The diet of harbour porpoises bycaught or washed ashore in Belgium: exploratory study and results of initial analyses

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Title:

The diet of harbour porpoises bycaught or washed ashore in Belgium: exploratory study and results of initial analyses

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Bones of a plaice *Pleuronectes platessa.*

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Summary

After decades of absence in the southern part of the North Sea, the harbour porpoise is again considered as common in this area. In Belgian waters, seasonally thousands of individuals occur. There are clear indications that the return of the porpoise to the southern North Sea is not the consequence of an increase in the population size, but rather of a shift of part of the population. The underlying reasons for this phenomenon are not clear, but the most plausible is that it is prey related. Research has indicated that porpoises through time have changed diet, probably under pressure of a diminished availability of preferred prey items caused by overfishing, but such changes could also be provoked indirectly by climate change.

Studies of impacts of climate on plankton abundance and distribution, with correlations to species higher up in the food chain, are rare, but important as they can help us understand changes in the past, and can be used to predict further consequences of climate change effects. The diet analyses of species at different levels in the food chain, including the diet analysis of the harbour porpoise, are a necessary element in such studies, together with studies on distributional changes of prey and predator. Studies on the diet of porpoises, combined with abundance and distribution data, can also be used to make a scientifically sound assessment of the existence or not of a competition between marine mammals and fishermen.

ASCOBANS, in its Harbour Porpoise Conservation Plan for the North Sea, recommends the study of the feeding ecology of the porpoise. With this report, a first step has been taken in the analysis of the diet of harbour porpoises in Belgian waters on the basis of the available stomach contents. No in depth analysis of all stomach contents of harbour porpoises collected so far in Belgium is made; rather, the possible information contained in those stomach contents, an initial testing of methods, the necessary investment in setting up a collection of reference material, and the results of the analysis of a small number of stomach contents is described. It will be useful to compare the results of stomach content analysis, now for a large part in grey literature, through time, and over a wide geographical level.

1. Introduction

After decennia of absence in the southern part of the North Sea, the harbour porpoise *Phocoena phocoena* is again common in this area. It is the most abundant marine mammal occurring in the Belgian part of the North Sea, with seasonally up to 4.000 individuals (Haelters, 2009). This number can be considered of international significance in the framework of the European Habitats Directive (92/43/EEC) (Degraer *et al.*, 2009). There are clear indications that the return of the porpoise to the southerly waters of the North Sea is not a consequence of an increasing population, but rather of a shift of part of the population towards the south (Hammond et al., 2002; Camphuysen, 2004; SCANS II, 2008). The underlying reasons for this shift are not clear, although the most plausible one is that it is prey related: an increase in suitable prey in the southern part of the North Sea, or a decrease in prey in the northern part, or a combination thereof. As the harbour porpoise is a small endothermic animal, with a small energy stocking capability, it needs to eat every day. It has been demonstrated that captive harbour porpoises consumed 4% to 9.5% of their body weight in food - in the wild food requirements might even be higher, given the colder water and the higher effort needed to catch live prey (Kastelein *et al.*, 1997). If a harbour porpoises cannot obtain sufficient food, it has to rely on its fat reserve. In theory, it could survive for three to five days without feeding, depending on its initial condition. However, given that its fat reserve also serves as thermal insulation, it can die due to hypothermia before the fat reserve is completely depleted. After 24 hours without food, the animal will be severely weakened. Therefore the actual life expectancy for a harbour porpoise not able to feed is estimated at three days in water of 20°C (Kastelein *et al.*, 1997).

Research has indicated that through time harbour porpoises have changed diet, probably under pressure of a diminished availability of preferred prey items, caused by overfishing (Santos, 1998). Such changes could also be provoked indirectly by climate change (MacLeod *et al.*, 2005; 2007; Simmonds & Isaac, 2007). An example of such phenomenon is the case of the sandeel *Ammodytes marinus*, an abundant staple food species in the northern part of the North Sea. The distribution of the copepod *Calanus finmarchicus*, an important prey species for sandeel and other pelagic or semi-pelagic fish in the North Sea, has shifted to the north, (indirectly) due to an increase in water temperature. This has negatively affected the recruitment success of the sandeel and other species, or had an impact on their distribution (van Deurs et al., 2009; Perry et al., 2005; Beaugrand et al., 2003; Frederiksen *et al.*, 2006; Hays *et al.,* 2005; MacLeod et al., 2007). In turn this affects predators which rely on the availability of sandeels. Impact studies of climate on plankton abundance and distribution, with correlations to species higher up in the food chain, are rare but important, as they can help us understand changes in the past, and can be used to predict further consequences of climate change effects. The diet analysis of species at different levels in the food chain, including the diet analysis of the harbour porpoise, are a necessary component of such studies, together with the investigation of distributional changes of prey and predator. Studies of the diet of porpoises, combined with abundance and distribution data, can also be used to make a scientifically sound assessment of the existence or not of a competition between marine mammals and fisheries.

Indirectly, information on the prey of marine mammals can be revealed through dietary signals present in their tissues: stable isotopes (information on the diet of days to years, depending on the tissue considered), trace elements (information on the diet of weeks to years), and fatty acids (information on the diet of weeks to months) (e.g. Das et al., 2001; Lahaye et al., 2005; Drouget et al, 2007). Since fatty acids originate mainly from the diet, fatty acid patterns (or signatures) are prey species dependent. Stable isotope ratios indicate the trophic level (analysis of δ_{15}N) and, given that they vary geographically, point towards the feeding location (analysis of δ_{13}C) (Das et al., 2003; Christensen & Richardson, 2008; Witteveen et al., 2011). For instance, a high cadmium content indicates a high proportion of cephalopods in the diet (e.g. Law et al., 1997). Although these relatively new methods give information about the general pattern in prey items over a long period of time, and the level of the predator in the food chain, they give no detailed indication on the prey species itself nor on the size of the prey. The identification of the most recent food uptake can be done directly through investigating the stomach content. Although a *high-tech* method exists, through a protein or DNA analysis of stomach remains, it is more straightforward to try and visually identify the remains of prey items in the digestive tract of marine mammals.

ASCOBANS recommends the study of the feeding ecology of the porpoise in its *Harbour Porpoise Conservation Plan for the North Sea* (Reijnders et al., 2009; Action 10 of the Plan: *Investigation of the health, nutritional status and diet*). The implementation timeline for parties is 'ongoing', with a regular (every 3 to 5 years) review of results. While in neighbouring countries stomach content analyses have been, or are being undertaken (e.g. Santos & Pierce, 2003; Leopold & Camphuysen, 2006), virtually no stomach content analysis has been made on porpoises washed ashore or bycaught in Belgium. Van Beneden (1889) gave an early overview of some small-scale investigations of the stomach of some harbour porpoises, however these harbour porpoises did not originate from Belgian waters.

