The diet of harbour porpoises *Phocoena phocoena* in Dutch waters

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IMARES WAGENINGEN UR

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Preface

For the past five months we have been working on our final thesis of the study 'Wildlife Management' at the University of Applied Sciences Van Hall Larenstein in Leeuwarden. We studied the diet of harbour porpoises in Dutch waters. This research is carried out at the Institute for Marine Resources and Ecosystem Studies (IMARES) on Texel.

Throughout our thesis we have got a lot of support and help. Our special thanks go to Mardik Leopold (IMARES, Texel) for giving us the opportunity to work on this project. He guided us through this research and taught us many things about the diet of harbour porpoises and the aspects of doing research. We would also like to thank Guido Keijl for helping us with the stomach content analysis and Erik Meesters and Henry Kuipers for helping us with the statistics. We would also like to thank Lineke Begeman and Lonneke IJseldijk for giving us the opportunity to watch and help with the necropsies of porpoises at the University of Utrecht, and providing stomachs and morphological data of the porpoises used in this study.

Finally, we also want to express gratitude to our supervisors at the Van Hall Larenstein, Okka Bangma-Jansen and Patrick Bron, for their endless effort and commitment, support, advice and assistance during our final thesis.

June 23rd 2014, Leeuwarden

Tara Schelling and Lizzy van der Steeg

Summary

The harbour porpoise *Phocoena phocoena* is one of the smallest odontocetes, rarely reaching a length of 1.8 meters. Harbour porpoises are the most numerous cetacean in the North Sea. Over the past years, the harbour porpoise population has shifted from the northern part towards the southern part of the North Sea. This increase in porpoise numbers in the southern North Sea resulted in an increase of strandings and by-catch along the Dutch coast. This increase raised concern about the conservation status of the harbour porpoise. In order to develop effective protection measures, a better understanding of the porpoises' function within the marine ecosystem is needed. Therefore diet studies are necessary. It is not possible to study the diet of porpoises in the wild with visual observations, due to their small size and elusive behaviour. Therefore, stranded porpoises are used. Since 2003, stranded porpoises in the Netherlands were collected for necropsies and diet studies. Stomach content analysis provides detailed information on relatively locally consumed prey on a short-term basis. In this study, the stomach contents of 600 harbour porpoises were collected from 2003 until 2014. They were analysed and sagittal otoliths (fish hearing bones) and other hard parts (e.g. bones, jaws, claws) were used to identify prey species. The length and width of all hard prey remains were measured and by means of regression equations recalculated into a total prey length and mass. Mainly sagittal otoliths were used for determination of prey. Due to acid fluids in the stomachs of porpoises, otoliths wear down. Correction factors were used to compensate for this loss. During determination, all hard prey remains were assigned to a prey group. In this study, twelve prey groups were used. The numeric values used were the percentage of occurrence (%O), the percentage by number (%N) and the percentage by mass (%M). In total, 104,051 prey of 66 different species were found, with a total estimated mass of 362 kg. Calves and juveniles feed mainly on gobies. As they age, the diet shifts to gadoids, clupeids and sandeels. Juvenile males and females show no significant difference in their diet. Adults do show a difference in their diet. Females eat fattier fish than males. This might be due to the fact that they need more energy during pregnancy and lactation. In North-Holland, South-Holland and South West, the diet of porpoises is very similar. In the Wadden Sea, more estuarine roundfish are consumed. In the Eastern Scheldt, mainly gobies are consumed. In winter, porpoises feed mainly on clupeids, in spring they feed mainly on gobies and in autumn they feed mostly on pelagic roundfish. There is no trend in diet seen over the years 2006 until 2013, however, this is probably due to fluctuation in prey availability. Porpoises which stranded in a fresh condition contained more clupeids in their stomachs. It is assumed that these fresh porpoises probably died near shore, which also explains the clupeids (which are known to live near shore) in their stomachs. Decomposed porpoises contained more sandeels and estuarine roundfish in their stomachs. Rotten porpoises had fed mostly on pelagic roundfish. This could mean that rotten porpoises came from far from the coast. Porpoises which stranded in a good condition (with a thick blubber layer) fed mostly on sandeels and clupeids, which are known to be fatty fish. Porpoises in an emaciated condition (thin or almost no blubber layer) fed mostly on gadoids and gobies, which are known to be lean fish.

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1. INTRODUCTION

This report describes a research on the diet of harbour porpoises in Dutch waters. Data was used of harbour porpoises stranded in the years 2003 until 2014. The project was carried out as part of an IMARES research project.

1.1 Problem description

The harbour porpoise *Phocoena phocoena* is one of the smallest odontocetes, rarely reaching a length of 1.8m (Harrison, 1971). They are widely distributed in the Pacific and Atlantic parts of the northern hemisphere and in coastal and continental shelf waters, such as the North Sea (Haug *et al*., 2003). Harbour porpoises are, with more than 200.000 individuals, the most numerous cetacean of the North Sea (Hammond *et al*., 2013). The harbour porpoise population has increased in the southern North Sea over the last decade (Haelters & Camphuysen, 2009; Camphuysen, 2011; Camphuysen & Siemensma, 2011; Hammond *et al.,* 2013). This recent increase seems to be the result of a population movement towards the southern North Sea, as the total population size has not changed significantly (Thomsen *et al.,* 2006). This increase in porpoise numbers in the southern North Sea resulted in an increase of strandings along the Dutch coast (see figure 1.1) (Haelters & Camphuysen, 2008). It is not known what caused the sudden increase of strandings in 2006 and 2011.

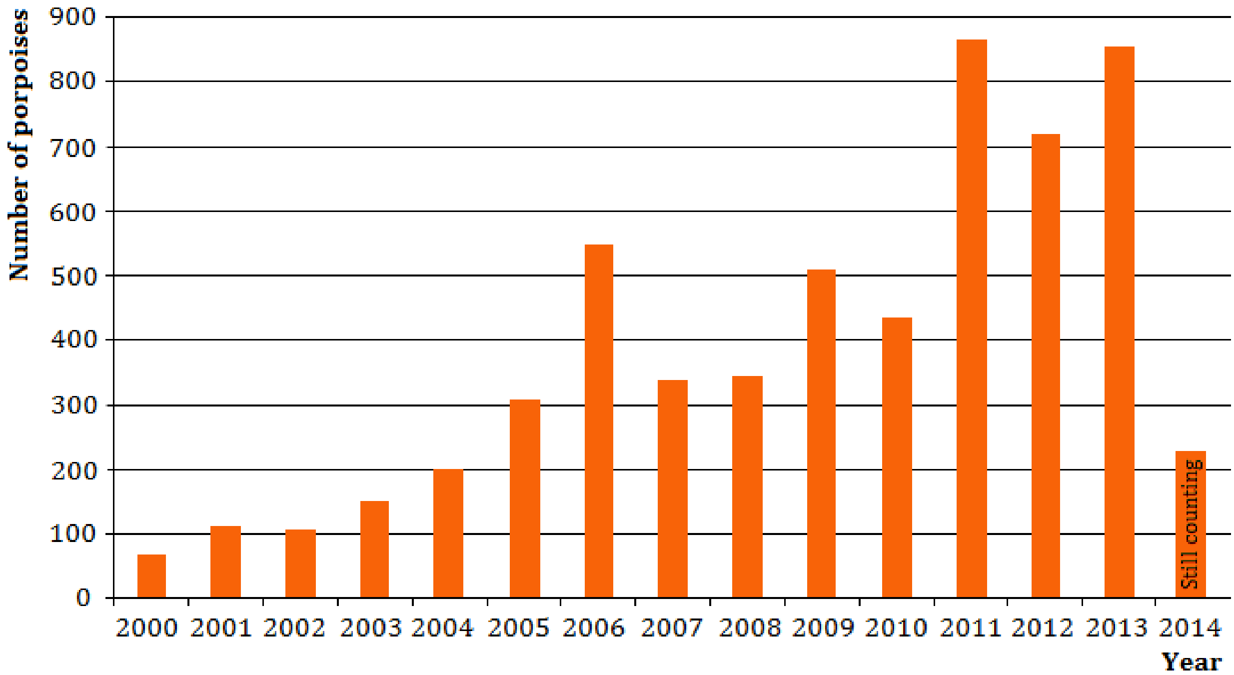

Figure 1.1. Reported strandings of harbour porpoises in the Netherlands (2000-2012) (www.walvisstrandingen.nl)

Based on the traumas of stranded porpoises, it appears that more than 40% of the stranded animals died of by-catch and trauma from fisheries, although lower by-catch rates have been reported (Leopold *et al.*, 2011). The combination of the population shift and high incidents of by-catch has raised concern about the conservation status of porpoises in Dutch waters. Currently, harbour porpoises are listed as threatened or endangered in several international conservation instruments (e.g. European Habitat Directive, Convention on International Trade in Endangered Species of Wild Fauna and Flora, Convention on the Conservation of European Wildlife and Natural Habitats, Convention on Migratory Species and the IUCN Red List of Threatened Species) (Reijnders *et al.*, 2009). In order to develop effective protection measures, information on the general health status and causes of death of stranded porpoises, sources of by-catch, population size, seasonal distribution and diet is required (Leopold *et al*., 2011).

To understand the porpoises' function within the marine ecosystem, diet studies (feeding strategy, predator-prey relationships, and responses of marine mammals to changes in food web dynamics) are necessary (Jansen, 2013). Diet should consist of the correct proportions of all food requirements of porpoises and should supply enough energy for its basal metabolism, thermoregulation, activity, digestion, growth and reproduction. It is not possible to study the diet of porpoises in the wild with visual observations due to their small size and elusive behaviour (Haug *et al*., 2003). Therefore, stranded and by-caught porpoises are used.

Stranded animals, however, often have an unknown cause of death which might provide biased information on the stomach contents (Jansen *et al.,* 2013). Since 2003, stranded porpoises in the Netherlands were collected for necropsies and diet studies (Leopold & Camphuysen 2006; Jauniaux *et al.,* 2008; Gröne *et al*. 2012). There are different methods to provide information on the diet consumed by harbour porpoises. A method which can determine diet, is measuring stable-isotopes (e.g. in skin, blubber, muscle and bone) and fatty-acid signatures (in milk, blood and blubber) (Fontaine *et al*., 2007; Christensen & Richardson, 2008; Gilles, 2008; Jansen, 2013). This method provides long-term dietary information on trophic position and foraging location through predictable changes in isotopic composition with each trophic transfer (Jansen, 2013). The advantage is that it gives a close approximation of the porpoises' diet composition. The disadvantage is that there is a possibility that not all prey species are identified (Jansen *et al.*, 2013). Another method is stomach content analysis which provides detailed information on relatively locally consumed prey on a short-term basis. The disadvantage is that only the last meal is observed and due to fast digestion, it might underestimate species with more fragile remains (Jansen, 2013). The advantage of this method is that it gives a more accurate result in determining prey species and sizes, which is why in this study the stomach contents were analysed and sagittal otoliths (fish hearing bones) and other hard parts (e.g. bones, jaws, claws) were used to identify prey species.

Sagittal otoliths are the most important objects for identifying fish species. Otoliths have unique shapes, which differ per species. Otoliths are located in the labyrinths inside the head of a fish. Figure 1.2 shows a dorsal view of the head of a generalized bony fish, with the location of the labyrinths. These labyrinths generally serve the same purpose as the structure in the human inner ear, which is giving the animal a sense of stability and balance. A bony fish has two labyrinths, each containing a set of three different types of otoliths: the sagitta, which is the largest; the lapillus and the asteriscus, both of which are very small. The sagittal otoliths are the ones which have been used for identification in this study. Figure 1.3 shows a lateral view of the inner ear structure, with the location of the otoliths indicated. The inside surface of an otolith, the sulcus, has distinguishing characteristics, which makes it very useful for identification.

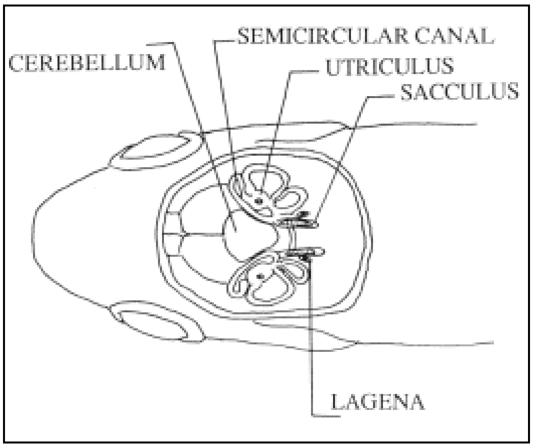

Figure 1.2. Dorsal view of a generalised head of a bony fish (Härkönen, 1986).

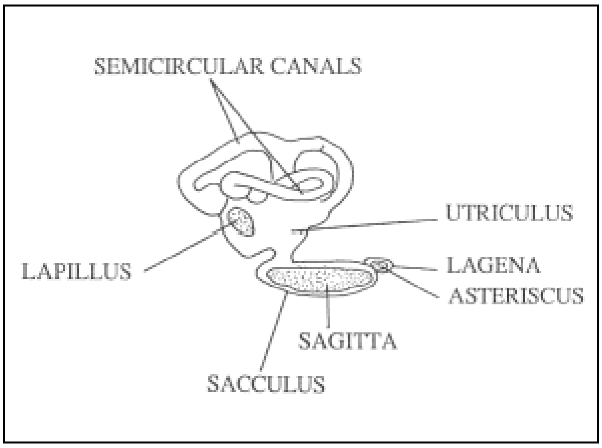

Figure 1.3. Lateral view of a labyrinth of a bony fish (Härkönen, 1986).

Several studies indicate that the diet of neonates, calves, juveniles and adult harbour porpoises differs (Börjesson *et al*., 2003; Santos & Pierce, 2003; Haelters *et al*., 2012). Due to their small size, limited body fat stores and high energy expenditure, the harbour porpoise requires a constant high energy input (Yasui & Gaskin, 1986; Kastelein *et al*., 1997; Koopman *et al*., 2002; Bjørge, 2003) and therefore a high quality diet (Spitz *et al*., 2012). Neonates, or new-borns, are very young porpoises which are not yet weaned and thus do not take solid food. Calves are slightly older, but still young animals that combine drinking milk with taking small fish like gobies *Gobiidae*. Juveniles are completely weaned and feed on gobies in particular, combined with small numbers of different species of fish, such as whiting *Merlangius merlangus* (Santos & Pierce, 2003; Leopold *et al.*, 2011). Once adulthood is reached, the diet of harbour porpoises in the North Sea consists of a broad prey spectrum. They need about ten per cent of their body weight in energy-rich food per day (Lockyer *et al*., 1999). In the North Sea, porpoises feed on a combination of fish with a high and low fat content (e.g. sandeels *Ammodytidae*, herring *Clupea harengus*, sprat *Sprattus sprattus*, mackerel *Scomber scombrus* and smelt *Osmerus eperlanus* which have a high fat content and gobies, whiting and cod *Gadus morhua,* which have a low fat content) (Sveegaard, 2010; Leopold *et al.*, 2011; Jansen *et al*., 2012; Haelters *et al*., 2012).

Different studies show that there is also geographical variation within the diet of harbour porpoises, probably due to differences in prey availability (Santos & Pierce, 2003). In Iceland, capelin *Mallotus villosus* was the main prey (Víkingsson *et al*., 2003). In the Baltic and northern Denmark, the main prey was herring and cod (Koschinski, 2001; Sveegaard, 2010). Gobies were the most important prey in German Baltic waters, but flatfishes, particularly sole *Solea solea,* were also remarkably important here (Lick, 1991[A]; 1993). Further east into the Baltic, Malinga *et al*. (1997) found mostly herring, sprat and gobies in the stomachs of 19 by-caught animals in Polish waters. Atlantic hagfish *Myxine glutinosa* is a relatively important prey for porpoises in Sweden (Aarefjord *et al.*, 1995). In northern Scotland whiting and Norway pouts *Trisopterus esmarkii* were the most important prey (Martin *et al*., 1990; Martin, 1996; Santos *et al.,* 2004). In the Netherlands, Belgium and France, porpoises eat mainly gobies and whiting (Santos, 1998; De Pierrepont *et al*., 2005; Leopold *et al.*, 2011; Haelters *et al.*, 2012). However, the harbour porpoise population has increased in the south eastern North Sea since these studies (Reijnders *et al.*, 2009; Camphuysen & Siemensma, 2011), so prey availability and, as a reflection of this, porpoise diet may have changed.

Several fish species in the North Sea have shown major fluctuations in abundance due to large fishing pressure, climatic changes and/or food web interactions. Several gadoid populations in the North Sea were extremely high from 1963 to 1983, during the so-called gadoid outbreak. After 1983, these populations decreased rapidly (Beaugrand *et al*., 2003). Likewise, the herring population collapsed around 1970, and the herring fishery was shut down. Unlike the situation with the gadoids, the herring population veered back to high stock sizes and the fisheries were re-opened. Ever since, the population size fluctuates (Dickey-Collas *et al*., 2010). These fluctuations are likely to have influenced harbour porpoise distribution patterns (Evans, 1990). In addition, sandeel stocks have fluctuated greatly in the North Sea, ultimately resulting in a partial fisheries closure in 2000 (Greenstreet *et al*., 2006). Due to the decreased population of sandeel, the likelihood of starvation and southward migration of harbour porpoises increased (MacLeod *et al*., 2007; Leopold *et al*., 2011). Harbour porpoises are known to have seasonal movements as well, presumably due to variations in prey availability (Tomilin, 1957; Gaskin *et al.*, 1974; Berggren & Arrhenius, 1995; Read & Westgate, 1997).

