

# FISHING FOR FOOD

Feeding ecology of harbour porpoises *Phocoena phocoena* and white-beaked dolphins *Lagenorhynchus albirostris* in Dutch waters



Okka Eike Jansen



# FISHING FOR FOOD

Feeding ecology of harbour porpoises *Phocoena phocoena* and white-beaked dolphins *Lagenorhynchus albirostris* in Dutch waters

Okka Eike Jansen

## **Thesis committee**

### **Promotor**

Prof.dr.ir. P.J.H. Reijnders  
Professor of Ecology and Management of Marine Mammals  
Wageningen University

### **Co-promotor**

Prof.dr. M. Scheffer  
Professor of Aquatic Ecology and Water Quality Management  
Wageningen University

### **Other members**

Prof.dr. J.B.M. Middelburg, Utrecht University  
Prof.dr. A.D. Rijnsdorp, Wageningen University  
Prof.dr. U. Siebert, University of Veterinary Medicine, Hannover, Germany  
Dr. C. Smeenk, Naturalis, Leiden

This research was conducted under the auspices of the Netherlands Research School for the Socio-Economic and Natural Sciences of the Environment (SENSE).



# FISHING FOR FOOD

Feeding ecology of harbour porpoises *Phocoena phocoena* and white-beaked dolphins *Lagenorhynchus albirostris* in Dutch waters

Okka Eike Jansen

## **Thesis**

submitted in fulfilment of the requirements for the degree of doctor  
at Wageningen University  
by the authority of the Rector Magnificus  
Prof. dr M.J. Kropff,  
in the presence of the  
Thesis Committee appointed by the Academic Board  
to be defended in public  
on Friday 8 March 2013  
at 4 p.m. in the Aula.

Okka Eike Jansen

Fishing for Food, Feeding ecology of harbour porpoises *Phocoena phocoena* and white-beaked dolphins *Lagenorhynchus albirostris* in Dutch waters,  
174 pages.

PhD thesis, Wageningen University, Wageningen, NL (2013)

With references, with summaries in Dutch and English

ISBN 987-94-6173-422-8

# CONTENTS

## Chapter 1

Introduction..... 6

## Chapter 2

Feeding ecology of harbour porpoises: Stable isotope analysis of carbon and nitrogen in muscle and bone ..... 18

## Chapter 3

Diet of harbour porpoises along the Dutch coast: A combined stable isotope and stomach contents approach ..... 38

## Chapter 4

Harbour porpoises *Phocoena phocoena* in the Eastern Scheldt: A resident stock or trapped by a storm surge barrier? ..... 54

## Chapter 5

The diet of harbour porpoises along the Dutch coast based on QFASA analysis: A combined fatty acid and stomach contents approach ..... 66

## Chapter 6

Are white-beaked dolphins *Lagenorhynchus albirostris* food specialists? Their diet in the southern North Sea ..... 86

## Chapter 7

Learning to eat: Juvenile white-beaked dolphins *Lagenorhynchus albirostris* take different prey than older individuals ..... 102

## Chapter 8

Synthesis..... 118

## Addendum

References ..... 138

Summary ..... 158

Nederlandse samenvatting ..... 161

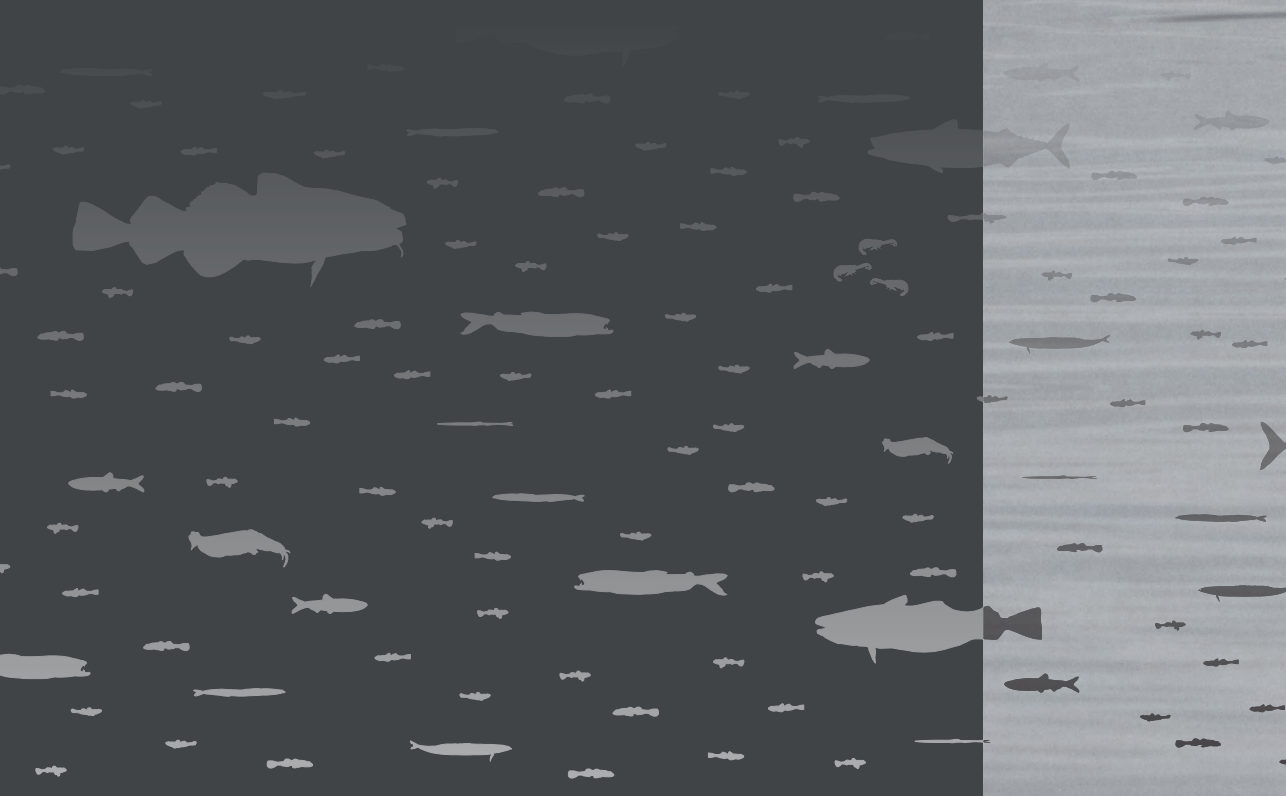
Publications ..... 166

ITSP (opleidingsplan) ..... 168

Acknowledgements..... 170

# CHAPTER 1

## INTRODUCTION





## 1.1 PROBLEM DEFINITION

Shelf waters are rich marine ecosystems. World-wide, major changes in number and distribution of species have occurred and raised concern about the state and stability of these ecosystems (Worm et al. 2006). Questions are arising as to what factors are causing these changes. Understanding these factors and underlying mechanisms and the adaptability of species can enable us to improve our conservation measures as the environments change.

As a contribution to such understanding I studied the feeding ecology of porpoises, a sentinel species in many coastal marine systems. The diet of marine mammals is fundamental to understand their ecology and their role and function in the marine ecosystem. It serves to investigate scientific questions on feeding strategy, predator-prey relationships, and responses of marine mammals to changes in food web dynamics, thereby delivering insight in functional relationships to community structure and ultimately state and stability of the ecosystem they inhabit. In that way marine mammals can be regarded as sentinel species for the state of the ecosystem they live in and can contribute to the assessment of e.g. the responses of the system to a topical issue such as climate change or fishery interactions (Moore 2008; Bossart 2011). Apart from the direct competition for resources, marine mammals and fisheries can have an impact on each other, due to by-catch of marine mammals during fishery activities or the damage of fishing gear. The effect of by-catch on marine mammal populations is relatively well documented (Northridge 1991; Matthiopoulos et al. 2008), while studying the competition for resources remains difficult due to problems in quantifying marine mammal diets that are often variable in space and time.

In this thesis, the focus lies on the ecosystem of the north-eastern Atlantic, in particular of the North Sea. Large changes have been documented in the abundance and distribution of marine mammals in this area. Quantitative abundance estimates of marine mammals in this area have only recently been done (Hammond et al. 2002; Hammond 2006), therefore earlier trends in abundance are mainly based on archived and anecdotal documentation of strandings. Strandings of marine mammals are usually rather well documented in densely populated areas and can yield information on species diversity as well as local occurrence and densities of marine mammals in the area (Pyenson 2010; Peltier et al. 2012). Dedicated surveys can be used to obtain information on changes in abundance and distribution of marine mammals. Stranded animals provide ample opportunity to study a whole suite of parameters, including diet.

In the North Sea, distinct changes in stranding frequencies of several cetacean species have occurred during the twentieth century. On the Dutch coast (Figure 1.1), numbers of stranded harbour porpoises *Phocoena phocoena* increased gradually again since the 1990s, and showed an abrupt increase in 2006





Figure 1.1 Location of the study area. WS = Wadden Sea, ES = Eastern Scheldt (dashed area).

with numbers ranging between 336 and 860 animals per years between 2006 and 2011 (walvisstrandingen.nl 2012). Strandings of white-beaked dolphins *Lagenorhynchus albirostris* have become more abundant since the 1960s. Before this, bottlenose dolphins *Tursiops truncatus* used to strand more frequently than white-beaked dolphins, but they have disappeared from the Dutch coastal waters in this period (Camphuysen et al. 2008). Since 2006, between one and four white-beaked dolphin strandings are recorded each year (walvisstrandingen.nl 2012).

## 1.2 STUDY OBJECTS

Harbour porpoises inhabit the temperate to cold waters throughout the Northern hemisphere (Hammond et al. 2002; Bjørge & Tolley 2009) of which the Dutch coastal waters may represent an important habitat for e.g. reproduction and foraging ecology. Harbour porpoise abundance and distribution has changed significantly over the past decades. This small cetacean became virtually extinct in the Netherlands in 1970-1990 (Smeenk 1987; Addink & Smeenk 1999; Reijnders & Lankester 1990; Camphuysen 2004). Increased numbers were observed from the end of the twentieth century onwards (Camphuysen 2004; Reijnders et al. 2005; Camphuysen et al. 2008), but the cause(s) for these changes in abundance remain uncertain (Reijnders 1992; Camphuysen 2004). Currently, porpoises are the most common cetacean species in the North Sea and Dutch waters (Camphuysen 2004; Reijnders et al. 2005). During a North Sea wide survey (SCANS II) the population was estimated at ca. 350,000 animals in the North Sea (Hammond 2006; Scheidat et al. 2012). Large numbers of harbour porpoises strand each year on the Dutch North Sea coast and the percentage of bycaught animals is high, ranging from 16-32% in the middle and eastern Wadden Sea (Osinga et al. 2008) to 64-70% (Leopold & Camphuysen 2006) in the western Wadden Sea and Dutch North Sea coast (Figure 1.1).

White-beaked dolphins inhabit the cold-temperate waters throughout the North Atlantic Ocean, including the northern North Sea (Kinze 2009). Since the late twentieth century, increased numbers are observed in the southern and eastern North Sea where white-beaked dolphins are currently the second most numerous cetaceans, both in sightings and in strandings (Kinze et al. 1997; van der Meij & Camphuysen 2006). The SCANS II survey in 2004 provided a population estimate of ca. 8000 white-beaked dolphins in the North Sea and Channel (Hammond 2006).

Harbour porpoises and white-beaked dolphins are listed as endangered in several international, European and national legislations, and are listed in several conventions, agreements and action plans such as the Habitats Directive (92/43/EEC), the Bern Convention, CITES and the ASCOBANS North Sea conservation plan under the Convention of Migratory Species (Reijnders et al. 2009). The conservation of species requires that we know enough about their ecology (e.g. migration, abundance, distribution, feeding ecology, reproduction, etc.) and their habitat in order to develop effective protection measures. This study responds to the requirements and recommendations of international regulations (e.g. Habitats Directive, OSPAR) and corresponding Dutch laws (e.g. Habitats Directive) to protect these species and their habitat.

### 1.3 STUDY OBJECTIVES

The distribution and relative abundance of harbour porpoises and white-beaked dolphins from the Dutch coastal waters has changed significantly over the past decades. My hypothesis is that this may reflect changes in the foodbase provided by that system for these top predators. To address that hypothesis, the objective of this thesis is to analyse in a comprehensive way the past and present feeding ecology, including trophic interactions, of both harbour porpoises and white-beaked dolphins.

Our second hypothesis is that combining several methods of diet analysis gives a more complete picture of the diet of the predator in time and space due to the advantages and disadvantages related to each technique. Thus, one additional objective of this thesis is to apply and compare three techniques of diet analysis to study the foraging ecology of harbour porpoises.

### 1.4 STUDY APPROACH AND METHODS

Traditionally diets have been deduced from stomach contents and faecal remains, being analysed for hard parts (e.g. otoliths, vertebrae, jaws and squid beaks) (e.g. Santos & Pierce 2003; MacLeod et al. 2007a). Stable-isotopes (e.g. in skin, blubber, muscle and bone) and more recently, fatty-acid signatures (in milk, blood and blubber), have been used as well (e.g. Fontaine et al. 2007; Christensen & Richardson 2008; Gilles 2008). Each of these techniques provides different detail and temporal clues on diet consumed (Figure 1.2). Most previous diet studies of porpoises and white-beaked dolphins were carried out using one method of diet analysis. In some cases, two lines of evidence have been combined.

For harbour porpoises, a comprehensive diet analysis was chosen using stomach content analysis (identification of hard prey remains), fatty acid analysis and stable isotope analysis. This integrated approach should theoretically give a more complete picture of the animal's diet, with each technique providing different spatial and temporal clues. For the interpretation of fatty acid signatures and isotopic compositions it is necessary to also analyse these in both predator and prey species.

For white-beaked dolphins, stomach content analysis has been applied. In addition to samples from recently stranded animals, historic material from museum collections was used, allowing the reconstruction of past dietary composition. We have analysed shifts in the diet of white-beaked dolphins over time and the development of foraging in young white-beaked dolphins towards the feeding in adult dolphins.

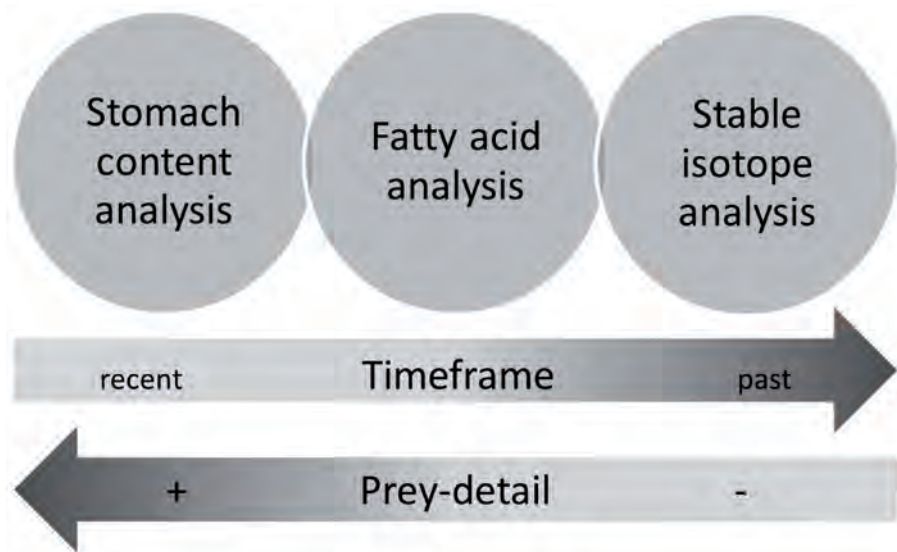


Figure 1.2 Schematic representation of methods for diet analysis and corresponding timeframes and detail.

#### 1.4.1 STOMACH CONTENT ANALYSIS

Stomach content analysis provides detailed, short-term (days) dietary information on relative locally consumed prey by identifying prey remains (e.g. otoliths, vertebrae, jaws, beaks). Species-specific prey items reveal number, size and weight of ingested species. Due to fast digestion, this method holds a possible bias, underestimating species with fragile remains and those ingested prior to the last feeding trip (Pauly et al. 1998). Stomachs were cut open and rinsed, subsequently collecting all hard prey remains. These remains included mostly sagittal otoliths, of which the species, size (length and width to the nearest 0.01mm) and wear-class (Leopold et al. 2001) were recorded. In addition, vertebrae, jaws, operculae and lenses were identified and measured. All these remains were identified to the lowest taxonomic level using published guides for prey remains and the reference collection of IMARES and the NIOZ. With this data, a minimum prey number estimation and prey size/weight estimation was carried out. Besides the calculation of common indices for diet analysis, multivariate analysis of diet composition was performed to relate diet composition to other environmental descriptors (e.g. age, cause of death, stranding date).

#### 1.4.2 FATTY ACID ANALYSIS

Fatty acid analysis is based on the fact that after passing the digestive tract, some fatty acids of prey species are deposited in adipose tissue of a predator with little modification. Therefore consumer fatty acid signatures mirror the signatures of their prey in a predictable way. They basically reflect the animals diet, providing

mid-term (months) dietary information on relative importance of prey species on a potentially larger spatial scale (Iverson et al. 2004). Porpoise blubber and whole fish samples were analysed for their fatty acid composition in accordance to standard techniques (Iverson et al. 2004; Budge et al. 2006). In short, individual samples were ground and homogenized before lipids were extracted and converted into fatty acid methyl esters by gas liquid chromatography. Individual fatty acids were expressed as percentage of the total weight of fatty acids. The obtained fatty acid signatures of the blubber were then compared to the signatures of prey species. Fatty acid data was analysed using Quantitative Fatty Acid Analysis (QFASA) and cluster analysis, providing estimates of the proportions of prey species in the diets of the individual porpoises.

### 1.4.3 STABLE ISOTOPE ANALYSIS

Stable isotope analysis of carbon and nitrogen provides long term (years) trophic position (e.g. demersal *versus* pelagic) and foraging location (e.g. estuarine *versus* open sea) of predators through predictable changes in isotopic composition with

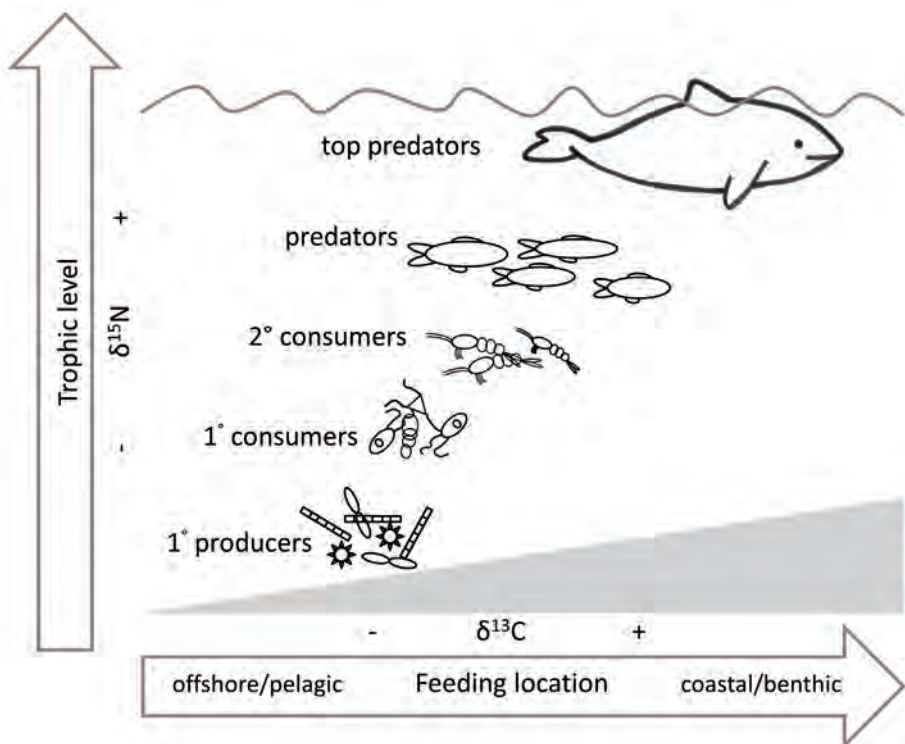


Figure 1.3 Schematic representation of  $\delta^{15}\text{N}$  enrichment with increasing trophic level and geographic differences in stable carbon between offshore/pelagic and coastal/benthic organisms.

each trophic transfer (Hobson 1999; Kelly 2000) (Figure 1.3). This technique is based on the fact that  $^{15}\text{N}/^{14}\text{N}$  systematically enriches from primary producers to every next trophic level. Stable isotope ratios are thus useful in estimating the trophic level of a predator.

In addition, differential fractionation of stable isotopes of carbon during photosynthesis causes C4 plants and C3 plants to have distinct carbon-isotope signatures. These plants occur in different geographical regions. As a result, carbon isotope ratios  $^{13}\text{C}/^{12}\text{C}$  can be used to obtain information on the area the species was ingested. In general,  $\delta^{13}\text{C}$  values are higher in coastal and estuarine systems compared to those from offshore marine systems.

Stable isotope analysis of historic (past 4-5 decades) samples from museum collections enables the comparison between historic and present populations. Porpoise muscle and bone and whole fish samples were analysed for their stable carbon ( $\delta^{13}\text{C}$ ) and stable nitrogen ( $\delta^{15}\text{N}$ ) ratios in accordance to standard techniques. In short, individual samples were dried, ground and combusted at very high temperature, converting organic material into nitrogen and carbon dioxide. This gas was analysed by a stable-isotope mass spectrometer. Carbon and nitrogen stable isotope ratios were expressed as parts per thousand (‰) relative to the PeeDee Belimnite (PDB) and atmospheric nitrogen (AIR). The stable isotope ratio for the animals tissue were directly related to that of its diet using Stable Isotope Analysis in R (SIAR), accounting for elemental composition and for the trophic enrichment from prey to consumer.

## 1.5 OUTLINE OF THIS THESIS

This thesis consists of chapters based on separate journal papers (published, accepted, or submitted) contributing to the knowledge of the feeding ecology of harbour porpoises and white-beaked dolphins in Dutch coastal waters. These articles cover qualitative and quantitative descriptions of the past and present diet composition of these two species, explore spatial differences in the foraging ecology and the development of feeding from young animals towards adult individuals. Chapter 2-5 relate to harbour porpoises, while chapters 6 and 7 are based on white-beaked dolphins. Chapter 8 combines the information on both species gathered in the previous chapters.

**Chapter 1** presents the research background and the problem definition that has led to this study. It further introduces the study objects and the approach of combining several methods of diet analysis.

**Chapter 2** examines the diet of harbour porpoises stranded between 2006 and 2008 using stable isotope analysis of carbon and nitrogen in muscle and bone. Stable isotope analysis provides data on foraging location and trophic level. It also yields information between short- and long-term diet by analysing tissues with varying turn-over times (Newsome et al. 2010). The results provide evidence of higher trophic level feeding by larger porpoises and seasonal varia-



tion in isotopic composition. We were able to identify porpoises from the Eastern Scheldt tidal bay by their distinct isotopic composition.

**Chapter 3** presents the diet of harbour porpoises as deduced from stable isotope analysis in muscle of porpoises stranded on the Dutch coast between 2006 and 2008 using SIAR modelling. The SIAR model has the advantage over other mixing models that it can deal with more sources than isotopes and that it incorporates trophic enrichment and elemental concentrations, allowing a quantitative estimation of prey composition using carbon and nitrogen. In comparison to stomach content analysis, the SIAR model highlighted a higher importance of pelagic, schooling species in the diet of porpoises.

**Chapter 4** looks in detail at the distinct isotopic composition of porpoises from the Eastern Scheldt to get insight in the habitat use and residency of these porpoises. Since the building of a storm surge barrier, movement of porpoises in and out of the Eastern Scheldt tidal bay may be limited. The results indicate that the storm surge barrier may indeed influence the residency of porpoises in the Eastern Scheldt, and that the area might act as an ecological trap for porpoises entering it.

**Chapter 5** describes the diet of porpoises stranded between 1990 and 2007 based on fatty acid analysis, using fatty acid signatures of porpoises and those of 30 potential prey species. QFASA models the most probable combination of prey species that can explain the fatty acid signature of the predator and gives information on its accumulated diet over longer time-scales (Iverson et al. 2004; Budge et al. 2006). By comparing the diet as modelled by QFASA to the diet as deduced from stomach contents, we found significant differences between the short- and long-term diet of porpoises in Dutch coastal waters.

**Chapter 6** describes the stomach contents of white-beaked dolphins stranded on the Dutch coast between 1968 and 2005. Only few studies of the diet of white-beaked dolphins in European waters have been published, most based on small sample sizes and anecdotal data. We found a lasting predominance of Gadidae (i.e. whiting and cod) in the diet of white-beaked dolphins, without distinct changes over time or differences between sexes or size-classes. This study gives the most comprehensive report of the species' diet in the south-eastern North Sea to date.

**Chapter 7** presents stomach contents data on the diet of white-beaked dolphin calves stranded on the Dutch coast between 1992 and 2010. As the previous study found a predominance of relatively large Gadidae in the diet of white-beaked dolphins of all age-classes but did not include very young individuals, the question is raised whether there is a transition in prey composition from post-weaned dolphins to those that consume those large prey species. We found a transition from feeding on small vertebrate and invertebrate prey of smaller size, gradually towards similar but smaller species.

**Chapter 8** gives a synthesis of this thesis. This chapter brings together the results described in the previous chapters and other published studies on the past and present diet of porpoises and white-beaked dolphins. It also discusses whether dietary information reflects food availability, thus linking abundance and distribution of these animals with the foodbase provided by Dutch coastal waters. Another element is the discussion on the diet of porpoises as deduced from the various methods of diet analysis, focusing on differences in detail and in time periods and the complementary value of using multi-method dietary analyses.



# CHAPTER 2

## FEEDING ECOLOGY OF HARBOUR PORPOISES: STABLE ISOTOPE ANALYSIS OF CARBON AND NITROGEN IN MUSCLE AND BONE

Published as: Jansen, O.E., Aarts, G.M., Das, K., Lepoint, G., Michel, L., Reijnders, P.J.H. (2012) Feeding ecology of harbour porpoises: stable isotope analysis of carbon and nitrogen in muscle and bone. *Marine Biology Research* 8: 829-841.







## 2.1 ABSTRACT

Harbour porpoises are the most common small cetaceans in the North Sea and Dutch coastal waters. To study their trophic level and feeding location, stable carbon and nitrogen isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) were analysed in muscle and bone samples collected from 157 porpoises stranded along the Dutch coast (2006-2008). In addition, samples from 30 prey species were analysed. Prey samples showed high  $\delta^{15}\text{N}$  values in species of higher trophic level. In addition, geographic differences in isotopic composition were found, with higher  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in prey from more southern, coastal and estuarine areas. Based on muscle  $\delta^{15}\text{N}$  values, we found neonatal enrichment and that larger porpoises, in particular males, seem to feed on lower trophic level species, compared to smaller individuals. Also bone  $\delta^{15}\text{N}$  values show that larger animals had fed on lower trophic levels in distant times. Porpoises from the Eastern Scheldt reveal distinct  $\delta^{13}\text{C}$  values in muscle, but not in bone. This shows that these animals had foraged in the Eastern Scheldt for a longer time period but were not born there. Seasonal variation in bone  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values revealed two distinct groups of porpoises along the Dutch coast, a winter group (mainly males) that migrated from neighbouring regions and a Dutch subpopulation in summer. These results furthered our insight about shifts in trophic level and feeding location of harbour porpoises from the southern North Sea over time.

## 2.2 INTRODUCTION

The harbour porpoise (*Phocoena phocoena* Linnaeus, 1758) is widely distributed throughout the temperate and cold waters of the Northern hemisphere (Gaskin 1984; Hammond et al. 2002) and is the most common small cetacean in the North Sea and Dutch coastal waters. Population estimates for the North Sea at large, are approximately 350 thousand individuals in 1994 and 2005 (Hammond 2006; SCANS-II 2008). Their abundance and distribution in the southern North Sea has changed significantly over the past decades (Camphuysen 2004; Thomsen et al. 2006). A southern shift in distribution has been documented (SCANS-II 2008), which is also reflected in Dutch coastal waters with a peak in sightings and strandings in 2006 (Camphuysen et al. 2008; Reijnders et al. 2009; Camphuysen 2011). Changes in porpoise abundance and distribution are hypothesized to result from changes in prey availability (Camphuysen 2004; MacLeod et al. 2007a).

As direct observations of feeding marine mammals are extremely rare, commonly used methods to study the feeding ecology in marine mammals are the analysis of stomach contents, fatty acids and stable isotopes (e.g. Hyslop 1980; Hobson 1999; Iverson et al. 2004). Whereas stomach contents provide



information on recently ingested prey (Pierce & Boyle 1991), fatty acids in organisms reflect the assimilated diet over weeks to months (Budge et al. 2006) and stable isotopes over periods varying from hours to years, depending on the tissue analysed (Tieszen et al. 1983; Dalerum & Angerbjörn 2005; Phillips & Eldridge 2006).

Isotopic ratios of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ , expressed as  $\delta^{15}\text{N}$ ) and carbon ( $^{13}\text{C}/^{12}\text{C}$ , expressed as  $\delta^{13}\text{C}$ ) have been used to analyse diet composition, trophic level and origin in terrestrial and marine species (Michener & Kaufman 2007; Newsome et al. 2010). Predators are generally enriched in  $^{15}\text{N}$  compared to their prey (approximately 3-4‰ higher; DeNiro & Epstein 1981; Caut et al. 2009), and  $\delta^{15}\text{N}$  values can therefore be used as indicators of relative trophic level (Post 2002). In general,  $\delta^{13}\text{C}$  values are more similar between predator and prey (approximately 0.1-1‰ higher in predator; DeNiro & Epstein 1978; Caut et al. 2009), but geographic differences in  $\delta^{13}\text{C}$  can be used to indicate feeding location (e.g. offshore *versus* inshore) (Hobson 1999; Barnes et al. 2009). Isotopic discrimination of carbon and nitrogen, however, has shown to vary between tissues, diet and taxa (Caut et al. 2008; Bond & Diamond 2011). Diet-tissue fractionation rates are relatively well studied for muscle (Hobson & Clark 1992a,b; Hobson et al. 1996) but less so for bone. Isotopic discrimination rates have not been estimated specifically for porpoises.

Tissues integrate isotopic composition of diet at different rates depending on their own turnover rate. Muscle tissue reflects assimilated diet of weeks or months prior to sampling (Kurle & Worthy 2002). Bone tissue, in contrast, displays a more long-term integration, reflecting assimilated diet of 8-12 months in young animals, with an increasingly larger time period in older animals (Sealy et al. 1995; Richards et al. 2002; O'Regan et al. 2008). This offers the opportunity to examine shifts in diet or feeding location within the same individuals over time.

The purpose of the present study is to use isotope analysis on porpoises and their prey 1) to gain insight into the trophic level and feeding location (e.g. coastal *versus* offshore) of harbour porpoises from the southern North Sea during a period of high porpoise abundance and stranding frequency (2006-2008), 2) to assess how individual characteristics (e.g. sex, age and location) can explain variability in isotopic composition between individuals, and 3) to define possible shifts in trophic level or feeding location by comparing the isotopic composition between muscle and bone. To that end we have analysed 157 harbour porpoises and 30 prey species and have assessed sex- and age-related, seasonal and geographic effects on the isotopic composition ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) of porpoise muscle and bone, and the difference between these tissues.

## 2.3 MATERIAL AND METHODS

### 2.3.1 SAMPLE COLLECTION

This study was conducted on 157 harbour porpoises stranded on the Dutch coast between 2006 and 2008 (Figure 2.1). Stranding date and location were reported for each animal and during post-mortem examinations, general morphometric data were collected, e.g. sex (male, female, unknown) and length (cm). For each animal, age was determined based on total body length: neonates <90 cm,

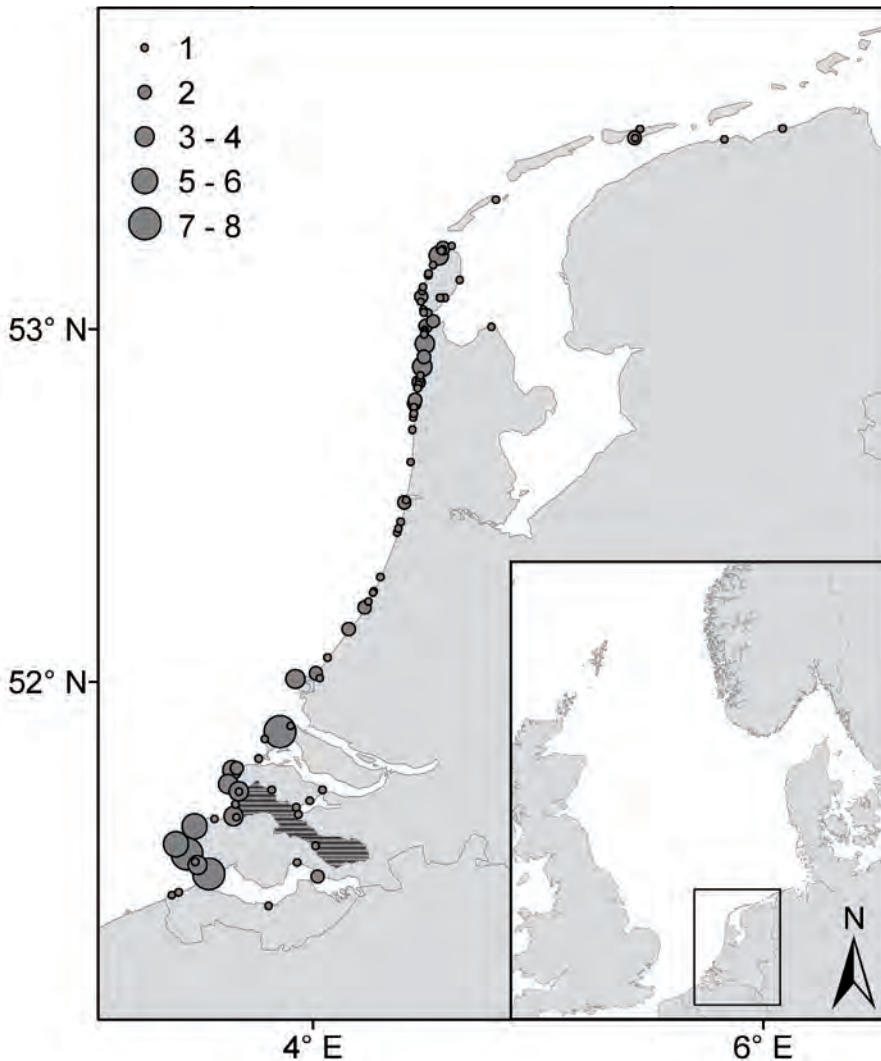


Figure 2.1 Porpoise *Phocoena phocoena* stranding locations and numbers along the Dutch coast analysed in this study (2003-2008). The dark grey area indicates the Eastern Scheldt.

juveniles 90-130 cm and adults >130 cm; unless teeth or reproductive organs indicated differently (Table 2.1). Muscle samples were taken from the ventral mid-region, while for bone tissue, the 5<sup>th</sup> rib was collected. Sixty-three porpoises were sampled concurrently for both muscle and bone, allowing the comparison between the two tissue types. Depending on the state of decomposition and/or sampling protocol, some animals were only sampled for muscle (n = 39) or only for bone (n = 55), resulting in a total of 102 muscle samples and 118 bone samples which were available for the separate analyses of these tissues. Muscle was sampled mostly from very fresh and fresh animals, and only some samples were considered putrefied or very putrefied. Bone was sampled regardless of decomposition state, ranging from very fresh to very putrefied.

Table 2.1 *Porpoise Phocoena phocoena length and weight measurements, grouped by age-class (adult/juvenile/neonate) and sex (female/male/unknown) with number of samples (n), mean, standard deviation (SD) and range (Min/Max).*

Age-class	Sex	n	Length (cm)				Weight (kg)			
			Mean	SD	Min	Max	Mean	SD	Min	Max
Neonate	Female	6	76.0	4.3	71	80	7.2	1.2	6	9
Juvenile	Female	45	112.2	10.9	92	133	19.7	6.2	9	41
	Male	63	109.1	12.0	87	141	18.6	5.5	8	37
	Unknown	3	105.7	15.5	88	117	-	-	-	-
Adult	Female	20	152.1	7.3	140	165	41.8	6.7	31	58
	Male	14	141.1	6.7	131	153	38.4	7.3	28	49

Table 2.2 *Range of latitude and longitude of the fishing localities of prey samples (n = 624).*

Area	n	Latitude (°)		Longitude (°)		δ <sup>15</sup> N (‰)		δ <sup>13</sup> C (‰)	
		Min	Max	Min	Max	Mean	SD	Mean	SD
Central North Sea	32	53.91	56.24	1.41	6.16	13.08	1.78	-20.02	1.31
Dutch Coastal Zone	404	35.50	53.91	4.20	6.91	16.14	1.56	-18.26	1.94
Eems Delta	18	53.29	53.49	7.16	7.47	18.76	1.13	-17.96	0.80
English Channel	28	50.79	50.79	0.86	0.86	15.14	1.09	-18.45	0.75
Firth of Forth	20	56.32	56.71	-1.44	-0.24	14.09	0.54	-18.31	0.48
German Coastal Zone	30	54.00	54.53	8.11	8.32	16.68	1.14	-18.53	1.23
Northern North Sea	22	57.59	58.12	0.55	1.90	12.17	1.10	-20.08	0.88
NW Moray Firth	18	57.89	58.21	-3.24	-2.70	11.60	1.36	-20.13	0.96
SE UK Coast	8	51.48	53.11	1.73	1.89	15.63	1.25	-18.57	1.09
Southern Bight	27	52.21	53.21	2.12	3.61	15.76	1.19	-18.01	1.10
Outer Delta	17	51.79	51.79	3.52	3.52	18.84	1.24	-18.46	1.82

Thirty species of fish and squid were collected during ongoing surveys in the North Sea between 2002 and 2008 by the Centre for Fishery Research CVO (cvo.wur.nl 2009). Species, length, fishing locality (latitude/longitude) and date were available for each sample ( $n = 624$ ). Fishing localities were grouped into 11 areas (Table 2.2). White muscle tissue samples were collected, then prepared and analysed for stable isotope analysis in the same way as porpoise samples, except that no lipid extraction was performed. Porpoise and prey samples were stored frozen at  $-20^{\circ}\text{C}$  until analysis.

### 2.3.2 SAMPLE PREPARATION

Lipids are depleted in  $^{13}\text{C}$  relative to proteins (DeNiro & Epstein 1978; Lidén et al. 1995). Variation in  $\delta^{13}\text{C}$  among animals thus primarily reflects fat content of tissues due to differences in nutritional status, masking possible underlying differences in prey preferences. Lipids were therefore extracted from samples prior to analysis, both in muscle and bone tissue. Bone samples were also acidified to remove non-dietary carbonates and to extract collagen. Muscle samples were freeze-dried for ca. 20 hours and homogenized with a pestle and mortar. Ribs were cleaned with a scalpel and bone marrow was removed. Bone fragments were sonicated in Milli-Q purified water and dried overnight at room temperature. Bone samples were then homogenized with an automatic grinder (Retsch MM301), and demineralized in a weak acid solution (2% HCl) for 20 minutes or until no more gas bubbles were produced (Ambrose 1990). They were then rinsed with Milli-Q purified water to neutralize and dried at  $35^{\circ}\text{C}$  overnight (Moore et al. 1989). Lipids were extracted from muscle and bone powder in a 2:1 chloroform-methanol solution (Folch et al. 1957). Because pre-treatment may sometimes alter  $\delta^{15}\text{N}$  (Lidén et al. 1995), samples were analysed in twofold: one time before pre-treatment to measure  $\delta^{15}\text{N}$  and a second time after lipid extraction and acidification to measure  $\delta^{13}\text{C}$  values.

### 2.3.3 STABLE ISOTOPE ANALYSIS

Muscle and bone samples (1.5 mg for muscle and 2 mg for bone tissue) were weighed into tin cups. Stable isotope measurements were performed by isotope-ratio mass spectrometry using a mass spectrometer (V.G. Optima Isoprime, UK) coupled to an N-C-S elemental analyser (Carlo Erba) for automated analyses at the Laboratory for Oceanology, Liège University, in Belgium. Stable isotope abundances are expressed in conventional delta ( $\delta$ ) notation in parts per thousand (‰), and are expressed relative to the international standards Vienna-PeeDee Belimnite limestone (V-PDB) for  $^{13}\text{C}$  measurements and atmospheric nitrogen for  $^{15}\text{N}$  measurements according to the following equation:

$$\delta X = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \right] \times 1000$$
 where  $R_{\text{sample}}$  is the  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  isotopic ratio of the sample and X is  $^{13}\text{C}$  or  $^{15}\text{N}$ . Reference materials used were: IAEA-N1 ( $\delta^{15}\text{N} = 0.4 \pm 0.2\text{‰}$ ) (mean  $\pm$  SD) and IAEA-C6 ( $\delta^{13}\text{C} = -10.4 \pm 0.2\text{‰}$ )

(IAEA, Vienna, Austria). Internal standards (glycine) were inserted into all runs at regular intervals to assess drift over time. Standard deviation on repeated measurements on glycine and replicated samples was  $\pm 0.1\text{‰}$  for carbon and  $\pm 0.3\text{‰}$  for nitrogen, respectively.

### 2.3.4 STATISTICAL ANALYSIS

Generalized Additive Models (GAMs) were fitted to test for age-related and temporal trends in isotope values and to examine whether variation in isotope values is associated with sex or stranding location. A total of 18 sets of models were fitted using six possible response variables (i.e. bone  $\delta^{13}\text{C}$ , muscle  $\delta^{13}\text{C}$ , bone  $\delta^{15}\text{N}$ , muscle  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}_{\text{bone}} - \delta^{13}\text{C}_{\text{muscle}}$  expressed as  $\Delta^{13}\text{C}_{\text{bone} - \text{muscle}}$  and  $\delta^{15}\text{N}_{\text{bone}} - \delta^{15}\text{N}_{\text{muscle}}$  expressed as  $\Delta^{15}\text{N}_{\text{bone} - \text{muscle}}$ ) and using data from all individuals, excluding outliers. Outliers were identified using the Chauvenet's criterion (Chauvenet 1863). Samples of unknown sex or without length measurements were also removed from the analysis. The explanatory variables included are a smooth function of Length (in cm, acting as proxy for age), Month, Sex (female, male) and whether the individual was found in the Eastern Scheldt (an inshore tidal bay) or along the Dutch coast. The smooth function (with a maximum number of 4 degrees of freedom) enables the estimation of a non-linear relation between the response and the explanatory variable. For Month, a cyclic smoother was used which ensures that the model estimates at the beginning and end are identical. To arrive at the best model, forward model selection based on AIC was used. The model with the lowest AIC was used, but only if the change in AIC from one to the next was larger than 2 (Burnham & Anderson 2002). Data is presented as mean  $\pm$  SD unless stated otherwise. Statistical analysis was carried out in the computing environment R (R 2.9.2; R Development Core Team 2009).

## 2.4 RESULTS

### 2.4.1 PORPOISES

This study included a total of 157 porpoises stranded in three consecutive years, 36, 50 and 71 animals in 2006, 2007 and 2008 respectively. There were slightly more male ( $n = 83$ ) than female porpoises ( $n = 71$ ), and of three animals the sex was not determined. Most animals were juveniles ( $n = 111$ ), compared to 34 adults and 12 neonates. Length and weight measurements per age-class and sex are given in Table 2.1. In summary, length ranged from 71-165 cm ( $116 \text{ cm} \pm 22$ ) and weight ranged from 6-58 kg ( $23 \text{ kg} \pm 11$ ). Porpoises were collected along the entire Dutch coast, including 16 animals that have stranded inside the Eastern Scheldt (Figure 2.1). Samples were available for each month but two distinct stranding periods can be recognized, comparable with the seasonal pattern of all recorded strandings along the Dutch coast (Figure 2.2). The first stranding

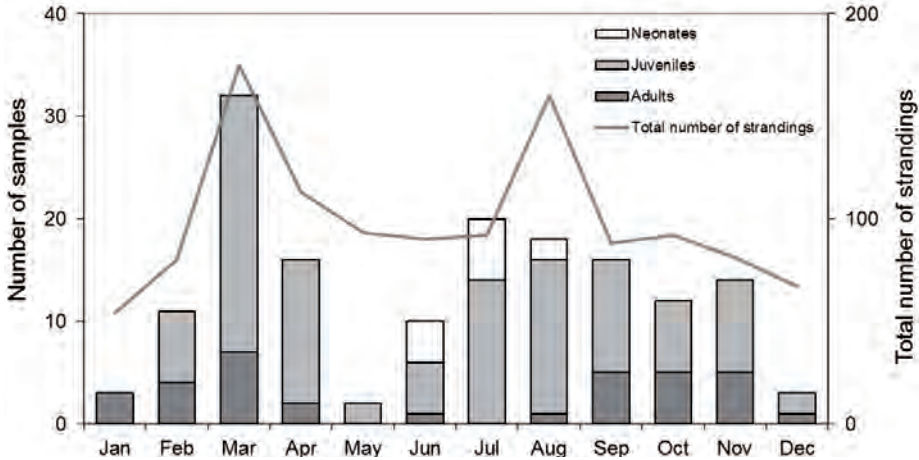


Figure 2.2 *Porpoise Phocoena phocoena* strandings per calendar month ( $n = 157$ ) analysed in this study (2006-2008), separated by age-class. The solid line shows the total number of strandings on the Dutch coast from 2006-2008 ([www.walvisstrandingen.nl](http://www.walvisstrandingen.nl)).

period includes animals stranded in winter and spring (December until May) with a distinct peak of strandings in March. The second period includes animals stranded in summer and autumn (June until November). In this latter period, samples used in our study were more evenly distributed compared to the total number of recorded strandings along the Dutch. Neonates were only found in summer (June until August).

Based on criteria in Kuiken (1996), 21 porpoises were diagnosed as by-catch (13.3%) and another 30 porpoises that were diagnosed as possible or probable by-catch (19.1%), animals that were mostly also suffering from infectious disease and lung oedema. The remaining 104 animals mostly died of emaciation, infectious diseases and lung oedema (66.2%), while two fairly emaciated porpoises were life strandings (1.3%). Most porpoises showed signs of emaciation (75.1%), 19 animals slight, 26 moderate and 73 severe emaciation.

Three outliers were identified in the response data, one in the bone  $\delta^{15}\text{N}$  values (17.7‰), one in the  $\Delta^{13}\text{C}_{\text{bone-muscle}}$  values (3.3‰) and one in the  $\Delta^{15}\text{N}_{\text{bone-muscle}}$  values (-0.1‰). In- or excluding these outliers did not lead to different models being selected in the forward model selection procedure. The final six models presented here were based on all animals, excluding outliers.

#### 2.4.2 ISOTOPIC COMPOSITION IN MUSCLE

Muscle  $\delta^{15}\text{N}$  ranged from 13.4 to 19.1‰ ( $16.4 \pm 1.4$ ‰), with  $\delta^{13}\text{C}$  ranging from -19.7 to -16.8‰ ( $-18.2 \pm 0.5$ ‰) (Table 2.3). GAMs revealed that length and sex explained a significant part of the variation of  $\delta^{15}\text{N}$  ( $R^2 = 0.243$ , deviance explained = 26.1%). The  $\delta^{15}\text{N}$  value of an individual of 80 cm was ca. 2.6‰ lower compared to an individual of 160 cm, and females had on average ca. 0.5‰ higher  $\delta^{15}\text{N}$  values than males (Figure 2.3A). GAMs revealed that the area of



Table 2.3 Porpoise *Phocoena phocoena* isotopic composition ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) of muscle samples, grouped by age-class and sex with number of samples (n), C:N ratio (C:N), mean, standard deviation (SD) and range (Min/Max).

Muscle	Age-class	Sex	n	C:N	$\delta^{15}\text{N}$ (‰)			$\delta^{13}\text{C}$ (‰)			
					Mean	SD	Min	Max	Mean	SD	Min
Neonate	Female	3	3.50	17.28	1.76	15.46	18.98	-17.99	0.88	-19.00	-17.46
	Male	3	3.51	18.23	0.91	17.27	19.07	-17.76	0.08	-17.82	-17.68
Juvenile	Female	33	3.34	16.66	1.24	14.18	18.73	-18.19	0.51	-19.31	-17.26
	Male	41	3.38	16.41	1.26	13.92	18.56	-18.29	0.49	-19.69	-17.21
Adult	Female	11	3.38	16.17	1.35	13.68	17.65	-18.11	0.61	-19.09	-16.82
	Male	11	3.29	15.15	1.34	13.45	17.52	-18.32	0.57	-19.21	-17.02

stranding (Eastern Scheldt *versus* Dutch coast) explained a significant part of the variation of  $\delta^{13}\text{C}$  ( $R^2 = 0.0733$ , deviance explained = 8.25%). Animals stranded along the Eastern Scheldt had on average 0.5‰ higher  $\delta^{13}\text{C}$  values compared to animals stranded along the Dutch coast (Figure 2.3B).

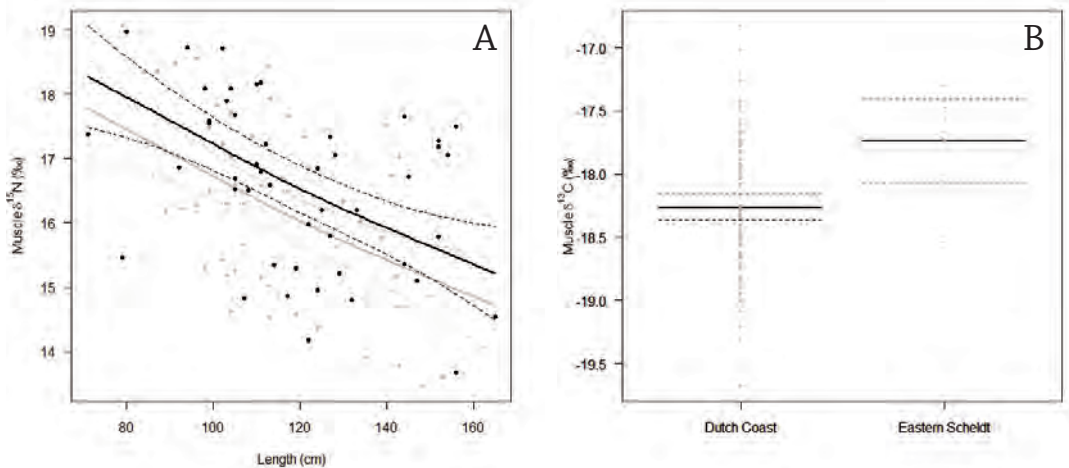


Figure 2.3 Porpoise *Phocoena phocoena* isotopic composition of muscle; effect of length and sex (black = females, grey = males) on  $\delta^{15}\text{N}$  (A) and the effect of stranding location (Dutch coast versus Eastern Scheldt) on  $\delta^{13}\text{C}$  (B). Solid line presents the mean model estimate and the dotted lines the 95% confidence intervals.

#### 2.4.3 ISOTOPIC COMPOSITION IN BONE

Porpoise bone  $\delta^{15}\text{N}$  ranged from 11.3 to 20.7‰ ( $16.3\text{‰} \pm 1.7$ ), with  $\delta^{13}\text{C}$  ranging from -17.3 to -13.8‰ ( $-15.4\text{‰} \pm 0.7$ ) (Table 2.4). GAMs revealed that length and month explained a significant part of the variation of both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  ( $R^2 = 0.331$ , deviance explained = 35.1% and  $R^2 = 0.19$ , deviance explained = 22.1%, respectively). The  $\delta^{15}\text{N}$  value of an individual of 80 cm was on average 3.0‰

Table 2.4 *Porpoise Phocoena phocoena* isotopic composition ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) of bone samples, grouped by age-class and sex with number of samples (n), C:N ratio (C:N), mean, standard deviation (SD) and range (Min/Max).