Santos (1998) and Santos & Pierce (2003) made an in-depth overview of the diet of the harbour porpoise throughout the North-East Atlantic, mostly based on published reports. They found large regional differences. Predominant prey species of porpoises in the southern North Sea, including The Netherlands and the North Sea shores of Denmark, were whiting *Merlangius merlangus* and cod *Gadus morhua* (Gadidae), sandeels (Ammodytidae) and gobies (Gobiidae). Harbour porpoises collected in The Netherlands had consumed significantly more gobies, dragonets *Callionymus* sp. and squid *Loligo forbesi* than harbour porpoises collected in Scotland, while Dover sole *Solea solea* was an important prey item in porpoises stranded on German North Sea shores. Santos & Pierce (2003) also reviewed seasonal and long-term trends, differences between males and females, and differences between juveniles and adults, and they furthermore explored the possible competition for prey with other predators and fisheries.

Finally, stomach content investigation allows an assessment of the ingestion, and possible effects of litter on marine mammals (e.g. Kastelein & Lavaleije, 1992).

The aim of this project was to look into the stomach contents available for research and explore the possible information they contained, to test methods for analyzing these stomachs, and to initiate a collection of reference material. An overview of initial results is presented. This report does not present the results of an in depth analysis of all stomach contents of harbour porpoises collected so far in Belgium.

2. Material and methods

2.1. Collecting and preparing stomach contents

Harbour porpoises washed ashore or bycaught in Belgium during the last fifteen years have been, to the extent possible, collected for research purposes by the Royal Belgian Institute of Natural Sciences (RBINS), department Management Unit of the North Sea Mathematical Models (MUMM). Only few, very decomposed carcasses were transferred immediately to a destruction facility. All data relevant to the stranding (location, date, circumstances, external characteristics of the animal, etc.) were taken up in a database. The collected animals were temporarily stored in a freezer at Ostend, after which they were transported to the University of Liège, where they were subjected to an extensive necropsy using a standardized methodology (Kuiken & Hartmann, 1991; Jauniaux et al., 2002). Some selected very fresh animals were transported immediately upon the stranding, especially for microbiological sampling and inner ear investigation.

During the autopsy of the harbour porpoises, the first and second stomach were cut open to be checked for parasites and lesions. The general nature of the stomach content itself was described in a few sentences. Afterwards, the stomach was cut open further where necessary, and rinsed over a 315 µm square meshed sieve, which retains the saccular otoliths (see further) of even the smallest fish. The complete content was collected. Only when no visible remains of prey were present, no sample was retained for further analysis. In some cases, the whole stomach was collected in a plastic bag. The presence of prey in the oesophagus was noted, and these prey remains were added to the stomach content (figure 1). After the autopsy, the stomach content or whole stomach was frozen (-20°C) pending further treatment.

To prepare the collected stomach contents for analysis, the following methods were tested:

1) Destruction of soft remains in a hot sodium hydroxide (NaOH) solution:

   Stomach contents were put in a hot sodium hydroxide NaOH solution for some minutes to destroy soft remains. After cooling, the remains were rinsed with water over a 315 µm square meshed sieve, and dried. This method dissolves all fish remains except for otoliths. Also squid beaks and decapod carapaces remain fairly intact. The result is a clean sample which can be analyzed rapidly.

2) Macerating the stomach content with enzymatic washing powder:

Stomach contents were rinsed in a bucket, and visually inspected for invertebrate remains. They were poured over a square meshed 315 µm sieve, and transferred to a beaker to which Biotex Green™ was added. Biotex Green is a washing powder which contains enzymes that break down fat, protein and polymers of carbohydrates such as starch. The remains were left for maceration for 1 to 3 days at 40 °C (*au bain marie*). Afterwards, the content of the beaker was rinsed again over a 315 µm sieve. The content was transferred to a recipient, and rinsed with a small amount of ethanol. After drying, a fairly clean sample remains; it includes otoliths, but also other fish bones.

The harbour porpoises of which the stomach content was investigated for this exploratory study are listed in table 1. In this table data on length, weight, sex, cause of death, and health status prior to death are included, as these may be determining factors in the last feeding of the animals, and as such in the stomach content remains.

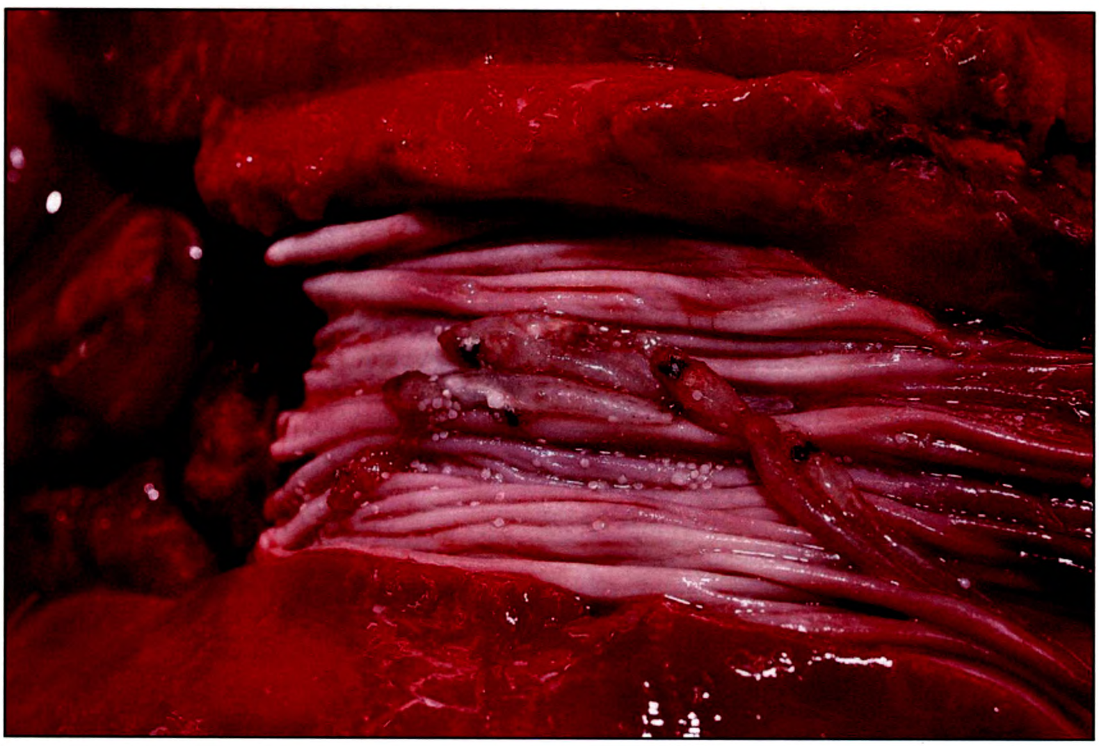

Figure 1. Four small gobies and many goby otoliths present in the oesophagus of a bycaught harbour porpoise.