Previous studies on the diet of harbour porpoises in Dutch waters showed that diets were in general rather similar along different sections of the Dutch coastline. However, there was a slight difference in the diet of porpoises from the Eastern Scheldt, which showed more gobies. Furthermore, in North Holland, there were higher numbers of sandeels and clupeids found in the stomach contents (Leopold *et al*., 2011). In two other studies of Leopold *et al.* (in prep.[A, B]) the effect of Decomposition Condition Code (DCC) and Nutritive Condition Code (NCC) on the diet of harbour porpoises was studied. The DCC study compared the diet of fresh, decomposed and very rotten porpoises. There were significant differences between all groups. The diets of fresh and very rotten porpoises differed most. In all groups, the importance of gobies, gadoids and clupeids gradually decreased with advancing decomposition. However, with the rotten porpoises included in the DCC diet study, a wider prey spectrum was found. The NCC study indicated that the diet of porpoises in good condition differed significantly from the diet of emaciated porpoises. In porpoises in good condition a mixture of energy-rich prey (sandeels, clupeids, pelagic and estuarine roundfish) and leaner prey types (gobies and gadoids) were found. Emaciated porpoises had mostly consumed gadoids and gobies and there was a lack of energy-rich prey types. In both studies differences were found in diets between adult male and female porpoises, with e.g. more gadoids and less sandeels and roundfish found in the stomach contents of males. The same studies also indicated a difference in diet between harbour porpoises of different age classes, with e.g. mostly gobies in the diet of calves, and a decrease in number of gobies when the porpoise ages. Adult harbour porpoises mainly fed on gadoids.

1.2 Research goal

This study on the diet of harbour porpoises in Dutch waters combines the above mentioned diet studies of Leopold *et al.* (2011) and Leopold *et al.* (in prep.[A, B]) and used data from the past eight years (2003-2014). New data was added to the results of these previous studies in the database collection of IMARES, Texel. The primary objective of this study was to give a detailed description of the harbour porpoises' diet composition in Dutch waters, considering the following covariates: age, sex, stranding location, stranding month, decomposition condition and nutritive condition. In addition, a comparison was made between the years 2006-2013.

2. MATERIAL AND METHODS

This chapter describes the steps taken between a reported stranding of a harbour porpoise and the processing of the data obtained from stomach content analysis. Also the covariates are explained and how the data was statistically analysed.

2.1 Sample collection

Often, when a stranded harbour porpoise is reported, it is taken from the beach and brought to a freezer. This is done by volunteers or employees from IMARES, Neeltje Jans or Pieterburen. There are four places with large freezers where stranded porpoises are brought to: IMARES at Texel, Petten, Katwijk and Neeltje Jans (see figure 2.1). Around five times a year, a truck from the University of Utrecht comes to these freezers to take the stranded porpoises to the University of Utrecht. In Utrecht, the porpoises are stored in a freezer at a temperature of -30 degrees Celsius and are thawed before necropsies. The necropsies are carried out by veterinary pathologists, which follow a standard necropsy protocol (based on Kuiken & García Hartmann, 1991; Jauniaux *et al.*, 2002; Jauniaux & Jepson, 2006; Leopold & Camphuysen, 2006). First, the porpoises are photographed and the date of stranding, the location and morphological data are recorded. If the bodies are still intact, they are also measured (from the tip of the beak to the notch in the tail fluke), weighed and sexed. After this, the autopsies follow and the stomachs are taken out, bagged, stored frozen and transported to IMARES, Texel.

Figure 2.1. Locations of large storage freezers where stranded porpoises can be brought to.

2.2 Sample preparation

The gastrointestinal system of porpoises contains out of four stomachs (see figure 2.2). The forestomach (1), the main stomach (2), the pyloric stomach (4) and the main stomach is attached to the pyloric stomach by a narrow connecting channel (3)(Smith, 1972). All stomach compartments are cut open and their contents are rinsed into a 2 litre beaker. The dense heavy parts that are used for the determination of prey species need to be separated from the soft stomach contents. For this, the beaker is placed under a gently running tap, which makes the beaker overflow, taking the soft particles with it. The heavy particles remain at the bottom. This procedure ensures that only the otoliths, fish bones, squid jaws, ragworm jaws and other relevant parts remain in the beaker. Subsequently, these particles are transferred into a petri dish and dried.

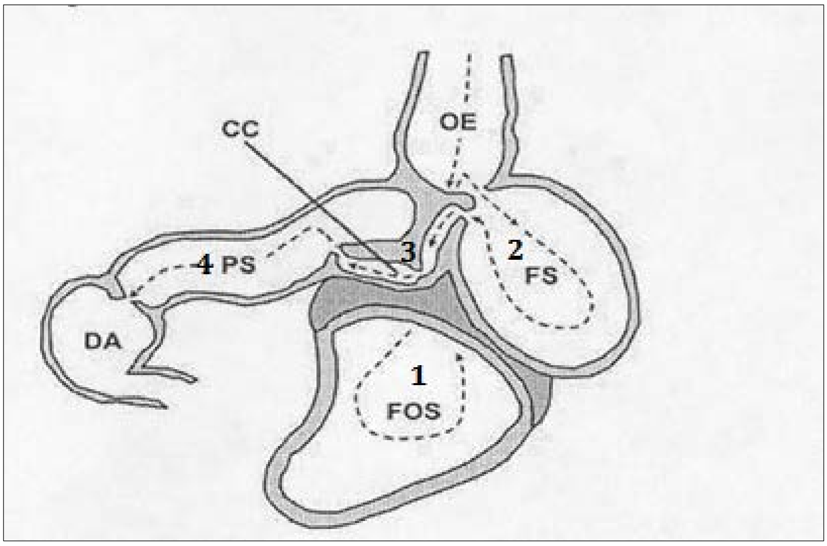

Figure 2.2. Schematic overview of all stomach compartments of odontocetes. OE, Oesophagus; FOS, forestomach; FS, the main stomach; CC, connecting channel; PS, pyloric stomach; DA, beginning of the small intestine (Aznar et al., 2006).

2.3 Stomach content analysis

The prey taken by the porpoises are determined by analysing the hard parts (e.g. otoliths, vertebrae, jaws and squid beaks) found in the stomach contents (e.g. Santos & Pierce, 2003; MacLeod *et al.*, 2007). All remains are measured (length, width and wear-class) with the computer program AxioVision 4.8.2 which is connected to a microscope, and then identified to the lowest taxonomic level using published guides for prey remains (e.g. Härkönen, 1986; Clarke, 1986; Leopold *et al.*, 2001) and the reference collection of IMARES and the NIOZ. With this data the minimum prey number, prey size, prey mass and caloric content is estimated using published regressions (Leopold *et al.*, 2001).

The data collected from these measurements has been added to the already existing porpoise diet database of IMARES, Texel. In total, 1,308 porpoise stomachs have been collected between the years 2003-2014.

2.3.1 Otoliths

All otoliths found in the porpoises stomachs are paired (left and right of similar size) as much as possible, in order to determine a minimum number of individuals.

In this study, otolith wear class has been carefully assessed by M.F. Leopold. Five grading classes are used; wear 0, wear 1, wear 2, wear 2.5 and 3 (figure 2.3).

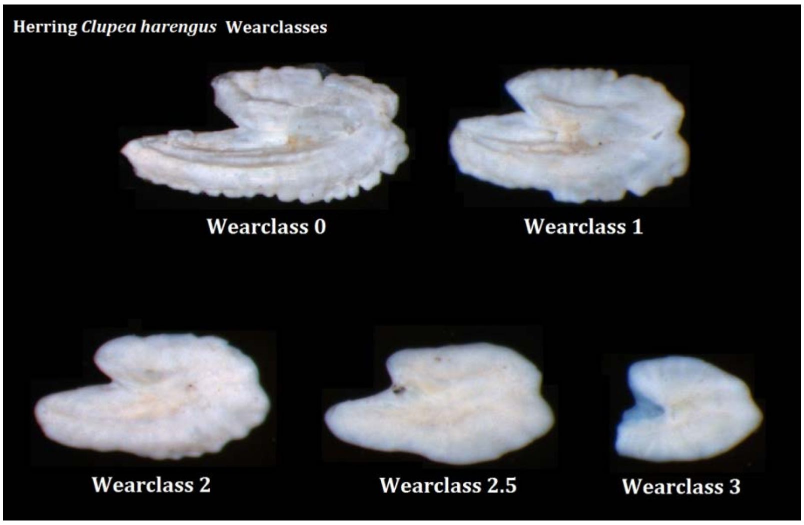

Figure 2.3. Otolith of herring Clupea harengus in five different wear classes.

Species specific regression coefficients, which account for wear, can then later be applied when determining the prey size. This is done in order to minimize the underestimation of the prey size due to otolith digestion.

Wear 0 means the otolith is not affected by digestion at all and is in pristine condition. It is corrected with factor 1.0 and the true fish length and mass can be calculated. Wear 1 means that the otolith is lightly affected and has some wear at margins but was still largely intact. This is corrected with factor 1.05. Wear 2 means that there is moderate wear and the otolith is rounded. Shape and sometimes sulcus are still visible. This is corrected with factor 1.1. Wear 2.5 means that the otolith has severe wear. Shape and size are severely affected. The sulcus is hardly or not visible at all. However, the otolith is still measurable. This is corrected with factor 1.2. Wear 3 means the otolith is worn down to such an extent that the size of the otolith has no longer a relation to the original fish size. The length and mass of fish with otoliths in wear class 3 cannot be calculated, so they get a mean total fish length (TFL), calculated by using otoliths of the same species present in the same stomach. Whiting otoliths wear faster in length than width so different correction factors are taken into account. Wear 0 is corrected with 1.0, wear 1 is corrected with 1.06, wear 2 is corrected with 1.14 and wear 2.5 is corrected with 1.24. When no otoliths in the same stomach and of the same species are available, the mean fish length and/or mass is used of all similar fish species in the whole spreadsheet of the same month (Leopold *et al.*, 2001).

Researchers at IMARES have been working to translate prey remains found in piscine carnivores to their original size for many years. These studies have been done on prey remains found in stomachs and faeces of several species of sea birds, seals and cetaceans. All these studies used the same techniques and extensive reference collections present at IMARES and the NIOZ. In all studies, hard particles were measured to calculate the original prey size and mass with a regression analysis (see table 2.1). All required regression equations are present on the cd-rom “Fish identification key by means of otoliths and other hard parts” which is based on 10.000 fish (Leopold *et al.*, 2001). An overview of all regression formulas used in this study can be found in appendix II.

Table 2.1. Example of regression factors of herring Clupea harengus, and sprat Sprattus sprattus, of which fish length and mass can be calculated according to otolith length and width, which were already corrected for wear. FL = Fish length (cm); OL = otolith length (mm); OW = otolith width (mm) and M = mass (g).

	Regression	
Species	**Equation**	**X range**
Herring *(Clupea harengus)*	FL= -1.93+6.29*OL	0.5-5.5
	FL= -6.36+15.51*OW	0.4-2.7
	M= $(0.18*FL)^{3.11}$	4.0-31.5
Sprat *(Sprattus sprattus)*	FL= 0.00+6.87*OL	1.1-2.3
	FL= -1.41+11.92*OW	0.8-1.5
	M= $(0.18*FL)^{3.78}$	6.8-15.0

2.3.2 Squids and ragworms

A squid has an upper and lower beak or jaw, which is more resistant to digestion than other body parts. The number of squids in a porpoise stomach is obtained by counting the jaws. The species could not be identified and size and mass of the whole prey could not be calculated because there is not enough known about the different squid species found in porpoise stomachs. Therefore, all squids were given a fixed mass of 3 gram (Croxall & Prince, 1982).

Ragworms have hard jaws which are highly resistant to digestion. The number of prey is determined on the basis of the number of left and right jaws. The jaws were measured in length, which could determine the original size of the ragworms.

2.3.3 Crustaceans

Shrimp also have measurable hard parts: their claws and tail (see figure 2.4). The original size of partially digested shrimp can be estimated by measuring tail flaps and claw length (Doornbos, 1984). The number of shrimp found per stomach was estimated on the basis of the number of claws and tails found.

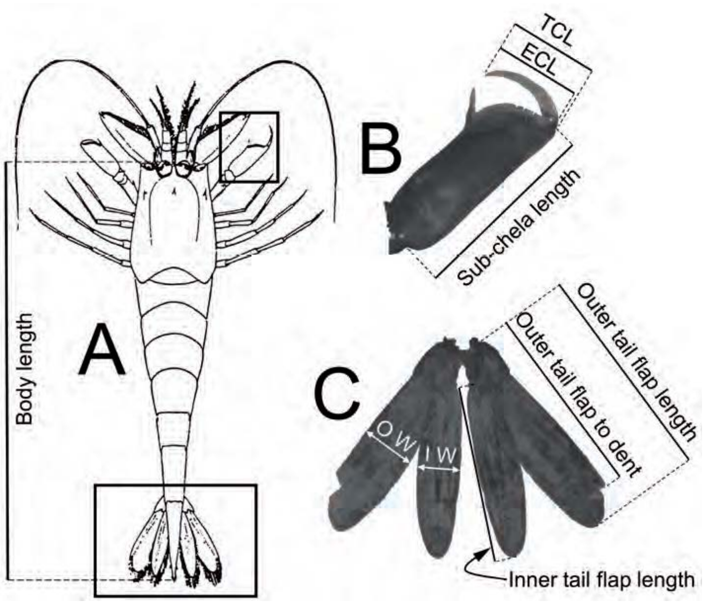

Figure 2.4. Brown shrimp Crangon crangon measurements: body length from tip of rostrum to tip of telson (A), sub-chela and claw lengths (B), tail flaps (outer/lower and inner/upper uropods) (C). Drawing of whole shrimp taken from de Ruijter & Schoenmaker (1989), TCL and ECL are total and exposed claw lengths, OW and IW are outer and inner tail flap widths, respectively.

2.3.4 Other hard prey remains

These include vertebrae, denticles (skin fragment of pipefish), premaxilla (fish jaws) and sub- and preopercula (skull bones). Vertebrae are measured by length. Pipefishes were determined by denticles. However, there is not enough information about how to calculate the full size of pipefish by their denticles. This is why they were all given an average length of 10cm (Leopold, unpublished data). Premaxilla are a pair of curved bones located at the front of the upper jaw of fish, which contain their teeth.

Reference material for most types of prey was present at IMARES/NIOZ in the form of boiled-out fish skeletons with a pre-measured length. On the basis of this reference material, a comparison could be made between the skeleton parts found in the porpoise stomachs and the homologous parts of already identified fish species of which the length was known. In this way, an estimation could be made of the original prey size.

2.3.5 Foreign objects

Other objects such as leaves, plastics, papers, nylon threads, stones, wood and shells were measured by length and width and weighed. They were not considered prey.

2.4. Covariates

In this paragraph it is explained how the stomachs are categorized according to each covariate.

2.4.1 Porpoise age

In order to assess whether porpoises of different age differed in diet, the stomachs were divided into three categories of age: calves, juveniles and adults. Age class was determined by the veterinary pathologists of the University of Utrecht and the biologists of IMARES and determined according to body length (see figure 2.5). Foetuses, stillborns and neonates were excluded from this research, because they had no food remains in their stomachs. Empty stomachs of juveniles and adults were also excluded from this research, because they did not contribute to this diet study. Porpoises smaller than 90cm were considered calves. Porpoises between 90cm and 130cm were considered juveniles. Porpoises larger than 130cm were considered adults.

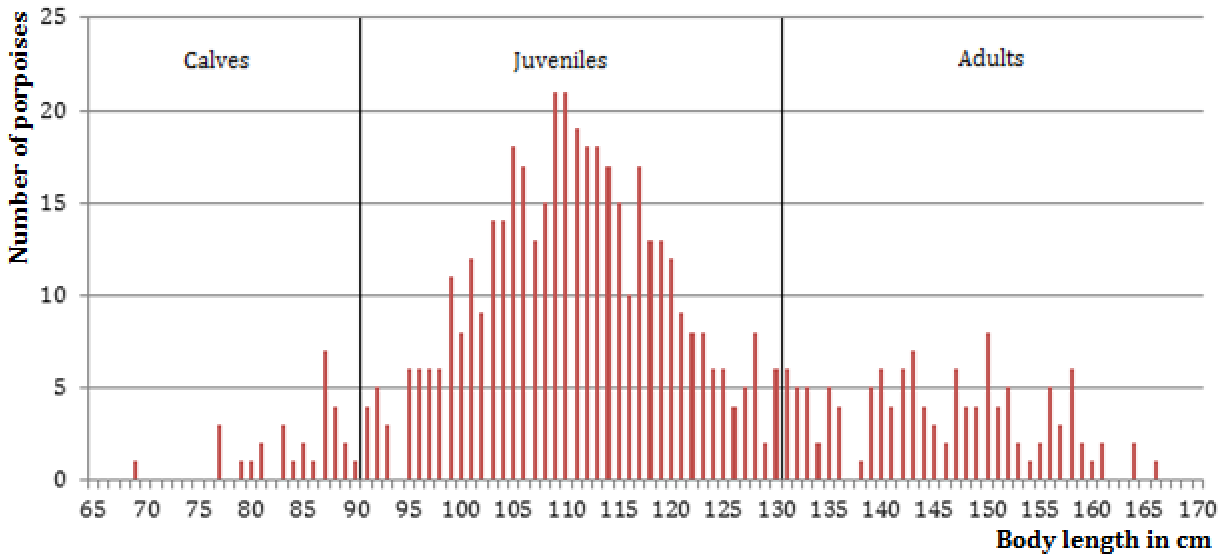

Figure 2.5. The number of porpoises (y) stranded along the Dutch coast between 2003 until 2014 with food remains in their stomachs (n=600) divided in age classes according to their body length in cm (x). Calves <90cm, Juveniles >90cm - <130cm and adults >130cm.