Bone	Age-class	Sex	n	C:N	$\delta^{15}\text{N}$ (‰)				$\delta^{13}\text{C}$ (‰)			
					Mean	SD	Min	Max	Mean	SD	Min	Max
Neonate	Female		5	3.35	19.10	1.89	15.93	20.69	-15.03	0.89	-16.59	-14.46
	Male		7	3.33	17.93	1.32	16.17	19.77	-15.21	0.62	-16.14	-14.56
Juvenile	Unknown		3	3.68	14.75	1.21	13.77	16.11	-16.46	0.21	-16.68	-16.27
	Female		37	3.48	16.24	1.65	12.97	19.21	-15.58	0.68	-17.31	-14.01
	Male		39	3.44	16.08	1.37	12.31	18.67	-15.47	0.66	-17.05	-14.29
Adult	Female		18	3.56	15.64	1.55	11.33	17.49	-14.85	0.63	-15.77	-13.81
	Male		9	3.47	15.87	1.17	13.69	17.72	-14.77	0.48	-15.68	-14.29

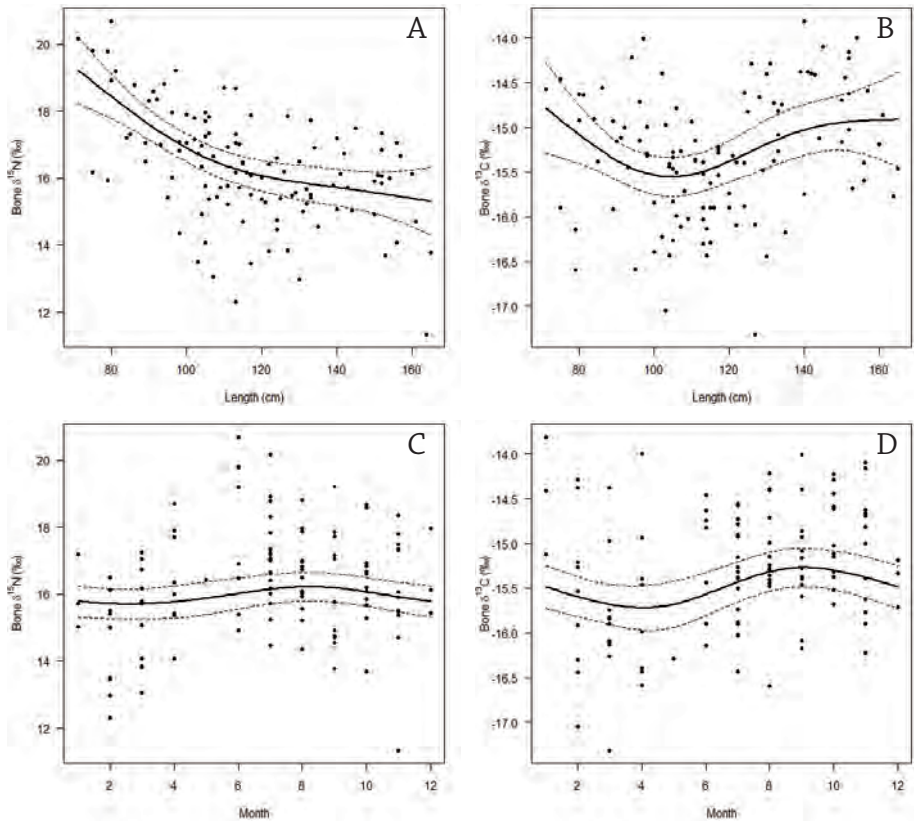


Figure 2.4 *Porpoise Phocoena phocoena* isotopic composition of bone; effect of length on  $\delta^{15}\text{N}$  (A) and  $\delta^{13}\text{C}$  (B), effect of month on  $\delta^{15}\text{N}$  (C) and  $\delta^{13}\text{C}$  (D). Solid line presents the mean model estimate and the dotted lines the 95% confidence intervals.

higher compared to an individual of 160 cm (Figure 2.4A). Highest  $\delta^{15}\text{N}$  values were found in August (Figure 2.4C). Individuals of 105 cm had the lowest  $\delta^{13}\text{C}$  values, 0.5‰ lower than an individual of 80 cm and 0.7‰ lower than an individual of 160 cm (Figure 2.4B). Lowest values for  $\delta^{13}\text{C}$  were found in April and highest  $\delta^{13}\text{C}$  values in September (Figure 2.4D).

#### 2.4.4 MUSCLE VERSUS BONE

Porpoises showed slightly but not significantly lower values ( $-0.3\text{‰}$ , t-test,  $df = 45$ ,  $p = 0.062$ ) in bone  $\delta^{15}\text{N}$  ( $16.1\text{‰} \pm 1.5$ ) relative to muscle  $\delta^{15}\text{N}$  ( $16.4\text{‰} \pm 1.3$ ). In contrast, bone  $\delta^{13}\text{C}$  ( $-15.2\text{‰} \pm 0.7$ ) was significantly higher ( $+3.1\text{‰}$ , t-test,  $df = 45$ ,  $p < 0.001$ ) relative to muscle  $\delta^{13}\text{C}$  ( $-18.3\text{‰} \pm 0.4$ ). GAMs revealed that length explained a significant part of the variation of  $\Delta^{15}\text{N}_{\text{bone-muscle}}$  and  $\Delta^{13}\text{C}_{\text{bone-muscle}}$  ( $R^2 = 0.139$ , deviance explained = 17.9% and  $R^2 = 0.214$ , deviance explained = 22.7%, respectively). Lowest  $\Delta^{15}\text{N}_{\text{bone-muscle}}$  values were found in animals of 105 cm with higher  $\Delta^{15}\text{N}_{\text{bone-muscle}}$  values in both smaller and larger animals (Figure 2.5A). The  $\Delta^{13}\text{C}_{\text{bone-muscle}}$  of an individual of 80 cm was 1.2‰ lower compared to an individual of 160 cm (Figure 2.5B).

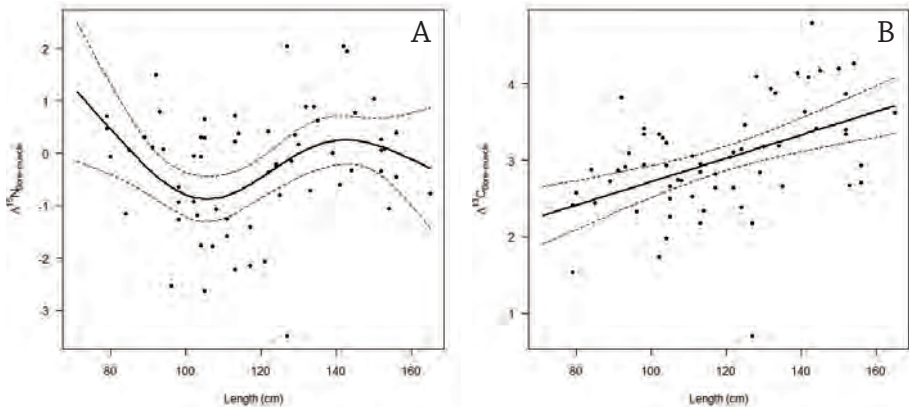


Figure 2.5 Porpoise *Phocoena phocoena* isotopic composition, effect of length on  $\Delta^{15}\text{N}_{\text{bone-muscle}}$  (A) and  $\Delta^{13}\text{C}_{\text{bone-muscle}}$  (B). Solid line presents the mean model estimate and the dotted lines the 95% confidence intervals.

#### 2.4.5 PREY SAMPLES

This study included a total of 624 prey samples of 30 fish and squid species, collected throughout the North Sea. About two third of all prey samples were collected from the Dutch Coastal Zone (64.7%,  $n = 404$ ), while other locations each accounted for between 1.3% and 5.1% of the samples (Table 2.2). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of each prey species are presented in Table 2.5. Mean  $\delta^{15}\text{N}$  values of potential prey species ranged from 11.6 to 18.8‰ ( $15.8 \pm 2.4\text{‰}$ ), with mean  $\delta^{13}\text{C}$  values ranging from  $-20.1$  to  $-18.0$  ( $-18.5 \pm 0.8\text{‰}$ ).

Table 2.5 Isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of prey species collected from the North Sea between 2002 and 2008.

Species		n	C:N	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)	
				Mean	SD	Mean	SD
<i>Arnoglossus laterna</i>	Scaldfish	20	3.3	-18.28	0.83	15.98	1.03
<i>Alloteuthis subulata</i>	Common squid	19	3.6	-18.52	0.52	13.85	0.74
<i>Ammodytes tobianus</i>	Lesser sandeel	10	3.2	-18.76	0.80	15.08	1.02
<i>Buglossidium luteum</i>	Solenette	20	3.3	-17.93	0.77	16.51	0.59
<i>Clupea harengus</i>	Herring	20	3.2	-18.59	0.37	15.82	1.18
<i>Callionymus lyra</i>	Dragonet	15	3.1	-19.26	1.32	15.75	0.61
<i>Ciliata mustela</i>	5b-rockling	20	3.2	-17.14	0.56	16.96	0.63
<i>Dicentrarchus labrax</i>	Seabass	21	3.3	-15.13	1.22	18.18	1.14
<i>Gadus morhua</i>	Cod	33	3.1	-18.75	1.50	15.68	2.54
<i>Gobius niger</i>	Black goby	13	3.3	-18.34	0.60	19.46	0.48
<i>Hyperoplus lanceolatus</i>	Greater sandeel	20	3.2	-18.07	0.95	15.19	0.63
<i>Hippoglossoides platessoides</i>	Long rough dab	19	3.2	-18.70	1.00	14.40	3.06
<i>Loligo forbesi</i>	Veined squid	20	3.5	-20.54	0.95	12.46	0.45
<i>Limanda limanda</i>	Dab	31	3.3	-18.47	0.65	14.21	1.74
<i>Merlangius merlangus</i>	Whiting	30	3.1	-18.19	0.86	16.38	1.60
<i>Osmerus eperlanus</i>	Smelt	20	3.4	-18.50	0.97	18.48	0.28
<i>Platichthys flesus</i>	Flounder	30	3.2	-19.98	3.44	17.20	0.67
<i>Pomatoschistus microps</i>	Common goby	20	3.5	-15.66	0.72	16.73	0.48
<i>Pomatoschistus minutus</i>	Sand goby	20	3.3	-16.61	1.00	16.96	0.41
<i>Pleuronectes platessa</i>	Plaice	27	3.1	-18.06	0.80	15.53	0.68
<i>Syngnathus acus</i>	Greater pipefish	5	3.3	-18.33	0.83	15.21	0.81
<i>Scophthalmus maximus</i>	Turbot	20	3.6	-17.63	0.74	16.71	0.38
<i>Syngnathus rostellatus</i>	Nilsson's pipefish	21	4.3	-19.05	1.21	15.97	0.51
<i>Scomber scombrus</i>	Mackerel	20	6.4	-22.42	0.63	13.45	1.50
<i>Solea solea</i>	Sole	15	3.2	-17.72	1.12	16.34	1.56
<i>Sprattus sprattus</i>	Sprat	20	4.4	-20.57	0.89	14.96	0.71
<i>Trisopterus esmarkii</i>	Norway pout	20	3.3	-19.68	0.83	11.56	1.34
<i>Trisopterus luscus</i>	Bib	20	3.2	-17.65	0.49	18.02	1.96
<i>Trisopterus minutus</i>	Poor cod	35	3.1	-18.54	0.63	14.58	0.77
<i>Trachurus trachurus</i>	Scad	20	3.7	-18.91	1.30	16.18	0.73

Prey samples showed large geographic differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (Table 2.2). There were three clusters of locations with similar, partly overlapping isotopic composition (Figure 2.6). The main cluster included locations from the southern North Sea, including the Dutch-, German- and south-eastern UK

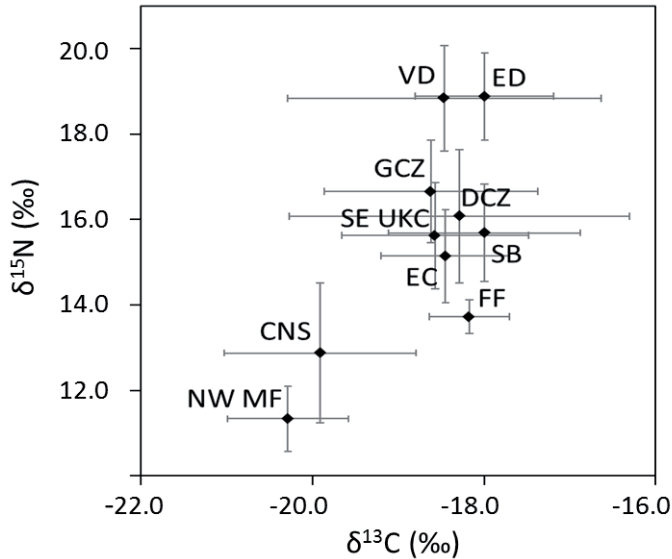


Figure 2.6 Isotopic composition  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of prey species grouped by fishing localities. VD = Voordelta, ED = Eems Delta, GCZ = German Coastal Zone, DCZ = Dutch Coastal Zone, SB = Southern Bight, SE UKC = South-eastern UK Coast, EC = English Channel, FF = Firth of Forth, CNS = Central North Sea, NW MF = North-western Moray Firth.

coasts and the Southern Bight and English Channel. One cluster included the two delta areas, the Outer and Eems Delta, both characterized by relatively high  $\delta^{15}\text{N}$  values. Another cluster included the northern locations Northwest Moray Firth and the Central North Sea, characterized by comparatively low  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. The Firth of Forth had somewhat lower  $\delta^{15}\text{N}$  values compared to the main cluster of locations, comparable to the northern locations but similar  $\delta^{13}\text{C}$  values as the main cluster. This shows that estuarine areas are characterized by relatively high  $\delta^{15}\text{N}$  values while coastal areas are characterized by relatively high  $\delta^{13}\text{C}$  values. Northern and central areas from the North Sea are characterized by relatively low  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values, while the remaining areas fall into one large cluster.

Excluding samples from the northern and central North Sea and the delta areas, top predators such as smelt (*Osmerus eperlanus*), seabass (*Dicentrarchus labrax*), whiting (*Merlangius merlangus*) and cod (*Gadus morhua*) showed the highest  $\delta^{15}\text{N}$  values, characteristic for their high trophic level, feeding on other fish species. Flounder (*Platichthys flesus*) and long rough dab (*Hippoglossoides platessoides*) also showed relatively high  $\delta^{15}\text{N}$  values. Although their main food source are small invertebrates, mature specimen have been shown to feed on small fish species (Knijn et al. 1993). In contrast, mackerel (*Scomber scombrus*) showed the lowest  $\delta^{15}\text{N}$  values, characteristic for the low trophic level of a filter feeder. Com-

mon squid (*Alloteuthis subulata*) and dab (*Limanda limanda*) also showed relatively low  $\delta^{15}\text{N}$  values, as small individuals feed mainly on small crustaceans. Despite feeding on plankton, herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) did not show distinct low  $\delta^{15}\text{N}$  values (Knijn et al. 1993; Pierce et al. 2010). This suggests that a coastal distribution is also associated with higher  $\delta^{15}\text{N}$  values. This is confirmed by other typical coastal species such as gobies (*Pomatoschistus minutus* and *Pomatoschistus microps*) and 5-bearded rockling (*Ciliata mustela*) that show relatively high  $\delta^{15}\text{N}$  in combination with high  $\delta^{13}\text{C}$  values, even though they mostly feed on small benthic prey species (Knijn et al. 1993). Flounder showed a large variation in  $\delta^{13}\text{C}$  values, covering both its winter distribution along the coast and its summer distribution in the brackish waters. Cod and long rough dab showed a large variation in  $\delta^{15}\text{N}$  values, due to the large size range, covering small individuals feeding mainly on benthic species and large individuals that also feed on small fish (Knijn et al. 1993). The remaining species can all be found throughout the entire North Sea, covering both coastal and deeper waters. They feed mainly on benthic species such as crustaceans, molluscs, polychaetes and sometimes very small fish, resulting in relatively average  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Knijn et al. 1993).

## 2.5 DISCUSSION

This study included porpoises stranded in a period of high porpoise abundance and stranding frequency. Samples covered both sexes, all age-classes, and animals stranded along the Dutch coast throughout the year. Individual characteristics (i.e. length, sex, stranding area and month) were accountable for part of the variation in isotopic composition of the animals and gave insight in the trophic level and feeding location.

### 2.5.1 AGE-RELATED EFFECTS

Age-related changes in isotopic composition (derived from length, Lockyer 2003) were identified both in muscle and bone tissue. We found that neonatal enrichment for nitrogen values was very distinct from older age-groups. Generally, foetal tissue has the same isotopic composition as the mother's tissue (Richards et al. 2002). After birth, when the young suckles, neonatal enrichment in  $^{15}\text{N}$  occurs relative to their mothers' isotopic values as the offspring is theoretically 'feeding' on their mothers tissues (Jenkins et al. 2001; Witt & Ayliffe 2001).

In muscle, smaller individuals had higher  $\delta^{15}\text{N}$  values but similar  $\delta^{13}\text{C}$  values. Studies based on stomach contents have shown smaller, more benthic, coastal prey in young porpoises (e.g. shrimp, small fish and squid) compared to larger more pelagic, offshore prey in adult porpoises (e.g. gadoids and flatfish)



(Smith & Read 1992; Börjesson et al. 2003; Santos et al. 2004). It is also assumed that young animals start preying on small prey species such as gobies, small flatfish and shrimp before preying on larger species (Santos & Pierce 2003). Our data confirms that young porpoises stay in coastal waters and feed mainly on small species, i.e. gobies. These prey are small in size, are found in high numbers along the Dutch coast and show high  $\delta^{15}\text{N}$  values. Even though  $\delta^{13}\text{C}$  values of adult porpoises were similar to those of younger individuals, the lower  $\delta^{15}\text{N}$  values reflect offshore feeding. We cannot confirm that adult porpoises feed mainly on larger gadoids (e.g. cod and whiting) as the high trophic level of large gadoids is not reflected in porpoise tissues.

A relation between length and  $\delta^{13}\text{C}$  was less distinct than for nitrogen and found in bone only, presumably caused by differences in trophic enrichment between carbon and nitrogen. We found that muscle showed temporal differences in prey choice faster (Figure 2.4A and Figure 2.5A,B), resulting in more individual variation in isotopic composition compared to bone. The gradual decrease in signal acquired during suckling when animals become older, is caused by a faster dilution in muscle compared to bone due to differences in turnover times (Jenkins et al. 2001; Habran et al. 2010). In bone,  $\delta^{13}\text{C}$  values were high in young individuals and in animals with a length of approximately 135 cm and longer, suggesting that they were feeding more coastal. Higher  $\delta^{13}\text{C}$  values in young animals can be explained both by neonatal enrichment due to recent suckling as well as differences in the foraging ecology of porpoises of different age-classes based on their diving and hunting experience or feeding location.

### 2.5.2 SEXUAL SEGREGATION

In muscle, females generally showed slightly higher  $\delta^{15}\text{N}$  values compared to males, suggesting that they, to some extent, fed on higher trophic level prey. Intersexual differences in nitrogen values were more explicit in adult porpoises and only reflected in muscle but not in bone tissue, indicating sexual segregation at maturity, where females feed at a relatively higher trophic level. This has previously been documented for porpoises (Das et al. 2004) and for other marine mammal species (Hobson 1999; Lesage et al. 2001). This is confirmed by Smith & Gaskin (1983), who suggest that adult females stay with their young while adult males migrate further offshore, possibly preying on different prey species. Even though previous studies on the diet of porpoises (Aarefjord et al. 1995) suggest that a higher consumption need of lactating females may result in feeding at larger and different prey species, stomach contents analysis on porpoises from Dutch coastal waters has shown that female porpoises had ingested more small gobies compared to males that fed more on larger gadoids (Santos & Pierce 2003). The high  $\delta^{15}\text{N}$  values of gobies in our study confirm the findings from stomach contents analysis that adult females feeding more coastal and on similar prey together with their young.

### 2.5.3 SEASONAL EFFECTS

Bone showed slightly higher  $\delta^{15}\text{N}$  values in porpoises stranded in August, while  $\delta^{13}\text{C}$  values were lowest in April and highest in September. As bone is considered a long-term integrator and since the effect of length is corrected for by the model, this does not reflect a seasonal effect or merely the occurrence of neonates in summer months, but suggests that animals from each respective period belong to two groups composed of different animals that have used a different habitat during their period of rapid growth. Genetic analyses of porpoises support this hypothesis as they found that porpoises (mainly males) stranded along the Dutch coast in winter had migrated from neighbouring regions, most probably from British and Danish coastal waters (Andersen et al. 2001). Porpoises stranded along the Dutch coast in summer are considered to be part of a Dutch subpopulation of the south-eastern North Sea population (Yurick & Gaskin 1987; Walton 1997).

### 2.5.4 PORPOISES IN THE EASTERN SCHELDT

Porpoises stranded within the Eastern Scheldt had distinct (higher)  $\delta^{13}\text{C}$  values in muscle compared to porpoises stranded along the Dutch coast. The Eastern Scheldt is a tidal bay, created by dams isolating the former estuary from freshwater input of the river Scheldt (Nienhuis & Smaal 1994). Although no baseline isotopic values are available for the Eastern Scheldt, our data confirms that prey from the delta areas differ significantly from the marine system (Clementz & Koch 2001). The distinct isotopic composition of porpoises stranded in the Eastern Scheldt indicates that they have been feeding in the area long enough to integrate this distinct isotopic pattern and that they do not frequently leave the area to forage offshore. It is plausible that movement of marine mammals is limited since the building of the storm-surge barrier. Having entered the Eastern Scheldt, porpoises may stay there for most of the time. As this distinct Eastern Scheldt isotopic signature was not observed in bone tissue, these animals were not born in the Eastern Scheldt but entered the area relative recently.

### 2.5.5 SHIFTS IN TROPHIC LEVEL AND FEEDING LOCATION (MUSCLE VERSUS BONE)

Muscle  $\delta^{15}\text{N}$  values were on average only slightly higher compared to bone (0.29‰), while  $\delta^{13}\text{C}$  values were significantly higher in bone compared to muscle (3.02‰). Differences in isotopic composition between muscle and bone can be due to two factors: tissue-dependent fractionation between diet and tissues, and/or a recent shift in feeding locality before the stranding.

Diet-tissue fractionation rates are well studied for muscle and other tissues (Hobson & Clark 1992a,b; Hobson et al. 1996), but not for bone in marine mammals. In general,  $\delta^{15}\text{N}$  values are considered very similar among different tissue types. However,  $\delta^{13}\text{C}$  values are expected to differ between muscle

and bone, even in animals on a constant diet. This difference can be estimated around 3‰ as  $\Delta^{13}\text{C}_{\text{muscle-diet}}$  and  $\Delta^{13}\text{C}_{\text{collagen-diet}}$  are approximately 1-2‰ and 4-5‰, respectively (Hedges 2003; Koch 2007). The difference in  $\delta^{13}\text{C}$  values between muscle and bone found in this study could therefore be explained purely by tissue-dependent fractionation rates.

However,  $\Delta^{13}\text{C}_{\text{bone-muscle}}$  values also showed a relation with length,  $\Delta^{13}\text{C}_{\text{bone-muscle}}$  values were lowest in very young animals and increased with length. This shows that the difference between muscle and bone cannot be generalized and has to be considered age-specific, based on different turnover rates of muscle and bone. Until more information is available on specific turnover rates in tissues of porpoises, it is difficult to unmask a growth or metabolic effect and to expose possible dietary changes later in life.

### 2.5.6 INDIVIDUAL VARIATION

There was a large amount of unexplained variation by the GAM models, pointing towards a high individual variation in diet. In general, porpoises are considered opportunistic, generalist feeders, relying for their main dietary intake on few species that are easily available in high numbers (Teilmann & Dietz 1998; Christensen & Richardson 2008). Most diet descriptions are based on stomach contents analysis that often fails to account for individual variation. Our study on a population level suggests that porpoises may be considered an opportunistic, generalist feeder but that there is large individual variation. That there may be individual variation in feeding preference has also been suggested by Recchia & Read (1989) and Santos & Pierce (2003).

### 2.5.7 GENERAL ASSUMPTIONS AND CAUTIONS

Stable isotope analysis is widely used to study the feeding ecology of marine species. Isotopic composition in a predator is subjective to three factors: [1] tissue composition and lipid content (e.g. Sotiropoulos et al. 2004; Jacob et al. 2005), [2] tissue turnover-rates (Tieszen et al. 1983) and [3] tissue-dependent fractionation (DeNiro & Epstein 1978, 1981). Given these assumptions, the ability to infer information on trophic level and feeding location of porpoises and possible changes over time depends on the knowledge of the specific influence of these factors on porpoise isotopic composition.

We have extracted lipids prior to analysis to eliminate the influence of differences in nutritional status (varying lipid content of tissues) and to enable the comparison between muscle and bone tissue. Specific turnover rates in porpoise tissues are yet unknown but can be considered similar to or slightly higher than in other marine mammals, due to their small size (Worthy & Edwards 1990; Kastelein et al. 1997a). Diet-collagen fractionation in marine mammals is still poorly understood. It is therefore difficult to examine whether differences in isotopic composition between muscle and bone is reflecting differences in frac-

tionation, or temporal changes in feeding ecology. The identification of temporal changes in trophic level or feeding location thus remains difficult until specific turnover rates and tissue dependent fractionation of carbon and nitrogen in porpoises are better understood.

About three quarter of the porpoises analysed were emaciated, many of them severely. About two third of the animals had died of emaciation, sometimes in combination with infectious diseases and lung oedema. Approximately one third of the animals were diagnosed as by-catch, either as definite by-catch or possible/probable by-catch. These animals showed no signs of infections or emaciation. With their fast metabolism, porpoises suffer from emaciation relatively fast (Worthy & Edwards 1990; Kastelein et al. 1997a) but emaciation is not considered to influence  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (Das et al. 2004; Gómez-Campos 2011).

### 2.5.8 COMPARISON TO OTHER STUDIES

There are two previous studies on the isotopic composition of porpoises in Dutch coastal waters. Das et al. (2003) analysed muscle tissue of 46 porpoises stranded on the French, Belgian and Dutch coasts between 1994 and 2000. The  $\delta^{15}\text{N}$  values of porpoises from our study were 0.2‰ lower compared to those documented by Das et al. (2003). This shows that porpoises from Dutch and adjacent coastal waters are feeding on similar trophic level prey. The  $\delta^{13}\text{C}$  values from our study were 1.8‰ lower compared to those documented by Das et al. (2003). This difference is probably due to regional differences in  $\delta^{13}\text{C}$  baseline values that are reflected in porpoises from France and Belgium, which are included in their study.

Christensen & Richardson (2008) analysed bone tissue of 88 porpoises stranded on the Dutch coast between 1848 and 2002, most animals from 1940 onwards. The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of porpoises from our study were approximately 1‰ and 3‰ lower to those reported by Christensen & Richardson (2008) for the period 1978 to 2002, respectively. This supports that porpoises have gradually been feeding on lower trophic level prey over the last century. They argue that this is due to changes in food web structure with progressively lower trophic prey available to porpoises. The gradual decrease in  $\delta^{13}\text{C}$  can only partially be explained by anthropogenic changes in carbon composition in the atmosphere (Cullen et al. 2001), but indicates that the food web structure of the North Sea has also changed over the past century. Similar to our study, both studies (Das et al. 2003; Christensen & Richardson 2008) found sexual segregation in adult porpoises with higher  $\delta^{15}\text{N}$  in females. In contrast to our study, Das et al. (2003) found that female porpoises had also slightly higher  $\delta^{13}\text{C}$  values, which could reflect a bias due to different sex ratios of porpoises from the three countries.

## 2.6 CONCLUSION

We have shown that stable isotope analysis can yield important information on the feeding ecology of harbour porpoises. We have found 1) differences in trophic level and feeding location between animals of different ages, 2) sexual segregation between adult porpoises, and 3) have identified different groups of porpoises that stranded during the summer and winter months. We have also shown that  $\delta^{13}\text{C}$  values can be used to identify porpoises that have been feeding in the Eastern Scheldt for a longer period of time. We have found no evidence that any of the animals we analysed was born in the Eastern Scheldt, indicating that they have recently entered the Eastern Scheldt. Future stable isotope analysis in bone has the potential to assess whether animals born in the Eastern Scheldt stay there. The difference in isotopic composition between muscle and bone, however, cannot be used for determining shifts in porpoise feeding ecology over time until we have better insight into differences in turnover times and isotopic routing of these two tissues.

## 2.7 ACKNOWLEDGEMENTS

Samples were collected by staff and volunteers of the Dutch strandings network, coordinated by the National Museum of Natural History (now NCB Naturalis) in Leiden. The authors would like to thank M.F. Leopold (IMARES), C.J. Camphuisen (NIOZ), T. Jauniaux (Liège University), A. Gröne (Utrecht University) and L. Wiersma (Utrecht University) for organizing and executing the post-mortem examinations that have provided samples and information on the animals. K. Das and G. Lepoint are F.R.S.-FNRS Research Associates. L. Michel was a F.R.S.-FNRS Research Fellow. We would like to thank G.O. Keijl (NCB Naturalis) for providing long-term data of porpoise stranding records and R.S.A. van Bemelen for creating the map of strandings localities. We also thank C. Smeenk, R.W.P.M. Laane and M.J. van den Heuvel-Greve for their valuable comments on the manuscript. This work was funded by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV), BO Project 4308201019.

# CHAPTER 3

## DIET OF HARBOUR PORPOISES ALONG THE DUTCH COAST: A COMBINED STABLE ISOTOPE AND STOMACH CONTENTS APPROACH

Published as: Jansen, O.E., Michel, L., Lepoint, G., Das, K., Couperus, A.S., Reijnders, P.J.H. (2012) Diet of harbour porpoises along the Dutch coast: A combined stable isotope and stomach contents approach. *Marine Mammal Science*. DOI: 10.1111/j.1748 7692.2012.00621.x







### 3.1 ABSTRACT

High stranding frequency of porpoises *Phocoena phocoena* along the Dutch coast since 2006 has led to increased interest in the ecology of porpoises in the North Sea. Stranded porpoises were collected along the Dutch coast (2006-2008) and their diet was assessed through stomach content and stable isotope analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of porpoise muscle and prey. Stable isotope analysis (SIAR) was used to estimate the contribution of prey species to the porpoises' diet. This was compared to prey composition from stomach contents, to analyse differences between long- and short-term diet. According to stomach contents, 90.5% of the diet consisted of gobies, whiting, lesser sandeel, herring, cod and sprat. Stable isotope analysis revealed that 70-83% of the diet consisted of poor cod, mackerel, greater sandeel, lesser sandeel, sprat and gobies, highlighting a higher importance of pelagic, schooling species in the porpoises' diet compared to stomach contents. This could be due to prey distribution as well as differences in behaviour of porpoises and prey between the coastal zone and offshore waters. This study supports the need for multi-method approaches. Future ecological and fishery impact assessment studies and management decisions for porpoise conservation should acknowledge this difference between the long- and short-term diet.

### 3.2 INTRODUCTION

Strandings of harbour porpoises *Phocoena phocoena* along the Dutch coast have become increasingly more frequent since 2006 (Camphuysen et al. 2008). Hence, the abundance, distribution, and ecology of porpoises in these waters have become subjects of ecological as well as resource management interest. Understanding their diet can contribute considerably towards the understanding of how the southern North Sea and Dutch coastal waters are supporting the increasing numbers of this species.

Diets of harbour porpoises have generally been reconstructed from stomach contents of stranded or bycaught animals, and based on identification of undigested prey remains such as otoliths, vertebrae, jaws and squid beaks (Börjeson et al. 2003; Santos & Pierce 2003; Víkingsson et al. 2003). Their diet consists mostly of pelagic and demersal species (mainly clupeids (Clupeidae), sandeels (Ammodytidae) and gadoids (Gadidae), although geographical variation in preference of specific prey species has been documented (Santos & Pierce 2003). In Dutch coastal waters, a large variety of prey species have been documented but here porpoises tend to consume mainly whiting *Merlangius merlangus*, sandeels and gobies *Pomatoschistus* spp. (Santos 1998; Santos & Pierce 2003; Santos et al. 2005). Christensen & Richardson (2008) analysed bone tissue of porpoises

stranded on the Dutch coast between 1848 and 2002 and have found a decrease in  $\delta^{15}\text{N}$  values over time, suggesting that porpoises have gradually been feeding on lower trophic level prey. They argue that this reflects a change in the food web structure of the North Sea with progressively lower trophic prey available to porpoises.

Stomach content analysis has some inherent biases, e.g., differential recovery rates, degradation and passage times of prey remains (Prime & Hammond 1987; Bowen 2000). Due to fast digestion rates, stomach contents of stranded animals only provide information on recently ingested prey, possibly over emphasizing the relevance of near shore species (Pierce & Boyle 1991).

To overcome these problems, stable isotope ratios of nitrogen ( $^{15}\text{N}/^{14}\text{N}$  or  $\delta^{15}\text{N}$ ) and carbon ( $^{13}\text{C}/^{12}\text{C}$  or  $\delta^{13}\text{C}$ ) can be used to analyse past diet composition (Kelly 2000; Crawford et al. 2008; Newsome et al. 2010). Stable isotope analysis provides insight into feeding ecology over longer time periods and reflects the general diet assimilated over time (Budge et al. 2006; Newsome et al. 2010). In general, predators are enriched in  $^{15}\text{N}$  compared to their prey ( $\pm 3.5\text{‰}$  per trophic level, e.g., Kelly 2000; Michener & Kaufman 2007). In contrast,  $\delta^{13}\text{C}$  is very similar between predator and prey ( $\pm 0.5\text{--}1\text{‰}$  per trophic level, e.g., Post 2002; Michener & Kaufman 2007) but rather reflects geographic differences throughout the food web to indicate foraging location (offshore *vs.* inshore, pelagic *vs.* benthic) (Hobson 1999; Barnes et al. 2009). However, factors such as age, type of diet, composition of food, nutritional status, environment, identity of nitrogenous waste product and taxonomical position can notably influence trophic fractionation (Minagawa & Wada 1984; McCutchan et al. 2003; Vanderklift & Ponsard 2003). Depending on their specific turnover time, tissues reflect various timeframes, from very short-term (e.g., liver and plasma) to relatively long-term or life-time (e.g., bone tissue and teeth) (Dalerum & Angerbjörn 2005). Muscle tissue, as analysed in this study, reflects assimilated diet of several months (Tieszen et al. 1983; Hobson et al. 1996).

Application of stable isotope analysis relies on the fact that stable isotope composition of a consumer is the weighted mixing of the stable isotopic composition of its food sources, modified by isotopic fractionation (Newsome et al. 2010). Therefore, several isotopic mixing models have been developed to link isotopic signatures of predators to isotopic signatures of potential prey species, taking into account isotopic fractionation between prey and predator (Phillips 2001; Phillips & Gregg 2001, 2003). Via these models, the proportional contribution of each source (prey species) to the isotopic signature (accumulated diet) of the predator is estimated. Simple linear or Euclidean distance-based models are limited in their application, as only few prey species can be included in the model due to the small number of measured isotope ratios (Phillips & Gregg 2001). More recent models are able to deal with more prey species (e.g., IsoSource, Phillips & Gregg 2003) or variability within sources (e.g., IsoError, Phil-



lips & Gregg 2001). In this study, SIAR (Stable Isotope Analysis in R, Parnell et al. 2010) was used. This Bayesian stable isotope mixing model is not only able to deal with more sources than variables, but also includes uncertainties (natural variation and analytical error), producing results as probability distributions with residual errors (Parnell et al. 2010).

The primary objective of this study was to estimate the diet composition of harbour porpoises using SIAR on muscle  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from porpoises stranded on the Dutch coast between 2006 and 2008 (Jansen et al. 2012a) and using the isotopic composition of their potential prey sources. We then compare the diet as estimated by SIAR with the diet as deduced from stomach contents of the same individuals, enabling a comparison between long- and short-term dietary information.

### 3.3 MATERIALS AND METHODS

#### 3.3.1 PORPOISE AND PREY SAMPLES

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values analysed in the muscle of harbour porpoises were extracted from a database ( $n = 160$ ) published by Jansen et al. (2012). They have identified suckling neonates by their neonatal enrichment and porpoises stranded within the Eastern Scheldt tidal bay by their distinct isotopic composition. These animals were excluded from this study. They have found no interannual or seasonal variation in isotopic composition but there were differences between juveniles and adults and between males and females. Therefore, the remaining 90 porpoises were analysed by their age-class and sex.

Details of sample collection, preparation, and isotopic analysis are described in Jansen et al. (2012). In short, muscle samples were freeze-dried and homogenized before lipids were extracted in a 2:1 chloroform-methanol solution (Folch et al. 1957). Prey samples used for SIAR ( $n = 202$ ) were extracted from a larger database published by Jansen et al. (2012). These samples were selected using the following criteria: samples from the southern North Sea (*i.e.*, the Dutch, German and southeastern UK coastal zone, the English Channel, and the southern Bight), and prey covering the size-classes found in stomach contents. Prey samples were either analysed including lipids, or prey  $\delta^{13}\text{C}$  values were corrected ( $\delta^{13}\text{C}'$ ) using arithmetic lipid normalization as described by McConnaughey & McRoy (1979) where:

$$\text{Lipid (L)} = 93 / [1 + (0.246 \text{ C/N} - 0.755)^{-1}]$$

$$\delta^{13}\text{C}' = \delta^{13}\text{C} + 6 * [-0.207 + 3.90 / (1 + 287 / \text{L})]$$

Samples were analysed for carbon (lipid extracted) and nitrogen (untreated) stable isotope ratios using continuous flow EA-IRMS (Optima, Isoprime, UK). Data were expressed in delta ( $\delta$ ) notation (hereafter, noted as  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , for nitrogen and carbon stable isotopic composition, respectively) in parts per thousand (‰) using Vienna Pee Dee Belemnite (vPDB) and atmospheric nitrogen as international standard (Coplen 2011). IAEA-C6 and IAEA-N1 were used as certified internal standards. Standard deviations on multibatch replicate measurements of glycine were 0.3 and 0.2 ‰ for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively.

### 3.3.2 STOMACH CONTENT ANALYSIS

Stomach content data were extracted from a wider study on harbour porpoises that stranded along the Dutch coast (Leopold & Camphuysen 2006). Stomach contents were re-analysed after selection ( $n = 76$ ) using the following criteria: stomachs with identifiable prey remains, stomachs of weaned animals (excluding neonates) and stomachs of animals analysed for their isotopic composition in this study. All prey remains were identified to the lowest taxonomic level possible, using a reference collection of IMARES and the Royal Netherlands Institute for Sea Research (NIOZ) and guides for otoliths as well as other identifiable remains such as vertebrae, jaw bones and lenses (Härkönen 1986; Watt et al. 1997; Leopold et al. 2001). Measurements of otoliths and other identifiable remains were used to reconstruct the length and weight of individual fish using published regressions of fish species (Härkönen 1986; Prime & Hammond 1987; Coull et al. 1989; Leopold et al. 2001), correcting for wear according to Leopold et al. (1998). Prey composition was described as reconstructed weight (%W), expressed as the mean of the weight of a given prey species as a percentage of the total prey weight in each stomach.

### 3.3.3 STABLE ISOTOPE MIXING MODEL

The stable isotope mixing model SIAR (Stable Isotope Analysis in R) was used to estimate the relative contribution of different prey species (isotopic sources) to the isotopic composition of porpoises. SIAR (Version 4.1.3) was fitted in R (R 2.9.2, R Development Core Team 2009) including isotopic compositions of the predator, isotopic composition and elemental concentrations of prey species (sources) and trophic enrichment factors (TEFs). In the model, individual porpoise isotope ratios were used while for prey species, means and SDs were entered. Prey species that accounted for more than 1% of the prey composition as determined from stomach contents were included in the SIAR models. Four previously published trophic enrichment factors (TEFs) for carbon ( $\Delta^{13}\text{C}$ ) and nitrogen ( $\Delta^{15}\text{N}$ ) were tested successively in different model runs, one specifically for seals and other marine mammals (Hobson et al. 1996; model run [A]), one as averaged from carnivores (Vander Zanden & Rasmussen 2001; model run [B]), one as averaged from lipid removed muscle samples (McCutchan et al. 2003;

model run [C]) and one specifically for cetaceans (Caut et al. 2011; model run [D]). The TEFs for these four model runs are given in Table 3.1. As TEFs determined by Caut et al. (2011) are based on lipid extracted  $\delta^{13}\text{C}$  values for predator and prey, lipid corrected prey  $\delta^{13}\text{C}$  values were used in model run [D]. SIAR model outcomes are described as mean percentage (%) with the 95% credibility interval ( $\text{CI}_{95}$ ).

The four resulting relative prey compositions were compared to the prey composition as determined from stomach contents using non-metric multi-dimensional scaling (NMDS). NMDS based on Bray-Curtis similarity coefficients was applied to the average percentage (SIAR outcomes) and %W (stomach contents) per prey species, using Primer Software (Clarke & Gorley 2006). To limit the influence of dominant prey species on the ordination, data were fourth-root transformed. Subsequently, SIAR was used to separately estimate the diet of porpoises grouped by their age-class and sex.

Table 3.1 Trophic enrichment factors (TEFs) as used in SIAR modelling

Reference	$\Delta^{13}\text{C}$ (‰)		$\Delta^{15}\text{N}$ (‰)	
	Mean	SD	Mean	SD
[A] Hobson et al. 1996	1.30	0.10	2.40	0.12
[B] Vander Zanden & Rasmussen 2001	0.91	1.04	3.23	0.41
[C] McCutchan et al. 2003	1.80	0.29	3.20	0.43
[D] Caut et al. 2011	1.26	-	1.23	-

## 3.4 RESULTS

### 3.4.1 PORPOISE SAMPLES COMPOSITION AND STABLE ISOTOPE ANALYSIS

This study included a total of 90 porpoises, of which 31, 13, and 46 animals stranded in 2006, 2007, and 2008, respectively (Figure 3.1). The male to female ratio was 1.1 and most animals were juveniles (77%). Juvenile lengths and weights ranged from 87-141 cm ( $111.5 \text{ cm} \pm 12.0$ ) and from 10-41 kg ( $20.4 \text{ kg} \pm 6.1$ ), respectively. Adult lengths and weights ranged from 134-165 cm ( $147.7 \text{ cm} \pm 7.2$ ) and from 33-58 kg ( $41.9 \text{ kg} \pm 7.1$ ), respectively. Samples were available from each month with two distinct stranding periods, one from January to May with a distinct peak of strandings in March and a second stranding period from June until December, comparable with the seasonal pattern of all recorded strandings along the Dutch coast (Jansen et al. 2012a).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values measured in the selected 90 porpoises ranged from -19.7 to -16.8‰ ( $-18.3 \pm 0.5\text{‰}$ ) for  $\delta^{13}\text{C}$  and from 13.4 to 18.7‰ ( $16.2 \pm 1.3\text{‰}$ ) for  $\delta^{15}\text{N}$ .  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values per age-class are given in Table 3.2.



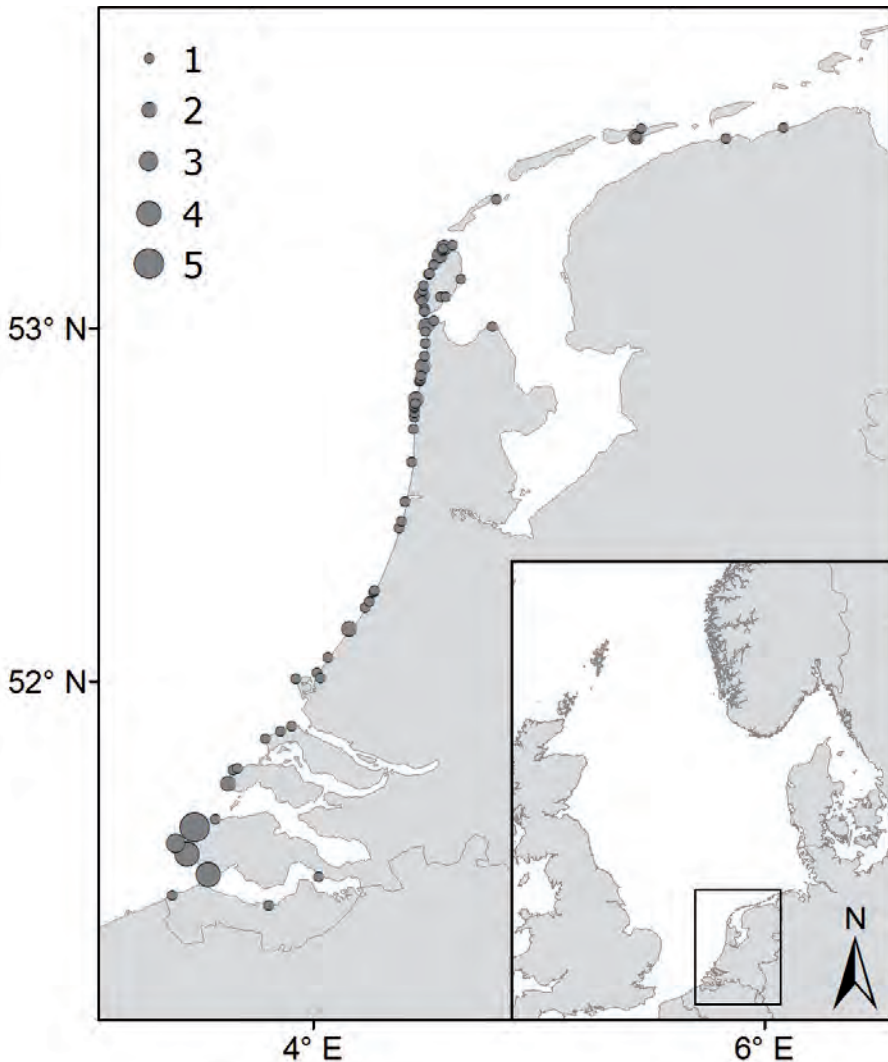


Figure 3.1 *Porpoise Phocoena phocoena* stranding locations and numbers ( $n = 90$ ) along the Dutch coast analysed in this study (2006-2008).

### 3.4.2 STOMACH CONTENT ANALYSIS

In total, 27 prey species were identified (Table 3.3), of which 10 species each accounted for more than 1%W in overall diet composition (indicated with an asterisk in Table 3.3). These 10 prey species together accounted for 97.4% of the total ingested prey weight. Gobies were the most important prey species (36.6%), followed by whiting (25.4%) and lesser sandeel *Ammodytes tobianus* (13.2%). Herring *Clupea harengus*, cod *Gadus morhua* and sprat *Sprattus sprattus* accounted for 5.9%, 5.2%, and 4.1%, respectively. For SIAR, gobies were in-

Table 3.4 Isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of prey species analysed in this study.

Species		n	C:N	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
				Mean	SD	Mean	SD
<i>Ammodytes tobianus</i>	Lesser sandeel	10	3.25	-18.76	0.80	15.08	1.02
<i>Clupea harengus</i>	Herring	20	3.22	-18.59	0.37	15.82	1.18
<i>Hyperoplus lanceolatus</i>	Greater sandeel	20	3.24	-18.07	0.95	15.19	0.63
<i>Merlangius merlangus</i>	Whiting	19	3.16	-17.91	0.76	17.38	0.96
<i>Osmerus eperlanus</i>	Smelt	14	3.36	-18.50	0.97	18.48	0.28
<i>Pomatoschistus microps</i>	Common goby	20	3.50	-15.66	0.72	16.73	0.48
<i>Pomatoschistus minutus</i>	Sand goby	20	3.27	-16.61	1.00	16.96	0.41
<i>Scomber scombrus</i>	Mackerel	17	6.48	-22.35	0.61	13.70	1.36
<i>Sprattus sprattus</i>	Sprat	19	4.36	-20.57	0.89	14.96	0.71
<i>Trisopterus minutus</i>	Poor cod	25	3.12	-18.54	0.63	14.58	0.77

Table 3.5 Diet composition as modelled by SIAR, using TEFs from A: Hobson *et al.* (1996), B: Vander Zanden & Rasmussen (2001), C: McCutchan *et al.* (2003) and D: Caut *et al.* (2011).

Model	A		B		C		D	
	Mean	CI <sub>95</sub>	Mean	CI <sub>95</sub>	Mean	CI <sub>95</sub>	Mean	CI <sub>95</sub>
<i>A. tobianus</i>	9.4	0.0-22.2	14.2	1.2-26.5	7.6	0.0-19.7	9.7	0.0-23.6
<i>C. harengus</i>	3.2	0.0-9.1	6.1	0.0-14.8	2.8	0.0-8.6	5.6	0.0-14.6
<i>G. morhua</i>	1.2	0.0-3.4	1.7	0.0-4.6	1.0	0.0-2.9	2.3	0.0-6.4
<i>H. lanceolatus</i>	8.9	0.0-22.9	13.7	1.9-25.4	6.8	0.0-17.6	15.5	0.0-32.4
<i>M. merlangus</i>	1.3	0.0-3.6	1.9	0.0-5.1	1.2	0.0-3.3	2.4	0.0-6.3
<i>O. eperlanus</i>	0.8	0.0-2.4	1.1	0.0-3.1	0.8	0.0-2.3	1.8	0.0-5.2
<i>P. microps</i>	2.4	0.0-6.8	4.4	0.0-10.5	2.0	0.0-5.9	9.5	0.5-18.3
<i>P. minutus</i>	1.7	0.0-4.8	2.8	0.0-7.9	1.4	0.0-4.2	5.7	0.0-14.7
<i>S. scombrus</i>	23.7	16.3-30.3	15.9	8.6-23.3	35.3	28.3-41.6	18.6	1.7-34.7
<i>S. sprattus</i>	7.1	0.0-19.3	9.2	0.0-19.0	7.1	0.0-19.9	11.8	0.0-25.8
<i>T. minutus</i>	40.2	18.8-60.5	29.0	15.3-43.0	34.0	15.3-51.9	17.1	0.1-34.1

Table 3.6 Bray-Curtis similarities (%) between prey compositions deduced from stomach contents (SC) and as estimated by SIAR modelling using different TEFs (1-4).

	[SC]	[A]	[B]	[C]	[D]
[SC] Stomach contents					
[A] Hobson <i>et al.</i> 1996	79.578				
[B] Vander Zanden & Rasmussen 2001	82.880	94.979			
[C] McCutchan <i>et al.</i> 2003	78.473	97.674	93.327		
[D] Caut <i>et al.</i> 2011	83.927	92.577	95.842	90.925	

cluded in the model separately as sand goby *Pomatoschistus microps* and common goby *Pomatoschistus minutus*. The isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of the resulting 11 prey species is given in Table 3.4.

### 3.4.3 SIAR MODELLING

The estimated relative contribution of the 11 prey species to the diet of porpoises differed slightly between model runs using different TEFs (Table 3.5). In all model runs poor cod *Trisopterus minutus* (17.1-40.2%) and mackerel *Scomber scombrus* (15.9-35.3%) were the most important prey species. In model run A and C, lesser sandeel, greater sandeel *Hyperoplus lanceolatus* and sprat accounted for 25.4% or 21.5% of the diet, respectively. In model run B, lesser sandeel, greater sandeel and sprat accounted for 37.1% of the diet. In model run D, greater sandeel, sprat, lesser sandeel, gobies and herring accounted for 57.8% of the diet. Outcomes of these four different model runs show similarity coefficients (s) ranging between 90.9% and 97.7% (Table 3.6). Prey composition using TEFs as published by Caut et al. (2011) most closely resembled the prey composition as determined from stomach contents (s = 83.9, Figure 3.2) as it estimated the highest importance of gobies and the lowest importance of poor cod out of all the models.