Table 1. Porpoises studied for this project, including a number of relevant parameters obtained upon the stranding and/or autopsy, and a brief description of the general nature of the stomach content. F: Female; M: Male; U: Unknown; N: Natural; BC: Bycatch or suspected bycatch.

Date	Place	Length (cm)	Weight (kg)	Sex	Cause of death	Emaciation	Blubber thickness (mm)	Parasite infestation	Pneumonia	Remarks	Short stomach content description
18/04/2000	Oostende	112	20,5	F	N	x	13	x	x		
18/01/2003	Oostende	124	25	F	U	x	15	x			Fish remains
5/03/2003	Middelkerke	120	23,7	M	BC		30	x			Grey mass with a lot of fresh gobies
23/03/2003	Wenduine	110	22,5	M	BC		11	x			
22/04/2003	De Panne	109	15	F	N	x	8				
13/05/2003	Middelkerke	152	42	M	N	x	17	x	x	Live stranded	Mass of digested fish
12/03/2004	Middelkerke	99	17	M	BC		17				
17/03/2004	Oostende	111	30	F	BC		18	x			One half-digested fish
3/04/2004	Nieuwpoort	94	15	F	BC	x	11	x			Fish in oesophagus; stomach almost empty
21/03/2010	Koksijde	113	21,5	M	BC		18				Fresh fish remains
22/03/2010	Middelkerke	104	24	F	BC		23				Fresh fish, many fish bones
26/03/2010	Oostduinkerke	101	14	F	BC	x	8				Fresh fish in oesophagus and stomach

Table 1 Continued.

Date	Place	Length (cm)	Weight (kg)	Sex	Cause of death	Emaciation	Blubber thickness (mm)	Parasite infestation	Pneumonia	Remarks	Short stomach content description
1/05/2010	Middelkerke	118	18	M	N	x	8	x			Empty (but sampled)
16/05/2010	Middelkerke	130	25,5	M	U	x	12	x			Few otoliths
31/05/2010	Middelkerke	157		F	N		10			With foetus	Empty
12/06/2010	Oostende	107	14	F	U		12	x			Empty
20/06/2010	Koksijde	80	4	F	N		5			Still-born	Empty
29/07/2010	Oostduinkerke	97	9,5	M	N	x	5	x			Empty
2/08/2010	Wenduine	104	15,5	M	N	x	8			Live stranded	Empty
5/08/2010	At sea	115	22,5	M	BC						Many otoliths, few fresh fish remains
14/08/2010	Koksijde	158		F	U			x		Lactating female	Many fish bones
16/08/2010	Middelkerke	114	16,5	M	N	x		x			Empty
25/08/2010	De Panne	98	9	F	U		4				Empty
29/08/2010	Bredene	159	39	F	U	x	10	x		Lactating female	Fresh fish, fish bones

2.2. Analyzing stomach contents

The main prey of harbour porpoises consists of different fish species. Otoliths, mineralised, solid compact structures forming part of the inner ear of fish, are particularly important for analyzing stomach contents. They predominantly consist of aragonite (calcium carbonate, $CaCO_3$), unlike fish bone, which makes them fairly resistant to chemicals such as stomach acid, that does affect other fish bones more severely. Given their small size, they can remain in the stomach of harbour porpoises for some time before being transported further down the digestive tract. Otoliths of a large number of prey items can frequently be found in the first stomach, which has many folds collecting otoliths of fish possibly digested a relatively long time ago.

Fish have three otoliths in each inner ear; the largest are the saccular otoliths (or the sagittae) (figure 2). The morphology of the saccular otoliths of many fish is very typical for the genus or even the species, and can therefore be used to identify recent prey items. The size of the saccular otoliths (length, width) is a measure of the original size and weight of the fish it belonged to, and as such these otoliths can be used to reconstruct at least part of the composition of the most recent prey of the porpoise. However, such reconstruction should be made with caution. Otoliths in digestive tracts are subjected to chemical and mechanical erosion, which may render them difficult to identify and measure. Some otoliths may be more vulnerable to chemical and mechanical erosion than others, or may move more rapidly through the digestive tract than others (Grellier & Hammond, 2005).

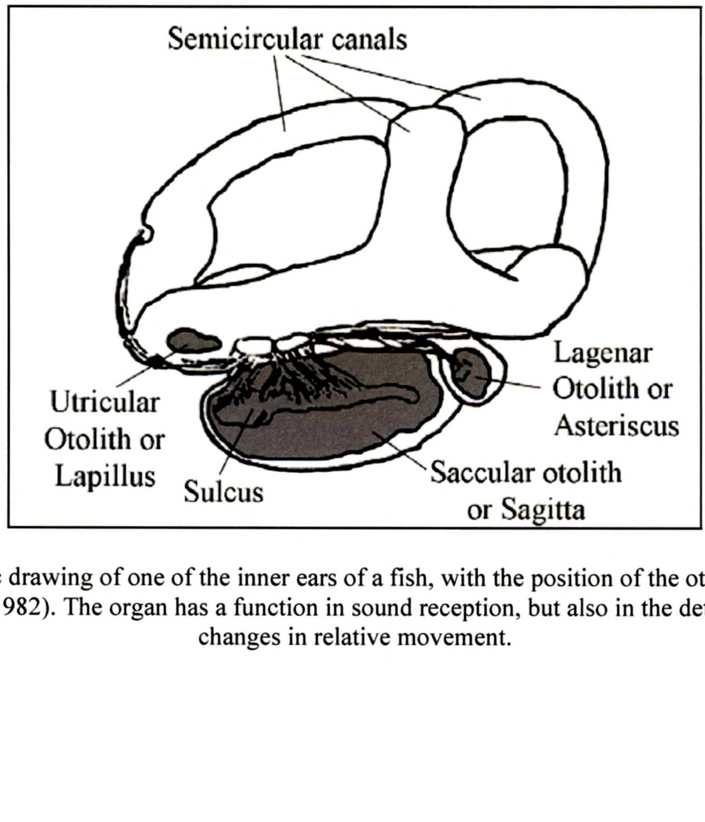

Figure 2. Schematic drawing of one of the inner ears of a fish, with the position of the otoliths (adapted from Popper & Coombs, 1982). The organ has a function in sound reception, but also in the detection of gravity and changes in relative movement.

To complement for saccular otoliths, also some fish bones can be used for identifying prey items (figure 3). Especially vertebrae and head bones can be useful. For instance, dragonet *Callionymus* sp. otoliths are rarely found in marine mammal stomachs, but the spines on its preopercule are very typical, and even enable to distinguish between *C.lyra* and *C.reticulatus* (Gema Hernández-Milián, personal communication, 3 September 2010). Other useful bones are the premaxillae, maxillae, urohyal (flatfish), cleithrum and vertebrae – however, such identifications are specialist work, and require the availability of a fish bone reference collection.

