Agenda Item 3.4

Investigation of the Health, Nutritional Status and Diet of Harbour Porpoises (Action 10)

Information Document 3.4

The Feeding Ecology of the Harbour Porpoise *Phocoena Phocoena* L. in a Changing Environment

Action Requested

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Belgium

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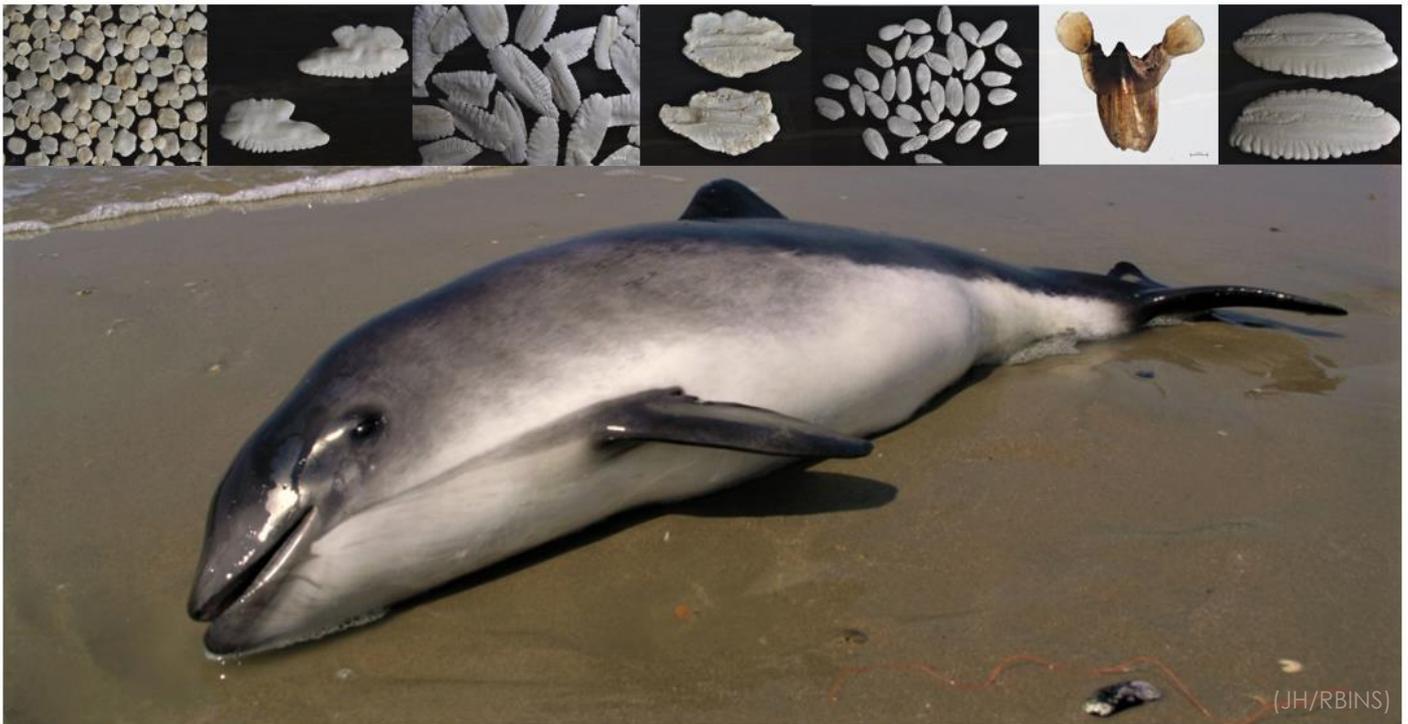
OCEANS & LAKES
Master of Marine and Lacustrine Science and Management

(JH/RBINS)

THE FEEDING ECOLOGY OF THE HARBOUR PORPOISE
Phocoena phocoena L. IN A CHANGING ENVIRONMENT

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June 2020

Thesis submitted in partial fulfillment for master's degree in Marine and Lacustrine Science and Management

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ABSTRACT

The stomach contents of 180 harbour porpoises (*Phocoena phocoena*) stranded or bycaught along the Belgian coastline between 1997 and 2018 were analysed to reconstruct the diet and study the factors shaping their feeding ecology. This was investigated combining two techniques used in diet studies: population averages (i.e. diet indices) and analysis of individual variation in the diet through multivariate analysing techniques (nMDS and PERMANOVA). More than 25 fish and invertebrate prey taxa were identified, highlighting the broad prey spectrum that these generalist predators can feed on. However, the majority of porpoises consumed between 1 and 4 prey groups. The diet was primarily dominated by four key prey guilds (i.e. "The big four"): gadoids (mostly whiting *Merlangius merlangus*), gobies (*Pomatoschistus sp.*), sandeels (*Ammodytes sp.*) and clupeids (both herring *Clupea* harengus and sprat *Sprattus* sprattus), whilst other taxa were of less importance. Harbour porpoises mainly consumed small prey species or the juveniles of larger sized gadoids (e.g. Atlantic cod). Even though the optimal foraging strategy expresses the need for porpoises to strive to feed on mainly prey with a high energetic return (i.e. high-quality prey), they do not solely feed on prey that are most profitable in Belgian and surrounding coastal waters (i.e. sandeels and clupeids) and tend to eat a considerable amount of relatively low-energy lean prey (i.e. gobies and gadoids). Our analysis recovered an ontogenetic development in prey choice, with juveniles eating mainly small lean gobies as opposed to larger gadoids, possibly complemented with energy rich sandeels in adult porpoises. Ample seasonal variation in the diet was also recovered, clearly linked to the changing availability of the different prey groups throughout the year in the Southern part of the North Sea. Our study could neither confirm nor reject an opportunistic or selective foraging strategy, though we present slightly more evidence for the former, with porpoises feeding on locally abundant and easy-to-access prey species. Though, more quantitative data on resource availability, especially for pelagic species, is needed in our waters to confirm or reject opportunistic feeding behaviour. A relatively low amount (15%) of empty stomachs was recovered during this study and preliminary analysis suggested that the highest chance of starving in harbour porpoises can be ascribed to juveniles during the summer months.

Key words: *Phocoena phocoena*; diet; individual variation; stomach content analysis; multivariate analysis; empty stomachs

ACKNOWLEDGEMENTS

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INTRODUCTION

HARBOUR PORPOISES IN THE SOUTHERN NORTH SEA

Apex predators, such as marine mammals, exert an important top-down control and play a crucial role in the ecosystem functioning and shaping of the food-web in marine systems[1–3]. Knowledge on the status of these top predators is imperative in order to have an idea on the status and health of the ecosystem and, as such, these predators are used as sentinels for monitoring aquatic ecosystem health[4,5].

Harbour porpoises (*Phocoena phocoena*) are among the smallest odontocetes, with sizes less than two meters and a mass of less than 100 kg[6]. Their small size enabled them to colonise waters rich in food, such as shelf seas and shallow coastal waters, at the higher cost of managing their energy budget. With a rapid life history and a small energy stocking capacity, they comprise one of the metabolic extremes amongst cetaceans[7]. Nevertheless, they inhabit the cold waters of the sub-arctic up to the temperate Northern hemisphere[8,9]. As a predominantly coastal dwelling species, harbour porpoises occur close to the coast and on continental shelves in depths under 200m. Though, offshore movements to deeper oceanic waters have been observed, which are mostly seasonal[10].

Harbour porpoises, like all marine mammals, contain a subcutaneous blubber layer or 'blubber coat' meant to keep them warm in the marine environment. Due to their smaller surface-to-volume ratio and thus a higher propensity to lose heat, harbour porpoises constantly have the need to manage their energy budget and avoid extended periods of fasting[11–14]. They adjust their blubber thickness throughout the year by regulating their energy intake. A seasonality in the thickness of this insulation layer was noticed, with thicker blubber coats found in winter as opposed to summer[11–13,15]. This because in autumn and winter they consume more food than is needed to compensate for the extra endothermic cost associated with colder water temperatures[7,16,17]. When food is lacking or scarce, porpoises can theoretically survive several days without feeding, only relying on their stored fat. Yet, they can suffer from hypothermia before these reserves are used up due to the cold waters surrounding them[13,14].

Porpoises thus have a high caloric demand in order to deal with the high endothermic costs, which is higher in winter due to colder water temperatures[2,7,16,17]. In order to survive in these colder waters, harbour porpoises are evolutionary adapted to a life constructed of short-term feast and famine periods[18]. They must feed frequently on large quantities of food or high quality prey every day relative to their body weight (Fig.1) to overcome energetic challenges[19,20]. These extreme traits and requirements make harbour porpoises very prone to starvation[6,21–23].
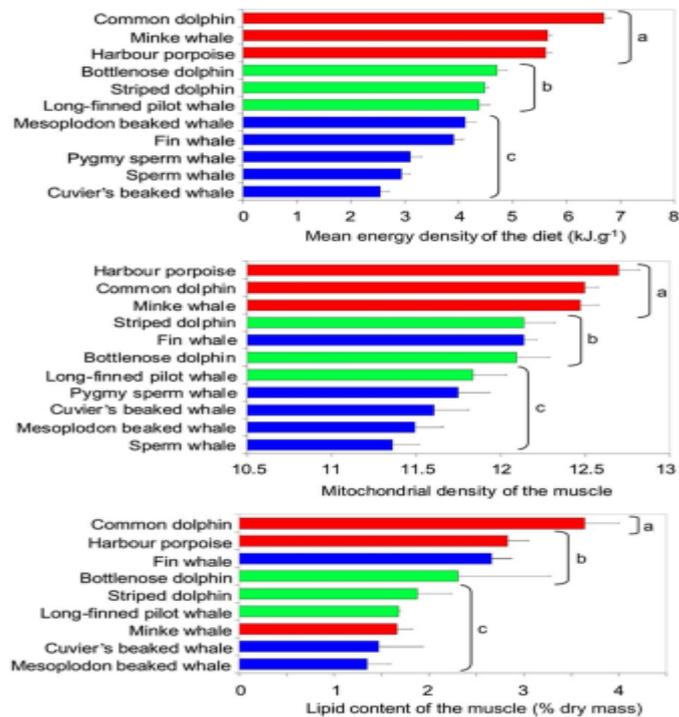
Figure 1: Graph showing the differences in energy requirements in different cetacean species, using calculated mean energy densities of the diet and two proxies for metabolic cost of living. Three significant groups of cetacean species can be distinguished corresponding to a, b and c in all graphs. Group a contains species with a high-quality diet (first graph), mitochondrial density (second graph) and a high lipid contents (third graph) and group b and c contain respectively species with a medium and low-quality diet, mitochondrial density and lipid content. The colours represent groups of similar quality diets. Note the position of the harbour porpoise in all graphs. (Spitz *et al.*, 2012)

The highest harbour porpoise densities are found in and around the North Sea, where they present the most common cetacean species with an estimated population size of over 345.000 individuals[24–30]. In the Belgian part of the North Sea, the abundance can seasonally be as high as 10.000 individuals[31,32]. Through the completion of small-scale surveys complemented with major aerial and shipboard surveys (SCANS –Small Cetaceans Abundance in the North Sea project), an increase in the harbour porpoise population in the Southern North Sea was found[32–34]. As the total population size did not change significantly, this increase seemed to be the result of a large-scale shift in the summer distribution of harbour porpoises (Fig.2) from the north-western North Sea to its south-eastern parts[25]. In 1994, most of the porpoises were concentrated in the north-western North Sea and along the West coast of Germany and Denmark, whilst being almost absent in the Southern North Sea (Fig.2, left panel)[25,35]. In 2005, most of the porpoises were found in Southern parts of the North Sea, especially in Dutch and Belgian coastal waters (Fig.2, right panel). This coincided with an increase in harbour porpoise strandings in this part of the North Sea, especially along the French to Dutch coastal zone[24,31,36,37]. This distributional shift was attributed either to changed environmental conditions, which indirectly affected prey availability, or to an indirect decline of available prey in the north, possibly as a consequence of climate change, that led these porpoises to depart their normal overwintering area because of food shortages[30,35,38–43].

As a consequence, these animals might be sentenced to eat 'junk food' (i.e. prey of a lower nutritious value) in order to prevent starvation as they shifted from regions with a high abundance of high-energy sandeels or herring to regions with high abundances of leaner gobies, gadoids and even flatfish[16,38,44,45]. This junk food for wild animals comprises the exact opposite of human junk food (i.e. fatty), as it indicates prey with too few fat and energy to maintain a good body condition[46].
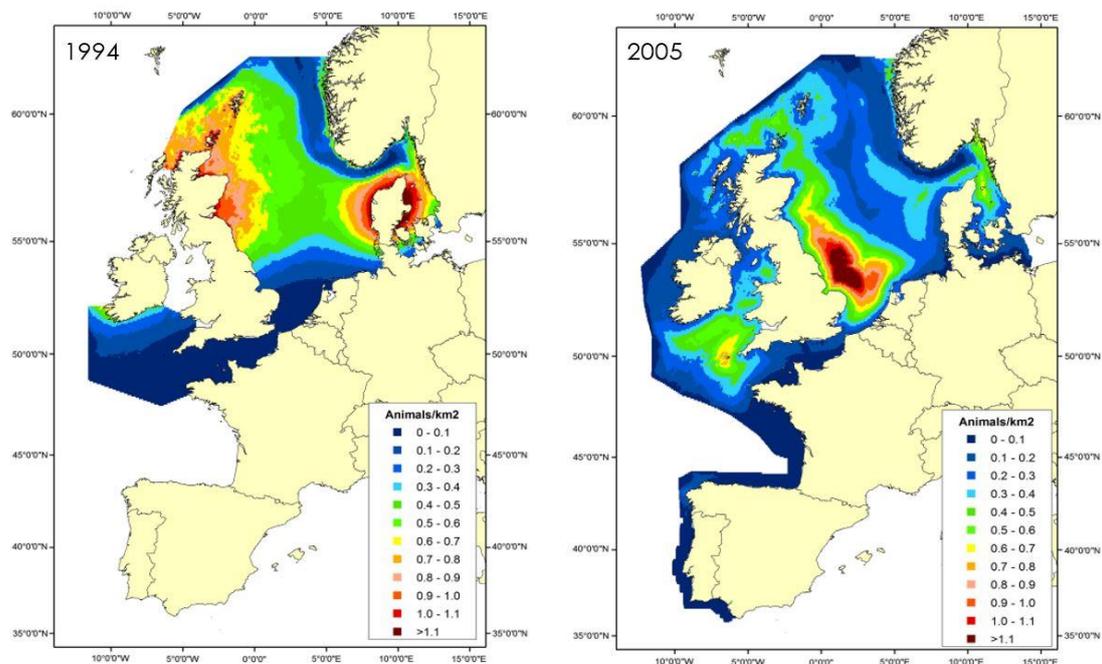
Figure 2: Density surface models of the harbour porpoise in 1994 compared to 2005 as predicted from respectively the SCANS-I and SCANS-II survey, displaying the distributional shift from a northerly concentration of porpoises in the 1994, to a more south-eastern distribution in 2005. (Modified from Hammond *et al.,* 2013)

Whilst many environmental variables structure harbour porpoise's habitat preference, their distribution and movement behaviour is most likely and ultimately directed by prey availability and its patchy distribution[9,47–51]. Though, it is certain that shifts in prey availability directly or indirectly played a role, evidence of the actual reason is lacking as our current understanding of prey resources in the southern North Sea is still limited[52]. After a virtual absence since the 1950s, porpoise strandings in Belgium increased from a few porpoises per year in the early 1990s, up to an average of more than 100 per year during the last decade[27,53]. Now, porpoises can be observed all year round in the Southern North Sea, with seasonal peaks in the late winter and late summer[28,54].

Throughout its distribution area, harbour porpoises are filed as threatened or vulnerable[55]. Due to their predominantly coastal distribution, concerns have been raised about their status and the sustainability of populations in the North-Atlantic due to the exposure to many natural and anthropogenic pressures. Several potential threats have been identified, with incidental bycatch remaining one of the major factors causing population decline and mortality in harbour porpoises throughout the North-Atlantic[5,55–57]. Though, numbers of bycaught animals might even be an underestimation as there are many biases associated with the identification of bycatch[58–62].

Fisheries using different types of gears are operating in the North Sea and adjacent waters, with bottom-set gillnets targeting demersal species posing the largest direct threat to harbour porpoises in these waters[63]. To verify the scope and the sustainability of incidental removals of harbour porpoises in fisheries, adequate information such as the abundance, natural mortality and the numbers of individuals removed as bycatch must be known. For example, in the "Agreement on the Conservation of Small Cetaceans of the Baltic and North Sea," member states are advised to reduce bycatch of cetaceans to below 1.7% of the best population estimate available[56,64]. Hence, highlighting the importance of efforts to estimate the abundance and distribution of harbour porpoises throughout the North-East Atlantic[25]. Additionally, fisheries can cause local depletions of key prey species, resulting in a decreased foraging efficiency of this apex predator. This constitutes a higher risk for smaller marine mammals such as porpoises, as they need to balance their energy requirements on a shorter spatial and temporal scale[65]. Next to the decrease in prey quantity, a decrease in prey quality by fisheries poses an additional threat[5].

Other anthropogenic and natural threats to harbour porpoise populations in the North-Atlantic include noise pollution (e.g. through pile driving in wind farms, vessel traffic and seismic surveys), changes in prey availability through a combination of overfishing and climate change related effects, marine litter and chemical pollution[34,38,39,61–74].

FEEDING ECOLOGY

The harbour porpoise's diet is mainly inferred from stomach content analysis of stranded or bycaught animals, using undigested prey remains such as otoliths, jaws, vertebrae and squid beaks in order to reconstruct their short-term diet and as such they still play a key role in understanding the feeding ecology of marine mammals and looking at long-term trends[29,127–129,138].

According to the *optimal foraging theory*, predators with a high cost of living should favour prey that provide them with more energy than they spend on catching and handling it[20,84]. Prey differ substantially in quality, with lean prey such as whiting containing 4.2 kJ/g wet mass as opposed to 7.6 kJ/g wet mass in fatty fish like sprat and these parameters varying with season and prey body size[85]. This theory is supported by some diet studies worldwide, showing that fatty schooling roundfish often compose a large part of the porpoise's diet[39,42,49,86]. Though, many diet studies showed that fatty fish are not the only prey they take. Dozens of other fish species, including leaner prey, are targeted, hampering the answer to the question whether harbour porpoises are selective or opportunistic feeders.

Most studies conclude that harbour porpoises take a mixture of high energy and leaner prey species, instead of only targeting prey that hand them the most energy causing harbour porpoises to be described as generalist or opportunistic predators, ingesting a wide variety of small schooling pelagic, demersal and even benthic fishes[13,14,42,76–89]. Next to the quality of ingested prey, the abundance and availability of prey in general also is an important factor for the survival of porpoises, with quality affecting their fitness in a more drastic way[85,97–100]. Yet, diets made up of exclusively lean prey might have serious repercussions for porpoise health.

This *junk food hypothesis* highlights the importance of some fatty prey in the diet spectrum and a diet of solely 'junk food' potentially leading to nutritional stress even when they can feed continuously on this leaner prey[38,46,85,86,97–101].

Irrespective from geographical and temporal variations, the most commonly encountered prey types in the harbour porpoise's diet in the North-East Atlantic include gadoids like Atlantic cod (*Gadus morhua*) and whiting (*Merlangius merlangus*), clupeids like sprat (*Sprattus sprattus*) and herring (*Clupea harengus*), sandeels (family Ammodytidae) and different goby species (family Gobiidae) with the latter being a key group depending on the region[90,95]. Occasionally, the diet is complemented with different crustaceans and cephalopods[81,82]. The diet of harbour porpoises throughout the North-East Atlantic exhibits ample variation between different regions, seasons and time periods as these predators need to feed on the available prey where and when they are foraging[80,87,90,91,102–104]. Some regional differences and similarities in the North-East Atlantic exist, with the most striking difference being the differential importance of more "qualitative" prey in the different areas. For example, clupeids and sandeels form an important part of the harbour porpoise's diet in respectively the waters around Sweden and Scotland as opposed to the dominance of leaner prey in the Southern North Sea[14,29,31,38,39,75,76,79,86,87,99-103]. Seasonal changes in the diet have been ascribed to the seasonal movements of harbour porpoises between inshore and offshore waters in combination with the specific life cycles of the different prey species. In addition to changes in prey composition, prey size and fat content (energy content) can also differ seasonally with differences in the size of the prey eaten throughout the year[106].

In our study area, the Southern North Sea, the prey consumed is dominated by one to four prey species, and at least one of these has a high energy density[13–15,75–77,82,85–88]. These key species include clupeids and sandeels and are found as prey in porpoise diet studies throughout the area, though not making up the dominant fraction[45]. As such, porpoises don't seem to restrict themselves to energy-rich high-quality prey, as large portions of their intake are dominated by rather lean prey such as gobies, gadoids or even squid[16,38,45,82,90]. A seasonality in their diet was observed as well, with the lowest proportion of energy rich prey in summer diets of porpoises bycaught and stranded along the Dutch coastline between 2006 and 2014 (based on examination of 829 stomachs)[107]. Additionally, analysis of 381 harbour porpoise stomachs showed the dominance of gadoids throughout the year with the highest contribution in autumn, the higher frequency of occurrence of sandeels and estuarine roundfish in summer, the scarcity of high-energy prey like herring in autumn and the highest contribution of gobies in autumn[45]. Thus, the summer was identified as being a time of scarcity in Dutch coastal waters, with a short supply of high quality prey and insufficient availability of gobies which are most frequently taken by juvenile harbour porpoises[45,107]. In combination with the blubber thickness being at its lowest during summer, a higher risk of starving during these summer months was observed[45,101].

Inter-individual diet variation has been examined in Dutch waters as well, with a focus on ontogenetic diet shifts and bycatch related diets. As illustrated by some studies in the Dutch and Belgian part of the North Sea, gobies tended to dominate the diet of calves and juveniles as compared to adults, which ate bigger fish and had a bigger variety of prey species in the stomach[16,82,106–108].

Explanations for this ontogenetic shift range from the more offshore feeding by adults, adults outgrowing the small and lean food source gobies present as they need a higher energetic return as they grow, up to gobies being an abundant and easy-to-catch food source for inexperienced young porpoises[45,107]. Thus, as porpoises get older they tend to shift their diet from gobies in calves and juveniles to sandeels and clupeids in mid-sized porpoises, and gadoids and pelagic roundfish in very large porpoises[45].

Differences in the diet between males and females are less frequently reported. Leopold *et al.*[45] only found slight diet variability between male and female porpoises in Dutch waters, with adult males taking relatively more clupeids and sandeels compared to females, which take more gadoids and other pelagic roundfish. It is hypothesized that these sex-related differences might be biggest when females are nursing calves, due to their increased energy demand and different foraging behaviour.[21,82] Sexual dimorphisms in the growth of harbour porpoises has been found, with adult females tending to grow larger than adult males, causing a difference in their energetic needs, potentially affecting their diet choices[109].

It is important to also take the cause of death into account as the analyses are performed on dead animals and this could be a confounding factor in diet studies[82]. Stranded porpoises could thus be samples of ill or injured harbour porpoises and bycaught animals could represent healthy samples, stressing the importance of a correct bycatch identification in post-mortem studies[61,110]. A number of defining characteristics to identify bycatch already exist, such as a good nutritional body condition and a full stomach[111,112]. Recently, it has been shown that porpoises dying of different causes also exhibited characteristic prey compositions. Identifying bycatch in porpoises thus becomes easier, as the prey consumed are indicative of the fishing gear used and its place in the water column[113]. Fisheries in the SE North Sea are dominated by bottom-trawling and bottom-set nets, explaining why stomach contents of bycaught porpoises predominantly contain demersal fish[56,61,114]. Though, some prey species (clupeids or sandeels) display a daily vertical migration, and as such they can be abundant near the seafloor during the day, complicating the assessment of bycatch in stranded harbour porpoises[61].

RESEARCH FRAMEWORK AND RELEVANCE

Due to their importance as apex predators, harbour porpoises are considered an 'species of conservation interest' in different international, regional, European and national conservation instruments, such as the EU Habitats and Species Directive (92/43/EEC), CITES, Bern Convention, Bonn Convention, ASCOBANS and the IUCN Red List of Threatened Species[5,55,114].

Furthermore, a plan was adopted in 2009 by the contracting parties of ASCOBANS, aimed at restoring and maintaining harbour porpoise populations at a favourable conservation status. This *Conservation plan of the harbour porpoise in the North Sea* identified some main threats that harbour porpoises are facing and translated these into a series of actions. To meet requirements of these conventions and regional agreements, resources are allocated towards prioritised fields of investigation[5].

The investigation of the health, diet and nutritional status of the harbour porpoise is one of the actions (action 10) written out under the framework of the conservation plan to assess harbour porpoise population health and to evaluate it in the future. Such investigations can aid us in understanding distributional changes of porpoises and even their prey species, as well as identifying the pressures causing these changes. Eventually, predictions can be made on the possible effects of climate change and even overfishing on harbour porpoise populations[56,114].

