods of travel, seals were observed to cover between 75 and 100 km per day. This study also revealed that 88% of trips to sea resulted in seals returning to the same haul-out from which they left; these trips were normally relatively short (mean 2.3 days) and not too distant (mean 39.8 km). Research on other grey seal populations has shown that the mean 95% kernel home range is $2658 \pm 508 \text{ km}^2$ for Baltic seals and $23976 \pm 9133 \text{ km}^2$ for sable island seals (Austin et al., 2004; Sjoberg et al., 2000). Juvenile home ranges tend to

be larger and more variable than those of adults (Harvey et al., 2008; Sjoberg et al., 2000; Harris, 2007)

Research has indicated that grey seals spend a high percentage of time at or near haul-out sites, with relatively short trips to localised off shore areas often with characteristic sediment type. Their distribution at sea tends to be relatively coastal (Matthiopoulos et al., 2004). They undertake mainly benthic dives in shallow waters (< 40 m, Harvey et al., 2008; McConnell et al., 1999). The seals tend to perform five different types of dives (Beck et al., 2003a; Lidgard et al., 2003) with square-shaped dives being the most common (accounting for more than 70% of dives (Beck et al., 2000)). It has been suggested that these dives (grey seals and other species) are foraging dives (Lidgard et al., 2003). However in shallow waters most dives reach the bottom and the distinction between foraging and other dives becomes problematic.

Grey seals are sexually size dimorphic. Body size and energy expenditure differs with sex resulting differences between the sexes in foraging ranges, dietary niche breadth and foraging behaviour (Iverson et al., 1993). Males and females accumulate and expend energy differently. During the breeding season females for example, expend energy at a faster rate than males, and generally tend to have smaller foraging ranges and a narrower niche breadth (Austin et al., 2004; Beck et al., 2003a; Beck et al., 2003b; Beck et al., 2003c; Mellish et al., 1999). However, research by Harvey et al., (2008) indicated that there was no difference in habitat selection between males and females at the home range scale unless season was taken into account (e.g. breeding season). In this study, for practical reasons, we tracked only females and relatively young males.

The distribution of the individually tagged seals in the Netherlands is used in this report to define the seals' preference for specific habitat characteristics defined by both abiotic factors (e.g. depth, sediment type and distance to shore) and human related factors such as shipping. This data and the underlying habitat model is also used to estimate seal density in Dutch waters. In addition, the effect of wind farming on the seals' distribution and, despite limited data, the effect of the construction of the wind farm is investigated.

In this way we can gain insight into why certain areas in the North Sea may be defined as preferred habitat (i.e. feeding, migration, rest).

5.2 Material and Methods

In order to achieve the aims of this study the following aspects of the study have been combined:

1. Define, based on tracking data for each seal, a general habitat preference based on environmental factors (physical and human).
2. Use aerial survey data (counts) of haul-outs, in combination with the results of 1. to predict seal distribution and abundance.

There is no *a priori* expectation of direct effects on the known haul-out sites of the seals, given the location of the two wind farms in the Dutch section of the North Sea. Therefore, research effort was concentrated on understanding the distribution of the

seals at sea. Seals are very cryptic animals; only needing to emerge their nostrils to breath and therefore they are seldom observed at sea. The only way to quantify their use of the aquatic environment is to track individual animals with telemetry devices (referred to as tagging).

With the data of seals tagged throughout the Netherlands the movements of the seals can was used to construct a model that describes their preference for particular environmental conditions. Then the number of seals counted during low tide, in the framework of the monitoring of the population (contracted by the ministry of Agriculture, Nature and Food Quality, LNV; paragraph 3), was used to validate model predictions. Consequently, maps were created to define the probability of seal presence.

Several types of tags were used in this study. Tags differed either in the way data was summarised and presented or in the transmission of the data or in how location data was obtained (Table 8).

Table 8. Overview of tag used in the analysis

	ARGOS (SDRL)	GPS phone tag
Used	2005-2006	2006-2008
Location and frequency	ARGOS (Doppler); average 7 loc/day	GPS; up to 1 loc/20 min
Data transmission	ARGOS	GSM (phone)
Dive data collected	6 hrs summary data individual + dive records	6 hrs summary data individual + dive records
Dive infliction points	Only deeper than 10m	All dives
Data quality	Variable	fixed and better than ARGOS
Data loss	Often	Less, but can still happen
Haul-out definition	sensor dry \leq 10 min.	

Dive data obtained by the tags was used to analyse behaviour. Both tag types collect data on individual dives and summary data. For individual dives dive shape may be recorded by infliction points (points where depth suddenly changes), however, ARGOS tags only record infliction points for dives deeper than 10 m. Because infliction points are only available for a limited amount of dives and the already mentioned fact that dive shape may not be informative as depth is not limiting for the animal we decided to use other parameters with which to describe different behaviour. An analysis was done to identify those periods in which the seals are assumed to spend the most of their time feeding. By mapping these periods to the locations where the data was collected, areas in which feeding probably occurs can be identified.

Finally, the possible effects of the wind farm in operation on the habitat preferences of the seals is explored and an attempt is made to evaluate the consequences of wind farm construction on the seals.

5.2.1 Maps on environmental conditions

A large quantity of spatial data has been collected on environmental conditions, including anthropogenic activities and man-made structures throughout the North Sea. Some spatial data, for example sediment type, has been collected using different

classification schemes for grain size, consequently, data from different countries cannot easily be merged. Differing data formats of different countries thus limit the possibilities of combining national data. Therefore, we concentrated our effort on the Dutch Continental Shelf (DCS, in dutch NCP). The data used in this study are shown in Table 9.

Table 9. Overview of maps used.

	Type of data	extent	Author/owner/
Depth	Depth grid in cm	NCP	TNO & RIKZ
Sediment type	Gridded percentage mud based on point measurements of particle size	NCP	TNO
Shipping activity	Number of ship hours per 5x5 km grid, based on the Automatic Identification System (AIS) carried by all vessels >300Gt	NCP	MARIN (Wageningen),
Location of haul-out site	Coordinates based on the Aerial surveys	The Netherlands, UK, Niedersachsen (Germany)	IMARES, Waterdienst (min. of Public Works), National Park Wattenmeer
At-sea distance to all haul-out sites	1x1 km grid of shortest at-sea (i.e. not crossing land features) distance to each individual haul-out site	North Sea	IMARES

5.2.2 Tagging of individual seals: Grey seals studied in the Netherlands

Seals were caught on haul-out sites with a large seine net, and tagged directly on location. The tags were glued to the fur on the neck using two component quick setting epoxy (Fedak et al., 1982). Captured seals were weighed and measured before release. Tags are lost as the seals moult in late summer. This project was given approval by the Dutch Animal Ethics Committee of the Royal Netherlands Academy of Sciences, and licences were obtained under the Flora en Faunawet & Natuurbeschermingswet.

Table 10. Overview of the grey seals tagged in the framework of this study, in the Dutch Wadden sea.

date	location	>160 cm		>180 cm		Total	Tag type
		F	M	F	M		
Apr-2005	Razende Bol	3		1	2	6	ARGOS tags
Nov-2005	Steenplaat			1	3	6	ARGOS tags
May-2006	Steenplaat			3	3	6	combination
Apr-2007	Steenplaat	2		2	1	5	GSM-tags
Sep-2008	Razende Bol			1	5	6	GSM-tags
total		5	1	10	13	29	

Both the ARGOS and the GPS phone tag used, were constructed by the Sea Mammal Research Unit (SMRU). Differences between the two type of tags is summarised in Table 8.

Data from a depth sensor (0.5 m resolution) and a submergence sensor were used to determine the activity of the seal: "diving" (deeper than 0 m for at least 4 s), "at surface" (no dives for 180 s) or "hauled out" (continuously dry for at least 600 s, stops when submerged after 40 s). Individual dive records include maximum dive depth, duration and previous surface interval durations. Dives were divided into shallow dives (<10m) and deep dives. From the deep dives, the shape was also recorded: four points per dive using dive characterisation algorithm, i.e. depth and time was recorded on four most significant flexing points in the dive.

The location of these tags is determined using the ARGOS satellite-system (<http://www.cls.fr/>). Argos locations are calculated by measuring the Doppler shift on the transmitter signals. This is the change in frequency of a wave when a source of transmission and an observer are in motion relative to each other. When the satellite "approaches" a transmitter, the frequency of the transmitted signal measured by the onboard receiver is higher than the actual transmitted frequency and lower when it moves away. Each time the satellite instrument receives a message from a transmitter, it measures the frequency and time-tags the arrival.

The accuracy at which the location is estimated depends on many factors such as the geometry of the satellite relative to transmitter, the number of uplinks received and the stability of the frequency. To indicate the level of accuracy, Argos supplements each location with a so called Location Quality (LQ).

From 2006 onwards the tags were equipped with GPS (Fastloc) and data was relayed through GSM. The main difference between the two tag types is that detailed dive behaviour information is collected and transmitted via ARGOS satellite or GSM, respectively. The average daily uplink rate of the ARGOS tags is seven (ranging from 2 to 12). In order to prolong battery life, the tags switches to an energy saving mode after 5 hrs when transmissions are continuous due to the seal being hauled out. The GPS tag is set to collect and store a location every 20 min. When in contact with a phone base, it sends the data as a text message. Data can be stored up to 3 months before being sent and received.

Both tags weigh 0.3 kg and can resist pressure up to a depth of 1000m. Data from a depth sensor (0.5 m resolution) and a submergence sensor were used to determine the activity of the seal: "diving" (deeper than 0 m for at least 4 s), "at surface" (no dives for 180 s) or "hauled out" (continuously dry for at least 600 s, stops when submerged after 40 s). Individual dive records include maximum dive depth, duration and previous surface interval durations. Dives were divided into shallow dives (<10m) and deep dives.

5.2.3 Animal tracking filtering procedure for ARGOS data

Some of the tracking devices used in this analysis relies on the ARGOS satellite system. In contrast to GPS locations, the ARGOS locations, as mentioned above, cannot estimate the exact location of the animal, i.e. the Argos estimates are known to have considerable errors. Consequently, in heterogeneous environments, such as coastal regions, some locations at-sea will appear to be on land. Traditionally, those locations are excluded from further analysis. This implies that locations close to the shore, are more likely to fall on land and thus to be removed, compared to those that are far from shore. This can lead to strong biases in estimates of spatial distribution of the species and their habitat preference, towards offshore. This is more dramatic in coastal species such as the harbour seal.

In this project, we developed a method that overcomes this problem by repositioning the ARGOS telemetry observations. The framework not only includes information on land-features, it also incorporates information on the magnitude of ARGOS error associated with each telemetry observation, and speed with which animals travel. Below we outline how this filtering algorithm works.

