**The potential threat of PFAs accumulation in the *Phocoena phocoena*: the North Sea food web.**

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# **Abstract**

This study present an overview on the contamination of poly- and perfluoroalkyl substances in the harbour porpoise and its foodweb of the North Sea. Perfluorooctane sulfonic acid (PFOS) is found to be the predominant compound in the harbour porpoise and in other marine apex predators globally. Studies in the North Sea suggest that PFOS remains stable in the food web or might even be decreasing in the last few years after regulations have been taken to limit the release and use of PFOS. However, long-chain Perfluoroalkyl carboxylates seem to be increasing in the harbour porpoise and other marine apex predators globally. The most important pathway that leads to contamination of PFAs into the North Sea are rivers that flow through urban, highly industrial and agricultural areas. The harbour porpoise feeds at a lower trophic level than other marine mammals in the North Sea but due to them being coastal foragers they might be more susceptible. Contamination is solely through their diet but there is a knowledge gap on trophic transfer in the harbour porpoise food web in the North Sea. New born porpoises and juveniles are the most contaminated of all life-stages, probably due to placental transfer and exposure through lactation. This makes the harbour porpoise particularly vulnerable, in laboratory animals is seen that exposure to high levels of PFOS results in developmental problems and reproductive failure. Recommendations on future research are given.

**Keywords: *Phocoena phocoena*****, poly- and perfluoroalkyl substances, accumulation, PFOS, food web**

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# **Introduction**

Poly- and perfluoroalkyl substances (PFAs) are man-made chemicals used for commercial or industrial applications. Since the 1950s PFAs have been produced on a global scale for their use in fire-fighting foams, textiles, food packaging, carpet protection and many other applications (Giesy & Kannan, 2001). The major producer of PFAs, the 3M Company, seized production in 2002. But PFAs remain in the environment due to other companies continuing production and the persistence of these chemicals (Cousins et al., 2020). PFAs end up in the environment through various ways such as disposal of products containing PFAs or by wastewater discharge from manufacturers (Liu et al., 2017).

PFAs can degrade to perfluorooctane sulfonate (PFOS), a persistent molecule with a half-life of 41 years in the environment (Hekster et al., 2002; Prevedouros et al., 2006). The first paper that found widespread distribution of PFOS in biota was published in 2001 (Giesy & Kannan, 2001). Since then PFOS and its related compounds have received global attention and have been listed as persistent organic pollutants (POPs) by the Stockholm convention (ONU, 2015). POPs are compounds that remain in the environment for long periods of time because of their resistance to degradation (Langenbach et al., 2013). In addition, measures to substitute long-chain PFAs with short-chain PFAs have been taken to limit PFOS pollution. Despite these efforts, PFOS is still detected in biota (Langberg et al., 2020; Teunen et al., 2021) and has been found to cause a variety of health effects such as: neurotoxicity (Johansson et al., 2008), implications on the immune system (McDonough et al., 2020) and reproductive impairments (Lau et al., 2003). PFOS is the predominant PFAs found in various food webs on a global scale due to its bioaccumulative characteristics (Chen et al., 2021; Giesy & Kannan, 2001; Law et al., 2008).

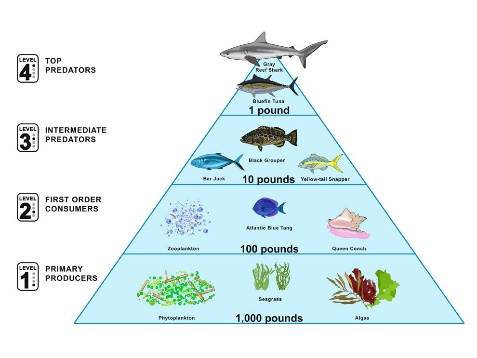
Bioaccumulation is a gradual increase of toxins in an individual and through the food chain. The food chain is divided in four levels to indicate at what order an organism feeds (Fig. 1). The first trophic level are the primary producers. Their energy source is sunlight and they conduct autotrophic feeding. The second order in the food chain are the first order consumers or herbivores. These organisms (meiofauna, herbivorous fish) feed on the primary producers. The third order are the intermediate predators (piscivorous fish). In aquatic food webs the highest trophic levels (4) are mainly marine mammals. Almost exclusively through their diet they attain the highest concentrations of POPs due to bioaccumulation in the food chain (McHuron et al., 2018). Therefore apex predators are more susceptible for bioaccumulative contaminants in the marine environment (Atwell et al., 1998; Jarman et al., 1996; Thompson, 1990;).

Figure 1 The marine food pyramid. Source: Tim Gunther, National Geographic 2021

One of the apex predators of the North Sea food web is *Phocoena phocoena* (Harbour porpoise)*,* a small cetacean that is found in shallow, temperate and sub-arctic waters in the Northern Hemisphere (Hammond et al., 2002; Reid et al., 2003). PFAs are present in the marine environment and potentially bioaccumulate in the food web of the North Sea. Several studies have shown that harbour porpoises are contaminated with PFAs (Galatius et al.,2011; Huber et al., 2012; Law et al., 2008). Porpoises with higher contamination levels are found to be linked to increased risk of infectious diseases and reproductive failures (Jepson et al., 2005; Pierce et al., 2008; Murphy et al., 2015). In the Danish North Sea PFAs have been also detected in neonates and calves (Galatius et al. 2011). This could have serious implications for the development of young harbour porpoises and could result in lower postnatal survival rates making it a potential threat for the newer generations of the population (Lau et al., 2003).

To understand the effects of PFAs in the harbour porpoise it is important to examine how PFAs enter their food web, their tropic position and location of foraging. The behaviour of PFAs and the potential health effects on the harbour porpoise population of the North Sea are of importance as well. There is a knowledge gap on the accumulation of PFAs and their potential harm in the North Sea food web. Therefore, a literature review could provide a better insight in the available knowledge on PFAs in the environment. In this study, I will provide a comprehensive literature review to elucidate (1) the background of PFAs (source, characteristics and behaviour in the environment) (2) the harbour porpoise food web of the North Sea, (3) the transfer and accumulation of PFAs in the North Sea food web, (4) the levels and effects of PFAS in Harbour porpoises and other relevant marine top predators, and as last (5) whether PFAs are a potential risk for the Harbour porpoise population in the North Sea. This overview of available literature on the accumulation of PFAs in the Harbour porpoise population of the North Sea could provide an understanding on the potential harmful effects of PFAs for the North Sea food web and might be used for future policy makers and scientific studies.

# **Chapter 1 The background of PFAs**

***What are PFAs?***

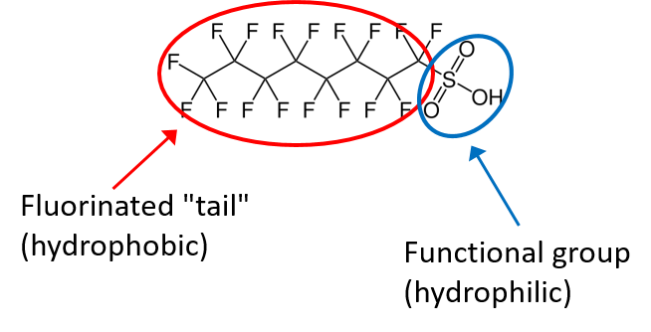
Poly- and perfluoroalkyl substances (PFAs) are man-made chemicals that are used in many industrial and commercial applications (Kissa, 2001). In 1938 Roy Plunkett invented polytetrafluorethylene (PTFE) or Teflon, the first ethylene polymer whereby all hydrogen atoms were replaced by fluorine atoms. After his invention many perfluorinated substances were made. According to the Dutch National Institute for Public Health and the Environment (RIVM) more than 4000 different types of PFAs have been made since by humans (RIVM, 2020). These chemicals consist of a partially or fully fluorinated carbon chain with a terminal sulfonate or carboxylate group (Fig. 2) (Beach et al., 2006). Perfluoroalkyl substances have fluorine atoms on every side chain and polyfluoroalkyl substances are partly fluorinated. PFA molecules are made of hydrophobic fluorinated carbon chains that can vary in length and in hydrophilic functional group. The Carbon-Fluorite bond is a very strong covalent bond and increases in strength when the C-F chain is longer (O’Hagan, 2008). Due to their strong C-F bond PFAs are persistent to heat, acids, bases, oxidants, and microbial and metabolic degradation (Kissa et al., 2001; Schultz et al., 2003). There are several groups of PFAs: Perfluoroalkyl sulfonates (PFSAs), Perfluoroalkyl carboxylates (PFCAs), Perfluorosulfonamides, Fluorotelomer alcohols, Fluorotelomer carboxylic acids etcetera (Table 1). These groups are classified by their chain length and functional groups (Buck et al., 2011). Furthermore, PFAs molecules can also be described as long-chain and short-chain. PFAs with carbon chains longer than or equal to 6–7 carbon atoms are referred to as long-chain PFAs, depending on their group (Buck et al., 2011).