Hence, understanding the harbour porpoise's diet can contribute considerably towards the understanding of how the southern North Sea and the Belgian coastal waters are supporting the increasing numbers of this species[16,83].

SPECIFIC OBJECTIVES OF THIS STUDY

This study aimed at assessing the seasonal, temporal and individual variation in the diet of the harbour porpoise *Phocoena phocoena* stranded or bycaught along the Belgian shoreline between 1997 and 2018 in order to:

1. Determine the preferred prey species in the Belgian part of the North Sea and whether they feed on the richest food source that is available in our waters.
2. Assess individual variation in the diet and whether a selective or an opportunistic feeding strategy is adopted. Or, in other words, investigate which factors dictate what individual porpoises could, and should eat[107].
3. Ascribe these dietary differences and/or preferences to distinct behaviours and relate them to variation in the stranding data to explain, for example, the high number of male porpoises stranding in our waters[36].

MATERIAL AND METHODS

COLLECTION AND PREPARATION STOMACH CONTENTS

To determine the diet of harbour porpoises in the Belgian part of the North Sea, 180 stomach contents were obtained from harbour porpoises washed ashore or bycaught along the 65 km Belgian shoreline between 1997 and 2018, of which 126 stomach contents were analysed during this thesis and complemented with data of 54 previously analysed samples. Since 1991, fresh stranded porpoise carcasses are collected by the Royal Belgian Institute of Natural Sciences (RBINS) and transferred to the University of Liège to be subjected to an extensive and standardised necropsy[115,116]. Porpoises which were too decomposed (decomposition code 4-5, as defined in the European Cetacean Society decomposition condition code system[115]) were sent away for destruction. During this necropsy, the first and second stomach of the harbour porpoises was cut open and checked for lesions, parasites and prey items. The stomach contents were collected for further analysis: the stomachs of porpoises with decomposition code 1-3 were almost always collected, unless they were empty. After removing the oesophagus and stomach as a whole from the carcass, it is cut open and the content of the oesophagus, the first and second stomach were rinsed over a square meshed sieve with a 315 µm mesh size. As such, the complete rinsed content was collected for further analysis. In rare cases, the whole stomach (including the stomach wall) was obtained. In seven cases prey remains were present in the oesophagus, they were noted and added to the stomach content. Afterwards, the content or full stomach was frozen (-20°C) awaiting further treatment and analysis.

Haelters *et al.*[16] was consulted regarding the protocol on stomach sample preparation. After a visual examination of the stomach content, the freshness of the prey remains was subjectively noted through the use of several categories (fresh pieces of fish, grey mass of digested fish remains and hard remains with few tissue). To prepare the stomachs for further processing and the determination of prey species, the stomach content was macerated with enzymatic washing powder. Rinsed stomach contents were transferred to plastic beakers to which Biotex Green™ or Biotex Blue™ was added. Biotex is a brand of washing powder containing enzymes that break down and dissolves organic matter and tissue. The closed plastic containers with the stomach content and Biotex were placed in a warm water bath with a constant temperature of around 40°C. Depending on the size of the sample and the amount of soft tissue, the maceration took between 3 days to a week. To speed up the process, the sample was rinsed and sieved multiple times during the maceration period. Finally, the remaining content of the beaker was rinsed over a 315 µm sieve. Sieving in combination with flotation ensures that the remaining digestible tissue, Biotex, or sediment could be discarded. Next, the rinsed content was transferred to a recipient and rinsed with ethanol in order to disinfect, eliminate smell and quick-dry the sample. In the end, a clean sample remains, including otoliths, fish bones and invertebrate remains such as cephalopod beaks (Fig.3). Cephalopod beaks were picked out and stored in 70% ethanol to prevent from deforming.

Figure 3: The end-product of the maceration process is a somewhat clean sample with fish bones, invertebrate remains and otoliths. Otoliths in samples are easily identified due to their brighter white colour.

A total of 180 stomachs were analysed, consisting of 3 neonates, 140 juveniles and 37 adults. The sex of six porpoises could not be determined and the sex ratio (M:F) of the remaining porpoises was 1.23:1 (n=174). Figure 4 gives an overview on the porpoises chosen in this thesis. Most of the stomach samples were from porpoises stranded or bycaught in winter (n=89) and spring (n=43), together comprising 73.3% of the samples with a smaller amount of samples derived from summer and autumn (respectively n=31 and n=17).
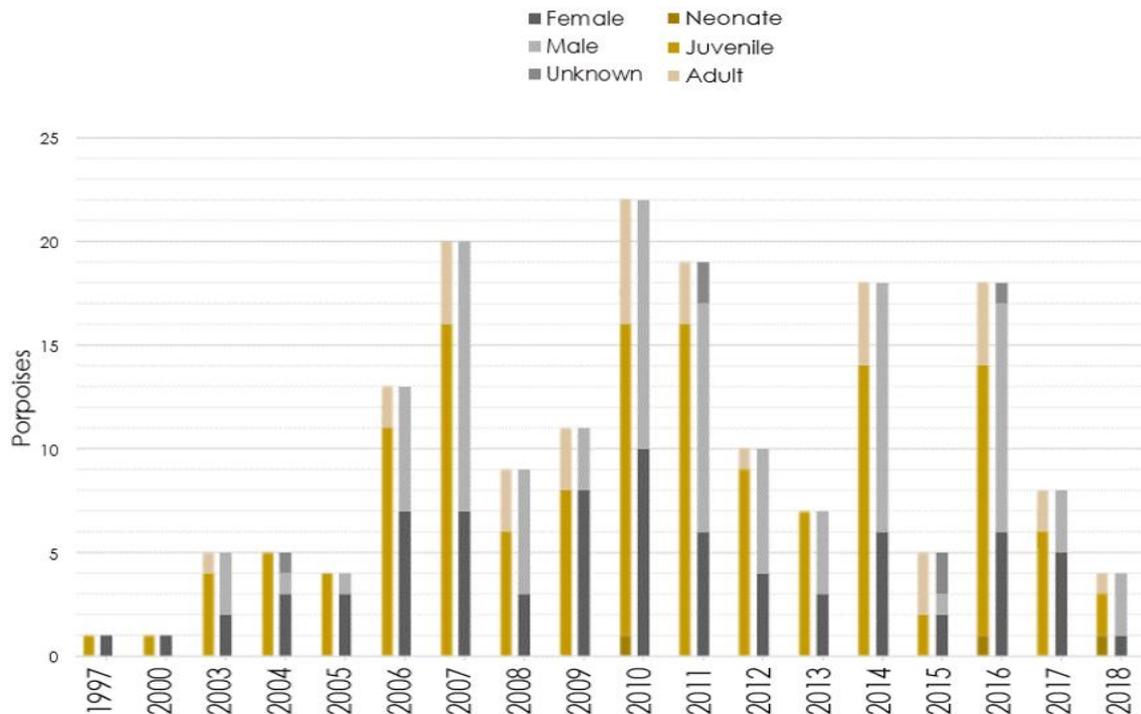
Figure 4: General overview of the 180 porpoises studied in this study with porpoises grouped per year according to their sex (orange shades) and life stage (grey shades).

The dorsal blubber thickness, as measured a few cm distally from the dorsal fin during the necropsies, can be used as a quantified proxy for the nutritional condition of the porpoises[15,117]. The thickness of the blubber layer tends to decrease with advancing decay, and as such it was only measured in porpoises with a decomposition code between 1 and 3. The blubber thickness was measured for 174 out of the 180 analysed porpoises. A summary of the porpoises, as well as additional data such as their sex, age class (juvenile/adult), cause of death, blubber thickness and a general description of the stomach content, are listed in Table 1 (Annex).

ANALYSIS OF THE STOMACH CONTENTS (SCA)

To determine the porpoise prey composition, the dried remains extracted from the stomachs were searched for characteristic structures using a stereo microscope with a SMZ25 Nikon camera attached (with a maximum magnification of maximum 315X).

The vast majority (99%) of all identified prey remains were otoliths. Otoliths, the solid mineralised structures found as a part of the inner ear of fish, are among the most widely used hard structures to identify fish prey in stomach contents analysis[118]. As they are the densest structures found in bony fish (made of aragonite), they are fairly resistant to stomach acid as opposed to fish bones. Additionally, their small size makes that their retention time in the stomach is considerably longer before being transported further down the digestive tract. Otoliths are frequently found in large number in the first stomach. This part consists of many folds, gathering otoliths of fish that might have been digested a long time ago. Each inner ear in fish contains three otoliths, with the saccular otoliths (or sagittae) being the largest (Fig.5)[119]. These sagittae are used to identify prey items in the stomach as their morphology is characteristic for many fish genera and in some cases even the species.
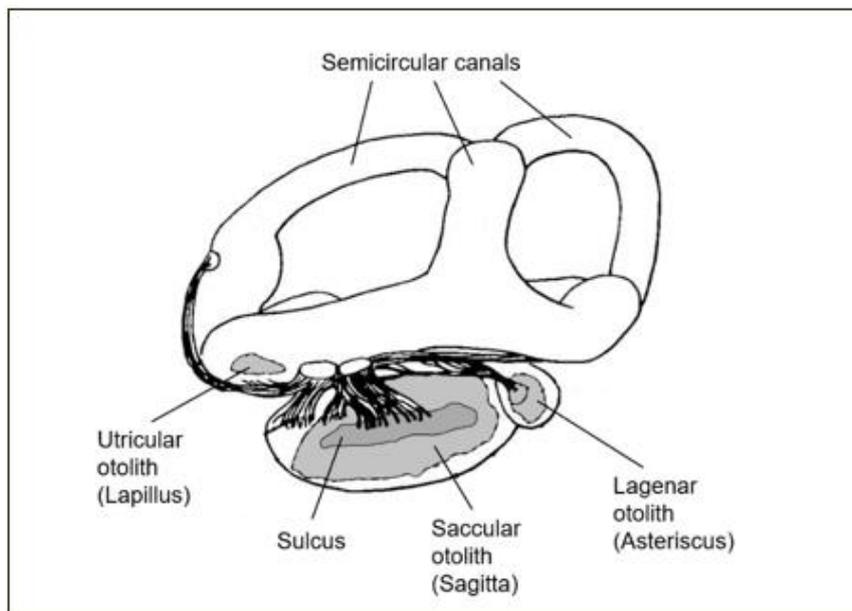
Figure 5: Diagram of one of the inner ears of a fish, with the position of the tree different otoliths. In this study, we will identify saccular otoliths present in the stomach. Figure adapted from Popper & Coombs, 1982.

Additionally, the size of the saccular otolith (length or width) can be utilised to estimate original characteristics of the fish they belonged to, such as the original length and biomass. All saccular otoliths were picked out, coupled into pairs, identified up to highest taxonomic level and measured to estimate original characteristics. Whenever many otoliths of the same species were present, their number was divided by two and rounded up in order to estimate the minimum number of this prey species present in the sample[16]. This was only the case for gobies (Gobiidae) and sand lances (Ammodytidae) as their prey remains often exceeded hundreds or thousands of items. For gobies and sand lances, the identification did not go up to species level, but instead they were classified as respectively *Pomatoschistus* sp. and *Ammodytes* sp. Brill/turbot otoliths were identified as *Scopthalmus sp.* as all otoliths originated from juveniles.

To count and measure all the otoliths of a species (or a subsample when there were too many), the Nikon's imaging software NIS-Elements (version 5.00) was used. Tools from the tab "Annotations and Measurements" were used to simultaneously count the otoliths and measure their length (Fig.6). In case of broken otoliths, the width of the otolith was measured.
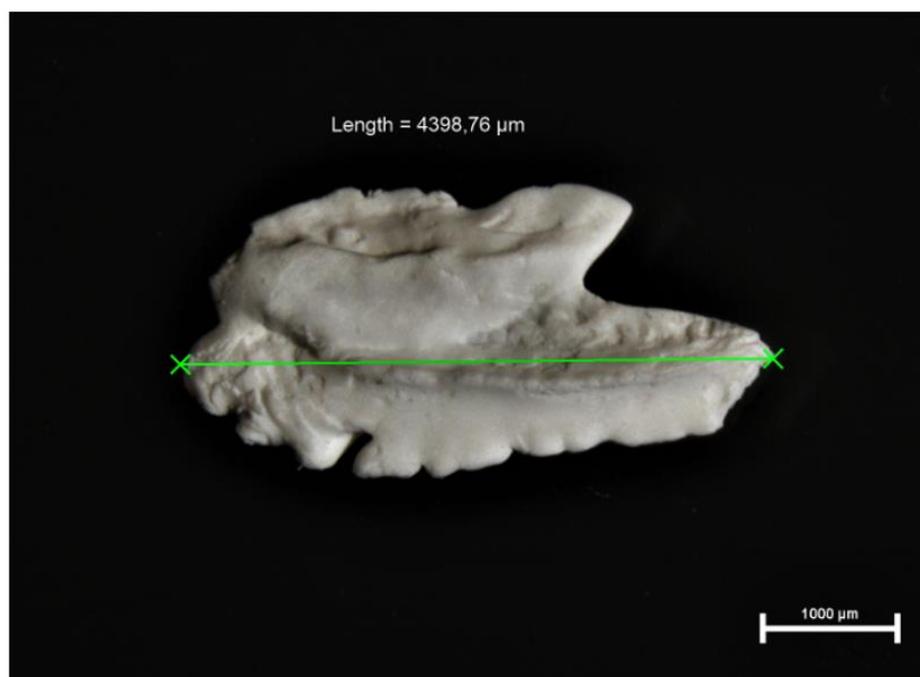
Figure 6: Example of a measurement of a slightly eroded herring (*Clupea harengus*) otolith using the measurement tool in the NIS-Elements imaging software. All measurements are saved and exported in text files. *format for*

As the stomach comprises an acid environment in which ingested prey is quickly digested, otoliths are also subject to chemical and mechanical erosion. Furthermore, some otoliths are more prone to this erosion than others[120,121]. In an attempt to tackle this, all otoliths are graded for wear and their size corrected, before calculating the prey's original characteristics (i.e. size and weight) to avoid underestimation[93]. The amount of wear of each otolith was assessed by ascribing an erosion factor to each otolith based on (the lack of) visual characteristics.

This was done according to Leopold *et al.*[93], with fresh un-eroded otoliths receiving no correction and otoliths of all species except whiting with slight, moderate or severe wear receiving a correction factor of respectively 1.05, 1.1 and 1.2. Correction factors for whiting were determined to be respectively 1.06, 1.14 and 1.24. After applying an erosion factor on the otoliths, the average length of two otoliths was taken when they clearly belonged to a pair, having belonged to the same fish. In case no pairs were formed (mostly with Ammodytidae and Gobiidae), the mean of all otoliths lengths was taken, without taking the average length of pairs of otoliths.

Other items, including fish bones such as vertebrae and some head bones, were used to complement the saccular otoliths in identifying different prey species or to confirm their presence, especially in case of heavily eroded otoliths[122]. For example, dragonet otoliths (*Calionymus sp.*) are rarely found in stomach content samples. Though, the preoperculum contains characteristic spines that even allow the distinction between *C. lyra* and *C. reticulatus* (Fig.7)[123]. However, the identification of these other useful bones is specialists work and can be more trustworthy by using a reference collection.

Figure 7: An example of used head bones and vertebrae to identify prey species. The top right corner shows the caudal vertebra and premaxillary of a *Calionymus sp*. The middle shows the coupled preopercula found in the same sample and showing the four characteristic spines, allowing us to identify this as *Calionymus lyra*. The scale in both pictures represents 1000 μm.

Next to otoliths, the bones most often used for identification of prey species in this thesis were the premaxillary, maxillary, dentary, preoperculum, cleithrum and vertebrae (Fig.8)[124].
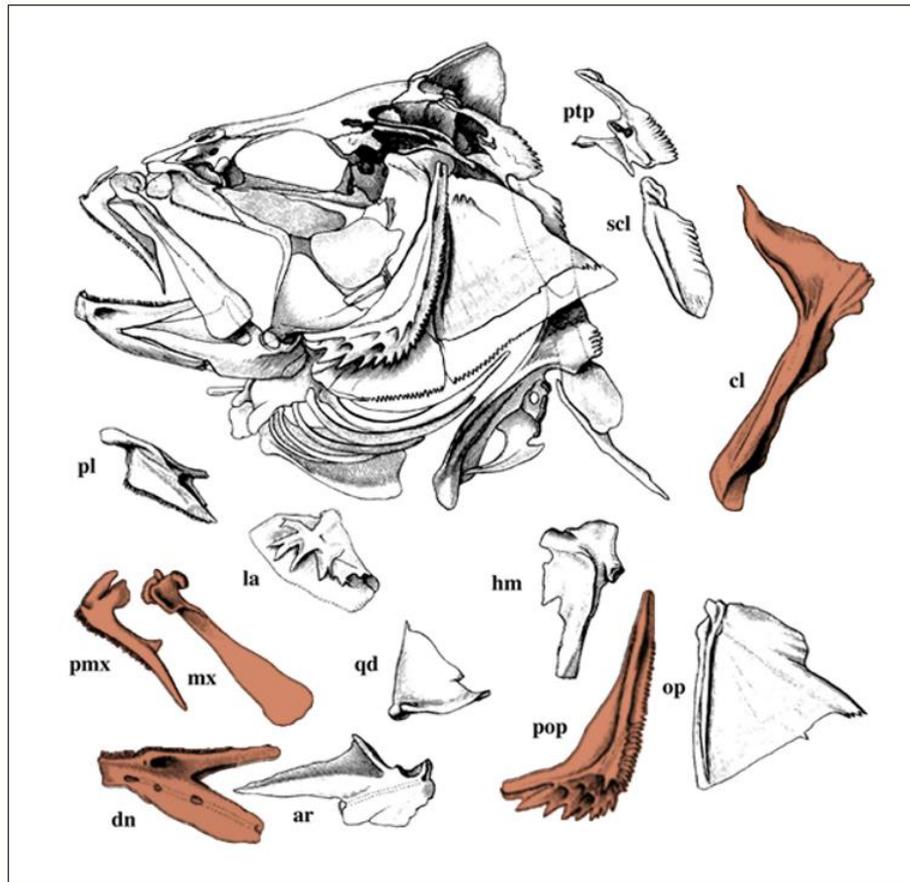
<u>Figure 8:</u> Skull of perch *Perca fluviatilis* displaying the numerous head bones that can be used for species identification as well as estimating original characteristics of the fish they belonged to. The head bones used for identifications during this thesis are highlighted [pmx: premaxillary, mx: maxillary, dn: dentary, pop: preoperculum ad cl: cleithrum]. Figure adapted from Muséum national d'Histoire naturelle – Osteobase : www.mnhn.fr (original from Cuvier & Valenciennes, 1828).

Cephalopods were identified based on their beaks and in one rare case on the gladius (*Loligo sp.*). The remains of polychaetes concerned chitinous jaws but were not further identified. For practical reasons, unidentified otoliths were left out of the analysis. In case of doubt, the sagittae and other bones were compared to a reference collection of fish bones of species commonly found in the Southern North Sea (which was prepared from fresh fish in 2011 by Jan Haelters and available for consultation at RBINS Ostend). The literature utilized for otolith and additional fish bones identification was Watt *et al.*[125], Leopold *et al.*[122], Tuset *et al.*[126], Svetocheva *et al.*[127] and Camphuysen & Henderson[123]; that on cephalopod beaks was Witteveen *et al.*[128], Xavier *et al.*[129] and Clarke[130]. A summary of the identified prey species that were included (fish and cephalopods) and left out (crustaceans and other invertebrates) of the analyses can be found respectively in Table 2 and 3 (Annex).

Stomachs were identified as being empty when there were absolutely no hard remains left in the sample, when there were few hard remains (yet no otoliths) and when fewer than ten otoliths were found in total belonging to one or multiple species if the total average reconstructed prey mass was less than 20 grams. In case only gobies were present, the stomach was classified as empty when the total reconstructed biomass did not exceed ten grams, regardless of the number of otoliths. Out of the 180 examined stomachs, 27 were found to be empty (15%).

ESTIMATING ORIGINAL CHARACTERISTICS

Otolith length is a good measure to reconstruct original characteristics of the fish such as fish length and fresh weight[131]. The regression models put forward by Leopold *et al.*[122] were used to estimate fish size and fresh biomass. The first regression of otolith length/width vs. fish length was under the form of $FL = a + bX$, with FL the original fish length, a and b the species-specific parameters (that differ when otolith length or otolith width was used) and X the otolith length/width. To estimate the fresh weight, regressions between fish length and fish weight were used under the form of $FW = (aX)^b$, with FW the original fish weight, a and b the species-specific parameters and X the fish length as estimated from the first regression.

For Gobiidae and Ammodytidae otoliths, regression coefficients of respectively *Pomatoschistus microps* and *Ammodytes tobianus* were used, as they are the most encountered species in each of the two families. The original length of squid prey in the stomach was not quantified.

Additionally, original prey size can also be estimated from allometric relationships between multiple fish bones and fish length, as obtained through reference collections[125,131]. This was outside the scope of this study and all fish lengths were obtained from otolith lengths. In the rare case that no otoliths were found, but a head bone or vertebra of a species was found, De Pierrepont *et al.*[132] was consulted and the length and mass of this prey individual was taken as the mean length or weight of this species found in stomach contents of seven harbour porpoises stranded between 1998-2003. When this prey species was not found in the seven analysed stomachs, the mean length and weight was taken from 26 analysed stomach contents of common dolphins or five analysed stomachs of grey seals. For example, the weight of the pelagic roundfish *Scomber scombrus* was taken from De Pierrepont *et al.*[132] as for this species only skull bones were found (premaxillary and dentary). The mean mass of squid prey was identified in the same manner using De Pierrepont *et al.*[132].

EXPRESSING PREY COMPOSITION: PREY GUILDS & DIET INDICES

In order to better understand potential selective behaviour and foraging decisions in marine predators, Spitz *et al.*[84] argued for grouping prey species into ecological guilds in diet studies rather than phylogenetically. Though their study primarily focussed on discerning dietary variation between multiple related predators, this recommendation is also applicable for understanding intra-specific differences in prey selection. Fish and squid species were subsequently grouped into nine prey guilds chosen as a compromise between taxonomical relation and ecological similarity. This mostly due to the limited ability to identify all otoliths up to species level (as is the case for most goby and some sandeel otoliths, with their similar size and shape complicating identification) and the lack of ecological knowledge on fish and porpoise behaviour, as well as their feeding ability[107]. Given that there is no evidence for different species of goby being dissimilarly available to a foraging porpoise, we can assume that, due to their similar features, size and caloric value (or energy density), one goby is as good as another, justifying their grouping into one prey guild based on both ecological and taxonomical grounds[84,107].

Similarly, other prey guilds were established with herring and sprat classified as clupeids; whiting, cod and pouts as gadoids; all species of sandeels as sandeels; smelt, sand smelt and European anchovy as estuarine roundfish; rocklings, sea bass and other bottom-dwelling species as demersal roundfish; mackerel and horse mackerel as pelagic roundfish; all different flatfishes as flatfishes and all species of cephalopods as squid (Table.2, Annex). We followed the lumping of prey species in guilds of Leopold *et al.*[107] except for the placement of seabass *Dicentrarchus labrax* which was a part of the pelagic roundfish in the former. As both prey guilds are of rather marginal importance, this change had very little effect on the outcome of the analyses. Clupeids, sandeels, estuarine roundfish (e.g. *Osmerus eperlanus*) and pelagic roundfish (e.g. *Scomber scombrus*) were considered in this study to be energy-rich prey, with more than 5 kJ•g^{-1} wet weight, and the other guilds were considered to be lean prey (<5kJ•g^{-1} wet weight), keeping in mind that these energy densities are not constant between seasons, years and different prey sizes[84–86].

Different indices were developed to measure and express the prey composition in the diet of marine mammals. These are normally used to calculate the relative importance of individual prey species. Though, in this thesis, we focussed on three indices and calculated them for each prey guild in order to evaluate the overall prey composition of the analysed porpoises. These include:

1. The numerical importance of a prey guild *i* in the stomach of a marine mammal. N_i is usually based on the number of otoliths remains per guild. Though, in this thesis, the estimated number of individuals per prey guild in each sample was used.

$$\%N_i = \frac{N_i}{N} \times 100$$ with N the total number of preys

2. Proportion of the prey guild *i* in the stomach by fresh weight, as estimated through regressions based on otolith remains.

$$\%W_i = \frac{W_i}{W} \times 100$$ with W the total estimated biomass of the prey

To acquire the total fresh weight per prey guild in each sample, the raw paired otolith measures were used. The mean otolith length of the pair was used to reconstruct the length of each prey taken and subsequently their mass. As such, the total weight per species could be calculated. When no pairs were formed (in case of too many otoliths), the measurements were ranked from lowest to highest value, and paired accordingly to calculate the associated weight per individual prey. In case of subsamples, a multiplication factor for the total weight was used (depending on whether half or a quarter of the sample was measured).