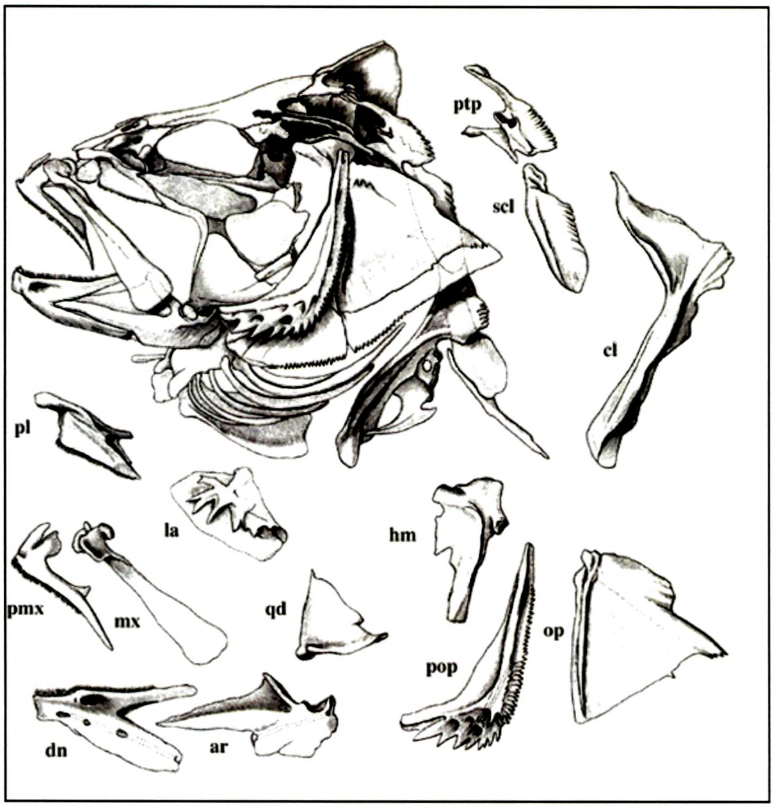

Figure 3. Many bones of the head of fish can be used to identify the fish species, and even to estimate the original length of the fish they belonged to (skull of perch *Perca fluviatilis*; taken from Muséum national d'Histoire naturelle – Osteobase: www.mnhn.fr; original in Cuvier & Valenciennes, 1828): cl: cleithrum, op: operculum, pop: preoperculum, hm: hyomandibular, qd: quadrate, ar: articular, dn: dentary, mx: maxillary, pmx: premaxillary, pl: paline, la: lacrimal, scl: supracleithrum, ptp: posttemporal.

The prepared samples of the stomach contents were investigated under a stereoscopic binocular microscope (magnification of 6X to 40X), and remains were, to the extent possible, identified to the species level. The literature on fish bone (including otoliths) identification used was Harkonen (1986); Nolf & Stringer (1992); Watt et al. (1997); Leopold et al. (2001); Conroy et al. (2003); Svetocheva et al. (2007); Tuset et al. (2008); Nolf et al. (2009).

Next to this, and certainly in cases of doubt, comparisons were made with saccular otoliths and other fish bones in a reference collection of fish bones prepared from fresh fish. For this study, a reference collection of the bones of fish species commonly occurring in the Southern North Sea was initiated (Annex 1). Collected fresh fish were identified, measured, and cooked (boiling water or microwave oven). After removing the largest fleshy parts, and the intestines to avoid contamination due to the presence of prey items, they were macerated in enzymatic washing powder, and treated further in a similar way as the stomach contents. The bones were further rinsed with ethanol, and stored dry. For fish species not obtained during this exploratory study, the reference collections present at the RBINS (fish otoliths at the Fossil Vertebrates Section, and fish bones at the Anthropology and Prehistory Section) remained available for consultation.

If possible, all saccular otoliths were counted per species. As otolith length is a good measure to reconstruct fish length (Harkonen, 1986), all otoliths, or a random subsample if there were too many, were measured. For estimating the length, a regression model using otolith length vs. fish length as proposed by Leopold et al. (2001) or Harkonen (1986) was used. In cases of broken otoliths, the width of the otolith was measured. Fish otoliths become smaller due to erosion – which evidently affects the estimate of the original length of the fish; for this study no correction factor for eroded otoliths was applied. If additionally the weight of the fish the otolith belonged to would need to be known, it could be estimated through a second regression (fish length – fish weight) – this was outside the scope of the present exploratory study.

Measurements of saccular otoliths were made on length-referenced photographs, using the software programme Photoshop. The average length of two otoliths was used in cases where it was clear that they concerned a pair, having belonged to one fish.

Besides from otolith length, the reconstruction of the original prey length could also be made through an allometric equation between other hard parts and length, as obtained through a reference collection (see eg. Harkonen, 1986; Watt et al., 1997). This also was outside the scope of the present study. However, the analysis was non destructive, and all stomach contents remain available for further investigation.

2.3. Expressing stomach content: diet indices

A number of different methods exist to measure and express the prey composition in the diet of marine mammals. They include:

- The total number of prey species in the stomach.

- The numerical importance of prey items per species *i* in the stomach of a marine mammal: N_i, usually based on the otolith remains (but also possible on the basis of cephalopod beaks), and the proportion of the species *i* in the stomach of a marine mammal by number, calculated on the basis of otolith (or cephalopod beak) remains:

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

- Proportion of the species *i* in the stomach by fresh weight (biomass), estimated on the basis of otolith or cephalopod beak remains:

$$\%W_i = \frac{W_i}{W} \times 100$$, with W the total estimated fresh weight of the prey.

- Frequency in occurrence of prey, or the presence/absence of fish or other prey species *i* in the stomach, as a percentage of the total number of stomachs analyzed (*n*), excluding those that were empty:

$$\%O_i = \frac{n_i}{n} \times 100$$ with n_i the number of stomachs in which prey item *i* was found.

- The average importance (expressed in %) of prey species *i* in the diet of the marine mammals investigated; this can be calculated differently, in which both methods yield different results, except with very uniform diets throughout the marine mammals investigated.

$$\frac{\sum_1^n \frac{N_i}{N} \times 100}{n} \quad \text{or} \quad \frac{\sum_1^n N_i}{\sum_1^n N} \times 100$$

- The average importance (expressed in %) by weight of prey species *i* in the diet of the marine mammals investigated; also here, different methods, yielding different results, can be used.

$$\frac{\sum_{1}^{n} \frac{W_i}{W} \times 100}{n} \quad \text{or} \quad \frac{\sum_{1}^{n} W_i}{\sum_{1}^{n} W} \times 100$$

The minimum number of fish present of a certain species was estimated as half the number of the otoliths of the species, except when single otoliths were present which clearly originated from different individuals. In case of an uneven number, the minimum number of fish of the species concerned was rounded to the higher number. For gobies it is possible that the number of otoliths should be divided by four, as also other otoliths than saccular otoliths could be present in the sample, and not easily distinguished from saccular otoliths - this remains to be investigated further.