Figure 2 The molecular structure of PFAs. The fluorinated carbon chain is hydrophobic and the functional group gives the molecule hydrophilic properties.

***Application***

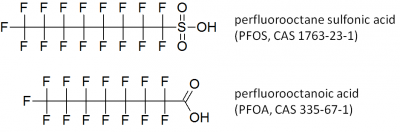
The 3M company started producing PFAs since the 1950s and contributed to 78 percent of the total PFAs emissions until 2002 (Wang et al., 2015). Since the 1950s, PFAs are widely applicated for industrial and commercial use because they are resistant to heat, and are grease and water repellent (Kissa, 2001). This makes them useful for example in firefighting foams, non-stick layers, cleaning products, paint, medical devices and food packaging (Kissa, 2001; Prevedouros et al., 2006). After rising global concerns due to contamination of biota and PFAs found in human blood the 3M company in the US voluntarily stopped producing long-chain PFAs in 2001 (Giesy & Kannan, 2001). However, the production continued at 3M in other countries. PFAs are released into the environment at various stages of their lifecycle (Boulanger et al., 2005; Huset et al., 2008). Due to their chemical properties they are also bioaccumulative and toxic (Martin et al., 2003; Austin et al., 2003; Oakes et al., 2004). Several studies have shown that biota are contaminated with PFAs, whereby PFOS is found to be the predominant molecule (Houde et al., 2006; Giesy & Kannan, 2001; Law et al., 2008; Galatius et al., 2013; Huber et al., 2012). PFOS is a long-chain perfluoroalkyl with hydrophobic, lipophobic, toxic and bioaccumulative characteristics (Fig. 3) (Table 1).

Figure 3 The molecular structure of perfluorooctane sulfonic acid (PFOS). (Geueke, 2016)

***Regulations***

Table 1 Poly- and perfluoroalkyl substances and their characteristics

Several actions have been taken to limit the release of PFOS into the environment. The 3 M Company substituted long-chain PFAs with short-chain PFAs e.g. perfluorobutane sulfonate (PFBS) (Prevedourous et al., 2006). PFBS is used as a substitute for PFOS because it has similar properties but a lower bioaccumulation potential (Table 1) (Cai et al., 2012). The OSPAR convention listed PFOS in their ‘’List of Chemicals for Priority Action’’ in 2003. OSPAR aims to minimize the use of PFOS and eventually to fall to zero. PFOS is also listed as persistent organic pollutant (POP) under Annex A of the Stockholm Convention. Parties that joined to this convention aim to eliminate the use of PFOS to protect the marine environment. Furthermore, the Water Framework Directive has established Environmental Quality Standards (EQS) for PFOS in biota. The EQS for PFOS and its derivatives is set on a 9.1 μg/kg wet weight (ww) as an acceptable contamination level in biota (Moermond & Verbruggen, 2010). In addition, the European Union restricts the use of PFOS under the EU POPs Regulation. However, due to the high persistence of PFOS it remains in the environment (Giesy & Kannan, 2001). Moreover, several precursors of PFOS (PFOSA, POSF, NEtFOSA) are produced by manufacturers that degrade in the environment or in the liver of biota to PFOS (Tomy et al., 2004; US EPA, 2002; Xu et al., 2004).

***Sources***

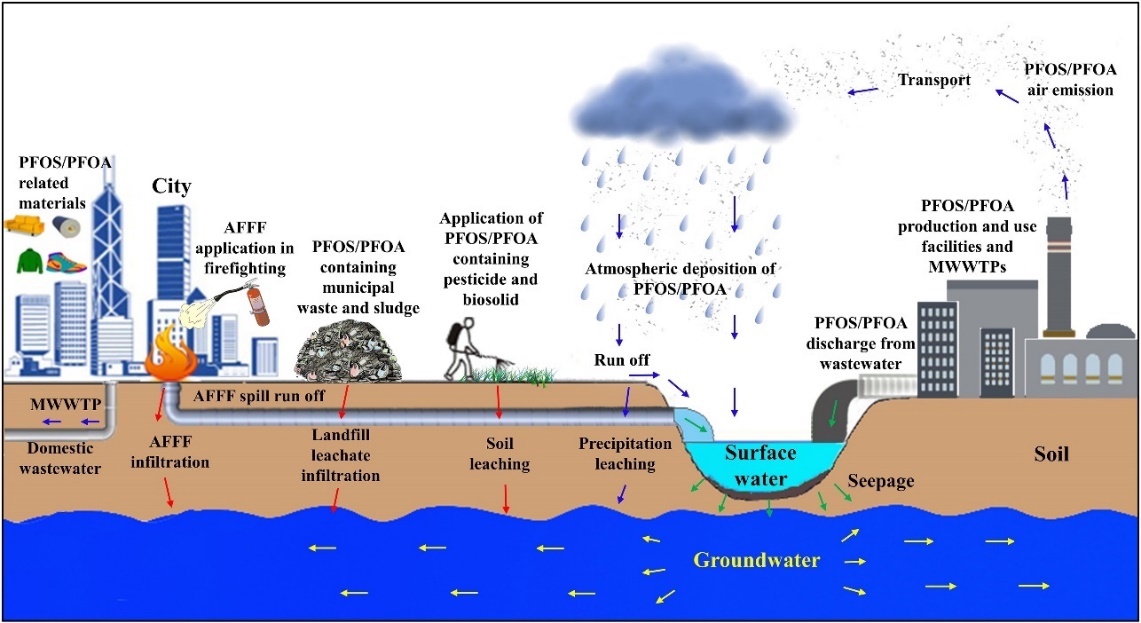
The major sources for the release of PFAs in the environment are waste water treatment plants (WWTPs), landfills, aqueous film forming foams (AFFFs), pesticides, municipal waste and commercial products (Fig. 4) (Exner & Färber, 2006; Kallenborn et al., 2006; Moody & Field, 2000; Schultz et al, 2006). From these sources PFAs leach into the environment via the atmosphere or are transported by water (Ahrens et al., 2009b,c; Ellis et al., 2004). After disposal of products containing PFAs or waste water discharge the PFAs are transported over long distances because of their water solubility (USEPA, 2012). The disposal of PFAs results in contamination of the surface water, soil and the atmosphere (Butt et al., 2010; Exner & Färber, 2006; Kallenborn et al., 2006). These sources combined eventually lead to the contamination of groundwater (Fig. 4) (Sharma et al., 2015; Liu et al., 2017). The atmosphere is contaminated by emissions in the air (Lui et al., 2017). The soil is contaminated by pesticides, leaching of landfills and AFFF’s (Fig. 4) (Xiao et al., 2015), and the surface water by atmospheric deposition, waste water discharge and run-off (Liu et al., 2017).

Figure 4 The sources and pathways of PFOS and PFOA into the environment. Source: Liu et al., (2017)

The most important pathway that leads to PFOS contamination of the North Sea are rivers like the Rhine, Elbe, Meuse and Scheldt (Ahrens et al., 2010). Many highly populated rivers (Rhine, Elbe, Thames, Meuse) discharge into the North Sea. The Rhine contributes to 1.6 tonnes/year of PFOS in the North Sea and the water has a concentration of 10 ng/L PFOS (Lindim et al., 2016). The river Elbe brings 0.01 tonnes/year and contains 2 ng/L (Ahrens et al., 2009b). The Thames is highly populated due to flowing through London where 13.8 million people live and contributes to 0.4 tonnes/year of PFOS and contains 6 ng/L water (Lindim et al., 2016). Meuse brings 0.03 tonnes/year of PFOS and contains 3.74 ng/L (Möller et al., 2010). The river Scheldt has a relatively low water discharge compared to the rivers mentioned before but it is important to include it as well because the 3M Company in Antwerp makes the Scheldt a regional hot-spot for PFOS emissions (van de Vijver et al., 2003). The river Scheldt is a relatively small river but contributes to 0.07 tonnes/year of PFOS into the North Sea and contains 154 ng/L PFOS in Belgium (Möller et al., 2010).

The major polluter of the river Rhine and Elbe are WWTPs (Ahrens et al., 2009b; Möller et al., 2009). WWTPs are a point source for PFAs due to their inefficient cleaning treatment systems. The effluents from WWTPs contain 5-10 times higher contaminants than river water (Ahrens et al., 2009b). The river Rhine is also polluted by runoff from agricultural lands. The river Moehne and Ruhr flow through areas with a lot of agriculture and bring polluted runoff from these lands into the river Rhine (Möller et al., 2010). The river Scheldt is highly contaminated due to waste water from the 3M company in Antwerp and has recently gained a lot of attention because PFOS was found in many biota, three times higher than the European safety standards (Teunen et al., 2021). Furthermore, the river Meuse flows through highly industrial areas as well leading to contamination by waste water discharge (Zafeiraki et al., 2019). The river Scheldt, Rhine, Meuse and including their deltas have a total flux of ±20 tonnes of PFAs per year (Möller et al., 2010).