In the past, studies have been conducted to get estimates of the magnitude of the error for each location class (Vincent et al., 2002). Given these error estimates it is now possible to generate any random location in space relative to the inaccurate Argos location, and calculate how likely it is that the animal was actually at that random location. When this random location falls on land, we know with some certainty that this is not correct. Finally, if the distance to the previous and next Argos location implies a travel speed beyond the animals' physiological capabilities, then we know that this location cannot be the true animal position. By repeatedly generating random locations, it is possible to find the location that is most likely to be the true animals' position. The final product of this algorithm is a new set of animal positions that are always at-sea and within the individuals' travel speed capabilities. All ARGOS tracks presented in this report were subjected to this treatment.

5.2.4 Analysis of trips

Definition of trips -To predict the spatial distribution of the entire population using the counts at the haul-out sites, it is essential to model the spatial distribution of individual tracked seals conditional on leaving from a known haul-out site. Therefore, each telemetry location should be part of a trip with a known start and end point. These points were notably haul-out sites, from which the trip would start and end. The treatment of the data was different depending on the tag used.

For the GPS locations, every location within 200 m of any known haul-out site was treated as haul-out event, therefore the beginning or end of a trip. This was different for the Data obtained by ARGOS. Defining whether a seal actually uses a haul-out is not straightforward, because the locations obtained through the ARGOS satellite system are not exact and there are a large number of haul-out sites in close proximity to one another. If we obtain an ARGOS location near a known haul-out site, the seal may in fact be swimming or lying several kilometres away from that site. The tag defines haul-out events, which consist of the start and end time of the period where the transmitter is dry for at least 10 minutes. If an ARGOS location falls within such a haul-out event, the seal is assumed to be on land and is given a value of 1. For this study it was hypothesised that individual grey seals would only use a limited number of sites to rest, instead of potentially all sites they might approach. High quality Argos locations ($LQ \geq 2$) and the wet-dry sensor were used to determine for each individual seal, which particular haul-out sites were used. From then on all other haul-out sites were disregarded for that particular animal. On the other hand, all haul-out periods, even if only bad quality locations were recorded, were allocated to one of the selected haul-out sites if it was within 5 km of such a individuals specific used haul-out site.

A trip starts at the mid point in time between the last location inside, and the first location outside this haul-out zone (5 km and 200 m, respectively). Similarly, a trip ends at the mid point between the last location outside and the first location inside this haul-out zone. For transitory trips, all locations obtained in the first and second half of the trip belong to the start and end haul-out respectively.

5.2.5 Spatial Modelling: Defining the habitat preference function

The spatial habitat analysis consist of two phases. First the seals preference for environmental variables is investigated, which results in a habitat preference model. Next this model and information on the number of seals on the haul-out sites can be

used to estimate the spatial distribution of the entire population. Details can be found in Aarts et al. 2008.

First we consider the estimation of the preference function. If all habitats are equally available, the seal will use habitats proportional to its preference (w) for those habitats. The preference can be any complex function of the environmental variables. For example, one could assume that log of preference is a linear function of the environmental variables x_1, \dots, x_k

$$w = e^\eta = e^{\beta_0 + \beta_1 x_1 + \dots + \beta_k x_k} \text{ eq. 1.}$$

However, animals often respond in a non-linear way to environmental variables, e.g. they might have a peak preference for a particular type of sediment. This non-linearity can be included in the model by including smooth functions of x

$$\eta = \beta_0 + s(x_1) \dots s(x_k) \text{ eq. 2}$$

Here we use b-spline smoothers consisting of four basic functions, each being a different cubic polynomial of the original explanatory variable x (function `bs()` within the R library 'splines') (de Boor 1978).

The wildlife telemetry locations come from different individuals, and most likely those individuals will differ in their preference for environmental conditions. Treating all telemetry locations as an independent sample of the entire population would therefore be inappropriate. To capture the hierarchical structure in the data (animal location \rightarrow individual \rightarrow (sub-)population) and to capture the non-independence in the observations within an individual, we used mixed-effect models. The idea is that each parameter in eq. 2 is treated as a random normally distributed variable (Pinheiro et al., 2000)

$$b_{j,m} = \beta_{j,0} + \nu_j \quad 3,$$

where m refers to the m^{th} individual and ν_j is the random effect which is assumed to have a joint multivariate normal distribution with a mean of zero and a variance-covariance matrix Ψ , representing within-class variability (Pinheiro et al., 2000).

The inclusion of individual specific random effects and b-spline smoothers means that it is not only possible to detect whether different individuals are affected by particular covariates but, also, whether the functional form of this relationship differs between individuals.

5.2.6 Accounting for unequal habitat availability

When all habitats are equally available, the observed use $f^u(x)$ of the different types of habitat is equal to preference $w(x)$. In nature, this is never the case. As a consequence it is most likely to observe seals at those environmental conditions that are most abundant. In mathematical notation

$$f^u(x) = w(x)f^a(x) \text{ eq. 4.}$$

For example, it is unlikely to observe grey seals from the Dutch Wadden Sea in deep conditions (e.g. > 80m), simply because such depths do not exist in this region. Not only total availability, but also the accessibility (i.e. the proximity to the haul-out site), influences the observed use of the different environmental conditions. To correct for the effect of habitat availability, it is necessary to compare the use of environmental conditions with those that are available to the study animal. The habitat availability is approximated by placing random points uniformly in space and to extract for each point the environmental conditions. One of those environmental conditions is the at-sea distance to the trip haul-out (5.2.1) which may differ for each seal location. Therefore each seal location is matched with a set (20 in our case) of such random points. Below outlines how both the seal locations and the 'control' locations are used to estimate the parameters of the preference function.

5.2.7 Likelihood function and parameter estimation

The previous section specifies the preference function. To estimate the parameters (β) of this function, the model needs to be linked to the data (the seal location and control points reflecting habitat availability), using a so-called likelihood function.

The likelihood of observing one animal observation at a point in space is based on the weighted likelihood function defined by Lele & Keim (2006) is

$$L(Y|\theta, X) = \prod_{i=1}^N \frac{w(X|\theta)}{\int_{\text{all } X} w(X|\theta) f^a(X)} = \prod_{i=1}^N \frac{w(X|\theta)}{M^{-1} \sum w(X^*|\theta)} \quad \text{eq. 5,}$$

where N is the total number of animal observations, $f^a(X)$ is the relative availability of the environmental conditions in the study area, X^* are the values of the environmental variable of a point randomly selected from space, and M is the total number of random points. Similarly, the log-likelihood can be defined as

$$\ell(Y|\theta, X) = \sum_{i=1}^N (\log(w(X|\theta)) - N \log(M^{-1} \sum w(X^*|\theta))) \quad 6.$$

Minimizing the negative of the log-likelihood function, leads to the maximum likelihood estimates of the parameters. Parameter estimation is done using Random Effects module of the Automatic Differentiation Model Builder (ADMB-RE, Skaug 2002, Skaug & Fournier 2003, Otter 2004)

5.2.8 Spatial prediction of usage and preference

The estimated function $w(x)$, quantifies the strength of the seals preference for the different environmental conditions. Although we may not observe seals in all areas throughout their North Sea range, there are maps of the environmental variables for the entire NCP. Using those maps and the preference function it is possible to estimate the spatial usage for this entire region. In addition, haul-out counts are available. For each haul-out site the expected distribution of one individual can be

predicted and multiplied by the total seal count at that site. This can be repeated for all sites to estimate the total at-sea distribution

Because seals are central-place foragers, the at-sea distribution is largely influenced by the distance to the haul-out site, which is included as a variable in the model. Using the preference function, and excluding this variable (i.e. assuming that it is 1 for all points in space) when making spatial predictions, results in the expected distribution of seals when they would move independent from their haul-out site.

5.3 Results

5.3.1 Tracking results; filtered data

Data from a total of 27 seals were used in the analysis. This resulted in a total of 3704 days of recording (Table 11).

Table 11. Overview of tracking results of grey seals 2005-2008 in relation to known pile driving activity for offshore wind farms.

Seal	Tag type	Start	Stop	Days recording	Days before OWEZ	Days during pile driving	Days operational	no. of data points
hg10_1_05	ARGOS	18 Apr 2005	04 Aug 2005	108	108			310
hg10_2_05	ARGOS	17 Apr 2005	29 Nov 2005	225	225			440
hg10_3_05	ARGOS	17 Apr 2005	07 Aug 2005	112	112			374
hg10_4_05	ARGOS	18 Apr 2005	06 Dec 2005	232	232			676
hg10_5_05	ARGOS	17 Apr 2005	10 Feb 2006	299	299			1347
hg12-1-05	ARGOS	13 Nov 2005	06 Mar 2006	113	113			463
hg12-2-05	ARGOS	12 Nov 2005	17 May 2006	185	139	46		476
hg12-3-05	ARGOS	14 Nov 2005	18 Jan 2006	64	64			109
hg12-4-05	ARGOS	12 Nov 2005	10 Feb 2006	90	90			426
hg12-5-05	ARGOS	13 Nov 2005	20 Mar 2006	127	127			357
hg12-6-05	ARGOS	28 Nov 2005	25 Apr 2006	148	123	24		550
hg14-1-06	ARGOS	06 May 2006	11 Sep 2006	128		128		871
hg14-2-06	ARGOS	06 May 2006	04 Nov 2006	182		182		1049
hg14g-1-06	GSM	03 May 2006	20 May 2006	18		18		113
hg14g-2-06	GSM	03 May 2006	29 Jul 2006	87		87		24
hg14g-3-06	GSM	03 May 2006	22 May 2006	20		20		412
hg16g-1-07	GSM	09 Apr 2007	16 Dec 2007	250		21	229	6340
hg16g-2-07	GSM	10 Apr 2007	04 Nov 2007	209		21	188	7718
hg16g-3-07	GSM	10 Apr 2007	19 Aug 2007	131		21	110	5043
hg16g-4-07	GSM	09 Apr 2007	31 Oct 2007	205		21	184	6163
hg16g-5-07	GSM	10 Apr 2007	15 Nov 2007	219		21	199	5552
hg21g-1-07	GSM	06 Sep 2008	29 Dec 2008	114			114	1354
hg21g-2-07	GSM	06 Sep 2008	11 Dec 2008	96			96	769
hg21g-3-07	GSM	06 Sep 2008	16 Dec 2008	101			101	939
hg21g-4-07	GSM	06 Sep 2008	19 Sep 2008	13			13	143
hg21g-5-07	GSM	06 Sep 2008	29 Dec 2008	114			114	1662
hg21g-6-07	GSM	06 Sep 2008	29 Dec 2008	114			114	1737
sum	27			3704	1633	609	1462	45417

From the results presented in 3 figures (Figure 15, Figure 16 and Figure 17), it may be clear that the seals may move over large distances. The first results represent the movements of grey seals before the pile driving activity at OWEZ (April 2006). The movements of seals during OWEZ and Princes Amalia park (April 2006-May 2007) and the third picture shows the movement after the construction was

terminated (May 2007). It should be noted that the quality of the data was ameliorated during this study: since 2007, the seals were equipped with GPS tags, yielding locations with much higher accuracy, furthermore the GSM in these tags allowed for higher data exchange.