Other interesting aspects of prey are seasonal and year to year differences, length-frequency distribution per prey species, importance of benthic, demersal and pelagic prey, and differences in prey according to age (size), sex and health or nutritional status of the harbour porpoise concerned. Given the small number of stomachs investigated in this study, no regional or seasonal differences were assessed.

2.4. Possible bias in results

The results of the analyses, both in their qualitative and quantitative expression, should be assessed with some caution, as several sources of bias are inherently connected to the methodology. Next to errors occurring through for instance the measurements of otoliths, and the inherent variability of fish (e.g. differences in weight vs. length before and after spawning), producing errors in the regression used for otolith length vs. fish length, the following problems, leading to bias and limitations, can occur (Pierce & Boyle, 1991; Wijnsma et al., 1999; Santos & Pierce, 2003):

- The sample itself is based on stranded and bycaught animals, and may as such be biased towards unhealthy individuals and animals more likely to be bycaught (eg. inexperienced juveniles), and a proportion of the population with a higher mortality rate.

- The remains in the stomach only represent the most recent prey items – as such, a 'prediction' of the diet on the basis of fatty acid analysis (long-term diet reconstruction) can be very different from the stomach content analysis.
- There is a different digestion rate of prey per species or per size (differential degradation), which leads to a different period that prey remains in the stomach (differential passage rates).
- Prey may only be ingested partly (e.g. without the head containing the otoliths).
- Otolith erosion, which depends on otolith size and species, leads to errors in the estimation of fish length, and accordingly, weight (but correction factors can be applied).
- Part of the stomach content may represent the digestive tract content of ingested fish (secondary prey).
- In some species of fish, the utricular otolith might not be very different in size than the saccular otolith – for instance in gobies this might be a problem.
- There is no information from animals with an empty stomach.
- In literature different relationships are presented for fish length and fish weight (Granedeiro & Silva, 2000).
- If the carcass of a washed ashore harbour porpoise is very decomposed, the location of where it died is highly uncertain.
- General patterns are blurred by differences in diet according to season, area and individual.

Still, the stomach content analysis, as described above, remains in most cases the best, and most widely used method to study and describe the diet of marine mammals and seabirds, and, in case of the harbour porpoise, virtually the only one available.

3. Results

3.1. Testing of the methods to prepare the samples

The method consisting of a destruction of soft remains of the stomach content in a hot sodium hydroxide (NaOH) solution was performed on a small number of stomach contents. However, it was abandoned, given it was perceived as possibly too destructive: interesting remains present in the untreated sample, such as fish jaws and soft remains of for instance shrimp, were in most cases completely destroyed. Moreover, otoliths themselves became somewhat brittle.

In contrast, the method in which the stomach content was rinsed thoroughly, and was macerated afterwards with enzymatic washing powder, yielded larger samples in which otoliths and fish bones, but also other parts, such as the chitinous remains of decapods, remained intact (figure 4).

Figure 4. After macerating the stomach content in enzymatic washing powder, a sample with identifiable fish bones remains.

3.2. Prey species present in the stomachs

Table 2 presents the prey species encountered in the stomachs of the harbour porpoises investigated. The numbers of otoliths of all fish, including gobies, were divided by two to obtain the minimum number of fish remains present, and the number of goby otoliths was estimated in cases when there were hundreds.

The remains of three to more than 1.000 fish were found in the stomachs (excluding the empty stomachs). Stomachs contained remains of one to six fish species or species groups – at least in the goby remains, more than one species is present. The juvenile animals investigated had mainly fed on small benthic fish, mostly gobies, of which they must have consumed hundreds per day (figure 5). Important prey items in adults were gadoids, Ammodytidae and in one animal scad *Trachurus trachurus*. Remarkable was the relatively large number of juvenile seabass *Dicentrarchus labrax* remains in two animals.

The remains of hermit crab *Pagurus bernhardus* and grey shrimp *Crangon crangon*, certainly when found together with gadoids, and also the gobies in the adult porpoise of 14 August 2010, probably constituted secondary prey items.

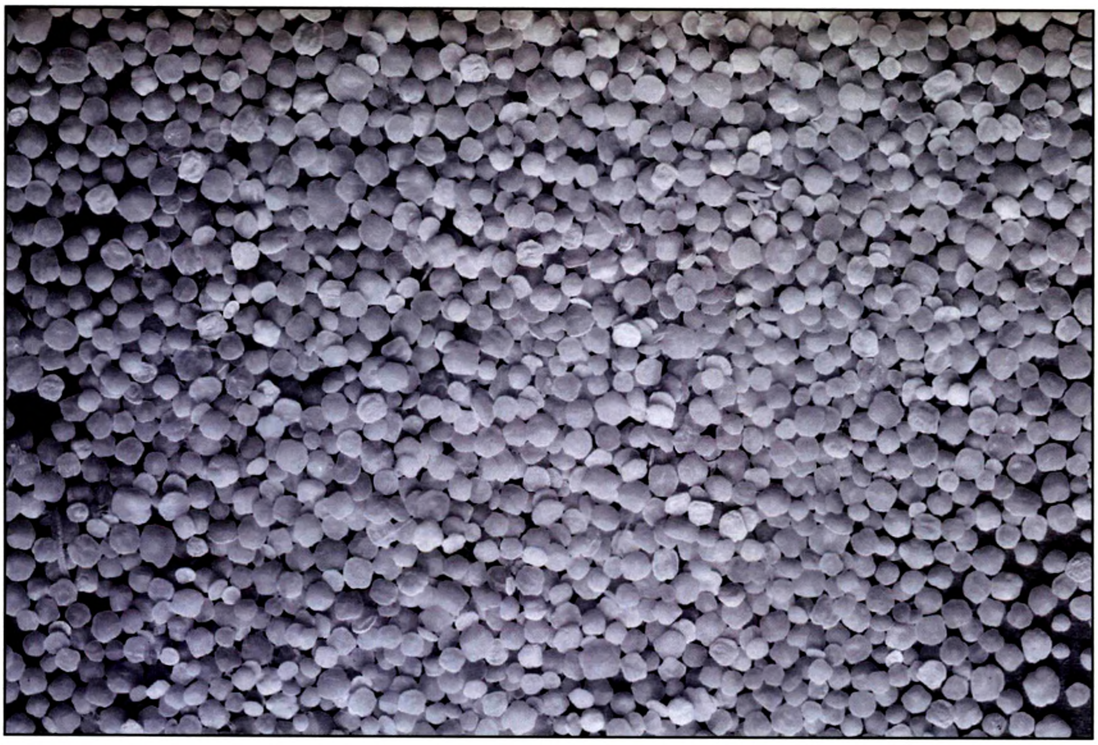

Figure 5. In many of the stomachs, hundreds of otoliths of gobies were present.