***Spatial distribution***

Short-chain PFBS and pentafluorobenzoic acid (PFBA) are the most abundant PFAs in the North Sea surface waters followed by long-chain Perfluorooctanoic acid (PFOA) and then PFOS (Ahrens et al., 2009a; Ahrens et al., 2010; Möller et al., 2010; Zhao et al., 2015). PFBS and PFBA are short-chain PFAs that are used as replacement compounds of PFOS and PFOA (Ritter et al., 2010). Since they are not efficiently removed at WWTPs they are found in high concentrations in the river Rhine watershed (Eschauzier et al., 2011; Möller et al., 2010). In the sediment of the North Sea, PFOS is the most abundant PFAs (Ahrens et al., 2009a; Joerss et al., 2019). The highest concentrations of total PFAs are found in the coastal areas and drop towards the open sea due to sedimentation and/or to dilution (Ahrens et al., 2010). PFAs concentrations might be higher in the coastal areas as well due to local point sources, such as the 3M Company in Antwerp in the western Scheldt, or due to the transportation of PFAs by ocean currents (Ahrens et al., 2009a; Tanisayu et al., 2003). For example, in the German Bight the river Elbe, Ems and Weser bring PFBS into the sea (Ahrens et al., 2009a). However, it is suggested that ocean currents bring additional PBS into the German Bight (Ahrens et al., 2009a). A more recent study has shown that the most abundant compound along the German coastline is hexafluoropropylene oxide dimer acid (HFPO-DA), a precursor of PFOA that is used as a replacement (Joerss et al., 2019). The river Rhine brings HFPO-DA into the coastal area of the Netherlands and it is hypothesized that from there HFPO-DA is transported by currents that flow along the coast towards Germany as well (Joerss et al., 2019). HFPO-DA is only found in the water column during this study due to its lower sorption to sediment (Sun et al., 2016).

***Behaviour in the environment***

PFAs are able to accumulate in biota, sediment and in water (Ahrens et al., 2009a; Ahrens et al., 2009d). They can be in the dissolved, particulate or gaseous phase (Ahrens et al., 2009c; Barber et al., 2007). Due to their hydrophobic fluorinated carbon chain they can adsorb easily to soil by interacting with organic material of the soil (Higgins & Luthy, 2006). Long-chain PFAs are more hydrophobic and therefore bind more easily to particles and end up in the sediment or in biota (Higgins & Luthy, 2006). Water solubility depends on the length of the carbon backbone as well, solubility decreases when the length of the carbon chain increases (Bhhatarai & Gramatica, 2011). The bioaccumulation potential also depends on the length of the carbon backbone, the longer the backbone the higher the bioaccumulation potential (Kwadijk et al., 2010). PFAs that can be in the gaseous phase are for example part of the Fluorotelomer alcohols group. They are highly volatile and therefore easily transported through the atmosphere and are able to degrade to PFCAs and PFSAs (Thackray et al., 2020).

PFOS belongs to the PFSA group and has several precursors that degrade to PFOS in the environment or liver of biota (Tomy et al., 2004; Xu et al., 2004). It is either in neutral or anionic state in the environment depending on the pH of the water (Martin et al., 2010; Gellrich et al., 2012). In seawater PFOS can be either in the particulate phase or the dissolved phase (Ahrens et al., 2010). PFOS that is negatively charged makes it more water soluble than neutrally charged PFAs (Martin et al., 2010). Neutrally charged PFAs are less persistent in the environment compared to PFCAs and PFSAs because they are able to fragment and biodegrade (Ellis et al., 2004; Rhoads et al., 2008; Martin et al., 2006). PFCAs and PFSAs are highly persistent and therefore mostly found in water (dissolved or bound to particles), sediment and bioaccumulate in the food web (Jahnke et al., 2007; Martin et al., 2003; Yamashita et al., 2005). Short-chain PFAs are mostly in the dissolved phase whereas long-chain PFAs such as PFCAs and PFSAs are more frequently bound to particles (Ahrens et al., 2009a).

# **Chapter 2 The harbour porpoise food web in the North Sea**

The harbour porpoise has a small body size compared to other cetecean species. Due to their small body and relatively thin blubber layer, the harbour porpoise needs a high caloric intake to prevent starvation (Bjørge et al., 2003; Koopman et al., 2002). Their large surface area to body volume ratio results in a higher rate of heat loss, especially in cold water (Spitz et al., 2012). To compensate for the heat loss, the harbour porpoise feeds on relatively large amounts of prey species for their body size (Bjørge et al., 2003). The harbour porpoise preys on a variety of mainly fish species but the composition of their diet varies per region, season, age and sex (Jansen et al., 2013; Leopold & Schelling, 2014; Santos & Pierce, 2003).

***Geographical variation in diet***

Due to differences in the availability of prey species the harbour porpoise diet varies geographically (Santos & Pierce, 2003). For example, in the Baltic Sea and Skagerrak the main prey species are *Clupea* *harengus* (herring) and *Gadus morhua* (cod) (Sveegaard et al., 2012). In Scottish waters *Merlangius merlangus* (whiting) and *Ammodytes tobianus* (lesser sand eel) form 80% of the porpoise diet (Santos, 2004; Santos et al., 1998). Furthermore, the earliest studies on the harbour porpoise’s diet in the northern North Sea report that herring is a significant species in their diet but this seems to have declined over the years (Rae, 1965, 1973). This might reflect a decrease in the availability of herring since the 1960s (Schelling & Leopold, 2014).

The diet of the harbour porpoise has been studied extensively in the southern North Sea with stomach content analysis and has the most recent published data on their diet (Haelters et al., 2012; Jansen et al., 2013; Leopold & Camphuysen, 2006; Mahfouz et al., 2017). Stomach content analysis is often used to determine the diet of the harbour porpoise of individuals that are washed upon the shore by identifying otoliths of prey species in the stomach of the harbour porpoise. However, this method can be biased towards species living close to the shore that are consumed before the stranding took place due to their high digestive rates (Pierce & Boyle, 1991). Multiple studies in the southern North Sea have shown that gadoids (whiting, cod), clupeids (herring, sprat), sand eels and *Solea Solea* (common sole) are the most important prey species for adult porpoises (Haelters et al. 2012; Jansen et al. 2013; Leopold & Camphuysen, 2006; Mahfouz et al. 2017; Schelling & Leopold, 2014). Although the diet proportion of species within the diet varies for every region in the North Sea, the same species seem to be part of the diet everywhere. Cod, whiting, gobies, sand eels, sole, sprat and herring are key for the diet of the harbour porpoise (Fig. 5) (Haelters et al. 2012; Jansen et al. 2013; Leopold & Camphuysen, 2006; Mahfouz et al. 2017; Schelling & Leopold, 2014). Cod and whiting forage at the highest trophic level due their predation on the first order consumers. Followed by sprat, sole and gobies that feed on benthic meiofauna (Das et al. 2003; Schückel et al. 2013). Herring and sand eels are feeding mainly on zooplankton and phytoplankton, thereby forming the lowest trophic level prey of the harbour porpoise (Fig. 5). Cod and whiting contribute to the highest relative mass in the diet of the harbour porpoise throughout the year in the southern North Sea (Leopold, 2011; Leopold & Schelling, 2014; Mahfouz et al., 2017).