2.4.2 Porpoise sex

In order to assess whether male and female porpoises showed a difference in diet, the porpoises were divided into three groups: male porpoises, female porpoises and porpoises of which the sex could not be determined because the porpoise was incomplete. The sex was determined by the veterinary pathologists of the University of Utrecht.

2.4.3 Stranding location

In order to assess whether porpoises stranded in different locations differed in diet, the Dutch coast was divided into six sub-regions (see figure 2.6), namely: the North Eastern part (NE), the Dutch Wadden Sea (WS), the north of Holland (NH), the south of Holland (SH), the southwest of Holland (SW) and the Eastern Scheldt (ES). These regions were chosen because the same regions were also used in previous studies (Leopold *et al.*, 2011 and Leopold *et al.* in prep.[A, B]).

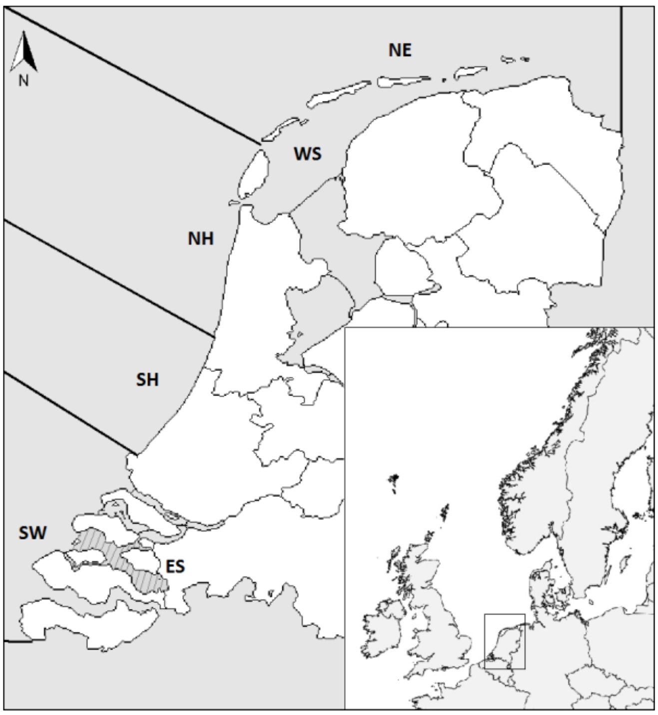

Figure 2.6. Location of the study area. NE = North East, WS = Wadden Sea, NH = North Holland, SH = South Holland, SW = South West, ES = Eastern Scheldt (dashed area).

2.4.4 Stranding month
In order to assess whether porpoises stranded in different times of year differed in diet, the year was divided into months (Jan-Dec), which made twelve categories.

2.5.5 Decomposition Condition Code (DCC)
In order to assess whether porpoises with a different decomposition condition differed in diet, the porpoises' carcass was rated by veterinary pathologists from very fresh to severe putrefaction on a 5-point scale defined by Kuiken & García Hartmann (1991) (1=live stranding but died on site, 2=very fresh, 3=decomposing, 4=advanced decomposition and 5=only remains left and very rotten). The diet was compared for three groups: fresh (1-2), decomposed (3) and very rotten (4-5).

To compare the influence of DCC on diet between winter and summer, the months with the mean coldest and mean warmest sea surface temperature (measured for the period 1861-2006) were taken. For winter, porpoises stranded in the months January until March were taken and for summer the months July and August were taken. These months were chosen with reference to figure 2.7.

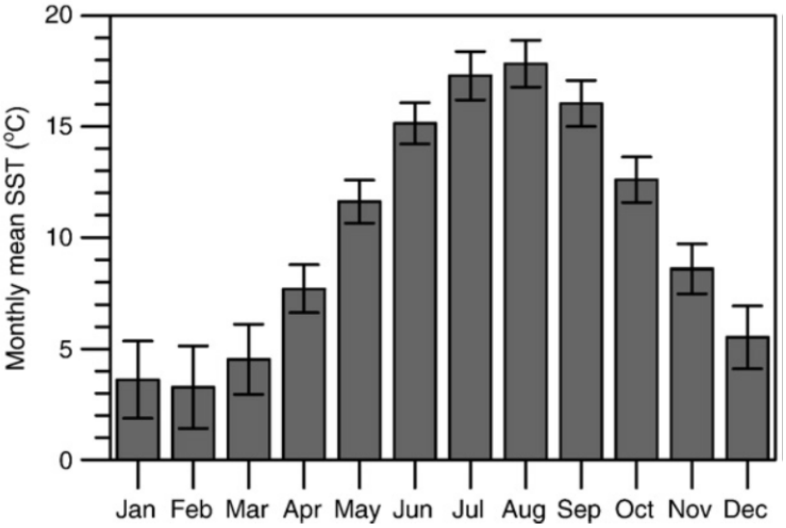

Figure 2.7. Averaged monthly mean sea surface temperature (SST) with standard deviation for the period 1861–2006. The error bars show the standard deviation, derived from the individual monthly averages (Aken, 2008).

2.4.6 Nutritive Condition Code (NCC)
In order to assess whether porpoises with a different nutritive condition differed in diet, the porpoises health was rated by veterinary pathologists from very fat to severely emaciated on a 6-point scale defined by Kuiken & García Hartmann (1991) (1=very fat, 2=fat, 3=normal, 4=emaciated, 5=very emaciated and 6=extremely emaciated). This was done by measuring the average thickness of the blubber layer on different reference points and by visually assessing relative muscle mass. The diet was compared for two groups: in good condition (1-3) and emaciated (4-6).

2.4.7 Stranding year
In order to assess whether porpoises stranded in different years differed in diet, the years 2006 until 2013 were analysed separately and then compared to each other.

2.5. Statistical analysis
This section reviews the various methods used for the statistical analysis of the data. The software that was used for these analyses was Microsoft Excel 2010, Corel Paradox, version 8 and PRIMER, version 6+.

Data of the stomach content analysis was first entered in an Excel database. With help of Paradox, a database management system, queries were made of the covariates to be tested against diet in both mass and number. These queries were transformed back to a spreadsheet in Excel. After this, the Excel datasheet could be transformed to the statistical program PRIMER.

Because PRIMER is sensitive for numbers which lay relatively far apart (e.g. 1 gadoid, 6000 gobies), differences in prey numbers were constrained by applying a fourth root transformation. After the fourth root transformation, a resemblance matrix was made using a Bray-Curtis dissimilarity to quantify the difference between samples.

After this, a PERMANOVA design was created. With the PERMANOVA routine (Anderson, 2001; McArdle & Anderson, 2001; Anderson *et al*., 2008) in PRIMER, version 6+ (Clarke & Gorley, 2006), diet, expressed as percentage mass, was put against one of the covariates (i.e.: age, sex, stranding location, stranding month, DCC, NCC and year). Diet was summarised per prey group. Twelve prey groups were used which were based on the results of former diet studies (see appendix I).

A covariate had a significant influence on porpoise diet if $P \leq 0,05$. The percentage in which a covariate determined diet was also calculated.

3. Results

Since 2003 stranded harbour porpoises are collected for among other diet research of which 600 contained prey remains and are therefore suitable for this study. The prey species are classified to their family names. Appendix I contains a table with all prey groups, including the species and scientific names.

3.1 Prey species

In total, the database consists of 1,308 stomachs collected from 2003 until 2014, of which 600 stomachs contained prey that were taken into account for this study. The number of prey is 104,051 with a total prey mass of 362.08 kg, divided over 66 different prey species. The prey species are divided into twelve prey groups as is shown in appendix I. Table 3.1 and figure 3.1 show the exact numbers per prey group.

Table 3.1. Of the 600 harbour porpoises stranded along the Dutch coast from 2003 until 2014, with prey remains in their stomachs, the number of prey and percentages by number (%N), the abundance of the prey groups in the stomachs (e.g. clupeids were present in 162 out of 600 stomachs) and the relative percentage of occurrence (%O), the total mass in kg and mass in percentage (%M) are shown.

	Number of prey	Percentage by number (%N)	Present in # of stomachs	Relative % of occurrence (%O)	Total mass (kg)	Percentage of mass (%M)
Clupeids	2,735	2.63	162	10.52	39.12	10.80
Demersal roundfish	84	0.08	25	1.62	1.42	0.39
Estuarine roundfish	3,071	2.95	95	6.17	18.56	5.12
Flatfish	120	0.12	35	2.27	1.26	0.35
Gadoids	1718	1.65	229	14.87	132.54	36.61
Gobies	86,903	83.52	377	24.48	82.03	22.66
Invertebrates	386	0.37	126	8.18	0.33	0.09
Other roundfish	218	0.21	23	1.49	0.48	0.13
Pelagic roundfish	227	0.22	42	2.73	14.16	3.91
Polychaetes	199	0.19	69	4.48	1.01	0.28
Sandeels	7,502	7.21	246	15.97	66.17	18.27
Squids	888	0.85	111	7.21	5.00	1.38

3.1.1 Percentage by number

Percentage by number shows the numeric importance (%N) of the prey groups. The number of gobies found in the stomachs was the highest with 83.52%. The percentage of sandeels came next with 7.21%, followed by estuarine roundfish with 2.95%. The least represented prey group is demersal roundfish with 0.08% (figure 3.1a)

3.1.2 Percentage of occurrence

Percentage of occurrence (%O) shows how frequently prey groups occur in porpoise stomachs. In total, 377 stomachs contained gobies, which is 24.48% of all porpoise stomachs. Next with 15.97% are sandeels, which were found in 246 stomachs, followed by gadoids found in 229 stomachs which is 14.87% (figure 3.1b).

3.1.3 Percentage by mass

Percentage by mass (%M) shows the percentage by mass of all prey groups found in the stomachs. With 36.61%, gadoids have the highest contribution of mass percentage of all prey groups. With 22.66%, gobies have the second highest contribution followed by sandeels with 18.27% (figure 3.1c).

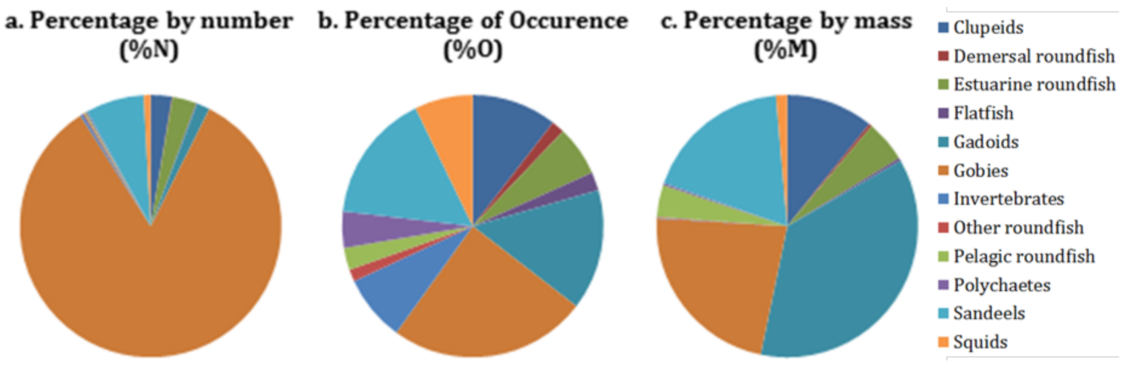

Figure 3.1. Of the 600 harbour porpoises stranded along the Dutch coast from 2003 until 2014 with prey remains in their stomachs, the percentages per prey group in numeric importance (%N) (a), the percentage of occurrence (%O) (b) and the percentage by mass contribution (%M) (c) are shown.

The Costello diagram (see figure 3.2) graphically represents the relative importance of each prey group in the diet by means of number and mass. This diagram shows that gadoids, gobies, sandeels and clupeids are the most important prey groups by means of mass. By means of number, gobies are the most important. The other prey groups were also frequently found but are less important as the above mentioned.

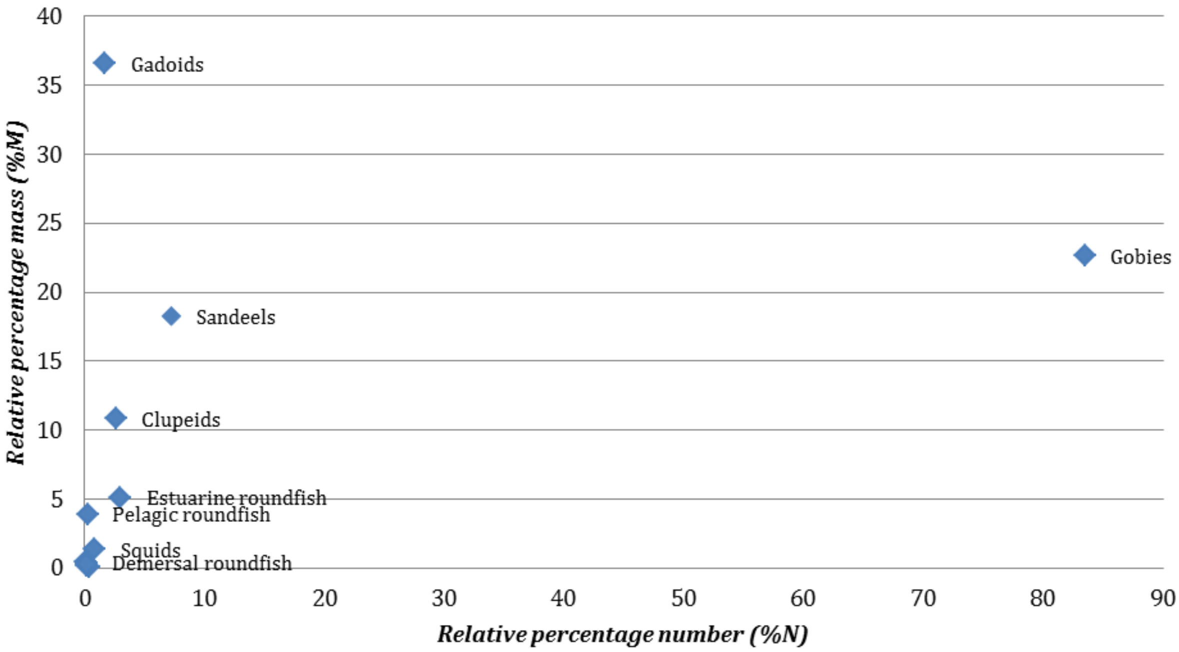

Figure 3.2. Costello diagram with relative percentage of mass (%M)(y) against relative percentage by number (%N)(x) of all prey groups of 600 harbour porpoises stranded along the Dutch coast between 2003 and 2014.

3.2 Covariates

The influence of porpoise age, sex, stranding location, month of stranding, Decomposition Condition Code, Nutritive Condition Code and year on the diet of harbour porpoises is presented in this paragraph. From here, the results and outcomes in PRIMER will only show percentages by means of relative percentage of mass. Numeric importance is not taken into account in this paragraph.

3.2.1 Porpoise age

Of all 600 porpoises with prey remains in their stomachs, 21 were calves with a total prey mass of 1,154g, 445 were juveniles with a total prey mass of 228,525g and 134 were adults with a total prey mass of 132,407g. PERMANOVA shows a significant influence of age on the diet of harbour porpoises (P=0.001), with age determining 9.06% of the diet. Table 3.2 shows the differences in diet between the three age classes, with the relative percentage of mass per prey group.

Table 3.2. This table shows the mass and relative percentage mass (%M) per prey group of harbour porpoises stranded along the Dutch coast from 2003 until 2014 found in each age class.