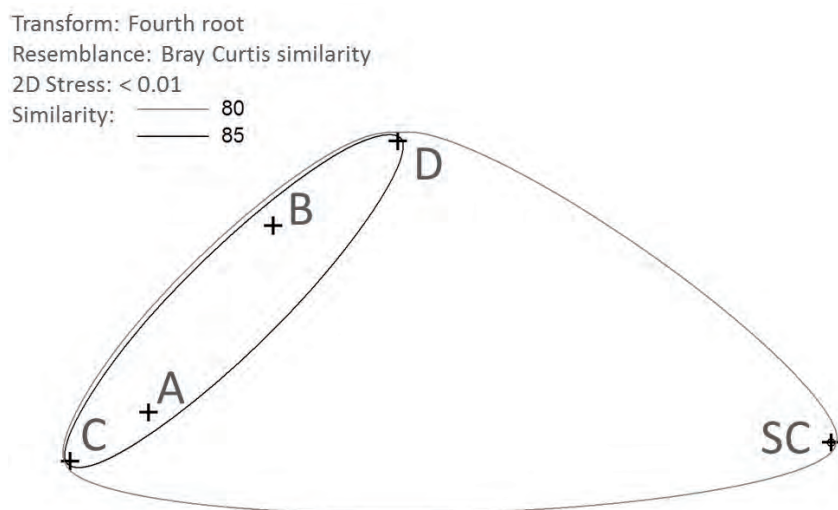


Figure 3.2 Non-metric multi-dimensional scaling (NMDS) graph, comparing relative prey composition in the diet of porpoises *Phocoena phocoena* as deduced from stomach content analysis [SC] and as modelled by SIAR using eleven prey species and four Trophic Enrichment Factors (TEFs): [A] by Hobson et al. (1996), [B] by Vander Zanden & Rasmussen (2001), [C] by McCutchan et al. 2003, and [D] by Caut et al. (2011).

Table 3.7 Diet composition of porpoises as modelled by SIAR, using TEFs from Caut et al. (2011), A: juvenile males, B: juvenile females, C: adult males and D: adult females.

Model	A		B		C		D	
	Mean	CI <sub>95</sub>	Mean	CI <sub>95</sub>	Mean	CI <sub>95</sub>	Mean	CI <sub>95</sub>
<i>A. tobianus</i>	11.2	0.0-23.7	11.1	0.0-24.4	10.5	0.0-21.0	10.1	0.0-19.8
<i>C. harengus</i>	8.6	0.0-19.6	7.2	0.0-18.8	9.2	0.0-19.1	9.0	0.0-18.6
<i>G. morhua</i>	4.9	0.0-12.7	3.3	0.0-9.1	7.4	0.0-17.3	7.4	0.0-16.6
<i>H. lanceolatus</i>	12.0	0.0-24.8	13.9	0.0-29.8	10.5	0.0-20.8	10.1	0.0-19.7
<i>M. merlangus</i>	4.8	0.0-13.0	3.4	0.0-9.8	7.1	0.0-16.5	7.4	0.0-16.4
<i>O. eperlanus</i>	3.9	0.0-10.3	2.7	0.0-7.5	6.6	0.0-15.7	6.6	0.0-15.8
<i>P. microps</i>	6.8	0.0-14.1	7.1	0.0-14.7	6.4	0.0-14.1	8.9	0.0-17.1
<i>P. minutus</i>	7.0	0.0-16.2	5.9	0.0-14.5	7.2	0.0-15.8	8.7	0.0-18.2
<i>S. scombrus</i>	14.4	1.2-27.4	17.9	1.6-33.1	12.2	0.3-22.5	11.0	0.0-20.9
<i>S. sprattus</i>	13.0	0.0-26.3	12.7	0.0-27.2	11.7	0.2-22.6	10.2	0.1-19.6
<i>T. minutus</i>	13.4	0.1-26.4	14.9	0.0-30.1	11.2	0.0-21.6	10.6	0.1-20.6

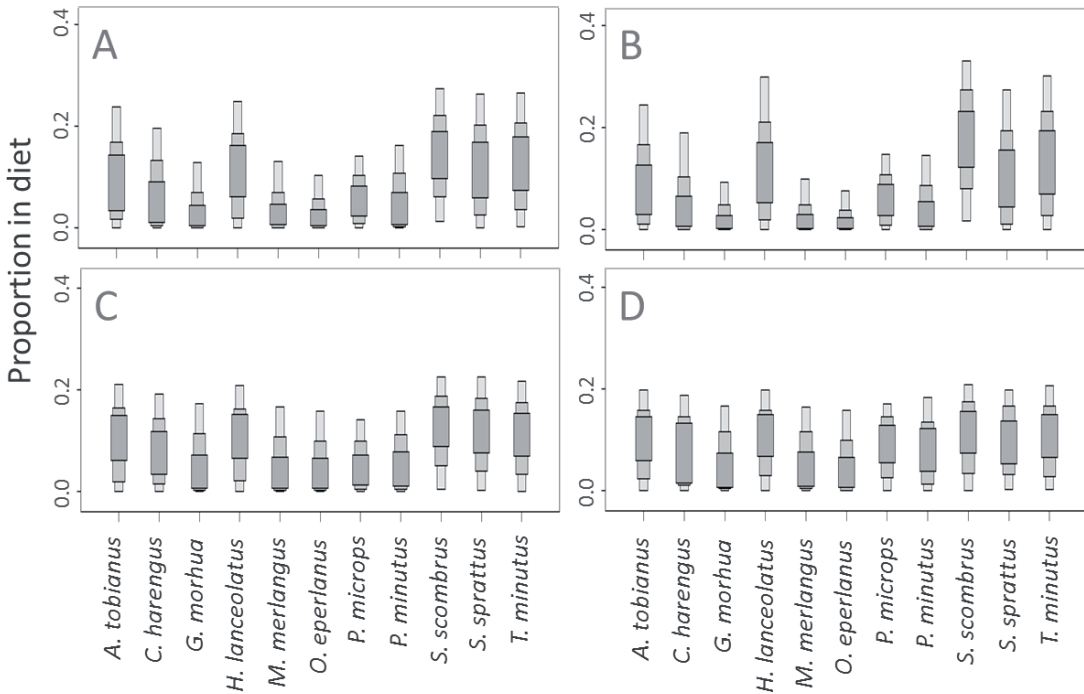


Figure 3.3 Boxplots of the relative contribution of prey sources to the diet of porpoises *Phocoena phocoena* as modelled by SIAR using TEFs by Caut et al. (2011). A: juvenile males, B: juvenile females, C: adult males and D: adult females. Credibility intervals (CI): CI<sub>50</sub> = dark grey, CI<sub>75</sub> = medium grey, CI<sub>95</sub> = light grey.

Using TEFs as published by Caut et al. (2011) we found slight differences in diet between porpoises grouped by their age-class and sex (Table 3.7, Figure 3.3). For all groups, mackerel was the most important prey species (11.0-17.9%). Mackerel is followed by poor cod (10.6-14.9%), sprat (10.2-13.0%), greater sandeel (10.1-13.9%) and small sandeel (10.1-11.2%). The remaining prey species all accounted for less than 10% of the estimated diet. For juvenile porpoises, greater sandeel, mackerel and poor cod were more important than for adults, especially for juvenile females. On the other hand, juvenile females fed less on herring compared to the other groups. Cod, whiting and smelt *Osmerus eperlanus* were less important for juvenile porpoises than for adults, being of lowest importance for female juveniles. Sprat and small sandeel were only slightly less important for adult porpoises compared to juveniles, this difference in importance being smaller for adult females. Herring was less important for juvenile females compared to the other groups while gobies were more important for adult females compared to the other groups. Gobies, both common goby and sand goby, were the more important for adult females than for the other groups.

#### 3.4.4 STABLE ISOTOPES VS. STOMACH CONTENTS

Using SIAR, mackerel was found to be the most important prey species (11.0-17.9%) while in stomach contents, it is only of minor importance (1.3%). Poor cod, sprat and greater sandeel, which are among the most important prey species as estimated by SIAR (together accounting for 30.9-41.8%), are only of minor importance in stomach contents (8.0%). In stomach contents, gobies were found to be the most important prey species (39.5%) followed by whiting (25.5%), while using SIAR, their importance was estimated to be much lower, between 12.9-17.6% for gobies and 3.4-7.4% for whiting.

### 3.5 DISCUSSION AND CONCLUSION

Using stable isotope analysis allows the estimation of past prey composition over a longer term than stomach content analysis (Newsome et al. 2010). Using the same individuals for both analyses, we have found profound differences in the dietary composition estimated by the two techniques, reflecting a genuine difference between the long- and short-term diet of harbour porpoises. The long-term diet outcome reveals that porpoises feed offshore on pelagic, schooling species (e.g., poor cod, mackerel, greater sandeel, and sprat) whereas the short-term diet outcome indicates that they feed closer to shore on more benthic and demersal species (e.g., gobies, whiting, herring, and cod).

### 3.5.1 STABLE ISOTOPE ANALYSIS

There are three possible methodological sources of variation that can influence the resulting diet estimate: [1] the number of prey sources included in the model (Phillips & Gregg 2003), [2] the TEFs used (Gannes et al. 1997; Bond & Diamond 2011) and [3] isotopic representation of sources (Parnell et al. 2010).

**Number of prey sources.** From stomach contents it has been shown that porpoises feed on a wide variety of prey species. Even though SIAR modelling can cope with more sources than isotopes (Parnell et al. 2010), reliably entangling the contribution of as many as 30 prey sources to the isotopic composition of the predator using just two stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) is impossible. In our study, we have only included prey species that have been shown to be of major importance to the diet of porpoises as deduced from stomach contents. Concentrating on only few species or grouping species with similar isotopic values will improve source differentiation but will also reduce distinction in quantitative diet estimation.

**Trophic Enrichment Factors.** TEFs are thought to be i.a. species-, tissue- and diet specific (DeNiro & Epstein 1981; Vanderklift & Ponsard 2003). It is common practice to use TEFs of other species or tissues when TEFs for the species analysed are not available yet (Bond & Diamond 2011). It has been shown that stable isotope mixing models are sensitive to variation in discrimination factors and can lead to misinterpretation when species- and tissue specific TEFs are unknown and general ones are applied instead (Martínez del Rio et al. 2009; Bond & Diamond 2011). Unfortunately, species- and diet-specific TEFs for porpoises are not available. We have therefore used several different published TEFs as calculated from seals (Hobson et al. 1996), as averaged for carnivores (Vander Zanden & Rasmussen 2001), averaged for lipid extracted muscle (McCutchan et al. 2003), and as derived from killer whales (Caut et al. 2011). Our study showed that for the porpoise, model outcomes using the different TEFs were in general very similar (Figure 3.2 and Table 3.6). The model using TEFs as deduced from cetaceans (i.e., killer whales, Caut et al. 2011) was most similar to the results from stomach contents. The fact that the cetacean derived TEFs show the highest similarity with stomach contents supports the need for the use and development of species-specific TEFs. The influence of diet-specific TEFs on the predictive power of SIAR is hard to evaluate. This issue would probably concern mostly mackerel and sprat, as other food items have similar C:N ratios, and therefore presumably similar nutritional quality. However, even prey showing similar C:N ratios can have different biochemical composition, leading to variability in trophic enrichment (Aberle & Malzahn 2007). Experimental measurements of species- and diet-specific TEFs would likely improve the accuracy of SIAR outputs, and efforts to produce these are desirable in this field of research.



**Isotopic representation of sources.** SIAR modelling is most useful when few prey species with distinct isotopic composition are used (Parnell et al. 2010). The isotopic composition of prey species, however, showed great spatial variation and large overlap between species. When dealing with a highly mobile predator that feeds on a multitude of species, sampling sufficient characteristic and representative prey is challenging, time consuming and expensive. Porpoises stranded along the Dutch coast are considered to have fed mainly in Dutch coastal waters, but satellite tracking has shown that they can range over considerable distances (Read & Westgate 1997; Johnston et al. 2005). Prey samples were therefore collected from the southern North Sea, with the majority of samples from Dutch coastal waters, covering size-classes that were identified in stomach contents (Leopold & Camphuysen 2006). Spatial variation in isotopic composition among prey from the southern North Sea has been shown to be low (Jansen et al. 2012a). In order to improve species differentiation a reduced set of prey sources (%W > 1) was used, but there was still some overlap in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between species.

### 3.5.2 STOMACH CONTENTS ANALYSIS

Stomach content analysis provides insight into the diet shortly before the stranding and may be biased towards species with large, robust hard parts (Hyslop 1980; da Silva & Neilson 1985). The otoliths of whiting and cod are large, robust and very distinct (Härkönen 1986), which makes them easy to identify, even in very digested or decomposed stomach samples. Otoliths of mackerel and sprat, however, are fragile (Härkönen 1986), and so may be less recognizable due to digestion and decomposition. This bias may lead to an overrepresentation of whiting and cod and an underrepresentation of species like mackerel and sprat in stomach contents (Bowen 2000). A second bias of stomach contents analysis is the confusion between fish species that are closely related and therefore have very similar otoliths, e.g., poor cod and bib *Trisopterus luscus*, lesser and small sandeel or different goby species. Including prey remains other than otoliths (Watt et al. 1997; Cottrell & Trites 2002) and correcting for wear (Jobling & Breiby 1986; Leopold et al. 1998) as we did, reduces this bias in stomach content analysis.

### 3.5.3 ECOLOGICAL IMPLICATIONS

Fish species identified in stomachs and by SIAR modelling are all very abundant in the North Sea, including pelagic, schooling species (e.g., mackerel, herring, and sprat), demersal species (e.g., whiting, poor cod, and sole) and typical coastal species (e.g., gobies, smelt, and bass). However, as stomach contents are likely biased towards near-shore species that are ingested shortly before the stranding, it is not surprising that gobies dominate the diet when only stomach contents are used (Knijn et al. 1993). It has been suggested that gobies are mainly prey

of juvenile porpoises (Addink et al. 1995), however, this was not the case for animals included in this study (Leopold & Camphuysen 2006).

Although SIAR is limited to the number and quality of prey sources included in the model, it covers a longer term diet, thus raising the chance to include prey taken during foraging trips further offshore. It is also able to recognize species with fragile hard prey remains and distinguishes between species with similar otoliths. Using SIAR resulted in a significant reduction in the importance of small benthic fish i.e., gobies while pelagic, schooling species such as mackerel became more important.

Gadoids are found to be the main prey in many studies, with regional differences in specific species (Santos & Pierce 2003). Poor cod can be found throughout the entire North Sea, although densities are generally lower in deeper parts (Knijn et al. 1993). Poor cod was identified among the most important prey species in Scottish and Irish waters (Rogan & Berrow 1996; Santos et al. 2004). Mackerel has also been identified in other studies (Santos & Pierce 2003); however, only in the coastal waters of Eastern Canada were they identified among the most important prey species (Smith & Gaskin 1974). Gadoids such as whiting and cod are more important in stomach contents than in the diet estimated by SIAR. They are both abundant and widely distributed species throughout the North Sea (Knijn et al. 1993). In almost all studies on porpoise diet, sandeels are found to be important prey species (Santos & Pierce 2003), also in our study, irrespective of the method used. The decline of sandeel stocks has been suggested as a reason for starvation and a southern migration of porpoises from Scottish waters (MacLeod et al. 2007a,b), underlining the impact that declines of certain fish stocks can have on the distribution of porpoises throughout the North Sea. Clupeids are among the most important prey species, using both SIAR (i.e., sprat) and stomach contents (i.e., herring). These energy-rich prey species seem to have become less important in the diet of porpoises over the years. It has been suggested that this is due to declines in their respective stocks (Santos & Pierce 2003).

The difference between the results of stomach contents and SIAR is not necessarily a result of the horizontal distribution of prey species but may also be caused by differences in the behaviour of fish species and porpoises in the turbid coastal waters compared to the clearer offshore waters. Pelagic fish tend to school during the day, while these aggregations become more dispersed in dark or turbid conditions (Glass et al. 1986; Turesson & Brönmark 2007). Dutch coastal waters are very turbid due to the outflow of big rivers (Eisma & Kalf 1979; Fettweis & van den Ende 2003). Pelagic fish are therefore highly dispersed in the coastal zone, rendering them less easy to catch. This could explain the higher occurrence of pelagic schooling prey species (e.g., mackerel) using SIAR compared to stomach content analysis. The comparison between the two methods suggests that porpoises are not limited to preying on demersal species in

the coastal zone, but also prey on pelagic schooling species in offshore waters. A future step in the interpretation of differences between diets as deduced from stable isotopes and from stomach contents should be the inclusion of age, seasonality in strandings, and/or prey availability.

### 3.5.4 CONCLUSION

Profound differences were found in the diet of harbour porpoises as estimated by SIAR and the diet as deduced from stomach content analysis. This points towards an ecological and not a methodological difference, because the prey species used in the isotope estimate were chosen on the basis of being most important in the stomach contents. This may indicate a difference between long-term diet where porpoises feed also offshore on pelagic, schooling species and their short-term diet where they feed closer to shore on more benthic and demersal species. This could be due to the distribution of prey species as well as differences in behaviour of porpoises and their prey between the coastal zone and offshore waters.

This difference between long- and short-term diet as deduced from applying two techniques is of relevance for e.g., ecological impact assessment studies, fishery impact assessments, and management decisions for the conservation of porpoises. When only one technique is used, key prey species in the predator-prey relation may be missed or underestimated, highlighting the need for multi-method approaches in diet reconstruction.

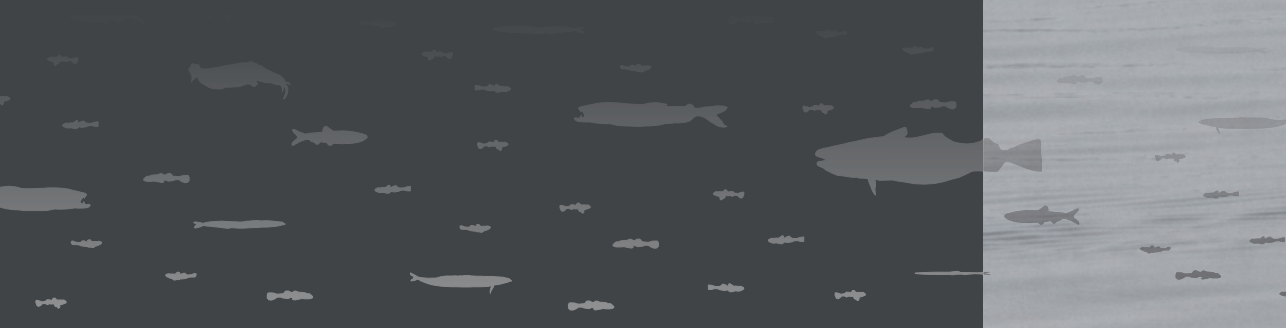
## 3.6 ACKNOWLEDGEMENTS

Porpoises were collected by staff and volunteers of the Dutch strandings network, coordinated by the NCB Naturalis in Leiden. We would like to thank M.F. Leopold (IMARES), C.J. Camphuysen (NIOZ), T. Jauniaux (Liège University), A. Gröne (Utrecht University) and L. Wiersma (Utrecht University) for porpoise sample collection. Fish samples were collected during IBTS, DFS and BTS surveys (IMARES) and we are thankful to staff and volunteers on board of the *Tri-dens* and *Isis*. We would like to thank E. Meesters (IMARES) and F. van Duyl (NIOZ) for fruitful discussions on stable isotopes. K. Das and G. Lepoint are F.R.S.-FNRS Research Associates (Liège University). L. Michel was a F.R.S.-FNRS Research Fellow (Liège University). We would also like to thank D. Miller and three anonymous reviewers for their helpful suggestions on this article. This work was funded by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV), BO Project 4308201019.

# CHAPTER 4

## HARBOUR PORPOISES *PHOCOENA PHOCOENA* IN THE EASTERN SCHELDT: A RESIDENT STOCK OR TRAPPED BY A STORM SURGE BARRIER?

Published as: Jansen, O.E., Aarts, G.M., Reijnders, P.J.H. (in press) Harbour porpoises *Phocoena phocoena* in the Eastern Scheldt: a resident stock or trapped by a storm surge barrier? PLOS ONE.





## 4.1 ABSTRACT

Coastal protection measures are planned and executed worldwide to combat the effects of global warming and climate change, in particular the acceleration of sea level rise, higher storm surge flooding and extensive coastal inundation. The extent to which these defensive measures may impact coastal and estuarine ecosystems is still poorly understood. Since the building of a storm surge barrier, movement of harbour porpoises *Phocoena phocoena* in and out of the Eastern Scheldt tidal bay (SW-Netherlands) may be limited. To measure residency, porpoises stranded along the Dutch North Sea coast between 2006 and 2008 were sampled for muscle (n = 102) and bone tissue (n= 118), of which 9 muscle (8.8%) and 12 bone samples (10.2%) were collected from animals stranded within the Eastern Scheldt. Stable carbon ( $\delta^{13}\text{C}$ ) was analysed to get insight into the habitat use and residency of porpoises in the Eastern Scheldt. Our data showed significantly higher  $\delta^{13}\text{C}$  values in the muscle of porpoises stranded within the Eastern Scheldt ( $\mu=-17.7\text{‰}$ ,  $\text{SD}=0.4\text{‰}$ ) compared to animals stranded along the Dutch coast ( $\mu=-18.3\text{‰}$ ,  $\text{SD}=0.5\text{‰}$ ). This suggests that most porpoises stranded in the Eastern Scheldt foraged there for a longer period. The distinct  $\delta^{13}\text{C}$  signature of animals from the Eastern Scheldt was not observed in bone tissue, suggesting a relatively recent shift in habitat use rather than life-long residency of porpoises within the Eastern Scheldt. The high number of strandings within the Eastern Scheldt suggests a higher mortality rate compared to the Dutch coastal zone. Our study indicates that along with other changes in the physical environment, the storm surge barrier may play an important role in determining the residency of porpoises in the Eastern Scheldt, and that the area might act as an ecological trap for porpoises entering it.

## 4.2 INTRODUCTION

The predicted consequences of climate change and global warming on human populations and coastal ecosystems, in particular the accelerated sea level rise, higher storm surge flooding and extensive coastal inundation (Meehl et al. 2005; Rahmstorf 2007), has led to increased coastal protection measures (Bosello et al. 2012; Hallegatte 2012). These include the construction of seawalls, levees and flood gates, tidal barriers and beach nourishment (IPCC 1990). For marine mammals, such defensive measures can lead to habitat loss, degradation and fragmentation. This can have important consequences for small populations due to chance effects and loss of genetic diversity (Harwood 2001).

For the Netherlands, where 60% of its land is situated below sea level (Kabat et al. 2005), these defensive measures are particularly necessary. One such measure is located at the entrance of the Eastern Scheldt, a tidal bay, situated



in the south-western part of the Netherlands. Two large auxiliary compartment dams were built between 1977 and 1987, isolating the former estuary from freshwater input of the river Scheldt and river Rhine. A storm surge barrier with gates was built between 1979 and 1986 at the entrance to the Eastern Scheldt to safeguard the tidal ecosystem while reducing the risk of flooding (Nienhuis & Smaal 1994). Comparable coastal protection structures are likely to increase world-wide in response to the effects of climate change (IPCC 1990; Hallegatte 2012), and even semi-open structures may have detrimental effects on coastal and estuarine ecosystems.

Harbour porpoises *Phocoena phocoena* are the most common small cetacean in Dutch coastal waters. Their abundance in Dutch coastal waters has changed significantly over the past decades. After a decline and near extinction in the 1950s and 1960s, numbers slowly recovered from the mid-1990s onwards, with a distinct peak in sightings and strandings in 2006 (Camphuysen et al. 2008; Reijnders et al. 2009). Recent population estimates for the Dutch Continental Shelf (DCS) are approximately 26,000 in summer and 30,000 in autumn, with peak densities of up to 86,000 animals in March (Geelhoed et al. 2011; Scheidat et al. 2012).

Harbour porpoises are listed as endangered in several international, European and national legislations. They are also listed in several conventions, agreements and action plans such as the Habitats Directive (92/43/EEC), the Bern Convention, CITES and the ASCOBANS North Sea conservation plan under the Convention of Migratory Species) (Reijnders et al. 2009). The conservation of species requires that we know enough about their ecology (e.g. migration, abundance, distribution, feeding ecology, reproduction, etc.) and their habitat in order to develop effective protection measures.

Anecdotal data shows that porpoises used to be common visitors in the Eastern Scheldt. However, for a few decades after the building of the storm surge barrier, no porpoises were observed in the area. Over the last ten years, small numbers of porpoises have been observed again in the Eastern Scheldt. They have become not only more abundant in the area but can now also be found there year round. Three dedicated annual surveys between 2009 and 2011 documented 37, 15 and 61 porpoises in the Eastern Scheldt, respectively, including 4–5 mother-calf pairs (Zanderink & Osinga 2010). Currently it is unknown whether these individuals feed in the Eastern Scheldt for longer periods, or whether there is a continuous exchange between the Eastern Scheldt and the adjacent North Sea.

To analyse diet composition, trophic level and origin in terrestrial and marine species, isotopic ratios of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ , expressed as  $\delta^{15}\text{N}$ ) and carbon ( $^{13}\text{C}/^{12}\text{C}$ , expressed as  $\delta^{13}\text{C}$ ) can be used (Michener & Kaufman 2007; Newsome et al. 2010). Generally, predators are enriched in  $^{15}\text{N}$  compared to their prey (approximately 3–4‰ higher; DeNiro & Epstein 1981; Caut et al. 2009)

while predator and prey are relatively similar in  $\delta^{13}\text{C}$  values (approximately 0.1–1‰ higher in predator; DeNiro & Epstein 1978; Caut et al. 2009).  $\delta^{15}\text{N}$  values can therefore be used as indicators of relative trophic level (Post 2002) while geographic differences in  $\delta^{13}\text{C}$  can be used to indicate foraging location and habitat use of animals (Hobson 1999; Barnes et al. 2009). Depending on the tissue analysed, stable isotopes reflect periods varying from hours to years (Tieszen et al. 1983; Dalerum & Angerbjörn 2005; Phillips & Eldridge 2006). Muscle tissue reflects assimilated diet of weeks or months (Kurle & Worthy 2002) while bone tissue displays a more long-term integration, reflecting assimilated diet of several years (Sealy et al. 1995; Richards et al. 2002; O'Regan et al. 2008). Stable isotope analysis thus enables the identification of (longer-term) residency in areas with contrasting isotopic composition (Harrod et al. 2005) such as the Dutch coastal zone *versus* the Eastern Scheldt.

A previous study based on stable isotope values of porpoises from Dutch coastal waters found differences in trophic level and feeding location between animals of different ages or sex, and identified different groups of porpoises that stranded during the summer and winter months. They also found differences in the isotopic composition between animals stranded within the Eastern Scheldt compared to animals stranded along the Dutch coast (Jansen et al. 2012a).

The purpose of the present study is to use the existing and new analysis to gain additional insight into the habitat use and residency of harbour porpoises from the Eastern Scheldt by inspecting stable isotope composition of different tissues. To that end we have analysed a large number of porpoises stranded along the Dutch coast and porpoises stranded within the Eastern Scheldt and have assessed residency based on the isotopic composition ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) of muscle and bone.

## 4.3 MATERIALS AND METHODS

### 4.3.1 SAMPLE COLLECTION

For this study, 102 muscle and 118 bone samples were collected from harbour porpoises stranded on the Dutch coast and in the Eastern Scheldt (Figure 4.1) between 2006 and 2008. Dead porpoises were collected by staff and volunteers of the Dutch stranding network, coordinated by the National Centre for Biodiversity (NCB) Naturalis in Leiden under the licence of the Dutch Ministry of Economic Affairs, Agriculture and Innovation (EL&I). Stranding date and location were reported for each animal, and during post-mortem examinations, general morphometric data were collected, e.g. sex (male, female, unknown) and length (cm). Muscle samples were taken from the ventral mid-region, while for bone tissue, the 5<sup>th</sup> rib was collected. Samples were stored frozen at -20°C until

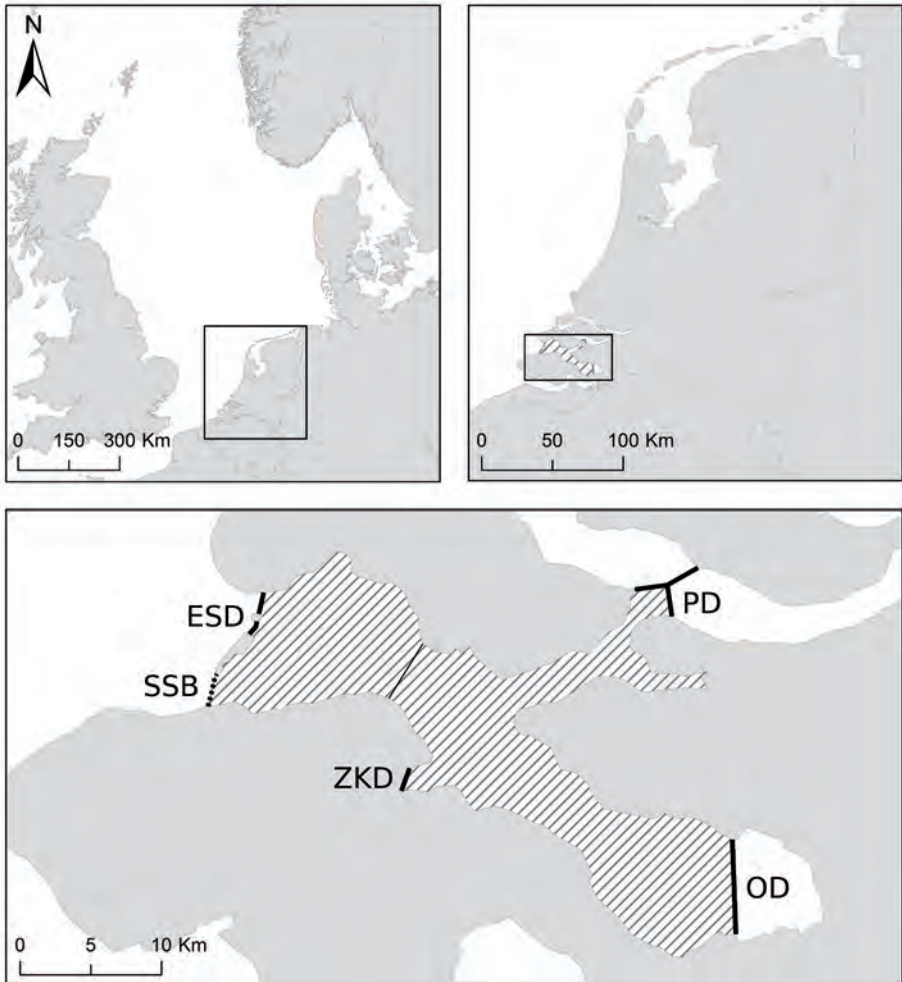


Figure 4.1 Eastern Scheldt tidal bay. The Eastern Scheldt tidal bay (dashed area), situated in the south-east of the Netherlands. Indicated are the Storm Surge Barrier (SSB), Eastern Scheldt Dam (ESD), Phillipsdam (PD), Oesterdam (OD) and Zandkreekdam (ZKD).

analysis. Stranding data of porpoises along the entire Dutch coast were taken from the database of NCB Naturalis ([walvisstrandingen.nl](http://walvisstrandingen.nl) 2012).

#### 4.3.2 SAMPLE PREPARATION

Sample preparation is described in detail in Jansen et al. (2012). In short, muscle samples were freeze-dried for ca. 20 hours, homogenized with a pestle and mortar and lipids were extracted using a 2:1 chloroform-methanol solution. Bone samples (ribs) were cleaned and bone marrow was removed, they were sonicated in Milli-Q purified water and dried overnight. Bone samples were then homogenized with an automatic grinder, demineralized in a weak acid solution

and dried overnight. Lipids were extracted from bone powder using a 2:1 chloroform-methanol solution. All samples were analysed twofold: before pre-treatment to measure  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values and after lipid extraction and acidification to measure  $\delta^{13}\text{C}$  values.

### 4.3.3 STABLE ISOTOPE ANALYSIS

Stable isotope measurements were performed by isotope-ratio mass spectrometry using a mass spectrometer (V.G. Optima Isoprime, UK) coupled to an N-C-S elemental analyser (Carlo Erba) for automated analyses at the Laboratory for Oceanology, Liège University, in Belgium. Stable isotope abundances are expressed in conventional delta ( $\delta$ ) notation in parts per thousand (‰), and are expressed relative to the international standards: Vienna-PeeDee Belin-nite limestone (V-PDB) for  $^{13}\text{C}$  measurements and atmospheric nitrogen for  $^{15}\text{N}$  measurements. The following equation is used:  $\delta X = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000$  where  $R_{\text{sample}}$  is the isotopic ratio of the sample and  $X$  is  $^{13}\text{C}$  or  $^{15}\text{N}$  and  $R$  the ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  (Hobson 1999; Newsome et al. 2010). Reference materials used were: IAEA-N1 ( $\delta^{15}\text{N}$ :  $\mu 0.4\text{‰}$ ,  $\text{SD}=0.2\text{‰}$ ) and IAEA-C6 ( $\delta^{13}\text{C}$ :  $\mu = -10.4\text{‰}$ ,  $\text{SD}=0.2\text{‰}$ ) (IAEA, Vienna, Austria). Internal standards (glycine) were inserted into all runs at regular intervals to calibrate the system and to assess drift over time. Measurement uncertainty, relative to true values of internal standards, was  $\text{SD}=0.1\text{‰}$  for carbon and  $\text{SD}=0.3\text{‰}$  for nitrogen. Further details can be found in Jansen et al. (2012).

### 4.3.4 STATISTICAL ANALYSIS

Homogeneity in variance was tested using the Bartlett test (Bartlett 1937). Generalized Linear Models (GLMs, McCullagh & Nelder 1989) were fitted to examine whether variation in isotope values was associated with stranding location. Models were fitted using four possible response variables (i.e. bone  $\delta^{13}\text{C}$ , muscle  $\delta^{13}\text{C}$ , bone  $\delta^{15}\text{N}$ , muscle  $\delta^{15}\text{N}$ ). The explanatory variable included stranding location (i.e. within the Eastern Scheldt or elsewhere along the Dutch coast). ANOVA F-tests were used to test if isotopic values differed between stranding locations. Statistical analysis was carried out in the computing environment R (R 2.9.2; R Development Core Team 2009).

## 4.4 RESULTS

### 4.4.1 PORPOISES

Between 2006 and 2008, 1189 stranded porpoises were documented along the Dutch coast and 37 within the Eastern Scheldt. The majority of bone and muscle samples were collected along the Dutch coast ( $n = 118$  and  $102$ , respectively). From the Eastern Scheldt, 12 bone samples (10.5%) and 9 muscle samples (8.8%) were available. Details on stranding location, sex ratio, age-classes and

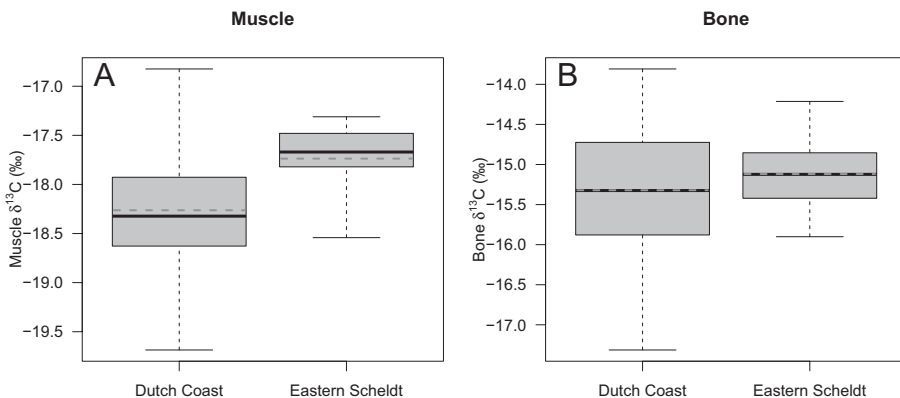


Figure 4.2 Porpoise  $\delta^{13}\text{C}$  values in muscle and bone tissue. Boxplot of  $\delta^{13}\text{C}$  values of porpoises *Phocoena phocoena* stranded within the Eastern Scheldt and elsewhere along the Dutch coast based on muscle tissue (A) and bone tissue (B). Solid line presents the median, dashed line represents the mean, the grey box represents the interquartile range (IQR, i.e. between 0.25 and 0.75 quantile) and the whiskers extend to the data extremes.

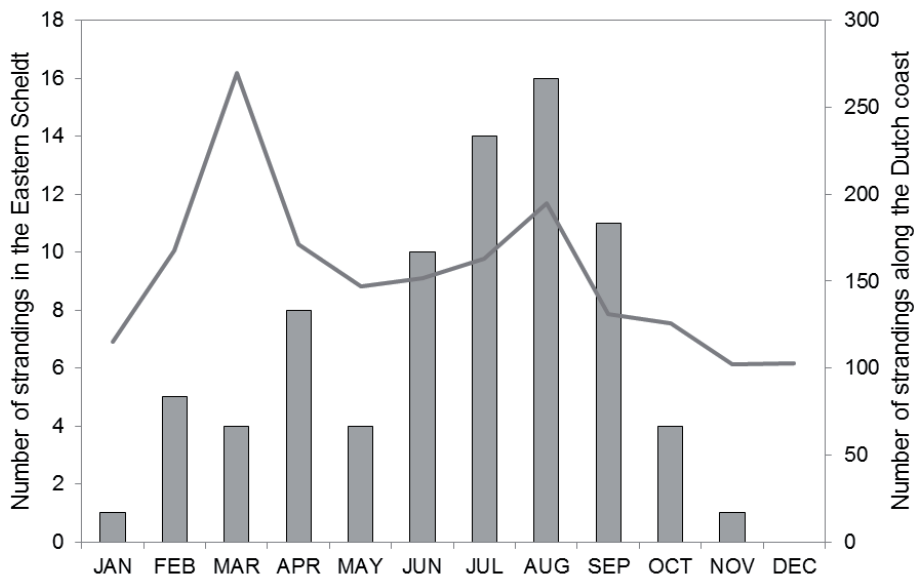


Figure 4.3 Porpoise strandings in the Eastern Scheldt. Porpoise *Phocoena phocoena* strandings in the Eastern Scheldt per calendar month (2006-2011). The solid line shows the number along the Dutch coast ([www.walvisstrandingen.nl](http://www.walvisstrandingen.nl)).

length and weight measurements are presented in Jansen et al. (2012). From 2006 to 2008, the seasonal pattern in strandings along the Dutch coast reveals a peak in March and a slightly lower peak in August. In the Eastern Scheldt, most animals stranded in the months around August, and relatively few strandings were recorded around March (Figure 4.3).

#### 4.4.2 ISOTOPIC COMPOSITION

$\delta^{13}\text{C}$  ranged from -19.7 to -16.8‰ ( $\mu=-18.2\text{‰}$ ,  $\text{SD}=0.5\text{‰}$ ) in muscle and from -17.3 to -13.8‰ ( $\mu=-15.4\text{‰}$ ,  $\text{SD}=0.7\text{‰}$ ) in bone (Figure 4.2). There was no evidence for heterogeneity in variance (muscle  $\delta^{13}\text{C}$ : Bartlett's  $K^2=1.13$ ,  $p\text{-value}=0.29$ ; bone  $\delta^{13}\text{C}$ :  $K^2=1.26$ ,  $p\text{-value}=0.26$ ). GLMs revealed that the area of stranding (Eastern Scheldt *versus* Dutch coast) explained a significant ( $p\text{-value}<0.01$ ) part of the variation of  $\delta^{13}\text{C}$  found in muscle tissue (ANOVA  $F=8.993$ ,  $p=0.0034$ ). In contrast, there was no significant effect of stranding location on bone  $\delta^{13}\text{C}$  ( $F=0.81$ ,  $p=0.37$ ).  $\delta^{15}\text{N}$  ranged from 13.4 to 19.1‰ ( $\mu=16.4\text{‰}$ ,  $\text{SD}=1.4\text{‰}$ ) in muscle tissue and from 11.3 to 20.7‰ ( $\mu=16.3\text{‰}$ ,  $\text{SD}=1.7\text{‰}$ ) in bone. There was also no significant effect of stranding location on  $\delta^{15}\text{N}$  in muscle and bone (respectively,  $F=3.6$ ,  $p=0.060$  and  $F=1.37$ ,  $p=0.25$ ).

## 4.5 DISCUSSION

Since isotopic composition in a predator depends on tissue composition and lipid content (Sotiropoulos et al. 2004; Jacob et al. 2005), tissue turnover-rates (Tieszen et al. 1983) and tissue-dependent fractionation (DeNiro & Epstein 1978, 1981), we have taken into account the influence of these factors in assessing diet and feeding location of our study animals. The ability to infer irrefutable information on diet and feeding location of porpoises depends on the knowledge of the specific influence of these factors on porpoise isotopic composition (Hobson 1999).

Isotopic data from porpoises stranded along the French, Belgian and Dutch coastal waters (Das et al. 2003) show similar  $\delta^{15}\text{N}$  values ( $\mu=16.2\text{‰}$ ) but lower  $\delta^{13}\text{C}$  values ( $\mu=-16.4\text{‰}$ ) compared to our study, suggesting that porpoises from these adjacent areas generally feed on a similar trophic level, but that regional differences in  $\delta^{13}\text{C}$  baseline values are found in porpoises. Our data supports the findings of Christensen & Richardson (2008) who have found gradually decreasing  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values during the last century, possibly due to porpoises feeding on lower trophic level prey due to changes in the food web structure of the North Sea.



#### 4.5.1 THE EASTERN SCHELDT

The Eastern Scheldt tidal bay was created by the building of compartment dams isolating the former estuary from freshwater input of the Scheldt and Rhine rivers (Nienhuis & Smaal 1994) (Figure 4.1). In addition, a storm surge barrier was built at the entrance of the Eastern Scheldt, reducing tidal amplitude in the bay. Since the existence of these constructions, changes in hydrodynamics (e.g. increased residency of the water mass), changes in hydrochemistry (e.g. increase of inorganic nutrients and particulate organic carbon) and related shifts in phytoplankton assemblage have occurred (Nienhuis & Smaal 1994). Though there are no baseline isotopic values available for the Eastern Scheldt, tidal estuaries are generally characterized by a long residence time of water and particles and a larger input of terrestrial organic matter. It can be expected that tidal estuarine systems differ significantly from other marine systems (Middelburg & Herman 2007), and Clementz & Koch (2001) have shown higher  $\delta^{13}\text{C}$  values in marine mammals from estuarine systems compared to those from offshore and near shore marine systems. Distinct (higher)  $\delta^{13}\text{C}$  values were found in muscle of porpoises stranded within the Eastern Scheldt compared to individuals stranded along the Dutch coast. This could either be caused by porpoises feeding on other prey species within the Eastern Scheldt, or by differences in baseline isotopic values of prey species of the Eastern Scheldt and the coastal waters. In any case, our data suggests that porpoises stranded in the Eastern Scheldt had fed in the area long enough to integrate the distinct isotopic pattern of this area and that they did not leave the area frequently to forage in the Dutch coastal zone. This distinct  $\delta^{13}\text{C}$  isotopic signature was not found in bone tissue, indicating a relatively recent shift in feeding location rather than life-long residency of porpoises within the Eastern Scheldt.

#### 4.5.2 DOES THE EASTERN SCHELDT CONTAIN A VIABLE POPULATION?

Three dedicated surveys of the Eastern Scheldt have documented between 15 and 61 porpoises, including 4-5 mother-calf pairs (Zanderink & Osinga 2010). Most harbour porpoises along the Dutch coast are present in early spring, with a peak in March, and have departed by the end of April. Similar patterns are observed for the Western Scheldt and Wadden Sea (Camphuysen 2011). This is most likely a seasonal migration as a result of prey availability. In contrast, in the Eastern Scheldt porpoises occur year round (Zanderink & Osinga 2010). Furthermore, the seasonal pattern in strandings within the Eastern Scheldt is rather different from that observed along the North Sea coast. The dissimilarity between the sighting and stranding pattern suggest a seasonal disconnection between the Eastern Scheldt and other regions.

In 2011, 61 individuals were counted (rugvin.nl 2012). However, there is no detailed information on the processing of the data and hence the accuracy of that survey. But given the excellent weather conditions during the 2011 survey, the total count is thought to be a realistic estimate of the number of porpoises present in this area (Zanderink & Osinga 2010). In that same year, 20 animals were found dead (Jansen et al. 2012a), suggesting that mortality is high and not sustainable if there would be no influx. It may be possible that some of these porpoises died in the North Sea and stranded in the Eastern Scheldt, but given the specific isotopic composition of porpoises stranded within the Eastern Scheldt, this is unlikely. Porpoises occurring in the adjoining North Sea are the likely source of this influx. Since the highest densities occur in March and April along the Dutch coast, most porpoises are expected to enter the Eastern Scheldt during that period. The fact that a specific isotopic composition in muscle tissue can be established in about 4-6 weeks (Tieszen et al. 1983), and that most strandings in the Eastern Scheldt occur between July and September, supports our conclusion that there must be a continuous influx of animals from the North Sea. The Eastern Scheldt may thus act as an ecological trap for porpoises.

This study shows how porpoises may be affected by coastal protection structures and how even semi-open structures may form a barrier for migration and transit of these animals. This in turn influences their habitat use, residency and possibly also their survival. As the number of coastal protection structures is increasing worldwide, it is becoming more important to take into account the potential effect of such structures on marine organisms in order to meet and improve management objectives and conservation measures.

## 4.6 CONCLUSIONS

Stable isotope analysis can provide important information on the feeding ecology and habitat use of harbour porpoises. Differences in isotopic composition were found between animals stranded along the Dutch coast and animals stranded within the Eastern Scheldt. We have shown that muscle  $\delta^{13}\text{C}$  values can be used to identify porpoises that have been feeding in the Eastern Scheldt for an extended period of time. Based on bone  $\delta^{13}\text{C}$  values, we have found no evidence that any of the animals analysed were born in the Eastern Scheldt, indicating that they have subsequently entered the area. Future stable isotope analysis of bone has the potential to assess whether animals born in the Eastern Scheldt stay there.

Mortality is exceptionally high in the Eastern Scheldt and both sighting rate and strandings differ seasonally compared to animals in the Dutch coastal zone. The increase in live animals counted in the past three years cannot be explained by calf production alone and observed relative mortality. Evidently,

regular influx of animals from the North Sea must occur. Our study indicates that along with other changes in the physical environment, the storm surge barrier may play an important role in determining the residency of porpoises in the Eastern Scheldt. Additionally, in view of the relative high percentage of animals found dead, the area may act as an ecological sink for some of those immigrants.

Worldwide, coastal protection measures increase in response to the effects of global warming and climate change. The effects of these defensive measures on marine and estuarine ecosystems are still poorly understood. This study is an example of the impact of such a protection structure and highlights that even semi-open structures, which are meant to ameliorate habitat loss, degradation and fragmentation, may still affect the abundance and distribution of individual species.

## 4.7 ACKNOWLEDGEMENTS

Porpoises were collected by staff and volunteers of the Dutch strandings network, coordinated by the NCB Naturalis in Leiden under the licence of the Dutch Ministry of Economic Affairs, Agriculture and Innovation (EL & I). Information on porpoise strandings on the Dutch coast and in the Eastern Scheldt was supplied by G. Keijl (NCB Naturalis) and J. van der Hiele (EHBZ ZuidWest). The authors would like to thank M.F. Leopold (IMARES), C.J. Camphuysen (NIOZ), T. Jauniaux (Liège University), A. Gröne (Utrecht University) and L. Wiersma (Utrecht University) for organizing and executing the post-mortem examinations that have provided samples and information on the animals. Stable isotope analysis was performed under supervision and with help from K. Das, G. Lepoint, L. Michel and S. Habran at the University of Liège. We would like to thank R.S.A. van Bemmelen for creating the map of strandings localities. We also thank D.C.M. Miller and four anonymous reviewers for valuable draft revisions. This work was funded by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV), BO Project 4308201019. G. Aarts is funded by the NWO-ZKO grant 'Effects of underwater noise on fish and marine mammals in the North Sea'.

# CHAPTER 5

## THE DIET OF HARBOUR PORPOISES ALONG THE DUTCH COAST BASED ON QFASA ANALYSIS: A COMBINED FATTY ACID AND STOMACH CONTENTS APPROACH

To be submitted as: Jansen, O.E., Meesters, E.H.W.G., Brasseur, S.M.J.M., Budge, S.M., Reijnders, P.J.H. The diet of harbour porpoises along the Dutch coast based on QFASA analysis: a combined fatty acid and stomach contents approach.







## 5.1 ABSTRACT

The diet of harbour porpoises stranded along the Dutch coast between 1990 and 2007 was estimated using quantitative fatty acid signature analysis (QFASA) and compared to the diet as deduced from stomach contents to analyse differences between short- and longer term diet composition. The diet as modelled by QFASA varied with the prey species included in the model, and the calibration coefficients and prey sets used. All QFASA models revealed a profound difference in diet composition compared to the diet as estimated by stomach contents. According to QFASA, the diet consists of 21.7% gobies, 16.7% mackerel, 14.4% smelt, 14.1% herring, 12.6% dragonet, and 20.6% other species. According to stomach contents, the diet consists of 38.5% gobies, 19.0% whiting, 9.6% herring, 7.5% lesser sandeel, 5.3% poor cod and 20.2% other species. The considerable differences between these estimates may reflect temporal differences in diet but may also be caused by methodological problems. To be able to distinguish between a possible real difference in longer term diets as revealed by fatty acid analysis compared to short term diets obtained by stomach content analysis, controlled feeding experiments are deemed to be necessary.

## 5.2 INTRODUCTION

Fatty acid analysis has been used to study the feeding ecology of marine mammals (Thiemann et al. 2008). This technique is based on the premise that consumers' fatty acids resemble those of their prey (Iverson et al. 1997; Beck et al. 2005). Fatty acids reflect the animal's accumulated diet over a period of up to several months and have therefore less (temporal and spatial) limitations of the more conventional method of stomach content analysis (Iverson et al. 2004; Budge et al. 2006). A specific modelling technique, Quantitative Fatty Acid Signature Analysis (QFASA, Iverson et al. 2004), is used to compute the most likely mixture of prey fatty acid signatures that most closely resembles that of the predator. This technique uses a selection of fatty acids that are derived from diet, and are either of strictly dietary origin or are also biosynthesized by the predator but still present in the diet. It also accounts and corrects for the effects of lipid metabolism within the predator on these fatty acids, using calibration coefficients. Calibration coefficients have been established for several seal species with feeding experiments, but have shown to be species-specific (Iverson et al. 2004). It is also important to acknowledge fatty acid variability within and among prey species (Iverson et al. 2004; Thiemann et al. 2004a), both in time and space. Although this model can give a close approximation of the diet composition of the species, misidentification of prey species has been observed in previous studies in marine mammals (Tucker et al. 2009; Williams & Buck 2010).