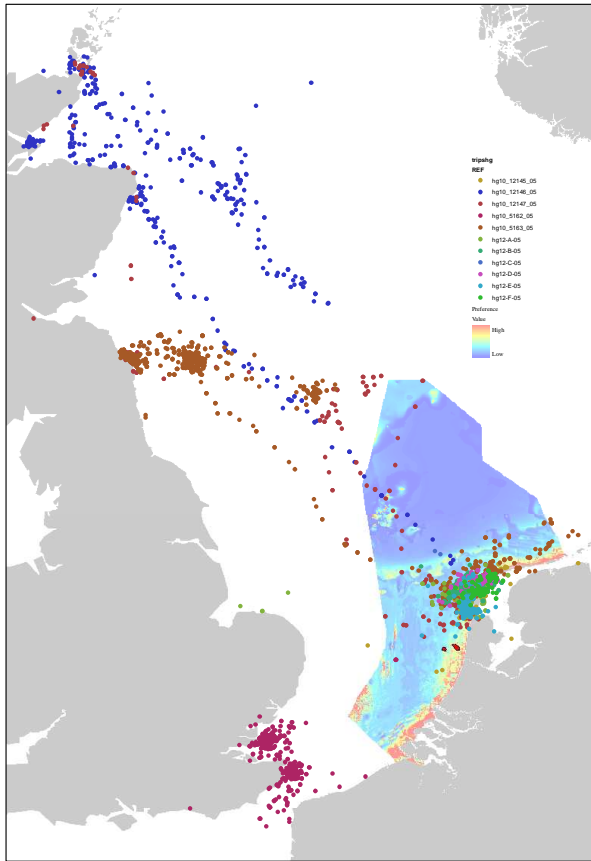


Figure 15. Tracking results of grey seals 2005- April 2006, commencement of pile driving for Dutch off shore wind farms

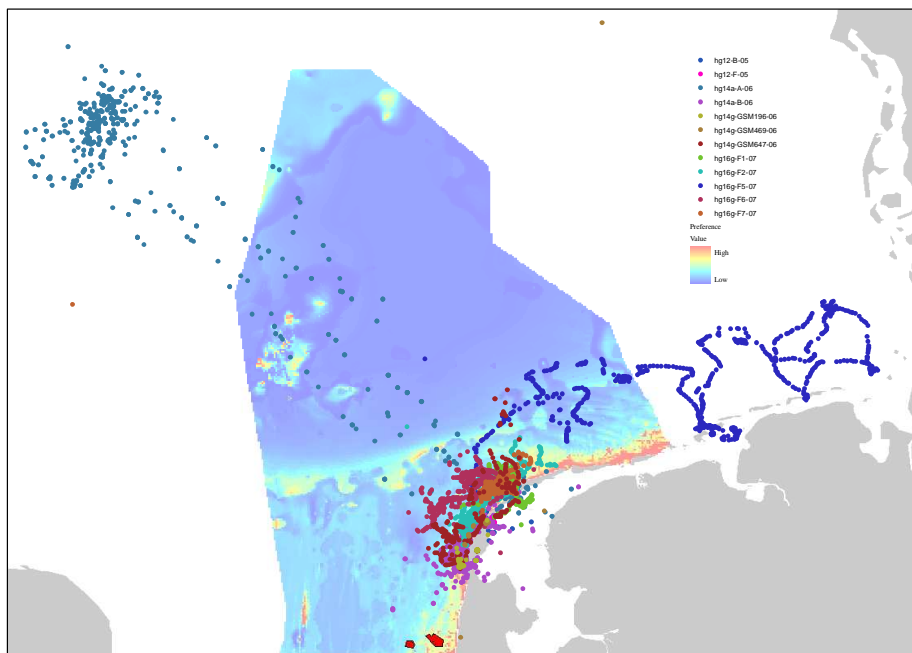


Figure 16 Tracking results of grey seals April 2006- May 2007, during pile driving for Dutch off shore wind farms

Though data lacks for statistical testing we should remark that during pile driving the seals clearly limit their movements to locations outside the direct vicinity of the wind farms. Three seals that were tracked after May 2007 demonstrated that it was possible for them to cross over to the Delta area; despite the presence of the wind farm.

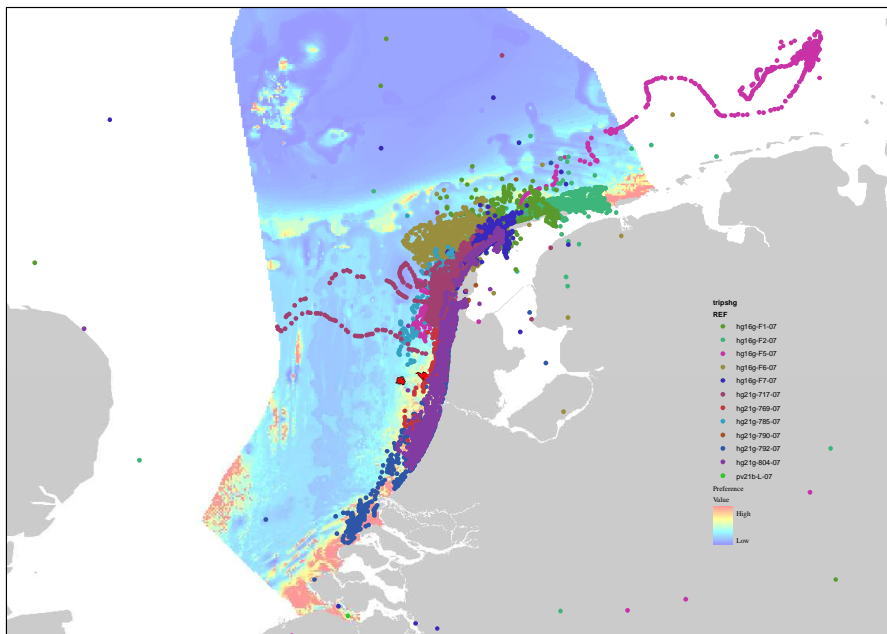


Figure 17 Tracking results of grey seals May 2007-2008, after pile driving for Dutch off shore wind farms had ceased.

5.3.2 Dive data

5.3.3 Behavioural data

During the same time span as the spatial data, the tags used also give insight into the diving behaviour of individual animals. Potentially, the location of feeding, resting and migration routes along which the seals commute between these areas can thus be identified. Here the methods to discern these different behavioural stages are investigated. First, frequency plots were made of the seals dives. For some individuals, these are displayed (Figure 18, Figure 19 and Figure 20)

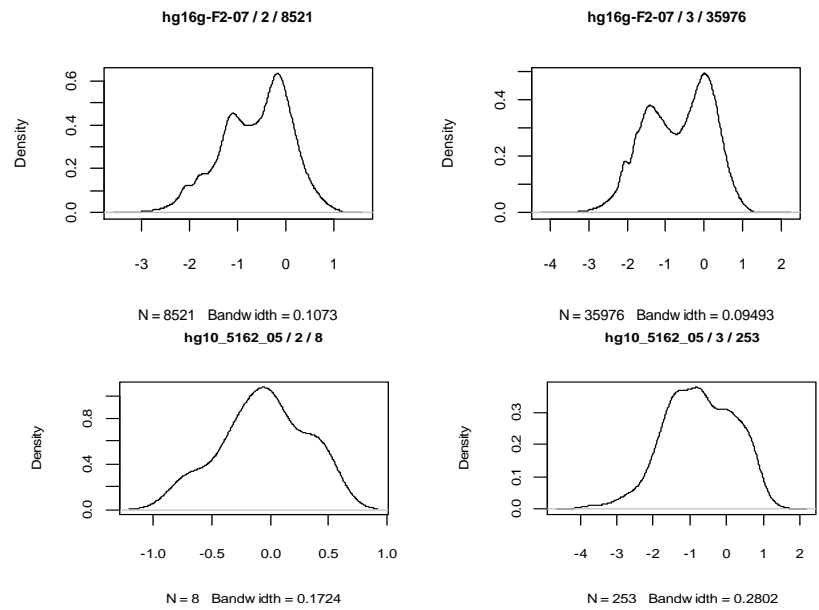


Figure 18 Relative frequency of dive duration in two adult females (hg16_f2-07 and hg10_5161_05). During spring (left) and summer (right).

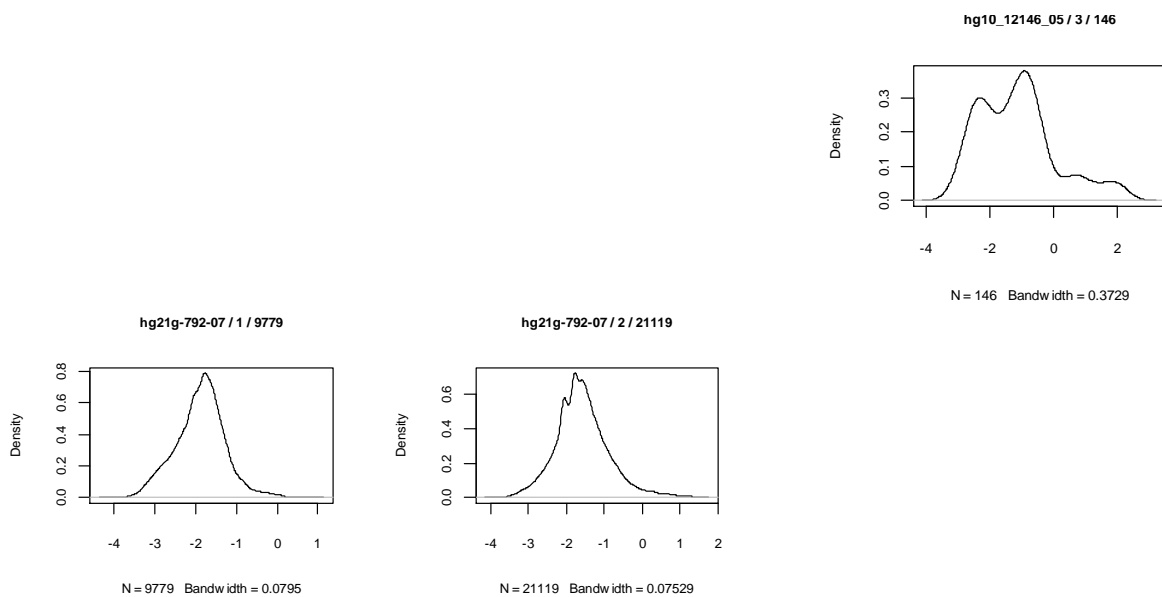


Figure 19 Relative frequency of dive duration in two adult males (hg10_12146_05 and hg21g-792-07). During autumn (left), spring(middle) and summer (right).