	Calves (n=21)		Juveniles (n=445)		Adults (n=134)	
	Prey mass (g)	Relative % mass	Prey mass (g)	Relative % mass	Prey mass (g)	Relative % mass
Clupeids	16.21	1.41	21,838.43	9.56	17,267.66	13.04
Demersal roundfish	9.66	0.84	308.81	0.14	1102.2	0.83
Estuarine roundfish	0.00	0.00	17,521.56	7.67	1,033.83	0.78
Flatfish	0.00	0.00	482.37	0.21	775.66	0.59
Gadoids	9.16	0.79	65,864.11	28.82	66,669.48	50.35
Gobies	1,084.34	94.00	78,193.09	34.22	2,754.83	2.08
Invertebrates	3.11	0.27	246.12	0.11	82.36	0.06
Other roundfish	0.08	0.01	438.55	0.19	43.48	0.03
Pelagic roundfish	0.00	0.00	3,237.38	1.42	10,918.63	8.25
Polychaetes	0.00	0.00	741.33	0.32	273.03	0.21
Sandeels	14.12	1.22	35,893.78	15.71	30,258.03	22.85
Squids	16.82	1.46	3,759.19	1.64	1,227.43	0.93

The diet of calves consists mainly of gobies and sometimes small numbers of species from other prey groups are consumed. Juveniles still consume gobies for the largest part of their diet, but the number of gadoids, sandeels and clupeids is increasing. The diet of adult harbour porpoises consists mainly of gadoids, sandeels and clupeids and the number of gobies has strongly decreased. Figure 3.3 gives an overview of differences in diet between porpoises of different age classes.

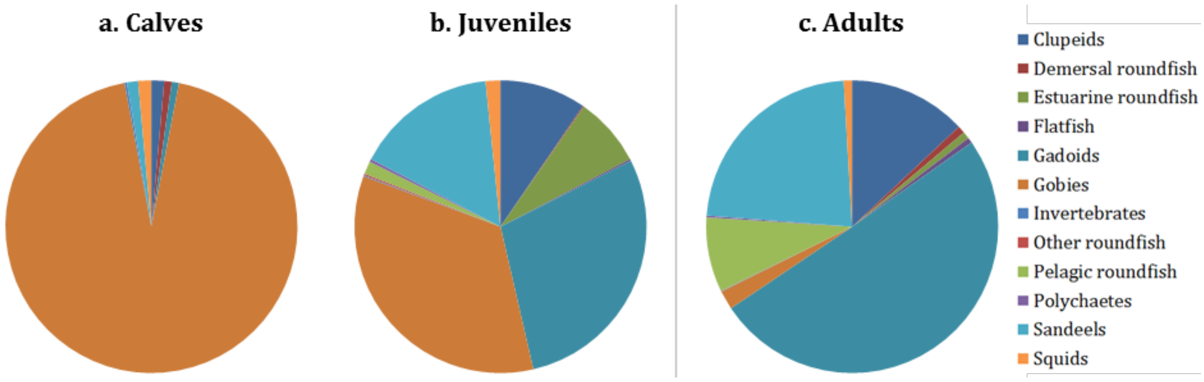

Figure 3.3. The relative percentage of mass (%M) per prey group of harbour porpoises stranded along the Dutch coast from 2003 until 2014 for calves (a), juveniles (b), and adults (c).

These results were based on data over the whole year. Because calves are only present in the months June until December, a new PERMANOVA was created, to show if there is a difference in diet for the juveniles and adults in these months. It showed that age class had a significant influence (P=0.001) on diet, with age determining 8.74% of the diet. Table 3.3 shows these differences between the three age classes, with the relative percentage of mass per prey group, for the months June until December. In these months, the amount of gadoids has increased for both juveniles and adults. For juveniles, the amount of gobies and clupeids has decreased. For adults, the amount of pelagic roundfish has increased and the amount of sandeels and clupeids decreased. Figure 3.4 gives an overview of the differences in prey group mass from June until December between the age classes.

Table 3.3. The mass and relative percentage of mass (%M) per prey group of harbour porpoises stranded along the Dutch coast from 2003 until 2014 per age class from June until December.

	Calves (n=21)		Juveniles (n=231)		Adults (n=85)	
	Prey mass (g)	Relative % mass	Prey mass (g)	Relative % mass	Prey mass (g)	Relative % mass
Clupeids	16.21	1.41	2,085.22	2.21	3,154.51	4.45
Demersal roundfish	9.66	0.84	149.61	0.16	1,037.47	1.46
Estuarine roundfish	0.00	0.00	6,788.95	7.19	393.44	0.55
Flatfish	0.00	0.00	40.19	0.04	230.42	0.32
Gadoids	9.16	0.79	48,830.63	51.68	45,317.51	63.86
Gobies	1,084.34	94.00	18,812.04	19.91	1,076.25	1.52
Invertebrates	3.11	0.27	64.41	0.07	70.73	0.10
Other roundfish	0.08	0.01	260.92	0.28	43.48	0.06
Pelagic roundfish	0.00	0.00	1,069.80	1.13	9,656.74	13.61
Polychaetes	0.00	0.00	67.97	0.07	217.55	0.31
Sandeels	14.12	1.22	12,757.96	13.50	9,035.84	12.73
Squids	16.82	1.46	3,559.03	3.77	733.33	1.03

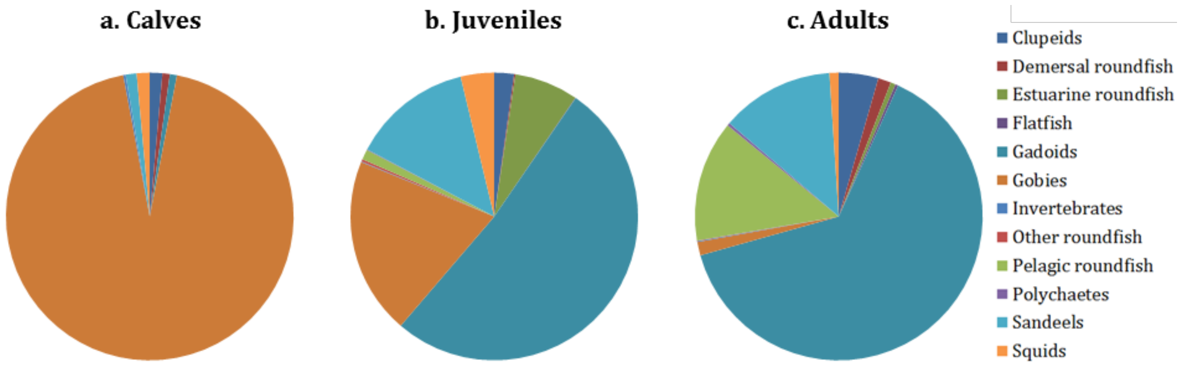

Figure 3.4. The relative percentage of mass (%M) per prey group of harbour porpoises stranded along the Dutch coast from 2003 until 2014 for calves (a), juveniles (b), and adults (c) with only the months June until December taken into account.

3.2.2 Porpoise sex

Of all 600 porpoises with prey remains in their stomachs, 334 were males with a total prey mass of 189,144g, 256 were females with a total prey mass of 160,151g. Of 10 porpoises the sex could not be determined, which is why they were not taken into account in this PERMANOVA. Sex had a significant influence (P=0.018) on diet, with 0.63% of the diet being determined by sex.

The same PERMANOVA test was done for juveniles and adults separately, because a difference in diet is expected between these age classes. These differences might be caused by the different needs of pregnant or lactating females, but also the number of juveniles is higher than the number of adults, which might give an overrepresentation of species consumed by juveniles. Of all 445 juveniles with prey remains in their stomachs, 226 were males with a total prey mass of 118,818g and 169 were females with a total prey mass of 96,917g. For juveniles, there is no significant influence (P=0.408) on diet between males and females. Of all 134 adults with prey remains in their stomachs, 55 were males with a total prey mass of 69,468g and 79 were females with a total prey mass of 62,939g. In adults, sex had a significant influence (P=0.012) on diet, with 3.24% of the diet being determined by sex.

Figure 3.5 shows the differences in diet for all males and all females, juvenile males and females and adult males and females. Overall, the amount of clupeids is higher in males than in females, whereas pelagic roundfish are consumed mainly by females compared to males. In juveniles, gobies and sandeels are less consumed by females compared to males, but gadoids are more consumed by females. In adults, gadoids are more consumed by males compared to females.

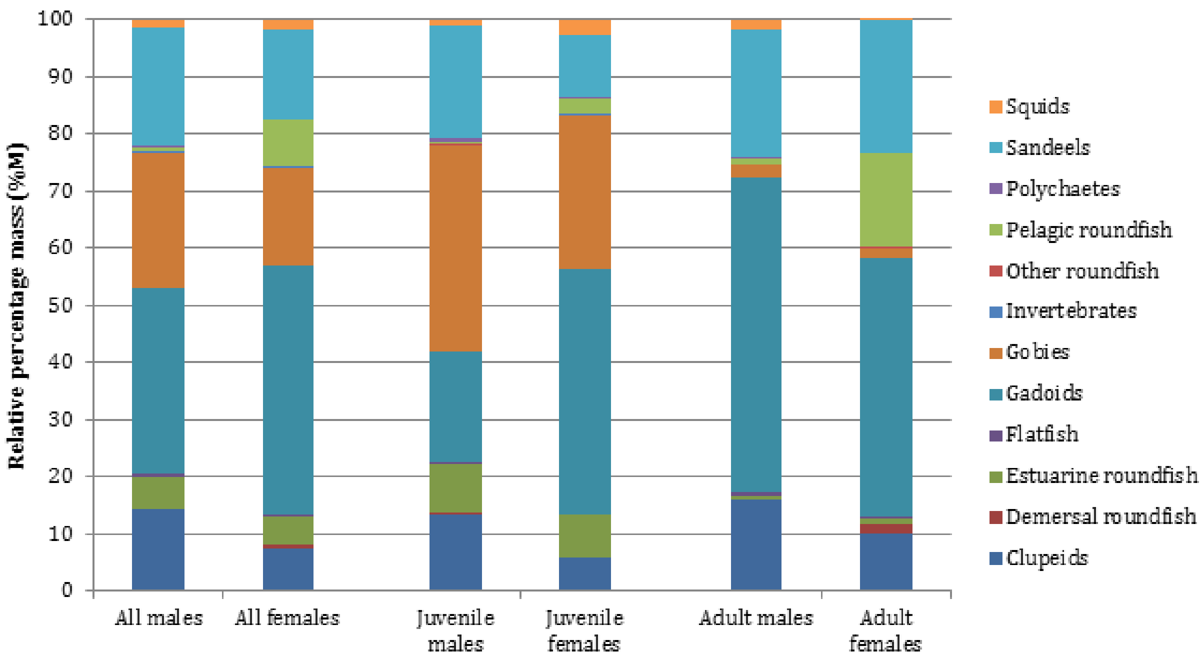

Figure 3.5. The differences in diet of harbour porpoises stranded along the Dutch coast from 2003 until 2014 in relative percentage of prey group mass (y) between males and females, divided in all males and females (P=0.018), juvenile males and females (P=0.408) and adult males and females (P=0.012) (x).

3.2.3 Stranding location

Of all 600 porpoises, 23 stranded in the Wadden Sea with a total prey mass of 13,822g, 26 stranded in the North East with a total prey mass of 8,702g, 179 stranded in North Holland with a total prey mass of 144,006g, 112 stranded in South Holland with a total prey mass of 67,082g, 238 stranded in South West with a total prey mass of 115,515g and 15 stranded in the Eastern Scheldt with a total prey mass of 7,841g. There were 7 porpoises which had an unknown stranding location and were therefore not taken into account in this PERMANOVA. The result is that stranding location has a significant influence (P=0.001), which determines 2% of the diet.

Figure 3.6 shows that estuarine roundfish is consumed mostly by porpoises stranded in the Wadden Sea. Besides this, gobies and pelagic roundfish also play an important role for porpoises in the Wadden Sea. The diet of porpoises stranded in the North East mainly consists of gobies followed by clupeids, sandeels and gadoids. The diet of porpoises in North Holland, South Holland and the South West are quite similar, almost half of the porpoise diet consists of gadoids. In North Holland, more clupeids are consumed; in South Holland, more gadoids are consumed; and in the South West, squids are consumed more than in the other locations. In the Eastern Scheldt, gobies are the most eaten prey group for porpoises.

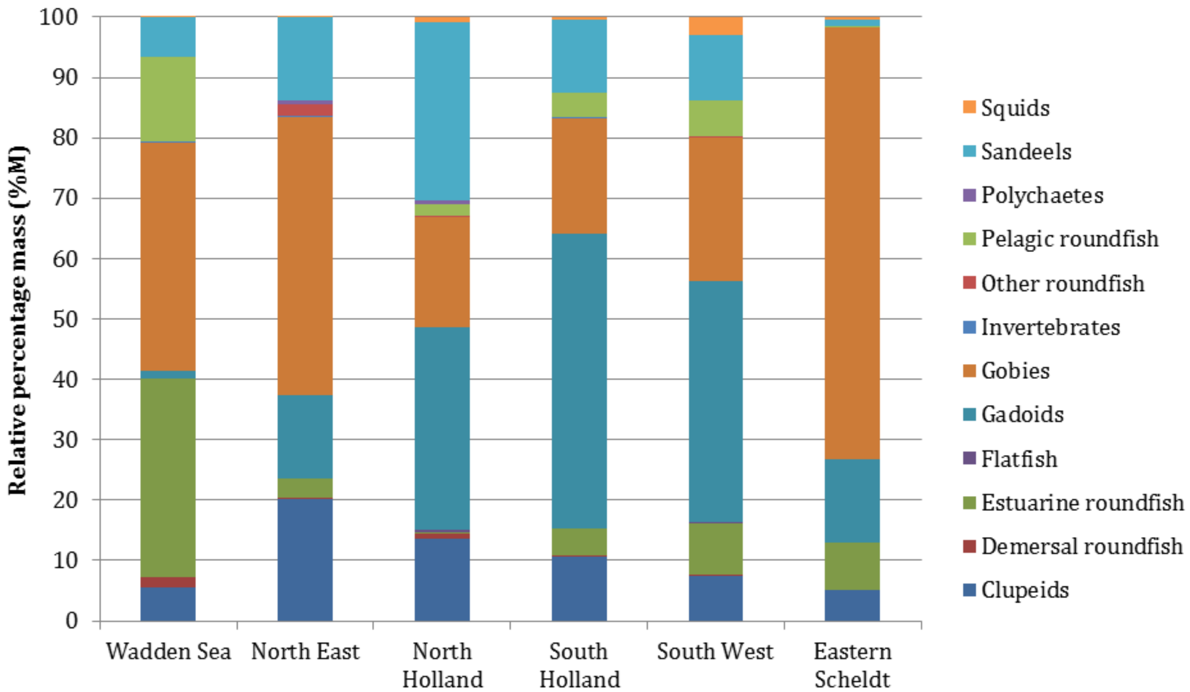

Figure 3.6. The differences in diet of harbour porpoises stranded along the Dutch coast from 2003 until 2014 in relative percentage of prey group mass (y) between the stranding locations (x) for all porpoises.

These results are based on all 600 porpoises of which the stranding location is known. Considering 445 of these porpoises were juveniles, the same PERMANOVA test was done for juveniles only, because a difference in diet is expected between juveniles and adults between locations. Eighteen juveniles stranded in the Wadden Sea with a total prey mass of 9,559g, 20 juveniles stranded in the North East with a total prey mass of 7,374g, 126 juveniles stranded in North Holland with a total prey mass of 83,269g, 82 juveniles stranded in South Holland with a total prey mass of 40,246g, 186 juveniles stranded in South West with a total prey mass of 77,012g and 8 juveniles stranded in the Eastern Scheldt with a total prey mass of 6,906g. The PERMANOVA showed that stranding location has a significant influence (P=0.001) on diet for juvenile porpoises, and 2.62% of the diet being determined by location.

Figure 3.7 shows the differences in diet of juvenile porpoises stranded in different locations. In all locations, juveniles mainly consume gobies, but in the Wadden Sea also estuarine roundfish has an important role in their diet. The amount of clupeids and sandeels is higher in the North East, North Holland, South Holland and the South West in comparison to the Wadden Sea and the Eastern Scheldt. In the last location mainly gobies (>96%) are consumed.

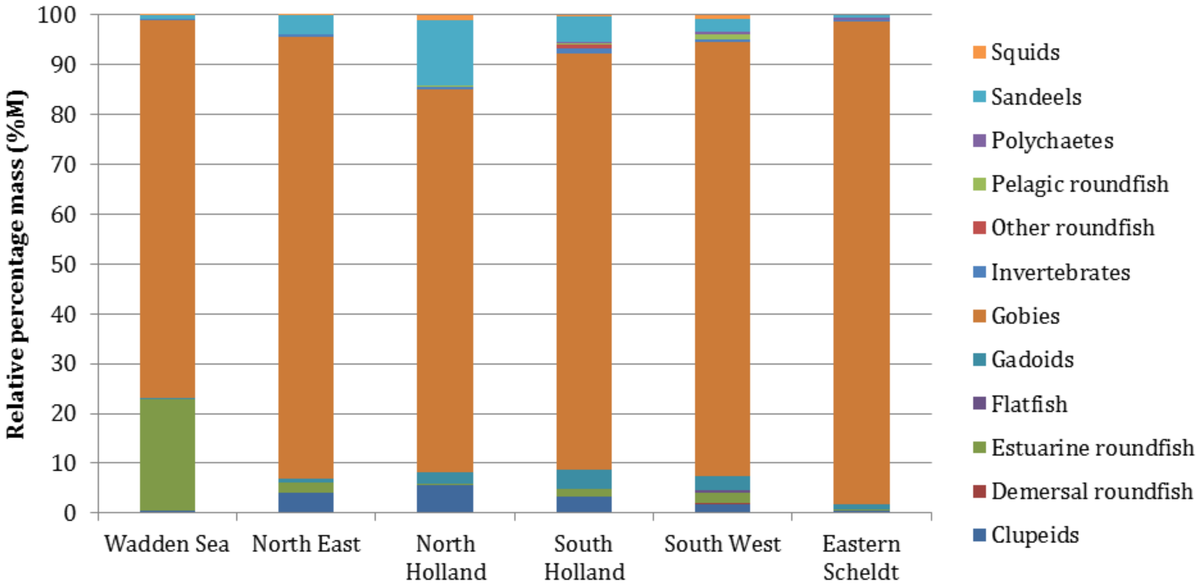

Figure 3.7. The differences in diet of harbour porpoises stranded along the Dutch coast from 2003 until 2014 in relative percentage of prey group mass (y) between the stranding locations (x) for all juvenile porpoises.