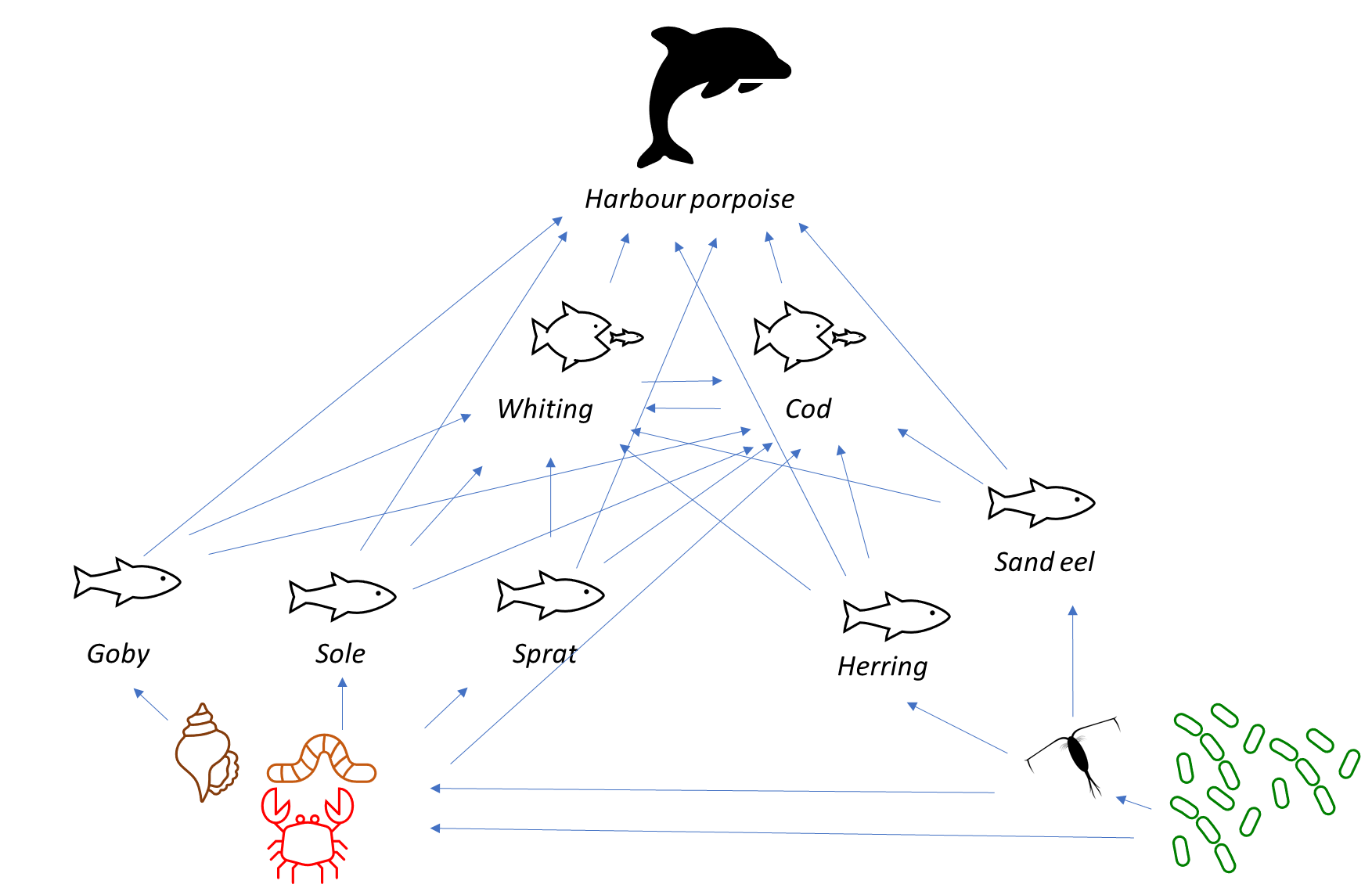


Figure 5 A Simplified food web of the harbour porpoise with its main prey species

**Age and sex-related diet**

A study with data from 2003 until 2014 has shown with stomach content analysis that gobies are consumed in the highest numbers of all prey species in the southern North Sea for all age classes and both sexes, but not in mass (Schelling & Leopold, 2014). Especially for calves the amount of gobies is very high because they are easy to catch for young inexperienced porpoises (Fig. 6) (Leopold et al., 2011; Schelling & Leopold, 2014). The calve is weaned approximately ten months and starts to learn how to prey on fish at 2-3 months old (Lockyer & Kinze, 2003). Because of this feeding behaviour, young porpoises stay closer to the shore with their mother. This also leads to a higher intake of gobies for female adult porpoises compared to male adults that tend to migrate towards open water (Santos & Pierce 2003). Juveniles also feed mainly on gobies but their diet is diversifying due to their intake of other fish species such as sand eels, herring and sprat (clupeids), cod and whiting (gadoids) (Leopold & Schelling, 2014). The relative importance of gobies decreases with age and the diet becomes more diverse into adulthood because adult porpoises need larger animals to sustain their caloric input (Fig. 6) (Leopold & Schelling, 2014; Leopold, 2011). Gobies are small fishes with a low caloric value, therefore, a more diverse diet is needed (Leopold, 2011). Another study shows that also *Sardina pilchardus* (sardine) are key species in the diet of juvenile harbour porpoises in the southern North Sea along the Belgium and French coast (Mahfouz et al., 2017). Sardines have not been shown in other studies yet as an important part of their diet and this could be due to their recent comeback in the North Sea (Mahfouz et al., 2017).

The most important prey in relative mass was found to be gadoids in adult porpoises followed by sand eels then clupeids (Fig. 7) (Schelling & Leopold, 2014). In calves and juveniles no difference is seen between sexes (Schelling & Leopold, 2014). Gadoids might be the most abundant in relative mass due to these fish being the biggest in length and weight of all prey species (Schelling & Leopold, 2014). Furthermore, it is found that gadoids seem to be more important in the diet of the male harbour porpoises compared to female porpoises in mass (Fig. 7) (Leopold & Schelling, 2014; Leopold, 2011). Male porpoises in all age classes have a higher total prey mass compared to female porpoises in the southern North Sea (Schelling & Leopold, 2014). This might be due to female harbour porpoises tend to feed more on fattier round fish (sole) that contain a higher caloric value and therefore might need to feed on less prey (Fig. 7) (Schelling & Leopold, 2014). In addition, during pregnancy and lactation the female porpoises increase their consumption of fatty pelagic round fish because a higher energetic intake is needed (Schelling & Leopold, 2014).

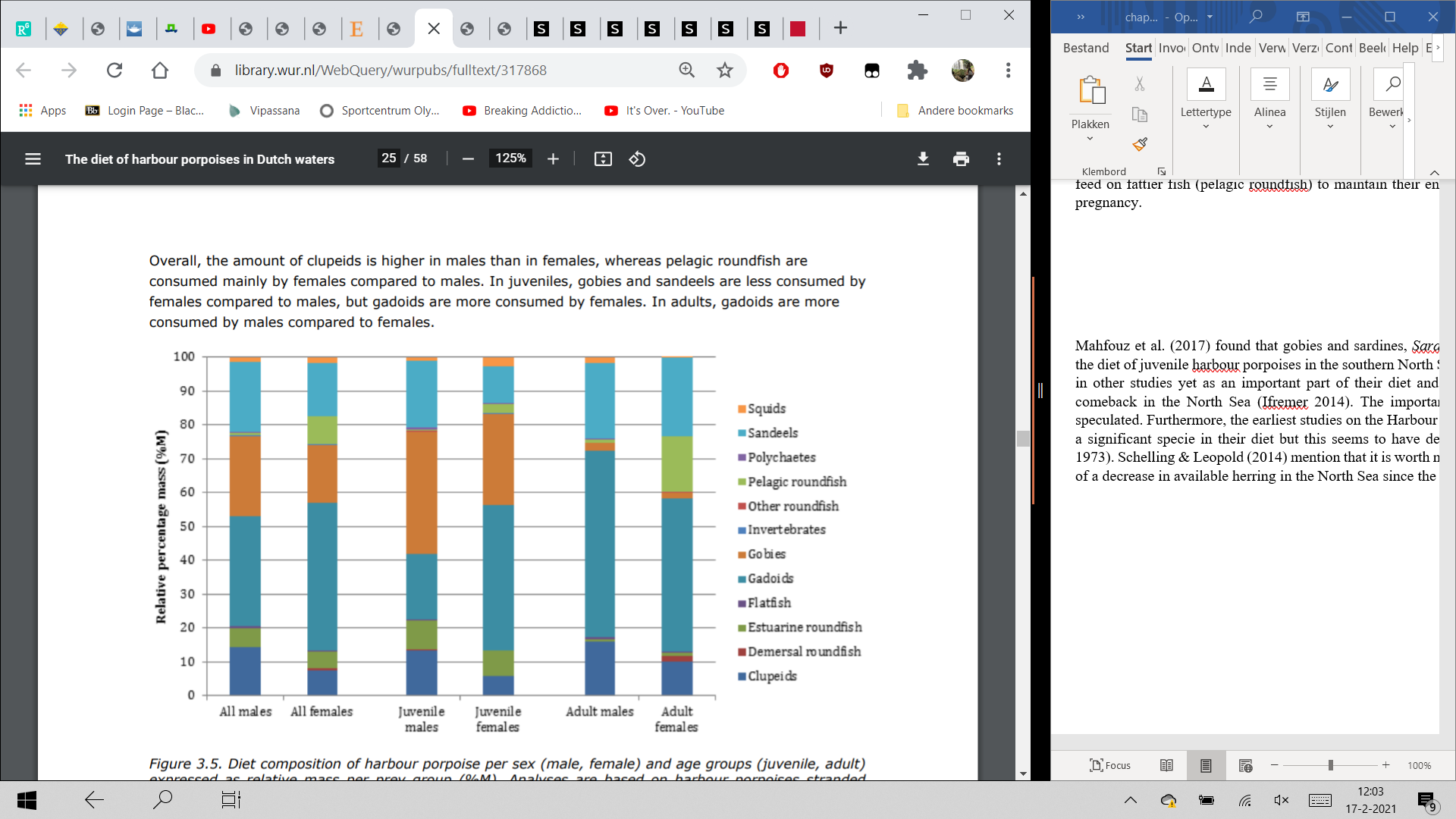
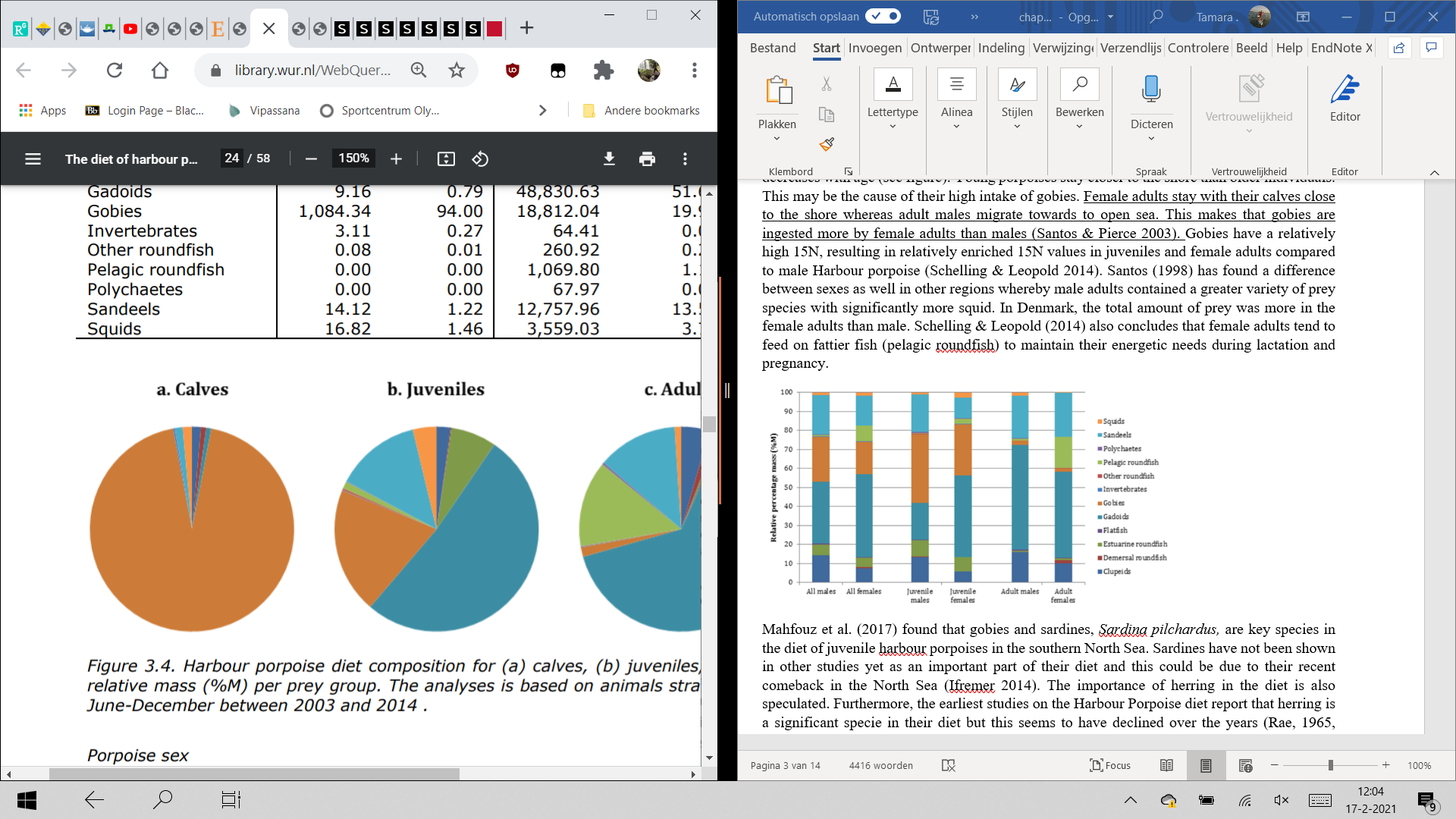