Harbour porpoises *Phocoena phocoena* are widely distributed throughout the temperate to cold waters of the Northern hemisphere (Gaskin 1984; Hammond et al. 2002). In the North Sea and Dutch coastal waters, they are the most common small cetacean (Reid et al. 2003), with population estimates at ca. 350,000 individuals (1994 and 2005). A southern shift in distribution has been documented (Hammond 2006), which is also reflected in Dutch coastal waters. After becoming virtually extinct by the 1960s (Reijnders 1992), numbers of sighted and stranded porpoises have remained low for approximately three decades (Camphuysen et al. 2008; Camphuysen 2011). Since 2003, an increase in porpoise sightings has been witnessed and also the number of stranded porpoises increased rapidly, with a maximum of 537 recorded strandings in 2006 (Camphuysen et al. 2008). It has been suggested that changes in harbour porpoise as well as prey abundance and distribution and a high incidence of by-catch may be causing the high stranding frequency along the Dutch coast (Camphuysen 2004). Diet composition is commonly based on stomach contents (e.g. Santos & Pierce 2003; Pierce et al. 2007) with its inherent biases such as differential recovery rates, degradation of prey remains and fast digestion (Prime & Hammond 1987; Bowen 2000), possibly overemphasizing the importance of near shore species (Pierce & Boyle 1991). In addition, stomach contents obtained from stranded animals with an often unknown cause of death might provide biased information. This bias may partly hold also for fatty acid analysis. Knowledge on the longer term feeding ecology as provided by fatty acid analysis will expectedly contribute towards the understanding of the abundance and distribution of porpoises in the southern North Sea.

The primary objective of this study was to use QFASA to estimate the diet of harbour porpoises stranded on the Dutch coast between 1990 and 2007. We compared the diet composition modelled by QFASA with the diet as deduced from stomach contents to analyse possible differences between the short- and longer term diet of porpoises.

## 5.3 MATERIAL AND METHODS

### 5.3.1 SAMPLE COLLECTION

Blubber samples from a total of 73 harbour porpoises stranded on the Dutch coast between 1990 and 2007 were used in this study (Figure 5.1). Of these animals, 16 blubber samples came from live stranded porpoises that died within the rescue facilities of Stichting SOS Dolfijn in Harderwijk between 1990 and 2003. They had died within the first week and are therefore perceived as having fatty acid profiles of porpoises from the wild. The additional 57 porpoises had stranded between 2003 and 2007 and were sampled during annual necropsy sessions organized by Wageningen IMARES, the Netherlands Institute for Sea

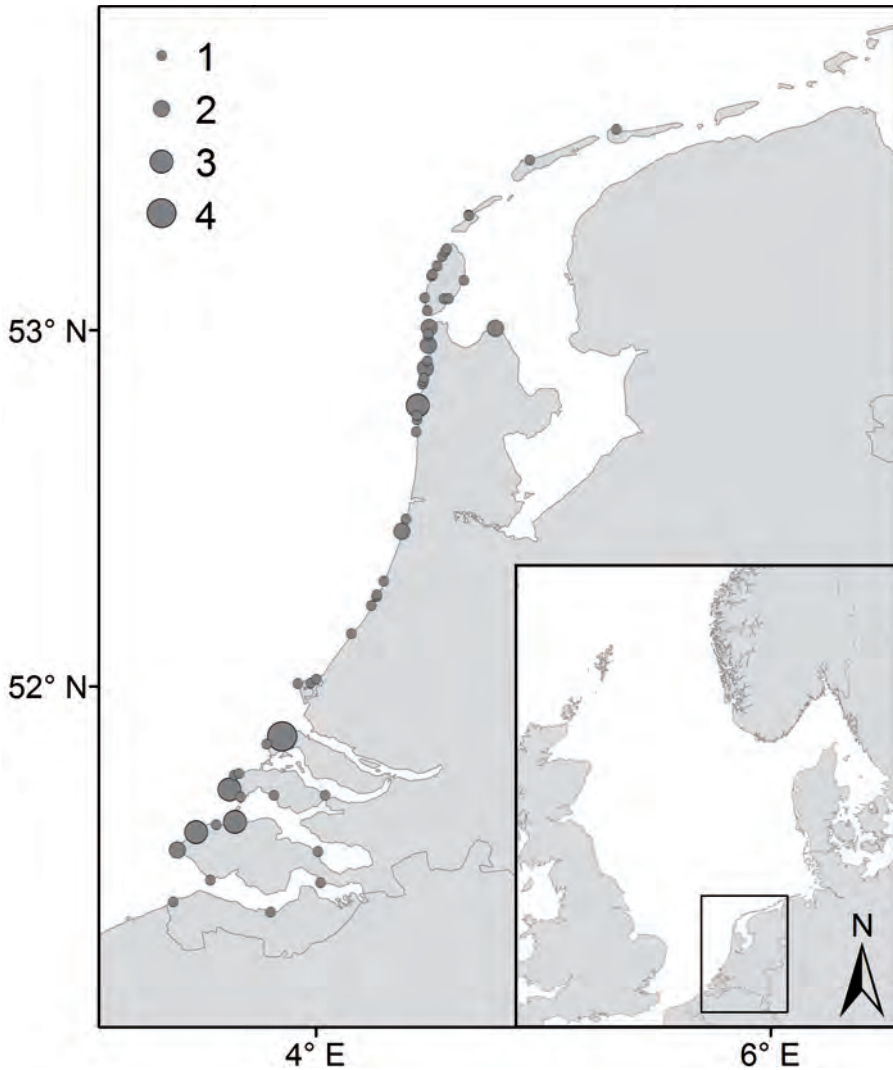


Figure 5.1 Stranding locations of harbour porpoises *Phocoena phocoena* along the Dutch coast (1990-2007).

Research (NIOZ) and Utrecht University. Blubber samples were taken dorsally, covering a full cross-section from muscle to skin and samples were kept frozen at  $-20^{\circ}\text{C}$  until lipid extraction. Animals were initially grouped into age-classes based on total body length: neonates  $< 90$  cm, juveniles 90-130 cm and adults  $> 130$  cm, unless teeth or reproductive organs indicated differently.

In order to relate fatty acid signatures of porpoises to potential prey species, 30 species of fish and squid (Table 5.1) were collected during ongoing surveys in the North Sea between 2002 and 2008 by the Centre for Fishery Research

THE DIET OF HARBOUR PORPOISES ALONG THE DUTCH COAST BASED ON QFASA ANALYSIS

Table 5.1 Summary of prey species analysed for fatty acid analysis (n=770). Species indicated with an asterisk are included in the 'selected' prey set, number (n), minimum-maximum (Min-Max), Standard deviation (SD).

Species		Standard length (cm)		
Scientific name	Common name	n	Min - Max	Mean (SD)
<i>Arnoglossus laterna</i>	Scaldfish	20	6.0 - 15.4	10.7 (3.2)
<i>Ammodytes tobianus</i> *	Lesser sandeel	21	10.7 - 17.7	14.3 (2.4)
<i>Buglossidium luteum</i>	Solenette	20	7.0 - 11.1	9.0 (1.3)
<i>Clupea harengus</i> *	Herring	20	5.9 - 8.9	7.7 (0.8)
<i>Callionymus lyra</i> *	Dragonet	20	6.7 - 21.3	14.6 (4.4)
<i>Ciliata mustela</i>	Five-bearded rockling	20	9.9 - 19.3	14.3 (2.1)
<i>Crangon crangon</i> *	Shrimp	58	2.4 - 5.7	3.9 - (0.9)
<i>Dicentrarchus labrax</i> *	Seabass	16	9.6 - 20.7	11.8 (2.7)
<i>Gadus morhua</i> *	Cod	33	7.0 - 31.7	19.6 (7.1)
<i>Hyperoplus lanceolatus</i> *	Greater sandeel	19	14.4 - 28.5	22.5 (4.3)
<i>Hippoglossoides platessoides</i>	Long rough dab	11	24.0 - 30.3	25.8 (1.8)
<i>Limanda limanda</i>	Dab	35	5.5 - 28.2	14.2 (8.9)
<i>Merlangius merlangus</i> *	Whiting	30	7.8 - 29.9	19.6 (7.0)
<i>Osmerus eperlanus</i> *	Smelt	21	12.5 - 16.2	13.7 (1.1)
<i>Platichthys flesus</i>	Flounder	30	9.9 - 32.0	18.9 (6.7)
<i>Pomatoschistus lozanoi</i>	Lozano's goby	5	3.7 - 4.5	4.1 (0.4)
<i>Pomatoschistus microps</i> *	Common goby	137	1.3 - 4.2	2.8 - (0.6)
<i>Pomatoschistus minutus</i> *	Sand goby	30	5.0 - 7.0	6.0 (0.5)
<i>Pleuronectes platessa</i>	Plaice	30	8.0 - 30.0	16.4 (6.6)
<i>Sepioloatlantica</i> *	Little cuttlefish	18	1.9 - 4.0	3.1 (0.6)
<i>Scophthalmus maximus</i>	Turbot	11	19.4 - 25.0	22.2 (2.0)
<i>Syngnathus rostellatus</i>	Nilsson's pipefish	16	7.7 - 13.9	11.6 - (1.6)
<i>Scomber scombrus</i> *	Mackerel	20	23.6 - 32.8	28.7 (2.9)
<i>Solea solea</i> *	Sole	27	7.4 - 18.6	10.8 (3.3)
<i>Sprattus sprattus</i> *	Sprat	20	6.6 - 11.4	9.1 (1.1)
<i>Trisopterus esmarkii</i> *	Norway pout	10	8.8 - 12.8	11.0 (1.2)
<i>Trisopterus luscus</i>	Pouting	20	1.3 - 20.2	15.9 (3.7)
<i>Trisopterus minutus</i> *	Poor cod	35	7.5 - 17.5	13.3 (2.7)
<i>Trachurus trachurus</i> *	Scad	17	18.9 - 30.0	22.6 (2.7)
<b>TOTAL</b>		<b>770</b>		

CVO (cvo.wur.nl 2009). Species, length, fishing location (latitude/longitude) and date were available for each sample ( $n=770$ ). From the animals analysed for their fatty acid signature in this study, stomach content data were extracted from a wider study on harbour porpoises stranded along the Dutch coast (Leopold & Camphuysen 2006). Only stomachs with identifiable prey remains ( $n=45$ ) were included in the analysis.

### 5.3.2 FATTY ACID ANALYSIS

Cetacean blubber has been described as vertically stratified with the inner layer being metabolically more active than the middle and outer layer. Thus, only the inner third of each blubber core was analysed as they best represented the fatty acid signature of the diet (Koopman et al. 1996; Koopman 2007). Most fish were analysed individually; only the common goby *Pomatoschistus microps*, Lozano's goby *Pomatoschistus lozanoi*, Nilsson's pipefish *Syngnathus rostellatus* and shrimp *Crangon crangon* were pooled to obtain samples large enough to process. Porpoise and prey samples were analysed for fatty acid composition in accordance to standard techniques following the protocol from Budge et al. (2002, 2006). In short, samples were homogenized and lipids extracted using the modified Folch procedure (Folch et al. 1957). Lipids were then converted into fatty acid methyl esters and analysed using gas chromatography.

### 5.3.3 STATISTICAL ANALYSIS

Analysis of similarity tests (ANOSIM, Clarke 1993) were used to examine whether fatty acid signatures of porpoise blubber significantly differed depending on sex, age-class, season, year, decomposition state, by-catch probability, stranding location and between stranded and rescued animals. ANOSIM tests were based on Bray-Curtis similarities between fatty acid signatures using Primer software (Clarke 1993; Clarke & Gorley 2006). Fatty acid signatures included all fatty acids and were fourth-root transformed, to limit the influence of dominant fatty acids on the ordination. Discrimination of prey fatty acid signatures was analysed by agglomerative, hierarchical clustering using group-average linking based on Bray-Curtis similarity coefficients ( $s$ ) of fourth-root transformed mean values. Similarity profile permutation tests (SIMPROF routine in PRIMER) were used to analyse differences between prey species. For all analyses in this study  $p < 0.05$  was the level for significance.

Subsequently, QFASA was used to estimate the relative contribution of different prey fatty acid signatures to the fatty acid profiles of porpoise blubbers. QFASA was fitted in R (R 2.92; R Development Core Team 2009). As calibration coefficients have not been established for porpoises specifically, two previously published sets were used in turn, one as derived from several seal species ('seal', Iverson et al. 2004; Nordstrom et al. 2008) and one derived from a porpoise ('porpoise', Gilles 2008) (Table 5.2). As there is some dispute on which fatty ac-

THE DIET OF HARBOUR PORPOISES ALONG THE DUTCH COAST BASED ON QFASA ANALYSIS

Table 5.2 *Fatty acids included in fatty acid sets (diet and extended) and calibration coefficients used for QFASA modelling of porpoise diet in this study.*

Source	'diet' (Iverson et al. 2004)	'extended'	Grey seal (Iverson et al. 2004; Nordstrom et al. 2008)	Harbour seal	Harp seal	Porpoise (Gilles 2008)
14:0		x	0.86	0.91	0.94	1.59
16:0		x	0.74	0.64	0.63	0.56
16:1n-7		x	1.52	2.34	1.62	2.3
16:2n-6	x	x	0.76	0.89	0.74	1.05
16:2n-4	x	x	1.5	1.13	0.95	0.71
16:3n-6	x	x	0.86	0.85	1.12	0.93
16:3n-4	x	x	0.68	0.75	0.87	1.15
16:3n-3	x	x	0.85	1	0.57	1.32
16:4n-1	x	x	0.59	0.74	0.77	0.74
18:0		x	0.84	0.54	0.79	0.55
18:1n-9		x	3.46	1.25	2.79	1.15
18:1n-7		x	1.41	1.1	1.44	0.73
18:2n-6	x	x	2.02	1.42	1.57	0.54
18:2n-4	x	x	0.98	1.09	0.86	0.32
18:3n-6	x	x	1.08	0.9	0.94	0.69
18:3n-4	x	x	2.32	1.92	2.59	0.53
18:3n-3	x	x	2.27	1.3	1.48	0.46
18:3n-1	x	x	0.95	1.1	0.95	0.64
18:4n-3	x	x	0.96	0.9	0.99	0.31
18:4n-1	x	x	1.1	1.77	1.39	1.13
20:1n-11	x	x	3.42	1.8	2.84	11.01
20:1n-9	x	x	0.81	0.86	1.00	3.63
20:1n-7	x	x	0.71	1.05	1.06	0.46
20:2n-6	x	x	1.65	1.33	1.39	0.32
20:3n-6	x	x	1.07	1	1.00	0.57
20:3n-3	x	x	1.16	1.25	0.98	0.26
20:4n-6	x	x	0.82	1.14	1.04	0.42
20:4n-3	x	x	2.11	1.36	1.50	0.8
20:5n-3	x	x	0.65	0.75	0.80	0.28
21:5n-3	x	x	1.37	1.02	1.45	0.47
22:1n-11	x	x	0.2	0.42	0.35	4.54
22:1n-9	x	x	0.27	0.57	0.59	0.62
22:1n-7	x	x	0.18	0.33	0.26	0.49
22:2n-6	x	x	1	1.04	1.00	1
22:4n-6	x	x	1	2.43	1.00	0.7
22:4n-3	x	x	2.58	1.51	1.55	1.18
22:5n-6	x	x	1.04	1.23	0.76	0.49
22:6n-3	x	x	1.11	1.01	0.94	0.38

ids should be included in the analysis, two fatty acid subsets were used as described by Iverson et al. ('diet' and 'extended diet', 2004; Table 5.2). The 'diet' set consists of 32 fatty acids that are considered strictly dietary in origin, while the 'extended diet' set includes fatty acids from the 'diet' and an additional 6 fatty acids that may also be synthesized by the predator (Iverson et al. 2004). We have also used two sets of prey species, one 'full' set, including all prey species and one 'selected' set, including species that were identified in stomach content analysis with a contribution of > 0.5% by mass (%W) with the addition of Norway pout *Trisopterus esmarkii*. Gobies *Pomatoschistus* sp. were included in the model separately as sand goby *Pomatoschistus microps* and common goby, and later pooled for comparison with stomach contents data.

### 5.3.4 STOMACH CONTENT ANALYSIS

All prey remains were identified to the lowest taxonomic level possible, using a reference collection of IMARES and the Royal Netherlands Institute for Sea Research (NIOZ) and guides for otoliths as well as other identifiable remains such as vertebrae and jaw bones (Härkönen 1986; Watt et al. 1997; Leopold et al. 2001). Measurements of otoliths and other identifiable remains were used to reconstruct the length and weight of individual fish using published regressions of fish species (Härkönen 1986; Prime & Hammond 1987; Coull et al. 1989; Leopold et al. 2001), correcting for wear according to Leopold et al. (Leopold et al. 1998). Prey composition was described as reconstructed weight (%W), expressed as the mean of the weight of a given prey species as a percentage of the total prey weight in each stomach.

## 5.4 RESULTS

### 5.4.1 PORPOISE SAMPLES

A total of 73 porpoise blubber samples were used in this study. There were more male (64%) than female porpoises. Most animals were juveniles (71%), compared to 22% adults and 7% neonates. Juvenile lengths and weights ranged from 87-135 cm (109.2 cm  $\pm$  10.5; mean  $\pm$  SD) and from 11-37 kg (19.7 kg  $\pm$  5.8), respectively. Adult lengths and weights ranged from 131-159 cm (146.8 cm  $\pm$  7.6) and from 28-52 kg (41.4 kg  $\pm$  6.6), respectively. Neonate lengths and weights ranged from 71-85 cm (77.9 cm  $\pm$  5.0) and from 6-9 kg (7.3 kg  $\pm$  1.2), respectively. Table 5.3 gives an overview of length and weight measurements of porpoises based on their age-class and sex.

Samples were available for each month with a distinct peak of strandings in March, comparable with the seasonal pattern of all recorded strandings along the Dutch coast. The second peak in strandings along the Dutch coast around August is not reflected in our samples as most animals stranded in that period



Table 5.3 Age-class, sex, length and weight of porpoises *Phocoena phocoena* stranded on the Dutch coast between 1990 and 2007 (n=73), M=male, F=female; number (n); total length (cm) and weight (kg).

Age-class	Sex	No.	Length (cm)		Weight (kg)	
			Min - Max	Mean (SD)	Min - Max	Mean (SD)
Neonate	M	1	79	-	8	-
	F	4	71 - 85	77.63 (5.74)	6 - 8.9	7.08 (1.26)
Juvenile	M	36	87 - 135	108.38 (10.76)	11 - 37	19.37 (6.28)
	F	17	98 - 127	111.71 (10.31)	12 - 29.4	20.85 (5.17)
Adult	M	9	131 - 157	144.95 (8.01)	28 - 49	41.14 (7.17)
	F	6	144 - 159	149.83 (6.31)	38 - 52	43.33 (5.52)

were too decomposed for fatty acid analysis. Based on criteria in Kuiken (1996), 12% of the animals were diagnosed as definite by-catch, 24% as possible or probable by-catch and 27% of the animals had died of other causes. For 36% of the porpoises, no conclusive cause of death could be determined.

#### 5.4.2 FATTY ACID ANALYSIS

ANOSIM pairwise tests showed a significant difference between neonates and juveniles ( $R=0.662$ ,  $p=0.001$ ) and neonates and adults ( $R=0.645$ ,  $p=0.003$ ) but no significant difference between juveniles and adults ( $R=0.063$ ,  $p=0.168$ ). ANOSIM showed no significant differences between the fatty acid signatures of animals from different sexes ( $R=0.011$ ,  $p<0.352$ ), seasons ( $R=0.082$ ,  $p<0.017$ ), years ( $R=0.168$ ,  $p<0.015$ ) or locations ( $R=-0.011$ ,  $p<0.589$ ). The fatty acid signature did not significantly differ depending on their chance of having been by-caught ( $R=0.061$ ,  $p<0.101$ ), their state of decomposition ( $R=0.04$ ,  $p<0.182$ ) and between wild and rescued animals ( $R=0.096$ ,  $p<0.11$ ).

Cluster analysis on prey including all fatty acids showed that most species can be discriminated well based on their fatty acid signature ( $p<0.05$ ) (Figure 5.2). However, there is large similarity in fatty acid signatures in some prey species, e.g. between whiting *Merlangius merlangus* and cod *Gadus morhua* ( $s=96.622$ ), also between mackerel *Scomber scombrus*, scad *Trachurus trachurus* and sprat *Sprattus sprattus*, between herring *Clupea harengus* and greater sandeel *Hyperoplus lanceolatus*, and between seabass *Dicentrarchus labrax* and shrimp, and finally between poor cod *Trisopterus minutus* and dragonet *Callionymus lyra* ( $p>0.05$ ).

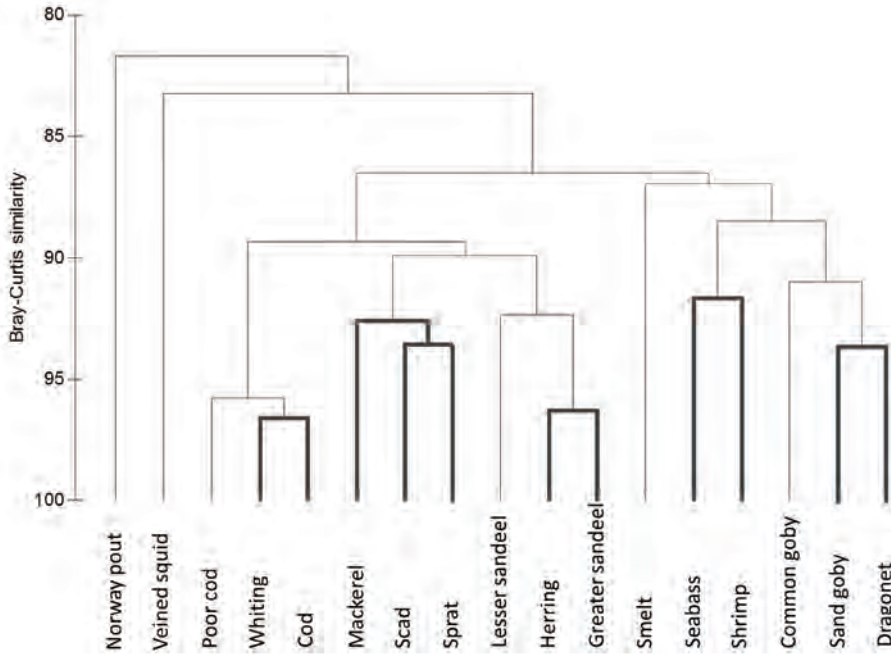


Figure 5.2 Hierarchical clustering of prey species using group-average linking on fatty acid signatures based on Bray-Curtis similarity of square root transformed mean values. Bold lines indicate homogeneous clusters ( $P > 0.05$ , SIMPROF).

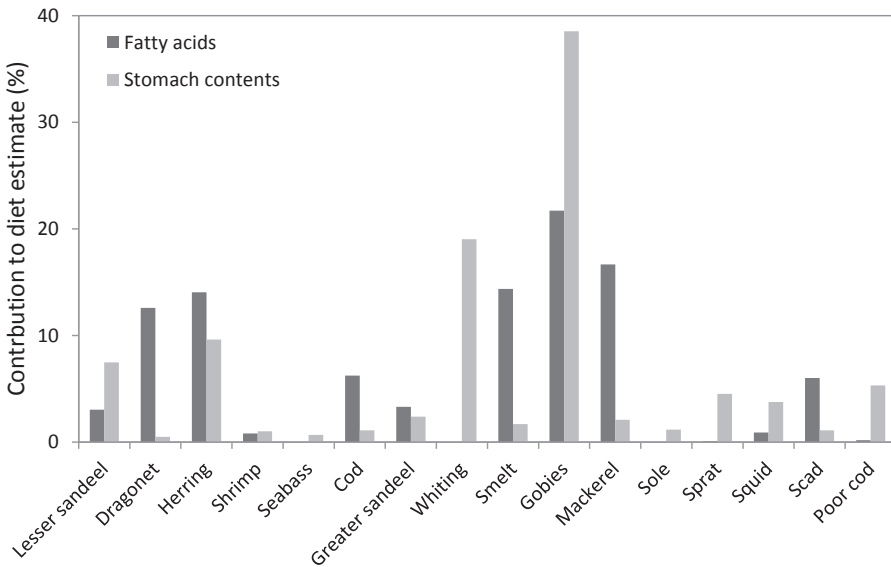


Figure 5.3 Diet composition of porpoises stranded on the Dutch coast as deduced from QFASA (Model 4; using porpoise calibration coefficients, selected prey and the extended fatty acid set) and from stomach contents.

Table 5.4 Diet of harbour porpoises *Phocoena phocoena* (n=45) as deduced from stomach contents, number (n), gram (g), percentage weight (%W). Species included in the 'selected' prey set are indicated with an asterisk.

Species	Name	n	Weight g	%W	
				Mean	SD
<i>Alosa fallax</i>	Twaite shad	1	151	0.4	0.0
<i>Ammodytes marinus</i>	Sandeel	13	92	0.3	0.0
<i>Ammodytes tobianus</i> *	Lesser sandeel	155	923	7.5	0.2
<i>Atherina presbyter</i>	Sand-smelt	12	48	0.1	0.0
<i>Callionymus lyra</i> *	Dragonet	6	48	0.5	0.0
<i>Clupea harengus</i> *	Herring	37	1095	9.6	0.3
<i>Crangon crangon</i> *	Shrimp	14	13	1.0	0.1
<i>Dicentrarchus labrax</i> *	Seabass	65	575	0.7	0.0
<i>Gadus morhua</i> *	Cod	12	814	1.1	0.0
<i>Hyperoplus lanceolatus</i> *	Greater sandeel	4	180	2.4	0.1
<i>Limanda limanda</i>	Dab	7	31	0.1	0.0
<i>Merlangius merlangus</i> *	Whiting	51	4149	19.0	0.3
<i>Osmerus eperlanus</i> *	Smelt	706	1703	1.7	0.1
<i>Platichthys flesus</i>	Flounder	6	18	0.1	0.0
<i>Pleuronectes platessa</i>	Plaice	2	12	0.1	0.0
<i>Pomatoschistus sp.</i> *	Gobies	6782	9419	38.5	0.4
<i>Scomber scombrus</i> *	Mackerel	3	1147	2.1	0.1
<i>Solea solea</i> *	Sole	32	264	1.2	0.1
<i>Sprattus sprattus</i> *	Sprat	47	634	4.5	0.1
<i>Squid spec.</i> *	Squid	44	121	2.5	0.2
<i>Syngnathus rostellatus</i>	Pipefish	12	7	0.2	0.0
<i>Trachurus trachurus</i> *	Scad	3	92	1.1	0.0
<i>Trisopterus luscus</i>	Bib	2	16	0.1	0.0
<i>Trisopterus minutus</i> *	Poor cod	23	667	5.3	0.2
Total		8039	22219	100	

### 5.4.3 DIET AS DEDUCED FROM STOMACH CONTENTS

In total, 24 species were identified, of which 16 each accounted for more than 0.5% of the total reconstructed weight, indicated with an asterisk in Table 5.4. These 16 prey species accounted for 98.7% of the total ingested prey weight. Figure 5.3 shows that gobies *Pomatoschistus spec.* were the most important prey species (38.5%), followed by whiting (19.0%). Herring, lesser sandeel *Ammodytes tobianus*, poor cod and sprat accounted for 9.6%, 7.5%, 5.3% and 4.5%, respectively. Another 10 species each accounted for more than 0.5% of the total reconstructed weight (Table 5.4), together accounting for 14.3%. The remaining

8 species were of minor importance and together accounted for the residual 1.4% of the total reconstructed prey weight. Details on the prey composition are given in Table 5.4.

#### 5.4.4 DIET COMPOSITION AS MODELLED BY QFASA

The estimated relative contribution of prey species to the diet of porpoises differed profoundly between the eight models using different calibration coefficients, fatty acid subsets and prey sets (Table 5.5). In all models using porpoise calibration coefficients (CCs) (models 1-4), gobies were identified as the most important prey species, accounting for 21.7-29.1% of the estimated diet. In model 1 (porpoise CCs, full prey, diet fatty acids), another five species were estimated to contribute to the bulk of the diet, including herring (24.5%), greater sandeel (12.4%), scad (9.1%), mackerel (7.9%) and flounder (6.9%). In model 2 (porpoise CCs, full prey, extended fatty acids), four additional species contributed to the bulk of the estimated diet, including smelt (20.2%), herring (18.1%), dragonet (7.3%) and mackerel (5.3%). In model 3 (porpoise CCs, selected prey, diet fatty acids), another four species were estimated to contribute to the bulk of the diet, including mackerel (21.2%), herring (18.9%), scad (16.4%) and greater sandeel (9.4%). In model 4 (porpoise CCs, selected prey, extended fatty acids), another six species were estimated to contribute to the bulk of the diet, including mackerel (16.7%), smelt (14.4%), herring (14.1%), dragonet (12.6%), cod (6.3%) and scad (6.0%).

In model 5 (seal CCs, full prey, diet fatty acids), three species were estimated to account for the bulk of the diet, including Norway pout (61.4%), mackerel (15.4%) and gobies (6.9%). In model 6 (seal CCs, full prey, extended fatty acids), three species were estimated to account for the bulk of the diet, including dragonet (39.5%), Norway pout (36.9%) and gobies (15.9%). In model 7 (seal CCs, selected prey, diet fatty acids), mackerel accounted for 87.9% of the total estimated diet. In model 8 (seal CCs, selected prey, extended fatty acids), three species were estimated to account for the bulk of the diet, including dragonet (46.6%), mackerel (45.2%) and gobies (6.0%). Dab *Limanda limanda*, plaice *Pleuronectes platessa*, bib *Trisopterus luscus* and whiting *Merlangius merlangus* were not identified in any of the models.

#### 5.4.5 QFASA VERSUS STOMACH CONTENTS

Gobies, identified as the most common prey species in stomach contents (38.5%) were also estimated by QFASA to contribute the most to the diet using porpoise calibration coefficients (mean=25.8%, sd=3.3). The importance of gobies was less pronounced when QFASA included seal calibration coefficients (mean=7.9%, sd=5.6), although they were also identified among the few species accounting for the bulk of the diet. The contribution of gobies to the diet as estimated by QFASA remains in all cases lower than deduced from stomach

THE DIET OF HARBOUR PORPOISES ALONG THE DUTCH COAST BASED ON QFASA ANALYSIS

Table 5.5 Diet (%) of harbour porpoises *Phocoena phocoena* (n=73) as estimated by QFASA; using porpoise or seal calibration coefficients, the 'full' or 'selected' prey set, and the diet fatty acid or extended (ext) fatty acid subset. Species not included in the analysis are marked by a dash (-).

Model		1	2	3	4	5	6	7	8
		Porpoise				Seal			
Calibration coefficients		full		selected		full		selected	
Prey set		diet	ext	diet	ext	diet	ext	diet	ext
Fatty acid set		diet	ext	diet	ext	diet	ext	diet	ext
<i>Ammodytes tobianus</i>	Lesser sandeel	1.8	1.5	3.8	3.0	1.6	2.1	1.2	0.8
<i>Arnoglossus laterna</i>	Scaldfish	0.0	0.0	-	-	0.0	0.5	-	-
<i>Buglossidium luteum</i>	Solenette	0.0	0.9	-	-	0.0	0.4	-	-
<i>Callionymus lyra</i>	Dragonet	0.6	7.3	0.9	12.6	0.1	39.5	0.0	46.6
<i>Ciliata mustela</i>	5-b. rockling	1.5	1.9	-	-	0.0	0.0	-	-
<i>Clupea harengus</i>	Herring	24.5	18.1	18.9	14.1	0.6	0.9	0.2	0.7
<i>Crangon crangon</i>	Shrimp	0.1	0.9	0.3	0.8	0.7	0.2	0.1	0.0
<i>Dicentrarchus labrax</i>	Seabass	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.0
<i>Gadus morhua</i>	Cod	0.1	3.1	1.4	6.3	0.0	0.0	0.3	0.0
<i>Hippoglossoides platessoides</i>	Long rough dab	0.0	2.6	-	-	0.0	0.0	-	-
<i>Hyperoplus lanceolatus</i>	Greater sandeel	12.4	4.7	9.4	3.3	0.0	0.0	0.0	0.0
<i>Limanda limanda</i>	Dab	0.0	0.0	-	-	0.0	0.0	-	-
<i>Merlangius merlangus</i>	Whiting	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Osmerus eperlanus</i>	Smelt	1.4	20.2	2.1	14.4	2.4	0.4	0.6	0.1
<i>Platichthys flesus</i>	Flounder	6.9	0.4	-	-	2.2	0.0	-	-
<i>Pleuronectes platessa</i>	Plaice	0.0	0.0	-	-	0.0	0.0		
<i>Pomatoschistus microps</i>	Common goby	29.1	27.7	24.5	21.7	6.9	15.9	2.9	6.0
<i>Pomatoschistus minutus</i>	Sand goby	0.0	0.0	0.0	0.0	1.8	0.0	1.0	0.0
<i>Scomber scombrus</i>	Mackerel	7.9	5.3	21.2	16.7	15.4	3.0	87.9	45.2
<i>Scophthalmus maximus</i>	Turbot	4.0	1.4	-	-	1.3	0.0		
<i>Sepiola atlantica</i>	Atlantic bobtail	0.2	0.4	0.4	0.9	0.0	0.0	0.0	0.0
<i>Solea solea</i>	Sole	0.1	0.0	0.1	0.0	1.1	0.0	0.4	0.0
<i>Sprattus sprattus</i>	Sprat	0.1	0.1	0.6	0.1	2.7	0.2	3.1	0.5
<i>Trachurus trachurus</i>	Scad	9.1	3.4	16.4	6.0	1.6	0.0	2.0	0.0
<i>Trisopterus esmarkii</i>	Norway pout	0.0	0.0	-	-	61.4	36.9	-	-
<i>Trisopterus luscus</i>	Bib	0.0	0.0	-	-	0.0	0.0	-	-
<i>Trisopterus minutus</i>	Poor cod	0.2	0.1	0.2	0.2	0.0	0.0	0.0	0.0
Total		100	100	100	100	100	100	100	100

contents. The better match between stomach content analysis and QFASA using porpoise calibration coefficients, confirms that the use of species-specific calibration coefficients is desirable.

Herring and greater sandeel showed similar fatty acid signatures (Figure 5.2), possibly resulting in misidentification between these species. Together, these species account for 17.4-36.9% of the diet when estimated by QFASA compared to 9.6% as deduced from stomach contents. Similarly, mackerel, scad and sprat showed similar fatty acid signatures (Figure 5.2), possibly resulting in misidentification between these species. Together, these species account for 8.8-22.8% of the diet when estimated by QFASA compared to 7.7% of the diet as deduced from stomach contents.

The contribution of lesser sandeel and poor cod to the total diet is estimated higher by stomach contents (7.5% and 5.3%, respectively) than estimated by QFASA (mean=2.5%, sd=1.1 and mean=0.2%, sd=0.0, respectively). Smelt and dragonet were among the five most important prey species in QFASA models including the extended diet fatty acid set (20.2-14.4% and 7.3-12.6%, respectively) while their contribution to the total diet was minor as deduced from stomach contents (1.7% and 0.5%, respectively). Dragonet and sand goby have similar fatty acid signatures (Figure 5.2), possibly leading to misidentification between these species. If this was the case, the diet as estimated by QFASA would have overestimated the importance of dragonet and underestimated the importance of gobies, giving a better match with stomach content analysis.

Dab, plaice and bib were not identified in any of the models, but their contribution to the diet as deduced from stomach contents was also relatively low with 0.1% each. Whiting, however, is not identified by QFASA in any model, while it is the second most common prey species in stomach contents (19.0%). It is unlikely that porpoises do not feed at all on whiting on a longer term while their short term diet is dominated by this species, pointing towards misidentification between whiting and cod due to similar fatty acid signatures (Figure 5.2). The contribution of whiting and cod to the diet as estimated by QFASA remains in all cases lower than as deduced from stomach contents. The higher importance of cod in QFASA models using the extended fatty acids gives a better match between the stomach content analysis and QFASA, suggesting that the extended fatty acid set is the most suitable set for porpoise diet estimation. Using both QFASA and stomach contents, only a few species contributed to the bulk of the diet of porpoises, authorizing the use of the selected prey set for porpoise diet estimation.

## 5.5 DISCUSSION AND CONCLUSION

Fatty acid analysis has the advantage that it estimates accumulated diet over a longer time-period than stomach contents. Although QFASA may not be able to identify prey items consumed only occasionally, it can determine prey species that the predator relies on for longer time periods and does so on a time scale



relevant to the animals' ecology. Reliable quantitative use of this method for marine mammals requires an extensive and characteristic prey fatty acid database and knowledge on how prey fatty acids are incorporated into the blubber.

### 5.5.1 PREY DATABASE

A variety of prey species has been identified in stomachs of porpoises (Santos & Pierce 2003). Porpoises were found to mostly rely on a few pelagic and demersal species (mainly clupeids, sandeels, gadoids and/or gobies) for their main dietary intake (Santos & Pierce 2003; Leopold & Camphuysen 2006). Using QFASA, we have estimated the diet of porpoises using two sets of prey species, comprised of all prey species sampled or only a selection of prey species identified as important prey species in stomach contents. In stomach contents and using QFASA, five species were identified respectively as the most important prey species, accounting for 84.4% respectively 76.1-92.4% of the estimated total diet. In stomach contents, these were gobies, whiting, herring, sandeel and poor cod; using QFASA these were gobies, mackerel, smelt, herring and dragonet. Using both QFASA and stomach contents confirm that porpoises rather rely on a few species for their dietary intake.

One of the requirements of QFASA is that prey species included in the model are characteristic of the prey ingested by the predator, with good understanding of the variation of fatty acid signatures between and among prey species (Iverson et al. 2004). Despite temporal and spatial variation in fatty acid signatures, as well as differences in fatty acid signatures between different age-classes and life-stages within prey species, fish species can in general be distinguished by their fatty acid composition (Budge et al. 2002; Iverson et al. 2002). In contrast, our study shows that based on their fatty acid signatures, not all prey species could be clearly distinguished from one another, possibly leading to misidentification in diet estimation using QFASA. Similar ecological niches for different species could cause such confusion. Sampling prey species with a perfect temporal and spatial correspondence to the predator samples might be part of the solution to this problem but is very complex, time consuming, expensive and in most cases impossible.

### 5.5.2 FATTY ACID METABOLISM

Currently, two important parameters are incorporated into the QFASA model to account for fatty acid metabolism within the predator: 1) the subset of fatty acids included in the model and 2) the set of calibration coefficients for individual fatty acids (Iverson et al. 2004). Calibration coefficients have been developed from feeding studies, and are calculated as the ratio of fatty acid proportion in blubber and in diet levels for each fatty acid (Iverson et al. 2004). It has been shown that QFASA tends to overestimate the importance of prey species that have been fed during feeding experiments in order to determine calibration co-

efficients (Budge et al. 2012). As porpoises in captivity are often fed fatty fish species (e.g. herring, sprat and mackerel, Kastelein et al. 1997a), this could explain the higher importance of these species in the diet as estimated by QFASA in our study compared to as deduced from stomach contents.

Our study shows as could be expected, that using different calibration coefficients, i.e. from seals and from porpoises, drastically changed the prey composition as estimated by QFASA. The diet estimates including porpoise calibration coefficients gave a better match with the diet as deduced from stomach contents, suggesting that it is better to use these porpoise calibration coefficients for porpoise diet estimation. The importance of using species-specific calibration coefficients has previously been emphasized (Nordstrom et al. 2008). As the porpoise calibration coefficients used in our study have been estimated from only one animal in captivity (Gilles 2008), more controlled feeding experiments are desirable in order to calculate species-specific calibration coefficients for porpoises.

Many fatty acids are routinely identified using gas chromatography, but fatty acids vary in the degree in which they actually reflect diet (Iverson et al. 2004). It is common practice to exclude fatty acids that are partly or entirely endogenous in origin and those found in trace amounts (Iverson et al. 2004; Wang et al. 2010). There is, however, large divergence between studies in the fatty acids used (Iverson et al. 2004; Gilles 2008; Nordstrom et al. 2008). Our study shows that the choice of fatty acids included in the QFASA model does influence the diet estimate. In order to make studies comparable, it could therefore be good to use the same fatty acid set in all studies. Certain fatty acids identified as biomarkers for certain predator-prey relationships may be added to this set (Budge et al. 2007).

### 5.5.3 ECOLOGICAL IMPLICATIONS

This study revealed differences between the diet composition as deduced from stomach contents and as estimated by QFASA (Figure 5.3). Fish species identified as porpoise prey using both methods are all very abundant in the North Sea (Knijn et al. 1993), suggesting that porpoises are opportunistic predators, preying on the most abundant and accessible prey.

As stomach content analysis has a spatial bias towards coastal species that have been ingested shortly before the stranding of the porpoises, a dominance of such coastal species should be expected. According to stomach contents, gobies, a typical coastal species (Fonds 1973; Knijn et al. 1993), are the most important prey species, both numerical and by weight. Using QFASA, gobies also contributed the most to the total diet, although their importance was less dominant. Also lesser sandeel and sprat, species that show higher densities in coastal areas (Knijn et al. 1993), were identified to contribute more to the diet

when estimated by stomach contents compared to QFASA. This shows that also on a longer term, porpoises rely for their food intake on these small (coastal) prey species, although less than they do shortly before they strand.

Despite being typical coastal species (Knijn et al. 1993), dragonet and smelt had a higher contribution to the total diet when estimated by QFASA. Figure 5.2 shows that dragonet and gobies have similar fatty acid signatures, possibly leading to the misidentification between these species. If this has been the case, the importance of gobies in these models would increase, resulting in a closer match between the diet as estimated by QFASA and as deduced from stomach contents. The fatty acid signature of smelt, however, has been shown to be significantly different to other prey species (Figure 5.2), so misidentification is not expected. This suggests that on a longer term, porpoises feed more on smelt than would have been concluded from stomach contents. In contrast, using QFASA suggests that porpoise rely for their longer term dietary intake less on poor cod than would be suggested by stomach contents.

The high importance of mackerel, scad, herring and sprat in the diet as estimated by QFASA compared to stomach contents may be due to differences in the behaviour of porpoises and fish between coastal and offshore waters. In coastal waters that tend to be more turbid than offshore waters, fish tend to disperse more (Glass et al. 1986; Turesson & Brönmark 2007). Porpoises may thus change their feeding strategy from preying on schooling prey species (e.g. mackerel and herring) in offshore waters to feeding more on non-schooling species. Stable isotope analysis of porpoises from Dutch coastal waters (Jansen et al. 2012b) also highlighted a higher importance of pelagic, schooling species, including mackerel, in the porpoises' longer term diet compared to the short term diet as deduced from stomach contents suggesting that this is an actual difference between the short- and longer term diet of porpoises.

The most striking difference between the diet as deduced from stomach contents and fatty acid analysis is seen in the importance of whiting. In stomach contents, whiting is the second most important prey species in its contribution to the total weight (19.0%). In contrast, none of the QFASA models identified whiting in the diet of porpoises. Whiting is one of the most abundant and widely dispersed gadoid species in the North Sea (Knijn et al. 1993). It would be expected that this species is preyed on both in offshore and coastal waters and one would expect to find at least some identification of whiting in the diet as estimated by QFASA. Results have shown that whiting and cod have very similar fatty acid signatures (Figure 5.2), possibly resulting in misidentification of these two species. If misidentification had taken place, the contribution of whiting would increase in the QFASA diet estimate, but not to the extent that it would mirror the importance of whiting found in stomach contents. As we do not have an ecological explanation for the low importance of whiting in the longer term

diet of porpoises in Dutch coastal waters it may suggest that there are other methodological problems using either QFASA or stomach content analysis that are not accounted for in this study.

#### 5.5.4 CONCLUSION

This study has revealed profound differences in the diet composition of porpoises as estimated by fatty acid analysis and as deduced from stomach contents. Although some differences could be explained by possible misidentification or methodological difficulties, others are thought to reflect an actual difference between the short- and longer term diet of porpoises in Dutch coastal waters. Our study revealed that the longer term diet of porpoises in Dutch coastal waters consists both of coastal species (e.g. gobies, smelt and dragonet) and also pelagic, schooling species (e.g. mackerel and herring). Although the use of QFASA for quantitative diet estimation is theoretically conclusive and has successfully been applied in some species, we emphasize the importance of applying different dietary analysis techniques, thereby combining several lines of evidence, when studying marine mammal diets. We also confirm the need for controlled feeding experiments in order to improve the interpretation of dietary analysis results.

#### 5.6 ACKNOWLEDGEMENTS

Most porpoise samples were collected by staff and volunteers of the Dutch stranding network, coordinated by the National Museum of Natural History (now NCB Naturalis) in Leiden. We thank the staff of Stichting SOS Dolfijn for additional porpoise samples. The authors would like to thank M.F. Leopold (IMARES), C. Camphuysen (NIOZ), T. Jauniaux (Liège University), A. Gröne (Utrecht University) and L. Wiersma (Utrecht University) for the organization and execution of the post-mortem examinations that have provided samples and information on the animals. We are grateful to Anne Timmins and Margaret Cooper (Dalhousie University) for sample preparation and the initial QFASA analysis. We would like to thank G. Keijl (NCB Naturalis) for providing longer term data of porpoise stranding records and R.S.A. van Bemmelen for creating the strandings locations map. This work was funded by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV), as part of the research program 'Harbour Porpoises: Abundance, strandings and feeding ecology' (BO-02-012-001), and many of the prey were analysed under the project 'Voedselectologie gewone zeehond' (BO-02-008 Mariene EHS).



# CHAPTER 6

## ARE WHITE-BEAKED DOLPHINS *LAGENORHYNCHUS ALBIROSTRIS* FOOD SPECIALISTS? THEIR DIET IN THE SOUTHERN NORTH SEA

Published as: Jansen, O.E., Leopold, M.F., Meesters, E.H.W.G., Smeenk, C. (2010) Are white-beaked dolphins *Lagenorhynchus albirostris* food specialists? Their diet in the southern North Sea. *Journal of the Marine Biological Association of the UK* 90: 1501-1508.







## 6.1 ABSTRACT

The white-beaked dolphin *Lagenorhynchus albirostris* is the most numerous cetacean after the harbour porpoise *Phocoena phocoena* in the North Sea, including Dutch coastal waters. In this study, the diet of 45 white-beaked dolphins stranded on the Dutch coast between 1968 and 2005 was determined by analysis of stomach contents. Although 25 fish species were identified, the diet was dominated by Gadidae (98.0% by weight, 40.0% in numbers), found in all stomachs. All other prey species combined contributed little to the diet by weight (2.0%W). The two most important prey species were whiting *Merlangius merlangus* (91.1% frequency of occurrence (FO), 30.5%N, 37.6%W) and cod *Gadus morhua* (73.3%FO, 7.4%N, 55.9%W). In numbers, gobies were most common (54.6%N), but contributed little to the diet by weight (0.6%W). Three stomachs contained different prey compared to the others: one animal had taken 2250 gobies, accounting for 96.4% of all gobies found; one animal had fed on 29 small sepiolids; and one animal had solely taken haddock *Melanogrammus aeglefinus*. Squid and haddock were not found in any other stomach. The overall diet showed a lasting predominance of whiting and cod, without clear changes over time (35 years) or differences between sexes or size-classes of dolphins. This study adds to earlier published and unpublished data for Dutch coastal waters and agrees well with studies of white-beaked dolphins from other parts of the species' range, in the North Sea and in Canadian waters, with Gadidae dominating the diet on both sides of the Atlantic.

## 6.2 INTRODUCTION

The white-beaked dolphin *Lagenorhynchus albirostris* Gray, 1846 inhabits the cold-temperate waters of the North Atlantic Ocean including the northern North Sea. It is usually seen in small groups of 5–15 animals, which sometimes form larger associations, occasionally with Atlantic white-sided dolphins *Leucopleurus acutus*. During the late 20<sup>th</sup> century, the species has extended its range into the southern and eastern North Sea, where it has become the most numerous cetacean after the harbour porpoise *Phocoena phocoena*, both in sightings and strandings. The white-beaked dolphin is now regularly recorded in German, Dutch and Belgian waters and has significantly increased in the strandings records for these countries since the 1960s (Kinze et al. 1997; Reeves et al. 1999; Reid et al. 2003; Camphuysen & Peet 2006). The population in the North Sea and adjacent Atlantic Ocean was estimated at ca. 10,000 individuals in 1994 and 2005, respectively (SCANS-I and II surveys: Hammond et al. 2002; Hammond 2006).

Because dolphins feed under water, direct observations of feeding are almost impossible. The most commonly used method of diet estimation is the analysis of stomach contents of dead animals (Pierce et al. 1993; Kinze et al. 1997; Pauly et al. 1998; Barros & Clarke 2009). This method is based on the identification of undigested hard prey remains such as otoliths, vertebrae, jaws and squid beaks. Few studies of the diet of white-beaked dolphins in European waters have been published. In a Scottish study by Canning et al. (2008), 22 stomachs were analysed. From other areas, only limited studies based on small sample sizes are available, and some anecdotal reports on single animals exist (van Bree & Nijssen 1964; de Smet et al. 1985; Smeenk & Gaemers 1987; Lick 1993; Berrow & Rogan 1996; Kinze et al. 1997; Reeves et al. 1999; de Pierrepont et al. 2005; Evans & Smeenk 2008).

This study is based on the analysis of stomach contents of 45 white-beaked dolphins stranded on the Dutch coast between 1968 and 2005, giving the most comprehensive description of the species' diet in the south-eastern North Sea to date.

## 6.3 MATERIAL AND METHODS

### 6.3.1 SAMPLE COLLECTION

Cetaceans stranded on the Dutch coast are recorded, collected and/or sampled through the Dutch national strandings network, coordinated by the National Museum of Natural History (now NCB Naturalis) in Leiden. The first stranded white-beaked dolphin from the Dutch coast was documented in 1886 (Weber 1887). Over the following 80 years, until 1967, 20 further cases were documented (van Deinse, 1931–1966). Since then, the stranding frequency has increased as another 175 strandings were recorded from 1968–2009, 45 of which are included in this study (Figures 6.1 & 6.2); see for details: van Utrecht & Husson (1968), Husson & van Bree (1972, 1976), van Bree & Husson (1974), van Bree & Smeenk (1978; 1982), Smeenk (1986–2003) and Camphuysen et al. (2008). When the state of decomposition allowed, animals were retrieved for post-mortem examination and/or sample collection. For most dolphins used in this study, stranding date and locality (n=40), body length (n=35) and sex (n=34) have been reliably recorded. The majority of samples were collected during 1986–2005 (n=39), and only six are from earlier years (1968–1976). Therefore, this study mainly reflects the period from 1986 onwards (Figure 6.1). Samples come from the entire Dutch coast, though with a higher abundance in the northern part of the country (Figure 6.3).

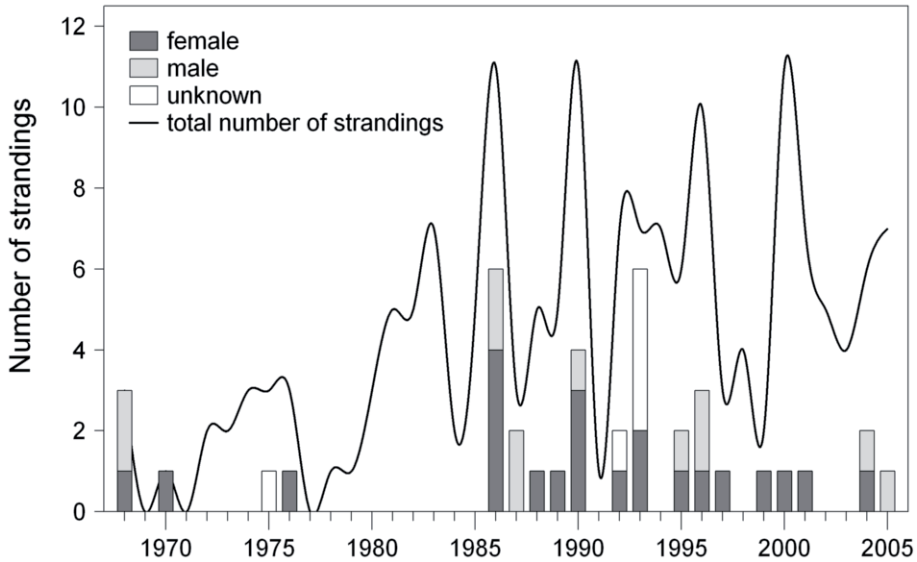


Figure 6.1 Number of strandings of *Lagenorhynchus albirostris* per year ( $n=40$ ) between 1968 and 2005, separated by sex. Five animals without known stranding dates were excluded. The line shows the total number of strandings recorded from the Dutch coast (data collected by the National Museum of Natural History (now NCB Naturalis), Leiden: [www.walvisstrandingen.nl](http://www.walvisstrandingen.nl)).