The same PERMANOVA test was done for the 134 adults porpoises. In the Wadden Sea, 5 adults stranded with a total prey mass of 4,263g, 2 adults stranded in the North East with a total prey mass of 355g, 51 adults stranded in North Holland with a total prey mass of 60,773g, 25 adults stranded in South Holland with a total prey mass of 27,045g, 45 adults stranded in South West with a total prey mass of 38,485g, and 4 adults stranded in the Eastern Scheldt with a total prey mass of 907g. Because most of the adult porpoises stranded in the locations North Holland, South Holland and South West, only these locations were taken into account for this PERMANOVA test. There is no significant influence (P=0.65) of the stranding locations North Holland, South Holland and South West on the diet of adult harbour porpoises.

To compare the regions North Holland, South Holland and South West, between adults and juveniles, the same PERMANOVA test with the same regions was done for juveniles. In North Holland 126 juveniles stranded with a total prey mass of 83,232g, in South Holland 82 juveniles stranded with a total prey mass of 40,058g and in the South West 186 juveniles stranded with a total prey mass of 77,003g. The stranding locations North Holland, South Holland and South West had a significant influence (P=0.001) on the diet of juvenile harbour porpoises, determining 2.18% of the diet.

Figure 3.8 shows the differences in diet between the regions North Holland, South Holland and South West for adult porpoises and juvenile porpoises separately. Although, a significant influence on diet between the regions and adult porpoises was not found, a difference is found in the amount of sandeels, gobies and clupeids. In North and South Holland, more sandeels were consumed, whereas in South West more gobies were consumed. Clupeids were mostly consumed in South Holland. The juvenile porpoises consumed mostly gobies in all three areas, but in North Holland, more sandeels and clupeids are consumed. In South West more estuarine roundfish is consumed and also other prey groups as pelagic roundfish and flatfish are consumed, more than in the other two areas.

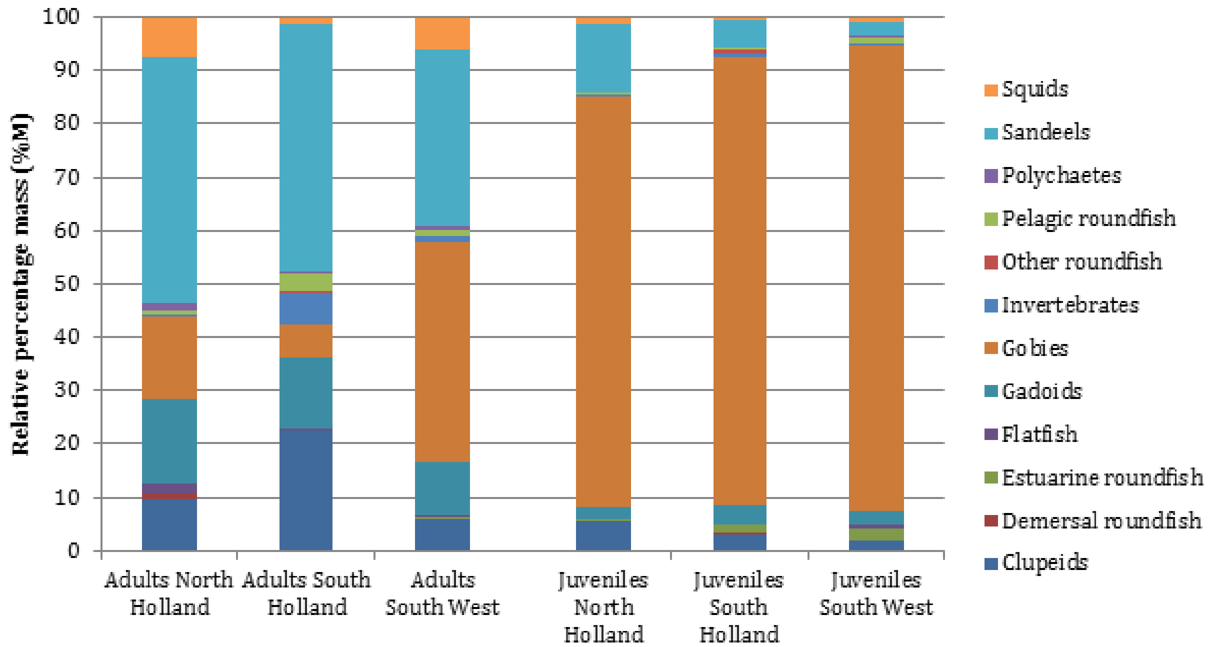

Figure 3.8. The differences in diet of harbour porpoises stranded along the Dutch coast from 2003 until 2014 in relative percentage of prey group mass (y) between adults (P=0.65) and juveniles (P=0.001) for the regions North Holland, South Holland and South west (x).

3.2.4 Stranding month

In table 3.4 the number of porpoises stranded, the total prey mass and relative percentage of prey mass is given for each month. The total prey mass of all months together is 356,975g. Of 6 porpoises the stranding month was unknown, so they were not taken into account in this PERMANOVA.

Table 3.4. The number of harbour porpoises stranded along the Dutch coast between 2003 and 2014, prey mass and relative percentage of mass per prey group for each month.

Month	N	Prey mass (g)	Relative % Mass
January	21	22,546.34	6.32
February	41	29,903.48	8.38
March	102	80,348.48	22.51
April	64	31,390.93	8.79
May	29	26,178.20	7.33
June	19	7,926.86	2.22
July	40	12,350.88	3.46
August	116	37,596.16	10.53
September	49	20,589.55	5.77
October	64	54,873.04	15.37
November	23	14,478.41	4.06
December	46	18,792.60	5.26

The PERMANOVA test revealed that stranding month had a significant influence (P=0.001) on the diet of harbour porpoises, determining 7.06% of the diet. Figure 3.9 shows the differences in relative percentage of mass per prey group between the months. The amount of clupeids is rather high in the winter months (December until March) but also in May. In March and April the amount of gobies is higher compared to the other months. The amount of sandeels is high in March, July and September, but even higher in February and May. The amount of estuarine roundfish, like smelt, is higher in April compared to the other months. The amount of pelagic roundfish, like mackerel or seabass, becomes higher in the months August and September. In September, demersal roundfish, like dragonets and gurnards, are consumed more compared to the other months. The amount of squids is highest in November. The amount of gadoids is rather high in most of the months, but less abundant in the months February until May.

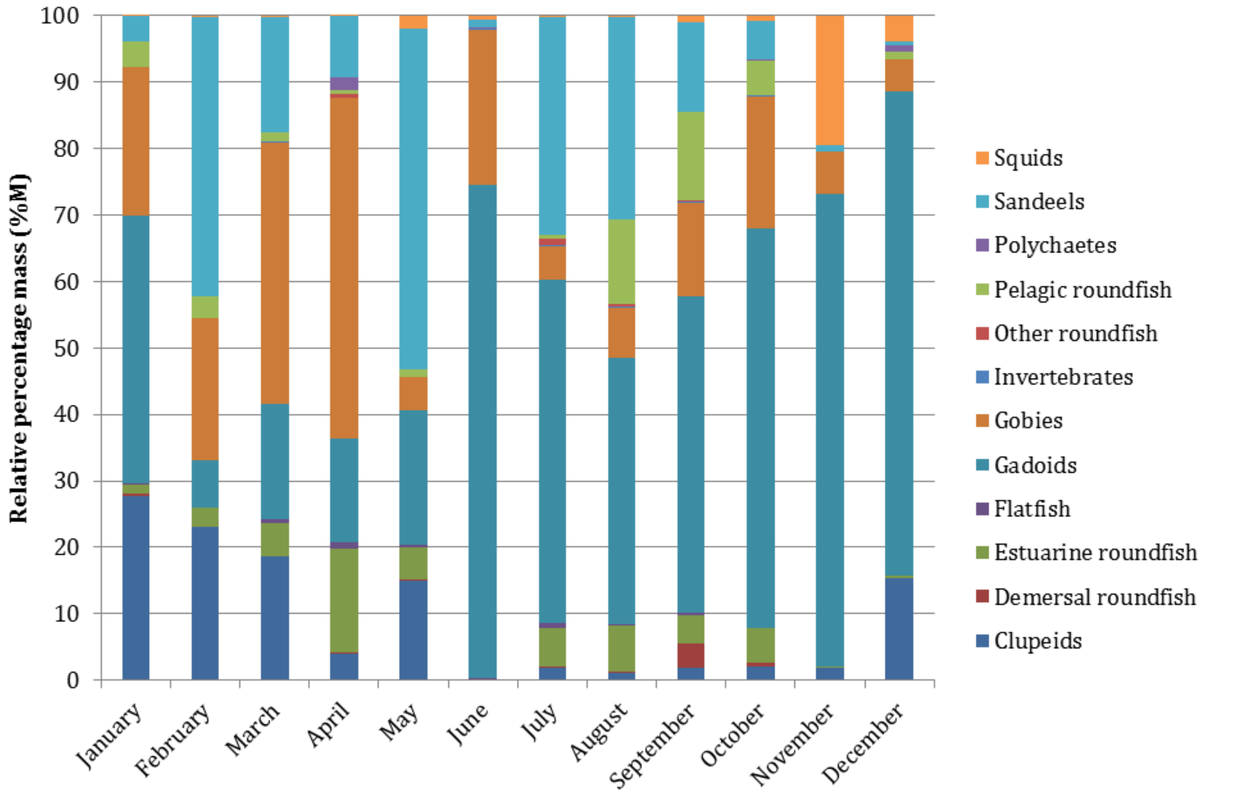

Figure 3.9. The differences diet of harbour porpoises stranded along the Dutch coast from 2003 until 2014 in relative percentage of prey group mass (y) between the months (x) for all porpoises.

3.2.5 Decomposition Condition Code (DCC)

Of all 600 porpoises, 188 were stranded while fresh with a total prey mass of 121,981g, 167 porpoises were stranded while decomposed with a total prey mass of 130,016g and 245 porpoises were stranded while very rotten with a total prey mass of 110,088g. DCC has a significant influence (P=0.001) on diet of harbour porpoises, determining 2.61% of the diet.

Figure 3.10 shows that the amount of sandeels and estuarine roundfish is higher and the amount of clupeids is lower when the porpoises are in a further state of decomposition. Porpoises in a very rotten condition show stomach contents with less gobies and gadoids, compared to porpoises stranded in a decomposed and fresh condition. Porpoises in a very rotten condition show stomach contents with more pelagic roundfish.

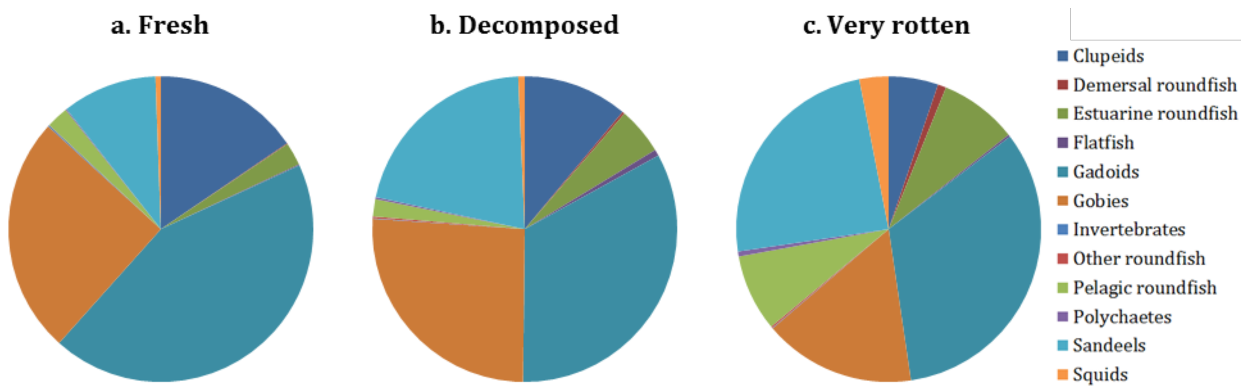

Figure 3.10. The relative percentage of mass per prey group for fresh (a), decomposed (b), and very rotten (c) harbour porpoises that stranded along the Dutch coast from 2003 until 2014.

As temperature varies during the season and may influence the speed of decomposition, a PERMANOVA was run to see if there is a difference in DCC between winter and summer months. For winter the months January until March are chosen and for summer the months July and August are chosen. In total, 164 porpoises stranded in winter of which 91 were fresh, with a total prey mass of 56,995g, 60 were decomposed with a total prey mass of 69,078g and 13 were very rotten with a total prey mass of 6,726g. In summer a total of

156 porpoises stranded of which 15 were fresh with a total prey mass of 3,334g, 40 were decomposed with a total prey mass of 16,222g and 101 were very rotten with a total prey mass of 30,392g. The PERMANOVA test showed that winter and summer had a significant influence (P=0.001) on the diet of harbour porpoises, determining 15.49% of the diet. Table 3.5 shows the prey mass and the relative percentage of prey mass per prey group per DCC for winter and summer.

Table 3.5. The mass and relative percentage of mass per prey group of harbour porpoises stranded along the Dutch coast from 2003 until 2014 per DCC for winter and summer.

	Winter					
	Fresh		Decomposed		Very rotten	
	Prey mass (g)	Relative % mass	Prey mass (g)	Relative % mass	Prey mass (g)	Relative % mass
Clupeids	15,419.36	27.05	12,494.88	18.09	133.60	1.99
Demersal roundfish	93.26	0.16	23.26	0.03	0.00	0.00
Estuarine roundfish	2,172.48	3.81	3,047.09	4.41	0.00	0.00
Flatfish	68.18	0.12	461.15	0.67	0.06	0.00
Gadoids	8,195.69	14.38	14,335.54	20.75	2,644.06	39.31
Gobies	17,590.18	30.86	23,234.73	33.64	2,165.13	32.19
Invertebrates	118.45	0.21	26.41	0.04	1.00	0.01
Other roundfish	1.19	0.00	0.96	0.00	0.00	0.00
Pelagic roundfish	2,733.47	4.80	245.12	0.35	0.39	0.01
Polychaetes	47.57	0.08	22.47	0.03	12.81	0.19
Sandeels	10,432.64	18.30	15,125.27	21.90	1,768.72	26.30
Squids	122.16	0.21	61.02	0.09	0.00	0.00
	Summer					
	Fresh		Decomposed		Very rotten	
	Prey mass (g)	Relative % mass	Prey mass (g)	Relative % mass	Prey mass (g)	Relative % mass
Clupeids	15.47	0.46	350.06	2.16	258.41	0.85
Demersal roundfish	0.00	0.00	11.98	0.07	81.04	0.27
Estuarine roundfish	156.28	4.69	817.32	5.04	2,372.71	7.81
Flatfish	0.00	0.00	82.54	0.51	92.78	0.31
Gadoids	1,827.33	54.81	4,500.74	27.75	15,136.04	49.80
Gobies	516.81	15.50	485.55	2.99	2,449.63	8.06
Invertebrates	9.47	0.28	17.21	0.11	28.33	0.09
Other roundfish	27.91	0.84	76.45	0.47	149.62	0.49
Pelagic roundfish	0.00	0.00	1,409.84	8.69	3,474.66	11.43
Polychaetes	7.54	0.23	0.28	0.00	2.09	0.01
Sandeels	759.10	22.77	8,440.27	52.03	6,293.10	20.71
Squids	13.96	0.42	29.49	0.18	53.04	0.17

Figure 3.11 shows the differences in relative prey group mass per DCC class for winter and summer. It is shown that in winter more gobies are consumed than in summer. Also clupeids are more consumed in winter, but the amount became less in porpoises in a further state of decomposition. In summer, porpoises in a decomposed condition showed stomach contents with more sandeels.