3. The percentage of stomachs in which prey guild *i* was found, also known as the frequency of occurrence.

$$\%FO_i = \frac{n_i}{n} \times 100$$ with n the total number of stomachs analysed

SOURCES OF BIAS ATTACHED TO METHODOLOGY

The collected stomachs originate from stranded and bycaught animals and as such the sample might be biased towards unhealthy and inexperienced individuals. It is estimated that only a small proportion of dead marine mammals do strand[62,133]. Thus, stranded porpoises are an 'opportunistic' source of data, affected by many factors. The use of only stranded animals inherently introduces bias and results of such analyses should be assessed with caution, both quantitatively and qualitatively[16,134]. Not all stomachs of collected carcasses are preserved, as a function of freshness, possibly introducing an extra source of bias as an incomplete sample of the population is obtained. Though, adding bycaught animals may supply samples of 'healthy' animals, these are also not free of bias with the diet being biased towards the target species of the fishery and juvenile porpoises being more prone to bycatch due to their inexperience[82,110]. Furthermore, stomach content analyses only covers the most recently taken prey, and as such might substantially differ from long-term diet reconstructions (e.g. fatty acid analysis)[16,83]. Both approaches differ in temporal and taxonomic resolution and might highlight differences between ingested and assimilated diets[135]. Additionally, the importance of some prey species might be overemphasized or downplayed due to differential residence and digestion time in the stomach[90,96,121,136,137]. This might lead to an overrepresentation of prey species with larger, heavier and more robust remains as they tend to remain longer in the stomach and are less affected by the acid gastric environment[121].

Studies to determine the rate of digestion beyond recognition between otoliths of different prey species have not been done yet and as such corrections could only be done on otoliths that were present in the stomachs[138]. Some prey items in the sample might have originated from the digestive tract of the prey itself (secondary prey), which is almost impossible to assess as secondary prey can only be identified without a doubt when the remains are found in the stomach of another prey[132]. Finally, some fish species have utricular otoliths of almost the same size as the saccular otoliths, complicating the estimation of prey numbers. This is especially true for different species of gobies[16]. Still, stomach content analysis is amongst the most detailed and most widely used techniques to estimate the diet in marine mammals and seabirds and remains very useful.

DATA ANALYSIS

The feeding ecology was reconstructed from the non-empty stomach data using two different methods: the overall prey composition and relative importance of each prey guild from unweighted population averages (through calculating diet indices) and the individual variation in the diet was investigated using multivariate techniques.

Basic comparisons of the diet using diet indices calculated on both the prey numbers and prey biomass were examined graphically using Microsoft Excel. Other plots were generated in Rstudio using both the lattice and ggplot2 packages[139,140]. Statistical analyses and coding were performed in the Rstudio environment under R version 4.0.0. Links between porpoise characteristics (porpoise weight and blubber thickness) were examined statistically using non-parametric tests (Spearman's rank correlation, Wilcoxon rank sum test and k-sample Kruskal-Wallis Rank test) as the data did not

comply with the assumptions of parametric tests, and just barely did after transformation. Significant results of the non-parametric tests were interpreted graphically and afterwards a Dunn's test was performed for pairwise comparisons (using the Bonferroni correction method) to examine the true differences between the different groups.

The main statistical analysis was performed on the reconstructed biomass data of the different prey guilds, as the prey number data is dominated by small and easy-to-catch prey species which potentially contribute little to the ingested prey mass or energy[107]. After filtering out the empty stomachs, samples of unknown sex and samples of underrepresented years (two samples from respectively 1997 and 2000) and life stages (three neonates), 155 samples remained as input for multivariate statistical testing. A non-parametric multivariate statistical test known as Permutational Multivariate Analysis of Variance (PERMANOVA) was chosen based on exploratory analyses and performed on the reconstructed biomass data of the different prey guilds (after log transformation and using the Bray-Curtis dissimilarity measure). Based on the outcomes of the exploratory analyses, four categorical predictors were chosen to be tested: year, season, sex and life stage. The fifth predictor, possible bycatch, was left out of the analysis due to the collinearity between season and possible bycatch.

The PERMANOVA test was performed using the adonis2 commando from the package 'vegan'[141-143]. This adonis2 commando uses type I tests (calculating sequential Sum of Squares) for the PERMANOVA analysis, and as such the order of the predictor variables matters. To determine the order of the predictors, we performed a marginal test using the adonis2 commando. Here, each predictor is tested one on one with the response variable by controlling for the other predictors. The predictors were ordered in the final PERMANOVA test based on the p-values of the marginal test.

The PERMANOVA test allows us to see significant differences between groups, but it does not distinguish between differences due to the factor effects or dispersion (variance). As such, a significant PERMANOVA can have multiple explanations: 1) The difference between the groups is due to a true shift in community composition 2) The difference between the groups is due to a difference in the variability of the community composition or 3) Both. Subsequently, a PERMDISP analysis was performed to test homogeneity of multivariate dispersion using distances among centroids calculated within groups that were significant in the PERMANOVA. The PERMDISP analysis has been written to only accept one grouping variable, hence the test was performed for different factors separately. A non-significant PERMDISP signifies equally dispersed distances to the centroids, hence the difference in community composition can be ascribed to the factor effect[144].

There is no function available yet to perform pairwise tests within each significant factor for the adonis2 commando. Thus, significant PERMANOVA results were interpreted using graphs. Furthermore, we performed a model selection by calculating the small-sample equivalent of the Akaike Information Criterion (AICc) for different PERMANOVA's in order to keep the simplest model, which still explains a large part of the observed variation. First, we tested the original model with four predictors against models where each time one of the predictors was removed.

Hence, the model with the lowest absolute value of the AICc should be kept and the previous step should be repeated until the AICc value remains stable. Though in our case, the model with the lowest AICc required us to remove a significant predictor from the model. Thus, we chose to keep the original model as the difference between the AICc values of the original model and the remaining models was negligible (<2). Visual representation of the porpoises with various diets was achieved by non-metric multidimensional scaling of log transformed reconstructed biomass data of the different prey guilds for non-empty stomachs.

Multiple categorical predictors were included in the statistical model to try to uncover what structures the diet of harbour porpoises in Belgian waters. These include season, year, lifestage and sex. Due to a specific collinearity between possible bycatch and the factor season we could not include it as a factor in our model.

PREY AVAILABILITY IN THE BELGIAN PART OF THE NORTH SEA

To investigate whether harbour porpoises are selective or opportunistic foragers, data on the prey availability is needed. Prey preference was investigated using raw unpublished data on the density per 100 m^2 of the most dominant prey species (gadoids, gobies, sandeels and clupeids) in Belgian waters, based on the average of seven coastal samples (3 m beam trawl-small meshed net, Simon Stevin) collected in the framework of the FWO B-FishConnect project (unpubl.data K. Hostens).

RESULTS

OVERALL PREY COMPOSITION

The majority of remains found in the stomachs belonged to fish. During this thesis 28.668 fish prey remains were analysed and identified and combined with 16.924 previously analysed fish prey remains. The amount of prey items was not equally distributed among the different prey species, with high numbers of prey items in a stomach sample often related to large quantity of goby remains. Other remains belonged to cephalopods, crustaceans and polychaetes and were present in 21% of the analysed stomachs, mostly in small quantities (<10 individuals). We identified 25 different fish species amongst the prey remains, belonging to 17 different families. An overview of identified fish and invertebrate prey species can be found in Table 2 and 3 (Annex). The porpoises in this study contained on average prey remains of 2.4 different fish species (SD 1.7), with both adults and juveniles containing 1 to 7 species/stomach.

Table 4: The relative importance in the overall diet of the studied harbour porpoises in Belgium between 1997 and 2018 in decreasing order of importance based on the 3 indices: frequency of occurrence (%FO), Numerical importance (%N) and importance by wet weight (%W). The shaded rows contain prey guilds with high energy densities. Prey mass in grams of wet weight.

Prey Guild	Number of stomachs	Number of prey	Summed prey mass	%FO	%N	%M
Gobies	124	35854	19385	74,70	89,54	18,65
Gadoids	74	676	38629	44,58	1,69	37,17
Sandeels	74	2911	29952	44,58	7,27	28,82
Clupeids	54	219	8301	32,53	0,55	7,99
Estuarine roundfish	21	158	2931	12,65	0,39	2,82
Other demersal roundfish	18	139	901	10,84	0,35	0,87
Pelagic roundfish	7	27	2611	4,22	0,07	2,51
Flatfish	3	6	217	1,81	0,01	0,21
Squid	22	53	994	13,25	0,13	0,96
Totals	**166**	**40043**	**103921**		**100**	**100**

Gobies dominate the diet in terms of prey numbers whereas gadoids and sandeels dominate in term of prey biomass (Table.4). Most of the prey caught by porpoises in this study is both by number (92%) and by mass (58%) dominated by relatively lean prey. Though, energy-rich high-quality prey was found in 58.8% of all the stomachs, and in 63.5% of the non-empty stomachs, albeit in small numbers and/or constituting a small fraction of total sample mass. Looking at contributions to the diet by relative mass, gadoids (mostly whiting), sandeels, gobies and clupeids are of primary importance. Estuarine and pelagic roundfish comprise the second most important group and other prey guilds are of minor importance (combined a little over 2% of total prey mass). Considering the quality of the different prey guilds, sandeels and clupeids gain importance relative to gadoids and gobies. As such, gobies, gadoids, sandeels and clupeids comprise the four key prey guilds in harbour porpoise diet in Belgian waters.

Though gobies, gadoids, sandeels and clupeids were found in a large percentage of the stomachs, their frequency of occurrence differed substantially between adults and juveniles (Fig.9). In stomachs of juvenile porpoises, gobies were the most commonly encountered prey type as opposed to sandeels and gadoids in stomach of adult porpoises.

Furthermore, estuarine species such as *Osmerus eperlanus,* demersal roundfish like *Dicentrarchus labrax* (only remains of juveniles were found) and remains of flatfishes were more present in juvenile stomachs. Additionally, pelagic roundfish like horse mackerel *Trachurus trachurus* were more commonly encountered in adult stomachs.
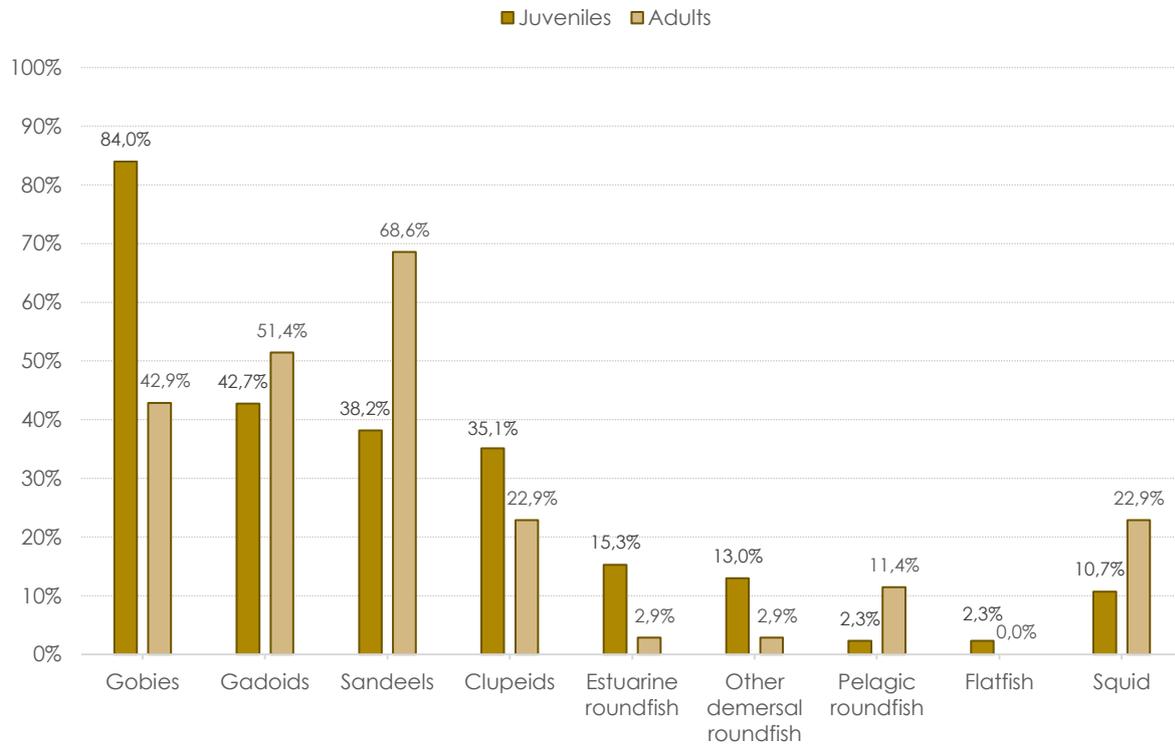
Figure 9: Frequency of occurrence (%FO) of the different prey guilds in the stomach of adult porpoises (n=37) and juvenile porpoises (n=143). The juvenile class includes three neonates.

Numerically, gobies dominated in juvenile stomachs, whilst gobies, sandeels and gadoids were numerically the most important in adults (Fig.10).
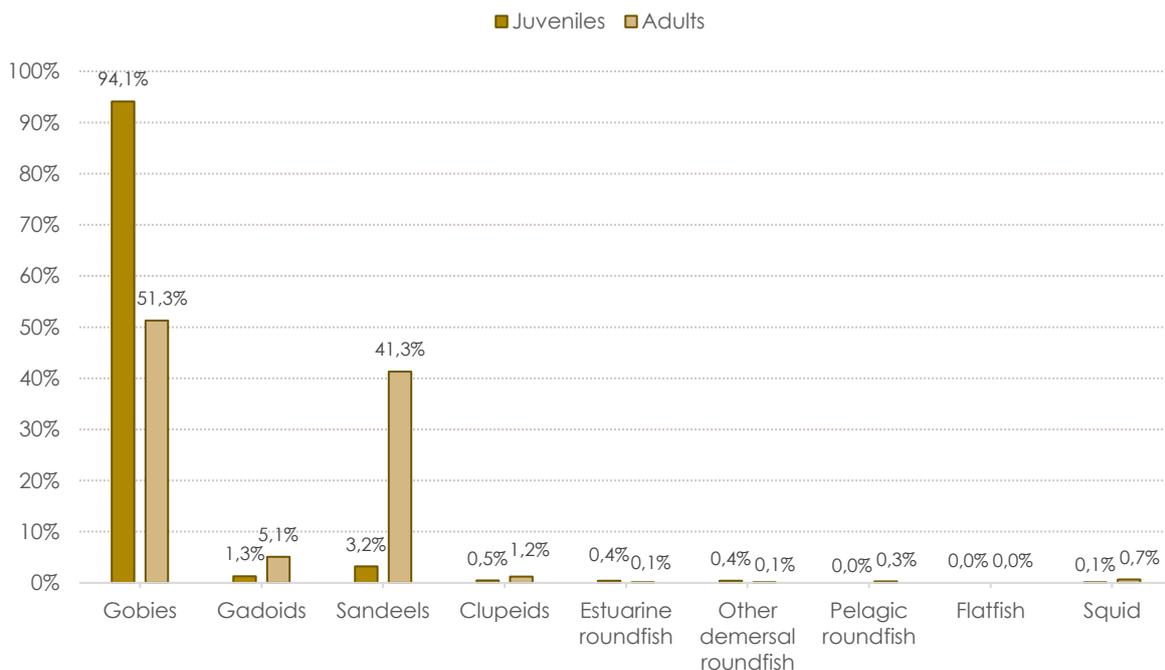
Figure 10: Numerical importance (%N) of the different prey guilds in stomachs of adult porpoises (n=37) and juvenile porpoises (n=143). The juvenile class includes three neonates.

The average reconstructed prey mass in all the stomachs, the adult stomachs and the juvenile stomachs was respectively 0.577 kg, 1.076 kg and 0.448 kg. The most important prey guilds in terms of weight are gadoids, gobies and sandeels in juveniles, whilst in adults this was mostly gadoids and sandeels (Fig.11).
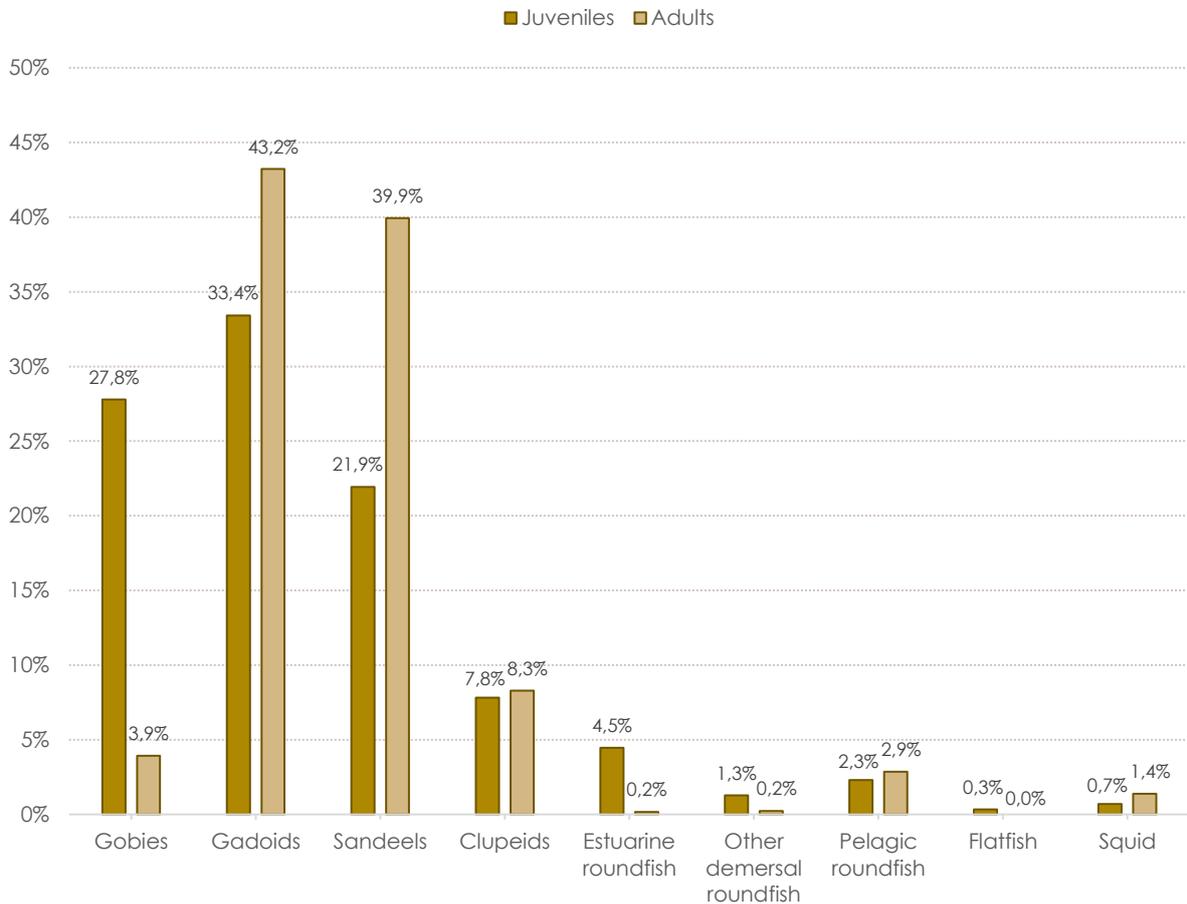
Figure 11: Importance by fresh weight (%W) of the different prey guilds in stomachs of adult porpoises (n=37) and juvenile porpoises (n=143). The juvenile class includes three neonates.

When comparing the prey composition between two time-periods (2007-2011 and 2014-2018) that combined contain 74.4% of the analysed stomachs, the numerical importance pattern was almost identical with gobies dominating in number, followed in lesser extent by sandeels and gadoids (Table.5). The other prey guilds were of minor numerical importance in both periods. Though, the reconstructed prey mass showed a different picture with some slight distinction between the two time periods. Here, the most important prey guilds in terms of weight were the same in both time periods, with gadoids, sandeels, gobies and clupeids comprising the most important groups in decreasing order. However, less sandeel mass and more clupeid and estuarine roundfish mass was consumed by porpoises in the most recent period (Fig.12). The amount of goby and gadoid mass consumed remained constant.

Table 5: Numerical importance (%N) of different prey guilds compared between two time-periods that contained 74.4% of

Prey Guild	%N 2007-2011	%N 2014-2018
Gobies	85,9%	88,0%
Gadoids	2,0%	2,7%
Sandeels	10,6%	7,1%
Clupeids	0,3%	1,0%
Estuarine roundfish	0,2%	0,9%
Other demersal roundfish	0,7%	0,1%
Pelagic roundfish	0,1%	0,1%
Flatfish	0,0%	0,0%
Squid	0,1%	0,1%
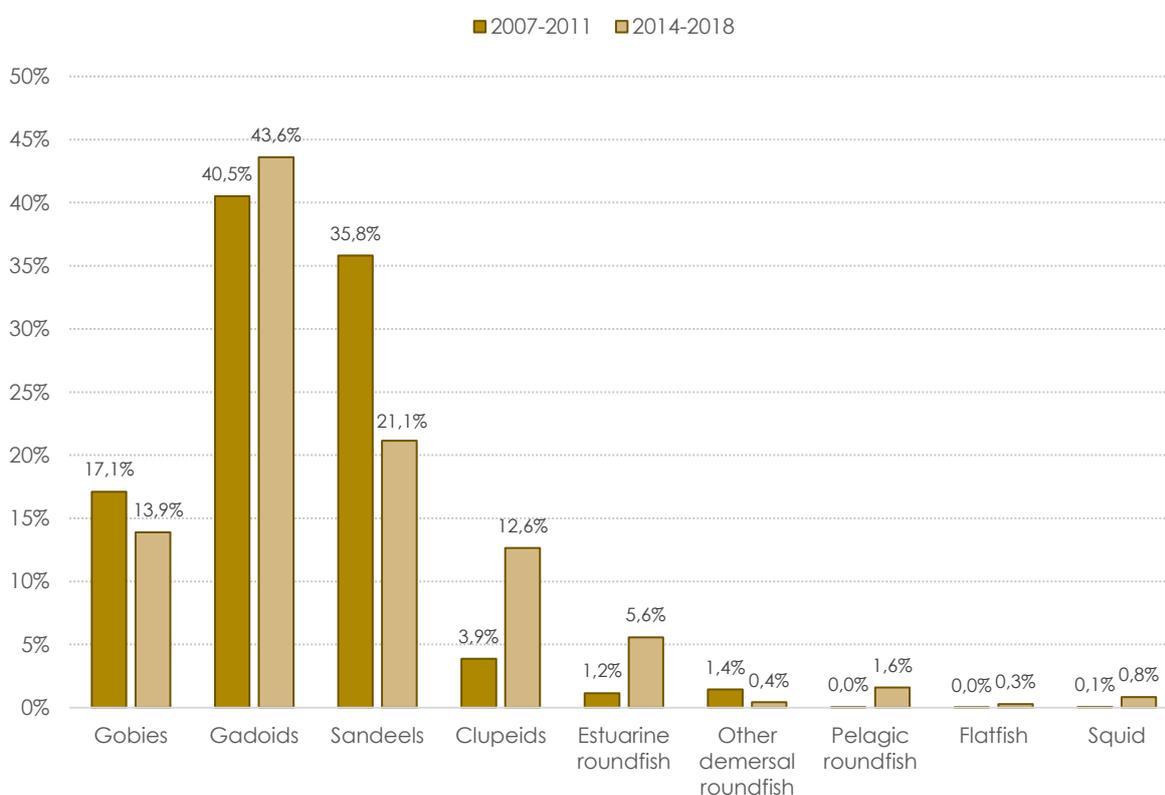
Figure 12: Importance by wet weight (%W) of different prey guilds compared between two time-periods that contained 74.4% of the samples (2007-2011 n=81; 2014-2018 n=53). The overall order of the most important prey guilds remained the same, with differences in the amount of mass consumed for some key prey guilds.