Table 2. Stomach content of the harbour porpoises investigated (minimum number of prey items; X indicates presence); the number of gobies present are estimated in cases with hundreds of otoliths.

Date	**Place**	**Length (cm)**	***Pomatoschistus sp.***	***Ammodytes sp.***	***Hyperoplus lanceolatus***	***Merlangius merlangus***	***Gadus morhua***	***Trisopterus minutus***	***Clupea harengus***	***Sprattus sprattus***	***Osmerus eperlanus***	***Trachurus trachurus***	***Dicentrarchus labrax***	***Scophthalmus sp.***	***Platichthys flesus***	***Pagurus bernhardus***	***Crangon crangon***	***Sepiola sp.***	***Loligo sp.***	***Sand/stones***
18/04/2000	Oostende	112	5			3														
18/01/2003	Oostende	124		3		2													1	
5/03/2003	Middelkerke	120	560																	
23/03/2003	Wenduine	110	310																	
22/04/2003	De Panne	109	600	2					1											
13/05/2003	Middelkerke	152		5		6			1									1		
12/03/2004	Middelkerke	99	350																	
17/03/2004	Oostende	111		6					4	2										
3/04/2004	Nieuwpoort	94						2	1				1		1					
21/03/2010	Koksijde	113	300			2				3	3		42	1		X	X			
22/03/2010	Middelkerke	104	1250								3		4				X			
26/03/2010	Oostduinkerke	101	10			2	1						17							

Table 2 continued.

Date	Place	Length (cm)	*Pomatoschistus sp.*	*Ammodytes sp.*	*Hyperoplus lanceolatus*	*Merlangius merlangus*	*Gadus morhua*	*Trisopterus minutus*	*Clupea harengus*	*Sprattus sprattus*	*Osmerus eperlanus*	*Trachurus trachurus*	*Dicentrarchus labrax*	*Scophthalmus sp.*	*Platichthys flesus*	*Pagurus bernhardus*	*Crangon crangon*	*Sepiola sp.*	*Loligo sp.*	*Sand/stones*
1/05/2010	Middelkerke	118																		
16/05/2010	Middelkerke	130	3																	X
31/05/2010	Middelkerke	157																		
12/06/2010	Oostende	107																		X
20/06/2010	Koksijde	80																		
29/07/2010	Oostduinkerke	97																		
2/08/2010	Wenduine	104																		
5/08/2010	At sea	115	300																	
14/08/2010	Koksijde	158	4	240								5								X
16/08/2010	Middelkerke	114																		
25/08/2010	De Panne	98																		
29/08/2010	Bredene	159	5		2															X

The frequency of occurrence of the prey is presented in table 3, which does not take account of the possible presence of secondary prey items. The figures are based on the stomach contents of 16 animals, excluding those with empty stomachs (eight out of the 24 stomachs investigated did not contain prey remains).

Table 3. Frequency of occurrence of species/species groups in the stomachs which contained prey remains.

Species/species group	**% of occurrence**
Gobiidae.	69%
Ammodytidae	38%
Gadidae	44%
Clupeidae	38%
Osmerus eperlanus	13%
Trachurus trachurus	6%
Dicentrarchus labrax	25%
Scophthalmus sp.	6%
Platichthys flesus	6%
Pagurus bernhardus	6%
Crangon crangon	13%
Sepiola sp.	6%
Loligo sp.	6%

3.3. Percentage of prey items in the stomach

Figure 6 presents the number of fish of each species or species group of which remains were present in the stomach content of the harbour porpoises investigated.

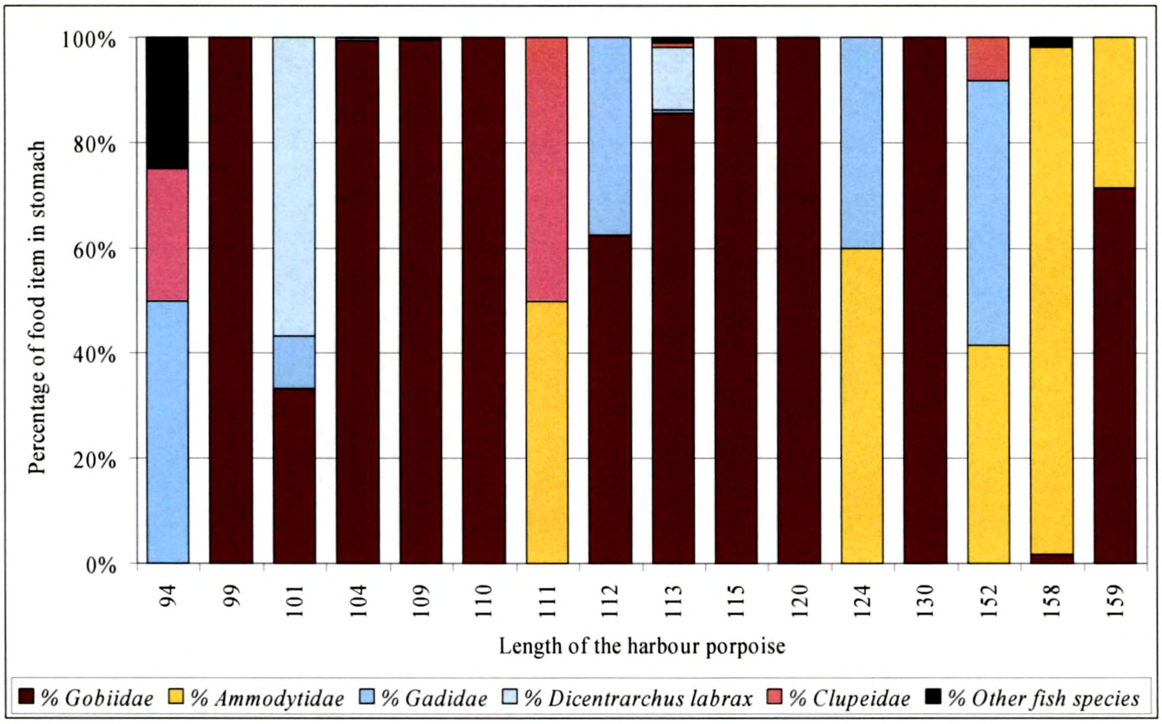

Figure 6. Number of fish remains of each species or species group (expressed as %) present in the stomachs of the harbour porpoises investigated.

3.4. Length of the individual fish in the stomach

In figure 7, the length of the individual (fish) prey species is presented vs. the length of the harbour porpoise in which it was found. When large numbers of certain otoliths were present, only a fraction was measured. For *Pomatoschistus sp.* and *Ammodytes sp.* the allometric (linear) equations presented by Leopold et al. (2001) of respectively *P. lozanoi* and *A. marinus* were used. No confidence values were applied to the measurements and estimates of the original length of the fish.