Figure 7 The relative percentage mass of prey species for different life stages and sexes in the Southern North Sea. Source: Schelling & Leopold, 2014

Figure 6 The diet composition of harbour porpoise calves. Source: Schelling & Leopold, 2014

***Seasonality***

The diet of the harbour porpoise also varies seasonally (Leopold, 2011; Santos & Pierce, 2003; Schelling & Leopold, 2014). These differences within seasons are largely explained by the availability of prey species due to their life cycle (Santos et al., 2004). The distribution of marine mammals is tightly linked with the distribution patterns of their prey (Gowans et al., 1995; Gannon et al., 1997; Sveegaard et al. 2012; Santos et al. 2004). For example, herring schools migrate from the Baltic sea towards the north-eastern North Sea during the summer, resulting in large groups of harbour porpoises gathering in this region to forage (Sveegaard et al., 2012). In the summer (April to October) cod is most important in terms of weight. In the winter herring was the most important prey source in terms of weight due to their abundance that is caused by their life cycle. A seasonal abundance in porpoises is seen during the summer (Sveegaard et al., 2012).

In spring and summer the amount of sand eels are the highest in the southern North Sea and a similar seasonal pattern is seen in the north-western North Sea along the coast of Scotland (Santos et al., 2004). The consumption of gadoids is highest in summer and autumn and lowest in spring (Leopold, 2011; Leopold & Schelling, 2014). Furthermore, goby and both pelagic and estuarine roundfish consumption is highest during spring, especially in April (Leopod, 2011; Leopold & Schelling, 2014). The amount of gadoids in the diet of the harbour porpoise is high throughout the year but is less in spring (Leopold & Schelling, 2014). Moreover, during the winter months the consumption of gadoids is especially high. In the month November the amount of squid in the diet increases and the amount of clupeids are highest in January (Fig. 8) (Leopold & Schelling, 2014). In addition, during the winter months sand eels tend to bury themselves in the sediment making them less available to prey upon. Instead, the relative percentage mass of whiting increases during the winter (Fig. 8) (Santos et al., 2004; Mcleod et al., 2007; Leopold & Schelling, 2014). In summary, the main prey species of the harbour porpoise in the North sea are whiting, cod, sole, sprat, goby and sand eel species (Schelling & Leopold, 2014; Mahfouz et al. 2017; Leopold & Camphuysen, 2006; Haelters et al. 2012; Jansen et al. 2013).