Breaking up overall prey composition (numerical importance as well as importance by biomass) by season, gave a contrasting importance of certain prey guilds during each season (Fig.13 ; Fig.14). The fraction of adults in winter, spring, summer and autumn was respectively 18.6%, 15.7%, 25.8% and 41.2%, thus mostly comparable with the exception of autumn.
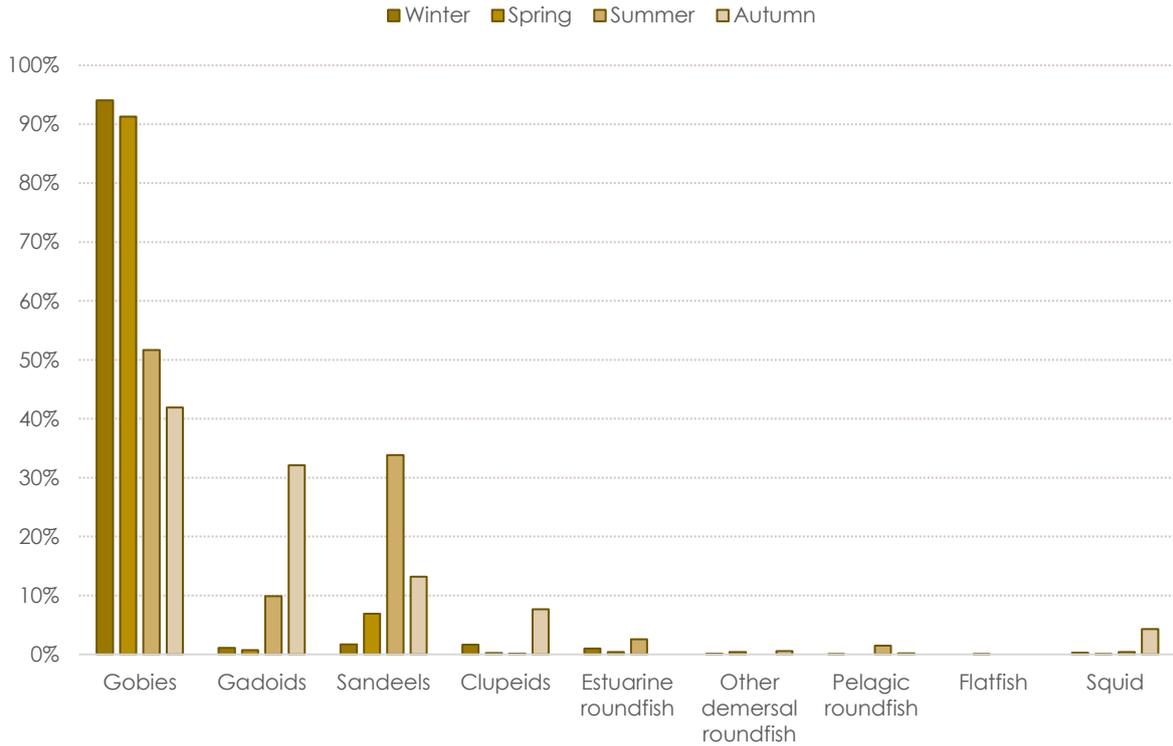
Figure 13: Numerical importance (%N) of different prey guilds over the seasons based on 180 stomach contents (Winter n=43; Spring n=89; Summer n=31; Autumn n=17).

Gobies were numerically more important in winter and spring as opposed to summer and autumn, in which gadoids as well as sandeels gained importance. Clupeids were only numerically important in autumn and estuarine roundfish were numerically most important in summer.
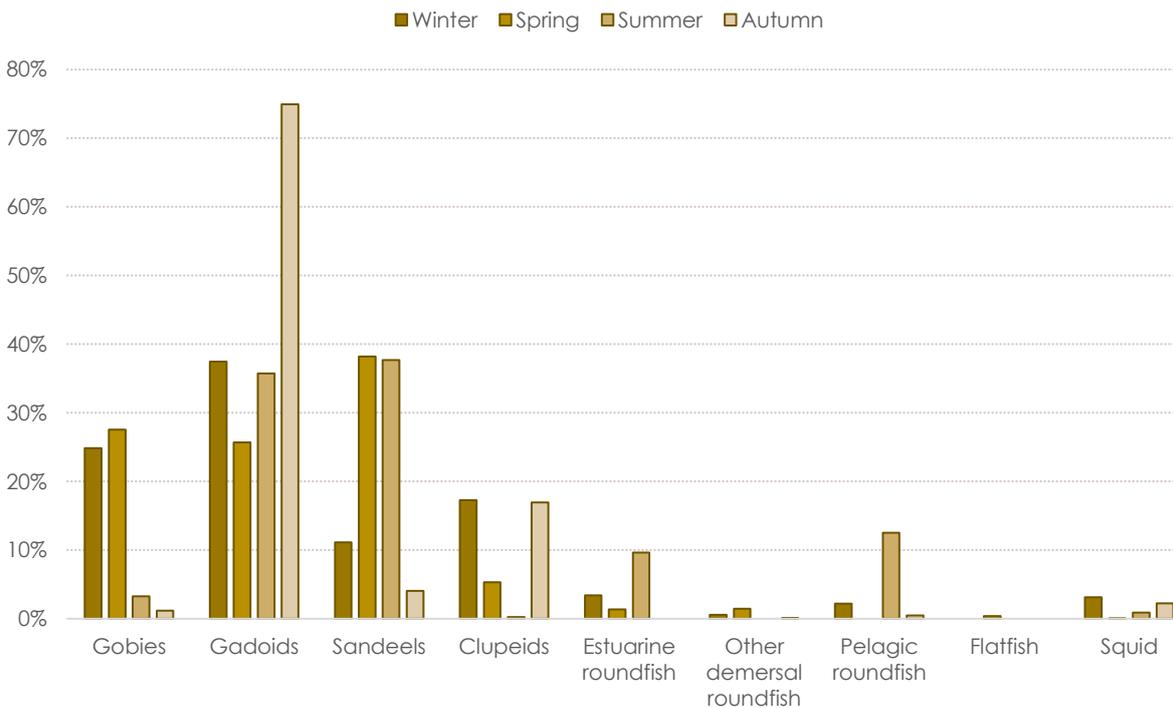
Figure 14: Importance by wet weight (%W) of the different prey guilds over the seasons based on 180 stomach contents (Winter n=43; Spring n=89; Summer n=31; Autumn n=17).

Gobies constituted an important amount of prey mass in winter and spring as opposed to summer and autumn where the contribution of gobies to the prey mass was very low. In Autumn, gadoids dominated the consumed prey mass in combination with clupeids. Energy rich clupeids constituted only a small amount of the prey mass caught in summer. They constituted an important portion of the prey mass in winter and autumn together with gadoids. Though they constituted a small fraction of the prey mass in summer, other energy rich prey guilds were consumed more in summer as opposed to the other seasons such as sandeels, estuarine roundfish and pelagic roundfish. Because the fraction of adults was highest in autumn, caution must be taken when comparing between the seasons.

Overall, relatively small differences can be seen between all males and all females, with the proportion of gobies and gadoids being most distinct: female porpoises consumed relatively more gadoids and less gobies and sandeels when compared to male porpoises, with the other prey guilds being of somewhat equal proportions (Fig.15, above). When comparing between juvenile males and females, the same small differences of the main plot are recovered. This contrasting importance of gobies and sandeels was more apparent when comparing adult males and females (Fig.15, below).
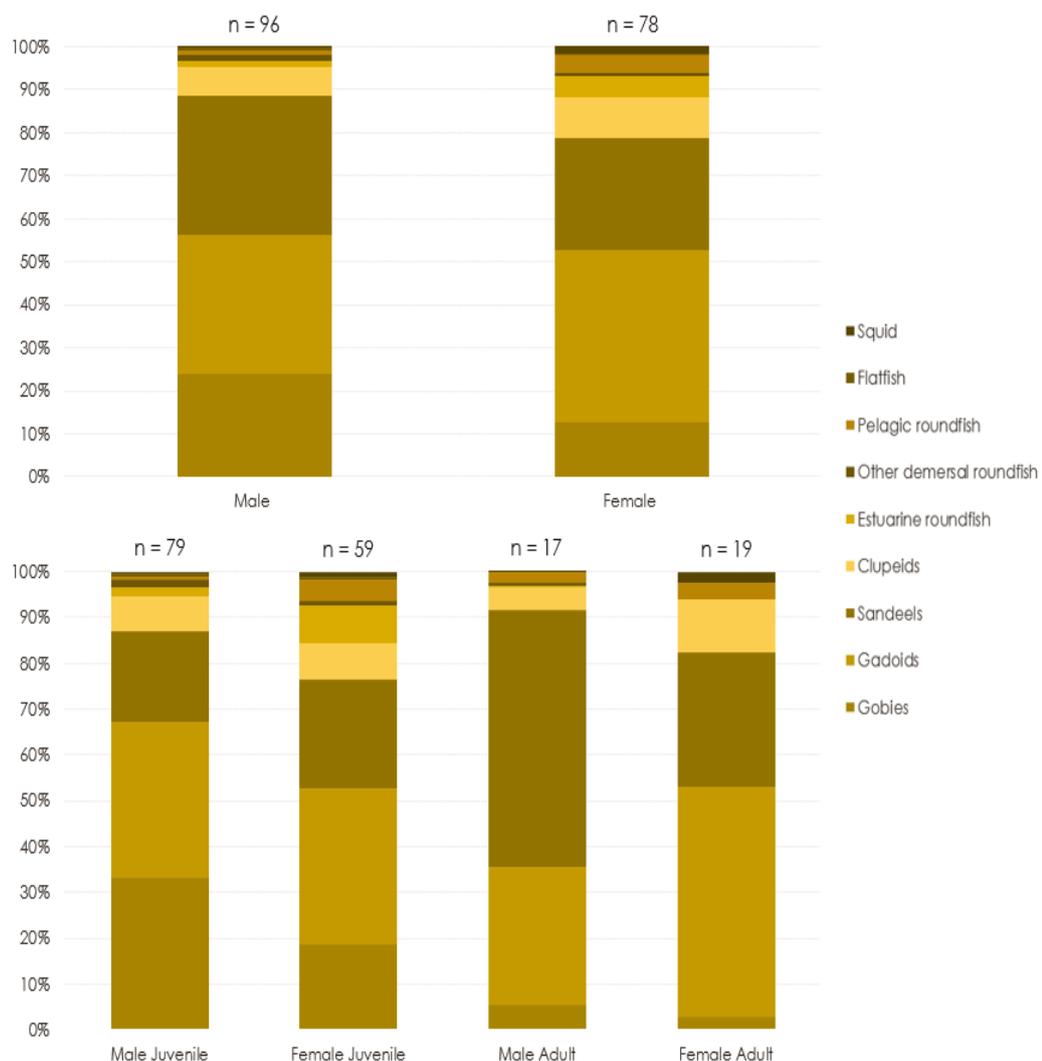
Figure 15: Importance by wet weight (%W) of the different prey guilds per sex (above) and per sex and age group (below). Based on 174 stomach contents (porpoises of unknown sex were left out; n=6).

SIZE AND WEIGHT OF PREY TAKEN

When plotting out the average length of the fish prey in the stomach of each porpoise against the length of that harbour porpoise, it is clear that larger porpoises tend to take larger fish (Fig.16). Though, the average length of samples with gadoid as well as goby remains might be skewed towards smaller sizes, as the small goby remains might have originated from the digestive tract of the larger gadoid prey (secondary prey).
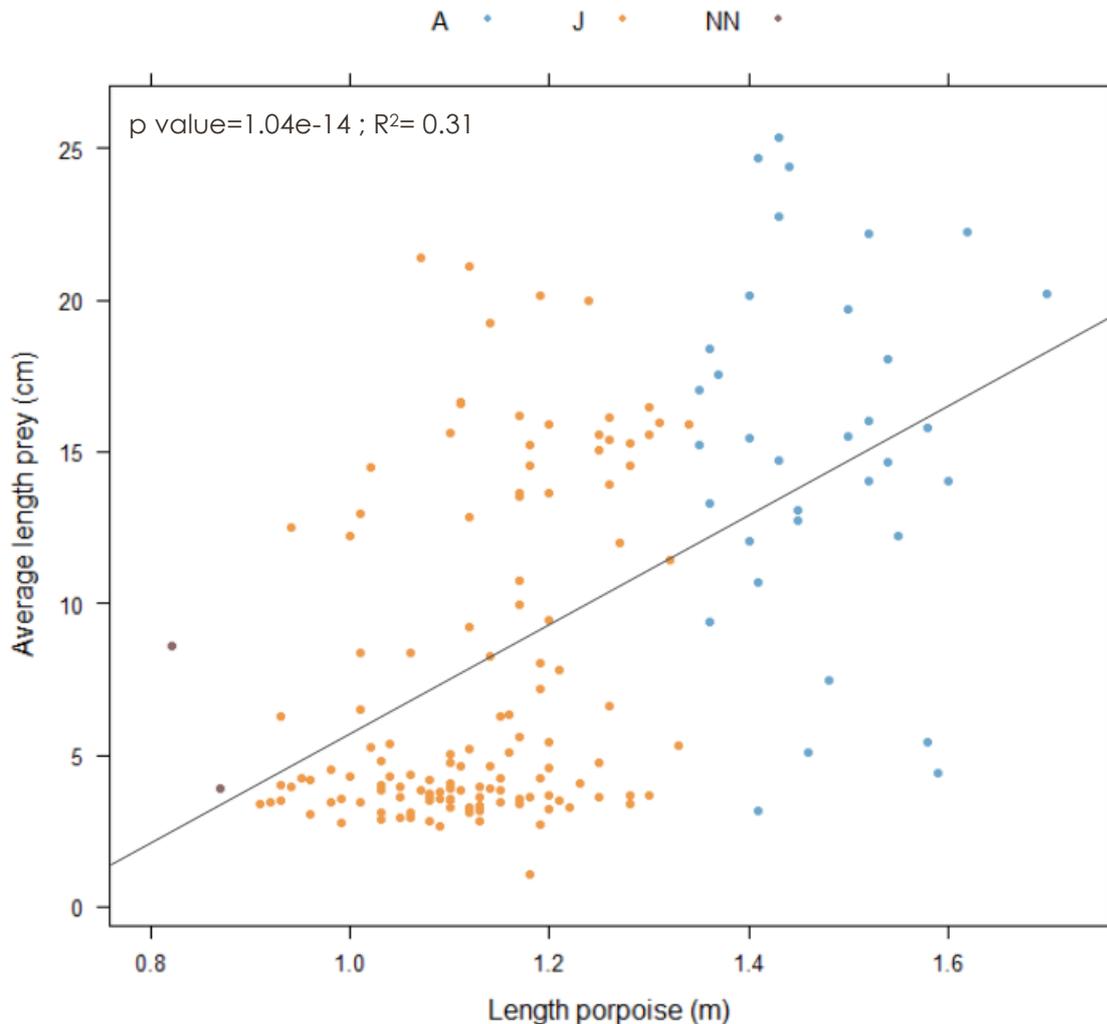
Figure 16: significant relationship between the average length of prey in the stomach of the examined porpoises against the length of that porpoise. Stomachs containing only squid prey remains (n=3) were left out of the analyses, as the original length of squid prey was not determined. The colour code indicates the life stage of the porpoise: adult (A); juvenile (J) and neonate (NN).

Excluding the empty stomachs (n=27), samples contained the highest amounts of reconstructed prey mass in autumn (October-November), whilst in winter (January-February) and summer (June and September) the lowest amount of reconstructed prey mass was present (Fig.17, upper panel).

The overall average reconstructed prey mass for non-empty stomachs was 678.8 gram (SD 1063.9). When comparing the average reconstructed prey mass per month between adults and juveniles, a different pattern is obtained between these two life stages (Fig.17, lower panel). Adult porpoise stomachs contained the highest prey mass in spring (April and May), August and November; as opposed to juveniles, where the highest amount of prey mass was found in October and July. Though, the prey mass might be biased in some months due to the presence of few samples there, as is the case in June, July and the period September-January with less than 10 porpoises sampled in each of these months (together holding just over a quarter of the sampled porpoises).
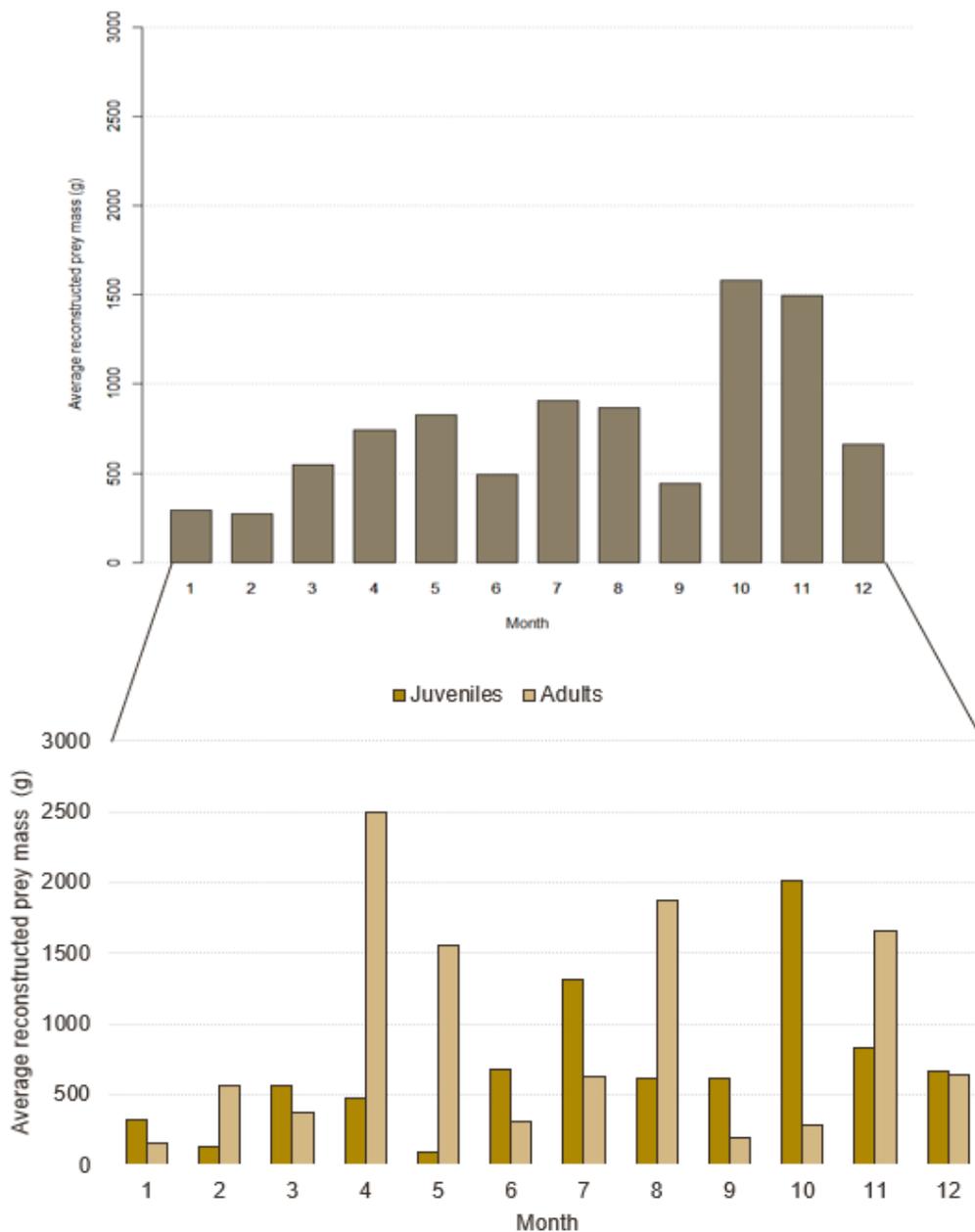
Figure 17: Average reconstructed prey mass of all non-empty porpoise stomachs (n=154) per month in the upper panel, and average reconstructed prey mass per month compared between adult and juvenile porpoises in the lower panel. The juvenile class includes three neonates. The error bars (SD) are not shown as they exceeded the limits of the graph.

CORRELATION WITH PORPOISE CHARACTERISTICS

A significant correlation exists between the weight and blubber thickness of porpoises of similar length (non-parametric Spearman's rank correlation on harbour porpoises with a length between 1.1 and 1.2m ; p = 1.312e-12). Heavier porpoises thus have a thicker blubber layer (Fig.18). Blubber thickness thus comprises a good indicator for nutritional status of the harbour porpoise.
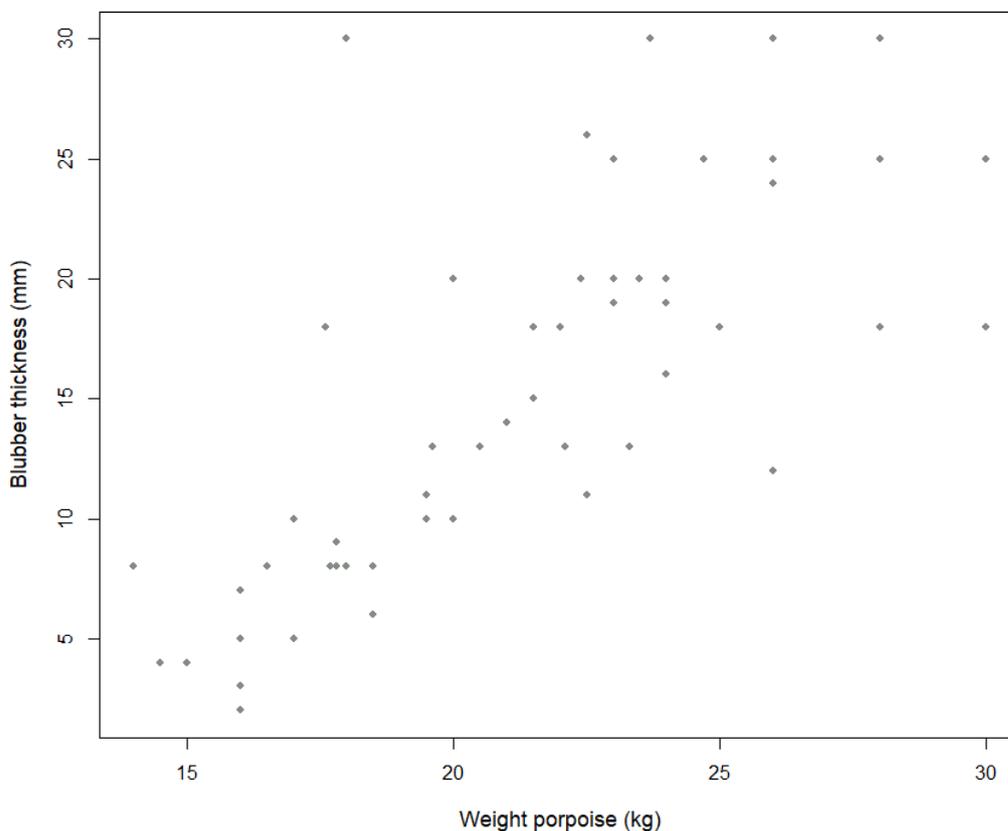
Figure 18: Scatter plot of blubber thickness measurements versus body weight of porpoises with a similar length (between 1.1 and 1.2 m ; n=55).

When comparing this indicator of nutritional status between porpoises with empty (n=26) and filled (n=148) stomachs, a significant difference was found (Wilcoxon rank sum test with continuity correction, p=0.000136, Fig.19). Note, the porpoises of the 'full stomach content class' did not necessarily have a lot of remains in their stomach as it includes all contents that were not classified as empty according to our criteria.

The same was tested between each combination of life stage and stomach content status. Again, a significant difference was found between the different groups (k-sample Kruskal-Wallis Rank test, p= 0.00296). Post-Hoc analysis (Dunn's test of multiple comparisons using rank sums) showed a significant difference between juvenile porpoises with full versus empty stomachs which was not found in the adult class (Fig.20, above). This is confirmed when making boxplots, showing a more outspoken difference in blubber thickness between porpoises with an empty versus a full stomach content in the juvenile class (Fig.20, below).
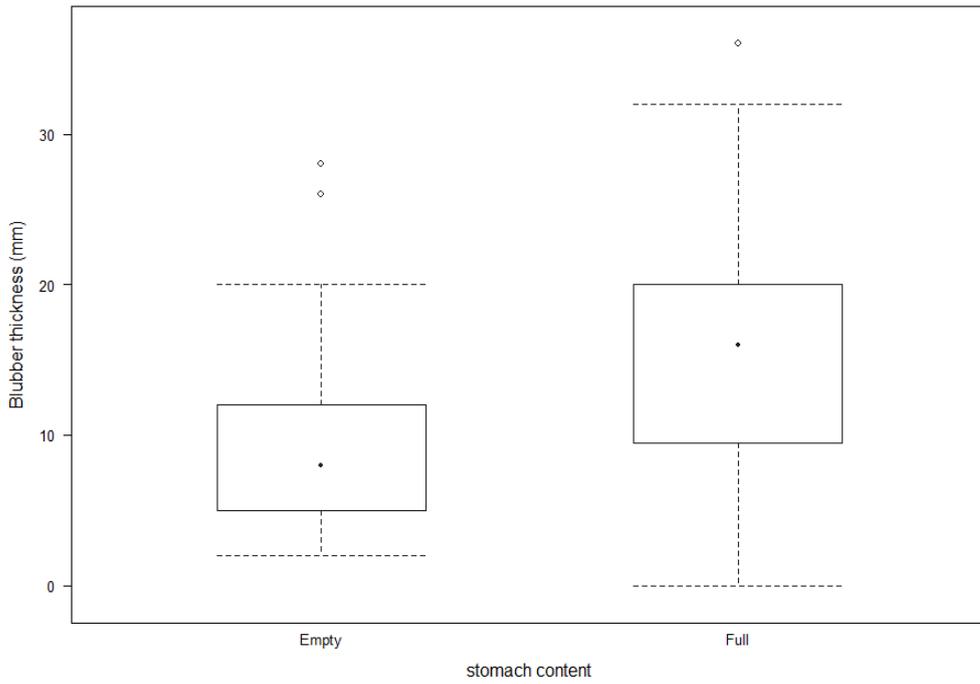
Figure 19: Boxplot of blubber thickness measurements between porpoises with an empty versus a filled stomach.