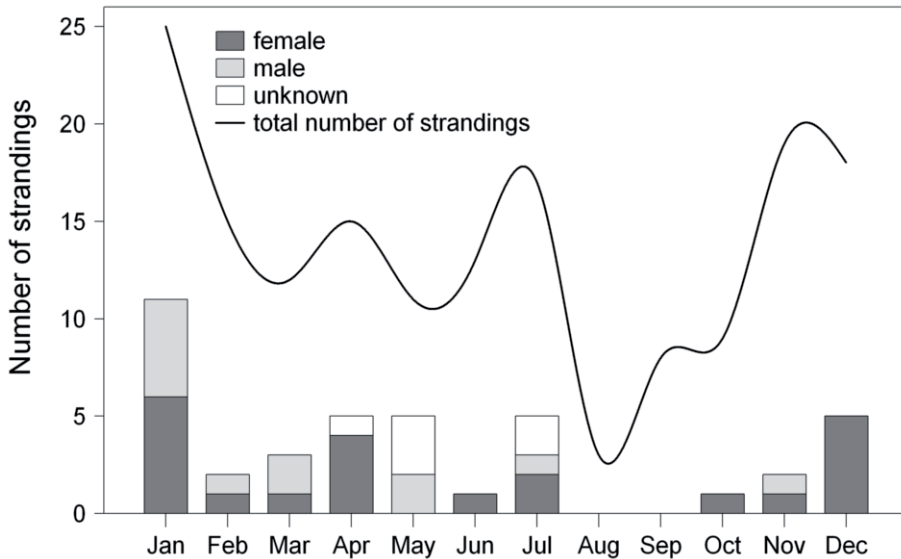


Figure 6.2 Number of strandings of *Lagenorhynchus albirostris* per calendar month ( $n=40$ ) between 1968 and 2005, separated by sex. Five animals without known stranding dates are excluded. The line shows the total number of strandings per calendar month on the Dutch coast (data collected by the National Museum of natural History (now NCB Naturalis), Leiden: [www.walvisstrandingen.nl](http://www.walvisstrandingen.nl)).

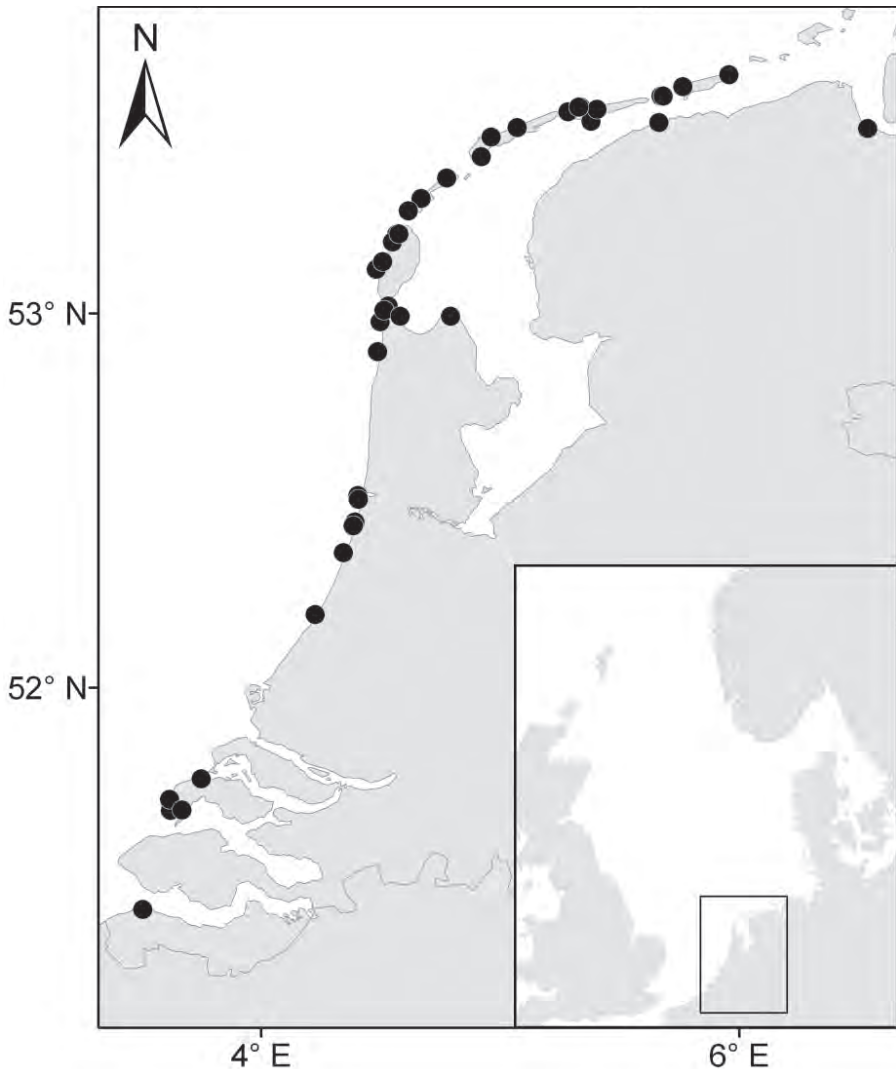


Figure 6.3 Map of *Lagenorhynchus albirostris* stranding localities ( $n=40$ ) on the Dutch coast between 1968 and 2005. Five animals without known stranding localities are excluded.

### 6.3.2 DIET ANALYSIS

Stomachs contents of white-beaked dolphins were collected during post-mortem examination and have been preserved dry in the collection of Naturalis. Only stomachs with prey remains are included in this study. The five stomachs analysed earlier by Smeenk & Gaemers (1987) are included. An animal studied by van Bree & Nijssen (1964) is excluded here, as fish weights could not be calculated from their data. All prey remains were identified to the lowest taxonomic level possible, using a reference collection (IMARES and the Royal Netherlands



Institute for Sea Research, NIOZ) and guides for otoliths and other identifiable hard prey remains (Härkönen 1986; Watt et al. 1997; Granadeiro & Silva 2000; Leopold et al. 2001). In order to improve prey identification and quantification, skeletal parts other than otoliths, such as vertebrae, jaw bones and lenses were also used (Tollit et al. 2003).

For each dolphin, the minimum number of individuals (MNI) per fish species in the samples was estimated by pairing left and right otoliths and other identifiable structures of similar size and wear (Tollit et al. 2003). Otolith length and width is proportional to fish length and weight. Therefore, otolith measurements were used to reconstruct the length and weight of individual fish using published regressions of fish species (Härkönen 1986; Prime & Hammond 1987; Coull et al. 1989; Leopold et al. 2001). To account for partial erosion in the stomach, otoliths were assigned to four 'wear-classes', i.e. pristine, slightly worn, moderately worn and severely worn. Correction factors for wear were derived from large samples, in which all four stages were present for a given fish species, by comparing median sizes (cf. Tollit et al. 2004; Grellier & Hammond 2006). Sizes of non-pristine (worn) otoliths were accordingly corrected before estimating fish length and weight, according to Leopold et al. (2001). For cephalopod remains, the MNI was estimated by pairing upper and lower beaks, but these prey were not identified to species. All squid beaks were tiny, with a hood length smaller than 3 mm, and were probably from small sepiolids. We assigned an average prey weight of 2.5 g per individual (the average weight of *Sepiolo atlantica* in our reference collection). The overall diet composition is quantified using three indices: [1] frequency of occurrence (%FO), expressed as the number of stomachs containing a given prey species as a percentage of the total number of stomachs examined; [2] numerical abundance (%N), expressed as the number of individuals of a given prey species as a percentage of the total number of all prey in the stomachs and [3] reconstructed weight (%W), expressed as the summed weight of a given prey species as a percentage of the total prey weight in all stomachs.

The North Sea stock size of cod, one of the two main prey species of white-beaked dolphins, decreased to an historic minimum in the early 1990s (Hislop 1996; Pope & Macer 1996). In order to compare the diet composition of animals before and after the "cod collapse", dolphins were grouped by their stranding date into two groups: [1] pre-1990 and [2] post-1990. Non-metric multi-dimensional scaling (NMDS) was used to compare prey composition between dolphins of different sex and age, between years and months of stranding, and before and after the cod collapse. NMDS based on Bray-Curtis similarities was applied to the MNI per prey species and the total reconstructed weight per prey species, using Primer software (Clarke & Gorley 2006). Data were fourth-root transformed, to limit the influence of dominant prey species on the ordination. Similarity tests (ANOSIM, Clarke 1993) were performed on the distance matrix



to investigate whether a grouping by any of the above mentioned factors was significantly different from random permutation of the distances.

Smaller dolphins have been found to feed on smaller prey (Dong et al. 1996). Newborn white-beaked dolphins are approximately 120 cm in length and sexually mature adults measure 240–310 cm (Kinze 2009). Accordingly, animals were grouped based on body length: [1] juveniles: <240 cm and [2] adults:  $\geq$ 240 cm. As dolphin lengths were all above 2 meters, the group of very young juveniles (120–200 cm) is not represented in this study.

Generalized additive mixed models (GAMMs) were fitted to the cod and whiting data, but AIC values and likelihood ratio tests indicated that the data were better described by a linear model. Consequently, we fitted a linear mixed model including an interaction between species and dolphin length. Inspection of the residuals indicated heterogeneity and several variance structures were tested (Pinheiro & Bates 2000; Zuur et al. 2009). The final model included a different exponential variance structure with dolphin length for each prey species and had the lowest AIC.

We also tested whether the fraction of cod in the diet (based on reconstructed weight) differed between individuals and was related to animal length. For this, a GAM with a so-called quasipoisson error distribution and with the total weight of cod and whiting as an offset proved to give the best results. Generalized additive models (GAMs) provide a flexible framework, allowing predictors to be fitted either as parametric or non-parametric smoothing terms (Hastie & Tibshirani 1987). The optimal amount of smoothing of GAMs was determined by cross-validation. Model assumptions were assessed visually. All calculations were carried out in the computing environment R (R 2.9.2., R Development Core Team 2009). Linear mixed models used the package ‘nlme’ (Pinheiro et al. 2009) and GAMMs were calculated using package ‘mgcv’ (Wood 2006).

## 6.4 RESULTS

### 6.4.1 SAMPLES

The majority of samples were collected during 1986–2005 ( $n=39$ ), against six earlier samples (1968–1976) and mainly reflect the period from 1986 onwards (Figure 6.1). Both the spatial and temporal patterns in the availability of stomachs correspond with the patterns in strandings on the Dutch coast (Kinze et al. 1997; Camphuysen & Peet 2006). Most stranded animals were found in the northern part of the country (Figure 6.3) and numbers of strandings peaked during the winter months (November–January), followed by a smaller surge from April to July (Figure 6.2). No samples were available from August and September.

Of the 45 white-beaked dolphins, reliable length measurements were available for 35 animals, showing an average of 249.7 cm (SD 23.6 cm, range 209–300 cm). Ten animals were juveniles (<240 cm) and the remaining 25 were adults ( $\geq$ 240 cm). Only 34 dolphins were sexed, 22 females (65%) and 12 males (35%).

#### 6.4.2 PREY SPECIES COMPOSITION

In total, 25 fish species were identified in the stomach contents (Table 6.1). Remains of small cephalopods were found in one stomach only. Items not considered prey and therefore excluded from further analysis were: remains of crustaceans, echinoderms, shells, worms, algae and foreign objects. Foreign objects found were: fishing line (in six animals), stones (in seven animals) and plastic debris (in one animal). None of these were found in such quantities as to be considered the cause of death.

The diet (Table 6.1) was dominated by Gadidae (40.0%N, 98.0%W), which were found in all stomachs. All other species contributed little to the diet (2%W, all combined). The two most important prey species were whiting *Merlangius merlangus* (91.1%FO, 30.5%N, 37.6%W) and cod *Gadus morhua* (73.3%FO, 7.4%N, 55.9%W). In numbers, gobies were most common (54.6%N); however, gobies contributed little to the overall diet by weight (0.6%W).

Three animals had very different prey in their stomachs compared to the others. In the stomach of a juvenile of 210 cm in length, stranded on 8 May 1993 near Den Oever, otoliths of 2250 gobies were found. Besides gobies, this stomach contained remains of 21 sandeels and 52 whittings. The gobies found in this animal's stomach account for 96.4% of all gobies identified in this study. The smallest juvenile in our study, an animal of 209 cm in length, stranded on 28 July 1996 near Bloemendaal, was the only animal that had fed on cephalopods. Besides the beaks of 29 small sepiolids, its stomach contained otoliths of 27 whittings, 12 sandeels, 6 cods and one herring.

One sample, labelled "L. albirostris" contained remains of haddock only and accounted for all of the haddock identified in this study. Because the stomach content of this dolphin was so different from all other dolphins, it dominated the NMDS graph, so that the remaining samples fell into one indistinguishable cluster. Without this individual, NMDS graphs showed no meaningful clusters in diet composition for dolphins grouped by either sex, age-class, year and month of stranding or whether they stranded before or after the cod collapse, neither in number nor in total weight per prey species. ANOSIM tests for sex, year and month of stranding, and before and after the cod collapse were not significant.

ARE WHITE-BEAKED DOLPHINS *LAGENORHYNCHUS ALBIROSTRIS* FOOD SPECIALISTS?

Table 6.1 Diet composition of *Lagenorhynchus albirostris* stranded on the Dutch coast between 1968 and 2005, identified from stomach contents, expressed by minimum number of individuals (MNI), frequency of occurrence (%FO), percent number (%N) and percent weight (%W).

Prey categories	Prey species	MNI	%FO	%N	%W
Gadidae		1711	100.00	39.99	98.03
	<i>Gadus morhua</i>	318	73.33	7.43	55.88
	<i>Melanogrammus aeglefinus</i>	14	2.22	0.33	0.56
	<i>Merlangius merlangus</i>	1307	91.11	30.54	37.62
	<i>Pollachius pollachius</i>	5	11.11	0.12	1.62
	<i>Trisopterus luscus</i>	62	31.11	1.45	2.36
	<i>Trisopterus minutus</i>	1	2.22	0.02	0
	<i>Trisopterus sp.</i>	4	6.67	0.09	0
Clupeidae		21	17.78	0.49	0.30
	<i>Clupea harengus</i>	17	15.56	0.40	0.29
	<i>Sprattus sprattus</i>	4	6.67	0.09	0.01
Ammodytidae		100	31.11	2.34	0.25
	<i>Ammodytes marinus</i>	3	4.44	0.07	0
	<i>Ammodytes tobianus</i>	43	11.11	1.00	0.12
	<i>Hyperoplus lanceolatus</i>	9	11.11	0.21	0.03
	<i>Ammodytes sp.</i>	45	17.78	1.05	0.09
Gobiidae		2335	31.11	54.57	0.60
	<i>Pomatoschistus microps</i>	2	2.22	0.05	0
	<i>Pomatoschistus minutus</i>	92	13.33	2.15	0.32
	<i>Pomatoschistus norvegicus</i>	1	2.22	0.02	0
	<i>Pomatoschistus pictus</i>	6	8.89	0.14	0
	<i>Gobiidae indeterminate</i>	2234	15.56	52.21	0.28
Flatfish		62	28.89	1.45	0.71
	<i>Buglossidium luteum</i>	1	2.22	0.02	0
	<i>Hippoglossoides platessoides</i>	1	2.22	0.02	0
	<i>Limanda limanda</i>	30	24.44	0.70	0.46
	<i>Pleuronectes platessa</i>	8	11.11	0.19	0.03
	<i>Solea solea</i>	17	11.11	0.40	0.20
	<i>Pleuronectidae indeterminate</i>	5	6.67	0.12	0.01
Other fish species		20	17.78	0.49	0.09
	<i>Callionymus lyra</i>	12	11.11	0.30	0.04
	<i>Enchelyopus cimbrius</i>	4	2.22	0.09	0.03
	<i>Mullus surmuletus</i>	1	2.22	0.02	0
	<i>Osmerus eperlanus</i>	1	2.22	0.02	0.01
	<i>Trachinus draco</i>	1	2.22	0.02	0
	<i>Fish indeterminate</i>	1	2.22	0.02	0
Cephalopoda	<i>Sepiolidae indeterminate</i>	29	2.22	0.68	0.02
Total		4278			

### 6.4.3 PREY SIZE AND WEIGHT

Prey sizes for cod and whiting for each dolphin are shown in Figure 6.4. The average size of cod is generally larger than that of whiting. For whiting, there is no relation with dolphin length, but dolphins smaller than 240 cm tended to eat relatively small cods. Average cod size in dolphins smaller than 240 cm was approximately 2 cm smaller (means 37.7 cm and 35.7 cm for adult and juvenile dolphins, respectively), however, this difference is not significant (likelihood ratio test:  $L_1=0.52$ ,  $p=0.47$ , linear mixed model).

The interactions between dolphin length and cod and whiting length were not significantly different from zero (respectively  $p=0.14$  and  $p=0.31$ ). The only difference that remained was the average length of the two prey species ( $L_1=243.8$ ,  $p<0.0001$ ), which was 35.6 cm (34.1, 37.2, 95% CL) for cod and 22.8 cm (21.7, 23.9) for whiting. The fraction of cod relative to whiting on a weight basis was investigated to see whether this changed with dolphin size (Figure 6.5). The model smoother was significant ( $p=0.026$ ) and indicates that up to approximately 250 cm the proportion of cod increases from approximately 0.3 to 0.6.

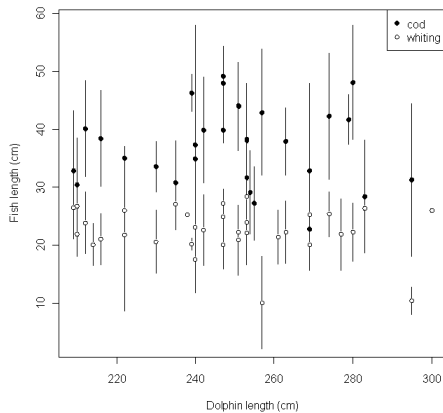


Figure 6.4 Length (cm) of cod *Gadus morhua* and whiting *Merlangius merlangus* (mean and SD) in the diet against body length of *Lagenorhynchus albirostris*.

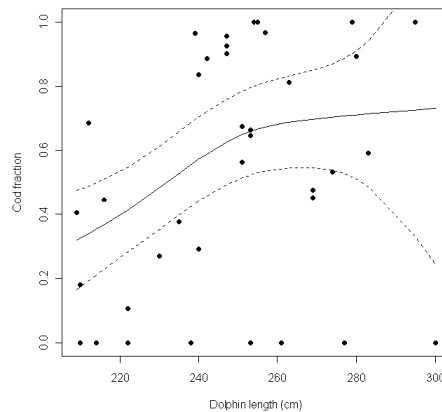


Figure 6.5 Relationship between the proportion of cod *Gadus morhua* (weight of cod relative to the total weight of cod and whiting) in the diet and body length of *Lagenorhynchus albirostris*. The line is the fitted regression line and the dashed lines are the 95% confidence limits.

## 6.5 DISCUSSION

### 6.5.1 SAMPLES

The number of stomach samples was limited, because not all stranded white-beaked dolphins in the Netherlands have been collected and/or sampled and some stomachs examined were empty. Nevertheless, this study comprises the largest sample size for this species to date, though it is restricted to the south-eastern North Sea. The 45 samples represent 27% of the white-beaked dolphins found stranded in the study period (1968–2005). There is a predominance of females among the samples, with 22 females to 12 males. This corresponds with the female dominance among all documented Dutch strandings of white-beaked dolphins to date (1886–2009,  $n=196$ , 89 females and 47 males) and within the study period (1968–2005,  $n=165$ , 70 females and 40 males) (walvisstrandingen.nl 2012). Female predominance among the strandings has also been documented for the Danish (42 females to 20 males, Kinze et al. 1997) and the German coast (12 females to 5 males, U. Siebert, unpublished data), though earlier, Kinze (1995) reported that all strandings on the German coast were females ( $n=9$ ). Kinze et al. (1997) suggest sexual segregation as a possible cause of this surplus of females. If males would generally stay further off the coast, they are less likely to become stranded or washed up in a fresh condition.

### 6.5.2 ANALYSIS OF STOMACH CONTENTS

The analysis of stomach contents enables us to identify the prey species ingested shortly before the animals died and, generally, not far from the place of stranding. This method has some disadvantages and inherent biases, such as: uncertainties of identification, passage time, retention and degradation and hence recovery rates of different prey, and partial and/or secondary ingestion of species. These problems have been reviewed in great detail elsewhere (Pierce & Boyle 1991; Cottrell et al. 1996; Tollit et al. 1997; Wijnsma et al. 1999; Bowen 2000; Arnett & Whelan 2001; Cottrell & Trites 2002; Tollit et al. 2003; Grellier & Hammond 2006). Despite these restrictions, the analysis of stomach contents gives the most solid and detailed information on ingested prey species and sizes if compared to other methods. Animals without hard parts will be underestimated, particularly when working on samples from museum collections, in most of which only hard parts are kept dry. However, it seems unlikely that such prey were of any significance for white-beaked dolphins, given the results of other diet studies of fresh stomachs (e.g. Dong et al. 1996; Canning et al. 2008).

### 6.5.3 DIET OF WHITE-BEAKED DOLPHINS FROM DUTCH COASTAL WATERS

The results show that the diet of white-beaked dolphins in Dutch waters is dominated by Gadidae, particularly whiting and cod (Table 6.1). Gobies, being the most numerous prey, contributed little to the diet by weight and most were

found in the stomach of only one animal. All prey species other than Gadidae and Gobiidae were only marginally important.

There is diet information for one additional animal. The stomach of an adult female (259 cm), stranded on the isle of Texel on 5 May 1964 was analysed by van Bree & Nijssen (1964) and contained otoliths of at least 4 cods and 30 whittings, along with 3 long rough dabs *Hippoglossoides platessoides* and 1 plaice *Pleuronectes platessa*. Although it had to be excluded from the present study, as fish weights could not be estimated from the available data, this animal too, had taken mainly gadids.

The proportion of the weight of a certain prey species in the dolphins' diet is a good indicator of the contribution of that species to total biomass of the stomach contents; obviously, large prey such as gadids contribute more to the diet than small species such as gobies. In this study, gobies were never found as the only prey in the stomachs, but always together with whiting and cod. Gobies are often considered secondary prey, ingested by larger fish, e.g. gadids (Pierce & Boyle 1991). However, the large number of gobies found in one juvenile dolphin show that gobies are in some cases preyed on directly by white-beaked dolphins. Cephalopods (small sepiolids) were only found in one other dolphin, the smallest juvenile included in our study. Both these squids and the gobies found in the other juvenile mentioned, contributed little to the overall diet.

As in most other dolphin species, cooperative feeding of white-beaked dolphins has been observed (Kinze et al. 1997; Evans & Smeenk 2008). In general, dolphin calves, including those of white-beaked dolphins, stay with their mothers for an extended period of time, during which they will learn which prey to take and how to catch them (Boran & Heimlich 1999). Dong et al. (1996) suggested that larger dolphins prey on larger cod. For the two main prey species, whiting and cod, we cannot confirm this, as we did not find a correlation between dolphin length and fish length. However, the fraction of cod in the diet in the combined weight of cod and whiting increased with dolphin length and cods were on average larger than whittings. In addition, the two dolphins that had taken large numbers of small prey, gobies and squid, respectively, were the smallest animals in our study. It may thus well be that young white-beaked dolphins prey on smaller and different prey species. As our smallest animal measured 209 cm, the possibility remains that still smaller individuals would indeed take smaller prey. The remaining animal with a different diet compared to the other dolphins had preyed solely on haddock, another gadid species. No further information is known for this individual, but its specific diet suggests that it may have been by-caught in the central North Sea where haddock is more common (Knijn et al. 1993).

Cetaceans are generally considered opportunistic foragers, selecting prey depending on availability (Trites 2009). The stocks of the main prey species of white-beaked dolphin: whiting and cod, have undergone drastic changes in



abundance in the North Sea during the 20th and 21st century (ICES 2006). Cod has been overexploited in the North Sea since the late 1960s (Cook et al. 1997; Bannister 2004) and stocks have failed to recover, even after severe catch restrictions. Whiting stocks have also significantly fluctuated over time, with clear peaks and troughs (Hislop 1996; Pope & Macer 1996). With cod and whiting accounting for most of the prey weight (93.7%W), being taken very frequently and in large numbers (40.6%FO, 80.4%N), differences in diet of white-beaked dolphins between years would be expected to occur. Such changes, however, are not reflected in the diet of the animals studied here, when arranged according to year (arranged by decade and by pre-1990 versus post-1990). The absence of a reflection of these drastic changes in prey abundance suggests that white-beaked dolphins are still able to exploit remaining concentrations of whiting and cod, staying highly selective in the choice of their prey.

In summary, our data show that the dolphins studied predominantly fed on whiting and cod, irrespective of their age-class, sex, season and year of stranding, before or after the “cod collapse”. It appears that white-beaked dolphins in the south-eastern North Sea are specialist feeders, with a strong preference for whiting and cod.

#### 6.5.4 COMPARISON WITH OTHER STUDIES

Most relevant to the Dutch situation are studies from elsewhere in the south-eastern North Sea. In agreement with the Dutch strandings, Gadidae predominated in stomachs of four white-beaked dolphins from Germany, particularly whiting, cod and poor cod *Trisopterus minutus* (Lick 1993). A single specimen from the French coast had Gadidae as the most important prey, including cod, *Trisopterus* sp. and pollack *Pollachius pollachius* (de Pierrepont et al. 2005).

White-beaked dolphins from northern British waters, the core distribution area of the species in the North Sea, were also found to feed mainly on cod and whiting, followed by haddock and hake *Merluccius merluccius* (Canning et al. 2008; Evans & Smeenk 2008). Herring *Clupea harengus*, mackerel *Scomber scombrus*, scad *Trachurus trachurus*, sandeel and long rough dab were occasional prey species. Cephalopods have also been identified in the diet of white-beaked dolphins in British waters, but as in our samples, only one animal had taken cephalopods, which were not identified to species. Additionally, Berrow & Rogan (1996) found two Gadidae and six scads in a white-beaked dolphin from Irish waters.

On the other side of the Atlantic Ocean, twenty animals from Newfoundland (Dong et al. 1996) and two from elsewhere in Canadian waters (Sergeant & Fisher 1957) contained remains of cod only. Despite slight differences in species composition within the Gadidae between regions and studies, gadids clearly account for most of the energetic intake of white-beaked dolphins, throughout their range.

## 6.6 ACKNOWLEDGEMENTS

Samples were collected by staff and volunteers of the Dutch strandings network, coordinated by the National Museum of Natural History (now NCB Naturalis) in Leiden. Thanks go to the staff of Naturalis, in particular to E. Gittenberger for supervision, M.J. Addink for necropsy data, A. Gittenberger for database setup and P.A.M. Gaemers for help on otolith identification. Thanks are also due to the staff of IMARES, in particular to R.S.A. van Bemmelen for creating the strandings locality map and P.J.H. Reijnders for valuable draft revisions. Thanks also to U. Siebert from the Forschungs- und Technologiezentrum Westküste (FTZ, University of Kiel) for contributing the German strandings data. Finally, we thank two anonymous reviewers for their valuable comments on earlier versions of this article.

ARE WHITE-BEAKED DOLPHINS *LAGENORHYNCHUS ALBIROSTRIS* FOOD SPECIALISTS?

# CHAPTER 7

## LEARNING TO EAT: JUVENILE WHITE-BEAKED DOLPHINS *LAGENORHYNCHUS ALBIROSTRIS* TAKE DIFFERENT PREY THAN OLDER INDIVIDUALS

Submitted as: Leopold, M.F., Jansen, O.E., Jansen, J.C., Kop, R., Keijl, G.O., Meesters, E.H.W.G, Reijnders, P.J.H. (submitted) Learning to eat: juvenile white-beaked dolphins *Lagenorhynchus albirostris* take different prey than older individuals. Journal of the Marine Biological Association of the UK.







## 7.1 ABSTRACT

White-beaked dolphins *Lagenorhynchus albirostris* are food specialists. Over 90% of their prey, in terms of prey mass, consists of rather large gadoid fishes. This raises the question how nursing or just-weaned calves learn to deal with such prey. Stomach contents of stranded juveniles of various ages were used to investigate their diet. Novel techniques were developed to reconstruct sizes of shrimp and whiting from tail flaps and eye lenses, respectively. Juvenile white-beaked dolphins, about 1.5 years old, appear to start feeding on solid food by taking a variety of small fish and invertebrate prey, mostly shrimp and squid. Immatures, estimated to be 2-4 years old, still take small prey, including small gadoids, but also take larger gadoids. Calves apparently gradually learn to eat big fish by taking prey that is much smaller than those normally taken by their mothers.

## 7.2 INTRODUCTION

White-beaked dolphins *Lagenorhynchus albirostris* are food specialists, preying mainly on rather large fishes. Depending on location, different fish species dominate the diet, but gadoids generally represent >90% of the total prey mass (Lick 1993; Dong et al. 1996; Kinze et al. 1997; Canning et al. 2008; Jansen et al. 2010). In Scotland, principal prey sizes ranged from 10.5 to 48.5 cm (mostly 26.5–28.5 cm) in haddock *Melanogrammus aeglefinus* and from 6.5–36.5 cm (mostly 15.5–16.5 cm) in whiting *Merlangius merlangus* (Canning et al. 2008). Whiting eaten in Dutch waters ranged from 5.6–39.5 cm (average  $\pm$  SD: 23.6  $\pm$  5.5 cm; n=1307) and cod *Gadus morhua* ranged from 9.0–62.7 cm (36.5  $\pm$  11.0 cm; n=318) (Jansen 2006; Jansen et al. 2010).

Dong et al. (1996) observed, in stomachs of twenty ice-entrapped white-beaked dolphins in Newfoundland, that “*in general, the largest otoliths*” [of cod, the principal prey in this study] “*came from the stomachs of the largest animals*”. Likewise, larger white-beaked dolphins in the size range of 209–300 cm (ranging from 3–24 years of age, n=45), most of them stranded on the Dutch coast, had eaten more cod than whiting, cod being on average larger than whiting (Jansen 2006; Jansen et al. 2010). Prey size thus appears to increase with dolphin size, or dolphin age.

New-borns do not take solid food, but only drink milk. The length of the lactation period is not known (Evans 1987) and neither is the age at which calves start taking solid food. It seems unlikely that juvenile white-beaked dolphins can switch overnight from drinking milk to catching, handling and swallowing large gadoid fish. Therefore, a transition period must exist during which juveniles learn to prey on fish. Information on prey taken by very young white-beaked dolphins is lacking, however. The three smallest individuals included in the



study of Jansen et al. (2010) measured 209, 210 and 210 cm and were immatures of 3-4 years old. All three had taken gadoids already, but two had also eaten significant numbers of much smaller prey.

Building on the data obtained from these three immatures, we put forward the hypothesis that juvenile white-beaked dolphins learn to eat big fish by starting out on small prey. To test this, we have studied stomach contents of five more animals that stranded in The Netherlands between 2005 and 2010 and that were all smaller and younger than the immatures in Jansen et al. (2010).

## 7.3 MATERIALS AND METHODS

### 7.3.1 STUDY ANIMALS

For this study data of 43 white-beaked dolphins with known prey sizes and body lengths are available. Most (41) animals had stranded on Dutch beaches between 1968 and 2012, the remaining two were by-caught by Dutch trawlers in the North Sea. Diet data for 33 of these (and 12 more for which exact body lengths were not known) have been published previously (Jansen et al. 2010), but for the present study the stomach contents of the three smallest animals studied by Jansen et al. (2010) were re-analyzed. In addition, five juveniles and five “adults” became available for necropsy and stomach contents analysis since Jansen et al. (2010) completed their study. The new juveniles were all smaller and younger than the first three immatures studied (Table 7.1).

*Table 7.1 Stranding locality, size, weight, sex and age of the white-beaked dolphins *Lagenorhynchus albirostris*, used in this study in chronological order of stranding. Age (years) was estimated based on length and sex according to Galatius et al. (in press). The first three dolphins stem from Jansen et al. (2010); dolphins WBD-J1 to J5 are new specimens.*

Dolphin	Location	North	East	Date	Length (cm)	Mass (kg)	Sex	Age
RMNH38327	Eems	53°21'	6°54'	16-03-1992	210	175	F	3
RMNH40071	Den Oever	52°56'	5°02'	08-05-1993	210	-	-	3-4
LA960728	Bloemendaal	52°24'	4°32'	28-07-1996	209	-	M	4
WBD-J1	Noordwijk	52°14'	4°25'	30-12-2005	170	-	M	1.5
WBD-J2	Maasvlakte	51°58'	4°04'	26-12-2009	201	107	F	2.5
WBD-J3	Ameland	53°28'	5°56'	27-12-2009	176	68.5	F	1.5
WBD-J4	Neeltje Jans	51°37'	3°40'	16-02-2010	172.5	72.5	F	1.5
WBD-J5	Ouddorp	51°49'	3°55'	07-03-2010	181	61	M	2

### 7.3.2 STOMACH ANALYSES

Stomach contents of 45 stranded white-beaked dolphins, kept in Naturalis Biodiversity Center (formerly National Museum of Natural History (Naturalis)) in

Leiden, were analysed by Jansen et al. (2010). Prey remains from the three immatures among these are reported in more detail here. In addition, complete stomachs of five young dolphins were analysed for this study (WBD J1-J5, Table 1). Each stomach (forestomach, fundic stomach and pyloric stomach; Smith 1972; Aznar et al. 2006) was cut open and the contents were washed into a large beaker. Prey remains from the oesophagi of dolphins WBD J2-J5 were also collected. Hard prey remains with near-neutral buoyancy, such as parts of brown shrimp *Crangon crangon*, were collected immediately from the beaker. To separate heavy, hard parts used for species identification, from all soft remains, the beaker was put under gently running tap-water, allowing the water to overflow. This procedure removed all soft remains, leaving otoliths, fish bones, eye lenses, squid beaks and polychaete jaws to settle at the bottom of the beaker, which were then collected. Hard prey remains were identified to the lowest taxonomic level possible, following Leopold et al. (2001) for otoliths and Clarke (Clarke 1986) for squid beaks. Minimum numbers of individual prey items (MNI) were assessed by pairing left and right otoliths, eye lenses of relatively large fish, uropods and claws of shrimp, and lower and upper squid beaks. When very large numbers of very similar prey items were present in a stomach complex, i.e. >500 otoliths of gobies, the highest number of either left or right sagittal otoliths were used as a conservative estimate of MNI. Original fish size and mass were estimated from otolith length and width, after correction for wear, following Leopold et al. (1998; 2001). New methods were developed to reconstruct sizes of partly digested shrimp and whiting, from tail flaps and eye lenses, respectively (see below).

**Shrimp tails.** Several stomachs contained remains of brown shrimp. Shrimp claws can be used to estimate original shrimp size (Doornbos 1984). However, from the stomachs in this study, relatively few claws were retrieved while a much larger number of complete shrimp tails and loose uropods (tail flaps) were found. The uropods found in the stomachs often had the terminal hairs missing due to digestion, but were otherwise not reduced in size. Regressions between uropod size and shrimp size, and between shrimp size and mass, were derived from a sample of fresh shrimps, collected from the Dutch Wadden Sea in March 2010. Ninety-six brown shrimps were used, evenly distributed over a size range of 20-55 mm, measured from the tip of the rostrum to the end of the telson (Figure 7.1). Exposed and total claw lengths were measured; the latter makes dealing with loose claws possible. Five measurements were taken from the tail flaps: 1) outer flap length from the joint to the tip, 2) outer flap length from the joint to the sub-terminal dent, 3) inner flap length excluding the joint, 4) maximum outer flap width and 5) maximum inner flap width (Figure 7.1). The size of the outer and inner tail flaps and claws all correlated strongly with shrimp length; as did shrimp length with shrimp fresh mass (Table 7.2).

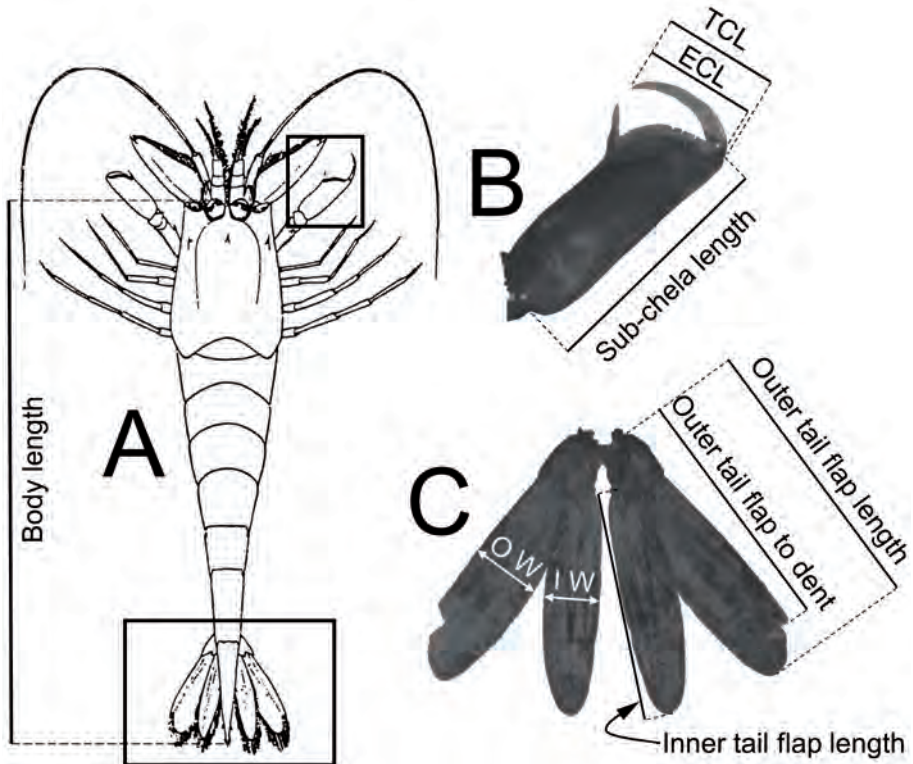


Figure 7.1 Brown shrimp *Crangon crangon* measurements: body length from tip of rostrum to tip of telson (A), sub-chela and claw lengths (B), tail flaps (outer/lower and inner/upper uropods) (C). Drawing of whole shrimp taken from de Ruijter & Schoenmaker (1989), TCL and ECL are total and exposed claw lengths, OW and IW are outer and inner tail flap widths, respectively.

Table 7.2 Regression parameters relating different measurements ( $X$ , mm), to shrimp body length (mm) according to:  $\text{body length} = aX + b$ . See also Figure 7.1. Shrimp fresh mass  $W$  (g) relates to shrimp length  $L$  (mm), according to:  $W = 0.000006L^{3.1664}$  ( $n=96$ ;  $R^2=0.9903$ ;  $P<0.001$ ).

Measurement	a	b	n	R <sup>2</sup>	P<
sub-chela length	7.2501	5.3692	93	0.9749	0.001
total claw length	14.7940	9.1950	96	0.9593	0.001
exposed claw length	17.3120	7.7635	96	0.9570	0.001
outer tail flap length	4.7152	0.6070	95	0.9710	0.001
outer tail flap to dent	5.2869	0.5548	95	0.9701	0.001
outer tail flap width	17.0430	7.1760	96	0.9667	0.001
inner tail flap length	5.8026	0.0431	95	0.9570	0.001
inner tail flap width	21.1660	6.5453	96	0.9408	0.001

**Fish eye-lenses.** Fifty fish eye lenses were retrieved from WBD-J2. The main prey of this dolphin, according to the otoliths found in the stomach, had been whiting (98.8% of prey mass, see Results section). Fish eye lenses, extracted from dolphin stomachs, are spherical and smooth initially, but soon degrade to a set of longitudinal parts, reminiscent of a peeled orange (Figure 7.2). Eye lens diameters were measured along the length axis. We compared the frequency distribution of eye lens lengths (Figure 7.2) with that of whiting lengths as derived from otolith measurements (Results section, Figure 7.4). We excluded the four smallest eye lenses, as they probably originated from other species, as well as the largest eye-lens (considered to belong to a single 20.2 cm long bib *Trisopterus luscus* that had been consumed along with the whiting; bib have considerably larger eyes than whiting). Matching the remaining smallest eye lens (1.9 mm) to the smallest whiting (16 cm) and the largest lenses (3.6 mm) to the largest whiting (30 cm), we found that intermediate peaks of lens and whiting lengths matched: peaks around 2.2 mm, and at 2.7, 2.9, 3.2 and 3.3 mm appeared to match with

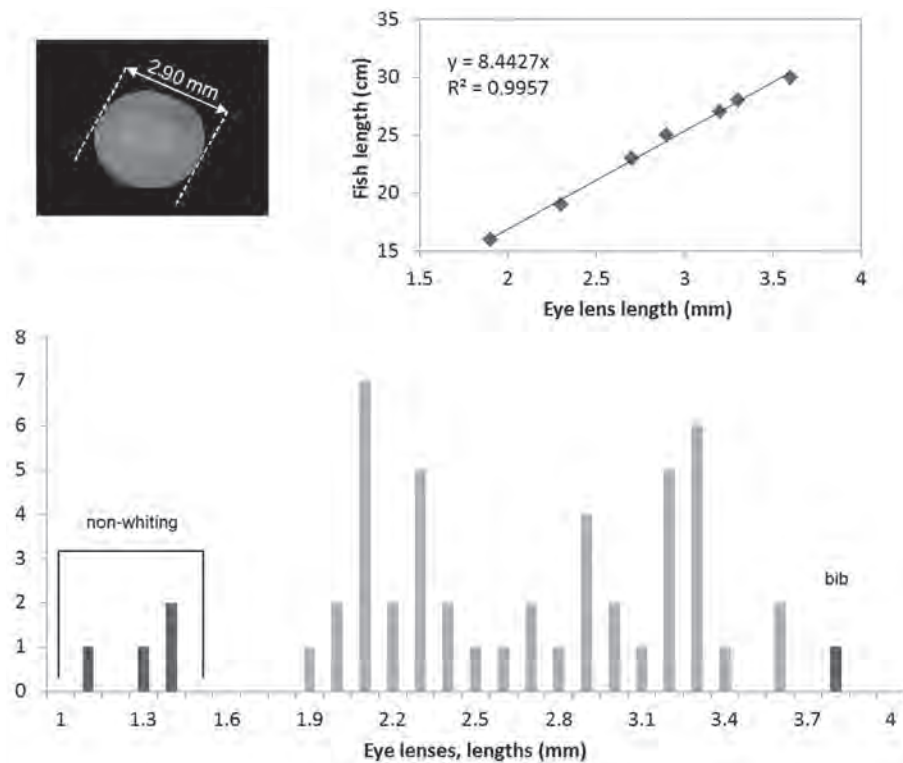


Figure 7.2 Length-frequency distribution of fish eye lenses ( $n = 50$ ; of which 45 are considered to be whiting; light gray bars) in the stomach of white-beaked dolphin WBD-J2. Inset left: eye lens, 2.90 mm long, presumably from whiting, 25 cm long. Inset right: peak-peak comparison of whiting total lengths (as derived from otoliths) and eye lens lengths.

peaks in fish lengths at 19, 23, 25, 27 and 28 cm, respectively. A peak-peak regression yields:

Fish length (cm) =  $8.4427 \times$  fish eye length (mm); ( $n = 7$ ,  $R^2 = 0.9957$ ; Figure 7.2).

This regression was used in another sample with eye lenses (WBD-J5), where antennary processes of *Lernaeocera branchialis* were found (see below), without any remaining fish hard parts, but eye lenses.

**Other remains.** Host-specific copepod parasites were used to identify whiting and sprat *Sprattus sprattus*. Remains of the parasitic copepod *Lernaeocera branchialis* were found in several stomachs. Gadoids are the prime final hosts of this species in the North Sea (Brooker et al. 2007) and whiting is probably the main final host in the south-eastern North Sea (van Damme & Hamerlynck 1999). The hard antennary processes of these parasites apparently remain for some time in the stomachs of dolphins after digestion of the host (fish). As the main gadoid prey of young white-beaked dolphins in Dutch waters is whiting (Jansen et al. 2010 and the present study), presence of *L. branchialis* antennary processes was considered proof that whiting had been eaten. In one case, otoliths were no longer present in stomach remains, while fish eye lenses did remain, and these were used to reconstruct whiting sizes (previous section).

One adult female *Lernaeenicus sprattae* was found in WBD-J4. *L. sprattae* have sprats as their only host in the North Sea (Rauck 1976; Schram 1987; Anstenrud & Schram 1988; Schram 1991; Groenewold et al. 1996) and may be found on sprat of all sizes. No otoliths of sprat were found in this stomach, only several clupeid vertebrae, that were used to estimate the size of the fish considered to be sprat, following Watt et al. (1997). Other remains encountered were squid, a jaw of the worm *Nereis succinea* and pincers of a decapod mudshrimp (length and body masses estimated by using our reference collection).

## 7.4 RESULTS

### 7.4.1 STOMACH CONTENTS FOUND IN JUVENILES

The smallest dolphin in our sample was a 170 cm long male, about 1.5 years old (WBD-J1; see Table 7.1). Its stomach contained 672 sagittal otoliths of sand gobies *Pomatoschistus minutus*: 336 left, 324 right, and 12 that were too worn to be assigned to either left or right. The MNI therefore, was 336 fish. Estimated fish lengths were similar for left and right otoliths (Figure 7.3; left:  $5.11 \pm 0.83$  cm, right:  $5.13 \pm 0.81$  cm,  $t=0.312$ , ns). The minimum total prey mass, based on left otoliths only, was 435.9 g.

The second-smallest dolphin was a 172.5 cm long female, also about 1.5 years old (WBD-J4). Its stomach contained remains of a variety of small prey: vertebrae of a sprat (5.0 cm, 0.7 g), otoliths of a lesser sandeel *Ammodytes mari-*

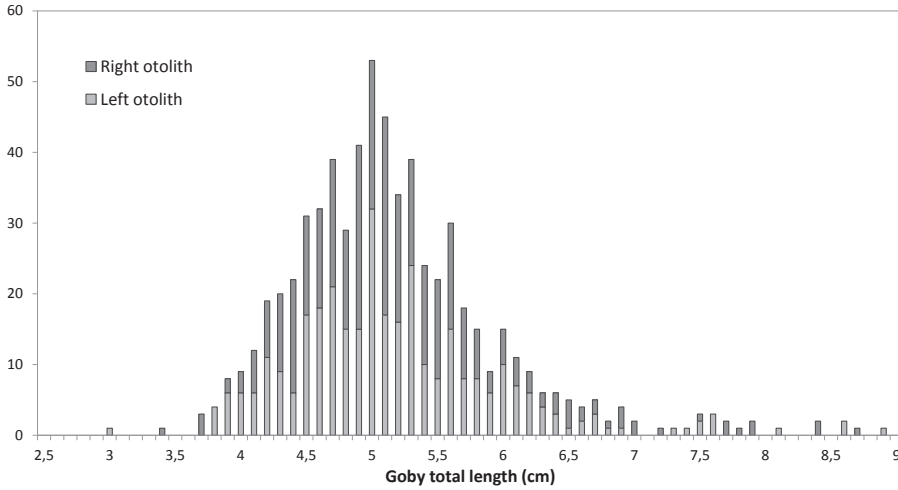


Figure 7.3 Sand goby *Pomatoschistus minutus* length-frequency distributions, estimated from left ( $n = 336$ ) and right ( $n = 324$ ) sagittal otoliths in white-beaked dolphin WBD-J1.

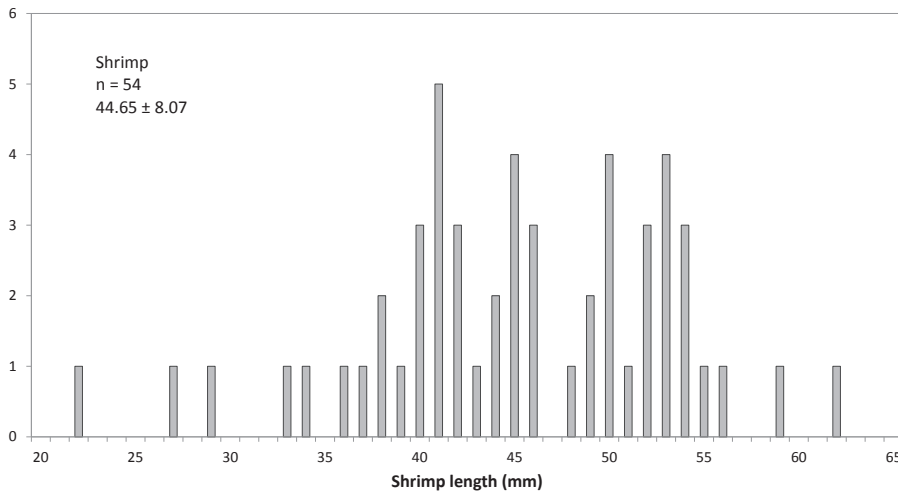


Figure 7.4 Estimated lengths of brown shrimps *Crangon crangon* from the stomach of white-beaked dolphin WBD-J3.

nus (18.6 cm, 19.7 g) and ten gobies, including nine *Aphia minuta* (ranging from 4.1-5.3 cm, together 5.8 g) and one *Pomatoschistus* spec. (estimated 3.7 cm and 0.4 g), sub-chelae and uropods of three brown shrimps (4.96, 5.23 and 5.55 cm body length, together 5.0 g), the upper and lower beak and eye lenses of one *Sepiolo atlantica* (1.8 cm mantle length, ca 2 g) and a jaw of *Nereis succinea* (0.3 g).

A third 1.5-years old, a 176 cm long female (WBD-J3, had the remains of dozens of brown shrimp in its stomach. No intact specimens were found, only



whole tails, loose uropods, sub-chelae and claws (Figure 7.1). Compiling the different items, we estimated an MNI of 53 shrimps with lengths ranging from 2.1 to 6.1 cm. Most shrimps were between 3.5 and 5.5 cm long, but some very small shrimps had also been taken (Figure 7.4). Together, the shrimps had an estimated mass of 59 g. The only other prey items found in this dolphin's stomach were the upper and lower beaks of a *Sepiolo atlantica*, with an estimated mantle length of 1.9 cm and mass of 2.75 g.

The next juvenile (in size) was 181 cm long male, aged 2 years old (WBD-J5). Its stomach contained at least 16 nearly digested brown shrimp (2.39 to 6.67 cm, 0.1 to 3.6 g; summed mass 28.6 g), and jaws of two *Sepiolo atlantica* (mantle lengths 2.0 and 2.3 cm, together 6.6 g). In addition, we found nine fish eye lenses and the heads and antennary processes of five *L. branchialis*. The eye lenses ranged from 1.5-2.7 mm in length and appeared to represent five different fishes (i.e. the same number as the number of *L. branchialis* found). If considered whiting, they would have been 13.0, 14.8, 17.2, 21.4 and 22.0 cm long (using the regression derived in Figure 7.2), with a summed mass of 239 g. The near-total digestion of the whiting suggested that these fish had been consumed some time before the smaller invertebrates were taken, implying that the shrimp and squid had been primary prey.