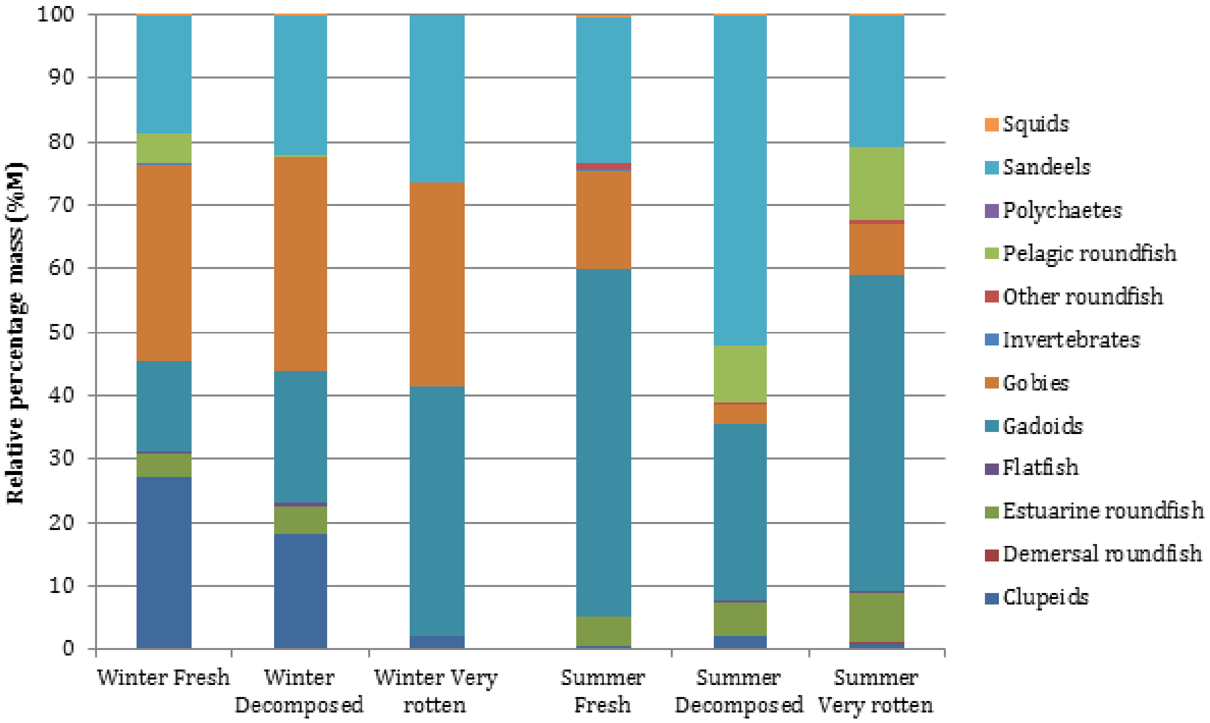

Figure 3.11. The differences in diet of harbour porpoises stranded along the Dutch coast from 2003 until 2014 in relative percentage of prey group mass (y) for DCC class between winter and summer (x) for all porpoises.

3.2.6 Nutritive Condition Code (NCC)

Of all 600 porpoises, 193 stranded in a good condition with a total prey mass of 162,676g and 193 stranded in an emaciated condition with a total prey mass of 103,313g. Of 214 porpoises the NCC class is unknown, which is why they were not taken into account in this PERMANOVA. The PERMANOVA test revealed that NCC class had a significant influence (P=0.001) on diet of harbour porpoises, determining 6.65% of the diet. The prey mass and relative percentage of mass per prey group per NCC class is shown in table 3.6.

Table 3.6. The mass and relative percentage of mass per prey group of harbour porpoises stranded along the Dutch coast from 2003 until 2014 per NCC class.

	Good condition		Emaciated		Unknown	
	Prey mass (g)	Relative % mass	Prey mass (g)	Relative % mass	Prey mass (g)	Relative % mass
Clupeids	31,111.41	19.12	5,287.91	5.12	2,722.98	2.83
Demersal roundfish	463.53	0.28	301.17	0.29	655.97	0.68
Estuarine roundfish	9,474.89	5.82	5,045.31	4.88	4,035.18	4.20
Flatfish	569.03	0.35	222.60	0.22	466.40	0.49
Gadoids	35,409.59	21.77	56,365.05	54.56	40,768.11	42.42
Gobies	37,408.67	23.00	22,250.30	21.54	22,373.29	23.28
Invertebrates	168.12	0.10	96.81	0.09	66.66	0.07
Other roundfish	103.72	0.06	297.29	0.29	81.10	0.08
Pelagic roundfish	5,060.70	3.11	2,352.32	2.28	6,743.00	7.02
Polychaetes	101.24	0.06	268.82	0.26	644.29	0.67
Sandeels	41,978.02	25.80	10,011.37	9.69	14,176.53	14.75
Squids	826.78	0.51	813.88	0.79	3,362.78	3.50

Because most of the porpoises were juveniles, and blubber thickness is known to be age related (Lockyer *et al.*, 2003[A]), the same PERMANOVA test was run for juveniles and adults separately. Of the 600 porpoises, there were 138 juveniles in a good condition, with a total prey mass of 113,283g and 143 juvenile porpoises in an emaciated condition, with a total prey mass of 49,340g. NCC had a significant influence (P=0.001) on diet of juvenile harbour porpoises, determining 10.25% of the diet.

There were 49 adults in a good condition, with a total prey mass of 49,064g and 44 adult porpoises in an emaciated condition, with a total prey mass of 53,200g. NCC had a significant influence (P=0.023) on diet of adult harbour porpoises, determining 3.54% of the diet.

Figure 3.12 gives an overview of the differences in relative percentage prey group mass between the NCC classes for all porpoises, juvenile porpoises and adult porpoises separately. For all porpoises, it is shown that the amount of clupeids and sandeels is higher for porpoises in a good condition compared to emaciated porpoises. In emaciated porpoises the amount of gadoids is very high. For juveniles, the diet of porpoises in a good condition looks similar to that of all porpoises in a good condition. The emaciated juvenile porpoises on the other hand, consumed more gobies and estuarine roundfish and less gadoids, compared to juveniles in a good condition. For adult porpoises, the difference lies in the amount of sandeels and clupeids, which were consumed more by porpoises in a good condition, and gadoids which were consumed more by porpoises in an emaciated condition.

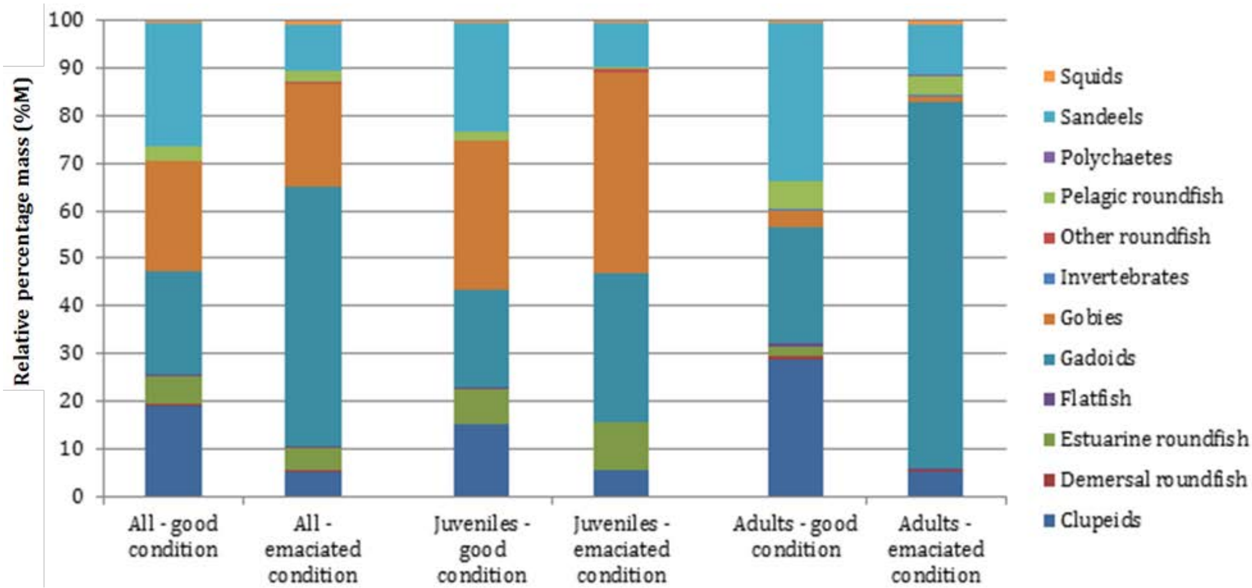

Figure 3.12. The differences in diet of harbour porpoises stranded along the Dutch coast from 2003 until 2014 in relative percentage of prey group mass (y) between NCC class, divided in all porpoises (P=0.001), juvenile porpoises (P=0.001) and adult porpoises (P=0.023) (x).

3.2.7 Stranding year

In table 3.7 the number of porpoises stranded, the total prey mass and relative percentage of prey mass is shown for the years 2006 until 2013. The total prey mass of the years 2006 until 2013 is 351,203g. In 2003 only 2 porpoises stranded, in 2005 only 3 porpoises stranded, in 2014 only 4 porpoises stranded so far with prey remains in their stomachs and of 5 porpoises the stranding year was unknown, so these were not taken into account in this PERMANOVA. Year had a significant influence (P=0.001) on diet of harbour porpoises. Figure 3.13 gives an overview of the differences in diet between the years. It is shown that the amount of gobies fluctuates throughout the years, as well as clupeids and sandeels. The amount of gadoids seems to increase from 2006 until 2008 but decreases after that. Pelagic roundfish and estuarine roundfish do not seem to have a certain trend over the years.

Table 3.7. The number of harbour porpoises stranded along the Dutch coast from 2006 until 2013, prey mass and relative percentage of mass per prey group for each year.

Month	N	Prey mass (g)	Relative % Mass
2006	51	20,019.07	5.53
2007	47	19,337.46	5.34
2008	69	61,287.96	16.93
2009	52	46,142.73	12.74
2010	35	44,685.42	12.34
2011	188	84,747.57	23.41
2012	74	32,166.34	8.88
2013	70	42,816.11	11.82

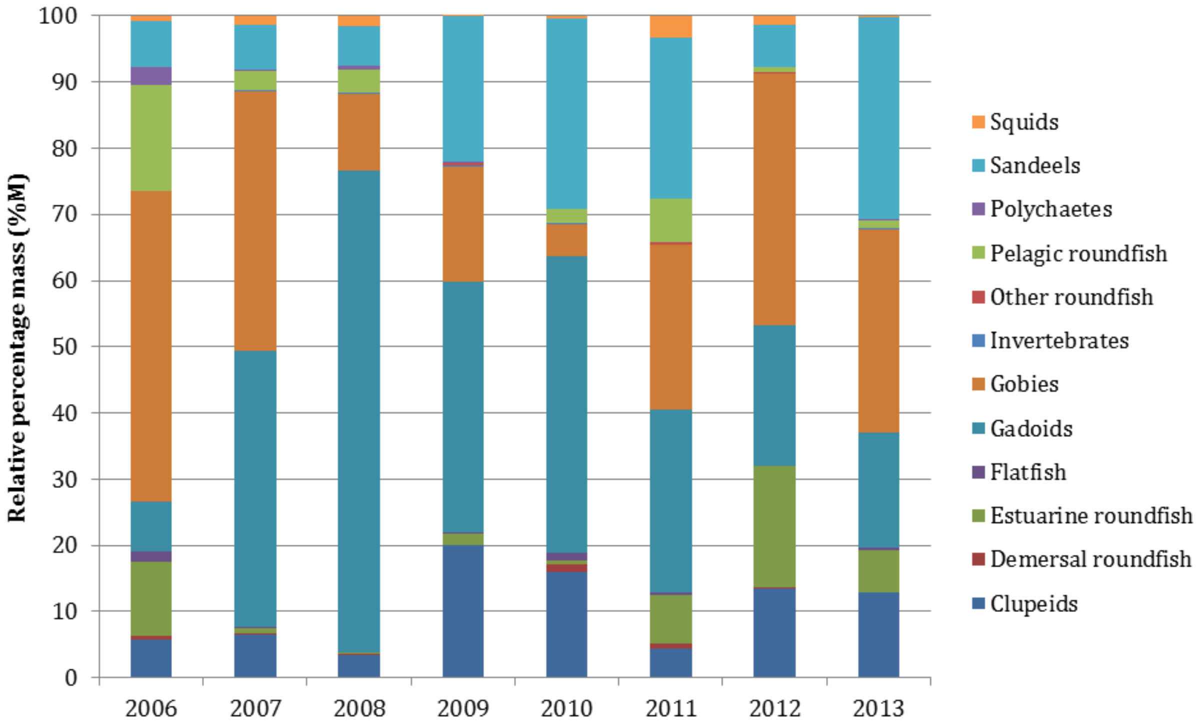

Figure 3.13. The differences in diet of harbour porpoises stranded along the Dutch coast from 2006 until 2013 in relative percentage of prey group mass (y) between the years (x) for all porpoises.

4. Discussion

This chapter describes how the methods used in this study could have affected the results. Furthermore the results are compared to similar studies.

4.1 The use of stranded and by-caught porpoises to determine diet

Using stranded porpoises for diet studies might cause an overrepresentation of porpoises that may not have been feeding normally or were sick. Kuiken *et al.* (1994) stated that the use of by-caught porpoises provides samples of 'healthy' animals. According to the study of Santos and Pierce (2003), by-caught porpoises can also create a bias towards diet, because many of these porpoises are juveniles, which might be caused by a lack of experience. Dunshea *et al.* (2013) collected faecal and gastric samples from healthy free-ranging dolphins of an extensively studied bottlenose dolphin *Tursiops aduncus* population. These samples were analysed by molecular prey detection and these data compared with stomach contents data derived from stranded bottlenose dolphins from the same population, collected over 22 years. The results from stomach content analysis and the faecal and gastric samples, showed a significant similarity. This was the first explicit test of the validity of stomach content analysis for accurate population-scale diet determination of an inshore cetacean. Other studies have found differences in diet between by-caught and stranded porpoises (Lick, 1991[B]; Aarefjord *et al*., 1995; Rogan & Berrow, 1996; Benke *et al.*, 1998), but this might be due to regional differences in prey abundance. In this study, both stranded and by-caught porpoises are used for stomach content analysis because it is still the most commonly used method and therefore most comparable to other studies. No distinction is made between these two groups.

4.2 Stomach content analysis

The pathologists at the University of Utrecht have performed necropsies on the porpoises of which the stomachs are used in this study. They have taken out the stomachs and cut them open to have a first look if the stomachs were full or empty. A disadvantage is that not all pathologists write down what they see at this first impression and it is possible that they consider a stomach as empty because small prey remains are not always visible by the naked eye. However, after rinsing these small prey remains might be found. Another disadvantage of cutting the stomachs open, is that a small part of the stomach contents can be lost, which leads to an underestimation of the number of small prey remains. In this study, these possible losses were not accounted for.

Stomach content analysis may cause an overrepresentation of prey species with large, robust hard parts. The otoliths of whiting are large, robust and very distinct. This makes them easy to identify, even if they are already affected by digestion. Otoliths of herring and sprat are more fragile and less recognizable due to digestion and decomposition. This bias may lead to an overrepresentation of whiting and an underrepresentation of species like herring and sprat (Grellier & Hammond, 2006). The assessment of accurate prey size is hampered due to gastric juices and mechanical wear. The otoliths will become progressively smaller in the acid environment of a porpoise stomach. Without a proper correction for otolith wear, it is very likely that prey size will be underestimated. It is therefore important to correct otoliths for wear in the process of estimating prey sizes (Santos *et al.*, 2004; Leopold *et al.*, 2011). Rates of digestion for different prey species or prey sizes, might also bias dietary estimates. For example, some otoliths might be digested beyond recognition faster than others. Such issues are very hard to address without experimental studies, for example with porpoises under controlled conditions in captivity. Such studies, however, have not yet been done on porpoises (Leopold *et al.*, 2011). Therefore, in this study, there could only be corrected for the otoliths which were actually found in the stomachs.

Furthermore, a distinction can be made between primary and secondary prey of harbour porpoises. Primary prey are prey eaten directly by the harbour porpoises and secondary prey are small prey species eaten by larger prey species (Pierrepoint *et al*. 2005) e.g. whiting or cod. These small prey species are secondary prey for harbour porpoises, but might be taken into account as primary prey, which can create an overrepresentation of the number of these prey. However, it is not possible to distinguish between primary and secondary prey, as it is never fully certain unless a prey remain is found in the stomach of another prey.

4.3 Caloric value of prey

Taking caloric value into account instead of mass, might give a clearer view on which prey species are more profitable to feed on. Eating 50 grams of an energy-rich prey species (such as herring), is probably more important in a porpoises' diet than eating 50 grams of an energy-poor prey species (such as whiting). Data on caloric values of prey species do exist, however, this is only one rough value per species without fish size and season taken into account. Therefore, only prey mass is taken into account in this study. There is a need for a better understanding about if variations in the caloric values of prey species occur (Leopold *et al.*, 2011). Therefore, for future studies, it might be interesting not to look at prey mass, as used in this study, but to also look at the caloric value of prey species. However, the caloric value of various prey species is likely to vary with fish size, location, season and year (Anthony, *et al.*, 2000). Only the effect of fish size on energy density can be accounted for to some extent.