Apparently seals show a very individual diving pattern in relation to their individual feeding strategies, this complicates the analysis needed to interpret the behaviour at sea.

Due to the fact that the Dutch coast, where the seals are active, is generally shallow, depth is not considered to be limiting or a good denominator for behavioural categories. In other studies (Baechler et al., 2002), U-shaped dives are often judged typical of foraging dives. Here the seal would make a relatively quick descent, spend some time at the bottom, then ascend relatively quickly. In so-called V-shaped dives all dive time is spent descending and ascending. Because of the shallow depth, almost

all dives in the Dutch waters are U-shaped and thus these dives cannot be used to define behavioural categories.

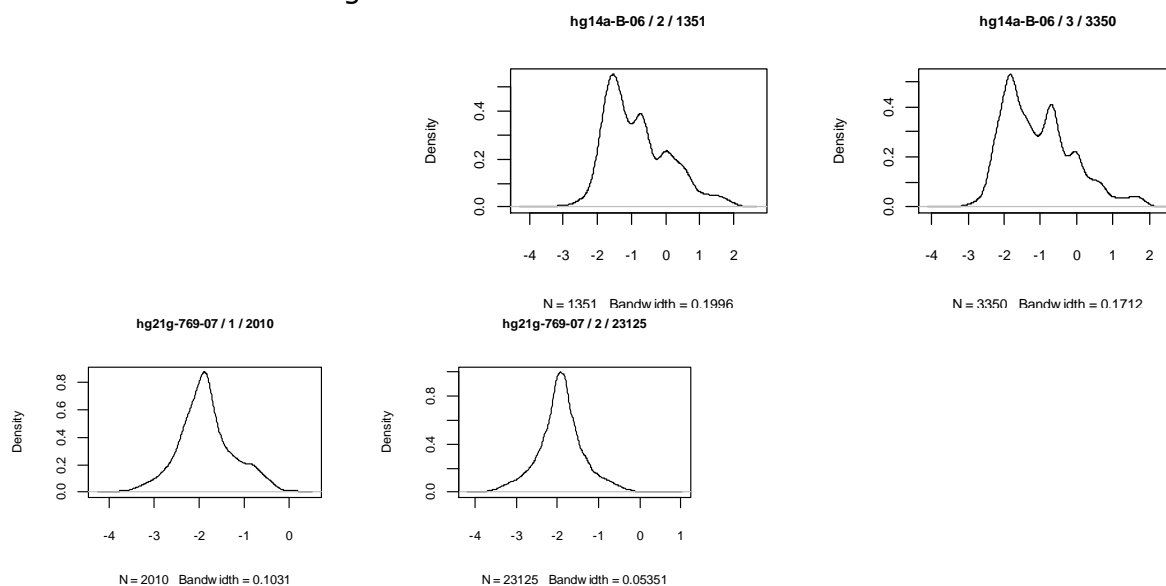


Figure 20 Relative frequency of dive duration in two subadults above: female (hg14_B_06) and below: male (hg21g-769-07). During autumn (left), spring(middle) and summer (right).

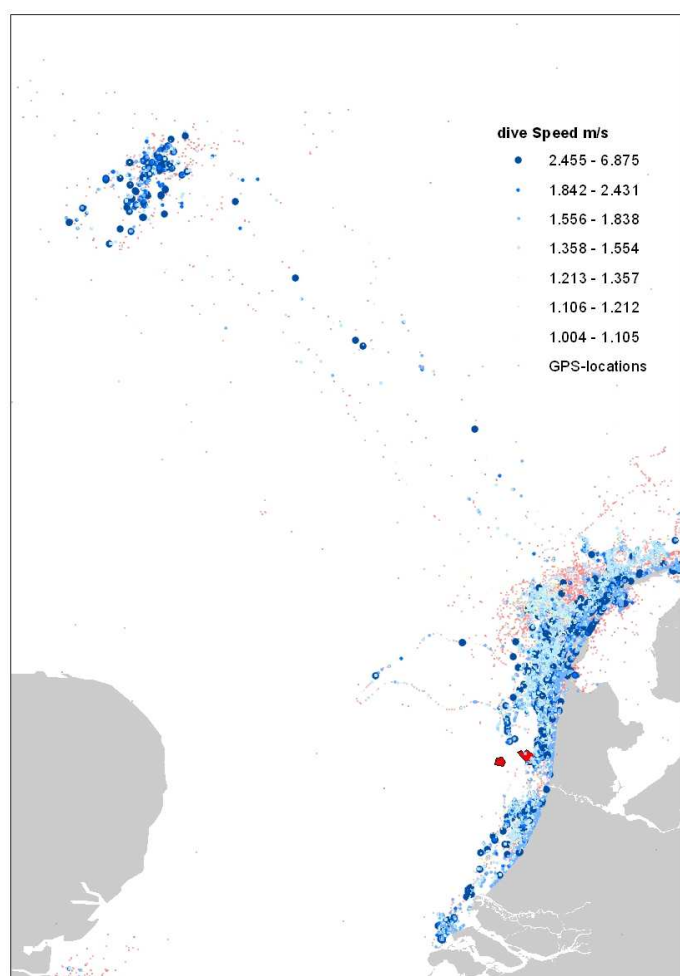


Figure 21. Detail of mapped tracks of grey seals. Diving activity defined as decent speed, shown in a size and colour gradient.

From the data obtained from individual dives, the decent speed was calculated which could potentially distinguish between dives at which the animal intentionally aims at reaching the bottom to feed (swimming at high speed) and dives where the animal sinks down slowly, or loses altitude when travelling underwater. As an example Figure 21 shows a map including the differentiated dive speeds. However promising, this method is still under construction and we have chosen not to elaborate on it here.

5.3.4 Habitat modelling

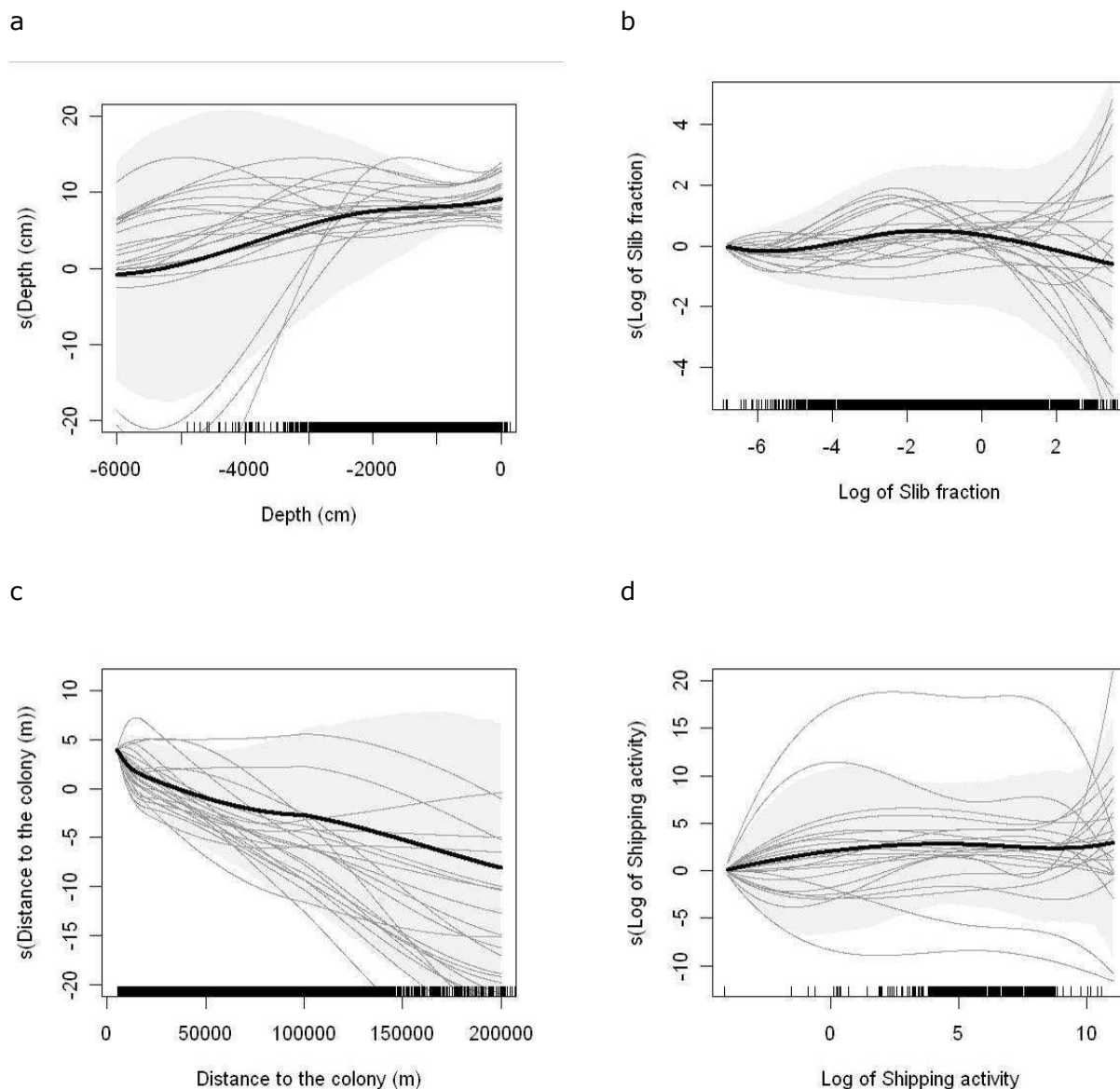


Figure 22. The relative preference for (a) depth, (b) log of %mud in the sediment, (c) distance to the haul-out, (d) shipping activity. The y-axis, represents preference. High and low values, represent preference and avoidance, respectively (see also eq. 1). The black line describes the mean population response; the grey lines the individual estimates for each seal and the grey area represent the 95% confidence limits for the entire population. On the x-axis a measure for the intensity of the available data is given.