A slightly larger and older dolphin, a 201 cm long female aged 2.5 years old (WBD-J2), had its stomach full of gadoid remains. The animal had been feeding shortly before death, as many fish bones, including 14 more or less intact skulls, and upper and lower jaws of 23 whiting and 1 bib were found in the stomach. The otoliths of these fish were only slightly worn. In addition, otoliths of 15 other whiting were found that showed more wear. Other otoliths in the stomach were of one herring *Clupea harengus*, two sandeels Ammodytidae and one plaice *Pleuronectes platessa*. Apart from fish remains, ten nearly intact brown shrimp were found and the claws of one *Pestarella tyrrhena* (formally known as *Callianassa tyrrhena*, Decapoda, Callianassidae). The herring (11.3 cm), sandeels (13.9 and 15.6 cm), plaice (9.2 cm), and the crustaceans (3.6-5.1 cm) may have been secondary prey. However, the fishes seem rather large for whiting prey, and the shrimps were still nearly intact, suggesting that these smaller prey may also have been dolphin prey. The bib (20.2 cm) must have been primary prey, like the whiting (16.6-30.8 cm; Figure 7.5). The whiting showed a bimodal length distribution, with one peak around 19 cm and a second peak around 27 cm. This bimodality was not due to otolith wear (as we corrected for this) and nearly unworn otoliths, that required no or very little correction, were present in both size-classes. Sizes of the whiting taken by this young dolphin were similar to the average size of whiting taken by immatures and adults (Figure 7.6), but large whiting ( $\geq 31$  cm) were missing in this sample. The gadoids (whiting and bib) constituted the bulk of the reconstructed prey mass (98.8%). The other fishes represented 1% of total prey mass and the crustaceans 0.2%.

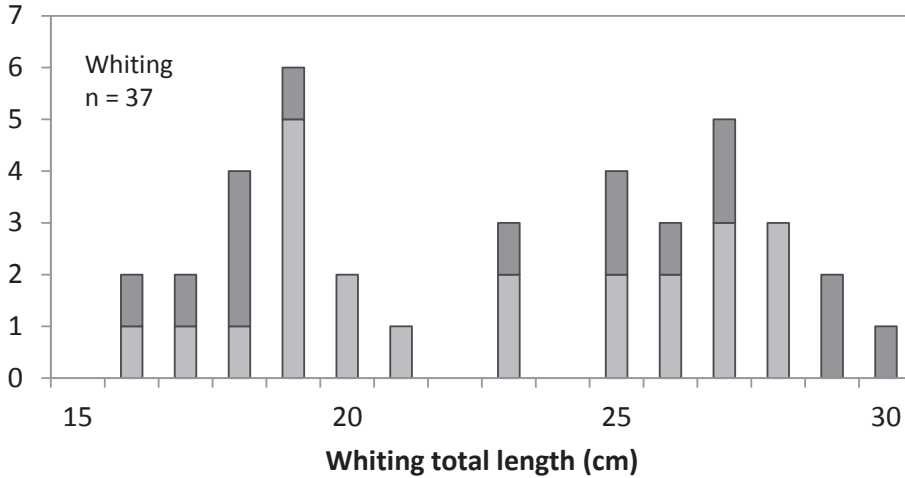


Figure 7.5 Whiting *Merlangius merlangus* lengths from dolphin WBD-J2, as estimated from (nearly) pristine otoliths (light gray) and from more worn otoliths (dark gray).

Three immatures, aged 3-4 years old and 209-210 cm long all had whiting as their principal prey in terms of prey mass. The first of these dolphins (RMNH38327; Table 7.1) had probably taken gadoids exclusively. Remains of 99 gadoids (whiting, cod and bib) were found, together with otoliths of two gobies and one sandeel, that may have been secondary prey.

In contrast, otoliths of 2220 gobies (MNI) were found in RMNH40071, together with otoliths of 52 whiting. These gobies had summed mass of 2880 gram and must have been mostly primary prey as the number of whiting in the same stomach was insufficient to account for the gobies as secondary prey. Together, the whiting weighed 9389 gram. Daily prey consumption of whiting is about 5% of their own body mass and about half of that is fish (Hislop et al. 1991; Hamerlynck & Hostens 1993). The whiting could thus be responsible for maximally 235 grams of gobies. Despite their large number, the contribution of the 2220 gobies to the total prey mass was outweighed by the 52 whittings, because were much larger prey.

The stomach of the third immature, LA960728, contained remains of 27 whittings and 6 cods, but also otoliths of herring and sandeels and the beaks of 29 unidentified squids (either *Sepietta*, *Sepiolo*, or *Rossia*). All three squid species occur off the Dutch coast and they are all small (mostly 2-3 cm mantle length; Lacourt & Huwae 1981; van Moorsel 2004; Groenenberg et al. 2009).

The whiting taken by these three immature dolphins were of similar size as those taken by larger white-beaked dolphins stranded in The Netherlands (Figure 7.6; T-test,  $t=1.27$ , ns). The cods, however, taken by two of these animals ( $n=11$ , average length  $31.8 \pm 9.07$  cm, range 19.9 to 45.9 cm) tended to be slightly smaller than the cods taken by larger and older dolphins ( $n = 312$ , average length  $36.6 \pm 11.04$  cm, range 9.0- 62.7 cm;  $t=1.73$ ,  $0.05 < P < 0.1$ ).

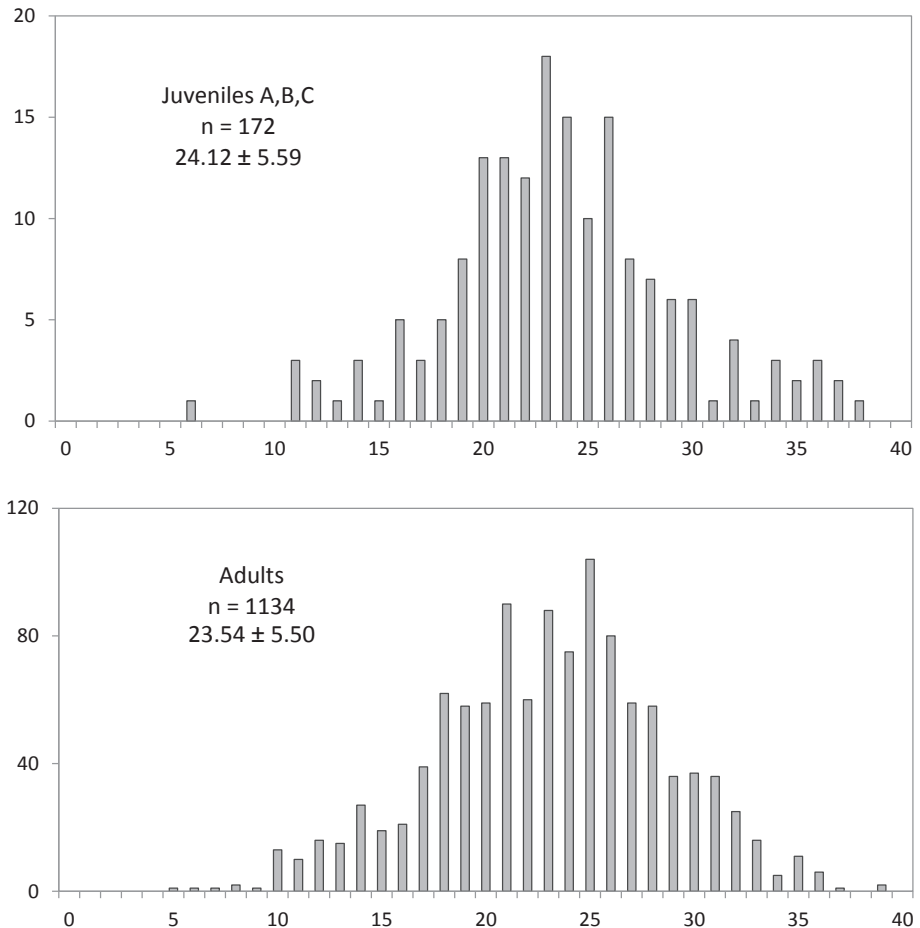


Figure 7.6 *Whiting* *Merlangius merlangus* length-frequency distributions in three juvenile white-beaked dolphins (upper panel) and in 42 larger white-beaked dolphins stranded in The Netherlands (lower panel).

## 7.4.2 DIET AND DOLPHIN AGE

Inter-age-class comparisons of diets can be made by plotting the various contributing prey species in two-dimensional planes, with the percentage frequency of occurrence (the fraction of stomachs containing that prey; %FO) on one axis and a measure of prey-specific abundance on the other (percentage of total prey volume, mass or number; Costello 1990; Amundsen et al. 1996). Such Costello diagrams have been used in earlier analyses of marine mammal diets (e.g. Ringelstein et al. 2006; Kavanagh et al. 2010), but by using only two axes, not all available information is incorporated. The diet of white-beaked dolphins appears to develop with age, while in addition to changes in %FO and %Mass of

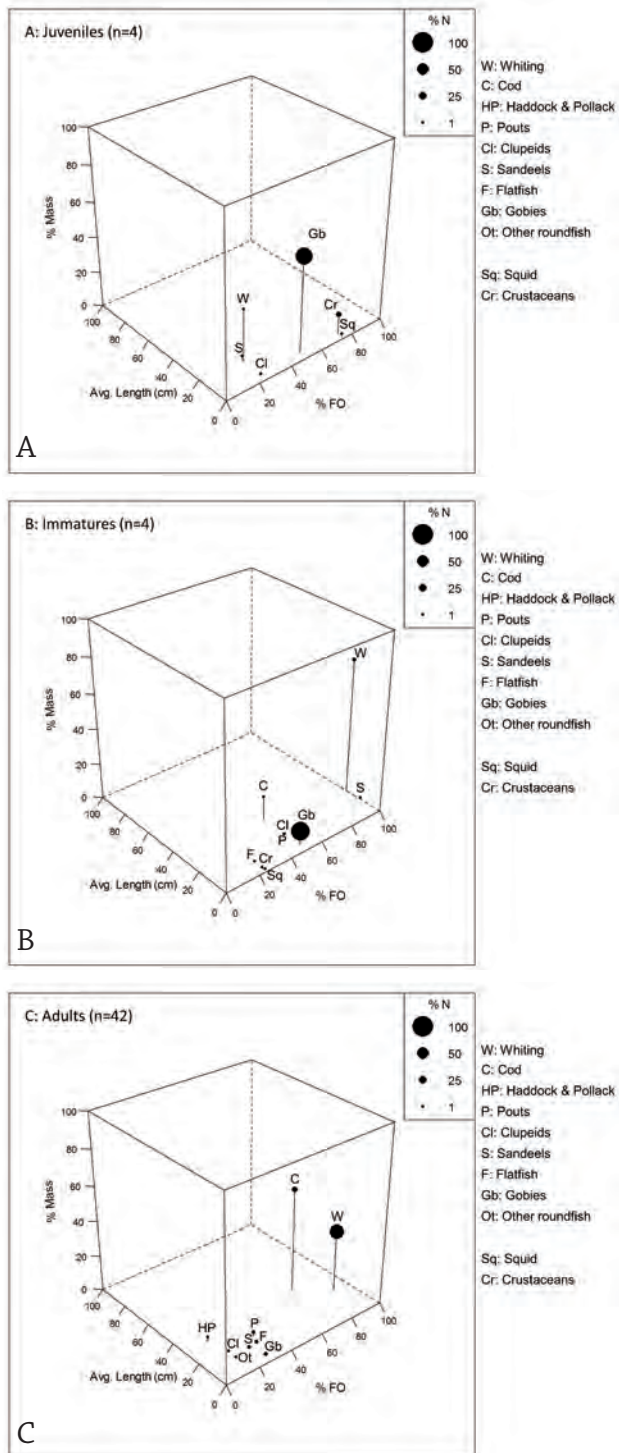


Figure 7.7  
 Summary of the diets of juvenile white-beaked dolphins, (A: body lengths 172-181 cm); immatures (B: 201-210 cm) and “adults” (C: >210 cm). X- and Y-axes are those of the ‘classic’ Costello diagram (see text): the proportion of stomachs with a particular prey (% FO) and the relative contribution to total estimated prey mass of that prey (%Mass), respectively. Average prey length (cm) is added along the Z-axis and the numerical contribution to total prey numbers (% N) is represented by symbol size (scale in upper-right panel).

contributing prey species, prey size and relative numerical abundances may also change. We have therefore added a third axis – average prey size – to the classic Costello diagram. By varying the size of the data points, we can also include relative numerical abundance.

The diet of four juvenile dolphins, 1.70 to 1.81 m long and estimated 1.5-2 years old, was characterised by a numerical dominance of small prey (gobies, shrimp, squid) and a high incidence (%FO) of the latter two. Gobies were numerically dominant and constituted most of the total prey mass (largely due to 336 gobies found in one dolphin). Whiting was numerically unimportant, and smaller in size than whiting in older dolphins, but still contributed substantially to total prey mass. Cod was missing in the diet (Figure 7.7A).

Cod appeared in the diet of immatures ( $n = 4$ , 201-210 cm long, 2.5-4 years old) and although this prey was still rather rare, its relatively large size made it important in terms of %Mass. Whiting and sandeels were found in all stomachs (100%FO). Whiting was the most important prey species by mass, while sandeels were unimportant in this respect. Shrimps and small squid were still taken by these immatures, while overall, gobies dominated the diet in terms of numbers. However, due to the increased importance of cod and whiting in this group, the gobies contributed little in terms of mass (Figure 7.7B). Adult diet is dominated by whiting and cod, with whiting being most abundant (both in %FO and in %N) and cod, as the largest prey, contributing most to total prey mass. All other prey species, including the gobies that are numerically dominant in the diets of younger dolphins, appear to be of marginal importance to adults (Figure 7.7C).

## 7.5 DISCUSSION

The gobies, shrimps and squids that were taken in relatively large numbers by juvenile and immature white-beaked dolphins, have individual prey masses that are 10-1000 times smaller than the mass of any individual gadoid, which make up the diet of grown dolphins. Taking many small prey instead of fewer large ones requires a lot of feeding effort, as a dolphin must catch and handle prey one by one. Moreover, the energetic return per prey item is very small, even though small prey may be easy to catch. Sand gobies caught in the Dutch Wadden Sea had  $4.80 \pm 0.88$  kJ/gram wet mass ( $n=42$ ) in January/February, but this value dropped to  $3.7 \pm 0.44$  kJ/g in April ( $n=20$ ; values assessed by bomb calorimetry, Beerman 2010; IMARES, unpublished data). Small squid and shrimp have similar energy densities, estimated as 3.6 kJ/g (Hammond & Fedak 1994) and 4.1-4.3 kJ/g (Massias & Becker 1990; Andersen 1999), respectively. For comparison, 22 whiting caught in the Wadden Sea in October/November (ranging from 10.4-24.5 cm) had  $4.92 \pm 1.67$  kJ/g (IMARES, unpublished data) while whiting

caught off Scotland had 3.9-5.4 kJ/g, depending on size and season (Pedersen & Hislop 2001). Considering these values, it would appear that dolphins should catch large prey, rather than small prey of similar or even lower energetic density. For instance, the 2220 gobies taken by dolphin RMNH40071 yielded 10,656 kJ, while the 52 whiting taken by the same animal yielded 41,010 kJ, nearly four times as much.

However, specific foraging skills are probably needed to feed on large goids. The three smallest dolphins in our sample may be too young to have mastered these, and had been feeding on small prey: small fish, shrimp, squid and a nereid worm. The smallest white-beaked dolphin feeding on whiting, albeit small ones, measured 181 cm and was estimated to be about two years old. In terms of prey numbers, however, shrimp and small squid were still its dominant prey. WBD-2 (201 cm long, 2.5 years old), was the smallest dolphin in our sample to have whiting as main prey, of similar size of whiting taken by adults. Even so, this immature also had a few smaller fishes and 10 shrimps its stomach. The collective evidence from these small dolphins suggests that small prey, such as gobies, shrimp and small squid comprise the first solid food of juvenile dolphins, while whiting is taken only when the animals get older.

Immatures of 3-4 years old and just over 2 m long appear to feed on whiting that are of similar sizes as whiting taken by adults, although the largest whiting found in adults were still missing in these dolphins. Two immatures of 209 and 210 cm long had also eaten cod but, like the whiting found in the smallest dolphins, these were small compared to cods taken by adults.

Gobies were found in 12 of the 42 adult white-beaked dolphin stomachs studied by Jansen et al. (2010), but mostly in low numbers ( $\leq 10$  fish) and always in the presence of whiting or cod, suggesting that they were the prey of these larger fishes. The maximum number of gobies found in adults was 66, found in a female of 251 cm. Her stomach also contained the remains of 99 whittings and 18 cods, so these gobies were probably secondary prey as well. Squid beaks or shrimp remains were not found in any of the 42 adult white-beaked dolphin stomachs studied by Jansen et al. (2010) and were thus unique prey to the juveniles and immatures in our sample. Small numbers of gobies were also found in white-beaked dolphins stranded in Germany (Lick 1993), and small numbers of gobies, squid and crustaceans in stranded animals in Scotland (Santos et al. 1994; Canning et al. 2008), but these authors did not give the sizes of the dolphins that had taken these small prey.

Juvenile dolphins may live for years in the social group in which they were born, and suckling may overlap for a long time with taking solid food (Herzing 1997; Archer & Robertson 2004; Mann 2009). Therefore, it may take juvenile dolphins years to reach true nutritional independence. Several studies have shown that prey size and prey species composition in juvenile dolphins gradually shift to those of adults (e.g. Archer & Robertson 2004). Similar gradual transi-



tions have been found in other large piscivores, such as sharks (e.g. Lucifora et al. 2006), seals (e.g. Mikkelsen et al. 2002) and porpoises: calves of harbour porpoises take a relatively high proportion of gobies (Lick 1991a,b; Benke et al. 1998; Börjesson et al. 2003; Santos et al. 2004) or euphausiids (Smith & Read 1992; Gannon et al. 1998), suggesting that small, slow and probably numerous and/or shoaling prey are good targets for marine piscivores learning to deal with solid food. The successive steps in the learning curve for young white-beaked dolphins may be from suckling to eating small fish and invertebrates (WBDs J1, J3 and J4), eating small whiting (WBD-J5), larger whiting, small cods (RMNH38327 and LA960728) and large cods, respectively. Prey size thus appears to increase with dolphin size over the entire length range of white-beaked dolphins.

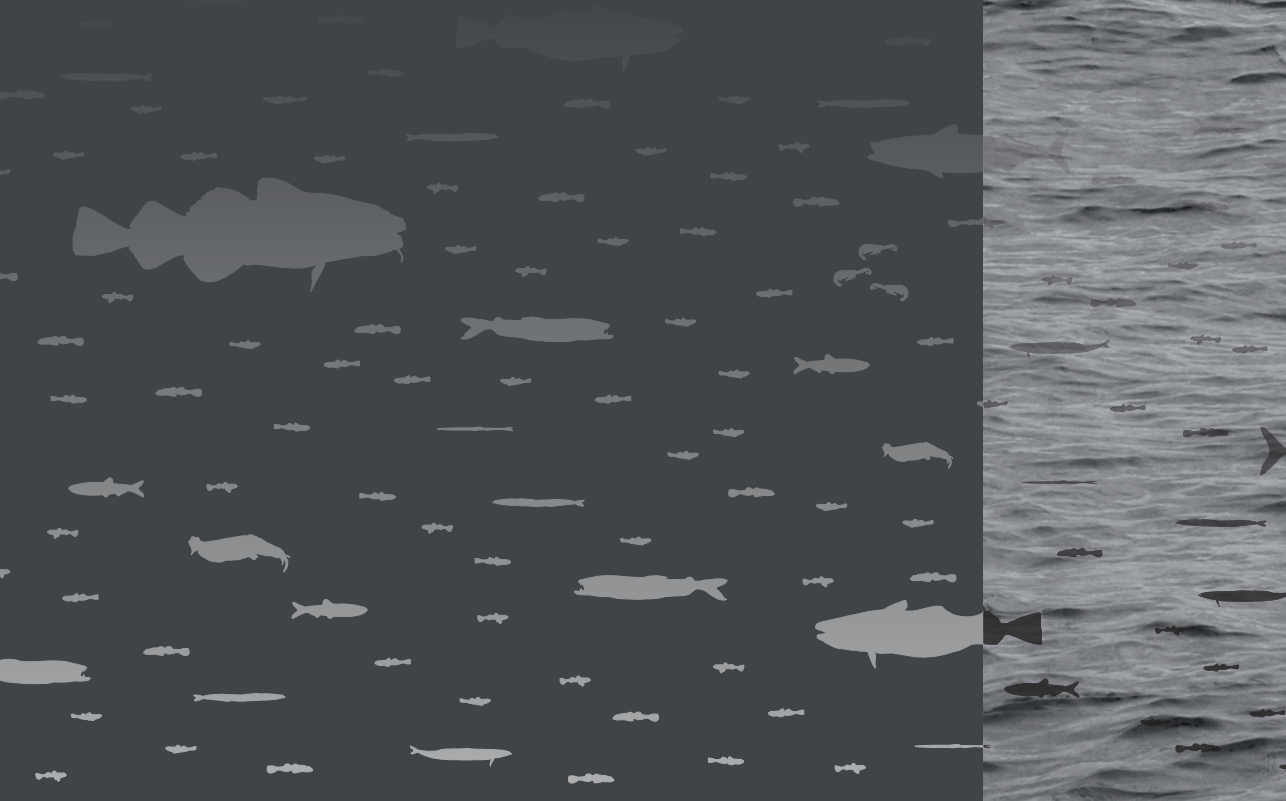
Transition from “juvenile” to “adult” diet is probably a gradual process in many animals. White-beaked dolphin calves may face particular problems in this respect, as prey fish typically taken by immatures and adults may be too large for juveniles to catch and/or swallow. Calves therefore, must take prey different from that of their mothers. Whether calves learn to eat by themselves, by trial and error or are taught by older conspecifics (social learning; Mann 2009; Campuysen & Krop 2011) remains a problem to be solved.

## 7.6 ACKNOWLEDGEMENTS

The dolphins were collected by staff and volunteers of the Dutch cetacean stranding network, coordinated by Naturalis Biodiversity Center (formerly National Museum of Natural History Naturalis) in Leiden and the Seal Rehabilitation and Research Centre in Pieterburen. Thanks go to Leen van Duijn, H.L.J. Eland, Marten Geerse, Jaap van der Hiele, Johan Krol, A. Schrauwen and Linda Wiersma for calling in and securing the young dolphins used for this study. Necropsies were carried out in Pieterburen (RMNH 40071), at Naturalis (RMNH 38327, LA960728 and WBD-J1) and in Utrecht (WBD-J2-5) by Thierry Jauniaux, Department of Veterinary Pathology of Liège University and Lidewij Wiersma, veterinary department of Utrecht University. The line-drawing of the shrimp in Figure 7.1 was taken from de Ruijter and Schoenmaker (1989), by permission of Stichting Jeugdbondsuitgeverij, 's Graveland. This work was supported by the National Museum of Natural History and Wageningen IMARES, was (co-) funded by the Dutch Ministry of Agriculture, Nature and Food Quality, and carried out by IMARES, Wageningen University and Research Centre as part of the research programme 'Harbour Porpoises: Abundance, strandings and feeding ecology' (BO-02-012-001).

# CHAPTER 8

## SYNTHESIS







Significant changes in distribution and relative abundance of harbour porpoises and white-beaked dolphins in Dutch waters have occurred over the past decades. I hypothesized that changes in the foodbase for these top-predators, could have driven these. To address that hypothesis we formulated two objectives: firstly to investigate past and present feeding ecology, including trophic interactions, of both species in a comprehensive way, and secondly to investigate whether the application of a multi-method diet analysis technique provides a more complete picture of the foraging ecology of harbour porpoises in time and space. This synthesis elucidates to what extent these objectives were met and, where appropriate, the outcome is put in a wider perspective.

## 8.1 THE FEEDING ECOLOGY OF HARBOUR PORPOISES

Harbour porpoises have a diverse diet, with spatial and temporal variation and differences between sexes and age-classes (e.g. Börjesson et al. 2003; Santos & Pierce 2003; Santos et al. 2004). In a review of the diet of porpoises in the North-east Atlantic, Santos & Pierce (2003) conclude that porpoises feed on a variety of prey, generally mainly on demersal species such as whiting, cod, sandeels, *Trisopterus* spp. (bib or poor cod) and gobies (Lick 1993; Martin 1996; Rogan & Berrow 1996; Santos 1998). In some areas a higher dependence on pelagic prey such as herring and capelin or pearlside *Maurolicus muellerii* (Aarefjord et al. 1995).

In Dutch coastal waters, there have been two larger studies on the diet of porpoises based on stomach contents (Santos 1998; Leopold & Camphuysen 2006, Table 8.1). These studies cover different time periods, respectively 1989-1995 and 2003-2006. Both studies indicate a large dietary overlap in the main prey species, i.e. whiting, sandeel and gobies. The older study (Santos 1998) showed that whiting and sandeel were the most important prey species in the diet of porpoises, but these species were of less importance in the diet of porpoises collected between 2003 and 2006 (Leopold & Camphuysen 2006). Gobies that were only of importance in terms of numbers present in the study by Santos (1998), were of high importance both in terms of numbers and weight in the study by Leopold & Camphuysen (2006).

Stomach content analysis has some inherent biases (Prime & Hammond 1987; Bowen 2000) and provides information on recently ingested prey, possibly overemphasizing the relevance of near-shore species (Pierce & Boyle 1991). Stable isotope and fatty acid analyses reflect the general diet assimilated over time, and thus provide insight in feeding ecology over longer time periods (Budge et al. 2006; Iverson 2008; Thiemann et al. 2008; Newsome et al. 2010). With the use of specific mixing models it is possible to estimate the proportional contribution of prey species to the accumulated diet of the predator (Phillips & Gregg 2001, 2003; Iverson et al. 2004).



Table 8.1 Diet studies on harbour porpoises from the Dutch coastal waters based on stomach content (SC), stable isotope (SI) and fatty acid analysis (FA).

Reference	N	Analysis	Primary prey items
Santos 1998; Santos & Pierce 2003	62	SC	Whiting, sandeel, gobies
Das et al. 2003	46	SI	Low trophic level prey
Leopold & Camphuysen 2006	64	SC	Whiting, sprat, sandeel, gobies
Christensen & Richardson 2008	88	SI	Increasingly lower trophic level prey
Jansen et al. 2012b	157	SI	Poor cod, mackerel, sandeel, sprat, gobies
Jansen et al. in prep.	73	FA	Gobies, mackerel, smelt, herring, dragonet

Stable isotope analysis on porpoises from Dutch coastal waters (Jansen et al. 2012b, Chapter 3) revealed that the long term diet consisted mainly of poor cod, mackerel, sandeel, sprat and gobies. Stomach contents of the same individuals show that porpoises fed on a shorter term mainly on gobies, whiting, sandeel, herring, cod and sprat. This reveals a profound difference between the long- and short-term diet of porpoises. On a longer term porpoises apparently depend more on pelagic, schooling species than would be expected if only stomach contents were analysed where more benthic, demersal species are dominating the short-term diet. As porpoises have shown to feed on abundant and easily available prey species (Knijn et al. 1993), this difference between the short- and longer-term diet may point towards differences between the behaviour of porpoises and their prey between the coastal zone and offshore waters.

Besides the assessment of the general diet composition via stable isotope analysis in muscle and bone tissue of porpoises stranded along the Dutch coast, stable isotope analysis provides more insight in the feeding behaviour of these animals. The results have revealed differences in trophic level and feeding location between animals of different ages, sexual segregation in feeding between adult porpoises, and have identified differences between groups of porpoises stranded during summer and winter months (Jansen et al. 2012a, Chapter 2). Based on higher  $\delta^{15}\text{N}$  values, female porpoises were found to have fed on prey with higher  $\delta^{15}\text{N}$  values compared to males. These intersexual differences were less profound in younger porpoises and only in the tissue reflecting the relatively shorter term diet (i.e. muscle), suggesting that segregation takes place after animals have reached maturity. Sexual segregation has also been suggested by a previous study on isotopic composition of porpoises from the southern North Sea (Das et al. 2003) and has been shown for other marine mammals (Hobson 1999; Lesage et al. 2001). As adult female porpoises stay with their calves in shelf waters for several months while adult males migrate further offshore, it can be expected that these two groups prey on different species (Smith et al. 1983). This is confirmed by stomach contents analysis on porpoises from Dutch coastal waters that has shown that adult female porpoises had fed more on small

gobies, while adult males had fed more on larger gadoids (Santos & Pierce 2003). In Jansen et al. (2012, Chapter 2), also prey species have been analysed, revealing higher  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in prey from more southern, coastal and estuarine areas. Higher  $\delta^{15}\text{N}$  values in adult female porpoises may thus support that they feed more coastally on gobies, as these prey species showed relatively high  $\delta^{15}\text{N}$  values.

As bone tissue reflects long term dietary input with a high bias towards the period of fast growth, it was surprising that seasonal variation was found in bone tissue. It could be rejected that this was a true seasonal variation in feeding preference or a reflection of higher  $\delta^{15}\text{N}$  values in neonate porpoises stranded in summer, suggesting that there are two distinct groups of porpoises occurring during the year along the Dutch coast. In combination with findings of DNA analysis on porpoises from the North Sea (Andersen et al. 2001), we conclude that there may be a winter group of mainly male porpoises that migrated from neighbouring regions and a group of porpoises that can be considered a Dutch subpopulation in summer (Yurick & Gaskin 1987; Walton 1997).

There are two previous studies on the isotopic composition of porpoises in Dutch coastal waters (Table 8.1). Das et al. (2003) analysed muscle tissue of 46 porpoises stranded on the French, Belgian and Dutch coasts between 1994 and 2000. The  $\delta^{15}\text{N}$  values of porpoises from our study were only slightly lower compared to those documented by Das et al. (2003), suggesting that porpoises from Dutch and adjacent coastal waters had fed on similar trophic level prey. The  $\delta^{13}\text{C}$  values from our study were significantly lower (-1.8‰) compared to those documented by Das et al. (2003). This difference may be partly due to regional differences in  $\delta^{13}\text{C}$  baseline values that are reflected in porpoises from France and Belgium, which are included in their study. In addition, it may also be a consequence of the gradually decreasing trend of  $\delta^{13}\text{C}$  in marine organisms Christensen & Richardson (2008). They analysed bone tissue of 88 porpoises stranded on the Dutch coast between 1848 and 2002, most animals from 1940 onwards. The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of porpoises from our study were approximately 1‰ and 3‰ lower than those reported by Christensen & Richardson (2008) for the period 1978 to 2002, respectively. They postulate that porpoises have gradually been feeding on lower trophic level prey over the last century and argue that this is due to changes in food web structure with progressively lower trophic prey available to porpoises. An additional explanation could be found in the increase of anthropogenic  $\text{CO}_2$  in the environment. This would in turn lower the  $\delta^{13}\text{C}$  of primary producers. However, the gradual decrease in  $\delta^{13}\text{C}$  can only partially be explained by anthropogenic changes in carbon composition in the atmosphere (Cullen et al. 2001). It is therefore concluded that the food web structure of the North Sea has also changed over the past century.

Similar to our study, both studies (Das et al. 2003; Christensen & Richardson 2008) found trophic sexual segregation in adult porpoises with higher



$\delta^{15}\text{N}$  in females. In contrast to our study, Das et al. (2003) found that female porpoises had also slightly higher  $\delta^{13}\text{C}$  values, which could reflect the differences in sex ratios between the two studies.

An unexpected outcome of the stable isotope analyses was the finding of a marked difference in isotopic signatures of porpoises found in the North Sea and those found in the adjacent estuary the Eastern Scheldt (Jansen et al. in press, Chapter 4).

Fatty acid analysis (QFASA) on porpoises from Dutch coastal waters (Chapter 5) revealed that the longer term diet consisted mainly of gobies, mackerel, smelt, herring and dragonet. The estimated relative contribution of the prey species differed, depending on the model parameters used but shows in all cases profound differences to the diet as deduced from stomach contents of the same individuals.

For gobies, lesser sandeel and sprat, the difference between the prey composition as deduced from stomach contents and as estimated by QFASA could be explained by the inherent bias of stomach content analysis towards coastal species.

In contrast, the higher importance of dragonet and smelt in the diet composition as estimated by QFASA contradicts this coastal bias in stomach contents analysis and may be caused by misidentification.

The higher importance of pelagic schooling fish (i.e. mackerel, scad and herring) in the diet as estimated by QFASA compared to stomach contents could be explained by differences between the hunting behaviour of porpoises and the schooling behaviour of fish between coastal and offshore waters.

The most striking difference between these two methods was seen in whiting. Whiting was not identified in any of the QFASA models, although it is the second most important prey species in stomach contents. Being one of the most abundant and widely dispersed gadoid in the North Sea, this difference cannot be explained by the coastal overrepresentation of near-shore species in stomach contents. Misidentification between whiting and cod due to similarities in fatty acid signatures would increase the importance of whiting in the diet as estimated by QFASA but not to the extent that it would reflect the importance of whiting in stomach contents. No ecological explanation was found that could account for this difference, suggesting that the diet of porpoises as estimated by QFASA in our study has to be cautiously interpreted (Chapter 5).

## 8.2 THE FEEDING ECOLOGY OF WHITE-BEAKED DOLPHINS

White-beaked dolphins prey on a wide variety of prey species, mostly fish species but also some cephalopod species and shrimp (Evans & Smeenk 2008; Kinze 2009). The diet of white-beaked dolphins in the wider North Sea is dominated

Table 8.2 *Diet studies on white-beaked dolphins from the North Sea based on stomach content analysis.*

Reference	Area	N	Primary prey items
van Bree & Nijssen 1964	The Netherlands	1	Cod, whiting, long rough dab, plaice
de Smet et al. 1985	Belgium	1	Fish unidentified
Smeenk & Gaemers 1987	The Netherlands	8	Whiting, cod, bib
Lick 1993	Germany	4	Whiting, cod, poor cod
de Pierrepont et al. 2005	France	1	Cod, bib, pollack
Canning et al. 2008	Scotland	22	Haddock, whiting, cod
Jansen et al. 2010	The Netherlands	45	Whiting, cod, gobies

by large Gadidae, despite regional differences in prey species composition (Table 8.2). Whiting and cod are found as important prey species in nearly all diet studies. Pollack and bib were found in white-beaked dolphins stranded in France (de Pierrepont et al. 2005), while in German waters poor cod was found (Lick 1993). Besides cod as main prey, also haddock and cephalopods were found in dolphins from Scottish waters (Canning et al. 2008).

In Dutch coastal waters, white-beaked dolphins prey on a wide variety of prey species, although their diet is heavily dominated by Gadidae, particularly whiting and cod (Jansen et al. 2010, Chapter 6). The diet showed a lasting predominance of these species, without distinct variation over time (35 years) or differences between sexes or size-classes of dolphins. Although gobies were also of importance in numbers compared to other prey species, nearly all gobies were identified in one young dolphin, together with the remains of sandeel and whiting. One white-beaked dolphin had solely preyed on haddock, a species that is more common in those waters (Knijn et al. 1993), suggesting that this animal did probably not strand in the Netherlands but had been by-caught in the central North Sea. Cephalopods have been identified in one young dolphin, that had fed on these sepiolids in addition to whiting, sandeel, cod and herring (Jansen et al. 2010). In the stomach content from another white-beaked dolphin stranded on the Dutch coast (van Bree & Nijssen 1964), long rough dab and plaice were identified as important prey species, although Gadidae also dominated the diet in this individual. A transition in the diet of white-beaked dolphins has been shown, first feeding on particularly small prey (gobies, shrimp and squid), through similar but smaller fish species (Gadidae), towards feeding on large Gadidae as preyed on by adult white-beaked dolphins (Leopold et al. submitted, Chapter 7). In summary it shows that in the south-eastern North Sea white-beaked dolphins can be considered specialist feeders, with a strong preference for whiting and cod.

### 8.3 ONTOLOGY IN FEEDING ECOLOGY OF SMALL CETACEANS

Differences in diet between age-classes have been reported for many cetacean species (e.g. Yasui & Gaskin 1986; Cockroft & Ross 1990; Smith & Read 1992), possibly due to physical restraints (diving ability), foraging skills or feeding location. In general, parental care occurs over a period of several months to several years in most cetaceans, where calves stay with their mothers (Wells et al. 1999). In this period the mother's milk is the initial nutritional source, but after a few months, young calves will have to learn which prey to take and how to catch them (Herzing 1997; Boran & Heimlich 1999; Archer & Robertson 2004). Harbour porpoises are weaned at the age of approximately 5-9 months. A gradual transition from liquid food (i.e. milk) to solid, larger food during the weaning period (i.e. fish) has been shown in several marine mammals species (e.g. Mikkelsen et al. 2002; Knoff et al. 2008), including harbour porpoises (Mohl-Hansen 1954; Read & Hohn 1995; Schulze 1996; Kastelein et al. 1997b). Based on stomach contents, porpoise calves have been found to feed on small crustaceans, and small fish while still being nursed, eventually feeding mainly on small crustaceans and fish species (Smith & Read 1992; Lick 1993; Gannon et al. 1998; Börjesson et al. 2003). Santos & Pierce (2003) show small prey species such as gobies, small flatfishes and shrimp in the diet of young porpoises. Gannon et al. (1998) describe small crustaceans, pearlside, and juvenile herring and hake as important species in the 'transitional diet' of porpoises from Canadian waters. Most studies have found that gobies are of greater importance in the diet of young porpoises than in adults (e.g. Aarefjord et al. 1995; Börjesson et al. 2003; Santos et al. 2004; de Pierrepont et al. 2005). Santos et al. (2004) equally found more haddock, saithe and pollack in the diet of larger porpoises. Based on stable isotope analysis on porpoises from Dutch coastal waters, suckling neonates could be identified by their high  $\delta^{15}\text{N}$  values in their tissues deriving from their mothers' milk (Jansen et al. 2012a, Chapter 2). We also found that young porpoises stay in coastal waters, feeding mainly on small prey species that are found in high numbers along the Dutch coast (i.e. gobies) and that adult porpoises with a length of more than 135 cm, tend to feed more in offshore waters. Larger porpoises, in particular males, have shown to feed on lower trophic level, also in distant times (Jansen et al. 2012a).

There are to our knowledge, no published data on either the average age at weaning or of solid food ingestion in white-beaked dolphins. Given the relation between mature female body length and interbirth interval (IBI) in cetaceans (Taylor et al. 2007), the similar IBI, age at first reproduction in bottlenose dolphins and a suckling duration in bottlenose dolphins of several years (Wells & Scott 2009), it is expected that the weaning age in white-beaked dolphins is considerably longer than in porpoises. It has been suggested that larger white-

beaked dolphins prey on larger cod (Dong et al. 1996). Although initially no correlation between dolphin length and fish length was found for whiting and cod, larger animals showed to have preyed on relatively more cod than whiting (Jansen et al. 2010, Chapter 6). Analysing specifically young white-beaked dolphins that were underrepresented in the previous study showed that very young white-beaked dolphins (estimated age of 18 months) start feeding on solid food by preying on a variety of small vertebrate and invertebrate species, e.g. feeding on gobies, shrimp and small squid species (Leopold et al. submitted, Chapter 7). Squid and shrimp remains were only found in the stomachs of immature and juvenile dolphins. Older juveniles (2-4 years old) were found to feed on similar but smaller prey than adult dolphins do, showing a clear transition in the feeding ecology of white-beaked dolphins from different and particularly small prey (gobies, shrimp and squid) through similar but smaller fish species (small Gadidae) towards feeding on large Gadidae as preyed on by full grown, adult white-beaked dolphins.

## 8.4 PORPOISES IN THE EASTERN SCHELDT

Prompted by the predicted climate change and future sea level rise, many authorities have developed plans for coastal defence against flooding. Those include embankment, and large constructions for temporarily or permanent damming up rivers and estuaries, and storm surge barriers (IPCC 1990; Bosello et al. 2012; Hallegatte 2012). The construction of the Eastern Scheldt storm surge barrier - finished in 1986 - was intended to protect the mainland coast against flooding and still preserve the tidal rhythm and connected ecosystem in that estuary. In those days harbour porpoises were rarely seen in that area and not considered to belong to the local fauna. Therefore, the possible consequences of such a partially blocked waterway for this species were not taken into account. However, the recently increased number of porpoises in Dutch coastal waters, has raised the questions whether the Eastern Scheldt storm surge barrier is limiting the movement of porpoises between the Eastern Scheldt and the adjacent North Sea coast. Acoustic monitoring has been applied to study the movement of porpoises through the gates, however, no results have been published yet. The increase in numbers of porpoises, and more recently the sightings of mother-calf pairs has led to the idea that porpoises have entered the Eastern Scheldt and may stay in this National Park because of good food availability.

We have found a distinct isotopic signature in porpoises stranded within the Eastern Scheldt tidal bay (Jansen et al. in press, Chapter 4). As this was found in muscle tissue, porpoises must have fed long enough in the area to integrate the distinct isotopic signature of the prey in the Eastern Scheldt. If there had been a constant exchange of animals between the North Sea coast and the

Eastern Scheldt, this signature should be less distinct or not detectable at all. That this Eastern Scheldt isotopic signature was not found in bone reveals that the porpoises investigated in this study were not born in the Eastern Scheldt.

Within the Eastern Scheldt, porpoises are seen all year through, while along the coast seasonal changes in porpoise abundance are recognized. Together with the high mortality of porpoises within the Eastern Scheldt as evidenced by stranding numbers, as compared to the Dutch coastal zone, suggests that the area might act as an 'ecological trap' for these animals (Jansen et al. in press, Chapter 4). It is theoretically possible that porpoises having died in the Eastern Scheldt, may have subsequently drifted into the North Sea and be recovered there, or vice versa. If this was the case the distinction between animals stranded within the Eastern Scheldt and along the Dutch coast would even be larger than we find now. It would also point towards an even higher mortality of porpoises within the Eastern Scheldt than presented by our data so far. Since 2010, porpoise neonates have been documented within the Eastern Scheldt. It would be interesting to retrieve stranded or bycaught neonates and use stable isotope analysis to determine whether these animals were actually born in the Eastern Scheldt.

With the predicted acceleration of climate change and global warming, coastal protection measures along the coast will increase in time worldwide. We show an example where the building of a storm surge barrier, a semi-open structure, has affected the residency of porpoises, creating a sub-group of animals with a high mortality (Jansen et al. in press, Chapter 4). This fragmentation of their habitat may well have consequences for the survival of such a small group of animals, due to chance effects and possibly loss of genetic diversity. As such, this example warrants that authorities include consequences of coastal defence structures for migratory species such as small cetaceans in environmental impact assessments.

## 8.5 PORPOISES AND WHITE-BEAKED DOLPHINS AS INDICATORS OF A FOODBASE CHANGE

Potential competition for resources between marine mammals and fisheries is a globally recurring management issue (Northridge 1991; Trites et al. 1997). This may involve reduced prey availability for marine mammals due to (over-) fishing or reduced catches by fisheries due to marine mammal predation. Investigating whether competition for resources exists is complicated by the fact that next to the exerted mortality on fish stocks by both fisheries and marine mammals, also natural mortality (including impact of predatory fish) occurs. In an attempt to unravel one piece of that puzzle, one might study whether changes in distribution and abundance of marine mammals might be related to food availability. To

find a quantitative and causal link between changes in the abundance and distribution of marine mammals and changes in their prey is difficult due to a deficiency in fine scale temporal and geographic information of the fish and marine mammal species involved (Reijnders 1992). A further complicating factor is that the relation between predator and prey abundance can also be caused by other confounding factors that are not directly food-related, such as e.g. pollution and climate change. Nevertheless, there are several examples where a relation was suggested between changes in marine mammal diets as a reflection of changes in prey abundance (e.g. Bowen & Harrison 1996; Thompson et al. 1996; Santos & Pierce 2003; MacLeod et al. 2007a,b).

Diet studies on small cetaceans have the potential to identify changes in lower trophic levels in the food web (Pauly et al. 1998), while changes in the abundance and distribution of these animals can yield information on the carrying capacity of the system studied (Christensen & Pauly 1998; Berggren et al. 2002). Rapid and unpredicted changes in numbers or health of cetaceans can be used as a signal for detrimental influences on these populations specifically, and possibly the ecosystem they inhabit. This signal can be expected to be strongest in food specialists, that depend for their main dietary intake on a few species. As opportunistic feeders are considered to prey on the most abundant or best available prey species, their diet can be used to analyse changes in species composition of the food web.

Porpoises are generally considered opportunistic, generalist feeders, apparently relying on few species that are easily available in high numbers (Martin 1996; Teilmann & Dietz 1998; Christensen & Richardson 2008). They are also considered to be very dependent on a relatively continuous food supply due to their high metabolism and high surface to volume ratio (Worthy & Edwards 1990; Brodie 1995; Kastelein et al. 1997c). They are thus reliant on the local food sources and should thus respond quickly and noticeably to changes in prey abundance and distribution in their food choice. In theory, their diet composition should therefore reflect the composition of prey species available and can thus be expected to show great spatial, temporal and individual variation.

Stomach contents analysis of porpoises from the North Sea has shown that porpoises do prey on species that are all very abundant in these waters (Santos 1998; Santos & Pierce 2003; Leopold & Camphuysen 2006). This is confirmed by stable isotope and fatty acid analysis, where porpoises' diet was found to include pelagic, schooling species (e.g. mackerel, herring and sprat) as well as demersal species (e.g. whiting, poor cod and sole) and species that are abundant in coastal waters (e.g. gobies, smelt and bass) (Jansen et al. 2012b, Chapter 3 and 5). Large individual variation in prey preference has been found in studies based on stomach contents on the one hand (Recchia & Read 1989; Santos & Pierce 2003; Leopold & Camphuysen 2006) and stable isotope analysis on the other (Jansen et al. 2012a, Chapter 2).



Santos & Pierce (2003) have shown geographic and interannual variation in prey composition of porpoises in studies from the Northeast Atlantic. A direct link between diet composition and prey abundances as deduced from fishery-catch data did not yield conclusive results as some prey species are of no commercial value or because fine-scale data on fish abundance is missing.

In many diet studies on porpoises, sandeels are found among the most important prey species (e.g. Santos & Pierce 2003; Jansen et al. 2012b, Chapter 3). Sandeels play a key role in the North Sea ecosystem as they are prey for many fish, seabird and other marine mammal species and concern has been raised about the possible effects of the intense sandeel fishery in the North Sea (Wright 1996; Furness & Tasker 2000; Frederiksen et al. 2004). MacLeod et al. (2007a,b) have suggested that starvation and a successive southern migration of porpoises from Scottish waters was caused by a decline in sandeel stocks. This suggests that porpoises do not inevitably switch to other, more abundant prey if certain fish stocks are declining, and that they are opportunistic predators only to some extent, but that declines in their main prey species may lead to starvation and/or migration to other areas. Porpoises can thus be recognized as indicators of changes in the foodbase.

White-beaked dolphins are highly selective relying for their main dietary intake mostly on a few species of large Gadidae (Jansen et al. 2010, Chapter 6). In theory, they should thus respond to structural changes in the abundance and distribution of these prey species either by changes in their own abundance and distribution or a drastic change in diet composition with a distinct switch to other prey species. The stocks of the two main prey species of white-beaked dolphins, cod and whiting, have shown drastic fluctuations in the North Sea during the 20<sup>th</sup> and 21<sup>st</sup> century (Hislop 1996; Pope & Macer 1996; Cook et al. 1997). During the so-called 'gadoid outburst' of the mid-1960s, population densities of cod, haddock, whiting and Norway pout were very high. With increasing fishing mortality, these stocks have subsequently been overexploited and declined rapidly from the mid-1980s onwards. Even after severe fishing restrictions, the cod stocks have failed to recover (Daan et al. 1990; Hislop 1996; Bannister 2004).

Remarkably, the fluctuations of these main prey stocks are neither reflected in the diet of white-beaked dolphins nor in a reduction in the abundance of these animals in the North Sea. This would indicate that they are capable of exploiting remaining prey of stocks even when these are under great pressure (Jansen et al. 2010, Chapter 6). It remains unclear what the effect on white-beaked dolphins will be if the stocks of their main prey species become even more depleted. It cannot be ruled out that they can switch to other prey species or may disappear from the North Sea completely. It is therefore concluded that white-beaked dolphins are less suitable as indicators of changes in their foodbase.

## 8.6 STRANDED CETACEANS: AN APPROPRIATE SOURCE FOR DIETARY INFORMATION?

National and international legislation and ethical considerations prevent the active collection of cetaceans for study purposes in the European Union. It is therefore common practice to use stranded cetaceans for various studies, including studies on their feeding ecology. As direct observations of feeding are rare and as the collection of regurgitation and defecation is difficult, if not impossible, stomach contents and tissue samples from stranded individuals are often the only source of valuable dietary information.

However, the use of stranded animals has advantages and limitations and may yield unexpected bias. It is often difficult or impossible to determine the cause of death in stranded cetaceans, especially when individuals are decomposed. Cetaceans strand for various reasons, they may either have died due to natural reasons and drifted ashore, or died due to e.g. environmental conditions, diseases, pollution, starvation or to fisheries activities and may therefore not be representative of the population at large. The first category of animals likely have suffered from a disease and, if this was severe and lasted for a longer time period, they may also be in a poor nutritional status. The sex- and age composition of those animals will probably not correspond to that of the 'healthy' population. Besides impacting the opportunities to forage, also the disturbed physiological processes in those animals might have influenced stable isotope and fatty acid composition in tissues such as blubber and muscle. In contrast, bycaught animals are basically good sample sources, with all the necessary associated biological data being relatively easy to obtain. However, the sex and age composition of the sample might also be biased when compared to the actual population.

The possible biases indicated afore might hold for our samples. About three quarter of the porpoises analysed were emaciated, many of them severely. About two third of the animals had died of emaciation, sometimes in combination with infectious diseases and lung oedema. Approximately one third of the animals were diagnosed as by-catch, either as definite by-catch or possible/probable by-catch. These animals showed no signs of infections or emaciation. With their fast metabolism, porpoises suffer from emaciation relatively fast (Worthy & Edwards 1990; Kastelein et al. 1997c), possibly resulting in (near) empty stomach samples and tissue composition that may be affected by emaciation. The effect of emaciation, decomposition and cause of death are important to understand and account for in order to use stranded cetaceans as a source for dietary information. Continuous stranding programs, thorough pathological examination, collection of samples and consequent documentation are essential in order to use stranded animals for scientific investigations and subsequently interpret analyses correctly.

## 8.7 THE VALUE OF USING MULTI-METHOD DIETARY ANALYSES

There are several techniques to analyse the diet of marine mammals. Most traditionally, the diet is deduced from stomach contents, identifying non-digested prey remains (Hyslop 1980; Barros & Clarke 2009). Indirect methods have been developed, including stable isotope analysis and fatty acid analysis, based on the principle that isotopic composition and fatty acid signatures of prey are reflected in the predator (Thiemann et al. 2008; Newsome et al. 2010). The combination of several techniques has become more common, but combining these three techniques has only been done a few times before (Hooker et al. 2001; Karnovsky et al. 2008), but not in small cetaceans. By itself, each technique has certain advantages, disadvantages and biases. Each technique integrates the signature of prey ingestion over a different time period.