	A:Empty	A:Full	J:Empty
A:Full	1,0000		
J:Empty	1,0000	0,0092*	
J:Full	1,0000	1,0000	0,0007*
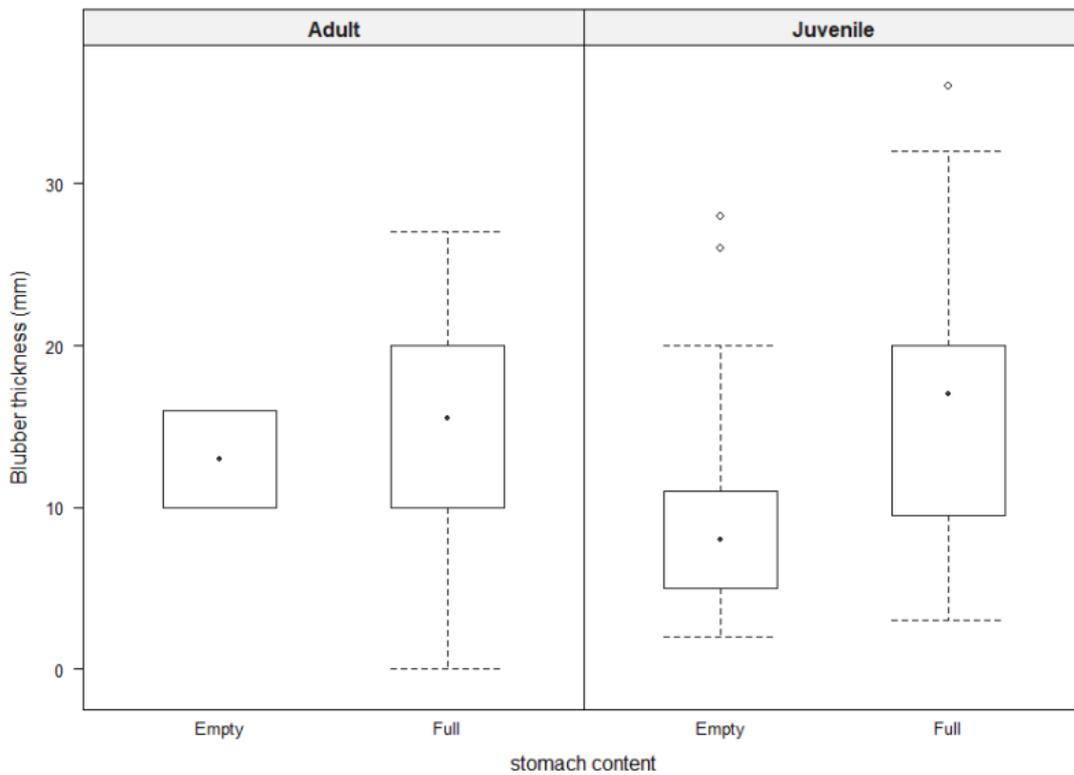
Figure 20: Boxplot of blubber thickness measurements compared between each combination of life stage and stomach content (below) and output of Dunn's test (above) with significant effects highlighted (*).

When looking at blubber thickness, one must also keep in mind any seasonal changes in the thickness of this insulation layer (Fig.21). The blubber thickness of porpoises in our study displayed this seasonal trend, which was confirmed as a significant difference in blubber thickness with season by the Kruskal-Wallis Rank test (p= 8.237e-07). The Dunn's test revealed that the blubber layer in winter and spring is significantly thicker from that in both summer and autumn, though not differing significantly between winter-spring and summer-autumn, as depicted in Figure 21.

	1	2	3
2	0,5199		
3	0,0000*	0,0005*	
4	0,0002*	0,0020*	1,0000
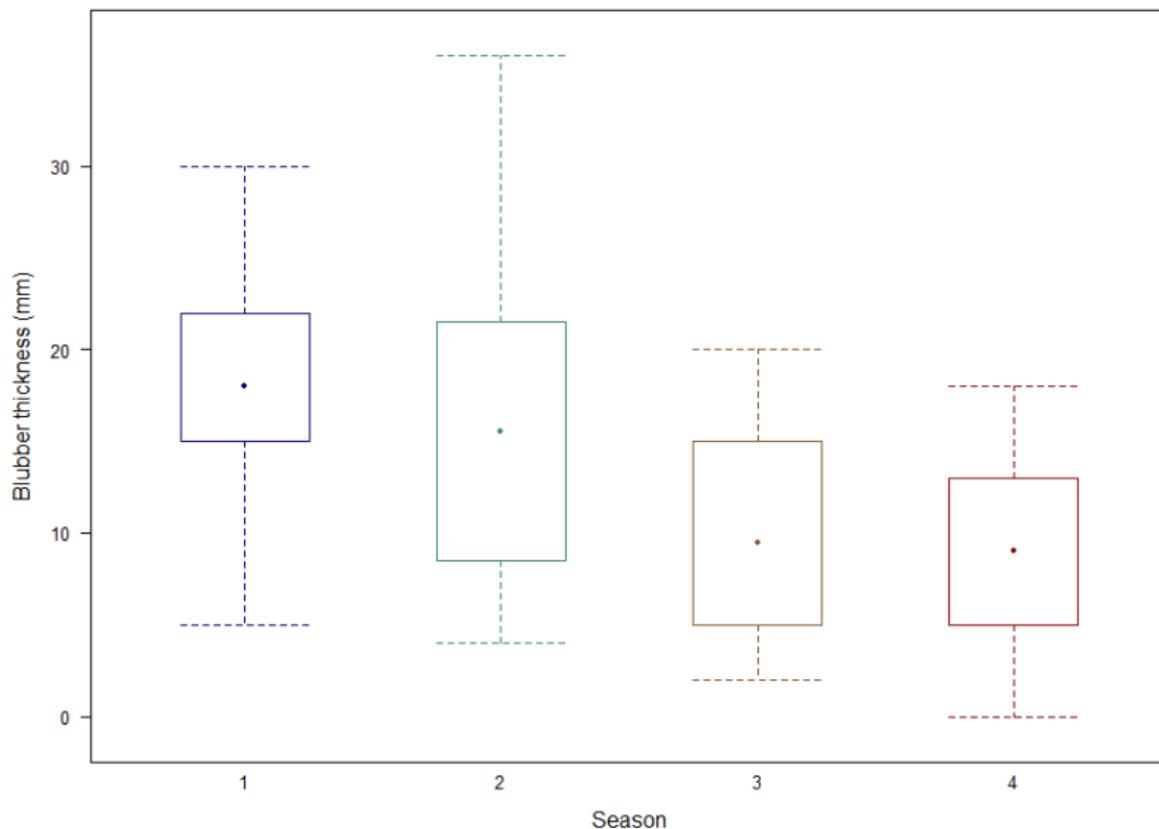
Figure 21: Boxplot of blubber thickness measurements compared between the four seasons (below) and output of Dunn's test (above) with significant effects highlighted (*). The season codes are 1: Winter; 2: Spring; 3: Summer and 4: Autumn.

Still, a significant difference in blubber thickness was found due to both season and stomach content status (k-sample Kruskal-Wallis Rank test, p=2.076e-07). The Dunn's test confirmed the seasonal trend in blubber thickness as stated above, though only the porpoises with a 'full' stomach showed this significantly thicker blubber layer in winter-spring as compared to summer-autumn (Fig.22). Though, it also revealed a significant difference in blubber thickness between porpoises with a 'full' and 'empty' stomach in spring (2:Full versus 2:Empty p=0.0213; Fig.22).

	1:Empty	1:Full	2:Empty	2:Full	3:Empty	3:Full	4:Empty
1:Full	1,0000						
2:Empty	1,0000	0,0087*					
2:Full	1,0000	1,0000	0,0213*				
3:Empty	0,4074	0,0015*	1,0000	0,0038*			
3:Full	1,0000	0,0065*	1,0000	0,0170*	1,0000		
4:Empty	1,0000	0,8623	1,0000	1,0000	1,0000	1,0000	
4:Full	1,0000	0,0018*	1,0000	0,0046*	1,0000	1,0000	1,0000
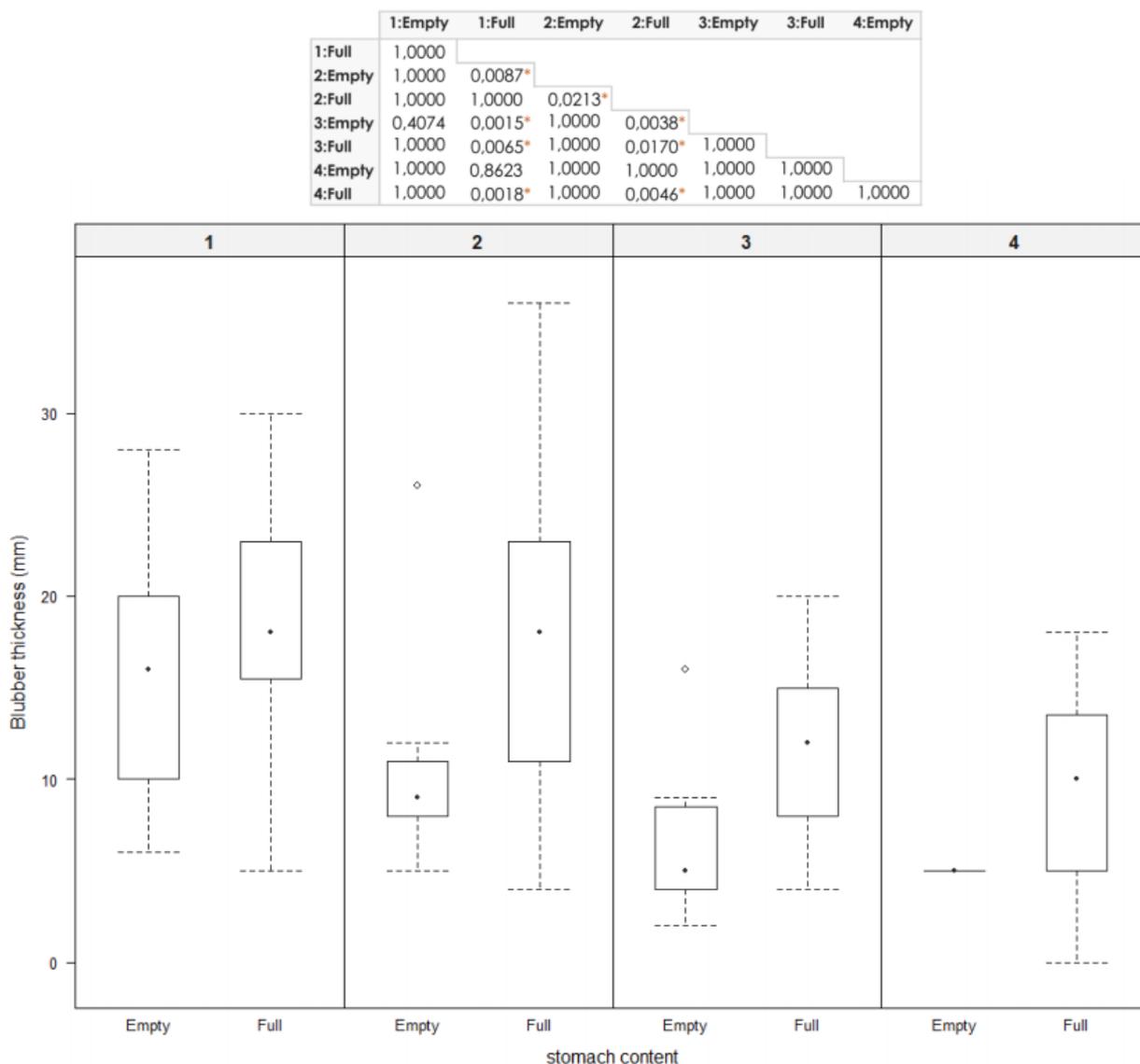
Figure 22: Boxplot of blubber thickness measurements compared between each combination of season and stomach content status (below) and output of Dunn's test (above) with significant effects highlighted (*). The season codes are 1: Winter; 2: Spring; 3: Summer and 4: Autumn.

EMPTY STOMACHS

Empty stomachs comprised 15% of the examined stomach samples (n=27). The distribution of examined stomachs over the years and seasons, with indication of the percentage of 'empty' stomachs are displayed in Table 6. As our data does not include all empty stomachs found between 1997-2018, no specific trends were discussed. Only general observations were made regarding the empty stomachs in our data.

Of the examined porpoises that died during the summer months, a quarter had an empty stomach (25.81%). This was followed by those that died in spring and winter (with respectively 14.6 and 11.63% of the stomachs being empty).

SEASON YEAR	Winter	Spring	Summer	Autumn
1997	0	1	0	0
2000	0	1	0	0
2003	1	4	0	0
2004	2	3	0	0
2005	1	1	1	1
2006	2	9	2	0
2007	6	7	4	3
2008	2	5	1	1
2009	3	5	3	0
2010	2	11	8	1
2011	4	9	3	3
2012	1	7	0	2
2013	2	5	0	0
2014	7	9	1	1
2015	1	0	2	2
2016	5	7	5	1
2017	4	2	0	2
2018	0	3	1	0
AVG % 'Empty'	11,63	14,6	25,81	5,88

Shading	
No data	
1 - 24	
25 - 49	
50 - 74	
75+	

Though, most of the sampled stomachs were from porpoises that died in spring, possibly leading to a higher proportion of empty stomachs there. When excluding empty stomachs from porpoises that died of either bycatch (n=4) or grey seal predation (n=2), the same trend was present with the highest proportion of empty stomachs in summer (23.33%), followed by spring and winter (respectively 11.63% and 7.32%). The proportion of empty stomachs in autumn remained the same. Thus, most of the porpoises seemed to be starving, in relative terms, during these summer months Furthermore, our limited data suggests that the lowest chance for dying with an empty stomach appears to be in autumn, with only one empty stomach sampled (comprising 5.88% of the stomachs sampled in autumn).

When looking at the proportion of empty stomachs between the combination of each season and sex, it becomes apparent that a higher percentage of males (46.67%) that died in summer had an empty stomach as compared to females (6.67%) in the same season (Fig.23). In winter and spring, the proportion of empty stomachs between both males and females was comparable. Thus, individuals starving in summer might be made up of mostly males, whilst in the other seasons these proportions are possibly more balanced whilst still maintaining the main trend of the highest proportion of empty stomachs in summer and the lowest in autumn.

The overall majority (92.59%) of empty stomachs in our dataset belonged to juveniles (24 juveniles and 1 neonate). Only two adults were sampled that had an empty stomach. To investigate whether smaller animals have a higher chance of starving during certain seasons, the length of animals with an empty stomach was plotted against the season in which they had died (Fig.24). Our limited data suggests that in summer the smaller porpoises seem to starve , as opposed to winter and spring in which larger (and thus older) animals also seem to starve.
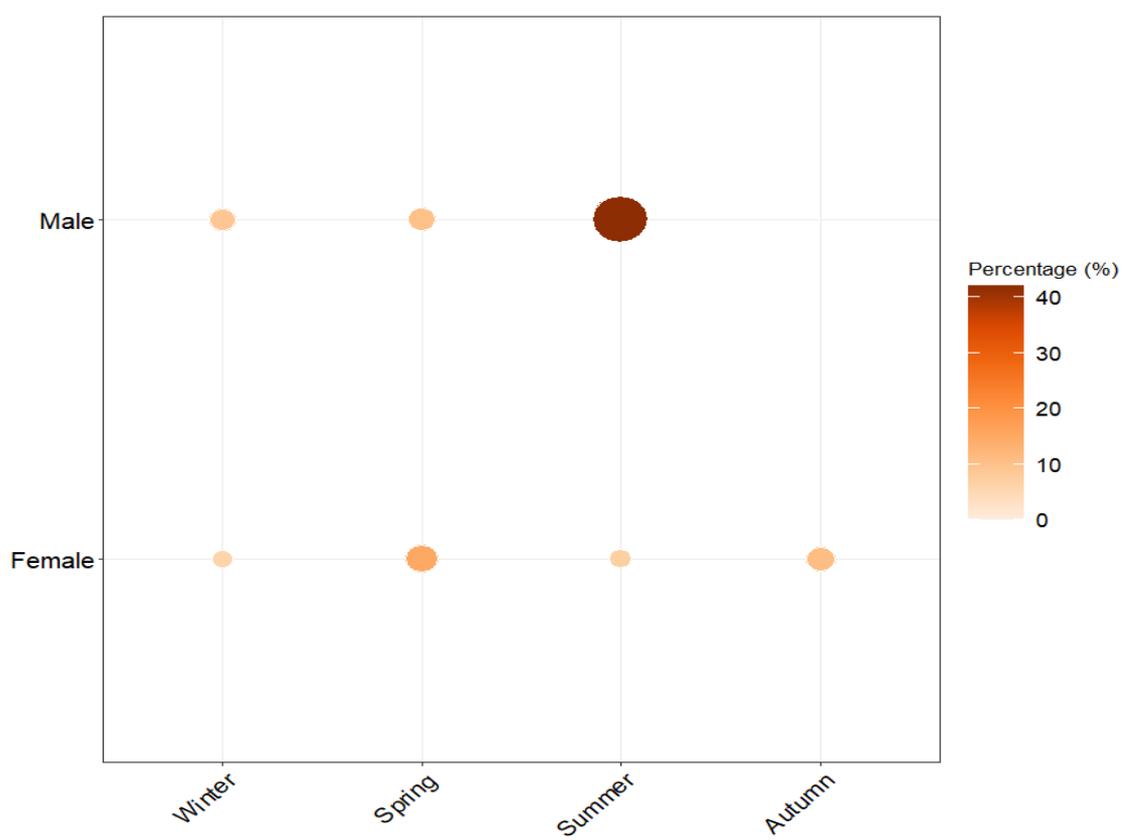
Figure 23: Bubble plot of the proportion of empty stomachs (n=21, excluding the porpoises that died of non-natural causes) in each season, subdivided by sex. The size and colour of the dots represents the percentage empty stomachs in that subgroup.
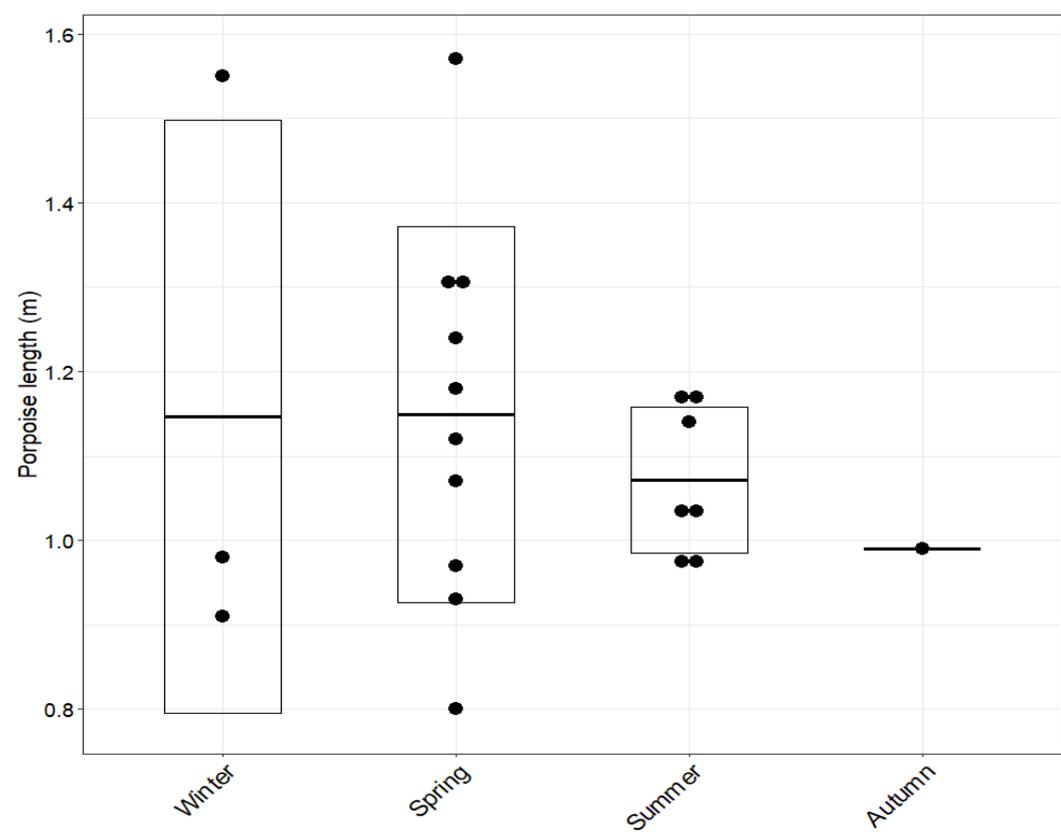
Figure 24: Dot plot of length of individual porpoises that had an empty stomach (n=21, excluding the porpoises that died of non-natural causes) by season in which they died. The line within the box plots on the data encompass the mean and standard deviation.