4.4 Porpoise age

This study showed that the diet of calves consists mainly of gobies and small number of species from other prey groups. Juveniles still feed mainly on gobies but the amount of gadoids, sandeels and clupeids is increasing. The diet of adult harbour porpoises consists mainly of gadoids, clupeids and sandeels and the amount of gobies has strongly decreased. Larger porpoises have fed more on whiting (and other relatively big and fatty prey) in their diets, possibly due to advanced foraging skills. Alternatively, larger porpoises need considerably more fish per day and a diet of only gobies probably cannot sustain larger animals (Smith & Read, 1992; Santos *et al.*, 2004).

Differences in diet between age classes in the Netherlands have also been found in several studies. In a study of Leopold *et al.* (2011) on porpoises in Dutch waters, the amount of gobies also became progressively less in older animals. In calves, gobies were all-abundant, both numerically and in terms of prey mass. The contribution of gobies to the total prey mass decreased in juveniles and decreased further in adults. Gadoids became progressively more abundant in older animals, both in numbers and in mass. The diet of adults also became more diverse. In a large diet study from Santos (1998), it was found that in the Netherlands, adults took bigger gobies and sandeels than juveniles. The author assumed that most of these differences were related to adult porpoises feeding further offshore than juveniles. In addition, the analysis showed that in the Netherlands, smaller porpoises took fewer whiting but more gobies than older porpoises did. In the same study it showed that in Scotland, adult harbour porpoises ate bigger whiting than juveniles, while in Denmark, juveniles ate bigger viviparous blennies and whiting than adults. In two studies of Lick (1991[A, B]) and Benke and Siebert (1996) on porpoises stranded and by-caught in Germany, differences in the diet of young (<120cm) and adult porpoises were found. Young porpoises ate more gobies, while adult porpoises ate more flatfish and gadoids and had a bigger variety of prey species in their stomachs. Börjesson & Berggren (1996) also found that gobies were important by means of number in the diet of calves (1 year old) from porpoises by-caught off Swedish waters. The authors concluded that the small size of gobies could make them a suitable prey for calves. In contrast with this study, no significant difference was found between the diet of calves and adults in a study of Martin (1996). This might be due to the small sample size of calves in the study of Martin, as also only presence or absence was taken into account for prey species and not prey mass. The same accounts for the study of Smith and Gaskin (1974) where no differences were found in the diet between juvenile and adult porpoises. However, only three juvenile porpoises were used in that study, and also only presence or absence of prey species was taken into account.

4.5 Porpoise sex

This study showed that in both sexes, gobies dominated the diet by numbers. In mass, however, the amount of gobies is low. The amount of clupeids is higher in males than in females, whereas the amount of pelagic roundfish is higher in females than males. In a study by Leopold *et al.* (2011) on harbour porpoises in Dutch waters, it was found that males and females have a very similar diet. Gobies dominated the diet of either sex, but clupeids and sandeels tended to be slightly more important in males than in females, which was also found in this study. Few differences were found between the diets of male and female porpoises in Scotland and Denmark. In Scotland, male porpoises fed more on squids and had a higher overall prey diversity than females. In Denmark, female porpoises had significantly more prey in their stomachs than males (Santos, 1998). In a Canadian study from Smith and Gaskin (1974) and a study along the northern coast of Washington State from Gearin *et al.* (1994), no significant differences between sexes were found. This might be due to the small sample size and a lack of pregnant or lactating females in their studies.

In this study, juveniles were taken separately from adults which showed that there is no difference in diet between juvenile males and females. However, in adults there is a significant difference between the sexes. Adult males feed more on gadoids than adult females. Pelagic roundfish is consumed more often by adult females than by adult males. Nevertheless, it should be noted that the differences in diet of lactating and non-lactating females are not distinguished, even though it seems that differences between the diet of males and females are most likely to be seen when females are nursing calves.

4.6 Stranding location

At the Wadden Islands (except Texel), the collection of stranded harbour porpoises is very low. The Seal Rehabilitation and Research Centre in Pieterburen studies stranded porpoises on the Wadden Islands on site and the autopsy reports or stomachs are not sent to or shared with IMARES. Therefore, written reports about harbour porpoises of IMARES represent mostly porpoises stranded on Texel and the west coast of the Netherlands. This might create an underrepresentation of the locations North East and the Wadden Sea, which might also be a reason that differences in diet were found between these locations.

In this study, a significant difference was found between the six stranding locations. In the Wadden Sea, estuarine roundfish stands out most of all locations. Besides this, gobies and pelagic roundfish also play an important role for porpoises in the Wadden Sea. The diet of porpoises stranded in the North East mainly consists of gobies followed by clupeids, sandeels and gadoids. The diet of porpoises in North Holland, South Holland and the South West are quite similar. Almost half of the porpoises' diet consists of gadoids. In North Holland, more clupeids are consumed, in South Holland, more gadoids are consumed and in the South West, squids are consumed more than in the other locations. In the Eastern Scheldt, gobies are the most important prey group for porpoises.

Juvenile porpoises taken into account separately, showed that gobies are the main prey species in every location. However, in the Wadden Sea, estuarine roundfish has also an important role in the diet. In adults, a difference is shown in the amount of sandeels, gobies and clupeids. In North and South Holland, more sandeels were consumed, whereas in South West more gobies were consumed. In South Holland, clupeids were consumed more in comparison with the other locations. In this study a relatively high amount of gobies was found in porpoises stranded in the Eastern Scheldt and the diets of porpoises stranded along the North Sea coastline were remarkably similar, which was also found in a study of Leopold *et al.* (2011). In the locations North Holland, South Holland and South West, gadoids dominated the diets in terms of %mass. The porpoises stranded in the North East had a high amount of gobies and clupeids. In a study by Aarefjord *et al.* (1995) on the diet of harbour porpoises along the Norwegian, Danish and Swedish waters, a difference between regions was found, which is also found in this study.

4.7 Stranding month

This study showed that the amount of clupeids is rather high in the winter months (December until March) but also in May. In March and April the amount of gobies is higher compared to the other months. The amount of sandeels is high in March, July and September, but even higher in February and May. The amount of estuarine roundfish, like smelt, is higher in April in comparison with the other months. The amount of pelagic roundfish, like mackerel or seabass, is higher in the months August and September. In September, the amount of demersal roundfish, like dragonets and gurnards, is higher in comparison with the other months. The amount of squids is highest in November. The amount of gadoids is rather high in most of the months, but less from February until May. These differences in diet might be due to seasonal movements, prey availability and migration of fish species, which have not been taken into account in this study. Seasonal movements are believed to be related to prey availability or to breeding habitat (Gaskin, 1977; Read & Westgate, 1997). Santos and Pierce (2003) pointed out that seasonal variation in harbour porpoise distribution has been described as a general inshore movement in summer and an offshore movement in winter.

In this study it was found that the amount of sandeels was higher during spring and summer and the amount of gadoids was higher in summer, autumn and winter, which was also found in a study of Santos *et al.* (2004) on porpoise diet in Scottish waters. Furthermore, in this study it was found that the amount of gadoids reduces in the months February until May and that the amount of clupeids is high in winter. This was also found in a study on harbour porpoises along the Dutch coast by Leopold *et al.* (2011). On the contrary, Leopold *et al*. (2011) found that the amount of gobies was high in all months and the amount of sandeels was high in winter. This study showed that the amount of gobies was high in spring and the amount of sandeels varies through the year.

4.8 Decomposition Condition Code

The average decomposition code (divided into three classes; fresh, decomposed and very rotten) varied over the months. This study showed that the amount of sandeels and estuarine roundfish is higher and the amount of clupeids is lower when the porpoises were found in a further state of decomposition. Gobies are less abundant in very rotten porpoises compared to decomposed and fresh porpoises, as well as gadoids. The amount of pelagic roundfish is higher in very rotten animals. In Leopold *et al.* (2011) it is assumed that seasonal temperatures, rather than time in the waters (of distance travelled since death) governs decomposition. Therefore, the months January until March (winter) and July and August (summer) are compared. It was found that the amount of gobies was higher in winter than in summer. Also clupeids are more consumed in winter, but the amount became less in porpoises in a further state of decomposition. For the decomposed porpoises in summer, the amount of sandeels is very high compared to the other groups. These results might be biased by a mass stranding of five harbour porpoises in 2013. These stranded porpoises were all in the same state of decomposition and all had a high amount of sandeels in their stomachs. In a study of Leopold *et al.* (in prep.[B]), it was found that DCC had the same significant influence on the diet of harbour porpoises.

A report by Haelters *et al.* (2012) stated that the decomposition rate of porpoises is influenced by water temperatures. Therefore, porpoises stranded during the summer months are often greatly decomposed or falling apart or not even washed ashore. Porpoises stranded during winter months are, most of the times, found fresh. And therefore it is also possible that the porpoises died far from the coast and floated for weeks or months. The prey items in very decomposed porpoises may not originate from porpoises that had their last meal in Dutch waters. It has been demonstrated that carcasses can float in from considerable distances (Haelters *et al.,* 2006). Therefore, if choice is possible or a selection is needed, prey analysis should by preference be performed on fresh animals found in the summer.

4.9 Nutritive Condition Code

This study showed that the amount of clupeids and sandeels is higher for porpoises in a good condition compared to emaciated porpoises. In emaciated porpoises the amount of gadoids is very high. Based on these results, it can be assumed that porpoises that had not eaten fat fish besides the standard diet of gobies and whiting, had a higher probability of being emaciated. Kastelein *et al.* (1997) argued that porpoises need to feed nearly constantly and cannot survive prolonged periods of fasting. They need a relatively large amount of food per day relative to their body weight. With their small body size and thin but essential blubber layer, they are quite vulnerable to starvation (Koopman *et al.,* 2002; Bjørge, 2003).

Because blubber thickness is related to porpoise age (Koopman, 1998; Leopold & Camphuysen, 2006), juveniles were considered separately, as these comprised the largest, and most homogenous sample. This showed that juvenile porpoises in good condition had eaten more sandeels and herring, which are known to be fatty fish. On the contrary, emaciated juvenile porpoises had consumed more gobies and gadoids, which are known to be leaner fish. The same results were found in a study by Haelters *et al*., (2012) on the diet of harbour porpoises along the Belgium coast. That study stated that the blubber layer of porpoises also serves as an energy storage. Therefore, it becomes thinner in animals failing to get enough food. However, there is also a seasonal aspect, with a blubber layer becoming thicker in the colder seasons, as was demonstrated by Lockyer *et al.* (2003[B]) in two captive harbour porpoises. In another study on the diet of harbour porpoises along the Dutch coast of Leopold *et al.* (2011), it was also found that juveniles in good condition tended to have more clupeids and sandeels in their diet than emaciated porpoises. Another study of Leopold *et al.* (in prep.[A]) mentions the 'junkfood hypothesis', which states that porpoises might starve by eating junk food: other, leaner prey than they should be taking in order to maintain a good body condition.

4.10 Stranding year

In this study an interesting interannual variation in diet was found. The amount of gobies fluctuates throughout the years, as well as clupeids and sandeels. The amount of gadoids seemed to increase from 2006 until 2008 but has decreased since. The amount of pelagic and estuarine roundfish do not seem to have a certain trend over the years. This was also found in another study on the diet of harbour porpoises along the Dutch coast of Leopold *et al.* (2011). However, this dietary difference over the years might be due to a different number of available stomachs over the years or a fluctuation of prey availability.

In a diet study on porpoises in Scottish waters from Santos *et al.* (2004), it was found that only clupeids showed significant interannual variation in abundance in porpoise diet. In another study from Santos (1998) on the diet of harbour porpoises in the northeast Atlantic, it was found that in terms of amounts eaten, significant interannual variation was found only for herring. The significance of this variation was strongly influenced by a single porpoise killed by bottlenose dolphins in the Moray Firth in November 1994. It was assumed that the interannual changes were unrelated to changes in herring abundance. However, it is worth noting that of the three main studies on porpoise diet in UK waters, only the earliest (Rae, 1965, 1973) records herring as of major importance in the diet, and this change could reflect the decline in herring abundance in the North Sea since the 1960s.

5. Conclusion

The primary objective of this study was to give a detailed description of the harbour porpoises' diet composition in Dutch waters, considering the following covariates: age, sex, stranding location, stranding month, decomposition condition and nutritive condition and year. For each variable the results are given concerning their influence on the porpoises' diet.

Age
This study showed that the diet of calves mainly consists out of gobies and small numbers of species from other prey groups. Juveniles still feed mainly on gobies but the amount of gadoids, sandeels and clupeids is increasing. The diet of adult harbour porpoises consists mainly of gadoids, clupeids and sandeels and the amount of gobies has strongly decreased. Larger porpoises include more whiting (and other relatively profitable prey) in their diets, possibly helped by their greater foraging skills. Alternatively, larger porpoises need considerably more fish per day and a diet of only gobies probably cannot sustain larger animals.

Sex
In both sexes, gobies dominated the diet in number but not in mass. The amount of clupeids is higher in males than in females, whereas the amount of pelagic roundfish is higher in females than in males. There is also a significant difference between the sexes if adults are considered separately. Adult males feed more on gadoids in comparison to adult females. Pelagic roundfish is more often consumed by adult females in comparison to adult males. Nevertheless, it should be noted that the differences in diet of lactating and non-lactating females are not distinguished, even though it seems that differences between the diet of males and females are most likely to be seen when females are nursing calves.

Stranding location
There is a significant influence of stranding location on the diet of harbour porpoises. Estuarine roundfish is consumed mostly by porpoises stranded in the Wadden Sea. Gobies and pelagic roundfish also play an important role for porpoises in the Wadden Sea. The diet of porpoises stranded in the North East mainly consists of gobies followed by clupeids, sandeels and gadoids. However, at the Wadden Islands (except Texel), the collection of stranded harbour porpoises is very low. The Seal Rehabilitation and Research Centre in Pieterburen studies stranded porpoises on the Wadden Islands on site and the autopsy reports or stomachs are not sent to or shared with IMARES. Therefore, written reports about harbour porpoises of IMARES represent mostly the porpoises stranded on Texel and the west coast of the Netherlands. This might create an underrepresentation of the locations North East and the Wadden Sea, which might also be a reason that differences in diet were found between these locations. The diet of porpoises in North Holland, South Holland and the South West are quite similar, with almost half of the porpoise diet consisting of gadoids. In North Holland, more clupeids are consumed; in South Holland, more gadoids are consumed; and in the South West, squids are consumed more than in the other locations. In the Eastern Scheldt, gobies are the most eaten prey group for porpoises. If only juveniles are taken into account, it showed that in all locations, mainly gobies are consumed, but in the Wadden Sea also estuarine roundfish has an important role in their diet. In North Holland, more sandeels and clupeids are consumed. In South West more estuarine roundfish is consumed and also other prey groups as pelagic roundfish and flatfish are consumed. In the Eastern Scheldt, mainly gobies are consumed.

Stranding month
The amount of clupeids is rather high in the winter months (December until March) but also in May. In March and April the amount of gobies is higher in comparison with the other months. The amount of sandeels is high in March, July and September, but even higher in February and May. The amount of estuarine roundfish, like smelt, is higher in April in comparison with the other months. Pelagic roundfish, like mackerel or seabass, becomes more abundant in the months August and September. In September, demersal roundfish, like dragonets and gurnards, are consumed more compared to the other months. The amount of squids is highest in November. The amount of gadoids is rather high in most of the months, but of less from February until May. These differences in diet might be due to seasonal movements, prey availability and migration of fish species.

Decomposition condition
The amount of sandeels and estuarine roundfish is higher and the amount of clupeids is lower when the porpoises are in a further state of decomposition. Porpoises in a very rotten condition show stomach contents with less gobies and gadoids, compared to porpoises stranded in a decomposed and fresh condition.

Porpoises in a very rotten condition show stomach contents with more pelagic roundfish. It is shown that in winter more gobies are consumed than in summer. Also clupeids are more consumed in winter, but the amount became less in porpoises in a further state of decomposition. Porpoises in a decomposed condition show stomach contents with more sandeels. However, this might be due to a mass stranding of five harbour porpoises, which were in the same state of decomposition in 2013 and had all been feeding on sandeels. However, it is stated that the decomposition rate of porpoises is influenced by water temperatures. Therefore, porpoises stranded during the summer months are often greatly decomposed or falling apart or are not even washed ashore. Porpoises stranded during winter months are, most of the times, found fresh. However, there is also the possibility that the porpoises died far from the coast and floated for weeks or months. Therefore, the prey items in very decomposed animals may not originate from animals that had their last meal in Dutch waters. It has been demonstrated that carcasses can float in from considerable distances. Therefore, if choice is possible or a selection is needed, prey analysis should by preference be performed on fresh animals found in summer.

Nutritive condition

For all porpoises, it is shown that the amount of clupeids and sandeels is higher when they were in a good condition compared those in an emaciated condition. In emaciated porpoises the amount of gadoids is very high. For juveniles, the diet of porpoises in a good condition looks similar to that of all porpoises in a good condition. Emaciated juvenile porpoises on the other hand, consumed more gobies and estuarine roundfish and less gadoids, compared to juveniles in a good condition. For adult porpoises, the difference lies in the amount of sandeels and clupeids, which were consumed more by porpoises in a good condition, and gadoids which were consumed more by porpoises in an emaciated condition. Based on these results, it can be assumed that porpoises that had not eaten fat fish besides the standard diet of gobies and whiting had a higher probability of emaciation. It can also be assumed that the blubber layer becomes thinner in animals failing to get enough food. These results confirm the ‘junkfood hypothesis’. Results showed that there was a significant influence of nutritive condition on diet between porpoises in a good body condition and porpoises in an emaciated body condition. However, there is also a seasonal aspect, with a blubber layer becoming thicker in the colder seasons, which could have caused a bias in the NCC results.