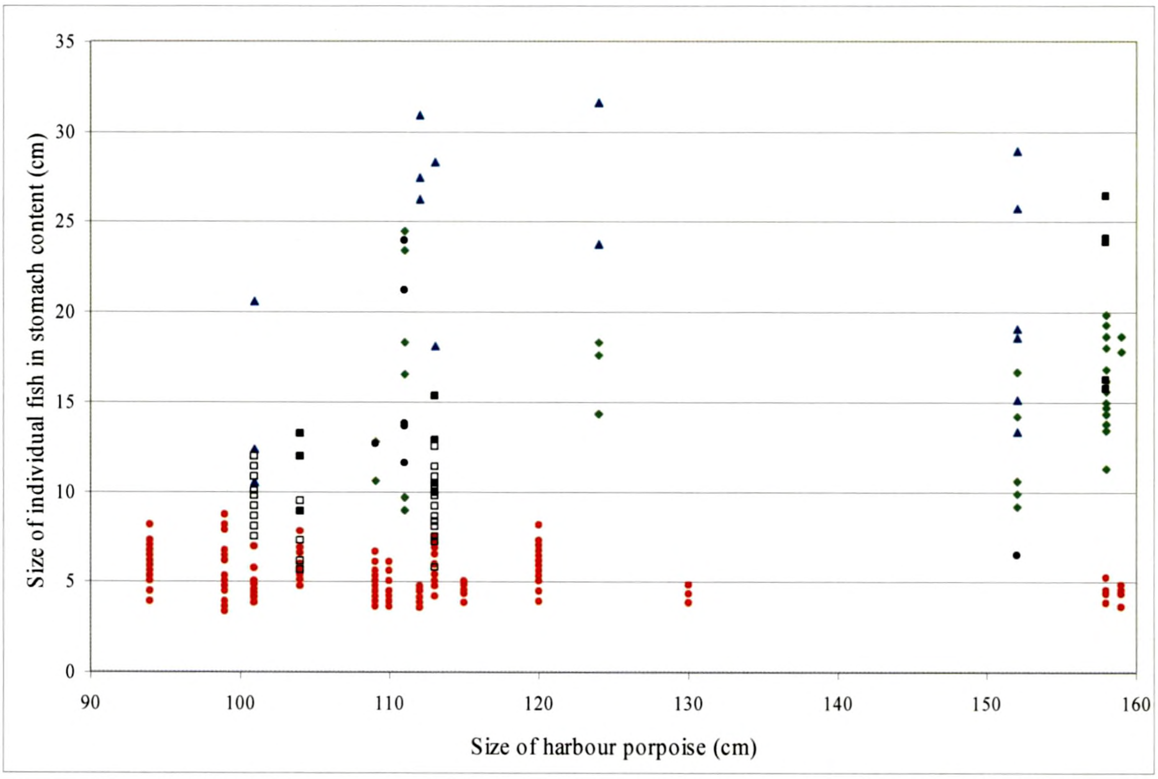

Figure 7. Length of the individual prey item (limited to fish, and extrapolated from the otoliths size) vs. length of the harbour porpoise in which it was found: gadoids: blue triangle; Ammodytidae: green square; clupeids: black circle; seabass: open square; other species: black square.

4. Summary of results

The methodology for analyzing stomach contents in future should be the 'soft' method, in which stomachs are rinsed in water over a sieve of 315 µm, after which the remains are macerated. The remains of the stomach contents remain available in a dry form for future analysis. A method could be developed in which stomach contents can be treated more rapidly, for instance by transferring them at an early stage to 315 µm sieve bags, in which they can macerated and dried without intermediate manipulation. The availability of a reference collection to assist in identifying fish remains was invaluable.

Initial results of the analyses indicate that porpoises feed on a large variety of fish. Only very few remains of cephalopods were found in the stomachs. The juvenile porpoises investigated had mainly consumed gobies and to a lesser extent other small benthic fish. In two cases juvenile seabass seemed to have been an important prey item, and in all four cases when juvenile seabass was found, it concerned animals that had washed ashore between the end of March and the beginning of April. From this initial analysis also Ammodytidae appear to be important food items. There were large individual differences in predominant prey, although with the limited number of stomach contents analyzed, no seasonal variability was assessed.

5. Discussion

In the past only very few stomach contents of harbour porpoises washed ashore in Belgium have been investigated. The stomach content of an unhealthy female of 1.36 m, stranded alive on 3 March 1984, was investigated by Dirk Nolf (RBINS). It contained otoliths of the following fish species (De Smet & Asselberg, 1985): sprat *Sprattus sprattus* (17 otoliths), cod (2 otoliths), whiting (37 otoliths), pout *Trisopterus luteus* (now *T. luscus*) (16 otoliths), *Gobius* sp. (2 otoliths), *Pomatoschistus* sp. (10 otoliths), *Ammodytes* sp. (4 otoliths). Unfortunately, no other stomach content analyses were performed on porpoises stranded during the 1970ies and 1980ies, hence comparing the diet of some decades ago with the current one is not possible.

Porpoises have been described as opportunistic feeders, given the large prey species spectrum and large geographical variations in prey. This would mean that their prey would predominantly depend on the availability of the prey species, and on the probability to encounter that species. However, this initial analysis gives, within the limited number of samples investigated, some indication of selective predation. The remains of species such as dragonet, lesser weever *Trachinus (Echiichthys) vipera*, sole, plaice *Pleuronectes platessa* and flounder *Platichthys flesus* were not recovered, although these species are very common in Belgian waters, and live among species of which the remains were abundantly present in the stomachs of harbour porpoises. Lesser weever might be avoided, given it has sharp spines connected to a poison gland. Dragonets and flatfish were however found during other studies – which investigated larger sample sizes. Still, the number of prey species is fairly high, and prey evidently depends for a large part on availability.

The feeding strategy might constitute one of the possible explanations for the fact that most bycaught porpoises in Belgium are juveniles (Haelters & Camphuysen, 2009). Bycatch almost exclusively occurs in static gear, set very low on the bottom. Porpoises feeding on gobies must stand vertically on the seafloor, and as such might be unaware of fishing gear in the vicinity.

This report only explores the possibilities of an in-depth analysis of the stomach contents available. It advises on the methods of preparing samples and analyzing stomach contents, and presents only a few initial results. The samples studied are still available, and might be revisited to possibly identify prey species from other fish bones than otoliths (such as dragonets). It should be possible – though time-consuming – to identify some remains to the species level (eg. Ammodytidae, Gobiidae). The number of untreated samples available (and growing) is appropriate to allow for a detection of individual differences, seasonal differences, trends throughout the years, and differences throughout age groups. Currently more than 100 stomachs are available for analysis (a list is available). It should be possible to relate the results of autopsies (such as cause of death, nutritional status, blubber thickness and age), present in the database on strandings and results of autopsies currently developed by MUMM ('Biobank') to stomach content data. As part of the diet consists of species also consumed by humans, the relationship between the health status and level of organic pollutants in tissues, together with diet, is of relevance to man.