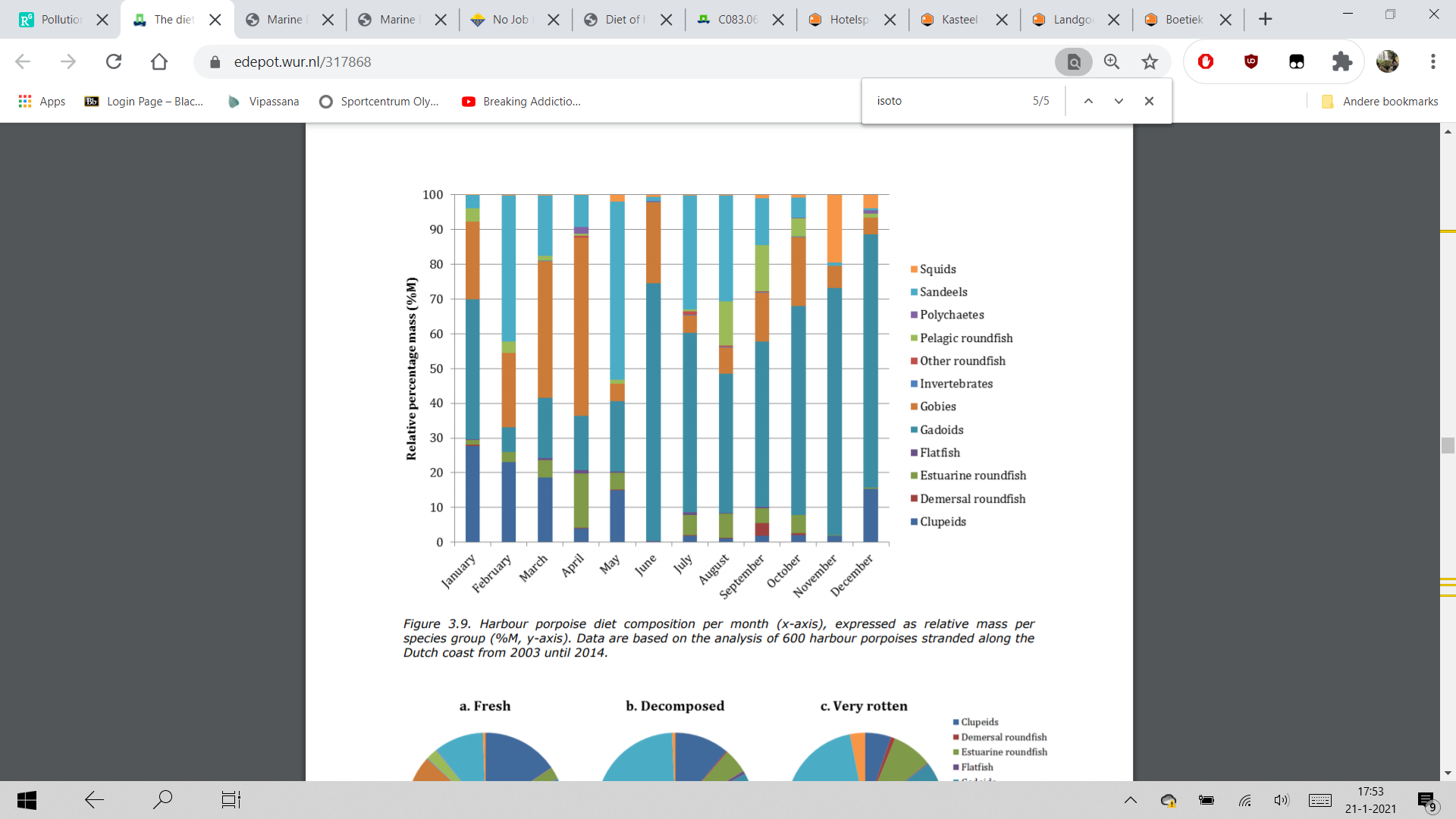


Figure 8 The diet of the harbour porpoise in the Southern North Sea annually. Based on a study that examined 600 stranded porpoises between 2003-2014. The amount of prey species is presented on the y-axis in relative percentage mass and on the x-axis the stomach content of the stranded porpoises are displayed. Source: Schelling & Leopold, 2014

***Trophic position***

To understand the effects of PFAs in the harbour porpoise it is important to examine its trophic position in the food web. Marine mammals occupy high trophic positions in marine food webs and are therefore more susceptible for bioaccumulative contaminants (Atwell et al., 1998; Jarman et al., 1996; Thompson, 1990). In feeding ecology studies the analysis of relatively enrichment of heavy isotope nitrogen 15 (15N) and carbon 13 (13C) in muscle tissue is often used to determine the diet composition over long time periods. The preferential excretion of lighter isotopes (14N) from tissue causes relative enrichment of 15N with an increase in every trophic level. The ratio of 14N/15N is a good indicator of the trophic position of an organism in the food web. The predator becomes relatively enriched ±3.5% with every trophic level compared to their prey (Kelly, 2000, Michener & Kaufman, 2007). Furthermore, the 12C/13C ratio is a good indicator whether the prey species are from coastal areas or more offshore sources in the marine environment. Studies conducted in the North Sea show that the harbour porpoise feeds at relative high trophic levels (Das et al., 2003; Jansen et al., 2013; Pauly et al., 1998).

A study with data from 1994 until 2000 examined the four most common marine mammals (Harbour porpoise, Grey seal, Harbour seal and White-beaked dolphin) in the southern North Sea (Das et al., 2003). Based on the stable isotopes in muscle tissue of all marine mammals indicated that the harbour porpoise has the lowest tropic level (3.4) compared with Grey seal (3.9), Harbour seal (4.1) and the White-beaked dolphin (4.2). This indicates that the harbour porpoise tends to feed more on herbivorous fish. Furthermore, the Harbour porpoise was less depleted in 13C than the other marine mammals (Das et al., 2003). This suggests that the Harbour porpoise feeds closer to the shore than other marine mammals in the North Sea. Female adult Harbour porpoises feed on higher trophic levels compared with male porpoises, however, no difference is seen between sexes in juveniles (Das et al. 2003; Jansen et al. 2012; van de Vijver et al., 2003). In adults, male harbour porpoises were found to be more 13C depleted suggesting that they tend to feed further offshore than females (Das et al. 2003).

In contrary, a study from 1998 suggests that the harbour porpoise and the white-beaked dolphin had the highest trophic level of the four marine mammals (Pauly et al., 1998). This difference could be explained by the fact that the food web in the North Sea has changed over time due to overfishing. Analysis of bone tissue of harbour porpoises stranded from 1848 to 2002 along the Dutch coast showed that 15N decreased over the years (Christensen & Richardson, 2008). The diet preference shifted towards lower trophic levels in the last few decades due to intensive and size-selective fishing in the North Sea (Christensen & Richardson, 2008). Concluding from the studies described above the harbour porpoise feeds at relative high trophic levels.

# **Chapter 3 The transfer and accumulation of PFAs in the North sea food web**

***Contamination in the food web***

The main prey species of the harbour porpoise in the North sea are whiting, cod, sole, sprat, goby and sand eel (Haelters et al. 2012; Jansen et al. 2013; Leopold & Camphuysen, 2006; Mahfouz et al. 2017; Schelling & Leopold, 2014). Micronutrients are transported from the lower trophic levels to the higher levels in a food chain. Contaminants get transferred throughout the food chain in a similar manner and accumulate (Barclay et al., 1994; Gobas & Morrison, 2000). Trophic transfer has been studied for PFOS in several marine food webs (Du et al., 2021; Gao et al., 2020; Kelly et al., 2009; Miranda et al., 2021; Munoz et al., 2017; Pan et al., 2019; Simonnet et al., 2019). However, trophic transfer for PFOS in the harbour porpoise food web of the North Sea has not yet been studied. The higher trophic levels in marine food webs have been found contaminated with greater proportions of long-chain PFAs, whereby PFOS is the predominant compound (Du et al., 2021; Gas et al., 2020; Miranda et al., 2021; Munoz et al., 2017; Pan et al., 2021; Zafeiraki et al., 2019).

The primary producers form the base of every marine food web (trophic level 1). Studies in the tropical and subtropical regions of the Pacific, Atlantic and Indian Ocean as well as the northwestern Atlantic margin have shown that phytoplankton contain PFOS (Zhang et al., 2019; Casal et al., 2017). As of now there have not been studies conducted yet in the North Sea to determine whether algae contain bioaccumulative PFAs. Herbivores (trophic level 2) such as zooplankton, invertebrates, crustaceans and small fish consume these primary producers. A study in the North Sea has shown that invertebrates contain high levels of PFOS due to them living on the substrate and their close interaction with the sediment (van de Vijver et al., 2002; 2003). Benthic organisms contain more long-chain PFAs due to them living on the substrate compared to pelagic organisms (Munoz et al., 2017; van de Vijver et al., 2002;2003). In the South China Sea and the Philippines, however, it was found that the low trophic levels contain more short-chain PFAs compared to the higher trophic levels containing long-chain PFAs. Species feeding on plankton, gastropods and benthic bivalves are found to contain more short-chain PFAs (Du et al., 2021; Zhang et al., 2019b).

The herbivores are eaten by the first order of consumers (trophic level 2.5). In the harbour porpoise food web these are herring, sand eel species, goby, sprat and sole. Sole has been found with PFOS concentrations of 1 ng/g ww in muscle tissue in the central North Sea (Zafeiraki et al., 2019), 45 µg/kg ww in muscle tissue in the southern North Sea and 125 µg/kg ww in liver tissue (Table 2) (van Leeuwen et al., 2006). The Water Framework Directive has established an EQS of 9.1 μg/kg ww. In liver and muscle tissue of sole higher concentrations than the set EQS are found. Herring has been studied as well and their muscle tissue contained 8 µg/kg ww in the southern North Sea and 50 µg/kg ww in the central North Sea in 2004 (Table 2) (van Leeuwen et al., 2006). However, the mentioned PFOS concentrations are not a good representation of the contamination in these species in the North Sea due to the low sampling numbers used in the study of Van Leeuwen et al., (2006). Studies on PFOS concentrations in gobies, sand eels and sprat have not been found for the North Sea. In the Gironde estuary in France gobies have been found to contain PFOS (2-2,4 ng/g ww) (Munoz et al., 2017). In addition, demersal fish are expected to contain high PFOS concentrations because they are more likely to be exposed to them due to them foraging on benthic organisms (Munoz et al., 2017). The piscivorous fish in the harbour porpoise food web are cod and whiting. Low PFOS concentrations are found in cod (0,5 ng/g ww) and whiting (0,4 ng/g) in the North Sea (Zafeiraki et al., 2019). Due to their trophic position it was expected that these piscivorous fish have higher contamination levels than omnivorous fish.

Table 2 PFOS concentrations in the harbour porpoise prey species in the North Sea.