Year

It is shown that the amount of gobies fluctuates throughout the years, as well as clupeids and sandeels. The amount of gadoids seems to increase from 2006 until 2008 but decreases after that. Pelagic and estuarine roundfish do not seem to have a certain trend over the years. However, this dietary difference over the years might be due to a different amount of available stomachs and prey availability over the years.

6. Recommendations

The harbour porpoise is one of the smallest cetacean and its habitat and life history impose very high energy demands. Understanding its distribution in relation to its environment, especially its prey, is vital for the conservation of the species.

The effect of the various possible factors influencing porpoise diet (age, sex, stranding location, stranding date, DCC, NCC and year) are to this day still difficult to disentangle. In this study, the influence of all these factors on the diet of harbour porpoises were tested separately, because there was no statistical test available to test the influence of these factors altogether. It is recommended, whenever such a test will be available, to test the influences of these factors again so a clearer view on the diet of porpoises will be created.

In this study, prey mass is used to determine diet. It would be better to calculate the caloric contents of prey species and use this to determine diet, because this would give a more accurate result of the dietary needs of harbour porpoises and would explain why en when they eat certain prey species. However, there is not much known about the caloric contents of fish and other prey species. It is very likely that, for example, the caloric content of a juvenile herring contains fewer calories than an adult herring and that a herring in winter contains fewer calories than a herring in spring (when they reproduce). When more information is available about the caloric contents of porpoises' prey species, it is recommended to study and determine diet according to caloric contents of prey species instead of mass of prey species.

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APPENDIX I Prey species by name

Prey group	Species	Species (Latin)
Clupeids	Sprat Herring	*Sprattus sprattus* *Clupea harengus*
Demersal roundfish	Dragonet Reticulated dragonet Hooknose Grey gurnard Tub gurnard Viviparous blenny Butterfish Lesser weaver	*Callionymus lyra* *Callionymus reticulatus* *Agonis cataphractus* *Eutrigla gurnardus* *Trigla lucerna* *Zoarces viviparous* *Pholis gunnellus* *Echiichthys vipera*
Estuarine roundfish	Smelt Sand smelt European perch Golden grey mullet Roach Ruffe Twaite shad	*Osmerus eperlanus* *Atherina presbyter* *Perca fluviatilis* *Liza aurata* *Rutilus rutilus* *Gymnocephalus cernuus* *Alosa fallax*
Flatfish	Common sole Solenette Plaice Common dab Long rough dab Turbot Flounder Flatfish spec.	*Solea solea* *Buglossidium luteum* *Pleuronectes platessa* *Limanda limanda* *Hippoglossoides platessoides* *Psetta maxima* *Platichthys flesus* *Pleuronectidae spp.*
Gadoids	Whiting Atlantic cod Poor cod Bib Five-bearded rockling	*Merlangius merlangus* *Gadus morhua* *Trisopterus minutus* *Trisopterus luscus* *Ciliata mustela*
Gobies	Sand goby Lozano's goby Goby spec. Common goby Black goby Transparent goby Two-spot goby Painted goby	*Pomatoschistus minutus* *Pomatoschistus lozanoi* *Pomatoschistus spp.* *Pomatoschistus microps* *Gobius niger* *Aphia minuta* *Gobiusculus flavescens* *Pomatoschistus pictus*
Invertebrates	Brown shrimp Arch-fronted swimming crab Bristly crab Bryer's nut crab Common shore crab Dwarf swimming crab Swimming crab Hermit crab Thumbnail crab Gammarid spec. Crustacean spec. Blue mussel American razorclam	*Crangon crangon* *Liocarcinus arcuatus* *Pilumnus hirtellus* *Ebalia tumefacta* *Carcinus maenas* *Liocarcinus pusillus* *Liocarcinus holsatus* *Pagurus bernhardus* *Thia scutellata* *Gammarus spp.* *Crustacea spp.* *Mytilus edulis* *Ensis directus*
Other roundfish	Lesser pipefish Greater pipefish Sea lamprey	*Syngnathus rostellatus* *Syngnathus acus* *Petromyzon marinus*
Pelagic roundfish	Atlantic mackerel Atlantic horse mackerel European seabass	*Scomber scombrus* *Trachurus trachurus* *Dicentrarchus labrax*
Polychaetes	Euneris longissima King ragworm Common clam worm	*Euneris longissima* *Nereis virens* *Nereis succinea*
Sandeels	Small sandeel Lesser sandeel	*Ammodytes tobianus* *Ammodytes marinus*

	Sandeel spec. Greater sandeel	*Ammodytes spp.* *Hyperoplus lanceolatus*
Squid	Veined squid Stout bobtail Common bobtail European common squid Atlantic bobtail Squid spec.	*Loligo forbesi* *Rossia macrosoma* *Sepietta oweniana* *Allotheutis subulata* *Sepiola atlantica* *Sepiolidae spp.*

APPENDIX II Regression formulas

Fish species	Regression	
	Formula	**X range**
Herring (*Clupea harengus*)	FL = -1.93 + 6.29 * OL FL = -6.36 + 15.51 * OW $M = (0.18 * FL)^{3.11}$	0.5-5.5 0.4-2.7 4.0-31.5
Sprat (*Sprattus sprattus*)	FL = 0.00 + 6.87 * OL FL = -1.41 + 11.92 * OW $M = (0.18 * FL)^{3.78}$	1.1-2.3 0.8-1.5 6.8-15.0
Fint (*Alosa fallax*)	FL = -12.11 + 13.74 * OL FL = -14.61 + 26.50 * OW $M = (0.19 * FL)^{3.05}$	1.3-4.5 0.8-2.5 8.1-49.0
Sandeel (*Osmerus eperlanus*)	FL = -1.63 + 3.97 * OL FL = -4.29 + 7.51 * OW $M = (0.17 * FL)^{3.40}$	0.9-7.4 0.8-4.2 4.2-28.1
Cod (*Gadus morhua*)	FL = -6.64 + 3.49 * OL FL = -5.51 + 7.84 * OW $M = (0.19 * FL)^{3.26}$	2.5-18.4 1.1-8.6 6.0-34.2
Whiting (*Merlangius merlangus*)	FL = 0.81 + 1.73 * OL FL = -2.97 + 6.74 * OW $M = (0.19 * FL)^{3.09}$	1.2-21.8 0.6-5.7 3.0-37.7
Poor cod (*Trisopterus minutus*)	FL = -3.84 + 0.05 * OL FL = -2.98 + 5.22 * OW $M = (0.21 * FL)^{3.10}$ FL = 60.480 * VL + 13.51	2.7-9.4 1.4-4.5 4.9-21.5
Bib (*Trisopterus luscus*)	FL = -5.40 + 2.99 * OL FL = -3.21 + 5.82 * OW $M = (0.21 * FL)^{3.23}$	1.2-11.9 0.7-6.7 2.3-33.9
Sand smelt (Atherina presbyter)	FL = 0.00 + 3.11 * OL FL = -0.97 + 4.99 * OW $M = (0.18 * FL)^{3.17}$	2.5-4.8 1.7-3.1 7.3-15.3
Pipefish (*Syngnathus rostellatus*)	FL = 0.00 + 42.86 * OL FL = -4.91 + 81.86 * OW $M = (0.07 * FL)^{3.99}$	
Earopean seabass (*Dicentrarchus labrax*)	FL = -4.20 + 3.51 * OL FL = -9.88 + 8.61 * OW $M = (0.21 * FL)^{3.00}$	2.8-15.1 1.6-6.5 6.9-53.7
European perch (*Perca fluviatilis*)	FL = -2.54 + 3.44 * OL FL = -3.21 + 7.30 * OW $M = (0.20 * FL)^{3.44}$	2.4-11.4 1.3-6.1 5.6-40.4
Horse mackerel (*Trachurus trachurus*)	FL = -0.90 + 3.29 * OL FL = -3.10 + 7.67 * OW $M = (0.21 * FL)^{2.97}$	0.4-11.5 0.3-5.7 1.6-39.0
Lesser sandeel (*Ammodytes tobianus*)	FL = 1.16 + 5.00 * OL FL = 0.00 + 10.92 * OW $M = (0.13 * FL)^{3.46}$	0.7-4.1 0.4-1.9 4.6-20.2
Raitt’s sandeel (*Ammodytes marinus*)	FL = 2.10 + 4.91 * OL FL = 0.00 + 11.46 * OW $M = (0.13 * FL)^{3.37}$	1.4-2.9 0.8-1.6 9.0-17.4
Greater sandeel (*Hyperoplus lanceolatus*)	FL = -2.56 + 6.80 * OL FL = -5.81 + 16.80 * OW $M = (0.14 * FL)^{2.93}$	1.3-5.5 0.7-2.6 7.6-36.2
Common dragonet (*Callionymus lyra*)	FL = -5.48 + 8.41 * OL FL = -5.19 + 17.33 * OW $M = (0.19 * FL)^{2.96}$	0.9-3.7 0.5-1.9 3.3-26.9
Sand goby (*Pomatoschistus minutus*)	FL = -0.43 + 3.92 * OL FL = -1.74 + 5.27 * OW $M = (0.21 * FL)^{2.83}$	0.9-2.6 0.9-2.1 2.8-9.3
Lozano’s goby (*Pomatoschistus lozanoi*)	FL = 0.00 + 3.83 * OL FL = 0.00 + 3.94 * OW $M = (0.19 * FL)^{2.78}$	0.7-1.7 0.8-1.5 2.7-6.2

Atlantic Mackerel (*Scomber scombrus*)	FL = 0.00 + 8.09 * OL FL = -6.48 + 26.35 * OW $M = (0.22 * FL)^{2.85}$	2.3-5.4 0.9-1.9 18.0-46.0
Plaice (*Pleuronectes platessa*)	FL = -2.07 + 4.85 * OL FL = -4.70 + 8.15 * OW $M = (0.22 * FL)^{3.02}$	0.5-9.6 0.4-5.8 1.8-44.5
European flounder (*Platichthys flesus*)	FL = -3.65 + 5.61 * OL FL = -5.77 + 9.61 * OW $M = (0.22 * FL)^{3.00}$	1.0-7.6 0.7-5.0 3.6-40.9
Dab (*Limanda limanda*)	FL = -3.49 + 5.43 * OL FL = -5.40 + 8.88 * OW $M = (0.22 * FL)^{3.00}$	0.7-7.1 0.5-4.1 2.2-30.5
Common sole (*Solea solea*)	FL = -2.65 + 8.18 * OL FL = -4.72 + 10.32 * OW $M = (0.20 * FL)^{3.05}$	0.6-5.9 0.5-4.6 2.8-42.9
Brown shrimp (*Crangon crangon*)	SL = 0.73 + 1.60 * CL $M = 0.00699 * SL^{3.326}$	

Appendix III PERMANOVA Results

PERMANOVA results belongs to paragraph 3.2.1

Table a. Resemblance matrix with Bray-Curtis similarity considering diet and age class.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Ag	2	72116	36058	11.78	0.001	999
Res	594	1.818E6	3060.7			
Total	596	1.8902E6				

Source	Estimate	Sq. root
S (Ag)	277.4	16.655
V (Res)	3060.7	55.323
%	9.06	

Table b. Resemblance matrix with Bray-Curtis similarity considering diet and age class from June until December.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Ag	2	50211	25105	7.8125	0.001	999
Res	334	1.0733E6	3213.5			
Total	336	1.1235E6				

Source	Estimate	Sq. root
S (Ag)	280.83	16.758
V (Res)	3213.5	56.688
%	8.74	

PERMANOVA results belongs to paragraph 3.2.2

Table c. Resemblance matrix with Bray-Curtis similarity considering diet and sex.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Se	1	8933	8933	2.8227	0.018	999
Res	584	1.8482E6	3164.8			
Total	585	1.8571E6				

Source	Estimate	Sq. root
S (Se)	20.042	4.476
V (Res)	3164.8	56.256
%	0.63	

Table d. Resemblance matrix with Bray-Curtis similarity considering diet of juveniles and sex.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Se	1	2997.2	2997.2	0.97835	0.408	998
Res	433	1.3265E6	3063.5			
Total	434	1.3295E6				

Source	Estimate	Sq. root
S (Se)	-0.32093	-0.56651
V (Res)	3063.5	55.349
%	0.01	

Table e. Resemblance matrix with Bray-Curtis similarity considering diet of adults and sex.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Se	1	9347.9	9347.9	3.0982	0.012	999
Res	132	3.9827E5	3017.2			
Total	133	4.0762E5				

Source	Estimate	Sq. root
S (Se)	97.619	9.8802
V (Res)	3017.2	54.929
%	3.24	

PERMANOVA results belongs to paragraph 3.2.3

Table f. Resemblance matrix with Bray-Curtis similarity considering diet and stranding location.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Re	6	45424	7570.6	2.4213	0.001	998
Res	590	1.8447E6	3126.7			
Total	596	1.8902E6				

Source	Estimate	Sq. root
S (Re)	62.581	7.9108
V (Res)	3126.7	55.917
%	2.00	

Table g. Resemblance matrix with Bray-Curtis similarity considering diet of juveniles and stranding location.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Re	6	42691	7115.1	2.3736	0.001	999
Res	438	1.313E6	2997.6			
Total	444	1.3557E6				

Source	Estimate	Sq. root
S (Re)	78.518	8.861
V (Res)	2997.6	54.751
%	2.62	

Table h. Resemblance matrix with Bray-Curtis similarity considering diet of adults and the stranding locations North Holland, South Holland and South West.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Re	2	4744.4	2372.2	0.77792	0.65	998
Res	118	3.5983E5	3049.4			
Total	120	3.6458E5				

Source	Estimate	Sq. root
S (Re)	-17.453	-4.1777
V (Res)	3049.4	55.221
%	0.00	

Table i. Resemblance matrix with Bray-Curtis similarity considering diet of juveniles and the stranding locations North Holland, South Holland and South West.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Re	2	22175	11087	3.6572	0.001	999
Res	380	1.152E6	3031.6			
Total	382	1.1742E6				

Source	Estimate	Sq. root
S (Re)	66.093	8.1298
V (Res)	3031.6	55.06
%	2.18	

PERMANOVA results belongs to paragraph 3.2.4

Table j. Resemblance matrix with Bray-Curtis similarity considering diet and the time of year in which the porpoises stranded.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Mo	11	1.4417E5	13106	4.3678	0.001	998
Res	581	1.7434E6	3000.7			
Total	592	1.8876E6				

Source	Estimate	Sq. root
S (Mo)	211.78	14.553
V (Res)	3000.7	54.778
%	7.06%	

PERMANOVA results belongs to paragraph 3.2.5

Table k. Resemblance matrix with Bray-Curtis similarity considering diet and DCC.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
DC	2	38242	19121	6.1331	0.001	999
Res	594	1.8519E6	3117.7			
Total	596	1.8902E6				

Source	Estimate	Sq. root
S (DC)	81.484	9.0268
V (Res)	3117.7	55.836
%	2.61	

Table l. Resemblance matrix with Bray-Curtis similarity considering diet and DCC.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
Se	1	75765	75765	25.773	0.001	998
Res	318	9.3483E5	2939.7			
Total	319	1.0106E6				

Source	Estimate	Sq. root
S (Se)	455.44	21.341
V (Res)	2939.7	54.219
%	15.49	

PERMANOVA results belongs to paragraph 3.2.6

Table m. Resemblance matrix with Bray-Curtis similarity considering diet and NCC.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
NC	1	42220	42220	13.832	0.001	999
Res	384	1.1721E6	3052.5			
Total	385	1.2144E6				

Source	Estimate	Sq. root
S (NC)	202.94	14.246
V (Res)	3052.5	55.249
%	6.65	

Table n. Resemblance matrix with Bray-Curtis similarity considering diet and NCC of only juveniles.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
NC	1	44326	44326	15.39	0.001	999
Res	279	8.0357E5	2880.2			
Total	280	8.479E5				

Source	Estimate	Sq. root
S (NC)	295.08	17.178
V (Res)	2880.2	53.667
%	10.25	

Table o. Resemblance matrix with Bray-Curtis similarity considering diet and NCC of only adults.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
NC	1	7635.1	7635.1	2.6373	0.023	999
Res	91	2.6345E5	2895			
Total	92	2.7108E5				

Source	Estimate	Sq. root
S (NC)	102.23	10.111
V (Res)	2895	53.805
%	3.53	

PERMANOVA results belongs to paragraph 3.2.7

Table p. Resemblance matrix with Bray-Curtis similarity considering diet and year.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
NC	7	69696	9956.6	3.2105	0.001	996
Res	578	1.7925E6	3101.2			
Total	585	1.8622E6				

Source	Estimate	Sq. root
S (NC)	98.955	9.9476
V (Res)	3101.2	55.689
%	3.19	