The analysis of the habitat preference for depth, %mud in the sediment, distance to haul-out site, and shipping activity is shown in Figure 22. It is not possible to plot the

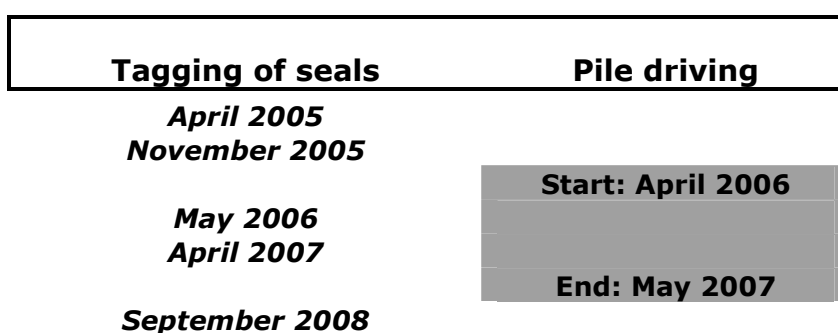
effect of a single explanatory variable relative to the spatial distribution of the seals. This is because the absolute effect of one variable directly depends, non-linearly, on the other explanatory variables (see eq. 1). Therefore, preference is represented as relative preference.

The graphs show that the grey seals in this study have a slight preference for shallow waters (a), whilst a very high and low mud fraction is avoided. As could be expected, the seals tend to prefer areas close to haul-out sites (c). Preference for shipping activity is not evident from (d), which is mostly due to large individual variation in preference

The functions obtained in the analysis for the seals' preference for the different environmental conditions described in Figure 22 are now used to predict the distribution of the Dutch seal population at sea (Figure 23). This is done by predicting distribution for every seal counted during an aerial survey of the haul-out areas, and then summing the results. Consequently, the seal relative density is estimated. At this stage it is not possible to estimate absolute density of seals at sea. The reason is that there are currently no exact estimates of the percentages of the total population of seals present during the aerial counts at the haul-out sites. As predicted, the at-sea distribution is largely influenced by the distance to haul-out site, which is included as a variable in the model.

Figure 18 shows the distribution of preference as predicted by the habitat preference model, after excluding the effect of distance to the haul-out. This map is mostly driven by the variables % mud and the depth. Some of the areas far offshore, such as the Doggersbank, are preferred areas, but the large distance to the colony results in a relative low density of seals.

continuous sound source of pile driving activity between April 2006 and May 2007. After filtering the ARGOS data, the OWEZ tag data was separated in three periods for visual inspection: I) September 2005 - April 2006 (absence of the wind farm); II) April 2006 - May 2007 (building period of subsequently OWEZ and Prinses Amalia Windpark); III) May 2007 - June 2008 (both wind farms operational).



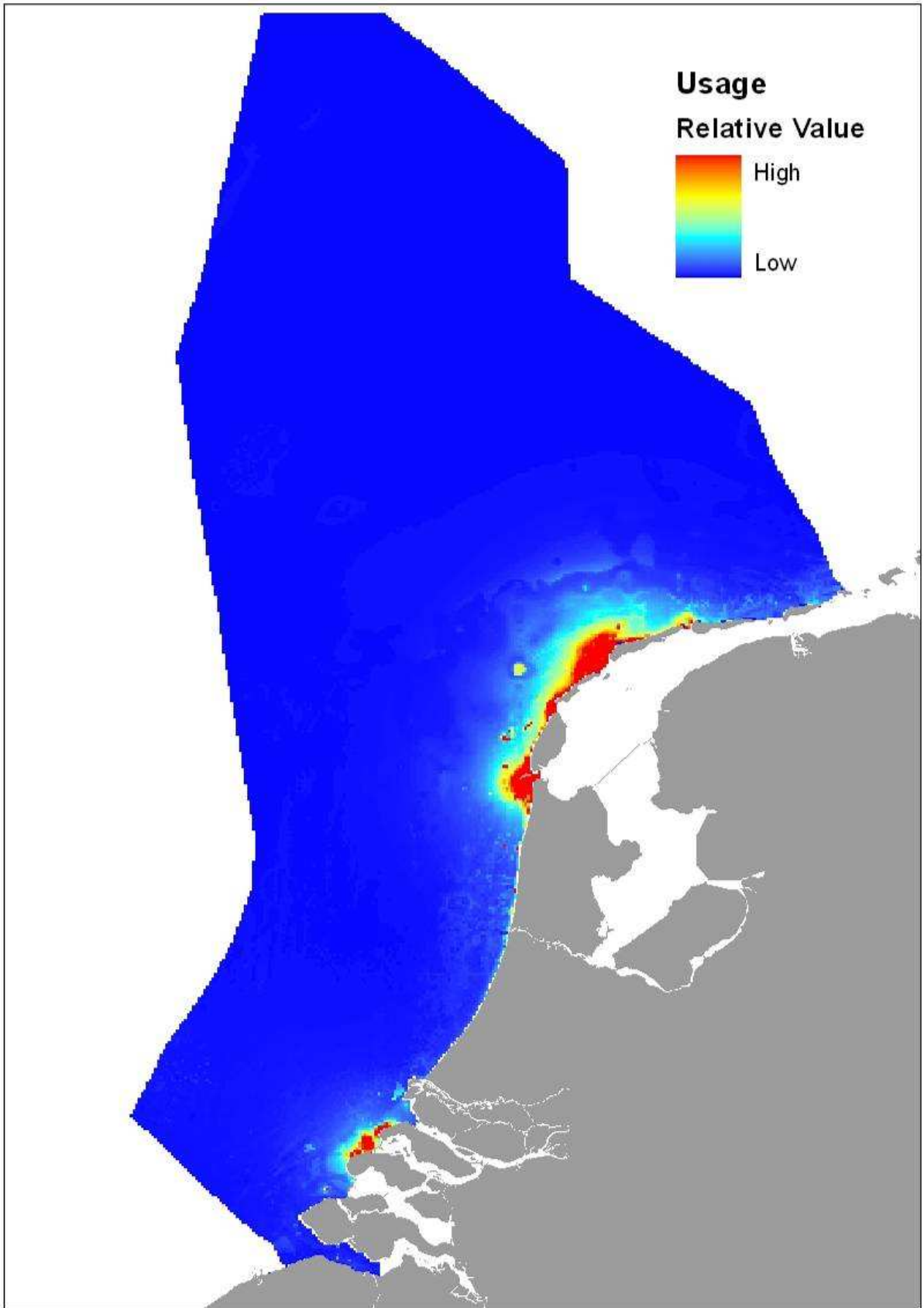


Figure 23. Modelled seal density using the preferences for the various environmental characteristics described in Figure 22 combined with the numbers of seals counted during the aerial surveys.

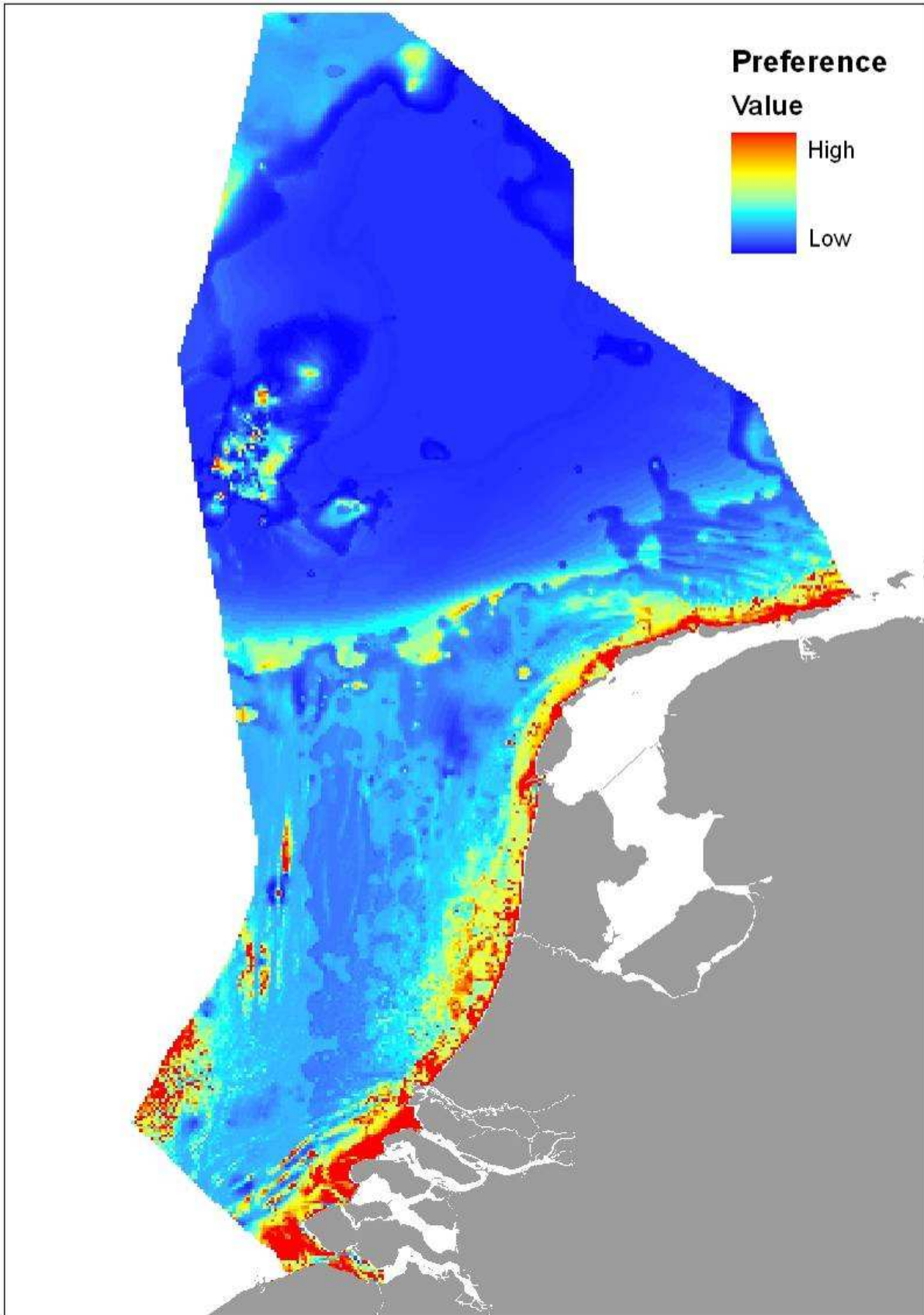


Figure 24 Modelled seal preference using the preferences for the various environmental characteristics described in Figure 22 , but after removal of the effect of distance to the haul-out site. This maps provides a rough indication of where seals would be if they would not be constrained to return to their haul-out

When designing the study it was not known that Prinses Amalia Windpark would be build during the study period. The two wind farms ensured that there was virtually a

When attempting to calculate the effect of pile driving activity at the wind farm the numbers of seals tagged at that moment was relatively low. Furthermore, many seals were too far from that area to perceive any activity. Though circumstantial, the seals seem to move more towards the wind farm area after the pile driving stopped this is shown by the tracks of the seals that were tagged during the pile driving activity (Figure 25).