***Geographical variation***

Geographical variation in PFAs concentrations of the harbour porpoise its prey can be observed, which are due to variation in PFAs pollution on a broad scale and by local emission sources (van de Vijver et al., 2003; van Leeuwen et al., 2006; Zafeiraki et al., 2019). For example, the harbour of Antwerp contributes to the contamination of invertebrates living in the western Scheldt. A pollution gradient is seen whereby the western Scheldt is most contaminated on the east side and contaminant levels in biota decline more offshore in the North Sea (van de Vijver et al., 2003). Furthermore, a contamination pattern is seen for the different regions in the North Sea. In general, fish caught in the northern North Sea are found to be contain lower PFOS concentrations compared to fish from the central and southern North Sea (Zafereiki et al., 2019; van Leeuwen et al., 2006). This is due to big rivers that debouch in the southern North Sea such as the Scheldt, Rhine, Elbe and Weser that are highly contaminated with PFAs (Möller et al., 2010). In addition, along the Dutch coast, fish caught close to the shore are found to be more contaminated than fish caught more offshore (Zafereiki et al., 2019; van Leeuwen et al., 2006). It is hypothesized that river effluents are transported by currents that flow along the coast create a “pollution plume” along the coast. Marine mammals that are coastal foragers have a higher PFOS contamination compared to offshore foragers (de Voogt et al., 2006). Therefore, the feeding location (inshore versus offshore) might determine the amount of pollution in the harbour porpoise.

# **Chapter 4 The levels and effects of PFAs in harbour porpoises and other relevant marine top predators**

***Contamination in the North Sea***

PFOS is the most frequently reported PFAs in marine apex predators followed by PFCAs with a backbone of 9 to 14 carbon atoms (Chen et al., 2021; Galatius et al., 2013). Perfluoroundecanoic acid (PFUnDA) is the most frequently reported PFCA in marine apex predators globally (Chen et al., 2021). The effects of PFUnDA on the health of marine mammals are not studied yet and this research is therefore urgently needed (Chen et al., 2021). In stranded porpoises from the Danish North Sea between 1980 and 2005 a stable trend was seen in PFOS contamination and an increasing trend was seen for PFCA’s (Galatius et al., 2011). In addition, another study on harbour porpoises stranded between 1991 and 2008 in the northern North Sea found that PFOS is decreasing and confirms that long-chain PFCAs are increasing (Huber et al., 2012). Sample sizes were low in these studies, therefore further research is needed.

Several studies have analysed tissue of stranded harbour porpoises from various regions of the North Sea and have shown contamination by PFOS (Table 3). The values in table 3 show that harbour porpoises have been contaminated since at least 1980 (Galatius et al., 2011), above the set EQS of 9.1 µg/kg ww by the Water Framework Directive. Though the EQS is set for biota at lower trophic levels and not for marine apex predators. The most recent study on the pollution of PFOS in stranded porpoises from 2006 until 2016 shows that a range was found of 50-3000 µg/kg ww (van den Heuvel-Greve et al., 2017). PFOS concentrations were found in this study that were much higher than the data from previous studies.

Table 3 PFOS concentrations in tissue of harbour porpoises of the North Sea.



***Contamination levels found in harbour porpoises in different life-stages***

PFAs are found in all life-stages of the harbour porpoise in the North Sea (Table 4) (Galatius et al., 2011; Law et al., 2008; van den Heuvel-Greve et al., 2017). New born porpoises and juveniles are the most contaminated with PFOS (Galatius et al., 2011; van den Heuvel-Greve et al., 2017). Fetuses are exposed to PFOS during the pregnancy already through placental transfer. Due to their lack of fecal excretion in the uterus PFOS is easily accumulated (Galatius et al., 2011). Furthermore, neonates are found to contain high PFOS due to their exposure through lactation by their mother as well (Houde et al., 2005; Pierce et al., 2013; van den Heuvel-Greve et al., 2017). In liver tissue the neonates had significant higher PFOS than the mother (Galatius et al., 2011), this has been reported for the (*Tursiops truncatues*) bottle nose dolphin (Houde et al., 2005) and (*Peponocephala electra*) melon-headed whale as well (Hart et al., 2008). The same study reports that the rate of placental transfer of PFAs are higher than those for other contaminants such as Polychlorinated biphenyl (PCB) and Polybrominated diphenyl ethers (PBDE) (Hart et al., 2008). Fetuses with higher PFAS concentrations than their mother have been reported in the Black Sea too (van de Vijver et al., 2007). Higher PFOS concentrations were found in fetus brain (92 ng/g ww) and kidney (1371 ng/g ww) whereas the mother had lower PFOS (17-21 ng/g ww) concentration in all tissues (van de Vijver et al., 2007). Similar results were also seen for porpoises in the southern North Sea and in Norway with the adult porpoises having lower contamination than juveniles (van de Vijver et al., 2003; van de Vijver et al., 2004). PFOS contamination decreases after the juvenile stage, probably due to the increase in length resulting in the spread of the contaminants (van den Heuvel-Greve et al., 2017). Age is found to be the most important factor influencing the contamination level (van den-Heuvel Greve et al., 2017).

Table 4 Studies in the North Sea on the contamination of PFOS between different life-stages.



Compared to all adult harbour porpoises, lactating females have the highest PFOS body burden followed by non-lactating female adults (Table 4) (Galatius et al., 2011; van den Heuvel-Greve et al., 2017). This might be due to their increased consumption while lactating combined with their decreased protein metabolism and urine excretion (Galatius et al., 2011). Another explanation could be (as described in chapter 2) that female porpoises forage close to the shore and at higher trophic levels than male adults. Furthermore, they prefer roundfish which contain higher PFOS burdens (Das et al., 2004). The sample sizes of these studies per class are low, further research is needed to draw conclusions. In addition, these findings are in contrast to other contaminants e.g. PCBs and PBDEs, which are transferred from mother to calve during pregnancy or lactation resulting in the offloading of the mother (Aguilar et al., 1999; van den Heuvel-Greve et al., 2021).

Furthermore, Law et al. (2008) published data on PFOS concentrations in 57 harbour porpoises of various life-stages stranded and caught around the United Kingdom. This review did not provide an analysis on the differences in contamination between the gender and life-stages. To examine whether a difference is seen, data were used to calculate the median and the standard deviation between populations (Table 5). To identify the different life-stages a length of 91-130 cm is taken to classify porpoises of this length as juveniles. Harbour porpoises longer than 130 cm are considered as adults. Neonates are classified as porpoises stranded between May and August with a length shorter than 91 cm (van den Heuvel-Greve et al., 2017). The data shows that juveniles contain higher PFOS concentrations than adults. Between sexes a difference is seen of 950 µg/kg ww for female juveniles and 578 µg/kg ww in male juveniles. No such difference is seen in adult female and males. In addition, PFOS concentrations in one male neonate is published showing low contamination. This is in contrast to the data of table 4. However, conclusions cannot be made due to the low number of porpoises that were analysed and the high variation per class.

Table 5 Analysis of the different life-stages and between sexes of the data published by Law et al. (2008)

***Harmful effects/distribution in the body***

In the harbour porpoise it was found that the liver is mostly enriched by PFAs followed by kidneys>muscle>brain in the Black Sea (van de Vijver et al., 2007). However, this study did not include blood and other organs meaning this does not give a whole perspective on the tissue distribution. Total body distribution in the harbour porpoise has not yet been studied. In the harbor seal (*Phoca vitulina*) in the German Bight the following order found of contamination by PFOS was found: blood>liver>muscle>lung>kidney>blubber>heart>brain>thymus>thyroid (Ahrens et al., 2009d). Another report on harbour seals in the Wadden Sea shows the following accumulation pattern: kidney>liver>blubber>skeletal muscle for PFOS (van de Vijver et al., 2005). PFAs have a high affinity to proteins, especially serum albumins and fatty acids, and are therefore easily accumulated in the blood and organs instead of blubber which is normally seen by POPs (Luo et al., 2012).

Studies on the effects of PFAs contamination in harbour porpoises or other marine mammals are absent but the effects of other POPs have been studied. A link between contamination of POPs in harbour porpoises and increased susceptibility to catch infectious disease or parasites are found in the North Sea (Hall et al., 2006; Jepson et al., 1999; 2005; Pierce et al., 2008). Reproductive failures are also linked to contamination by POPs (Murphy et al., 2015). In laboratory animals was found that exposure to PFOS resulted in prenatal mortality (Fuentes et al., 2006) and an impaired reproductive system (Lai et al., 2017; Qiu et al., 2013). PFOS exposure in mice fetuses impairs the development of the nervous system and the immune system (Johansson et al., 2009; Torres et al., 2021). Therefore the high contamination levels found in young porpoises is concerning because these studies suggest that mammals are irreversibly impacted during the development. A lower survival rate after birth is also seen in mice (Lau et al., 2003).

# **Chapter 5 Are PFAs a potential risk for the harbour porpoise population in the North Sea?**

**PFAs pathways and sources into the North Sea**

In this review several topics have been touched to get an insight whether the harbour porpoise population in the North Sea are at a potential risk due to PFAs. The behaviour, spatial distribution, pathways and sources of PFAs are important to understand because it shows how these chemicals might behave in the North Sea and potentially end up in the harbour porpoise food web. Rivers that flow through urban, industrial and agricultural lands such as the Rhine, Weser, Elbe and Thames are the most important pathways that contribute to the pollution of the North Sea (Ahrens et al., 2009b; Lindim et al.,2016). Rivers are mostly contaminated by waste water discharge, AFFF’s and agricultural run-off (Liu et al., 2017). In addition, local hot-spots of emission (3M in Antwerp) and ocean currents contribute to higher contamination levels along the coast (Ahrens et al., 2009a; Tanisayu et al., 2003).