nMDS

A non-metric multidimensional scaling of reconstructed prey guild biomass data for non-empty stomachs (after log transformation and using Bray-Curtis dissimilarity measure) separates porpoises with differing diets (Fig.25). Because of the use of reconstructed prey guild biomass, samples containing only one prey type were placed somewhat separated as the ingested biomass differs mostly between individuals. Samples with remains of only one prey guild can be found at the edges of the plot and stand out as they are most distinct from the other samples. About one third of the samples (33.5%) contained solely remains belonging to one prey guild. There are two porpoises that only contained squid remains (beaks of *Sepiola sp.* in one and remains of both *Sepiola sp.* and *Loligo vulgaris* in the other). The other single prey guild samples either only contained gobies, sandeels or gadoids; constituting respectively 63.5%, 21.2% and 11.5% of the 'one prey guild' samples (Fig.26). There were no porpoises in this study that solely contained remains of clupeids, estuarine roundfish, demersal roundfish, pelagic roundfish or flatfish. Most of the animals have a mixed diet and are placed roughly in the centre of the plot. The mixed samples contained prey remains belonging to minimally two and maximally seven different prey guilds, with the majority of the mixed diet samples including remains of maximum four different prey guilds (92.2%).
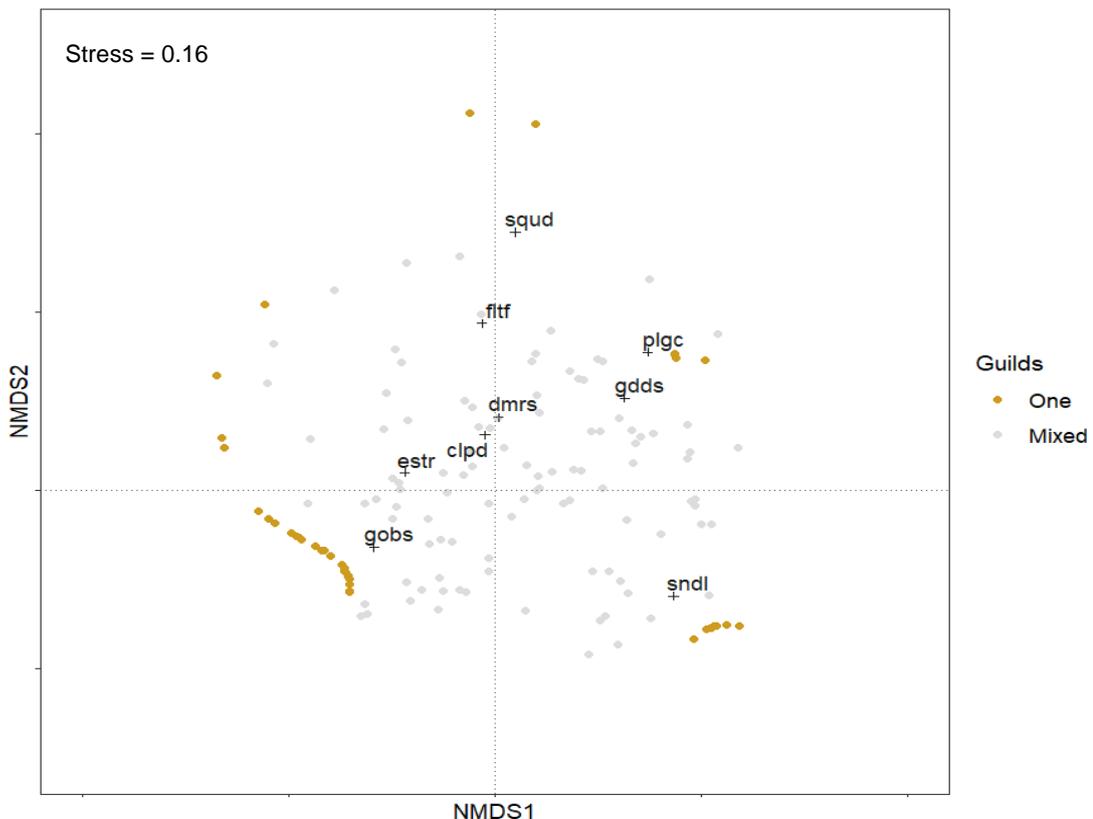
Figure 25: nMDS ordination based on Bray–Curtis dissimilarities of log-transformed biomass of different prey guilds in individual stomach samples. Prey guild names depicted as gobies (gobs); sandeels (sndl); estuarine roundfish (estr); clupeids (clpd); demersal roundfish (dmrs); gadoids (gdds); pelagic roundfish (plgc) and flatfish (fltf). The orange labels mark stomach contents with remains of only one prey guild and are placed off-centre, whilst grey labels denote mixed diets and have a somewhat central position.
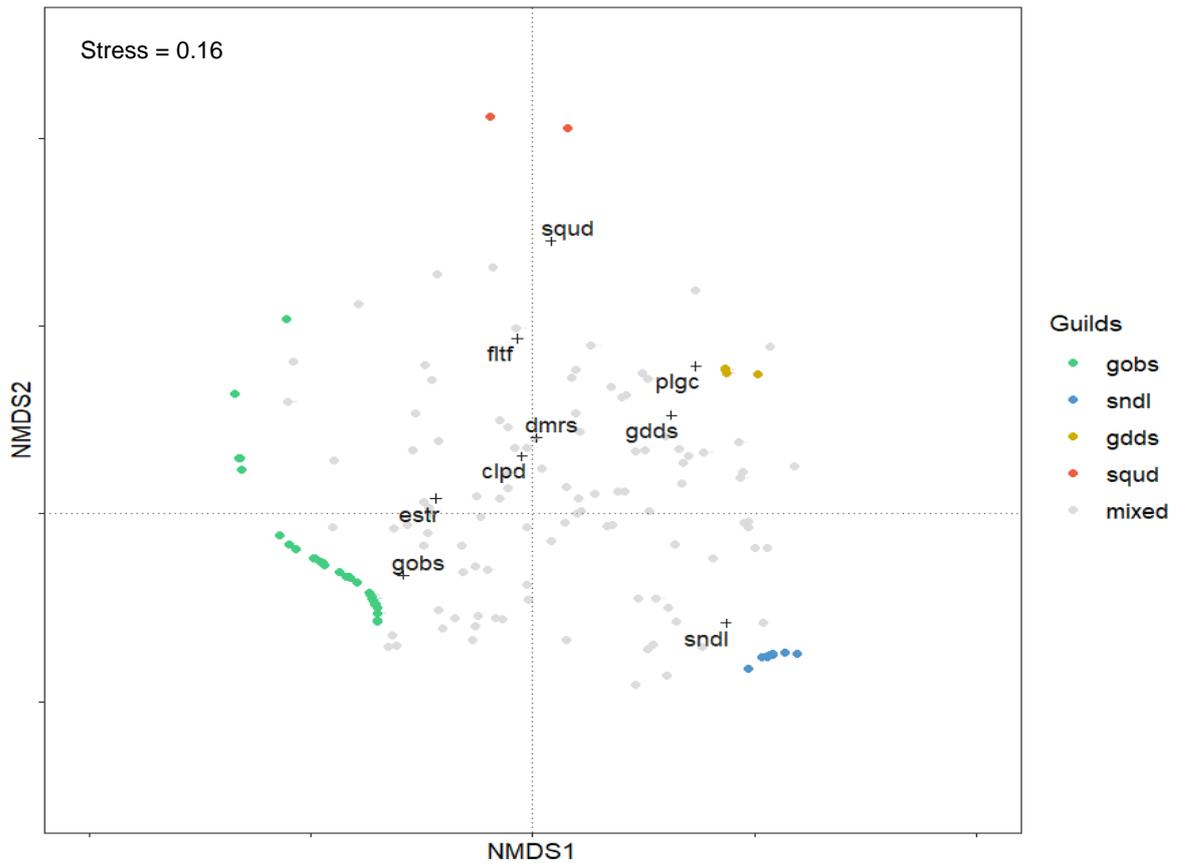
Figure 26: nMDS ordination of individual stomach samples with different labels for the samples dominated by one specific prey guild. The closer a mixed sample is to a 'single prey guild' sample, the more it is dominated by that specific prey guild.

PERMANOVA

The output of the marginal tests, on which the order of the predictors in the final model was based, can be found in table 7.

Table 7: output of PERMANOVA with marginal testing with significant effects highlighted (*).

	p
Lifestage	0,001*
Year	0,093
Sex	0,305
Season	0,028*

Only the marginal effect of lifestage and season was significant after controlling for the other variables. Next, the predictors in the model were arranged in ascending order according to their p-value. Thus, the final PERMANOVA model, even after model selection, included all four predictors: LogPreyMass=Lifestage+Season+Year+Sex. PERMANOVA partitioning using a sequential (Type I) sum of squares revealed that the greatest component of variation was the residual, followed by lifestage, season, year and then sex (Table 8, R^2).

The PERMANOVA model detected a strong significant effect of both lifestage and season (Table 8, p respectively 0.001 ; 0.011) even though together they only explain 10.9% of the observed variation in the data. Thus, even though a big part of the variation remains unexplained, both lifestage and season seem to have a clear effect on the diet of harbour porpoises.

Table 8: PERMANOVA partitioning and analysis of 155 individual stomach samples based on log-transformed prey guild biomasses and Bray–Curtis Dissimilarities with significant effects highlighted (*).

	p	R^2
Lifestage	0,001*	0,090
Season	0,011*	0,019
Year	0,080	0,012
Sex	0,293	0,007
Residual		0,872

The PERMDISP analysis performed on both significant factors revealed that their significant effect in the PERMANOVA is indeed due to a difference in community composition between the different life stages and seasons, as identifiable from the non-significant results of the PERMDISP analysis for both life stage and season (with an overall p-value of respectively 0.786 and 0.165). Though some of the pairwise comparisons embedded in the PERMDISP analysis of the factor season returned significant, the overall effect of season is most likely due to a true shift in prey guilds and not due to differences in the variability of the community composition between the different seasons.

As pairwise testing was not possible in our case, we examined the main effects of both significant factors graphically. Figure 27 depicts the repeated nMDS ordination, now with samples grouped according to their life stage. Though there is quite some overlap between the two groups, adult diets are clearly dominated by other prey guilds than juvenile diets as the centroids of both sample clouds are clearly separated (Fig.29).

The same plot was reconstructed with samples categorised by season (Fig.28). Here, discerning a pattern of differences between samples from different seasons was laborious due to the considerable amount of overlap. Still, significant differences in prey composition by season were found in the PERMANOVA and plotting the centroids allowed us to resolve the most important differences (Fig.29). This 'main effects' plot clearly showed that the life stage effect is the largest (greatest distance between centroids), with the adult centroid closest to gadoids and sandeels, whilst the juvenile centroid lies primarily in the vicinity of gobies and estuarine roundfish and secondarily clupeids and demersal roundfish. A seasonal effect was also apparent, especially between spring-winter and summer-autumn, with the smallest differences in prey composition during winter and spring due to their close placement on the plot. During this half of the year, gobies contribute largely to the diet as do clupeids and sandeels in respectively the winter and spring months. In autumn, gadoids and clupeids were major contributors to the diet; whilst in summer sandeels, pelagic- and estuarine roundfish were consumed more than in any other season. Gadoids were a key prey in all seasons, though in autumn they were consumed in larger amounts.
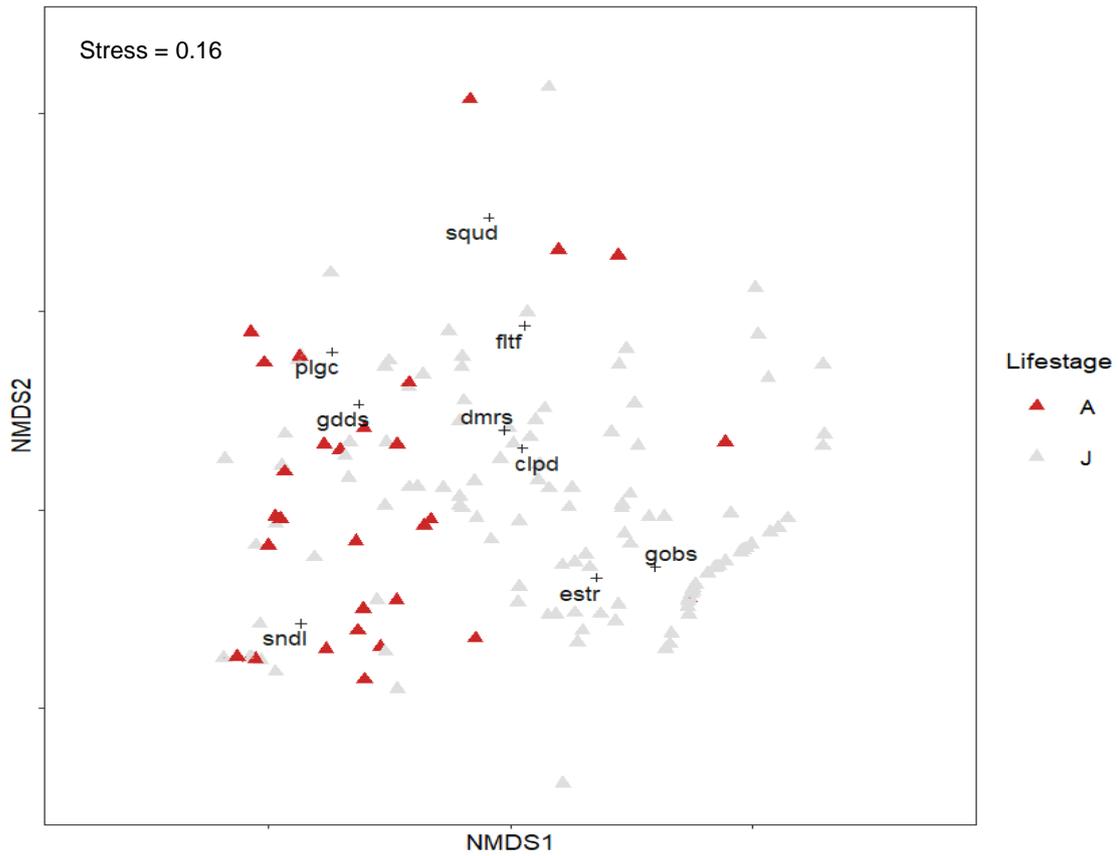
Figure 27: nMDS ordination based on Bray–Curtis dissimilarities of log-transformed biomass of different prey guilds in individual stomach samples with the colour of the samples denoting the life stage (based on the total length of harbour porpoises).
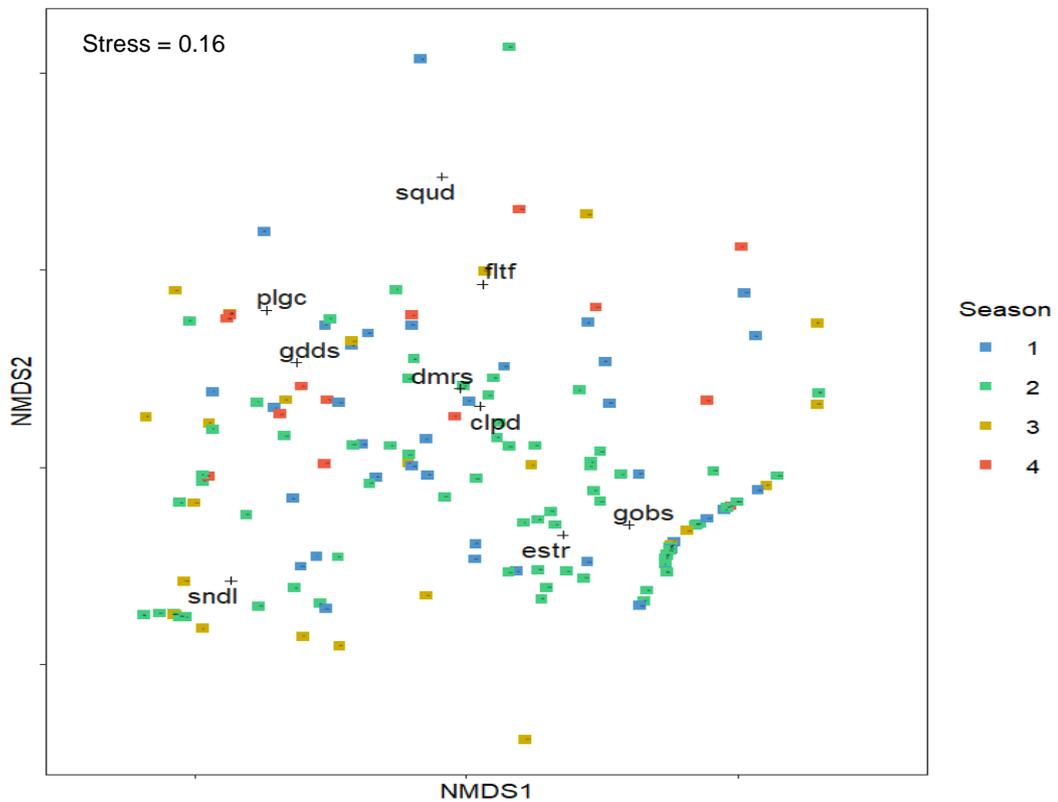

Figure 28: nMDS ordination based on Bray–Curtis dissimilarities of log-transformed biomass of different prey guilds in individual stomach samples with the colour of the samples denoting the season in which the porpoise as stranded/bycaught.
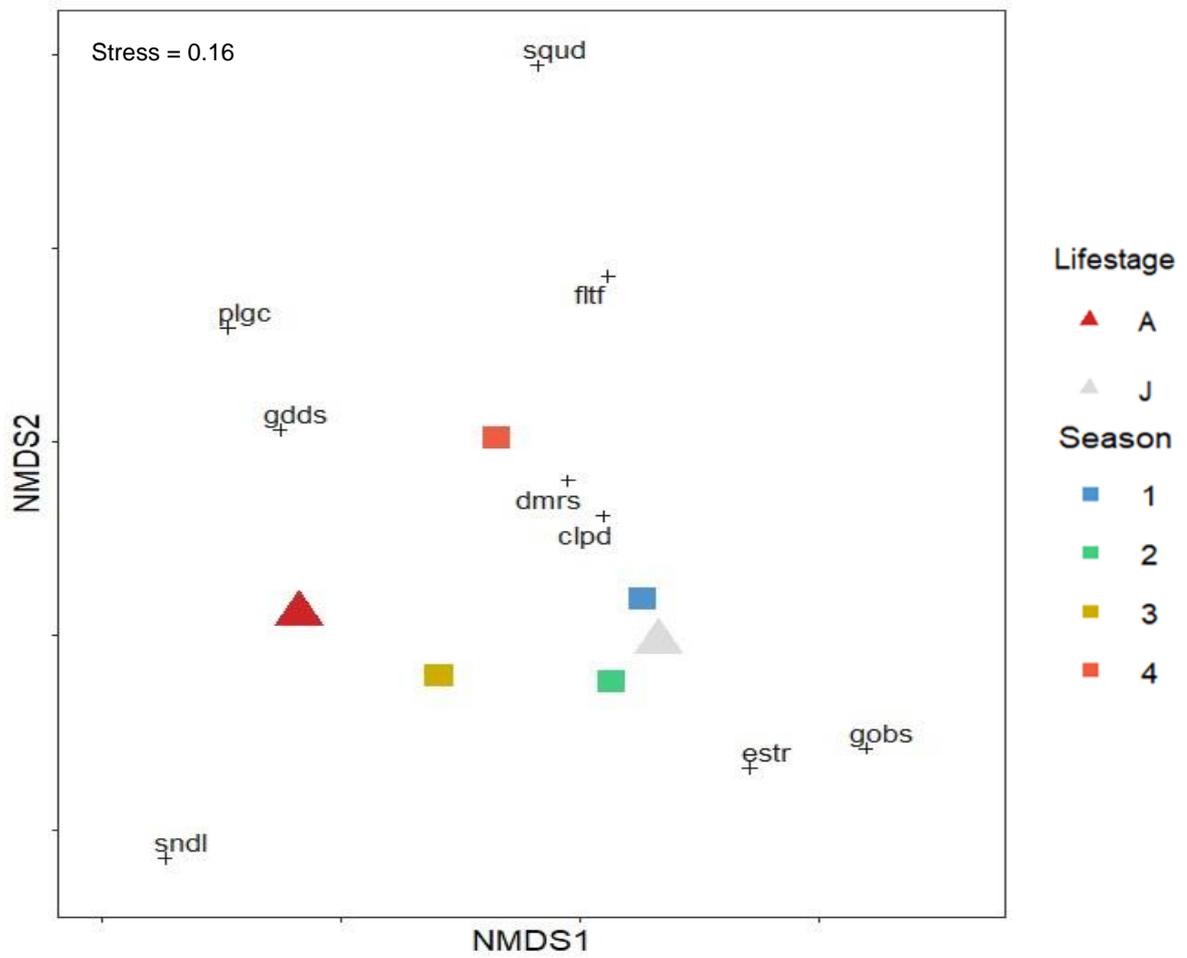
Figure 29: nMDS ordination of distances among the centroids for the individual levels of each of the main effects.

DISCUSSION

DIET PREDICTION FROM STRANDED AND BYCAUGHT PORPOISES

According to Pyenson[145,146], using strandings records as population indicator is justified, with strandings providing a unique sample of the free ranging population which is difficult to obtain through other means of observation. Thus, even though our samples are a subset of all the stranded and bycaught porpoises between 1997-2018, it represents a reliable snapshot of the harbour porpoise community in our waters and biological metrics, like diet, can be inferred from our samples[90,147].

As stated in the materials section, there are numerous sources of bias attached to the methodology. The use of stranded porpoises might lead to an overrepresentation of 'sick' animals in the dataset[16,134]. Though adding bycaught animals may provide samples of 'healthy' individuals, their use is not unbiased either, with the bycatch pool dominated by inexperienced juvenile porpoises and porpoises with a diet possibly biased towards the target species of the fishery[82,110]. Our sample pool consisted of 53.3% stranded individuals and 46.7% individuals marked as (potentially) bycaught.

Furthermore, stranded and bycaught porpoises are not equally available throughout the year. Along the Belgian coast, two stranding peaks are observed during the year, one in late winter-spring and another during the summer[16]. This spring peak was most probably the combined result of the highest porpoise densities in Belgian and surrounding coastal waters between February and May and the high fishing effort for Dover sole *Solea solea* in the same period (March-April), using set nets close to the shore and known to have a high bycatch[16,32]. Another explanation for this spring peak was proposed by Ijsseldijk & ten Doeschate[36] as a possible high density of juvenile mortality following starvation. New-born porpoises become independent in February-April when the sea water temperatures are at their lowest throughout the North Sea[15,148]. Thus, juveniles' first independent foraging in combination with the cold water temperatures could result in high nutritional and physiological stress, possibly leading to a high proportion of juvenile mortality due to hypothermia[36]. The summer peak is most probably the result of both the harbour porpoise's life history and seasonal prey availability, with inexperienced calves born between May-August having a higher mortality rate during this period of food scarcity, when their fat reserve and tolerance to starvation is at its lowest (see below)[6,9,10,11]. In addition to the probable differential mortality rate during the year, strandings are also affected by a multitude of processes, including physical, social and biological processes[147]. As in most marine mammal studies, we were not able to reach a balanced dataset due to the unequal availability of samples throughout the year. Though, we tried to account for this sample bias by using, to the extent possible, stratified sampling (i.e. sampling individuals that belong to categories for which still relatively little information is available)[94].

Additionally, we used the length of individual porpoises as a proxy for age (or in our case age class). However, dividing the porpoises into life stage classes remains an estimation, as there is individual variation in the growth rate of harbour porpoises[14,149]. Furthermore, the proportion of juveniles in our dataset might be higher as compared to other similar studies in Belgian and surrounding waters. This can be attributed to the more stringent definition of an adult used in this thesis.

In Jauniaux *et al.*[60] and Ijsseldijk & ten Doeschate[36], animals are considered adults when their length exceeded 130 cm. In our case, animals were considered adults starting from a length of 135 cm, leading to a smaller adult pool[117,149,150].

Additional biases in diet reconstruction were evaluated by Pierce *et al.*[151] using a dataset on the diet of harbour porpoises in Scottish waters from 1992-2003 (n=180). It concluded that sampling error, resulting from sampling only *n* stomachs from the population, had the biggest effect on the precision of the diet composition estimates. Börjesson *et al.*[80] recommended 35-71 stomachs as minimum sample size for diet reconstructions, as in that case all common prey species will be represented by at least one individual (95% confidence) in the samples. Our sample size of 180 samples should as such be large enough to recover the overall prey spectrum consumed by harbour porpoises in Belgian waters.

Furthermore, the importance of using the appropriate regressions that relate to the prey in the study area was emphasized in the study by Pierce *et al.*[151], as the use of alternative regressions constructed for the same species in other areas gave other results. In our study we used regression coefficients derived specifically from fish species present in our study area (the Southern part of the North Sea) to avoid over- or underestimation of the original characteristics (i.e. length and mass) of specific prey species[122]. Additionally, applying relative weight to different individual stomach contents by calculating indices should be carefully considered as each meal varies in size and porpoises will have died during different stages in the feeding cycle, inevitably leading to a more or less filled stomach. As such, an equal weighting of stomach contents from different individuals should be considered[151]. To partly deal with this problem, a combination of multivariate statistical analyses like nMDS and PERMANOVA were used, in which each stomach sample has an "equal weighting".

Another problem faced in diet studies is that otoliths from different prey species erode at different rates, reflecting the general shape and robustness of the otoliths[152,153]. Combined with differential residence times of prey remains from different species in the stomach, the importance of prey species with larger, heavier and more robust remains might be overemphasized as their remains tend to reside longer in the stomach as opposed to smaller (e.g. goby) and more fragile (e.g. clupeid) prey remains[90,96,121,136,137]. Specific digestion coefficients and numerical correction factors were developed for grey seals and harbour seals in captivity, though these did not exist for harbour porpoises[121,153,154]. We used erosion correction factors as described by Leopold *et al.*[93] to account for the partial digestion rates of prey hard remains in the stomach, and in order to avoid underestimation of the prey size and mass[80]. We were not able to account for the differential residence time of remains of different prey guilds in the stomachs of the harbour porpoises as only the prey remains found in the stomach samples were accounted for.

Stomach content analysis was the only technique used to reconstruct the diet of harbour porpoises in Belgian waters in this study. The effectiveness of stomach content analysis for accurate population-scale diet prediction was explicitly tested by Dunshea *et al.*[155] on another inshore cetacean. Specifically, it was investigated whether the estimated diet based on stranded dolphins was representative for the whole population.

Both gastric and faecal samples were collected from healthy free-ranging individuals of an extensively investigated bottlenose dolphin (*Tursiops aduncus*) population in Sarasota Bay (USA) and used for molecular prey detection. This data was compared against stomach content data as collected from stranded bottlenose dolphins of the same population over 22 years. Both datasets showed a significant similarity, both in prey species composition and their relative amounts, validating stomach content analysis as an important tool for cetacean research and management[155].

The results presented in this study should be assessed with the above-mentioned biases in mind. Though, by using erosion factors and regression coefficients constructed for species in our study area as well as looking at the individual variation in the diet, we partly dealt with some of the biases associated with stomach content analysis and diet reconstruction in general. Hence, we are confident that these only had a minor effect on the overall diet reconstruction.

DIET OF THE HARBOUR PORPOISE

OVERALL PREY COMPOSITION

The first specific objective of this study was to determine the preferred prey species in the Belgian part of the North Sea and whether they feed on the richest food source that is available in our waters.

This study confirms that the main portion of the harbour porpoise's diet is dominated by various fish species belonging to several prey guilds, which are known from previous studies[16,82,94] In this study, the maximum number of recorded prey species in an individual stomach was seven belonging to seven different prey guilds, suggesting that harbour porpoises in Belgian coastal waters have a broad diet niche, able to feed on a broad prey spectrum. This is in line with the commonly described generalist feeding strategy adopted by porpoises[81,82,156]. Though, most porpoises only consumed between one and four different prey guilds with on average prey remains of two species belonging to two different prey guilds present in individual stomachs.

In the first part of this thesis, the relative importance of each prey guild was investigated across all examined porpoises by lumping the stomach contents of all sampled porpoises and calculating three indices (Table.4). Each of these statistics has strengths and weaknesses[157]. %FO is an absence/presence measure describing the relative number of predators that have consumed a given prey, not considering the amounts eaten. %N is dominated by small prey species that are taken in large numbers as it represents the effort put into foraging for this certain prey, which often contributes little to ingested mass or energy. %M on the other hand grants more weight to larger prey, even if they are only taken rarely[107].