The stomach content analysis of the currently available samples can form a background for possible future changes in the distribution of the harbour porpoise, in the distribution of its current prey species, and in the preferred prey species themselves. It can also be used to compare current prey preferences throughout the distributional range of the harbour porpoise, and in the first place throughout the North Sea. This can be related to changes in prey distribution and abundance throughout the North Sea, and possibly with overfishing and/or climate change.

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Annex 1. Preliminary reference collection of fish bones, with details on species, fish length and fish bones (otoliths separated from whole skeletons) present.

Species	Number of fish	Fish length (cm)		Otoliths	Skeleton
		min	max		
Agonus cataphractus	*12*	6.0	11.7	X	X
Alosa fallax	*1*	35.0	35.0	X	X
Ammodytes marinus	*2*	18.0	18.5	X	X
Ammodytes tobianus	*12*	13.5	15.5	X	X
Arnoglossus laterna	*13*	5.5	14.0	X	X
Belone belone	*2*	10.0	19.5	X	X
Buglossidium luteum	*10*	9.0	11.5	X	X
Callionymus lyra	*2*	10.0	13.0	X	X
Ciliata mustela	*5*	12.5	21.0	X	X
Clupea harengus	*14*	6.5	11.0	X	X
Dicentrachus labrax	*1*	20.0	20.0	X	X
Enchelyopus cimbrius	*1*	23.0	23.0	X	
Engraulis encrasicolus	*2*	6.0	7.0	X	X
Eurtigla gurnardus	*1*	unkn	unkn	X	X
Gadus morhua	*4*	16.5	23.0	X	X
Gadus morhua	*1*	unkn	unkn	X	
Gaidropsarus vulgaris	*1*	unkn	unkn	X	
Gobius niger	*1*	9.0	9.0	X	X
Hyperoplus lanceolatus	*12*	15.0	30.0	X	X
Limanda limanda	*10*	15.0	23.0	X	X
Liparis liparis	*3*	8.0	9.0	X	X
Merlangius merlangus	*8*	13.0	25.0	X	X
Microstomus kitt	*6*	6.5	19.5	X	X
Molva molva	*1*	32.0	32.0	X	
Mullus surmuletus	*1*	9.4	9.4	X	X
Myoxocephalus scorpius	*3*	15.2	24.0	X	X
Myoxocephalus scorpius	*1*	unkn	unkn	X	
Pagrus pagrus	*1*	unkn	unkn	X	
Phrynorhombus norvegicus	*1*	8.0	8.0	X	
Platichtys flesus	*7*	14.0	35.0	X	X
Pleuronectes platessa	*12*	10.0	23.0	X	X
Pollachius virens	*1*	55.0	55.0	X	
Pomatoschistus minutus	*2*	8.5	8.8	X	X
Salmo salar	*1*	unkn	unkn	X	
Salmo trutta	*1*	unkn	unkn	X	
Scomber scomber	*1*	24.5	24.5	X	X
Solea solea	*6*	11.0	19.5	X	X
Sprattus sprattus	*11*	6.5	9.1	X	X
Trachinus vipera	*15*	5.0	12.5	X	X
Trachurus trachurus	*3*	8.5	22.0	X	X
Trigla lucerna	*1*	unkn	unkn	X	X
Trisopterus luscus	*1*	unkn	unkn	X	X
Trisopterus minutus	*2*	13.0	13.0	X	X
Trisopterus minutus	*1*	unkn	unkn	X	

Annex 2. Fish species (alphabetically according to scientific name) mentioned in the report, with scientific, English and Dutch name.

Scientific name	English	Dutch	French
Agonus cataphractus	Hooknose	Harnasmannetje	Aspidophore
Ammodytes marinus	Raitt's sandeel	Noorse zandspiering	Lançon nordique
Arnoglossus laterna	Scaldfish	Schurftvis	Arnoglosse
Belone belone	Garfish	Geep	Orphie
Buglossidium luteum	Solenette	Dwergtong	Solenette
Callionymus lyra	Dragonet	Pitvis	Lavandière - callionyme
Ciliata mustela	Five-bearded rockling	Vijfdradige meun	Motelle
Clupea harengus	Atlantic herring	Haring	Hareng
Dicentrarchus labrax	Seabass	Zeebaars	Bar
Enchelyopus cimbrius	Four-bearded rockling	Vierdradige meun	Motelle à quatre barbillons
Engraulis encrasicolus	Anchovy	Ansjovis	Anchois
Eurtrigla gurnardus	Grondin gris	Grauwe poon	Grey gurnard
Gadus morhua	Cod	Kabeljauw	Cabillaud
Gaidropsarus vulgaris	Three-bearded rockling	Driedradige meun	Motelle à trois barbillons
Gobius niger	Black goby	Zwarte grondel	Gobie noir
Hyperoplus lanceolatus	Greater sand eel	Smelt	Grand lançon
Limanda limanda	Dab	Schar	Limande
Liparis liparis	Sea snail	Slakdolf	Grande limace de mer
Merlangius merlangus	Whiting	Wijting	Merlan
Microstomus kitt	Lemon sole	Tongschar	Sole limande
Molva molva	Ling	Leng	Grande lingue
Mullus surmuletus	Red mullet	Koningsvis - Mul	Rouget
Myoxocephalus scorpius	Bull rout	Gewone zeedonderpad	Chaboisseau
Pagrus pagrus	Common seabream	Gewone zeebrasem	Pagre
Phrynorhombus norvegicus	Norwegian topknot	Dwergbot	Petit turbot de roche
Platichtys flesus	Flounder	Bot	Flet
Pleuronectes platessa	Plaice	Pladijs - schol	Carrelet - plie
Pollachius virens	Saithe	Koolvis	Lieu noir
Pomatoschistus minutus	Sand goby	Dikkopje	Bourgette
Psetta maxima	Turbot	Tarbot	Turbot
Salmo salar	Salmon	Zalm	Saumon
Salmo trutta	Trout	Forel	Truite
Scomber scomber	Mackerel	Makreel	Maquereau
Scophthalmus rhombus	Brill	Griet	Barbue
Solea solea	Dover sole	Tong	Sole
Sprattus sprattus	Sprat	Sprot	Esprot
Trachinus vipera	Lesser weever	Kleine pieterman	Petite vive
Trachurus trachurus	Horsemackerel - scad	Horsmakreel	Maquereau bâtard
Trigla lucerna	Tub gurnard	Rode poon	Grondin
Trisopterus luscus	Pout - bib	Steenbolk	Tacaud
Trisopterus minutus	Poor cod	Dwergbolk	Petit tacaud