Stomach content analysis is based on the identification of undigested hard prey remains e.g. otoliths, vertebrae and squid beaks. These can be used to identify species, and to calculate the number, weight and caloric contribution of each individual prey, giving the most concrete and detailed information on ingested prey. Due to the fast passage time of food through the animal, stomach contents give dietary information from a short period before the animal died, and thus generally not far from the place of stranding. Other biases associated with stomach content analysis are i.a. misidentification, species-specific retention and degradation and partial or secondary ingestion (Pierce & Boyle 1991; Cottrell et al. 1996; Tollit et al. 2003).

Stable isotope analysis has the advantage that it reflects the assimilated diet over time, providing insight in feeding ecology over longer time-periods. Depending on the turnover time of the tissue analysed it minimizes small-scale temporal and geographic variation (Kelly 2000; Crawford et al. 2008; Newsome et al. 2010). Due to predictable enrichment in isotopic values between the predator and prey, stable isotope analysis yields information of trophic level and feeding origin of the predator (Hobson 1999; Post 2002; Michener & Kaufman 2007; Barnes et al. 2009). The use of isotopic mixing models allows the estimation of the proportional contribution of prey to the assimilated diet of the predator (Crawford et al. 2008; Wolf et al. 2009; Parnell et al. 2010). Age, type of diet, nutritional status, environment and taxonomical position have shown to influence trophic fractionation (Minagawa & Wada 1984; McCutchan et al. 2003; Vanderklift & Ponsard 2003). The quantitative interpretation of isotopic signatures using mixing models depends on the number of isotopes included in the model, knowledge of species-specific trophic enrichment factors and the appropriate representation of prey sources (Gannes et al. 1997; Parnell et al. 2010; Bond & Diamond 2011).

Fatty acids also reflect the assimilated diet over a period of up to several months and have therefore less temporal and spatial limitations compared to stomach contents analysis (Iverson et al. 1997; Iverson et al. 2004; Beck et al. 2005; Budge et al. 2006). Using Quantitative Fatty Acid Signature Analysis (QFASA) allows the quantitative estimation of the proportional contribution of prey to the fatty acid signature of the predator (Iverson et al. 2004). Reliable quantitative use of this method for marine mammals depends on the appropriate representation of prey species and knowledge on the effects of lipid metabolism within the specific predator (Iverson et al. 2004; Thiemann et al. 2004b).

Combining these three methods offers the opportunity to analyse changes in diet over time and limitations of each technique can be compensated by the others. We have therefore used the combination of all three techniques, stomach content-, stable isotope- and fatty acid analysis to examine the feeding ecology of porpoises in Dutch coastal waters. An overview of the results from the different methods are given in Table 8.3. Within the limitations of the three methods as described in earlier chapters, some general conclusions can be drawn from this table. Firstly, no one method covers all species found in the short term as well as longer term diet. Secondly, gobies, sprat, herring, cod and lesser sandeel were all found as the most important prey species in both the short term and longer term diet. Thirdly, whiting was only found to be an important prey species in the short term diet, whereas mackerel, smelt and greater sandeel only appeared to be important prey species in the longer term diet. In all likelihood, weaned young porpoises start preying on small prey species such as gobies, small flatfish and shrimp before preying on larger species (Santos & Pierce 2003). Stable isotope analysis identified suckling in neonates and suggested that young

Table 8.3 *The six prey species that contribute most to the diet of porpoises analysed in this study, based on stomach content, fatty acid and stable isotope analysis.*

Analysis	Stomach content analysis	Fatty acid analysis (QFASA)	Stable isotope analysis (SIAR)
Time frame	short term days	mid term several months	mid term several months
Tissue	Stomachs	Blubber	Muscle
Sample n	76	73	90
Species	Gobies	Gobies	Poor cod
	Whiting	Mackerel	Mackerel
	Lesser sandeel	Smelt	Greater sandeel
	Herring	Herring	Lesser sandeel
	Cod	Dragonet	Sprat
	Sprat	Cod	Gobies
Total	84%	86%	70-83%

porpoises stay more in coastal waters, feeding mainly on small abundant prey species, i.e. gobies (Jansen et al. 2012a,b; Chapter 2 and 3). This is confirmed by other studies based on stomach contents. These have shown smaller, more benthic, coastal prey in young porpoises (e.g. shrimp, small fish and squid) compared to larger more pelagic, offshore prey in adult porpoises (e.g. gadoids and flatfish) (Smith & Read 1992; Börjesson et al. 2003; Santos et al. 2004; Jansen et al. 2012a,b; Chapter 2 and 3).

Stable isotope analyses also enabled to detect trophic sexual segregation in feeding of porpoises (Das et al. 2004) and for other marine mammal species (Hobson 1999, Lesage et al. 2001). Similarly, our stable isotope analyses indicated sexual segregation at maturity (Jansen et al. 2012a, Chapter 2), where females fed more coastal at a relatively higher trophic level compared to males. This segregation of mature animals is supported by Smith & Gaskin (1983), who proposed adult males migrate offshore to feed, while adult females stay with their young closer to the shore. Stomach contents analysis has shown that female porpoises preyed on comparably more small gobies, while male porpoises fed on relatively larger gadoids (Santos & Pierce 2003). This is surprising, as the high consumption need of lactating females should result in feeding at larger, energy-rich prey species (Aarefjord et al. 1995).

Stable isotope analysis also revealed that porpoises stranded in summer had used a different habitat compared to porpoises stranded in winter. This is confirmed by genetic analyses, which found that male porpoises, stranded in winter had migrated from neighbouring regions, most probably from British and Danish coastal waters (Andersen et al. 2001). In contrast, porpoises stranded in summer are considered to be part of the south-eastern North Sea population (Yurick & Gaskin 1987, Walton 1997).

Another example of the extra value of including stable isotope analysis lies outside its direct contribution to dietary studies. Only stable isotope analysis identified porpoises stranded within the Eastern Scheldt tidal bay based on their distinct isotopic composition. It also showed that these porpoises were not born there (Jansen et al. in press, Chapter 4). This specific group and the ecological significance of this finding would have been missed by analysing only stomach contents or fatty acids.

Fatty acid analysis (QFASA) revealed that the longer term diet consisted mainly of gobies, mackerel, smelt, herring and dragonet. The estimated relative contribution of the prey species differed, depending on the model parameters used, but showed in all cases profound differences to the diet as deduced from stomach contents of the same individuals. For some species (e.g. gobies, lesser sandeel and sprat), these differences reflect the bias of stomach content analysis towards coastal species. A higher importance of pelagic schooling fish (i.e. mackerel, scad and herring) in the diet as estimated by QFASA could be explained by differences between the hunting behaviour of porpoises and the schooling

behaviour of fish between coastal and offshore waters. Whiting, although possibly overestimated in stomach contents was not identified using QFASA, suggesting that the diet of porpoises as estimated by QFASA in our study has to be cautiously interpreted (Chapter 5).

Our study shows that none of the methods is essentially better than the others as they reflect different time periods and provide different specific dietary information. Combining techniques thus resulted in greater insight into the feeding ecology of porpoises in Dutch coastal waters than could have been inferred by using only one technique alone.

Porpoises and white-beaked dolphins are impacted by increasing anthropogenic influences such as pollution, habitat destruction, underwater noise and by-catch in fisheries. As top predators, they integrate changes lower in the food chain, making them potential indicators for ecosystem changes and ecosystem health. Close monitoring of their abundance and distribution, complemented by studies on feeding ecology aimed at assessing changes in their foodbase, render harbour porpoises a suitable sentinel species. It is recommended to include such investigations in the activities fulfilling the reporting obligations contained in (inter) national management instruments of the North Sea such as the Habitats Directive and OSPAR. That would bring the regular assessment of the state of the environment at a higher level and address ecosystem functioning and services.

Another recommendation for extending management strategies lies in transferring the information found in this study on blockage of migratory routes by coastal protection structures. Given the world-wide emerging and increasing potential effects of coastal protection measures, production platforms and wind-farms, the minimisation and mitigation of degradation, fragmentation and loss of habitat for marine mammals should have a more prominent place in Environmental Impact Assessment studies, prior to construction.

## 8.8 FUTURE PERSPECTIVES

The interpretation of results from dietary studies is inevitably limited by a lack of knowledge in species-specific parameters for porpoises, e.g. digestion rates, turnover rates of tissues, tissue-dependent isotopic fractionation between predator and prey and lipid metabolism within the animal. It is common practice to substitute these by parameters that have been established from controlled feeding experiments with other species. Including these proxies, however, makes it difficult to examine whether differences between the methods are actual temporal or spatial changes in the feeding ecology of porpoises or methodological artefacts. Large improvement in the diet analysis of marine mammals thus lies in the development of species-specific parameters (e.g. digestion rates, turnover times, isotopic fractionation, lipid metabolism, etc.) that are neces-



sary for each technique. These can in principle only be established by controlled feeding experiments with animals in captivity. These experiments, however, are difficult to conduct in marine mammal species due to considerable legal (e.g. the protected status of most marine mammals), ethical (e.g. confinement and husbandry conditions), logistic (e.g. catching animals) and financial considerations and restrictions. Studies on other species or on chemical processes (e.g. isotopic fractionation, lipid metabolism) that may help to understand the general patterns of these parameters and can be extrapolated to marine mammal species, are potential solutions to avoid numerous controlled feeding experiments and can greatly improve the interpretation of the results of diet analyses.

# ADDENDUM





## REFERENCES

- Aarefjord, H., A. Bjørge, C.C. Kinze, and I. Lindstedt. 1995. Diet of harbour porpoise (*Phocoena phocoena*) in Scandinavian waters. Report of the International Whaling Commission 16:211-222.
- Aberle, N., and A.M. Malzahn. 2007. Interspecific and nutrient dependent variations in stable isotope fractionation: experimental studies simulating pelagic multitrophic systems. *Oecologia* 154:291-303.
- Addink, M.J., M. García Hartman, and C. Smeenk. 1995. The harbour porpoise *Phocoena phocoena* in Dutch waters: life history, pathology and historical records. International Whaling Commission, Scientific Committee Document SC/47/SM5:1-8.
- Addink, M.J., Smeenk, C. 1999. The harbour porpoise *Phocoena phocoena* in Dutch coastal waters: analysis of stranding records for the period 1920-1994. *Lutra* 41:55-80.
- Ambrose, S.H. 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. *Journal of Archaeological Science* 17:431-451.
- Amundsen, P.A., H.M. Gabler, and F.J. Staldvik. 1996. A new approach to graphical analysis of feeding strategy from stomach contents data - modification of the Costello (1990) method. *Journal of Fish Biology* 48:607-614.
- Andersen, L.W., D.E. Ruzzante, M. Walton, P. Berggren, A. Bjørge, and C. Lockyer. 2001. Conservation genetics of harbour porpoises, *Phocoena phocoena*, in eastern central North Atlantic. *Conservation Genetics* 2:309-324.
- Andersen, N.G. 1999. The effect of predator size, temperature, and prey characteristics on gastric evacuation in whiting. *Journal of Fish Biology* 54:287-301.
- Anstenrud, M., and T.A. Schram. 1988. Host and site selection by larval stages of the parasitic copepod *Lernaenicus sprattae* (Sowerby) (Copepoda, Pennellidae) in the Oslofjord. *Hydrobiologia* 167/168:587-595.
- Archer, F.I., and K.M. Robertson. 2004. Age and length at weaning and development of diet of pantropical spotted dolphins, *Stenella attenuata*, from the eastern tropical Pacific. *Marine Mammal Science* 20:232-245.
- Arnett, R.T.P., and J. Whelan. 2001. Comparing the diet of cod (*Gadus morhua*) and grey seals (*Halichoerus grypus*): an investigation of secondary ingestion. *Journal of the Marine Biological Association of the United Kingdom* 81:365-366.
- Aznar, F.J., P. Fognani, J.A. Balbuena, M. Pietrobelli, and J.A. Raga. 2006. Distribution of *Pholeter gastrophilus* (Digenea) within the stomach of four odontocete species: the role of the diet and digestive physiology of hosts. *Parasitology* 133:369-380.
- Bannister, R.C.A. 2004. The rise and fall of cod (*Gadus morhua* L.) in the North Sea. In A.I.L. Payne, C.M. O'Brien, and S.I. Rogers, (eds). *Management of Shared Fish Stocks*. Blackwell, Oxford. 316-338.
- Barnes, C., S. Jennings, and J.T. Barry. 2009. Environmental correlates of large-scale spatial variation in the  $\delta^{13}\text{C}$  of marine animals. *Estuarine Coastal and Shelf Science* 81:368-374.



- Barros, N.B., and M.R. Clarke. 2009. Diet. In W.F. Perrin, B. Würsig, and J.G.M. Theewissen, (eds). *Encyclopedia of Marine Mammals*. Academic Press, Burlington/San-Diego/New York/London. 311-316.
- Bartlett, M.S. 1937. Properties of insufficiency and statistical tests. *Proceedings of the Royal Society of London Series A* 160:268-282.
- Beck, C.A., S.J. Iverson, and W.D. Bowen. 2005. Blubber fatty acids of gray seals reveal sex differences in the diet of a size-dimorphic marine carnivore. *Canadian Journal of Zoology* 83:377-388.
- Beerman, A. 2010. The diet of harbour porpoises (*Phocoena phocoena*) in the Dutch North Sea, with special reference to the prey family Gobiidae. MSc thesis. Wageningen University and IMARES-Texel, The Netherlands.
- Benke, H., U. Siebert, R. Lick, B. Bandomir, and R. Weiss. 1998. The current status of harbour porpoises (*Phocoena phocoena*) in German waters. *Archive of Fishery and Marine Research* 46:97-123.
- Berggren, P., P.R. Wade, J. Carlström, and A.J. Read. 2002. Potential limits to anthropogenic mortality for harbour porpoises in the Baltic region. *Biological Conservation* 103:313-322.
- Berrow, S.D., and E. Rogan. 1996. Stomach contents of harbour porpoises and dolphins in Irish waters. *European Research on Cetaceans* 9:179-181.
- Bjørge, A., and K.A. Tolley. 2009. Harbour porpoise *Phocoena phocoena*. In W.F. Perrin, B. Würsig, and J.G.M. Theewissen, (eds). *Encyclopedia of Marine Mammals*. Academic Press, Burlington/SanDiego/New York/London.
- Bond, A.L., and A.W. Diamond. 2011. Recent Bayesian stable-isotope models are highly sensitive to variation in discrimination factors. *Ecological Applications* 21:1017-1023.
- Boran, J.R., and S.L. Heimlich. 1999. Social learning in cetaceans: hunting, hearing and hierarchies. In H.O. Box, and K.R. Gibson, (eds). *Mammalian social learning. Comparative and ecological perspectives*. Cambridge University Press, Cambridge. 282-307.
- Börjesson, P., P. Berggren, and B. Ganning. 2003. Diet of harbor porpoises in the Kattegat and Skagerrak seas: accounting for individual variation and sample size. *Marine Mammal Science* 19:38-58.
- Bosello, F., R.J. Nicholls, R. J., R. Roson, and R.S.J. Tol. 2012. Economic impacts of climate change in Europe: sea level rise. *Climatic Change* 112:63-81.
- Bossart, G.D. 2011. Marine mammals as sentinel species for oceans and human health. *Veterinary Pathology* 48:676-690.
- Bowen, W.D. 2000. Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. *Canadian Journal of Fisheries and Aquatic Sciences* 57:898-905.
- Bowen, W.D., and G.D. Harrison. 1996. Comparison of harbour seal diets in two in-shore habitats of Atlantic Canada. *Canadian Journal of Zoology* 74:125-135.

## REFERENCES

- Brodie, P.F. 1995. The Bay of Fundy/Gulf of Maine harbour porpoise (*Phocoena phocoena*): some considerations regarding species interactions, energetics, density dependence and bycatch. Report of the International Whaling Commission:181-187.
- Brooker, A.J., A.P. Shinn, and J.E. Bron. 2007. A review of the biology of the parasitic copepod *Lernaecera branchialis* (L., 1767) (Copepoda: Pennellidae). *Advances in Parasitology* 65:297-341.
- Budge, S.M., S.J. Iverson, W.D. Bowen, and R.G. Ackman. 2002. Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences* 59:886-898.
- Budge, S.M., S.J. Iverson, and H.N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Marine Mammal Science* 22:759-801.
- Budge, S.M., S.N. Penney, and S.P. Lall. 2012. Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses; validation with controlled feeding studies. *Canadian Journal of Fisheries and Aquatic Sciences* 69:1033-1046.
- Budge, S.M., A.M. Springer, S.J. Iverson, and G. Sheffield. 2007. Fatty acid biomarkers reveal niche separation in an Arctic benthic food web. *Marine Ecology Progress Series* 336:305-309.
- Burnham, K.P., and D.R. Anderson 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer Verlag.
- Camphuysen, C.J. 2004. The return of the harbour porpoise (*Phocoena phocoena*) in Dutch coastal waters. *Lutra* 47:113-122.
- Camphuysen, C.J. 2011. Recent trends and spatial patterns in nearshore sightings of harbour porpoises (*Phocoena phocoena*) in the Netherlands (Southern Bight, North Sea), 1990-2010. *Lutra* 54:37-44.
- Camphuysen, C.J., and A. Krop. 2011. Maternal care, calf training and site fidelity in a wild harbour porpoise in the North Sea. *Lutra* 54:123-126.
- Camphuysen, C.J., and G. Peet 2006. Whales and dolphins of the North Sea. Fontaine Uitgevers, 's-Graveland.
- Camphuysen, C.J., C. Smeenk, M. Addink, H. Van Grouw, and O.E. Jansen. 2008. Cetaceans stranded in the Netherlands from 1998 to 2007. *Lutra* 51:87-122.
- Canning, S.J., M.B. Santos, R.J. Reid, P.G.H. Evans, R.C. Sabin, N. Bailey, and G.J. Pierce. 2008. Seasonal distribution of white-beaked dolphins (*Lagenorhynchus albirostris*) in UK waters with new information on diet and habitat use. *Journal of the Marine Biological Association of the United Kingdom* 88:1159-1166.
- Caut, S., E. Angulo, and F. Courchamp. 2008. Caution on isotopic model use for analyses of consumer diet. *Canadian Journal of Zoology* 86:438-445.
- Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors ( $\Delta^{15}\text{N}$  and  $\Delta^{13}\text{C}$ ): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology* 46:443-453.



- Caut, S., S. Laran, E. García-Hartmann, and K. Das. 2011. Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). *Journal of Experimental Biology* 214:538-545.
- Chauvenet, W. 1863. *A manual of spherical and practical astronomy*, Dover/New York.
- Christensen, J.T., and K. Richardson. 2008. Stable isotope evidence of long-term changes in the North Sea food web structure. *Marine Ecology Progress Series* 368:1-8.
- Christensen, V., and D. Pauly. 1998. Changes in models of aquatic ecosystems approaching carrying capacity. *Ecological Applications* 8:S104-S109.
- Clarke, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18:117-143.
- Clarke, K.R., and R.N. Gorley. 2006. *PRIMER v6.1.13: Multivariate statistics for ecologists*, Plymouth, UK: PRIMER-E.
- Clarke, M.R. 1986. *A handbook for the identification of cephalopod beaks*. Clarendon Press, Oxford.
- Clementz, M.T., and P.L. Koch. 2001. Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. *Oecologia* 129:461-472.
- Cockroft, V.G., and G.J.B. Ross. 1990. Food and feeding of the Indian Ocean bottlenose off southern Natal, South Africa. In S. Leatherwood, and R.R. Reeves, (eds). *The bottlenose dolphin*. Academic Press, San Diego. 295-308.
- Cook, R.M., A. Sinclair, and G. Stefánsson. 1997. Potential collapse of North Sea cod stocks. *Nature* 385:521-522.
- Coplen, T.B. 2011. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry* 25:2538-2560.
- Costello, M.J. 1990. Predator feeding strategy and prey importance: a new graphical analysis. *Journal of Fish Biology* 36:261-263.
- Cottrell, P.E., and A.W. Trites. 2002. Classifying prey hard part structures recovered from fecal remains of captive Steller sea lions (*Eumetopias jubatus*). *Marine Mammal Science* 18:525-539.
- Cottrell, P.E., A.W. Trites, and E.H. Miller. 1996. Assessing the use of hard parts in faeces to identify harbour seal prey: results of captive-feeding trials. *Canadian Journal of Zoology* 74:875-880.
- Coull, K.A., A.S. Jermyn, A.W. Newton, G.I. Henderson, and W.B. Hall. 1989. Length/weight relationships for 88 species of fish encountered in the North East Atlantic. *Scottish Fisheries Research Report*:81pp.
- Crawford, K., R.A. McDonald, and S. Bearhop. 2008. Applications of stable isotope techniques to the ecology of mammals. *Mammal Review* 38:87-107.
- Cullen, J.T., Y. Rosenthal, and P.G. Falkowski. 2001. The effect of anthropogenic CO<sub>2</sub> on the carbon isotope composition of marine phytoplankton. *Limnology and Oceanography* 46:996-998.

## REFERENCES

- [cvo.wur.nl](http://www.cvo.wur.nl/). 2009. Wageningen UR. January 2012. <http://www.cvo.wur.nl/>.
- da Silva, J., and J.D. Neilson. 1985. Limitations of using otoliths recovered in scats to estimate prey consumption in seals. *Canadian Journal of Fisheries and Aquatic Sciences* 42:1439-1446.
- Daan, N., P.J. Bromley, J.R.G. Hislop, and N.A. Nielsen. 1990. Ecology of North Sea fish. *Netherlands Journal of Sea Research* 26:343-386.
- Dalerum, F., and A. Angerbjörn. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia* 144:647-658.
- Das, K., L. Holsbeek, J. Browning, U. Siebert, A. Birkun, and J.M. Bouqueneau. 2004. Trace metal and stable isotope measurements ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in the harbour porpoise *Phocoena phocoena relicta* from the Black Sea. *Environmental Pollution* 131:197-204.
- Das, K., G. Lepoint, Y. Leroy, and J.M. Bouqueneau. 2003. Marine mammals from the southern North Sea: feeding ecology data from  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements. *Marine Ecology Progress Series* 263:287-298.
- de Pierrepont, J.F., B. Dubois, S. Desormonts, M.B. Santos, and J.P. Robin. 2005. Stomach contents of English Channel cetaceans stranded on the coast of Normandy. *Journal of the Marine Biological Association of the United Kingdom* 85:1539-1546.
- de Ruijter, E., and A. Schoenmaker 1989. Zeeboek. Jeugdbondsuitgeverij JBU and Stichting Uitgeverij KNNV, Utrecht.
- de Smet, W.M.A., R. Asselberg, and R. Duyndam. 1985. Gegevens over een witsnuitdolfijn, *Lagenorhynchus albirostris*, uit de zuidelijke Noordzee. *Marswin* 6:3-19.
- DeNiro, M.J., and S. Epstein. 1978. Influence of diet on distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42:495-506.
- DeNiro, M.J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:341-351.
- Dong, J.H., J. Lien, D.E. Nelson, and K. Curren. 1996. A contribution to the biology of the white-beaked dolphin, *Lagenorhynchus albirostris*, in waters off Newfoundland. *Canadian Field Naturalist* 110:278-287.
- Doornbos, G. 1984. Piscivorous birds on the saline lake Grevelingen, The Netherlands: abundance, prey selection and annual food consumption. *Netherlands Journal of Sea Research* 18:457-497.
- Eisma, D., and J. Kalf. 1979. Distribution and particle size of suspended matter in the southern bight of the North Sea and the eastern Channel. *Netherlands Journal of Sea Research* 13:298-324.
- Evans, P.G.H. 1987. The natural history of whales & dolphins. Christopher Helm, London.
- Evans, P.G.H., and C. Smeenk. 2008. Genus *Lagenorhynchus*. In S. Harris, and D.W. Yalden, (eds). *Mammals of the British Isles. Handbook*. The Mammalian Society, New Road, Southampton. 724-727.
- Fettweis, M., and D. van den Ende. 2003. The mud deposits and the high turbidity in the Belgian-Dutch coastal zone, southern bight of the North Sea. *Continental Shelf Research* 23:669-691.

- Folch, J., M. Lees, and G.H.S. Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* 226:497-509.
- Fonds, M. 1973. Sand gobies in the Dutch wadden Sea (*Pomatoschistus*, Gobiidae, Pisces). *Netherlands Journal of Sea Research* 6:417-478.
- Fontaine, M.C., K.A. Tolley, U. Siebert, S. Gobert, G. Lepoint, J. Bouquegneau, and K. Das. 2007. Long-term feeding ecology and habitat use in harbour porpoises *Phocoena phocoena* in Scandinavian waters inferred from trace elements and stable isotopes. *BMC Ecology* 7:1-12.
- Frederiksen, M., S. Wanless, M.P. Harris, P. Rothery, and L.J. Wilson. 2004. The role of industrial fisheries and oceanographic change in the decline of North Sea black-legged kittiwakes. *Journal of Applied Ecology* 41:1129-1139.
- Furness, R.W., and M.L. Tasker. 2000. Seabird-fishery interactions: quantifying the sensitivity of seabirds to reductions in sandeel abundance, and identification of key areas for sensitive seabirds in the North Sea. *Marine Ecology Progress Series* 202:253-264.
- Galatius, A., O.E. Jansen, and C.C. Kinze. in press. Parameters of growth and reproduction of white-beaked dolphins (*Lagenorhynchus albirostris*) from the North Sea. *Marine Mammal Science*. DOI: 10.1111/j.1748-7692.2012.00568.x
- Gannes, L.Z., D.M. O'Brien, and C.M. Del Rio. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78:1271-1276.
- Gannon, D.P., J.E. Craddock, and A.J. Read. 1998. Autumn food habits of harbor porpoises, *Phocoena phocoena*, in the Gulf of Maine. *Fishery Bulletin* 96:428-437.
- Gaskin, D.E. 1984. The harbour porpoise *Phocoena phocoena* (L.): regional populations, status, and information on direct and indirect catches. Report of the International Whaling Commission 34:569-586.
- Geelhoed, S., M. Scheidat, G. Aarts, R. van Bemmelen, N. Janinhoff, H. Verdaat, and R. Witte. 2011. Shortlist Masterplan Wind Aerial surveys of harbour porpoises on the Dutch Continental Shelf. Report C103/11, IMARES, Wageningen UR.
- Gilles, A. 2008. Characterisation of harbour porpoises (*Phocoena phocoena*) habitat in German waters. PhD thesis. Forschungs- und Technologiezentrum Westküste. Christian-Albrecht-Universität Kiel, Kiel.
- Glass, C.W., C.S. Wardle, and W.R. Mojsiewicz. 1986. A light intensity threshold for schooling in the Atlantic mackerel, *Scomber scombrus*. *Journal of Fish Biology* 29:71-81.
- Gómez-Campos, E. 2011. Nitrogen and carbon stable isotopes do not reflect nutritional condition in the striped dolphin. *Rapid Communications in Mass Spectrometry* 25:1343-1347.
- Granadeiro, J.P., and M.A. Silva. 2000. The use of otoliths and vertebrae in the identification and size-estimation of fish in predator-prey studies. *Cybiurn* 24:383-393.

## REFERENCES

- Grellier, K., and P.S. Hammond. 2006. Robust digestion and passage rate estimates for hard parts of grey seal (*Halichoerus grypus*) prey. *Canadian Journal of Fisheries and Aquatic Sciences* 63:1982-1998.
- Groenenberg, D.S.J., J. Goud, A. de Heij, and E. Gittenberger. 2009. Molecular phylogeny of North Sea Sepiolidae (Cephalopoda: Sepiolidae) reveals an overlooked *Sepiola* species. *Journal of Molluscan Studies* 975:361-369.
- Groenewold, S., R. Berghahn, and C.-D. Zander. 1996. Parasite communities of four fish species in the Wadden Sea and the role of fish discarded by the shrimp fisheries in parasite transmission. *Helgoländer Meeresuntersuchungen* 50:69-85.
- Habran, S., C. Debier, D.E. Crocker, D.S. Houser, G. Lepoint, J.M. Bouquegneau, and K. Das. 2010. Assessment of gestation, lactation and fasting on stable isotope ratios in northern elephant seals. *Marine Mammal Science* 26:880-895.
- Hallegatte, S. 2012. A framework to investigate the economic growth impact of sea level rise. *Environmental Research Letters* 7.
- Hamerlynck, O., and K. Hostens. 1993. Growth, feeding, production and consumption in 0-group bib (*Trisopterus luscus* L.) and whiting (*Merlangius merlangus* L.) in a shallow coastal area of the south-west Netherlands. *Ices Journal of Marine Science* 50:81-91.
- Hammond, P.S. 2006. Small Cetaceans in the European Atlantic and North Sea (SCANS-II). Page 54 pp. Sea Mammal Research Unit, St Andrews.
- Hammond, P.S., P. Berggren, H. Benke, D.L. Borchers, A. Collet, M.P. Heide-Jørgensen, S. Heimlich, A.R. Hiby, M.F. Leopold, and N. Øien. 2002. Abundance of harbour porpoise and other cetaceans in the North Sea and adjacent waters. *Journal of Applied Ecology* 39:361-376.
- Hammond, P.S., and M.A. Fedak 1994. Grey seals in the North Sea and their interactions with fisheries. Final Report to the Ministry of Agriculture, Fisheries and Food, contract MF0503, SMRU & NERC, Cambridge.
- Härkönen, T. 1986. Guide to the otoliths of the bony fishes of the Northeast Atlantic. Danbiu ApS, Hellerup.
- Harrod, C., J. Grey, T.K. McCarthy, and M. Morrissey. 2005. Stable isotope analyses provide new insights into ecological plasticity in a mixohaline population of European eel. *Oecologia* 144:673-683.
- Harwood, J. 2001. Marine mammals and their environment in the twenty-first century. *Journal of Mammalogy* 82:630-640.
- Hastie, T., and R. Tibshirani. 1987. Generalized additive models: some applications. *Journal of the American Statistical Association* 82:371-386.
- Hedges, R.E.M. 2003. On bone collagen - apatite-carbonate isotopic relationships. *International Journal of Osteoarchaeology* 13:66-79.
- Herzing, D.L. 1997. The life-history of free-ranging Atlantic spotted dolphins (*Stenella frontalis*): age classes, color phases, and female reproduction. *Marine Mammal Science* 13:576-595.

- Hislop, J.R.G. 1996. Changes in North Sea gadoid stocks. *ICES Journal of Marine Science* 53:1146-1156.
- Hislop, J.R.G., A.P. Robb, M.A. Bell, and D.W. Armstrong. 1991. The diet and food consumption of whiting (*Merlangius merlangus*) in the North Sea. *ICES Journal of Marine Science* 48:139-156.
- Hobson, K.A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120:314-326.
- Hobson, K.A., and R.G. Clark. 1992a. Assessing avian diets using stable isotopes 1: turnover of  $^{13}\text{C}$  in tissues. *The Condor* 94:181-188.
- Hobson, K.A., and R.G. Clark. 1992b. Assessing avian diets using stable isotopes 2: factors influencing diet-tissue fractionation. *The Condor* 94:189-197.
- Hobson, K.A., D.M. Schell, D. Renouf, and E. Noseworthy. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Canadian Journal of Fisheries and Aquatic Sciences* 53:528-533.
- Hooker, S.K., S.J. Iverson, P. Ostrom, and S.C. Smith. 2001. Diet of northern bottle-nose whales inferred from fatty-acid and stable-isotope analyses of biopsy samples. *Canadian Journal of Zoology* 79:1442-1454.
- Husson, A.M., and P.J.H. van Bree. 1972. Strandingen van Cetacea op de Nederlandse kust in 1970 en 1971. *Lutra* 14:1-5.
- Husson, A.M., and P.J.H. van Bree. 1976. Strandingen van Cetacea op de Nederlandse kust in 1974 en 1975. *Lutra* 18:25-32.
- Hyslop, E.J. 1980. Stomach contents analysis - a review of methods and their application. *Journal of Fish Biology* 17:411-429.
- ICES. 2006. Working Group on the Assessment of Demersal Stocks in the North Sea and Skagerrak. Pages 1-981.
- IPCC. 1990. Strategies for adaption to sea level rise. Page 122. Report of the Coastal Management Subgroup. IPCC Working Group III, Rijkswaterstaat, The Netherlands.
- Iverson, S. 2008. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In M.T. Arts, (ed.) *Lipids in Aquatic Ecosystems*. Springer Science + Business Media, LLC.
- Iverson, S.J., J.P.Y. Arnould, and I.L. Boyd. 1997. Milk fatty acid signatures indicate both major and minor shifts in the diet of lactating Antarctic fur seals. *Canadian Journal of Zoology* 75:188-197.
- Iverson, S.J., C. Field, W.D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological Monographs* 74:211-235.
- Iverson, S.J., K.J. Frost, and S.L.C. Lang. 2002. Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. *Marine Ecology Progress Series* 241:161-181.

## REFERENCES

- Jacob, U., K. Mintenbeck, T. Brey, R. Knust, and K. Beyer. 2005. Stable isotope food web studies: a case for standardized sample treatment. *Marine Ecology Progress Series* 287:251-253.
- Jansen, O.E. 2006. Ecological aspects of white-beaked dolphins, digestive tract contents analysis and age determination in teeth. Msc Thesis. Experimental Animal Sciences. Leiden University, Leiden.
- Jansen, O.E., G. Aarts, M.F. Leopold, and P.J.H. Reijnders. in press. Harbour porpoises *Phocoena phocoena* in the Eastern Scheldt: a resident stock or trapped by a storm surge barrier? PLOS ONE.
- Jansen, O.E., G.M. Aarts, K. Das, G. Lepoint, L. Michel, and P.J.H. Reijnders. 2012a. Feeding ecology of harbour porpoises: Stable isotope analysis of carbon and nitrogen in muscle and bone. *Marine Biology Research* 8:829-841.
- Jansen, O.E., M.F. Leopold, E.H.W.G. Meesters, and C. Smeenk. 2010. Are white-beaked dolphins *Lagenorhynchus albirostris* food specialists? Their diet in the southern North Sea. *Journal of the Marine Biological Association of the United Kingdom* 90:1501-1508.
- Jansen, O.E., E.H.W.G. Meesters, S.M.J.M. Brasseur, S.M. Budge, and P.J.H. Reijnders. in prep. The diet of harbour porpoises along the Dutch coast based on QFASA analysis: a combined fatty acid and stomach contents approach.
- Jansen, O.E., L. Michel, G. Lepoint, K. Das, A.S. Couperus, and P.J.H. Reijnders. 2012b. Diet of harbor porpoises along the Dutch coast: a combined stable isotope and stomach contents approach *Marine Mammal Science*. DOI: 10.1111/j.1748-7692.2012.00621.x
- Jenkins, S.G., S.T. Partridge, T.R. Stephenson, S.D. Farley, and C.T. Robbins. 2001. Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. *Oecologia* 126:336-341.
- Jobling, M., and A. Breiby. 1986. The use and abuse of fish otoliths in studies of feeding habits of marine piscivores. *Sarsia* 71:265-274.
- Johnston, D.W., A.J. Westgate, and A.J. Read. 2005. Effects of fine-scale oceanographic features on the distribution and movements of harbour porpoises *Phocoena phocoena* in the Bay of Fundy. *Marine Ecology Progress Series* 295:279-293.
- Kabat, P., W. van Vierssen, J. Veraart, P. Vellinga, and J. Aerts. 2005. Climate proofing the Netherlands. *Nature* 438:283-284.
- Karnovsky, N.J., K.A. Hobson, S. Iverson, and G.L. Hunt. 2008. Seasonal changes in diets of seabirds in the North Waters Polynya: a multiple-indicator approach. *Marine Ecology Progress Series* 357:291-299.
- Kastelein, R.A., J. Hardeman, and H. Boer. 1997a. Food consumption and body weight of harbour porpoises (*Phocoena phocoena*). In A.J. Read, P.R. Wiepkema, and P.E. Nachtigall, (eds). *The biology of the harbour porpoise*. De Spil Publishers, Woerden. 217-233.
- Kastelein, R.A., J. Hardeman, and H. Boer. 1997b. A method for tube-feeding juvenile harbour porpoises (*Phocoena phocoena*). In A.J. Read, P.R. Wiepkema, and P.E. Nachtigall, (eds). *The biology of the harbour porpoise*. De Spil Publishers, Woerden. 63-83.



- Kastelein, R.A., S.J. van der Sijs, C. Staal, and S.H. Nieuwstraten. 1997c. Blubber thickness in harbour porpoises (*Phocoena phocoena*) In A.J. Read, P.R. Wiepkema, and P.E. Nachtigall, (eds). The biology of the harbour porpoise. De Spil Publishers, Woerden. 179-199.
- Kavanagh, A.S., M.A. Cronin, M. Walton, and E. Rogan. 2010. Diet of the harbour seal (*Phoca vitulina vitulina*) in the west and south-west of Ireland. Journal of the Marine Biological Association of the United Kingdom 90:1517-1527.
- Kelly, J.F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1-27.
- Kinze, C.C. 1995. Danish whale records 1575-1991 (Mammalia, Cetacea). Review of whale specimens stranded, directly or incidentally caught along the Danish coast. Steenstrupia 21:155-196.
- Kinze, C.C. 2009. White-beaked dolphin *Lagenorhynchus albirostris*. In W.F. Perrin, B. Würsig, and J.G.M. Thewissen, (eds). Encyclopedia of Marine Mammals. Academic Press, Burlington/SanDiego/New York/London. 1255-1258.
- Kinze, C.C., M. Addink, C. Smeenk, M. García Hartman, H.W. Richards, R.P. Sonntag, and H. Benke. 1997. The white-beaked dolphin (*Lagenorhynchus albirostris*) and the white-sided dolphin (*Lagenorhynchus acutus*) in the North and Baltic Seas: review of available information. Report of the International Whaling Commission 47:675-681.
- Knijn, R.J., T.W. Boon, H.J.L. Heessen, and J.R.G. Hislop. 1993. Atlas of North Sea Fishes Based on bottom-trawl survey data for the years 1985-1987. Pages 1-268. ICES Cooperative Research Report, Copenhagen.
- Knoff, A., A. Hohn, and S. Macko. 2008. Ontogenetic diet changes in bottlenose dolphins (*Tursiops truncatus*) reflected through stable isotopes. Marine Mammal Science 24:128-137.
- Koch, P.L. 2007. Isotopic study of the biology of modern and fossil vertebrates. In R. Michener, and K. Lajtha, (eds). Stable isotopes in Ecology and Environmental Science. Blackwell Publishing, Oxford. 99-154.
- Koopman, H.N. 2007. Phylogenetic, ecological, and ontogenetic factors influencing the biochemical structure of the blubber of odontocetes. Marine Biology 151:277-291.
- Koopman, H.N., S.J. Iverson, and D.E. Gaskin. 1996. Stratification and age-related differences in blubber fatty acids of the male harbour porpoise (*Phocoena phocoena*). Journal of Comparative Physiology B-Biochemical Systemic and Environmental physiology 165:628-639.
- Kuiken, T. 1996. Review of the criteria for the diagnosis of by-catch in cetaceans. In T. Kuiken (ed.) Diagnosis of bycatch in cetaceans: proceedings of the second ECS workshop on cetacean pathology. European Cetacean Society Newsletter (Special Issue) 26. 38-43.
- Kurle, C.M., and G.A.J. Worthy. 2002. Stable nitrogen and carbon isotope ratios in multiple tissues of the northern fur seal *Callorhinus ursinus*: implications for dietary and migratory reconstructions. Marine Ecology Progress Series 236:289-300.

## REFERENCES

- Lacourt, A.W., and P.H.M. Huwae. 1981. De inktvissen (Cephalopoda) van de Nederlandse kust. Wetenschappelijke Mededelingen van de Koninklijke Nederlandse Natuurhistorische Vereniging 145:1-32.
- Leopold, M., and C.J. Camphuysen. 2006. Bruinvisstrandingen in Nederland in 2006: achtergronden, leeftijdsverdeling, sexratio, voedselkeuze en mogelijke oorzaken. IMARES: C083/06, NIOZ: 2006-5. Pages 1-136.
- Leopold, M.F., O.E. Jansen, J.C. Jansen, R. Kop, G. Keijl, E.H.W.G. Meesters, and P.J.H. Reijnders. submitted. Learning to eat: juvenile white-beaked dolphins *Lagenorhynchus albirostris* take different prey than older dolphins. Journal of the Marine Biological Association of the United Kingdom.
- Leopold, M.F., C.J.G. van Damme, C.J.M. Phillippart, and C.J.N. Winter 2001. Otoliths of North Sea fish: interactive guide of identification of fish from the SE North Sea, Wadden Sea and adjacent fresh waters by means of otoliths and other hard parts. ETI, Amsterdam.
- Leopold, M.F., C.J.G. van Damme, and H.W. van der Veer. 1998. Diet of cormorants and the impact of cormorant predation on juvenile flatfish in the Dutch Wadden Sea. Journal of Sea Research 40:93-107.
- Lesage, V., M.O. Hammill, and K.M. Kovacs. 2001. Marine mammals and the community structure of the Estuary and Gulf of St Lawrence, Canada: evidence from stable isotope analysis. Marine Ecology Progress Series 210:203-221.
- Lick, R. 1993. Nahrungsanalysen von Kleinwalen Deutscher Küstengewässer. Chapter 1 In H. Bohlken, H. Benke, and J. Wulf, (eds). Untersuchungen über Bestand, Gesundheitszustand und Wanderungen der Kleinwalpopulationen (Cetacea) in deutschen Gewässern. Institut für Haustierkunde der Universität Kiel und Forschungs- und Technologiezentrum Westküste Zentrale Einrichtung der Universität Kiel. 1-21.
- Lick, R.R. 1991a. Parasites from the digestive tract and food analysis of harbour porpoise (*Phocoena phocoena*) from German waters. In P.G.H. Evans (ed.), European Research on Cetaceans - 5. European Society, Cambridge. 65-68.
- Lick, R.R. 1991b. Untersuchungen zu Lebenszyklus (Krebse-Fische-marine Säuger) und Gefrierresistenz anisakider Nematoden in Nord- und Ostsee. PhD thesis. University of Kiel, Germany.
- Lidén, K., C. Takahashi, and D.E. Nelson. 1995. The effects of lipids in stable carbon isotope analysis and the effects of NaOH treatment on the composition of extracted bone collagen. Journal of Archaeological Science:321-326.
- Lockyer, C. 2003. Harbour porpoises (*Phocoena phocoena*) in the North Atlantic: Biological parameters. NAMMCO Scientific Publication 5:71-90.
- Lucifora, L.O., V.B. García, R.C. Menni, and A.H. Escalante. 2006. Food habits, selectivity, and foraging modes of the school shark *Galeorhinus galeus*. Marine Ecology Progress Series 315:259-270.
- MacLeod, C.D., G.J. Pierce, and M.B. Santos. 2007a. Starvation and sandeel consumption in harbour porpoises in the Scottish North Sea. Biology Letters 3:535-536.

- MacLeod, C.D., M.B.A. Santos, R.J. Reid, B.E. Scott, and G.J. Pierce. 2007b. Linking sandeel consumption and the likelihood of starvation in harbour porpoises in the Scottish North Sea: could climate change mean more starving porpoises? *Biology Letters* 3:185-188.
- Mann, J. 2009. Parental behaviour. In W.F. Perrin, B. Würsig, and J.G.M. Thewissen, (eds). *Encyclopedia of Marine Mammals*. Academic Press, Burlington/San Diego/ New York/London. 830-836.
- Martin, A.R. 1996. The diet of harbour porpoises (*Phocoena phocoena*) in British waters. International Whaling Commission, Scientific Committee Document SC/47/ SM48, Cambridge, UK.
- Martínez del Rio, C., N. Wolf, S.A. Carleton, and L.Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84:91-111.
- Massias, A., and P.H. Becker. 1990. Nutritive value of food and growth in common tern *Sterna hirundo* chicks. *Ornis Scandinavica* 21:187-194.
- Matthiopoulos, J., S. Smout, A.J. Winship, D. Thompson, I.L. Boyd, and J. Harwood. 2008. Getting beneath the surface of marine mammal - fisheries competition. *Mammal Review* 38:167-188.
- McConnaughey, T., and C.P. McRoy. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Marine Biology* 53:257-262.
- McCullagh, P., and J.A. Nelder 1989. *Generalized Linear Models*. Chapman & Hall, London.
- McCutchan, J.H., W.M. Lewis, C. Kendall, and C.C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102:378-390.
- Meehl, G.A., W.M. Washington, W.D. Collins, J.M. Arblaster, X. Hu, L.E. Buja, W.G. Strand, and H. Teng. 2005. How much more global warming and sea level rise? *Science* 307:1769-1772.
- Michener, R.H., and L. Kaufman. 2007. Stable isotope ratios as tracers in marine food webs: an update. Chapter 9 In R.H. Michener, and K. Lajtha, (eds). *Stable Isotopes in Ecology and Environmental Science*. Blackwell Publishing, Boston. 238-282.
- Middelburg, J.J., and P.M.J. Herman. 2007. Organic matter processing in tidal estuaries. *Marine Chemistry* 106:127-147.
- Mikkelsen, B., T. Haug, and K.T. Nilssen. 2002. Summer diet of grey seals (*Halichoerus grypus*) in Faroese waters. *Sarsia* 87:462-471.
- Minagawa, M., and E. Wada. 1984. Stepwise enrichment of  $^{15}\text{N}$  along food chains: further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochimica et Cosmochimica Acta* 48:1135-1140.
- Mohl-Hansen, U. 1954. Investigations on reproduction and growth of the porpoise (*Phocoena phocaena* (L.)) from the Baltic. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening* 116:369-396.
- Moore, K.M., M.L. Murray, and M.J. Schoeninger. 1989. Dietary reconstruction from bones treated with preservatives. *Journal of Archaeological Science* 16:437-446.

## REFERENCES

- Moore, S.E. 2008. Marine mammals as ecosystem sentinels. *Journal of Mammalogy* 89:534-540.
- Newsome, S.D., M.T. Clementz, and P.L. Koch. 2010. Using stable isotope biogeochemistry to study marine mammal ecology. *Marine Mammal Science* 26:509-572.
- Nienhuis, P.H., and A.C. Smaal. 1994. The Oosterschelde estuary, a case study of a changing ecosystem: an introduction. *Hydrobiologia* 282/283:1-14.
- Nordstrom, C.A., L.J. Wilson, S.J. Iverson, and D.J. Tollit. 2008. Evaluating quantitative fatty acid signature analysis (QFASA) using harbour seals *Phoca vitulina richardsi* in captive feeding studies. *Marine Ecology Progress Series* 360:245-263.
- Northridge, S.P. 1991. An updated world review of interactions between marine mammals and fisheries. Food and Agriculture Organization (FAO) Fisheries Technical Report 251:58.
- O'Regan, H.J., C. Chenery, A.L. Lamb, R.E. Stevens, L. Rook, and S. Elton. 2008. Modern macaque dietary heterogeneity assessed using stable isotope analysis of hair and bone. *Journal of Human Evolution* 55:617-626.
- Osinga, N., P. 't Hart, and D. Morick. 2008. By-catch and drowning in harbour porpoises (*Phocoena phocoena*) stranded on the northern Dutch coast. *European Journal of Wildlife Research* 54:667-674.
- Parnell, A.C., R. Inger, S. Bearhop, and A.L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. *PLOS ONE* 5:1-5.
- Pauly, D., A.W. Trites, E. Capuli, and V. Christensen. 1998. Diet composition and trophic levels of marine mammals. *ICES Journal of Marine Science* 55:467-481.
- Pedersen, J., and J.R.G. Hislop. 2001. Seasonal variations in the energy density of fishes in the North Sea. *Journal of Fish Biology* 59:380-389.
- Peltier, H., W. Dabin, O. Van Canneyt, G. Dorémus, M. Huon, and V. Ridoux. 2012. The significance of stranding data as indicators of cetacean populations at sea: modelling the drift of cetacean carcasses. *Ecological Indicators* 18:278-290.
- Phillips, D.L. 2001. Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia* 127:166-170.
- Phillips, D.L., and P.M. Eldridge. 2006. Estimating the timing of diet shifts using stable isotopes. *Oecologia* 147:195-203.
- Phillips, D.L., and J.W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171-179.
- Phillips, D.L., and J.W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136:261-269.
- Pierce, G.J., L. Allcock, I. Bruno, P. Bustamante, A. González, A. Guerra, P. Jereb, E. Lefkaditou, S. Malham, A. Moreno, J. Pereira, U. Piatkowski, M. Raserio, P. Sánchez, M.B. Santos, M. Santurtún, S. Seixas, I. Sobrino, and R. Villanueva. 2010. Cephalopod biology and fisheries in Europe. Page 175. ICES Cooperative Research Report.
- Pierce, G.J., and P.R. Boyle. 1991. A review of methods for diet analysis in piscivorous marine mammals. *Oceanography and Marine Biology* 29:409-486.

- Pierce, G.J., P.R. Boyle, J. Watt, and M. Grisley. 1993. Recent advances in diet analysis of marine mammals. *Marine Mammals: Advances in behavioural and population biology*. Symposia of the Zoological Society of London 66:241-261.
- Pierce, G.J., M.B. Santos, and S. Cerviño. 2007. Assessing sources of variation underlying estimates of cetacean diet composition: a simulation study on analysis of harbour porpoise diet in Scottish (UK) waters. *Journal of the Marine Biological Association of the United Kingdom* 87:213-221.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and the Core Team. 2009. nlme: Linear and Nonlinear Mixed Effects Models. R package version. 3:1-96.
- Pinheiro, J.C., and D.M. Bates 2000. *Mixed-effects models in S and S-PLUS*. Springer, New York.
- Pope, J.G., and C.T. Macer. 1996. An evaluation of the stock structure of North Sea cod, haddock, and whiting since 1920, together with a consideration of the impacts of fisheries and predation effects on their biomass and recruitment. *ICES Journal of Marine Science* 53:1157-1169.
- Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83:703-718.
- Prime, J.H., and P.S. Hammond. 1987. Quantitative assessment of gray seal diet from fecal analysis. In A.C. Huntley, D.P. Costa, G.A.J. Worthy, and M.A. Castellini, (eds). *Approaches to marine mammal energetics*. Special Publications 1. Society for Marine Mammology. 165-181.
- Pyenson, N.D. 2010. Carcasses on the coastline: measuring the ecological fidelity of the cetacean stranding record in the eastern North Pacific Ocean. *Paleobiology* 36:435-480.
- R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [www.R-project.org](http://www.R-project.org).
- Rahmstorf, S. 2007. A semi-empirical approach to projecting future sea-level rise. *Science* 315:368-370.
- Rauck, G. 1976. Starker Befall der Nordseesprotten durch den Parasiten *Lernaenicus sprattae* (Soverby) and *L. encrasicholi* (Turton). *Archiv für Fischereiwissenschaft* 26:151-153.
- Read, A.J., and A.A. Hohn. 1995. Life in the fast lane: the life-history of harbor porpoises from the Gulf of Maine. *Marine Mammal Science* 11:423-440.
- Read, A.J., and A.J. Westgate. 1997. Monitoring the movements of harbour porpoises (*Phocoena phocoena*) with satellite telemetry. *Marine Biology* 130:315-322.
- Recchia, C.A., and A.J. Read. 1989. Stomach contents of harbor porpoises, *Phocoena phocoena* (L), from the Bay of Fundy. *Canadian Journal of Zoology* 67:2140-2146.
- Reeves, R.R., C. Smeenk, C.C. Kinze, R.L. Brownell, and J. Lien. 1999. White-beaked dolphin, *Lagenorhynchus albirostris* Gray, 1846. In S.H. Ridgway, and S.R. Harrison, (eds). *Handbook of Marine Mammals*. Volume 6. Academic Press, London/San Diego. 1-30.