Table 4 shows that numerically gobies dominate the diet of harbour porpoises in Belgian waters, whilst based on biomass gobies, gadoids, sandeels and clupeids (in that order) were the main prey guilds consumed. Gadoids (mostly whiting *Merlangius merlangus*) made the largest contribution to the diet in terms of prey biomass, even though numerically they are dwarfed by other prey guilds. This is mainly because the average consumed gadoid is much larger and heavier (57 g) than the average goby (<1 g), clupeid (38 g) and sandeel (10 g).

These four prey guilds stand out over the whole study period and across all ages and life stages and can thus be seen as the "big four" of harbour porpoise's diet along the Belgian coast. This is perfectly in line with results from previous diet studies along the Northern French, Belgian and Dutch coastlines[16,44,83,106–108,158,159]. Other prey guilds were found to be of secondary importance in this study, with estuarine roundfish (mostly European smelt *Osmerus eperlanus*) and demersal roundfish (dominated by juvenile sea bass *Dicentrarchus labrax*) being the most noteworthy.

Osmerus eperlanus, or European smelt, was reported as rare in the Belgian part of the North Sea[160]. Though, our study and the study by Haelters *et al.*[16] show that this diadromous fish has become a common resident again in Belgian coastal waters since 2010 (as *Osmerus eperlanus* was the dominant estuarine round fish found in this study)[161]. Though, European smelt might be underreported in the results of general fish abundance studies that use mostly bottom trawls. This technique does not allow for adequate assessment of small pelagic species and as such quantitative data is missing for many pelagic species.

We were not able to distinguish primary from secondary prey items. Thus, some prey items in the samples might have originated from the digestive tract of the prey itself, which is almost impossible to assess as secondary prey can only be identified without a doubt when the remains are found in the stomach of another prey[132]. This might especially be common in stomachs with both gadoid and goby remains, as the smaller gobies might partly represent the stomach content of the much larger gadoids, potentially leading to an overestimation of the importance of gobies[16].

Throughout the North-East Atlantic, the diet of harbour porpoises is dominated by one to four prey species of prey groups, with at least one of these constituting a high quality food source[45,49,80,82,90]. This is in line with our findings, with the diet of harbour porpoises in Belgian waters dominated by the "big four" prey guilds, with two of them comprising high-quality energy-rich prey. According to the *optimal foraging theory* predators with a high cost of living, like harbour porpoises, should prefer prey that provide them with more energy than they spend on catching and handling it (i.e. prey with a high energy density)[84,91,162]. If, in light of this theory, opportunistic foraging is observed, it might imply that high quality prey are relatively rarely encountered. Overall, a mixture of seasonally available high quality prey and readily available leaner prey species is consumed[13–15,75–77,82–84,86 89–91,104]. Thus, harbour porpoises in Belgian and surrounding coastal waters do not solely feed on prey that are most profitable (i.e. high-quality prey like clupeids and sandeels). Specific reasons for the considerable amount of lean prey in the diet of harbour porpoises are lacking, though Leopold *et al.*[45] hypothesized that these lower quality prey might be more readily available, constitute an easy-to-catch food source and/or they might contain components essential for growth as they are rich in protein.

Harbour porpoises in this study generally fed on relatively small fish with mean sizes of the dominant prey species ranging between ca. 1 cm and 35 cm. Harbour porpoises perform suction feeding, swallowing their prey as a whole instead of breaking them down in to smaller pieces, limiting the size of prey they can consume[13].

A 50 cm whiting was the largest captured prey by a porpoise during this study, which is in line with the upper size limit of prey consumed by harbour porpoises reported in other studies (48-51 cm)[81,156].

Even though using otoliths to reconstruct prey lengths causes an underestimation of the true prey sizes, it probably reflects the true pattern of prey sizes taken, with e.g. most gadoid fishes consumed probably constituting juvenile fish[163,164]. Furthermore, we found that on average larger porpoises tended to consume larger prey (Fig.16), which might be explained by the fact that juvenile diets tended to be dominated by small and easy-to-catch gobies as compared to adults which consumed more larger fish like gadoids (Fig.10 ; Fig.11 ; see below).

INDIVIDUAL DIET VARIABILITY AND FEEDING STRATEGY

The second specific objective of this study was to assess individual variation in the diet and to uncover whether a selective or an opportunistic feeding strategy was adopted.

As stated in Thompson *et al*.[39] using data of separate individuals instead of unweighted population averages (as is the case with calculating diet indices) is preferred in diet analyses, especially as skills and needs to sustain their requirements can differ among individuals as well as diets can fundamentally differ between different groups of individuals within a population. Hence, individual variation might represent more than just random scatter around an optimal foraging strategy that should be adopted by the whole population, and as such should not be ignored[107]. Additionally, as predators are rarely sampled at random, the output of diet studies using these unweighted population averages will inevitably be biased, especially as prey composition most likely varies between individuals and this variation is dependent on a multitude of factors like sex, age, location, body condition, etc[118].

The potential factors shaping harbour porpoises' diet and food intake in Belgian coastal waters were examined using a combination of unweighted population averages (using diet indices) and multivariate statistical analyses like nMDS and PERMANOVA. The factors identified as possible contributors to the variation that were tested in this study were year, season, life stage and sex.

The nMDS plots teased apart porpoises with differing diets and highlighted the large amount of individual dietary variation of the analysed porpoises (Fig.25 ; Fig.26). This already illustrates that, as stated by Leopold *et al*.[107], individual differences in the diet matter.

The results of our PERMANOVA model indicated a significant contribution of both life stage and season to the observed variation in the diet of harbour porpoises in Belgian waters but did not recover an interannual or sex-related effect (Table.8). These results were backed by the trends seen in the graphs using population averages (i.e. diet indices).

The diet of harbour porpoises in Belgian waters is thus significantly structured by life stage (and indirectly age). This dietary difference is mostly attributed to a differing importance of gobies, gadoids and sandeels (Fig.10 ; Fig.11; Fig.27 ; Fig.29).

Young porpoises mostly consumed gobies, possibly because these comprise an abundant and easy-to-catch food source for these still unexperienced individuals, and larger species like gadoids simply cannot pass the mouth and/or digestive tract yet[107,138]. Young porpoises quickly learn to forage efficiently on these small bottom dwelling fishes, as exemplified by their large presence (both in number and mass) in examined stomachs of juveniles. Many of these stomachs contained hundreds of goby remains, and 13 stomachs even contained remains of more than a thousand gobies (the maximum amount of goby remains found in one stomach during this study was 5,300). As the energetic return of a goby is very low, it is hypothesized that learning to catch and eat larger fish is of utmost importance[107]. Adults probably consume more big lean prey and fatty prey due to their advanced foraging skills and caloric needs. Larger porpoises need a larger amount of food to sustain them, as it has been estimated that a porpoise needs to eat up to 10% of its own body weight on a daily basis[93]. A diet of solely gobies cannot sustain larger porpoises and as such they simply must switch to larger and/or higher quality prey, to keep up with their daily requirements[90,94,165]. This is in line with our findings, showing that adult porpoises feed on both larger and energy richer prey (gadoids and sandeels). Thus, the diet of the harbour porpoise in Belgian waters develops with size (~age) in order to keep up with their daily energy requirements. Several other studies in the North Sea also recovered this ontogenetic development in prey selection[15,40,78,82,87,101–103,174-176]. This possible shift in diet selection with age has already been recorded in Belgian waters by Haelters *et al.*[16] and Mahfouz *et al.*[108], in which juvenile porpoises consumed more bottom dwelling fish (mainly gobies) whilst adults took both pelagic and demersal species, with the contribution of gobies to the diet decreasing with age. The same ontogenetic trend was found in Dutch waters, with the proportion of gobies progressively decreasing with increasing age[94,138,158]. Furthermore, as porpoises get older their total prey intake increases, gadoids contribute more to the overall consumed prey mass and the diet becomes more diverse[94].

Secondly, a significant seasonal variability in the consumed prey species was found. This dietary difference was mostly attributed to the different consumption of the "big four" prey guilds throughout the year (Fig.13 ; Fig.14 ; Fig.28 ; Fig.29). The largest differences in diet can be found between winter/spring and summer and winter/spring and autumn (as seen from the placement of the seasonal centroids on the nMDS plot ; Fig.28). Gobies were both numerically and by biomass proportionally less consumed during summer and autumn, though they tend to be consumed in large amounts throughout the year. Gadoids were also consumed throughout the year, though they were consumed in larger amounts and seemed to dominate the consumed prey mass in autumn. Energy-rich clupeids tended to be almost absent in stomach samples of porpoises that died in summer, whilst they represented an important portion of the consumed prey biomass in both winter and autumn. Next to gadoids, sandeels seemed to constitute an important fraction of harbour porpoise diet during summer. These seasonal trends possibly mirror the seasonal availability of these different prey groups. Though, these seasonal results must be interpreted with caution as the proportion of adults and juveniles differed between each season, with the highest relative contribution of adults in the autumn months.

In Belgian waters, seasonal abundances of some of the dominant prey guilds are well known (unpubl.data, K. Hostens): clupeids, like sprat and herring, seem to be mostly present in late winter – early spring (February-April); gobies are present throughout the year with higher abundances in late summer – autumn (August-October); whiting is present throughout the year and cod mainly in the period between September and November; and sandeels are also available throughout the year, possibly even more during the summer months. This is almost perfectly in line with the seasonal differences of prey found in the analysed stomach contents, except for the proportion of gobies (Fig.13 ; Fig.14 ; Fig.29). This seasonality in prey availability is no surprise, as each fish species has its own life cycle, with species-specific spawning and migration periods , changing their availability and energy density throughout the year and potentially impacting the diet of their[82,107]. Additionally, seasonal variation in the diet might also be the result of described seasonal migrations of harbour porpoises, more specifically the observed inshore movement in summer and offshore movement during winter[82]. Other studies in the North Sea also found seasonal variation in the diet, most of them in line with what was found during this study[45,90,106,107].

Our analysis did not recover any sex-related dietary differences in Belgian waters. Only minor differences were revealed when using the diet indices, with a higher consumption of gobies and sandeels in male porpoises, whilst female porpoises appeared to take more gadoids, pelagic and estuarine roundfish. Though overall, the ranked importance of each prey guild was similar between males and females (Fig.15, above). However, a greater dietary difference was indicated between male and female adults (Fig.15, below) mostly due to the contrasting importance of gadoids, sandeels and clupeids between the two. Yet, this might be an artefact of the uneven distribution of adults over the seasons (18.7% in winter, 15.7% in spring 25.8% in summer and 41.2% in autumn), as well as the contrasting contribution of male and female adults within each season. This was especially true when we compared winter and spring, which contained respectively more female and male adults, whilst the adult sex ratio was more balanced in summer and autumn. The similar diet of males and females was also found in comparable studies in Dutch and Scottish waters[106,107,138]. Though, some slight differences between males and females were found in Leopold *et al.*[107], with adult males taking relatively more clupeids and sandeels compared to females, which take more gadoids and other pelagic roundfish. Furthermore, differences between male and female harbour porpoises might be the biggest when females are nursing calves, due to their different energy demands and foraging behaviour[21,82]. This dietary difference between adult males and females was recently reported in Dutch waters[158].

No interannual variation in the diet of the harbour porpoise in Belgian waters was found in this study. This was especially backed by the observation of fairly equal relative proportions of each prey guild, both numerically and based on mass, between 2007-2011 and 2014-2018 (Table.5 ; Fig.12). These long-term trends were also not recovered in most of the recent diet studies in and around the North Sea. The only well-known shift in prey species occurred after the collapse of the herring *Clupea harengus* stocks between the 1950s and 1970s[106]. This was most apparent in the studies by Rae[103,104], in which the contribution of clupeids in the harbour porpoise's diet was clearly greater than is seen in more recent studies[104].

Additionally, although herring stocks are partially recovering, their relative importance in the porpoise diet has not been observed to increase again[16].

Opportunistic foraging or predation *sensu stricto* entails that prey are taken as they are encountered and the diet choice is only shaped by the availability of the different prey species, which is the exact opposite of selective foraging strategies[169]. The dominance of just four key prey guilds in the diet of harbour porpoises in Belgian coastal waters may seem a restricted diet. However, this might hints at the existence of selective foraging on these key prey groups with possibly opportunistic feeding on the side on a variety of other prey, simply because they are available, or because they contain crucial components for maintenance[16,94,158].

To answer the question whether harbour porpoises are opportunistic foragers, with their diet composition following prey availability, or selective foragers, information on the distribution and seasonal availability of the prey and other possible target species is needed on a small spatial scale, which is often lacking in fisheries literature[57]. Santos, 1998 tried to answer this question by using fisheries catch data as an estimate of prey species abundance and availability in and around Scotland and comparing this with the ranked importance of prey species by biomass in harbour porpoise diets. A positive correlation in three out of the five analysed years yielded weak support for harbour porpoises as opportunistic foragers[82,106]. Furthermore, using fishery landings data to assess the availability of certain prey species should be done with caution, as these landings are subject to changes in the demand, fishing effort and the introduction of management measures[163]. Nevertheless, it can provide a reasonable indication of the availability and abundance of prey species that are commercially fished[170]. In another study by Santos *et al.*[171], relationships between annual prey abundance and diet did neither confirm nor reject either opportunistic or selective foraging in common dolphins, though there is more support for the former[171]. The study by Haelters *et al.*[16] in the Belgian part of the North Sea suggested that harbour porpoises are might not be feeding as opportunistically as often suggested, mostly due to the absence or low appearance of some commonly occurring species in the examined stomachs, such as dragonets, rocklings, sea-snails and different species of flatfish. These are generally found to be moderately (in the case of rocklings and sea-snails) or abundantly (dragonets and flatfishes) present on the bottom of Belgian waters in between gobies[123,172,173]. This study did find the above mentioned species, with the exception of sea snails *Liparis*, though in very low quantities (13 individuals in total: two individuals of common dragonet *Calionymus lyra*; four Fivebeard rocklings *Ciliata mustela*; seven flatfishes belonging to 3 different species), with all these species comprising only 0.39% of the total consumed prey mass. Possibly, their limited presence might be an artefact of the differential digestion and residence time of their hard parts (especially otoliths) in the stomach. As such, these remains might not be retained long in the stomach, leading to the underrepresentation of these species in the diet[96].

Our study, much like Santos *et al.*[82,106,171] and Leopold[94] neither confirmed nor rejected opportunistic or selective foraging of harbour porpoises, though we present more, though non-conclusive, evidence for harbour porpoises as opportunistic feeders, with ontogenetic and seasonal variation in the diet. Especially the seasonal variation was expected in harbour porpoises that forage opportunistically[82].

LINK WITH DISTINCT BEHAVIOURS AND VARIATION IN THE STRANDINGS DATA

The third and last specific objective of this study was to examine whether dietary differences and/or preferences could be ascribed to distinct behaviours or related to variation in the stranding data.

As stated above, the summer stranding peak of harbour porpoises along the Belgian coast is probably an indirect result of the differential seasonal prey availability. Porpoises mainly rely on lean gadoids and secondarily on sandeels and gobies in Belgian and surrounding waters during the summer, when their fat reserve (blubber layer) is at its lowest (unpubl.data, K. Hostens ; our data, see above)[45,101]. Finding insufficient quantities of gadoids and/or sandeels in this period could explain the higher chance of starvation and dying with an empty stomach that has been ascribed to these summer months (see below). This highlights the importance of some energy-rich species in their diet[45].

The majority of our stomach samples were derived from juvenile porpoises (Fig.4), comprising 77.8% of all samples. In many cetacean species, mortality rates are reported to be the highest during this life stage, leading to their dominance in strandings and bycaught animals[24,174]. As juvenile porpoises mainly feed on lean prey (gobies), they are very prone to starvation if they do not find sufficient food. Summer is described as a time of scarcity for harbour porpoises, and the lower proportion (both in number and in mass) of gobies consumed during these months might represent a lower availability of their dominant food source during this period (Fig.13 ; Fig.14). This might explain why the smallest porpoises seem to starve during the summer (Fig.24 ; see below)[107].

The overall sex ratio (M:F) of the analysed porpoises in our study was 1.23:1 (n=174). Though, this ratio differed with age: with a fairly even sex ratio in adults (52.8% females; 47.2% males) and more juvenile males than females (42.2% females; 57.8% males). As such, our dataset to some extent reflects the trend of a higher number of juvenile male strandings in Belgian and surrounding waters, as the higher proportion of males is not likely the consequence of a higher bycatch rate in males[27,31,36,53,175,176]. One hypothesis to explain the high number of male porpoises stranding along our coast was the Southern North Sea constitutes a lower quality habitat and acts as a sink for the whole North Sea population, being inhabited by the weakest part of the population: the juvenile males, sentencing them to eat more junk food as compared to females[36,94,177,178]. Though, as no dietary differences were resolved between male and female porpoises during this study, the higher male mortality cannot be ascribed to diet-related causes. Thus, as stated in Ijsseldijk & ten Doeschate[36], this warrants further investigation. Other plausible explanations for the higher proportion of male strandings could be sexual segregation caused by differential predation risks, resource competition and/or differential activity budgets[179–184].

OTHER POSSIBLE (CONFOUNDING) FACTORS STRUCTURING THE DIET

Even though prey availability is likely to be uneven throughout Belgian waters, the stranding location was not considered as a factor in this diet study due to the high mobility of harbour porpoises, uncertainties about the precise origin of many carcasses and due to the short nature of the Belgian coastline (merely 65 km)[16]. Though, some small regional differences in diet were found in a study in Dutch waters (based on 600 stomach contents from 2003-2014) with porpoises stranded and bycaught along North-East Holland and the Wadden Sea showing differences in the diet as compared to the other locations[158]. Yet, this difference could also be attributed to the underrepresentation of samples from the North East and the Wadden Sea as the collection of stranded porpoises is low and/or sharing of information is hampered. Furthermore, the overall diets of stranded porpoises along the North Sea were found to be remarkably similar, as pointed out by Leopold *et al.*[138]. This was confirmed again in a study by Leopold *et al.*[107] on 829 harbour porpoises stranded and bycaught along the Dutch coast between 2006 and 2014, in which no significant effect of region was recovered. Thus, even though stranding location was not included in this study, we can argue that the diet of harbour porpoises along the Southern North Sea is very similar and any regional differences would be too small to recover, especially as the key prey guilds ("big four") did not differ along the Northern French, Belgian and Dutch coastline[16,44,83,106–108,132,138,158,159]. On a larger geographical scale, variation in the diet was often found, with only a few studies reporting regional variation in the main prey consumed (e.g. Aarefjord *et al.*[156])[82,90,106,189].

Porpoises dying of different causes also exhibited characteristic prey compositions, with the difference between stranded and bycaught animals most noteworthy. Bycatch rates of harbour porpoises in French, Belgian and Dutch coastal waters are reported to be highest between late Winter and Spring[64,175,182,183] and because of this specific collinearity between possible bycatch and the factor season we could not include it as a factor in our model. Though, likelihood of bycatch was also identified as a possible factor structuring the diet of harbour porpoises in other studies, as it results from fishing gear set or towed in specific parts of the water column which might be mirrored by specific food remains in stomach samples of bycaught animals[61]. This has been reported for other cetaceans including dolphins, showing that stomach contents of dolphins bycaught in mid-water trawls or seines differed from those bycaught in bottom trawls, with a higher contribution of pelagic prey species in the former and a higher proportion of demersal prey in the latter[113,184,185]. Several other studies also revealed a difference in the diet of bycaught versus stranded porpoises, though they indicated that this difference might also be ascribed to regional differences in prey abundance[156,166,168]. Bycatch of porpoises in the Southern part of the North Sea is mostly attributed to fisheries employing bottom-set gill nets, leading to the hypothesis that most porpoise bycatches in this region probably occur near the sea floor and the prey species composition in their stomach might be indicative of the type of fishing gear held responsible for the bycatch i.e. dominated by different types of demersal prey species[114,182,186]. This has been extensively studied by Leopold *et al.*[61] in Dutch coastal waters in which stomach contents of porpoise carcasses with high indication of bycatch (n=24; based on the presence of net marks, full stomach and other signs used to diagnose bycatch[110,111,115]) were dominated by demersal prey.

They concluded that a high prey mass alone is a poor indicator of likelihood of bycatch, but the use of prey species composition as an additional bycatch classification characteristic can aid to better identify bycatch in stranded porpoises.

EMPTY STOMACHS

In most diet studies, the empty stomachs are discarded without further investigation. However, these might also carry important information about foraging conditions in our waters[107].

The relatively low proportion of empty stomachs in this study (15%) indicates that most of our studied porpoises had foraged relatively recently. As we were not able to sample all empty stomachs in the examined time-period, we could only describe possible trends in our data.

Leopold et al.[107] studied empty stomach data in more detail and found that the probability of dying with an empty stomach is highest in summer. This was demonstrated by a higher percentage of empty stomachs during the summer, in line with what we found (Table.6). Harbour porpoises contain a blubber layer to preserve their energy balance in the marine environment[187]. Next to its insulative function, blubber coats in cetaceans also function as a fat reserve which can be exploited in times of nutritional stress or fasting[117,188,189]. Figure 18 shows that heavier porpoises seemed to have a thicker blubber layer, justifying its use as a proxy for nutritional condition in harbour porpoises. Furthermore, our data shows that blubber thickness in porpoises containing an empty stomach were significantly lower than porpoises with prey remains found in their stomachs (Fig.19). This is in line with findings by Jauniaux et al.[60], in which it was shown that severe emaciation in porpoises is mostly associated with weight loss and a reduced blubber layer thickness. Though, when we incorporate the seasonality in the thickness of the blubber layer (Fig.21), we only found a significant difference in blubber thickness between porpoises with empty and filled stomachs in spring (Fig.22). This might be an artefact of the small sample sizes in the other seasons, as the overall majority of the examined porpoises died in spring.

Apparently, the summer represents a difficult time for harbour porpoises. As stated by Leopold et al.[45], harbour porpoises seem to have a higher chance of starving during the summer months, due to the combined lack of sufficient (energy rich) prey and a lower energy reserve. Harbour porpoises are shown to have a thinner blubber layer during the summer as a response to the higher ambient water temperature (Fig.21) and thus cannot endure extended periods of fasting, leading to a lower tolerance to starvation in summer[12–14]. Kastelein et al.[12] examined the reduction in body mass and blubber thickness of two captive porpoises during a near-fasting period of 24 hours and found and concluded that a fasting period lasting longer than 24 hours, especially when food is not abundant, could already have detrimental effects on harbour porpoises that could be long-lasting[12]. During this 24-hour period a reduction of up to 3 mm of blubber thickness was observed. Leopold et al.[45] reported that both energy-rich prey and gobies seemed to be in short supply during the summer months in Dutch coastal waters, leading to the hypothesis that the temporary shortage in high quality prey is the most likely cause of starvation. This scenario is most likely, as harbour porpoises don't drive prey availability but are limited by prey availability[190].

A similar pattern is present in the Belgian part of the North Sea, with porpoises mainly relying on lean gadoids and secondarily on sandeels in Belgian and surrounding waters during the summer (unpubl.data, K. Hostens ; our data)[45,101]. Reubens et al.[191–193] also found a higher abundance of both pout and Atlantic cod during the summer at offshore wind farms in the Belgian part of the North Sea.

Even though at least one high quality prey guild is available in summer, the availability of sandeels in Belgian coastal waters (as is also true for Dutch coastal waters) tends to vary substantially from month to month (unpubl.data, K. Hostens)[36]. Furthermore, sandeels move from a benthic to a pelagic life stage during the course of one day, possibly affecting their availability[16,194]. It is not clear whether harbour porpoises take sandeels from the water column, the sand or from both. As such, finding insufficient quantities of sandeels and/or gadoids in this period could explain the higher chance of starvation that has been ascribed to these summer months[45].

Moreover, feeding on solely lean prey or 'junk food' can cause nutritional stress and even starvation, even when they can feed continuously on this low quality prey[46,85,97–100]. This lower consumed mass of energy-rich prey was also found in starving porpoises during the summer in Scotland, leading to conclude that quantity of food cannot always replace its quality, especially in starving porpoises[20,38,86,162]. Or, in other words, harbour porpoises need high-quality prey in their diet to prevent starvation[45]. This highlights the importance of sandeels as a staple food in the diet of harbour porpoises along the Belgian coast, especially during the summer months.

The higher probability of starving during the summer months was also illustrated by the lower reconstructed prey masses in non-empty stomachs during these months in Dutch waters[107]. The reconstructed prey masses in non-empty stomachs in our study showed to be relatively low (but not the lowest) in the summer months, for both juveniles and adults, especially in June and September (Fig.17). Even though the reconstructed prey mass was not at its lowest during the summer in our study, it could still be that a large part of the population is starving during summer as they try to compensate for the lack of high quality prey by ingesting more of the available lean prey such as gobies and gadoids[45]. Furthermore, food consumption rates have been documented to be lower during the summer months as opposed to winter[11].