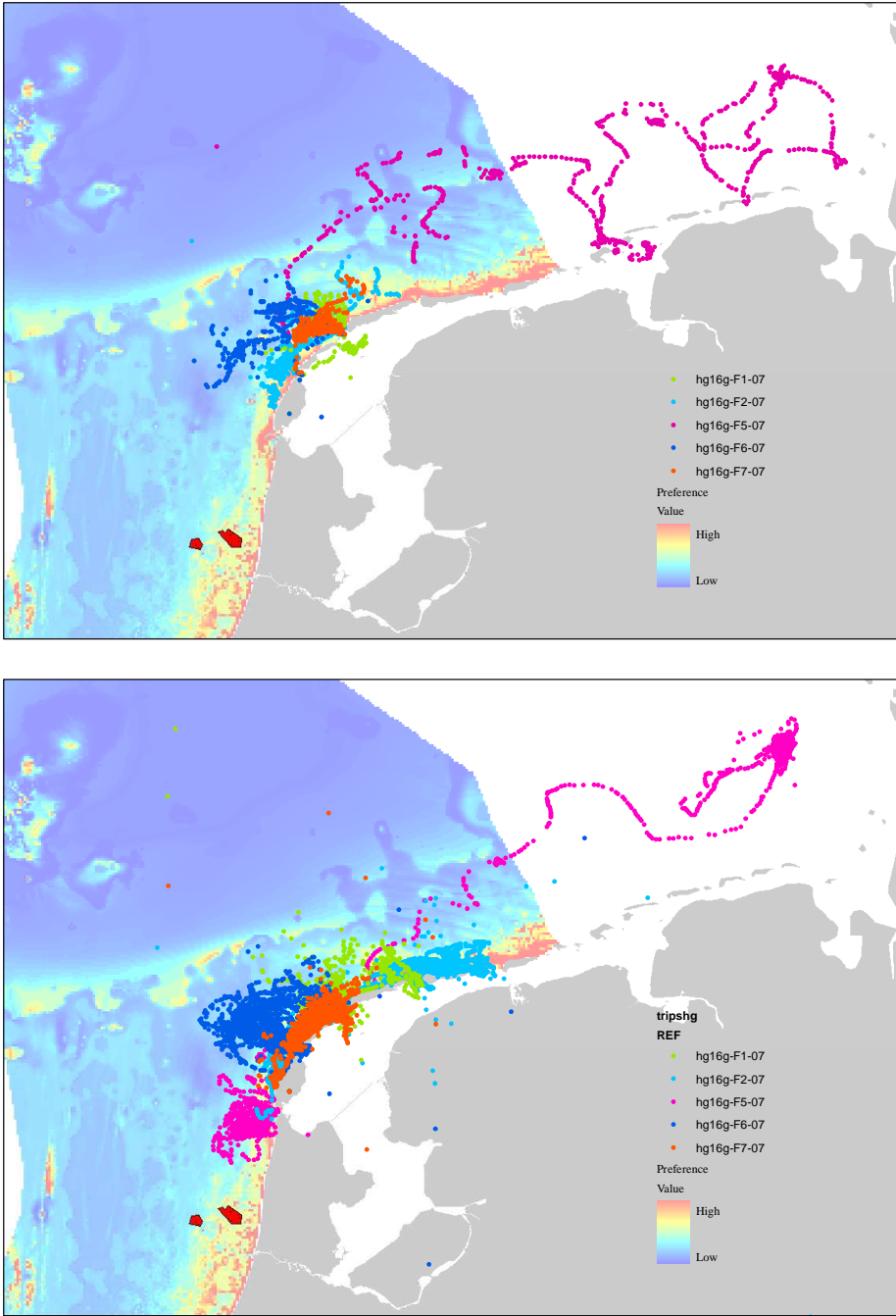


Figure 25 Tracks of the seals that were tagged during the pile driving activity. ABOVE: data collected before may 2007, BELOW: data collected after may 2007, when pile driving had ceased.

5.4 Discussion

The objective of this study is to increase our understanding of the biology of grey seals in the Dutch North Sea, to estimate effects of off shore wind farming: To understand where grey seals forage, what they feed on and which haul-out sites they use.

Defining spatial distribution and habitat preference of the population of grey seals is difficult due to the enormously large individual variability and our inability to correctly quantify the distribution of their food resources. Nevertheless, the data collected in this study has greatly improved our understanding of how grey seals use their marine environment. The data from the telemetry devices has indicated that most spatial usage occurs in proximity to their haul-out sites, but often they also forage further offshore such as the Frisian front and the Doggersbank. In addition, the data shows occasional large-distance migration between the Wadden Sea and the province of Zeeland, but also between the Netherlands and the UK. The relative small proportion of seals being tagged, suggests that such large-scale movement is common among other members of the population.

This paragraph of the report was concentrated on trying to understand which environmental conditions grey seals prefer. The habitat model shows that grey seals avoid areas with high mud content and deeper areas. These results are very similar to studies in the UK (Aarts et al. 2008) and harbour seals in the Netherlands (Brasseur et al. 2009). The resulting model and information on the observed numbers of seals at the haul-out sites is used to estimate the relative density at sea. Due to the fact that not all seals are present at the haul-out site during the aerial counts, it is at this stage not possible to estimate the absolute population density at sea. Furthermore, the relative density estimate does illustrate where seals occur, but not how many individuals are dependent upon some area in space.

The telemetry data collected and analysis has improved understanding on the spatial distribution and habitat preference, but due to the seals' complex behaviour, there are still many uncertainties.

This study has shown that the Dutch North Sea zone plays an important role for grey seals. It serves as a foraging area, but it is also a migration route. The data from tagged seals show that they travel along the coast to exchange between the Wadden Sea and the Delta area and the different areas within the Wadden Sea. Grey seals even migrate to the UK coast. The two existing wind farms, Offshore Windpark Egmond aan Zee (OWEZ) and Prinses Amalia Windpark, located 8-23 km offshore with a area of approximately 54 km² in total, may therefore potentially act as a barrier. The locations of the wind farms that may be constructed in the near future (Figure 1) are located further offshore. Since most seals depart from the haul-out sites in proximity to the Wadden Sea and Zeeland, the expected use of some areas by seals will be lower than the coastal zone. However, some areas such as areas 6, 5 and 14 (Figure 1) are closer to the Wadden Sea and the construction (e.g. pile driving) and operational use of these wind farms may hamper foraging and exchange with the world's largest grey seal population in the UK. In particular, the accumulated effect of wind farms in the already intensively used area is still unclear.

6 Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 57846-2009-AQ-NLD-RvA). This certificate is valid until 15 December 2012. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2013 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

References

- Aarts G., M. MacKenzie, B. McConnell, M. Fedak, J. Matthiopoulos (2008) Estimating space-use and habitat preference from wildlife telemetry data. *Ecography* 31: 140-160
- Anderson SH (1990) *Seals*. Whittet Guilford, U.K.
- Anderson SS (1992) *Halichoerus grypus* (Fabricius, 1791) - Kegelrobbe. In: Niethammer J, Krapp F (eds) *Handbuch der Säugetiere Europas*. Akademische Verlagsgesellschaft, Wiesbaden, p 97-115
- Austin D, Bowen WD, McMillan JI (2004) Intraspecific variation in movement patterns: modeling individual behaviour in a large marine predator. *Oikos* 105: 15-30
- Austin D, Bowen WD, McMillan JI, Boness DJ (2006) Stomach temperature telemetry reveals temporal patterns of foraging success in a free-ranging marine mammal. *Journal of Animal Ecology* 75: 408-420
- Baechler J, Beck CA, Bowen WE (2002) Dive shapes reveal temporal changes in the foraging behaviour of different age and sex classes of harbour seals (*Phoca vitulina*). *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 80: 1569-1577
- Beck (1994) Managing Antarctic Tourism: a Front Burner Issue. *Annals of Tourism Research* 21: 375-386
- Beck CA, Bowen WD, Iverson SJ (2000) Seasonal changes in buoyancy and diving behaviour of adult grey seals. *J Exp Biol* 203: 2323-2330
- Beck CA, Bowen WD, Iverson SJ (2003a) Sex differences in the seasonal patterns of energy storage and expenditure in a phocid seal. *Journal of Animal Ecology* 72: 280-291
- Beck CA, Bowen WD, McMillan JI, Iverson SJ (2003b) Sex differences in diving at multiple temporal scales in a size-dimorphic capital breeder. *Journal of Animal Ecology* 72: 979-993
- Beck CA, Bowen WD, McMillan JI, Iverson SJ (2003c) Sex differences in the diving behaviour of a size-dimorphic capital breeder: the grey seal. *Animal Behaviour* 66: 777-789
- Beck CA, McMillan JI, Bowen WD (2002) An algorithm to improve geolocation positions using sea surface temperature and diving depth. *Marine Mammal Science* 18: 940-951
- Benoit D, Bowen WD (1990) Seasonal and geographic variation in the diet of grey seals (*Halichoerus grypus*) in eastern Canada. *Can Bull Fish Aquat Sci* 222: 215-226
- Bjorge A, Oien N, Hartvedt S, Bothun G, Bekkby T (2002) Dispersal and bycatch mortality in gray, *Halichoerus grypus*, and harbor, *Phoca vitulina*, seals tagged at the Norwegian coast. *Marine Mammal Science* 18: 963-976
- Bonner WN (1989) *The natural history of seals*. London
- Bowen WD, Harrison GD (1994) Offshore Diet of Grey Seals *Halichoerus-Grypus* near Sable Island, Canada. *Marine Ecology-Progress Series* 112: 1-11
- Bowen WD, Lawson JW, Beck B (1993) Seasonal and Geographic-Variation in the Species Composition and Size of Prey Consumed by Gray Seals (*Halichoerus-Grypus*) on the Scotian Shelf. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 1768-1778
- Bowen WD, McMillan J, Mohn R (2003) Sustained exponential population growth of grey seals at Sable Island, nova Scotia. *Ices Journal of Marine Science* 60: 1265-1274
- Brasseur SMJM, Reijnders PJH (2001) *Zeehonden in de Oosterschelde, fase 2 : effecten van extra doorvaart door de Oliegeul*. Alterra, Research Instituut voor de Groene Ruimte, Wageningen