**Trophic position and location of foraging in the North Sea**

Harbour porpoises prefer to forage closer to the shore, especially young porpoises with their mothers (Santos & Pierce 2003). Analysis of carbon isotope 13 C has shown that the harbour porpoise feeds closer to the shore compared to the other marine mammals in the North Sea (Das et al., 2003). Marine mammals that forage close to the shore have been found with higher PFOS concentrations compared to offshore foragers (de Voogt et al., 2006). In general, fish caught in the northern North Sea are found to contain lower PFOS concentrations compared to fish from the central and southern North Sea (Zafereiki et al., 2019; van Leeuwen et al., 2006). The location of foraging therefore could significantly impact the contamination levels of the harbour porpoise. Harbour porpoises foraging in the central and southern North Sea might risk higher contamination by PFOS.

The trophic position is important to examine whether the harbour porpoise is susceptible for contaminants. Marine mammals feeding at high trophic levels are more susceptible for contaminants (Atwell et al., 1998; Jarman et al., 1996; Thompson, 1990). The harbour porpoise feeds at a lower trophic level (3.4) compared to the grey seal (3.9), harbour seal (4.1) and the white-beaked dolphin (4.2) in the North Sea (Das et al., 2003). It is also suggested that female porpoises forage at higher levels than male adult porpoises (Das et al., 2003). Research in the past has suggested otherwise, the harbour porpoise was together with the white-beaked dolphin the highest trophic feeder (Pauly et al., 1998). It might be that due to overfishing in the North Sea the harbour porpoise diet has shifted towards more herbivorous fish. However, according to diet studies in the southern North Sea cod and whiting are the most important prey species in mass (Schelling & Leopold, 2014). Cod and whiting forage at relatively higher trophic levels due to them being piscivorous fish.

**Transfer and accumulation in the harbour porpoise food web**

The main prey species of the harbour porpoise in the North sea are whiting, cod, sole, sprat, goby and sand eel species (Haelters et al. 2012; Jansen et al. 2013; Leopold & Camphuysen, 2006; Mahfouz et al. 2017; Schelling & Leopold, 2014). The diet of the harbour porpoise seems to differ for age, sex, season and region (Jansen et al., 2013; Schelling & Leopold, 2014; Santos & Pierce, 2003). It is important to understand their diet to elucidate the sources of PFAs in the food web. Calves and juveniles feed mostly on gobies and with age the diet is diversifying towards gadoids (cod and whiting) (Schelling & Leopold, 2014). In adult porpoises gadoids are the most important prey specie in mass and gobies in numbers in the southern North Sea (Schelling & Leopold, 2014). Male adult porpoises tend to feed more on gadoids whereas female porpoises feed more on pelagic and estuarine roundfish (sole). Especially in April the consumption of roundfish and gobies increase for female adult porpoises (Leopold & Schelling, 2014). Gadoid consumption increases in the winter and in summer but is less in spring and autumn for all age classes (Leopold & Schelling, 2014).

The preferred prey species of the harbour porpoise in mass are cod and whiting. It is therefore important to examine the the amount of pollution. Cod and whiting seem to be relatively low in terms of pollution compared to other species (van Leeuwen et al., 2006; Zafeiraki et al., 2019). However, the number of samples in these studies were low and only muscle tissue is measured which makes it seem lower due to all the PFOS being accumulated in the liver, further research is needed on the pollution of gadoids. Sole is a preferred prey species by pregnant and lactating porpoises and was found in the literature to exceed the EQS, but the number of samples were low as well (van Leeuwen et al., 2016; Zafeiraki et al., 2019). The contamination levels in sole found are concerning because female porpoises increase their intake during pregnancy and lactation. This feeding behaviour might lead to increased PFOS exposure to the fetus and weaning calves (Das et al., 2003). Trophic transfer has not been studied yet in the North Sea but has been studied in many other marine food webs (Du et al., 2021; Gao et al., 2020; Kelly et al., 2009; Miranda et al., 2021; Munoz et al., 2017; Pan et al., 2019; Simonnet et al., 2019). The higher trophic levels in marine food webs have been found contaminated with greater proportions of long-chain PFAs, whereby PFOS is the predominant compound (Du et al., 2021; Gas et al., 2020; Miranda et al., 2021; Munoz et al., 2017; Pan et al., 2021; Zafeiraki et al., 2019).

**Contamination in the harbour porpoise in different life-stages and both sexes**

Globally, PFOS is the predominant compound in marine apex predators followed by PFCAs with a backbone longer than 9-14 carbon atoms (Chen et al., 2021; Galatius et al., 2013). PFUnDA is the most frequently reported chemical of the PFCA group in marine apex predators (Chen et al., 2021). All studies on the harbour porpoise in the North Sea have shown that they are contaminated with PFOS (Huber et al., 2012; Galatius et al., 2011; 2013; Law et al., 2008; van de Vijver et al., 2003; van den Heuvel-Greve et al., 2017). A decreasing trend of PFOS is seen between 1999 and 2002 in the harbour porpoise population in the Danish North Sea, however, an increasing trend is seen for long-chain PFCAs (Galatius et al., 2013). Another study from the Danish North Sea in stranded porpoises between 1980 and 2005 a stable trend was observed of PFOS contamination and an increasing trend was seen for PFCA’s (Galatius et al., 2011). Even though a decreasing trend is seen for PFOS in the harbour porpoise population the contamination remains higher than the set 9.1 μg/kg EQS by The Water Framework Directive. In contrary, the EQS may not be the appropriate standard to determine the risk for marine apex predators since it is developed for biota at lower trophic levels. New born porpoises and juveniles are the most contaminated with PFOS of all age classes, no difference in sexes is seen (Galatius et al., 2011; van den Heuvel-Greve et al., 2017). Fetuses are exposed to PFOS during the pregnancy already through placental transfer (Galatius et al., 2011). Furthermore, neonates are found to contain high PFOS due to their exposure through lactation by their mother as well (Pierce et al., 2013; van den Heuvel-Greve et al., 2017; Houde et al., 2005). Compared to all adult harbour porpoises, lactating females have the highest PFOS body burden followed by non-lactating female adults (Galatius et al., 2011; van den Heuvel-Greve et al., 2017).

**Distribution and effects of PFAs in the harbour porpoise**

Due to its proteinphilic behaviour PFOS accumulates in blood and organs (especially liver) instead of blubber which is normally seen by POPs (Luo et al., 2012). Research on the health effects of PFAs in the harbour porpoise are absent. However, in laboratory animals was found that PFOS exposure leads to increased reproductive failures (Fuentes et al., 2006; Lau et al., 2003; Lai et al., 2017; Qiu et al., 2013). The development of the nervous and immune system in fetuses also seem to be disrupted due to PFOS (Johansson et al., 2009; Torres et al., 2021).

In conclusion, the levels and effects of PFAs found in the harbour porpoise and its food web in the North Sea are concerning. There might be a potential risk for the harbour porpoise due to the possible reproductive failures and developmental problems after exposure of PFOS that is seen in pregnant mice. These findings in mice are concerning because fetuses and young porpoises are exposed to high levels of PFOS as well. It might be that over a few decades the population numbers could decrease due to their lower reproductive success rate and a disruption in their development. Also due to them being coastal forager they are more susceptible even though they feed at lower trophic levels than other marine mammals in the North Sea.

**Recommendations for future research**

This review elucidated the current knowledge on PFAs in the harbour porpoise food web in the North Sea. Many knowledge gaps have been found, therefore research on the following topics are suggested:

* To understand the contamination of bioaccumulative PFAs it is important to research the trophic transfer in the harbour porpoise food web, as this has not yet been done, to identify the sources of PFAs in the food web.
* Tissue distribution and the harmful effects of PFAs accumulation in the harbour porpoise are not fully understood. To examine their potential effects it is needed to fill this knowledge gap for the health status of the population in the North Sea. Especially on long-chain PFCAs that seem to be increasing in the environment.
* As of now a quality standard is only set by The Water Framework Directive for lower trophic biota. An EQS for marine apex predators could provide a better framework for policymakers to determine the health status of the harbour porpoise population. This could help with future restrictions for manufacturers to strive for a healthier marine environment.
* The contamination of bioaccumulative PFAs in different life-stages is not fully understood yet. Research with a higher number of individuals per class is needed for the North Sea to obtain a better view on the effects of PFAs in the different life-stages and between sexes.
* Currently, most research is done for the southern North Sea of PFOS contamination in the North Sea. To get a clear understanding of the distribution of PFOS and other bioaccumulative PFAs other regions of the North Sea should be studied as well. This could provide a better basis for policymakers where new policies should be made to limit emissions.

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