## REFERENCES

- Reid, J.B., P.G.H. Evans, and S.P. Northridge 2003. Atlas of cetacean distribution in north-west European waters. Joint Nature Conservation Committee, Peterborough.
- Reijnders, P.J.H. 1992. Harbour porpoises *Phocoena phocoena* in the North Sea: numerical responses to changes in environmental conditions. *Netherlands Journal of Aquatic Ecology* 26:75-85.
- Reijnders, P.J.H., S.M.J.M. Brasseur, T. Borchardt, K. Camphuysen, R. Czeck, A. Gilles, L. Fast Jensen, M. Leopold, K. Lucke, S. Ramdohr, M. Scheidat, U. Siebert, and J. Teilmann. 2009. Marine Mammals. Thematic Report 20 in H. Marencic, and J. de Vlas (eds.), Quality Status Report 2009. Wadden Sea Secretariat, Wilhelmshaven.
- Reijnders, P.J.H., and K. Lankester. 1990. Status of marine mammals in the North Sea. *Netherlands Journal of Sea Research* 26:427-435.
- Reijnders, P.J.H., B. Reineking, K.F. Abt, S.M.J.M. Brasseur, C.J. Camphuysen, M. Scheidat, U. Siebert, M. Stede, J. Tougaard, and S. Tougaard. 2005. Marine Mammals. In K. Essink, C. Dettman, H. Farke, K. Lauersen, G. Lüerssen, H. Marencic, and W. Wiersinga, (eds). Wadden Sea Quality Status Report 2004. 317-330.
- Richards, M.P., S. Mays, and B.T. Fuller. 2002. Stable carbon and nitrogen isotope values of bone and teeth reflect weaning age at the medieval Wharram Percy Site, Yorkshire, UK. *American Journal of Physical Anthropology* 119:205-210.
- Ringelstein, J., C. Pusineri, S. Hassani, L. Meynier, J. Nicolas, and V. Ridoux. 2006. Food and feeding ecology of the striped dolphin, *Stenella coeruleoalba*, in the oceanic waters of the north-east Atlantic. *Journal of the Marine Biological Association of the United Kingdom* 86:909-918.
- Rogan, E., and S.D. Berrow. 1996. A Review of Harbour Porpoises, *Phocoena phocoena*, in Irish Waters. Report of the International Whaling Commission 46:595-605.
- rugvin.nl. 2012. Stichting Rugvin. April 2012. <http://www.rugvin.nl/>.
- Santos, M.B. 1998. Feeding ecology of harbour porpoises, common and bottlenose dolphins and sperm whales in the Northeast Atlantic. PhD thesis, University of Aberdeen, Aberdeen.
- Santos, M.B., and G.J. Pierce. 2003. The diet of harbour porpoise (*Phocoena phocoena*) in the northeast Atlantic. *Oceanography and Marine Biology: an Annual Review* 41:355-390.
- Santos, M.B., G.J. Pierce, E.N. Ieno, M. Addink, C. Smeenk, C.C. Kinze, and M. Sacau. 2005. Harbour porpoise (*Phocoena phocoena*) feeding ecology in the eastern North Sea. in ICES CM 2005/Theme Session R:15 (Marine Mammals: Monitoring Techniques, Abundance Estimation, and Interactions with Fisheries)
- Santos, M.B., G.J. Pierce, J.A. Learmonth, R.J. Reid, H.M. Ross, I.A.P. Patterson, D.G. Reid, and D. Beare. 2004. Variability in the diet of harbor porpoises (*Phocoena phocoena*) in Scottish waters 1992-2003. *Marine Mammal Science* 20:1-27.
- Santos, M.B., G.J. Pierce, H.M. Ross, R.J. Reid, and B. Wilson. 1994. Diets of small cetaceans from the Scottish coast. *International Council for the Exploration of the Sea* 11.



- Scheidat, M., H. Verdaat, and G. Aarts. 2012. Using aerial surveys to estimate density and distribution of harbour porpoises in Dutch waters. *Journal of Sea Research* 69:1-7.
- Schram, T.A. 1987. Prevalence of *Lernaenicus sprattae* (Soverby) and *L. encrasicoli* (Turton) (Copepoda, Pennellidae) infection of sprat from Oslofjorden. *Sarsia* 72:279-289.
- Schram, T.A. 1991. Eye-maggot (*Lernaenicus sprattae*) and body-maggot (*L. encrasicoli*) of the sprat. ICES identification leaflets for diseases and parasites of fish and shellfish 43:5pp.
- Schulze, G. 1996. Die Schweinswale. Spektrum Akademischer Verlag, Heidelberg/Berlin/Oxford.
- Sealy, J., R. Armstrong, and C. Schrire. 1995. Beyond lifetime averages: tracing life histories through isotopic analysis of different calcified tissues from archaeological human skeletons. *Antiquity* 69:290-300.
- Sergeant, D.E., and H.D. Fisher. 1957. The smaller cetacea of eastern Canadian waters. *Journal of the Fisheries Research Board of Canada* 14:83-115.
- Smeenk, C. 1986. Strandingen van cetacea op de Nederlandse kust in 1981, 1982 en 1983. *Lutra* 29:268-281.
- Smeenk, C. 1987. The harbour porpoise *Phocoena phocoena* (L., 1758) in The Netherlands: stranding records and decline. *Lutra* 30:77-90.
- Smeenk, C. 1989. Strandingen van cetacea op de Nederlandse kust in 1984, 1985 en 1986. *Lutra* 32:164-180.
- Smeenk, C. 1992. Strandingen van cetacea op de Nederlandse kust in 1987, 1988 en 1989. *Lutra* 35:51-66.
- Smeenk, C. 1995. Strandingen van cetacea op de Nederlandse kust in 1990, 1991 en 1992. *Lutra* 38:90-104.
- Smeenk, C. 2003. Strandingen van cetacea op de Nederlandse kust in 1993-1997. *Lutra* 46:45-64.
- Smeenk, C., and P.A.M. Gaemers. 1987. Fish otoliths in the stomach of white-beaked dolphins *Lagenorhynchus albirostris*. *European Cetacean Society Newsletter* 1:12-13.
- Smith, G.J.D. 1972. The stomach of the harbour porpoise *Phocoena phocoena* (L.). *Canadian Journal of Zoology* 50:1611-1616.
- Smith, G.J.D., and D.E. Gaskin. 1974. The diet of harbor porpoises (*Phocoena phocoena* (L)) in coastal waters of Eastern Canada, with special reference to Bay of Fundy. *Canadian Journal of Zoology* 52:777-782.
- Smith, G.J.D., and D.E. Gaskin. 1983. An environmental index for habitat utilization by female harbor porpoises with calves near Deer Island, Bay of Fundy. *Ophelia* 22:1-13.
- Smith, G.J.D., A.J. Read, and D.E. Gaskin. 1983. Incidental catch of harbour porpoise, *Phocoena phocoena* (L.), in herring weirs in Charlotte County, New Brunswick, Canada. *Fishery Bulletin* 81:660-662.

## REFERENCES

- Smith, R.J., and A.J. Read. 1992. Consumption of euphausiids by harbor porpoise (*Phocoena phocoena*) calves in the Bay of Fundy. *Canadian Journal of Zoology* 70:1629-1632.
- Sotiropoulos, M.A., W.M. Tonn, and L.I. Wassenaar. 2004. Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. *Ecology of Freshwater Fish* 13:155-160.
- Taylor, B.L., S.J. Chivers, J. Larese, and W.F. Perrin. 2007. Generation length and percent mature estimates for IUCN assessments of cetaceans. Southwest Fisheries Science Center. La Jolla, USA.
- Teilmann, J., and R. Dietz. 1998. Status of the harbour porpoise in Greenland. *Polar Biology* 19:211-220.
- Thiemann, G.W., S.M. Budge, W.D. Bowen, and S.J. Iverson. 2004a. Comment on Grahl-Nielsen et al. (2003) 'Fatty acid composition of the adipose tissue of polar bears and of their prey: ringed seals, bearded seals and harp seals'. *Marine Ecology Progress Series* 281:297-301.
- Thiemann, G.W., S.M. Budge, and S.J. Iverson. 2004b. Determining blubber fatty acid composition: a comparison of *in situ* direct and traditional methods. *Marine Mammal Science* 20:284-295.
- Thiemann, G.W., S.J. Iverson, and I. Stirling. 2008. Using fatty acids to study marine mammal foraging: The evidence from an extensive and growing literature. *Marine Mammal Science* 25:243-249.
- Thompson, P.M., D.J. Tollit, and S.P.R. Greenstreet. 1996. Between-year variations in the diet and behaviour of harbour seals *Phoca vitulina* in the Moray Firth; causes and consequences. *Aquatic Predators and their Prey*. Blackwells Scientific Publications, Oxford. 44-52.
- Thomsen, F., M. Laczny, and W. Piper. 2006. A recovery of harbour porpoises (*Phocoena phocoena*) in the southern North Sea? A case study off Eastern Frisia, Germany. *Helgoland Marine Research* 60:189-195.
- Tieszen, L.L., T.W. Boutton, K.G. Tesdahl, and N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for  $\delta^{13}\text{C}$  analysis of diet. *Oecologia* 57:32-37.
- Tollit, D.J., S.G. Heaslip, T.K. Zeppelin, R. Joy, K.A. Call, and A.W. Trites. 2004. A method to improve size estimates of walleye pollock (*Theragra chalcogramma*) and Atka mackerel (*Pleurogrammus monopterygius*) consumed by pinnipeds: digestion correction factors applied to bones and otoliths recovered in scats. *Fishery Bulletin* 102:498-508.
- Tollit, D.J., M.J. Steward, P.M. Thompson, G.J. Pierce, M.B. Santos, and S. Hughes. 1997. Species and size differences in the digestion of otoliths and beaks: implications for estimates of pinniped diet composition. *Canadian Journal of Fisheries and Aquatic Sciences* 54:105-119.
- Tollit, D.J., M. Wong, A.J. Winship, D.A.S. Rosen, and A.W. Trites. 2003. Quantifying errors associated with using prey skeletal structures from fecal samples to determine the diet of Steller's sea lion (*Eumetopias jubatus*). *Marine Mammal Science* 19:724-744.

- Trites, A.W. 2009. Predator-prey relationships. In W.F. Perrin, B. Würsig, and J.G.M. Thewissen, (eds). *Encyclopedia of Marine Mammals*. Academic Press, Burlington/SanDiego/New York/London. 933-936.
- Trites, A.W., V. Christensen, and D. Pauly. 1997. Competition between fisheries and marine mammals for prey and primary production in the Pacific Ocean. *Journal of the Northwest Atlantic Fishery Science* 22:173-187.
- Tucker, S., W.D. Bowen, S.J. Iverson, W. Blanchard, and G.B. Stenson. 2009. Sources of variation in diets of harp and hooded seals estimated from quantitative fatty acid signature analysis (QFASA). *Marine Ecology Progress Series* 384:287-302.
- Turesson, H., and C. Brönmark. 2007. Predator-prey encounter rates in freshwater piscivores: effects of prey density and water transparency. *Oecologia* 153:281-290.
- van Bree, P.J.H., and A.M. Husson. 1974. Strandingen van cetacea op de Nederlandse kust in 1972 en 1973. *Lutra* 16:1-10.
- van Bree, P.J.H., and H. Nijssen. 1964. On three specimens of *Lagenorhynchus albirostris* Gray, 1846 (Mammalia, Cetacea). *Beaufortia* 11:85-93.
- van Bree, P.J.H., and C. Smeenk. 1978. Strandingen van cetacea op de Nederlandse kust in 1976 en 1977. *Lutra* 20:13-18.
- van Bree, P.J.H., and C. Smeenk. 1982. Strandingen van cetacea op de Nederlandse kust in 1978, 1979 en 1980. *Lutra* 25:19-29.
- van Damme, P.A., and O. Hamerlynck. 1999. The infection dynamics and dispersion patterns of *Lernaeocera branchialis* L. on 0+ whiting (*Merlangius merlangus* L.) in the Oosterschelde (SW Netherlands). *Journal of Fish Biology* 41:265-275.
- van Deinse, A.B. 1931. De fossiele en recente cetacea van Nederland. PhD thesis. University of Utrecht, The Netherlands.
- van Deinse, A.B. 1946. De recente cetacea van Nederland van 1931 tot en met 1944. *Zoölogische Mededelingen* 26:130-210.
- van Deinse, A.B. 1951. Onze walvisachtige dieren van 1950. *Het Zeepaard* 11:64-73.
- van Deinse, A.B. 1955. De Nederlandse cetacea van 1954. *Lutra* 1:103-105.
- van Deinse, A.B. 1956. Walvissennieuws over 1955. *Mededelingen van de Vereniging voor Zoogdierkunde en Zoogdierbescherming* 12:127-131.
- van Deinse, A.B. 1963. Walvisnieuws over 1962. *Lutra* 5:31-35.
- van Deinse, A.B. 1966. Walvisnieuws over 1964. *Lutra* 8:22-26.
- van der Meij, S.E.T., and C.J. Camphuysen. 2006. The distribution and diversity of whales and dolphins (Cetacea) in the southern North Sea: 1970-2005. *Lutra* 49:3-28.
- van Moorsel, G. 2004. Dwerginktvissen (Sepiolidae) in Nederland. *Het Zeepaard* 64:78-91.
- van Utrecht, W.L., and A.M. Husson. 1968. Strandingen van cetacea in het voorjaar van 1967 op de Nederlandse kusten. *Lutra* 10:7-17.
- Vander Zanden, M.J., and J.B. Rasmussen. 2001. Variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  trophic fractionation: implications for aquatic food web studies. *Limnology and Oceanography* 46:2061-2066.

## REFERENCES

- Vanderklift, M.A., and S. Ponsard. 2003. Sources of variation in consumer-diet  $\delta^{15}\text{N}$  enrichment: a meta-analysis. *Oecologia* 136:169-182.
- Víkingsson, G.A., D. Olafsdottir, and J. Sigurjonsson. 2003. Geographical, and seasonal variation in the diet of harbour porpoises (*Phocoena phocoena*) in Icelandic waters. NAMMCO Scientific Publication 5:243-270.
- Walton, M.J. 1997. Population structure of harbour porpoises *Phocoena phocoena* in the seas around the UK and adjacent waters. Proceedings of the Royal Society of London Series B-Biological Sciences 264:89-94.
- walvisstrandingen.nl. 2012.NCB Naturalis. <http://www.walvisstrandingen.nl/>.
- Wang, S.W., T.E. Hollmén, and S.J. Iverson. 2010. Validating quantitative fatty acid signature analysis to estimate diets of spectacled and Steller's eiders (*Somateria ischleri* and *Polysticta stelleri*). *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* 180:125-139.
- Watt, J., G.J. Pierce, and P.R. Boyle. 1997. Guide to the identification of North Sea fish using premaxilla and vertebrae. Pages 1-231. ICES Cooperative Research Report.
- Weber, M. 1887. Über *Lagenorhynchus albirostris*, Gray. *Tijdschrift der Nederlandsche Dierkundige Vereeniging* 2:114-127.
- Wells, R.S., D.J. Boness, and G.B. Rathbun. 1999. Behaviour. In J.E. Reynolds, and S.A. Rommel, (eds). *Biology of Marine Mammals*. Smithsonian Institution Press Washington and London.
- Wells, R.S., and M.D. Scott. 2009. Common bottlenose dolphin *Tursiops truncatus* and *T. aduncus*. In W.F. Perrin, B. Würsig, and J.G.M. Thewissen, (eds). *Encyclopedia of Marine Mammals*. Academic Press, Burlington/SanDiego/New York/London. 122-128.
- Wijnsma, G., G.J. Pierce, and M.B. Santos. 1999. Assessment of errors in cetacean diet analysis: *in vitro* digestion of otoliths. *Journal of the Marine Biological Association of the United Kingdom* 79:573-575.
- Williams, C.T., and C.L. Buck. 2010. Using fatty acids as dietary tracers in seabird trophic ecology: theory, application and limitations. *Journal of Ornithology* 151:531-543.
- Witt, G.B., and L.K. Ayliffe. 2001. Carbon isotope variability in the bone collagen of red kangaroos (*Macropus rufus*) is age dependent: implications for palaeodietary studies. *Journal of Archaeological Science* 28:247-252.
- Wolf, N., S.A. Carleton, and C.M. del Rio. 2009. Ten years of experimental animal isotopic ecology. *Functional Ecology* 23:17-26.
- Wood, S.N. 2006. *Generalized additive models: an introduction with R*. Chapman and Hall/CRC, Boca Raton, Florida.
- Worm, B., E.B. Barbier, N. Beaumont, J.E. Duffy, C. Folke, B.S. Halpern, J.B. Jackson, H.K. Lotze, F. Micheli, S.R. Palumbi, E. Sala, K.A. Selkoe, J.J. Stachowicz, and R. Watson. 2006. Impacts of biodiversity loss on ocean ecosystem services. *Science* 314:787-790.

- Worthy, G.A.J., and E.F. Edwards. 1990. Morphometric and biochemical factors affecting heat loss in a small temperate cetacean (*Phocoena phocoena*) and a small tropical cetacean (*Stenella attenuata*). *Physiological Zoology* 63:432-442.
- Wright, P.J. 1996. Is there a conflict between sandeel fisheries and seabirds? A case study at Shetland. In S.P.R. Greenstreet, and M.L. Tasker, (eds). *Aquatic predators and their prey*. Fishing News Books, University Press, Cambridge.
- Yasui, W.Y., and D.E. Gaskin. 1986. Energy budget of a small cetacean, the harbor porpoise, *Phocoena phocoena* (L.). *Ophelia* 25:183-197.
- Yurick, D.B., and D.E. Gaskin. 1987. Morphometric and meristic comparisons of skulls of harbor Porpoise *Phocoena phocoena* (L.) from the North Atlantic and North Pacific. *Ophelia* 27:53-75.
- Zanderink, F., and N. Osinga. 2010. De bruinvis is terug in de Oosterschelde. *Zoogdier* 21:12-15.
- Zuur, A.F., E.N. Ieno, N. Walker, A.A. Saveliev, and G.M. Smith 2009. *Mixed effects models and extensions in ecology with R*. Springer, New York.

## SUMMARY

Harbour porpoises and white-beaked dolphins are the most common small cetaceans in the North Sea and Dutch coastal waters. The distribution and relative abundance of harbour porpoises and white-beaked dolphins from the Dutch coastal waters has changed significantly over the past decades. This thesis describes the past and present feeding ecology of these two species in Dutch coastal waters and investigates whether changes in abundance and relative distribution of porpoises reflect changes in their foodbase. For porpoises, three techniques for dietary analyses were combined, including stomach contents-, stable isotope- and fatty acid analysis, providing the most detailed description of their diet in time and space, elucidating differences between their short- and longer term diet.

Stable isotope analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) were carried out in bone and muscle samples collected from porpoises stranded along the Dutch coast. Muscle  $\delta^{15}\text{N}$  values revealed that neonatal enrichment occurred and that larger porpoises, in particular males, seem to feed on lower trophic level species, compared to smaller individuals. Also bone  $\delta^{15}\text{N}$  values show that larger animals had fed on lower trophic levels in distant times. Seasonal variation in bone  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values revealed two distinct groups of porpoises along the Dutch coast, a winter group (mainly males) that migrated from neighbouring regions and a Dutch sub-population in summer (Chapter 2).

To assess the contribution of prey species to the porpoises' diet, stable isotope analysis in both porpoise muscle and prey were carried out. With the use of a mixing model (Stable Isotope Analysis in R, SIAR), we revealed that 70-83% of the diet of porpoises consisted mainly of poor cod, mackerel, greater sandeel lesser sandeel, sprat and gobies. This highlights a higher importance of pelagic, schooling species in the porpoises' diet compared to stomach contents, where 90.5% of the diet consisted of gobies, whiting, lesser sandeel, herring, cod and sprat. Porpoises thus also feed offshore on pelagic, schooling species, while they feed closer to shore on more benthic and demersal species shortly before they strand. This could be due to the distribution of prey species as well as differences in behaviour of porpoises and their prey between the coastal zone and offshore waters (Chapter 3).

The use of Quantitative Fatty Acid Analysis (QFASA) showed that the diet of porpoises consisted mainly of gobies, mackerel, smelt, herring and dragonet, pointing towards profound differences between the diet as estimated by QFASA and as deduced from stomach contents. This study revealed that the longer term diet of porpoises in Dutch coastal waters consists both of coastal species (e.g. gobies, smelt and dragonet) and also pelagic, schooling species (e.g. mackerel and herring). The results also brought to dawn possible methodological problems in using QFASA for porpoise diet estimation, emphasizing the importance of



applying different dietary analysis techniques when studying marine mammal diets and the need for controlled feeding experiments in order to improve the interpretation of dietary analysis results (Chapter 5).

Besides new insights in the feeding ecology of porpoises, stable isotope analysis also elicited a non-food related conservation ecology issue. Distinct  $\delta^{13}\text{C}$  values in muscle of porpoises stranded in the Eastern Scheldt revealed that these porpoises foraged there for a longer period. This distinct  $\delta^{13}\text{C}$  signature of animals from the Eastern Scheldt was not observed in bone tissue, which suggests a relatively recent shift in habitat use rather than life-long residency of porpoises within the Eastern Scheldt. The high number of strandings within the Eastern Scheldt revealed a higher mortality rate compared to the Dutch coastal zone, indicating that along with other changes in the physical environment, the building of the storm surge barrier may play an important role in determining the residency of porpoises in the Eastern Scheldt, and that the area might act as an ecological trap for porpoises entering it. This is an example of the impact on marine species due to protection structures that emerge and respectively increase worldwide in response to the effects of global warming and climate change. It highlights that even semi-open structures, which are meant to ameliorate habitat loss, degradation and fragmentation, may still affect the abundance and distribution of migratory marine mammal species (Chapter 4).

The analysis of stomach contents of white-beaked dolphins showed that their diet was dominated by Gadidae. All other prey species combined contributed little to the diet by weight. The two most important prey species based in weight were whiting and cod. In numbers, gobies were most common, but these contributed little to the diet by weight. The overall diet showed a lasting predominance of whiting and cod, without clear changes over time (35 years) or differences between sexes or size-classes of dolphins and revealed that white-beaked dolphins in the south-eastern North Sea are specialist feeders, with a strong preference for whiting and cod (Chapter 6).

Stomach contents of juvenile white-beaked dolphins in our study revealed that at the age of about 1.5 years old, they had started feeding on solid food by taking a variety of small fish and invertebrate prey, mostly shrimp and squid. Immatures in our study, estimated to be 2-4 years old, still take small prey, including small gadoids, but also take larger gadoids. Calves apparently gradually learn to eat big fish by taking prey that is much smaller than those normally taken by their mothers. This study illustrates novel techniques for diet estimation to reconstruct sizes of shrimp and whiting from tail flaps and eye lenses, respectively (Chapter 7).

Most dietary studies on porpoises and white-beaked dolphins are deduced from stomach contents. This thesis has demonstrated that using indirect methods for studying the feeding ecology of marine mammals is a valuable addition to the more direct approach using stomach contents. It supports the need

## SUMMARY

for multi-method approaches because by using only one technique, key prey species in the predator-prey relation may be missed or underestimated. Future ecological and fishery impact assessment studies and management decisions for the conservation of porpoises and white-beaked dolphins should acknowledge a difference between their long- and short-term diet. Large improvement in the interpretation of the results from diet analyses can be established either by controlled feeding experiments with animals in captivity or by studies that help to understand the common principals in dietary analyses (e.g. digestion rates, turnover rates of tissues, tissue-dependent isotopic fractionation between predator and prey and lipid metabolism within the animal) and variation of these between species.

## NEDERLANDSE SAMENVATTING

Bruinvissen en witsnuitdolfijnen zijn de meest voorkomende kleine walvisachtigen in de Noordzee en de Nederlandse kustwateren. De aantallen en verspreiding van bruinvissen en witsnuitdolfijnen in Nederlandse wateren zijn sterk veranderd in de afgelopen decennia. In dit proefschrift wordt de huidige en vroegere voedsleecologie van deze twee soorten in de Nederlandse kustwateren beschreven. Specifiek voor bruinvissen is verder onderzocht of ontwikkelingen in aantallen en verspreiding, veranderingen in hun voedselbasis weerspiegelen.

Voor bruinvissen werden drie dieet analyse technieken gecombineerd; maaginhoud analyse, stabiele isotopen analyse en vetzuur analyse. Samen geven die de meest gedetailleerde beschrijving van hun dieet in de ruimte (foerageer locatie) en in de tijd (verschillen tussen het korte- en langere termijn dieet). De verhouding van de stabiele isotopen  $^{13}\text{C}$  en  $^{12}\text{C}$  ( $\delta^{13}\text{C}$ ) is indicatief voor de locatie van foerageren en de verhouding van  $^{15}\text{N}$  en  $^{14}\text{N}$  ( $\delta^{15}\text{N}$ ) geeft de plaats in de voedselketen aan. Stabiele isotopen analyses naar  $\delta^{13}\text{C}$  en  $\delta^{15}\text{N}$  werden uitgevoerd in bot- en spiermonsters van bruinvissen die waren gestrand op de Nederlandse kust. De  $\delta^{15}\text{N}$  waarden in spierweefsel lieten zien dat neonatale verrijking zich heeft voorgedaan en dat grotere bruinvissen, in het bijzonder mannelijke dieren, foerageren op soorten van lager trofisch niveau, terwijl kleinere individuen op soorten van hoger trofische niveaus foerageren. Bot  $\delta^{15}\text{N}$  waarden toonden aan dat grotere dieren in het verleden ook op soorten van een lager trofisch niveau foerageerden. Seizoensgebonden variatie in bot  $\delta^{15}\text{N}$  en  $\delta^{13}\text{C}$  waarden bracht aan het licht dat er twee verschillende groepen bruinvissen langs de Nederlandse kust voorkomen, een winter groep (voornamelijk mannetjes) die migreren uit naburige regio's en een Nederlandse subpopulatie in de zomer (hoofdstuk 2).

Om de compositie van de verschillende prooisorten in het bruinvissen dieet te analyseren werd stabiele isotopen analyse in zowel bruinvis spierweefsel als in prooien uitgevoerd. Met het gebruik van een mixing-model (Stabiele Isotopen Analyse in R, SIAR), hebben we aangetoond dat 70-83% van het dieet van bruinvissen bestond uit dwergbolk, makreel, smelt, kleine zandspiering, sprot en grondels. Hieruit is geconcludeerd dat er met deze dieet analyse techniek meer pelagische en scholende soorten in het bruinvissen dieet voorkomen dan werd gevonden bij de dieet analyse gebaseerd op de maaginhoud. Daarbij bestond 90,5% van het dieet uit grondels, wijting, zandspiering, haring, kabeljauw en sprot. Onze hypothese als verklaring voor deze verschillen is dat bruinvissen op pelagische, scholende soorten verder op zee foerageren, terwijl ze dicht bij de kust meer op bentische en demersale soorten foerageren. Dit kan te wijten zijn aan de verspreiding van prooidieren en verschillen in zowel het gedrag van bruinvissen als van hun prooi (hoofdstuk 3).

Het gebruik van kwantitatieve vetzuur signatuur analyse (QFASA) toonde aan dat het dieet van bruinvissen voornamelijk bestond uit grondels, makreel,

spiering, haring en pitvis. Dit laat grote verschillen zien tussen het dieet zoals is berekend door QFASA en zoals afgeleid uit de maaginhouden. In deze studie werd aangetoond dat op de langere termijn het dieet van bruinvissen in de Nederlandse kustwateren zowel bestaat uit kust gebonden soorten (bijv. grondels, spiering en pitvis) maar ook uit pelagische, scholende soorten (bijv. makreel en haring). Deze bevindingen dienen met enige reserve te worden geïnterpreteerd, omdat de resultaten ook mogelijke methodologische problemen lieten zien bij het gebruik van QFASA voor de bepaling van het dieet van bruinvissen. Dat probleem is op te lossen door gecontroleerde voedingsexperimenten uit te voeren om daarmee de interpretatie van de resultaten te verbeteren. De conclusie die uit deze studie kan worden getrokken is dat het erg belangrijk is om verschillende dieet analyse technieken toe te passen bij het bestuderen van het dieet van zeezoogdieren (hoofdstuk 5).

Naast nieuwe inzichten in de voedseleecologie van bruinvissen, heeft de analyse van stabiele isotopen ook geleid tot niet-voedsel gerelateerde ecologische beleidsvraagstukken. Unieke  $\delta^{13}\text{C}$  waarden in de spieren van bruinvissen gestrand in de Oosterschelde lieten zien dat deze bruinvissen voor een langere periode in dit gebied verbleven. Deze herkenbare  $\delta^{13}\text{C}$  waardes van dieren uit de Oosterschelde werden niet waargenomen in botweefsel, hetgeen een relatief recente verschuiving in hun habitat gebruik van de Nederlandse kustzone naar de Oosterschelde suggereert. Kennelijk waren die dieren daar niet geboren. Deze studie laat zien dat de stormvloedkering behalve veranderingen in de fysische processen in het Oosterschelde estuarium veroorzaakt, ook grote invloed heeft op het verblijf van de bruinvissen aldaar. Gezien de hogere sterfte daar in vergelijking met de Nederlandse kustzone, zou het gebied kunnen fungeren als een ecologische val voor bruinvissen die het gebied binnenkomen. De relevantie van deze constatering gaat uit boven het belang van de Oosterschelde alleen. Hier wordt immers aangetoond dat zelfs half-open structuren, die het verlies van habitat en de achteruitgang en versnippering van gebieden zouden moeten beperken, toch nog een grote invloed kunnen hebben op de aantallen en verspreiding van trekkende zeezoogdieren. We zien een wereldwijd steeds meer toenemend aantal kustbeschermings maatregelen ten gevolge van de klimaatverandering en verwachte zeespiegelstijging. Daarbij is het van belang dat bij grote infra-structurele werken in het mariene gebied de mogelijke effecten op zeezoogdieren en andere mariene organismen worden meegenomen in milieueffect rapportages (hoofdstuk 4).

De analyse van de maaginhoud van witsnuitdolfijnen toonde aan dat hun dieet werd gedomineerd door kabeljauwachtigen. Alle andere prooi-soorten droegen weinig bij aan het totale gewicht van het dieet. In aantallen kwamen weliswaar grondels het meest voor, maar ook deze droegen nauwelijks bij aan het dieet op basis van het gewicht. De twee belangrijkste prooi-soorten op basis van hun gewicht waren wijting en kabeljauw. Voor deze soorten werden geen

duidelijke veranderingen in de tijd (35 jaar) geconstateerd. Er waren tevens geen verschillen tussen mannetjes en vrouwtjes, noch tussen grootteklassen. Het bleek dat witsnuitdolfijnen in het zuidoostelijke Noordzee gespecialiseerde predatoren zijn, met een sterke voorkeur voor genoemde wijting en kabeljauw (hoofdstuk 6).

Uit de maaginhoud van jonge witsnuitdolfijnen in onze studie is gebleken dat jonge dieren van ongeveer 1,5 jaar oud, waren begonnen met het opnemen van vast voedsel, door te foerageren op verschillende kleine vissen en ongewervelde prooidieren, vooral garnalen en inktvis. Onvolwassen dieren in onze studie, geschat op 2-4 jaar oud, foerageerden nog steeds op kleine prooien, vooral op kleine maar ook op grotere kabeljauwachtigen. Kalveren leren blijkbaar geleidelijk aan grotere vissen te eten door te beginnen met prooi die veel kleiner is dan die waarop hun moeders foerageren. In deze studie zijn ook nieuwe technieken voor dieet studies ontwikkeld en beschreven. Daarmee is nu ook de grootte van garnalen en wijting te reconstrueren, aan de hand van respectievelijk staartflappen en ooglenzen (hoofdstuk 7).

De meeste dieet studies over bruinvissen en witsnuitdolfijnen zijn gebaseerd op de maaginhoud. In dit proefschrift is aangetoond dat het gebruik van indirecte methoden (zoals stabiele isotopen en vetzuuranalyse) voor het bestuderen van de voedsel-ecologie van zeezoogdieren een waardevolle aanvulling is op de meer directe benadering door middel van de maaginhoud. Daarmee wordt de noodzaak voor het combineren van verschillende methodes aangetoond, omdat door het gebruik van slechts één techniek belangrijke prooi-soorten kunnen worden gemist of onderschat. Voor toekomstige ecologische en visserij-effect beoordelingsstudies en beslissingen over de te nemen beheersmaatregelen ter bescherming van bruinvissen en witsnuitdolfijnen, is het van belang om het verschil tussen hun langere en korte termijn dieet te erkennen. Verbetering van de interpretatie van de resultaten van dieet analyses kan worden bereikt door middel van gecontroleerde voedingsexperimenten met dieren in gevangenschap of door studies naar chemische processen zoals weefsel-afhankelijke isotopische fractionering tussen roofdier en prooi en vet-metabolisme.

## CONTRIBUTING AUTHORS

### **DR. IR. GEERT M. AARTS**

IMARES Wageningen UR, Department of Ecosystems  
P.O. Box 167, 1790 AD Den Burg, The Netherlands  
Geert.Aarts@wur.nl

### **DRS. SOPHIE M.J.M. BRASSEUR**

IMARES Wageningen UR, Department of Ecosystems  
P.O. Box 167, 1790 AD Den Burg, The Netherlands  
Sophie.Brasseur@wur.nl

### **ABRAHAM S. COUPERUS**

IMARES Wageningen UR, Department of Fish  
P.O. Box 68, 1970 AB IJmuiden, The Netherlands  
Bram.Couperus@wur.nl

### **DR. KRISHNA DAS**

University of Liège, Laboratory of Oceanology (Mare Center)  
B6C, BE4000 Liège, Belgium  
K.Das@ulg.ac.be

### **CLASINA (J.C.) JANSEN**

University of Applied Sciences Van Hall Larenstein  
P.O. Box 1528, 8901 BV Leeuwarden, The Netherlands  
Clasina.Jansen@wur.nl

### **GUIDO O. KEIJL**

Naturalis Biodiversity Center  
P.O. Box 9517, 2300 RA Leiden, The Netherlands  
Guido.Keijl@naturalis.nl

### **ROBIN KOP**

University of Applied Sciences Van Hall Larenstein  
P.O. Box 1528, 8901 BV Leeuwarden, The Netherlands  
Robin.Kop@wur.nl

### **DRS. MARDIK F. LEOPOLD**

IMARES Wageningen UR, Department of Ecosystems  
P.O. Box 167, 1790 AD Den Burg, The Netherlands  
Mardik.Leopold@wur.nl



**DR. GILLES LEPOINT**

University of Liège, Laboratory of Oceanology (Mare Center)  
B6C, BE4000 Liège, Belgium  
G.Lepoint@ulg.ac.be

**DR. ERIK H.W.G. MEESTERS**

IMARES Wageningen UR, Department of Ecosystems  
P.O. Box 167, 1790 AD Den Burg, The Netherlands  
Erik.Meesters@wur.nl

**DR. LOÏC MICHEL**

University of Liège, Laboratory of Oceanology (Mare Center)  
B6C, BE4000 Liège, Belgium  
Loic.Michel@ulg.ac.be

**PROF. DR. IR. PETER J.H. REIJNDERS**

IMARES Wageningen UR, Department of Ecosystems, and Wageningen University, Department of Aquatic Ecology & Waterquality Management  
P.O. Box 167, 1790 AD Den Burg, The Netherlands  
Peter.Reijnders@wur.nl

**DR. CHRIS SMEENK**

Naturalis Biodiversity Center  
P.O. Box 9517, 2300 RA Leiden, The Netherlands  
Chris.Smeenk@naturalis.nl

## PUBLICATIONS

Camphuysen C.J., Smeenk, C., Addink, M., van Grouw, H. and Jansen, O.E. 2008. Cetaceans stranded in the Netherlands from 1998 to 2007. *Lutra* 51:87-122.

Jansen, O.E., Leopold, M.F., Meesters, E.H.W.G. and Smeenk, C. 2010. Are white-beaked dolphins *Lagenorhynchus albirostris* food specialists? Their diet in the southern North Sea. *Journal of the Marine Biological Association of the UK* 90:1501-1508.

Jansen, O.E., Das, K., Lepoint, G., Aarts, G.M., Michel, L. and Reijnders, P.J.H. 2012. Feeding ecology of harbour porpoises: stable isotope analysis in muscle and bone. *Marine Biology Research* 8:829-841.

Galatius, A., Jansen, O.E. and Kinze, C.C. in press. Parameters of growth and reproduction of white-beaked dolphins (*Lagenorhynchus albirostris*) from the North Sea. *Marine Mammal Science*. DOI: 10.1111/j.1748-7692.2012.00568.x

Jansen, O.E., Michel, L., Das, K., Lepoint, G. and Reijnders, P.J.H. in press. Diet of harbor porpoises along the Dutch coast: a combined stable isotope and stomach contents approach. *Marine Mammal Science*. DOI: 10.1111/j.1748-7692.2012.00621.x

Jansen, O.E., Aarts, G.M. and Reijnders, P.J.H. in press. Harbour porpoises in the Eastern Scheldt: a resident stock or trapped by a storm surge barrier? *PLOS ONE*.

Bravo Rebolledo, E.L., Franeker, J.A., Jansen, O.E. and Brasseur, S.M.J.M. in press. Plastics in stomachs of marine animals. *Marine Pollution Bulletin*.

Leopold, M.F., Jansen, O.E., Jansen, C.J. and Kop, R. submitted. Learning how to eat: juvenile white-beaked dolphins *Lagenorhynchus albirostris* take much smaller, and different prey than more mature dolphins. Submitted to the *Journal of the Marine Biological Association of the UK*.

Jansen, O.E., Meesters E.H.W.G., Brasseur, S.M.J.M., Budge, S. and Reijnders, P.J.H. in prep. The diet of harbour porpoises along the Dutch coast based on QFASA analysis: A combined fatty acid and stomach contents approach.

Pierce, G.J., Santos, M.B., Jansen, O.E., Addink, M., Leopold, M.F., Kinze, C., Smeenk, C., Iverson, M., Fernández, R., Ieno, E.N. and Lockyer, C. in prep. Long-term trends in diet of harbour porpoises (*Phocoena phocoena*) in the Eastern North Sea.



Netherlands Research School for the  
Socio-Economic and Natural Sciences of the Environment

# C E R T I F I C A T E

The Netherlands Research School for the  
Socio-Economic and Natural Sciences of the Environment  
(SENSE), declares that

***Okka Eike Jansen***

born on 18 April 1981 in Leer, Germany

has successfully fulfilled all requirements of the  
Educational Programme of SENSE.

Wageningen, 8 March 2013

the Chairman of the SENSE board

Prof. dr. Rik Leemans

the SENSE Director of Education

Dr. Ad van Dommelen

The SENSE Research School has been accredited by the Royal Netherlands Academy of Arts and Sciences (KNAW)



KONINKLIJKE NEDERLANDSE  
AKADEMIE VAN WETENSCHAPPEN



The SENSE Research School declares that **Mrs. Okka Eike Jansen** has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 40 ECTS, including the following activities:

#### SENSE PhD Courses

- o Environmental Research in Context
- o Research Context Activity: Writing and publishing article on research data of white-beaked dolphin diet in the southern North Sea

#### Other PhD and Advanced MSc Courses

- o Analysis of Multivariate Data from Ecology and Environmental Science using PRIMER v6
- o Estuarine Ecology, NIOO
- o Cursus Proefdierkunde, KNAW
- o Techniques for Writing & Presenting Scientific Papers

#### External training at a foreign research institute

- o Stable Isotope Analysis, 2009, Liège University, Belgium
- o Fatty Acid Analysis, 2008, Dalhousie University, Canada

#### Oral Presentations

- o *Different results in diet of porpoises using fatty acids and stomach contents: Genuine temporal changes in diet or an artefact?* 24<sup>th</sup> Annual Conference of the European Cetacean Society (ECS), 20-25 May 2010, Stralsund, Germany

SENSE Coordinator PhD Education and Research

Mr. Johan Feenstra

## ACKNOWLEDGEMENTS

Terugkijkend op mijn promotietraject vraag ik me vaak af hoe ik hier eigenlijk in verzeild ben geraakt. Er zijn een aantal mensen die mijn weg hier naartoe hebben beïnvloed, mogelijk gemaakt, begeleid, gevormd en vergezeld.

Na mijn eindexamen in Duitsland ben ik in 2000 naar Nederland gekomen om Diermanagement te gaan studeren. Op dag één aan het Van Hall Instituut heb ik Linda Bouma ontmoet. Linda, je hebt mij niet alleen tijdens de introductieweek door heel Friesland gefietst, maar mij ook door de hele studie begeleid. Dank je wel voor je vriendschap en dat je ook tijdens mijn verdediging aan mijn zijde staat als mijn paranimf.

Voor mijn stage- en afstudeeropdracht heb ik voor Sophie Brasseur aan zeehondenpoep en magen gewerkt. Ik mocht mee met het zeehonden vangen, de autopsies en één keer mocht ik mee met de vliegtuigtellingen (waarom het maar één keer was hou ik hier voor me). Daarnaast was ik altijd welkom voor een babbel, een hapje of borrel. Sophie, bedankt voor al deze kansen, de begeleiding en de gezelligheid.

Na mijn Bachelor ben ik in 2004 Biologie gaan studeren aan de Universiteit van Leiden. Onder begeleiding van Chris Smeenk heb ik bij Naturalis geholpen met de dissecties van bruinvissen, witsnuitdolfijnen, de bultrug op Vlieland en een spitssnuitdolfijn op Ameland. Mijn Masterscriptie ging over het dieet en de leeftijd van witsnuitdolfijnen, en ondanks kleine tegenslagen heeft dit ertoe geleid dat ik *cum laude* mocht afstuderen. Chris, bedankt voor deze kansen, de vriendschap en het vertrouwen dat ik de witsnuit gegevens ook echt zou gaan publiceren.

Met mijn bul in handen heeft Peter Reijnders mij gevraagd om bij IMARES (vestiging Texel) te komen werken. In 2006 ben ik als Junior Onderzoeker begonnen en heb ik mee geholpen aan diverse projecten. Toen ik het aanbod kreeg om te promoveren heb ik meteen toegezegd. De kans om 4 jaar lang aan één onderwerp te mogen werken leek mij geweldig. In 2007 kon ik beginnen aan mijn promotietraject. Peter, ik wil je graag bedanken voor deze kansen, het vertrouwen in mijn wetenschappelijke ontwikkeling en de begeleiding als mijn promotor. Elke zin in dit proefschrift is meerdere malen door jou gelezen, beoordeeld en verbeterd.

Graag wil ik ook al mijn co-auteurs bedanken die hun bijdrage aan de manuscripten, vaak in hun eigen tijd, hebben geleverd. Door jullie enthousiasme, bijdragen en kritische kijk is het gelukt om bijna alle stukken te publiceren. Ik heb veel van jullie geleerd. Mijn dank gaat ook uit naar mijn co-promotor Marten Scheffer en zijn team van de leerstoelgroep Aquatische Ecologie en Waterkwaliteitsbeheer van de Wageningen Universiteit die mij ondanks de afstand tussen Texel en Wageningen tijdens mijn incidentele bezoeken welkom hebben



geheten. Verder wil ik de leescommissie en mijn opponenten bedanken dat zij deze taak op zich hebben genomen.

Tijdens mijn verblijf op Texel en in IJmuiden heb ik met veel leuke en inspirerende collega's mogen samenwerken. Mijn dank gaat uit naar het hele team, zowel naar de onderzoekers, de aio's, de assistenten, de studenten en alle helpende handen. Ik heb me binnen deze groepen echt thuis gevoeld, mijn dank hiervoor.

Ik heb het grote geluk gehad dat ik bij IMARES mooie vriendschappen heb opgedaan. Mijn werkkamer op Texel heb ik gedeeld met Tamara van Polanen Petel. Tamara, bedankt voor de gezellige momenten op het werk (en ook daarna) en voor je vriendschap die ook nu nog, naar het andere einde van de wereld, stand houdt.

Meike Scheidat heeft op Texel niet alleen gezorgd voor een betere Feng Shui. Meike, bedankt voor je vriendschap, je peptalks, dat je altijd ruimte en begrip had voor mijn frustraties, mijn zorgen maar ook mijn geluk. De inhoudelijke begeleiding valt in het niet bij de bijzondere momenten die wij hebben mogen delen. Bedankt dat je mijn getuige was en mijn paranimf wil zijn.

Verder heb ik op Texel de kamer en de snoep-pot mogen delen met Rob van Bemmelen en Steve Geelhoed. Heren, bedankt voor het spotten van bruinvissen en vogels op de dijk. Jullie enthousiasme voor de natuur en het onderzoek is erg aanstekelijk. Rob, bedankt voor al die mooie kaartjes voor mijn manuscripten en dit proefschrift. Steve, jij ook bedankt voor de verbeteringen van mijn stukken en de twee leuke dienstreizen naar Stralsund en Cadiz. Samen zijn wij er achter gekomen dat vele wegen naar Rome leiden, ... ik bedoel naar het Amstel station.

Geert Aarts en Erik Meesters van IMARES waren onmisbaar om de statistiek in dit proefschrift niet te laten eindigen in basis statistiek. Bedankt dat jullie zo veel begrip voor mijn onbegrip hebben gehad en dat jullie mij hebben geholpen om het meeste uit mijn resultaten te halen. Mardik Leopold ben ik dankbaar voor de gezellige bruinvis snijdagen en het eeuwige spoelen en determineren van maaginhouden op Texel. Je hebt een heerlijk duidelijke en kritische kijk op de dingen, en dat waardeer ik zeer.

IMARES wil ik graag bedanken voor de directe en indirecte mede-financiering van mijn promotieonderzoek.

Oscar Bos wil ik bedanken als mijn IJmuiden/Texel lotgenoot en voor de hulp met het maken van een aantal figuren in dit proefschrift. Een aantal ideeën van Bram Couperus zijn cruciaal geweest om de resultaten van het stabiele isotopen onderzoek in context te plaatsen en weer vertrouwen te krijgen in de uitkomsten, bedankt hiervoor. Willem van Duin wil ik bedanken voor zijn onuitputtelijke persoonlijke aandacht en het regelen van kaartjes en cadeautjes voor de collega's. Willem Dekker en zijn moeder wil ik bedanken voor hun advies bij mijn stellingen. David Miller voor de correcties op mijn Engels. De collega's die na de lunch met mij een rondje om de haven liepen wil ik bedanken voor de

leuke gesprekken en de verse zuurstof (nou ja, het blijft IJmuiden). Ik zou geen monsters voor dit onderzoek hebben gehad zonder de inzet van de collega's in IJmuiden, de vrijwilligers en de mensen van het strandingsnetwerk. Daarnaast was de expertise van de pathologen onmisbaar en waren de snij sessies minder leuk geweest zonder de dames van het snij-team in Utrecht. Op Texel heb ik bij een aantal mensen in huis mogen wonen. Hiervoor wil ik graag iedereen bedanken, met name Maarten Brugge, Dennis Waasdorp, Hans Verdaat, Esther Koorn en Margot Bik. Bedankt aan alle mede-Potvissers voor de gezelligheid, in het bijzonder Jaap de Vos. Wouter Jan Strietman wil ik bedanken voor de prachtige foto's die hij van bruinvissen heeft gemaakt en die ik in al mijn presentaties en ook in dit proefschrift mocht gebruiken.

During my stay in Halifax and Liège, the analyses were supervised by Sue Budge (Dalhousie University, Department of Process Engineering and Applied Science in Halifax, Canada), Krishna Das and Gilles Lepoint (University of Liège, Laboratory of Oceanology, Mare Center in Liège, Belgium). Sue and Krishna, thanks for letting me stay at your houses for a few weeks. Thanks to Sue and Damian for taking me out on the whale field trip, this was a lot of fun and the most wonderful encounter with whales I have ever made. Without the dedication, help and good humour of Loïc Michel I would probably still be stuck in the lab in Liège. Thanks also to the other students at the lab, especially to Aurélie Dupont for taking me out of the lab for activities, to Christelle Dyc for sharing Krishna's house, and Sara Habran, Dorothee Pete and Joseph Schnitzler for the distraction from work during coffee, lunch, dinner and Belgian beer breaks.

De prachtige cover en opmaak van dit proefschrift heb ik te danken aan Rachel van Esschoten. Mijn 'kindje' was bij haar in goede handen en ik ben haar ontzettend dankbaar voor de organisatie, planning, zorgvuldigheid, creativiteit en het schitterende eindresultaat.

Naast mijn werk heb ik altijd de steun van familie en vrienden gehad. Zonder hen was het mij nooit gelukt om dit tot een goed einde te brengen. Ik wil bij deze graag iedereen bedanken die mij met interesse, flauwe vragen, kritiek en motivatie hebben begeleidt en gesteund.

Mama en Papa, ik ben jullie ontzettend dankbaar voor de steun tijdens mijn studie en dit promotietraject. Ik ben blij dat ik in zo'n liefdevol en beschermd nest ben opgegroeid. Jullie hebben mij altijd de vrijheid en steun gegeven die ik nodig had, zowel emotioneel als door bijvoorbeeld op Femke te passen. Daarnaast wil ik ook mijn oma Ingrid, Imke, Jacobus en Yuki bedanken voor hun interesse en steun. Dank ook aan Klaas, Paula, Hilde, Dorien, Jolt, oma en beppe.

Lieve Maarten, ik heb vaak grapjes gemaakt dat je eigenlijk geen bijdrage hebt geleverd aan mijn proefschrift, maar dat is niet juist. Zonder jou zou dit boekje hier vandaag gewoon niet liggen. Je bent een onmisbaar persoon in alles wat ik doe en daardoor ook in mijn werk. Je hebt mij geholpen om niet in de

stress van mijn promotie te verdrinken, je hebt alle begrip getoond voor mijn frustraties, mij geholpen om het einddoel voor ogen te blijven houden, te relativeren en hebt waar mogelijk ruimte gecreëerd om aan dit proefschrift te werken. Daarnaast hebben wij vooral veel mooie momenten samen beleefd die niets te maken hadden met dit werk, een uitstekende balans die nodig was om deze promotie af te ronden.

Lieve Femke, je bent nog zo klein, maar je hebt een grotere invloed gehad op dit werk dan ik ooit had kunnen bedenken. De meningen over het krijgen van kinderen tijdens een promotietraject zijn verdeeld. Door jouw geboorte heb ik eerst een aantal maanden vertraging opgelopen. Sinds jij bij ons bent valt echter alles op zijn plek en is stress over het werk maar relatief. Daardoor heb ik in het laatste jaar ontzettend veel werk kunnen verzetten en heb ik die verloren maanden makkelijk weer ingehaald. Het spijt me dat je daardoor zo vroeg naar de crèche moest, maar ik ben ontzettend blij dat we niet op jou hebben gewacht tot na mijn promotie. Lieve meid, je bent zo'n vrolijk, blij, gezellig en ondeugend ding. Je bent een lang gekoesterde wens die meer dan in vervulling is gekomen.

Naast blijdschap heeft het afsluiten van deze periode ook een bittere bij-smaak. Ik ben verdrietig dat mijn opa, Kurt Jansen, mijn promotie niet meer mee kan maken. Als er iemand was die mijn eigenaardigheden goed kon begrijpen dan was jij dat. Jouw onuitputtelijke interesse in mijn werk en het geloof in mij, hebben mij altijd enorm trots gemaakt. Ik weet dat jij dit proefschrift met plezier van voor naar achter door zou hebben gelezen. Lieve opa, ik mis je enorm.

## **Colofon**

### **Graphic design cover and inside:**

Rachel van Esschoten ([www.divingduck.nl](http://www.divingduck.nl))

### **Photography:**

Luc Hoogenstein: cover, p. 7, p. 19

Wouter Jan Strietman: p. 39, p. 55, p. 67

Steve Geelhoed: p. 87, p. 103, p. 137

Marijke de Boer: p. 119

### **Printed by:**

Ipskamp Drukkers, Enschede ([www.ipskampdrukkers.nl](http://www.ipskampdrukkers.nl))