Leopold et al.[107] also found that the smallest porpoises seemed to starve during the summer as opposed to larger and older animals during the winter. Figure 24 also hints at the existence of this trend in our data. It was hypothesized that as a new generation of porpoises enters our waters during the summer, these inexperienced porpoises might fail to fulfil their nutritional requirements as they switch from nursing to taking solid food during this time of scarcity[15,45]. Larger (older) animals then had a higher probability of starving during winter, possibly because of diseases that prevent them from eating properly[107]

Our limited data suggested that during these summer months, disproportionally more males died with an empty stomach than females (Fig.23), whilst these proportions are more balanced in other seasons. This could possibly be attributed to males being the more dispersing sex in harbour porpoises: due to the increased exercise they tend to lose more blubber, eventually causing poorer diving skills due to a less positive buoyancy.

This possibly affects their foraging efficiency as they are less able to catch the most profitable prey, leading to males being more prone to starvation in this period of scarcity[101,195].

All these studies indicate that the summer is a time of scarcity that harbour porpoises need to bridge which is overall in line with what our limited data suggests.

FUTURE WORK

To get a clearer picture of which prey species grant the highest energetic return, and as such are most profitable to feed on, prey-specific caloric values could be included in diet studies. In this study, reconstructed prey biomass was mainly used to investigate the diet of harbour porpoises in Belgian waters. Adding caloric values in the study might give an additional dimension, especially as eating 50 grams of herring (high energy content) is more important in the diet than 50 grams of whiting (lean prey)[158]. We classified prey guilds as 'energy rich' when they contained more than 5 kJ•g^{-1} wet weight, and the other guilds were considered to be lean prey (<5kJ•g^{-1} wet weight). Though, data on caloric values of different prey species consist of single roughly estimated values, not taking into account variation between prey of different size, different seasons and/or different reproductive stages, and over the years[38,84,85]. More research is thus needed to detect and better understand possible variation in energy densities of different prey species in order to gain a better picture of the most profitable prey for harbour porpoises in the Southern North Sea[138].

During this study, as many porpoises were sampled as possible and supplemented with data of previous diet studies in our waters to increase the sample size[16]. As we tested a few different factors (season, sex, year and life stage), sample sizes within each of these subgroups were also looked at using, to the extent possible, stratified sampling. For example, as it was apparent that the overall majority of the examined animals were juveniles, we devoted more time to analyse available adults. Due to time constraints, we were not able to sample all adults available for examination. These adult samples, together with other untreated samples are still available for further analysis. By including more adults in future studies, dietary differences between males and females might be recovered, as it has been suggested that adult females (especially nursing females) and adult males exhibit contrasting diets[158]. The distribution of samples was also different between the seasons, years and between males and females. future studies should strive for equal sample sizes within each factor and between combinations of factors, as it might cancel out some of the biases and help to uncover more solid trends (e.g. a large proportion of the porpoises sampled in autumn were adults, whilst other seasons had a higher proportion of juveniles).

This study indicated that harbour porpoises in Belgian coastal waters most probably feed opportunistically, with ontogenetic and seasonal variation in the diet. Prey preference was investigated using preliminary data on the density of the most dominant prey species (gadoids, gobies, sandeels and clupeids) in Belgian waters. Most resource studies in our study area use techniques that mostly target demersal species (e.g. beam trawls), and as such little quantitative data is available on the abundance and distribution of pelagic species in Belgian waters.

As resource and diet data cannot be gathered concurrently, future diet research should aim at using quantitative data of both demersal and pelagic species derived from resource studies from around the time of sampling.

Stomach content analysis of stranded and bycaught individuals is currently the most widely (and often only) method used to investigate in detail the feeding ecology of harbour porpoises throughout their distribution range. They are extremely useful as they provide both qualitative and quantitative data that is difficult to obtain using other methods. Though, stomach content samples merely represent a snapshot of the diet as they contain prey remains of the most recently caught prey species with a potential overemphasis on nearshore species[82,118]. Furthermore, stomach content analysis is inherently associated with additional biases, as described earlier[86,92,116,131–133]. Long-term diet reconstructions using stable-isotope and/or fatty acid analysis can be used to overcome some of these problems, as it can provide a picture of the assimilated diet on a larger temporal scale (time period determined by the type of tissue and its turnover rate)[159]. However, just like with stomach content analysis, these techniques are not free of limitations and biases[196]. These less traditional methods can mostly only resolve the trophic position of the predator as well as the origin and number of prey sources[83,108,152]. More and more diet studies adopt a polyphasic approach: through the combination of different methods, an integrated view on the porpoise's foraging behaviour is obtained, highlighting potential differences in the long- and short-term diet. Finally, feeding ecology research could be enhanced through the use of new and rapidly developing methods using DNA, either for full diet reconstruction or to identify prey remains up to species level that could not be distinguished using the traditional hard-remains approach[196–199]. To conclude, the use of multiple complementary methods is highly recommended in future diet research to obtain the most complete and detailed picture.

CONCLUSIONS

The main objective of the research done in this thesis was to gain insights in the feeding ecology of harbour porpoises in Belgian coastal waters.

Our study supports indications from previous studies that the harbour porpoises is a flexible and generalist predators, feeding on a broad prey spectrum, primarily dominated by four key prey guilds (i.e. "The big four"): gadoids, gobies, sandeels and clupeids. We found that both age (~ontogenetic development) and season are important factors structuring the diet of harbour porpoises in our waters. This ontogenetic diet shift can mainly be ascribed to physical constraints in juveniles as well as the increasing caloric needs with increasing porpoise length, obliging larger porpoises to switch from easy-to-catch prey with a low energetic return to larger and/or higher quality prey to keep up with their increasing daily intake requirements. The seasonal differences in the diet is clearly linked to the changing availability of the different prey guilds throughout the year in the Southern part of the North Sea. Though, potential confounding effects of the uneven sample design cannot be excluded. Finally, our study could confirm neither an opportunistic nor selective feeding strategy, though our results present more support for the former.

The results of this research are important in light of harbour porpoise conservation as well as monitoring the populations' health. Though, as our sampling design was non-random, major gaps remain in our understanding of the feeding ecology of harbour porpoises in Belgian waters. Future studies with a larger sample size and a more balanced design in combination with a better understanding of the resource availability and the adoption of a polyphasic approach are necessary to fill in these gaps and increase our understanding of the ecological role of harbour porpoises in Belgian and surrounding waters.

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ANNEX

Table 1: Porpoises of which the stomachs were analysed, including information gathered upon stranding/autopsy and a short description of the stomach content. The age class was determined based on the length of the porpoise. Juveniles had a length ranging from 0.9 up to 1,34m, neonates were smaller and from 1.35m onwards they were considered adults (Lockyer[149]; Karakosta et al.[150]). (F: Female; M: Male; U: Unknown; J: Juvenile; A: Adult; N: Natural; (P)B: (Possibly) Bycatch; GSP: Grey seal predation)

Date	Location	Sex	Age class	Cause of death	Blubber thickness (mm)	Description stomach content
4/04/1997	Koksijde	F	J	B	25	Partly digested fish remains
18/04/2000	Raversijde	F	J	N	13	
18/01/2003	Oostende	F	J	-	15	Fish remains
5/03/2003	Middelkerke	M	J	B	30	Grey mass with a lot of fresh gobies
23/03/2003	Wenduine	M	J	B	11	
22/04/2003	De Panne	F	J	N	8	
13/05/2003	Westende	M	A	N	17	Grey mass of digested fish
12/03/2004	Middelkerke	M	J	B	17	
17/03/2004	Oostende	F	J	B	18	One half-digested fish
27/03/2004	Oostende	F	J	B	22	
3/04/2004	Nieuwpoort	F	J	B	11	Fish in oesophagus; stomach almost empty
12/04/2004	De Panne	U	J	-	20	
17/01/2005	Koksijde	M	J	N	8	
12/04/2005	Oostduinkerke	F	J	PB	8	
20/08/2005	Wenduine	F	J	N	4	Many otoliths, few other remains
5/10/2005	Found at sea	F	J	N	5	Stomach almost empty
2/03/2006	Nieuwpoort	F	A	B	24	
26/03/2006	Oostduinkerke	M	J	B	18	Many otoliths in oesophagus
30/03/2006	Blankenberge	F	J	B	24	Grey mass of digested fish, many otoliths
9/04/2006	Bredene	F	J	B	30	Grey mass of digested fish, many small fish bones and otoliths
27/04/2006	Nieuwpoort	M	A	PB	20	Grey mass with fish bones but few otoliths
27/04/2006	De Haan	F	J	PB	32	
28/04/2006	Nieuwpoort	F	J	PB	36	
28/04/2006	At Sea	M	J	-	20	
8/05/2006	Oostduinkerke	F	J	N	4	Stomach almost empty
9/05/2006	Oostende	M	J	-	13	
3/08/2006	Knokke	M	J	N	18	
8/09/2006	Oostende	F	J	PB	7	
29/12/2006	Oostduinkerke	M	J	B	25	
6/02/2007	De Panne	M	J	B	20	
15/02/2007	Nieuwpoort	M	J	-	8	
22/02/2007	Oostende	M	J	N	6	Empty (but sampled)
26/02/2007	Blankenberge	M	A	N	22	
6/03/2007	Oostduinkerke	M	J	B	19	Few fresh fish remains, fish bones and otoliths
16/03/2007	Oostende	M	J	B	17	
25/03/2007	Oostende	F	J	N	5	Empty (but sampled)
30/03/2007	Oostende	M	J	B	27	Grey mass of digested fish, many small bones and otoliths
9/04/2007	Oostduinkerke	M	J	B	13	
16/04/2007	Oostende	M	J	-	14	Stomach almost empty
20/04/2007	De Haan	M	J	N	8	
4/05/2007	Oostduinkerke	M	A	PB	20	Mass of half-digested fish, many fish bones and otoliths
17/05/2007	Koksijde	F	J	B	18	
30/07/2007	De Panne	M	J	-	10	Grey mass of digested fish, many fish bones and bigger otoliths
20/08/2007	Westende	F	J	-	8	
3/09/2007	Koksijde	F	J	N	8	Grey mass, few fish bones and some otoliths

Date	Location	Sex	Age	Code	Size	Notes
9/09/2007	Koksijde	F	J	-	5	Few tissues, many bones and otoliths
26/09/2007	Mariakerke	F	J	-	5	Stomach almost empty
23/10/2007	Koksijde	F	A	N	9	
11/11/2007	Koksijde	M	A	-	5	Grey mass of digested fish, many bones and otoliths
27/01/2008	Oostende	M	J	N	20	Few digested masses but bigger fish bones and otoliths
7/02/2008	De Haan	M	J	B	19	Few fresh fish remains, some bones and otoliths
21/03/2008	Middelkerke	M	A	B	20	Stomach full
25/03/2008	Middelkerke	M	J	PB	24	Grey mass of digested fish, many bones and otoliths
9/04/2008	Blankenberge	M	J	B	11	Grey mass of digested fish, many small bones and otoliths
10/04/2008	Middelkerke	F	A	-	7	Few hard remains
17/04/2008	Middelkerke	M	A	PB	20	Few digested masses but many small bones and otoliths
29/08/2008	Koksijde	F	J	N	5	Few hard remains
1/10/2008	Wenduine	F	J	N	10	Grey mass of digested fish, many fish bones and otoliths
2/01/2009	Koksijde	F	J	N	8	Semi-fresh fish remains, many bones and otoliths
21/01/2009	Oostende	F	J	N	8	
10/03/2009	Bycatch (Buiten Ratel)	M	J	B	28	Stomach almost empty (few vertebrae)
29/03/2009	De Panne	F	J	PB	31	Grey mass of digested fish, bones and otoliths
30/03/2009	Oostduinkerke	M	J	B	23	
20/04/2009	Wenduine	F	J	B	30	Few digested masses, bones and otoliths
3/05/2009	Middelkerke	F	A	B	8	Many otoliths in stomach
6/05/2009	Middelkerke	F	A	PB	21	
11/07/2009	De Haan	F	A	N	5	Grey mass of digested fish, many small bones and otoliths
10/08/2009	Bycatch (at sea)	F	J	B	12	Few hard remains
13/08/2009	Koksijde	M	J	B	16	
16/03/2010	Nieuwpoort	M	J	B	18	Fresh (pieces of) fish in stomach
21/03/2010	Koksijde	M	J	B	18	Semi-fresh fish remains, many bones and otoliths
22/03/2010	Middelkerke	F	J	B	23	Fresh fish, many fish bones
26/03/2010	Oostduinkerke	F	J	PB	8	Fresh fish in oesophagus and stomach
4/04/2010	De Haan	M	J	N	10	Few hard remains
25/04/2010	Blankenberge	M	J	-	30	Semi-fresh fish remains, bones and otoliths
1/05/2010	Middelkerke	M	J	N	8	Empty
10/05/2010	Bredene	M	A	N	16	Few hard remains, some otoliths
16/05/2010	Middelkerke	M	J	N	12	Few otoliths
31/05/2010	Middelkerke	F	A	N	10	Empty
12/06/2010	Oostende	F	F	-	12	Empty
20/06/2010	Koksijde	F	NN	N	5	Empty
29/07/2010	Oostduinkerke	M	J	N	5	Empty
2/8/2010	Wenduine	M	J	N	8	Empty
5/08/2010	Bycatch (at sea)	M	J	B	-	Many otoliths, few fresh fish remains
13/08/2010	St. Idesbald	F	A	N	-	Many fish bones
16/08/2010	Middelkerke	M	J	N	-	Empty
25/08/2010	De Panne	F	J	N	9	Empty
29/08/2010	Bredene	F	A	N	10	Fresh fish and many bones
12/09/2010	At Sea	F	A	N	12	
13/12/2010	Nieuwpoort	M	J	GSP	10	Fresh pieces of fish in stomach
30/12/2010	Wenduine	F	A	PB	18	
29/01/2011	Lombardsijde	F	A	PB	27	Empty (but sampled)
28/02/2011	Oostende	M	J	GSP	5	
9/03/2011	Koksijde	M	J	B	24	
11/03/2011	Mariakerke	F	A	N	16	
24/03/2011	Blankenberge	M	J	B	13	Fresh gobies and goby otoliths in oesophagus, full stomach
26/03/2011	De Haan	F	J	B	23	
1/04/2011	De Panne	F	J	PB	17	
7/04/2011	Blankenberge	M	J	N	13	
9/04/2011	Bredene	M	J	-	15	Digested prey remains in oesophagus, many otoliths in stomach

Date	Location	Sex	Age	Code	Number	Remarks
17/04/2011	Bredene	M	J	GSP	18	
29/04/2011	Wenduine	U	J	N	4	
14/05/2011	Wenduine	U	J	PB	18	
24/05/2011	Middelkerke	M	J	B	10	Empty
25/06/2011	De Haan	M	J	N	4	Stomach almost empty (only 3 otoliths)
29/07/2011	Knokke-Heist	M	J	N	2	Few hard remains
9/08/2011	Oostende	M	J	B	16	Grey mass of digested fish, bones and otoliths
2/11/2011	De Panne	M	J	GSP	14	Semi-fresh fish remains, few bones and otoliths
5/12/2011	Koksijde	F	A	-	18	Grey mass of digested fish, bones and few otoliths
6/12/2011	Oostende	F	J	N	5	Fresh pieces of fish and 1 intact fish
15/03/2012	Blankenberge	M	J	B	18	Semi-fresh fish remains, many bones and otoliths
22/03/2012	Zeebrugge	F	J	B	6	Fresh gobies and many otoliths
23/03/2012	De Panne	M	J	B	19	Grey mass of digested fish, bones and otoliths
29/03/2012	Bredene	M	J	B	5	Fresh pieces of fish, many bones and otoliths
31/03/2012	Knokke	M	J	N	5	Stomach almost empty
1/04/2012	De Panne	F	J	B	10	Semi-fresh fish remains (gobies)
8/04/2012	Koksijde	F	J	B	30	Grey mass of digested fish, many bones and otoliths
13/04/2012	Wenduine	M	J	GSP	15	Fresh gobies and otoliths in oesophagus and stomach
28/09/2012	Westende	F	A	N	0	Few hard remains, only otoliths
2/12/2012	Middelkerke	M	J	N	18	Parasites, few otoliths
5/03/2013	Wenduine	M	J	B	14	Fresh pieces of fish in stomach, many fish bones and otoliths
18/03/2013	De Panne	F	J	B	25	
30/03/2013	Oostende	M	J	PB	18	Grey mass of digested fish, many bones and otoliths
13/04/2013	Koksijde	F	J	B	14	Grey mass of digested fish, many small bones and otoliths
24/04/2013	De Haan	M	J	PB	9	Few hard remains, mostly otoliths
27/04/2013	De Haan	F	J	B	25	Grey mass of digested fish, fish bones and otoliths
13/05/2013	De Panne	M	J	PB	8	Grey mass of digested fish, fish bones and otoliths
8/01/2014	Nieuwpoort	M	J	PB	15	Only otoliths
15/02/2014	Oostende	F	A	N	10	Semi-fresh fish remains, few bones and otoliths
26/02/2014	De Haan	M	J	N	10	Stomach almost empty
1/03/2014	Koksijde	F	J	B	25	Semi-fresh fish remains, many bones and otoliths
15/03/2014	Raversijde	M	J	B	20	Grey mass of digested fish, fish bones and otoliths
16/03/2014	De Panne	F	J	B	25	Grey mass of digested fish, many fish bones and otoliths
17/03/2014	Nieuwpoort	M	J	B	18	
27/03/2014	Nieuwpoort	M	J	N	8	Grey mass of digested fish, fish bones and otoliths
30/03/2014	De Haan	M	J	GSP	14	Semi-fresh fish remains, many bones and otoliths
8/04/2014	Westende	M	A	PB	23	
11/04/2014	Bredene	M	J	PB	15	Fresh pieces of fish in stomach, many fish bones and otoliths
12/04/2014	Westende	M	J	N	30	Grey mass of digested fish, fish bones and otoliths
13/04/2014	De Haan	M	A	N	15	Semi-fresh fish remains, many bones and otoliths
18/04/2014	Nieuwpoort (harbour)	M	J	B	18	Grey mass of digested fish, many fish bones and otoliths
4/05/2014	Koksijde	M	A	N	20	Grey mass of digested fish, many bones and otoliths
20/05/2014	De Haan	F	J	GSP	8	
28/08/2014	De Haan	F	J	GSP	13	Semi-fresh fish remains, fish bones and otoliths

Date	Location	Sex	Age	Code	Count	Notes
10/12/2014	De Haan	F	J	N	3	Grey mass of digested fish, few fish bones and otoliths
1/02/2015	Oostende	F	A	N	16	Empty (but sampled)
22/06/2015	Koksijde	U	J	PB	19	Fresh pieces of fish in stomach, many small bones and otoliths
27/06/2015	De Haan	M	J	N	4	Stomach almost empty
14/11/2015	Middelkerke	F	A	-	13	Grey mass of digested fish, fish bones and otoliths
17/11/2015	Nieuwpoort	U	A	-	12	Few digested masses, many bones and otoliths
12/02/2016	Koksijde	M	A	B	25	Grey mass of digested fish, fish bones and otoliths
5/03/2016	Koksijde	M	J	GSP	18	
6/03/2016	De Panne	M	J	PB	-	Few hard remains
10/03/2016	Middelkerke	U	J	GSP	-	Grey mass of digested fish, fish bones and otoliths
12/03/2016	De Panne	F	J	GSP	30	Grey mass of digested fish, fish bones and otoliths
21/03/2016	Koksijde	M	J	B	19	
26/03/2016	De Panne	M	J	B	22	Few digested masses and bones but many otoliths
27/03/2016	De Panne	M	J	-	-	Fresh pieces of fish in stomach, many fish bones and otoliths
28/03/2016	Koksijde	M	J	B	26	Semi-fresh fish remains, fish bones and otoliths
8/04/2016	Middelkerke	F	J	-	18	Only otoliths
20/04/2016	Koksijde	F	J	GSP	26	Stomach almost empty (only cephalopod beaks present)
25/04/2016	Middelkerke	F	J	N	6	Only several otoliths
10/07/2016	Koksijde	M	A	N	15	Few digested masses, big fish bones and several otoliths
21/07/2016	De Panne	F	J	GSP	20	
26/07/2016	Middelkerke	F	A	N	15	Grey mass of digested fish, fish bones and otoliths
4/09/2016	Middelkerke	M	A	B	15	Stomach almost empty
15/09/2016	De Panne	M	J	PB	13	Grey mass of digested fish, fish bones and otoliths
7/10/2016	Wenduine	M	NN	N	9	Few hard remains, almost exclusively otoliths
21/02/2017	De Panne	F	J	B	18	Only several bones and otoliths
21/02/2017	Oostduinkerke	F	J	B	18	Semi-fresh fish remains, many fish bones and otoliths
9/03/2017	Bycatch	F	J	B	20	Few hard remains
15/03/2017	Koksijde	F	J	GSP	18	Grey mass of digested fish, fish bones and otoliths
22/03/2017	Wenduine	F	J	N	8	1 flatfish in oesophagus, Fresh pieces of fish (no otoliths found)
12/06/2017	Middelkerke	M	A	B	18	Few hard remains
26/10/2017	Bredene	M	J	N	8	Few digested masses, many fish bones and otoliths
11/11/2017	Oostende	M	A	N	14	Grey mass of digested fish, fish bones and few otoliths
29/04/2018	De Haan	F	NN	-	8	Semi-fresh fish remains, many fish bones and otoliths
31/05/2018	Wenduine	M	J	GSP	5	Grey mass of digested fish, fish bones and otoliths
8/06/2018	Oostende	M	J	N	8	Empty except for some parasites (sampled)
10/09/2018	De Haan	M	A	N	8	Few digested masses, big fish bones and several otoliths

Table 2: Overview of the prey species (fish and cephalopods) that were included in the analyses and the prey guild they were assigned to, ordered alphabetically according to the scientific name. The identification of sand lances and gobies did not go up to species level due to time restrictions. Possible species present in these groups could be Ammodytes tobianus, Hyperoplus lanceolatus and Ammodytes marinus for sand lances; Pomatoschistus microps, Pomatoschistus lozanoi, Pomatoschistus minutus and to a smaller extent Gobius niger for gobies.

Scientific name	Common name	Order	Family	Prey guild
Ammodytes sp.	Sand lances	Perciformes	Ammodytidae	Sandeels
Atherina presbyter	Sand smelt	Atheriniformes	Atherinidae	Estuarine roundfish
Callionymus lyra	Dragonet	Perciformes	Calionymidae	Demersal roundfish
Ciliata mustela	Fivebeard rockling	Gadiformes	Lotidae	Demersal roundfish
Clupea harengus	Atlantic herring	Clupeiformes	Clupeidae	Clupeids
Dicentrarchus labrax	European Seabass	Perciformes	Moronidae	Demersal roundfish
Echiichthys vipera	Lesser weever	Perciformes	Trachinidae	Demersal roundfish
Engraulis encrasicolus	European anchovy	Clupeiformes	Engraulidae	Estuarine roundfish
Gadus morhua	Atlantic cod	Gadiformes	Gadidae	Gadoids
Pomatoschistus sp.	Gobies	Perciformes	Gobiidae	Gadoids
Loligo vulgaris	European squid	Myopsida	Loliginidae	Gobies
Merlangius merlangus	Whiting	Gadiformes	Gadidae	Gadoids
Mullus surmuletus	Surmullet	Perciformes	Mullidae	Demersal roundfish
Osmerus eperlanus	European smelt	Osmeriformes	Osmeridae	Estuarine roundfish
Platichthys flesus	European flounder	Pleuronectiformes	Pleuronectidae	Flatfish
Pollachius virens	Saithe	Gadiformes	Gadidae	Gadoids
Scomber scombrus	Atlantic mackerel	Perciformes	Scombridae	Pelagic roundfish
Scophthalmus sp.	Brill	Pleuronectiformes	Scophthalmidae	Flatfish
Sepia officinalis	Common cuttlefish	Sepiida	Sepiidae	Squid
Sepiola sp.	Bobtail squid	Sepiida	Sepiolidae	Squid
Solea solea	Common sole	Pleuronectiformes	Soleidae	Flatfish
Sprattus sprattus	European sprat	Clupeiformes	Clupeidae	Clupeids
Trachurus trachurus	Atlantic horse mackerel	Perciformes	Carangidae	Pelagic roundfish
Trisopterus luscus	Pouting	Gadiformes	Gadidae	Gadoids
Trisopterus minutus	Poor cod	Gadiformes	Gadidae	Gadoids

Table 3: Overview of the other prey species (crustaceans and other invertebrates) found during this study, but not included in the analyses, ordered according to their presence in the different samples.

Scientific name	Common name	Order	Family
Crangon crangon	Brown shrimp	Decapoda	Crangonidae
Nereididae	ragworms	Phyllodocida	Nereididae
Liocarcinus navigator	/	Decapoda	Polybiidae
Pagurus bernhardus	Common hermit crab	Decapoda	Paguridae
Palaemon serratus	Common prawn	Decapoda	Palaemonidae
Diogenes pugilator	Small hermit crab	Decapoda	Diogenidae
Parasitic nematoda	/	/	/