- Brasseur SMJM, Scheidat M, Aarts GM, Cremer JSM, Bos OG (2008) Distribution of marine mammals in the North Sea for the generic appropriate assessment of future offshore wind farms. In: IMARES,
- Bree van P, Vedder L, Hart tL (1992) De grijze zeehond in Nederland - terug van weggeweest .(The return of the grey seal in the Netherlands). Zoogdier 3(4): 3: 11-15
- Caudron AK (1997) The structure and behavior of the grey seal *Halichoerus grypus* breeding group of the Dutch Wadden Sea. *Ambio* 26: 404-404
- Clarke KR, Gorley RN (2006) Primer v6: user manual/tutorial. Primer-e Ltd, Plymouth
- Clarke KR, Green RH (1988) Statistical desing and analysis for a 'biological effects' study. *Mar Ecol Prog Ser* 46: 213-226
- Clason AT (1988) De grijze Zeehond *Halichoerus grypus* (Fabricius 1971). In: Bierma M, Clason AT, Kramer E, de Langen GJ (eds) *Terpen en wierden in het Fries-Groningse kustgebied* (Dwelling mounts in the Friesian-Groningen coastal area.). Wolters-Noordhoff/Forsten, Groningen, p 234-240
- Constantine R, Brunton DH, Dennis T (2004) Dolphin-watching Tour Boats Change Bottlenose Dolphin (*Tursiops truncatus*) Behaviour. *Biological Conservation* 117: 299-307
- De Jong GDC, Brasseur S, Reijnders P (1979) Grey seal *Halichoerus grypus*. In: Reijnders P, Veriopoloulos G, Brasseur S (eds) Status of Pinnipeds Relevant to European Union IBN Scientific Contributions 8. Wageningen, The Netherlands
- de Jong GDC, Brasseur SMJM, Reijnders PJH (1997) Grey Seal *Halichoerus grypus*. In: Reijnders PJH, Verriopulos G, Brasseur SMJM (eds) Status of Pinnipeds relevant to the European Union. DLO Institute for Forestry and Nature Research, Wageningen, p 58-75
- Duck CD, Mackey, B.L. (2008) Grey seal pup production in Britain in 2007. . In: NERC Sea Mammal Research Unit, Gatty Marine Laboratory, University of St Andrews, KY 16 8LB: 31-42.,
- Edrén SMC, Teilmann J, Dietz R, Carstensen J (2004) Effect from the construction of Nysted Offshore Wind Farm on seals in Rødsand seal sanctuary based on remote video monitoring. In: Ministry of the Environment, Denmark, p 21
- Fedak MA, Anderson SS (1982) The Energetics of Lactation - Accurate Measurements from a Large Wild Mammal, the Grey Seal (*Halichoerus-Grypus*). *Journal of Zoology* 198: 473-479
- Fedak MA, Hiby AR (1985) Population energy requirements of seals. In: Hammond PS, Harwood J (eds) The impact of grey and common seals on North Sea resources. Sea Mammal Research Unit Cambridge, p 50-58
- Greenstreet SPR, Hall SJ (1996) Fishing and the ground-fish assemblage structure in the north-western North Sea: An analysis of long-term and spatial trends. *Journal of Animal Ecology* 65: 577-598
- Grellier K, Hammond PS (2006) Robust digestion and passage rate estimates for hard parts of grey seal (*Halichoerus grypus*) prey. *Canadian Journal of Fisheries and Aquatic Sciences* 63: 1982-1998
- Griffen AEv (1913) Die Fauna der Wurten (The fauna of the 'Wurten'). E.J.Brill, Leiden
- Haaften JLv (1974). Zeehonden langs de Nederlands kust (Seals along the Dutch coast). *Wet Med KNNV, Hoogwoud* 101: 1-36
- Hammond PS, Hall AJ, Prime JH (1994a) The Diet of Gray Seals around Orkney and Other Island and Mainland Sites in North-Eastern Scotland. *Journal of Applied Ecology* 31: 340-350
- Hammond PS, Hall AJ, Prime JH (1994b) The Diet of Grey Seals in the Inner and Outer Hebrides. *Journal of Applied Ecology* 31: 737-746

- Hammond PS, Prime JH (1990) The diet of British grey seals (*Halichoerus grypus*) in eastern Canada. *Can Bull Fish Aquat Sci* 222: 234-254
- Harkonen T, Harding KC, Heide-Jorgensen MP (2002) Rates of increase in age-structured populations: a lesson from the European harbour seals. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 80: 1498-1510
- Härkönen TB, S.M.J.M.; Teilmann, J.; Vincent, C.; Dietz, R.; Abt, K.; Reijnders, P.J.H. (2007) Status of grey seals along mainland Europe from the Southwestern Baltic to France. In: *Grey Seals in the North Atlantic and the Baltic*. - Tromsø, Norway : NAMMCO, Polar Environmental Centre, (NAMMCO Scientific Publication). p p. 57 - 68.
- Harris RN (2007) Assessing grey seal (*Halichoerus grypus*) diet in western Scotland. In: *School of Biology. University of St Andrews, St Andrews*, p 101
- Harvey V, Cote ´ SD, Hammill MO (2008) The ecology of 3-D space use in a sexually dimorphic mammal *Ecography* 31: 371-380
- Haug T, Hammill M, Ólafsdóttir D (2007) Introduction. *NAMMCA Scientific Publications* 6: 7-12
- Hayward JL, Henson SM, Logan CJ, Parris CR, Meyer MW, Dennis B (2005) Predicting numbers of hauled-out harbour seals: a mathematical model. *Journal of Applied Ecology* 42: 108-117
- Iverson SJ, Bowen WD, Boness DJ, Oftedal OT (1993) The Effect of Maternal Size and Milk Energy Output on Pup Growth in Gray Seals (*Halichoerus-Grypus*). *Physiological Zoology* 66: 61-88
- Jepson PD, Arbelo M, Deaville R, Patterson IAP, Castro P, Baker JR, Degollada E, Ross HM, Herraes P, Pocknell AM, Rodriguez F, Howie FE, Espinosa A, Reid RJ, Jaber JR, Martin V, Cunningham AA, Fernandez A (2003) Gas-bubble lesions in stranded cetaceans. *Nature* 425: 575-576
- Joensen AH, Søndergaard NO, Hansen EB (1976) Occurrence of seals and seal hunting in Denmark. . *Dan Rev Game Biol* 10: 1-20
- Kastak D, Schusterman RJ (1998) Low-frequency amphibious hearing in pinnipeds: Methods, measurements, noise, and ecology. *Journal of the Acoustical Society of America* 103: 2216-2228
- Koschinski S, Culik BM, Henriksen OD, Tregenza N, Ellis G, Jansen C, Kathe G (2003) Behavioural reactions of free-ranging porpoises and seals to the noise of a simulated 2 MW windpower generator. *Marine Ecology-Progress Series* 265: 263-273
- Kruskal J (1964) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika* 29: 1-27
- Lidgard DC, Boness DJ, Bowen WD, McMillan JI (2003) Diving behaviour during the breeding season in the terrestrially breeding male grey seal: implications for alternative mating tactics. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 81: 1025-1033
- Lidgard DC, Boness DJ, Bowen WD, McMillan JI (2005) State-dependent male mating tactics in the grey seal: the importance of body size. *Behavioral Ecology* 16: 541-549
- Madsen PT, Wahlberg M, Tougaard J, Lucke K, Tyack P (2006) Wind turbine underwater noise and marine mammals: implications of current knowledge and data needs. *Marine Ecology-Progress Series* 309: 279-295
- Matthiopoulos J, McConnell B, Duck C, Fedak M (2004) Using satellite telemetry and aerial counts to estimate space use by grey seals around the British Isles. *Journal of Applied Ecology* 41: 476-491
- McConnell BJ, Chambers C, Nicholas KS, Fedak MA (1992) Satellite Tracking of Gray Seals (*Halichoerus-Grypus*). *Journal of Zoology* 226: 271-282

- McConnell BJ, Fedak MA, Lovell P, Hammond PS (1999) Movements and foraging areas of grey seals in the North Sea. *Journal of Applied Ecology* 36: 573-590
- McMahon CR, Bester MN, Burton HR, Hindell MA, Bradshaw CJA (2005) Population status, trends and a re-examination of the hypotheses explaining the recent declines of the southern elephant seal *Mirounga leonina*. *Mammal Review* 35: 82-100
- Meesters E, Reijnders P, Brasseur S, Siebert U, Stede M, Tougaard S, Härkönen T (2009) An effective survey design for harbour seals in the Wadden Sea: tuning Trilateral Seal Agreement and EU-Habitat Directive requirements. Abstract, 12th International Scientific Wadden Sea Symposium, 30 March - 2 April, 2009, Wilhelmshaven, Germany.
- Mellish JAE, Iverson SJ, Bowen WD (1999) Variation in milk production and lactation performance in grey seals and consequences for pup growth and weaning characteristics. *Physiological and Biochemical Zoology* 72: 677-690
- Mohr E (1952) *Die Robben der europäischen Gewässer* (The seals of the European waters). Verlag, Frankfurt/Main
- Murie DJ, Lavigne DM (1992) Growth and Feeding-Habits of Gray Seals (*Halichoerus grypus*) in the Northwestern Gulf of St-Lawrence, Canada. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 70: 1604-1613
- Nedwell J, Howell D (2004) A review of offshore windfarm related underwater noise sources. In: Cowrie Report. p 1-57
- Nordstrom CA (2002) Haul-out selection by Pacific harbor seals (*Phoca vitulina richardii*): Isolation and perceived predation risk. *Marine Mammal Science* 18: 194-205
- Pierce GJ, Boyle PR (1991) A Review of Methods for Diet Analysis in Piscivorous Marine Mammals. *Oceanography and Marine Biology* 29: 409-486
- Pinheiro JC, Bates DM (2000) *Mixed-Effects Models in S and S-PLUS*. Springer
- Pomeroy PP, Fedak MA, Rothery P, Anderson S (1999) Consequences of Maternal Size for Reproductive Expenditure and Pupping Success of Grey Seals at North Rona, Scotland. *Journal of Animal Ecology* 68: 235-253
- Quedens G (1988) Kegelrobbe bei Amrum Amrum-Chronik 44-48
- R Development Core Team (2008) R: a language and environment for statistical computing In: R Foundation for Statistical Computing, Vienna, Austria
- Rae BB (1973) Further observations on the food of seals. *Journal of Zoological Society of London* 169: 287-297
- Reijnders PJH (1978a) De grijze zeehond in de Waddenzeegebied (The Grey seal in the Wadden Sea area). *Waddenbulletin* 13: 500-502
- Reijnders PJH (1978b) Recruitment in the harbour seal (*Phoca vitulina*) population in the Dutch Wadden Sea. *Netherlands Journal of Sea Research* 12: 164-179
- Reijnders PJH, Brasseur SMJM (1997) Xenobiotic induced hormonal and associated developmental disorders in marine organisms and related effects in humans; An overview. *Journal of Clean Technology Environmental Toxicology and Occupational Medicine* 6: 367-380
- Reijnders PJH, Brasseur SMJM, Brinkman AG (2000) Habitatgebruik en aantalsontwikkelingen van gewone zeehonden in de Oosterschelde en het overige Deltagebied. Alterra, Research Instituut voor de Groene Ruimte, Wageningen
- Reijnders PJH, S.M.J.M. Brasseur & A.G. Brinkman (2003) The phocine distemper virus outbreak of 2002 amongst harbour seals in the North Sea and Baltic Sea: spatial and temporal development, and predicted population consequences. In: Wadden Sea Secretariat W, Germany. (ed) CWSS (eds), Management of North Sea harbour and grey seal populations. Proceedings of the International

- Symposium at EcoMare, Texel, The Netherlands, November 29-30, 2002. Wadden Sea Ecosystem. . p 19-25
- Reijnders PJH, van Dijk, J. & Kuiper, D. (1995) Recolonization of the Dutch Wadden Sea by the Grey Seal *Halichoerus grypus*. *Biological Conservation* 71: 231-235
- Reijnders PJH, Vandijk J, Kuiper D (1995) Recolonization of the Dutch Wadden Sea by the Grey Seal *Halichoerus grypus*. *Biological Conservation* 71: 231-235
- Requate H (1956) Die Jagdtiere in den Nahrungsresten einiger frühgeschichtlicher Siedlungen in Schleswig-Holstein. (Hunted animals in foodremains of some prehistoric settlements in Schleswig-Holstein). . *Schrft naturwiss Ver Schleswig-Holstein* 28: 21-41
- Ries EH, Hiby LR, Reijnders PJH (1998) Maximum likelihood population size estimation of harbour seals in the Dutch Wadden Sea based on a mark-recapture experiment. *Journal of Applied Ecology* 35: 332-339
- Shepard R (1962) The analysis of proximities: multidimensional scaling with an unknown distance function. *Psychometrika* 27: 125-140
- Sjoberg M, Ball JP (2000) Grey seal, *Halichoerus grypus*, habitat selection around haulout sites in the Baltic Sea: bathymetry or central-place foraging? *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 78: 1661-1667
- Sparling CE, Smout SC (2003) Population energy budget for UK North Sea grey seals. In: Briefing paper 03/9 to NERC Special Committee on Seals.
- Strucker R.C.W. AFA, Lilipaly S., Berrevoets C.M. & Meininger P.L. (2007.) Watervogels en zeezoogdieren in de Zoute Delta 2005/2006. . In: Rapport RIKZ/2007.005. Rijksinstituut voor Kust en Zee, Middelburg,
- Teilmann J, Tougaard J, Miller LA, Kirketerp T, Hansen K, Brando S (2006) Reactions of captive harbor porpoises (*Phocoena phocoena*) to pinger-like sounds. *Marine Mammal Science* 22: 240-260
- Thompson D, Fedak MA (1993) Cardiac Responses of Gray Seals during Diving at Sea. *Journal of Experimental Biology* 174: 139-164
- Thompson D, Hammond PS, Nicholas KS, Fedak MA (1991) Movements, Diving and Foraging Behavior of Gray Seals (*Halichoerus grypus*). *Journal of Zoology* 224: 223-232
- Thomsen F, Laczny M, Piper W (2006) A recovery of harbour porpoises (*Phocoena phocoena*) in the southern North Sea? A case study off Eastern Frisia, Germany. *Helgoland Marine Research* 60: 189-195
- Tougaard J, Tougaard S, Jensen RC, Jensen T, Teilmann J, Adelung D, Liebsch N, Muller G (2006) harbour seals on Horns Reef before, during and after construction of Horns Rev Offshore Wind Farm. In: Esbjerg, Denmark
- Trites AW, Joy R (2005) Dietary analysis from fecal samples: How many scats are enough? *Journal of Mammalogy* 86: 704-712
- van Polanen Petel TD, Terhune JM, Hindell MA, Giese MA (2006) An assessment of the audibility of sound from human transport by breeding Weddell seals (*Leptonychotes weddellii*). *Wildlife Research* 33: 275-291
- Vincent C, McConnell BJ, Ridoux V, Fedak MA (2002) Assessment of Argos location accuracy from satellite tags deployed on captive gray seals. *Marine Mammal Science* 18: 156-166
- Walton MJ, Henderson RJ, Pomeroy PP (2000) Use of blubber fatty acid profiles to distinguish dietary differences between grey seals *Halichoerus grypus* from two UK breeding colonies. *Marine Ecology-Progress Series* 193: 201-208
- Wright PJ, Jensen H, Tuck I (2000) The influence of sediment type on the distribution of the lesser sandeel, *Ammodytes marinus*. *Journal of Sea Research* 44: 243-256

List of tables and figures

- Table 1. Regression coefficients of a regression of $\log(\text{counts}) \sim I + \text{year.dec} * \text{Type}$, with $\log(\text{total})$, the total number counted logarithmically transformed; year.dec, the time as year with decimals; Type, as the three data collections Moulting, Other, and Pupping. The * denotes that the model also contains an interaction which allows for a different regression coefficient for each Type. 19
- Table 2. Results from 999 bootstraps of the model in Table 1. Estimated median values and 95% confidence limits are also given. 20
- Table 3. Overview of scat samples of grey seals (top) and harbour seals (below) included in this study. 35
- Table 4. Results of grey seal' scat analysis of samples collected in Dutch waters between 2002-2008. Including were possible species, number of individual fish found and size (average, median maximum and minimum length) 36
- Table 5. ANOSIM pairwise tests 38
- Table 6. Result of a SIMPER analysis for the dissimilarity between the contents of grey seal scats in two different seasons. Only the 10 most important species are shown. Av.Diss, is the average dissimilarity between samples of the 2 groups. Diss/SD is a measure of the constancy of the difference between the samples. The higher the more samples share the same characteristic. Contrib% is the percentage contributed to the total dissimilarity. 39
- Table 7. Result of a SIMPER analysis for the dissimilarity between the contents of harbour and grey seal scats. Av.Diss, is the average dissimilarity between samples of the 2 groups. Diss/SD is a measure of the constancy of the difference between the samples. The higher the value the more samples share the same characteristic. Contrib% is the percentage contributed to the total dissimilarity. 40
- Table 8. Overview of tag used in the analysis 45
- Table 9. Overview of maps used. 46
- Table 10. Overview of the grey seals tagged in the framework of this study, in the Dutch Wadden sea. 46
- Table 11. Overview of tracking results of grey seals 2005-2008 in relation to known pile driving activity for offshore wind farms. 52
- Figure 1. Planned locations for wind farms in the Netherlands (yellow) and existing wind farms (blue).
(Source:http://www.rijkswaterstaat.nl/actueel/nieuws_en_persberichten/2009/maart2009/huizingabehandelt17aanvragenwindturbineparkopzee.aspx#) 12
- Figure 2 Exponential growth of grey seals in the Dutch Wadden Sea during the moult (March/April -18.74%), the pupping period (December – February -19.76%) and during the remaining time 'other' - 14.65%. 19
- Figure 3. Residuals of pup (above) moult (middle) and other (bottom) counts of grey seals in the Dutch Wadden Sea. Residuals should be randomly distributed around zero with no obvious pattern discernable. A smoothing line for pattern detection has been added to each plot. Absolutely larger residuals are denoted by their date of collection. 21
- Figure 4. Monthly maximum of the number of pups counted in the Dutch Wadden Sea (Dec-Feb). 22
- Figure 5 The distribution of grey seals in the Dutch Wadden Sea during the moult (April) 2009 when the greatest number of hauled out seals are observed. 23
- Figure 6 The haul-out location of grey seals in the Dutch Wadden Sea during the pupping season (Dec-Feb) 1984/85-1994/95, showing the maximum number of

- pups counted during the season (green dots) as well as the maximum number of all grey seals (coloured circles). The white area shows the survey area. 24
- Figure 7 The haul-out location of grey seals in the Dutch Wadden Sea during the pupping seasons from 1999/00 - 2005/06, showing the maximum number of pups counted, as well as the maximum number of all grey seals. The colour indicates the actual number of seals and the white square shows the survey area 25
- Figure 8 The haul-out location of grey seals in the Dutch Wadden Sea during the pupping season of 2006/07 - 2008/09 showing the maximum number of pups counted as well as the maximum number of all grey seals. The colour indicates the actual number of seals and the white square shows the survey area. 26
- Figure 9 The maximum number of grey seals counted during the moult (March/April) between 1985 - 1995, showing the location of haul-out sites in the Dutch Wadden sea. The colour indicates the total number and the white areas indicates surveyed area. 27
- Figure 10 The maximum number of grey seals counted during the moult (March/April) between 2000 - 2006, showing the location of haul-out sites in the Dutch Wadden sea. The colour indicates the total number and the white areas indicates surveyed area. 28
- Figure 11 The maximum number of grey seals counted during the moult (March/April) between 2007 - 2009, showing the location of haul-out sites in the Dutch Wadden sea. The colour indicates the total number and the white areas indicates surveyed area. 29
- Figure 12. MDS-plot showing the differences between scat contents of grey seals collected in different years. (Fourth root transformed; Resemblance: S17 Bray Curtis similarity) 38
- Figure 13 MDS-plot showing the differences between grey and harbour seal prey remains derived from the scats. The stress value indicates that 2 dimensions is not enough to distinguish the species well enough. (Fourth root transformed; Resemblance: S17 Bray Curtis similarity) 40
- Figure 14. Length-frequency plots of fish species eaten by both harbour and grey seals in the Netherlands. Bleu :grey seal; Pink: harbour seal 41
- Figure 15. Tracking results of grey seals 2005- April 2006, commencement of pile driving for Dutch off shore wind farms 53
- Figure 16 Tracking results of grey seals April 2006- May 2007, during pile driving for Dutch off shore wind farms 53
- Figure 17 Tracking results of grey seals May 2007- 2008, after pile driving for Dutch off shore wind farms had ceased. 54
- Figure 18 Relative frequency of dive duration in two adult females. During spring and summer. 55
- Figure 19 Relative frequency of dive duration in two adult males. During spring and summer and autumn. 55
- Figure 20 Relative frequency of dive duration in two subadults above:female below: males. During spring and summer and autumn. 56
- Figure 21. Detail of mapped tracks of grey seals. Diving activity defined as decent speed, shown in a size and colour gradient. 56
- Figure 22. The relative preference for (a) depth, (b) log of %mud in the sediment, (c) distance to the haulout, (d) shipping activity. The y-axis, represents preference. High and low values, represent preference and avoidance, respectively (see also eq. 1). The black line describes the mean population response; the grey lines the individual estimates for each seal and the grey area represent the

95% confidence limits for the entire population. On the x-axis a measure for the intensity of the available data is given. 57

Figure 23. Modelled seal density using the preferences for the various environmental characteristics described in Figure 22 combined with the numbers of seals counted during the aerial surveys. 59

Figure 24 Modelled seal preference using the preferences for the various environmental characteristics described in Figure 22 , but after removal of the effect of distance to the haulout site. This maps provides a rough indication of where seals would be if they would not be constrained to return to their haulout 60

Figure 25 Tracks of the seals that were tagged during the pile driving activity. left: data collected before may 2007, right: data collected after may 2007.61