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Molecular determination of grey seal diet in the Baltic Sea in relation to the current seal-fishery conflict

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Photo: Morten Tange Olsen

Master's thesis (60 ECTS)

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Title:	Molecular determination of grey seal diet in the Baltic Sea in relation to the current seal-fishery conflict
Emnebeskrivelse:	The grey seal population in the Baltic Sea has increased rapidly in the last decades and this has led to conflicts with the fisheries. Seals eat the catch from the fishing gear and new action plans to avoid this problem has been introduced. In this study the diet of the seals are investigated using Next Generation Sequencing on faecal samples. Samples from two localities in the Southern Baltic Sea were collected in 2014 – 2016 and the fish species present in the scats could be determined.
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CONTENTS

Abstract

The conflict between seals and fisheries has been present for centuries. The seal population in the Baltic Sea has increased rapidly since the 1970s where a total protection was introduced in Denmark and Sweden. In recent years, many fish stocks have declined because of overexploitation and now it is relevant to investigate how much the seals can affect fish abundance and cause damage to fishing gear. Previous studies on seal diet have relied on identification of otoliths found in scats or digestive tracts, but this method can be inaccurate and cause underrepresentation of certain fish species.

In this study we used DNA barcoding to analyse the diet of grey seals (*Halichoerus grypus*) from faeces collected on Tat, Denmark and Måkläppen, Sweden over several seasons. The diet analysis relied upon next generation sequencing, which allows identification of numerous species in samples yielding several thousand sequences per PCR product.

The prey species were significantly different between the two locations and twice as many species were found in scats from Måkläppen than from Tat. The most common species at Tat were cod (*Gadus morhua*), sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) while garfish (*Belone belone*), cod and herring were the most common species from Måkläppen. The seasonal variation was not as evident, but at Måkläppen the seals consumed significantly more garfish during spring. No yearly variation was observed between 2014 and 2015 on Måkläppen. Comparisons between data from the Atlas of Marine Fishes of Denmark showed a preference for cod in the seal diet, which is in conflict with the Danish National landings data, as cod is the far most valuable species for the fisheries.

Sammenfatning

Konflikten mellem fiskere of sæler har varet i århundreder. Sælpopulationen i Østersøen er steget hastigt siden 1970erne hvor en total fredning blev introduceret i Danmark og Sverige. I de seneste år er mange fiskebestande gået tilbage på grund af overfiskeri og nu er det relevant at undersøge hvordan sæler påvirker antallet af fisk og tilfører skader på fiskeredskaber. Tidligere studier har brugt morfologiske analyser af øresten fundet i ekskrementer og mavesæk til at identificere fiskearter i diæten, men denne metode kan være unøjagtig og underrepræsentere nogle arter.

I dette studie bruger vi molekylære metoder til at analysere diæten af gråsæl (*Halichoerus grypus*) fra ekskrementer samlet på Tat, Danmark og Måkläppen, Sverige i løbet af flere sæsoner. Diætanalysen blev baseret på Next Generation Sequencing, som gør det muligt at identificere adskillige arter i prøverne som giver mange tusinde sekvenser per PCR produkt.

Fiskearterne i sælernes diæt var signifikant forskellige mellem de to lokaliteter og dobbelt så mange arter blev fundet på Måkläppen i forhold til Tat. De mest almindelige arter fra Tat var torsk, brisling of sild, mens hornfisk, torsk of sild var de mest almindelige på Måkläppen. Sæsonvariationen var ikke så tydelig, men på Måkläppen spiste sælerne signifikant flere hornfisk i foråret. Ingen årlig variation blev observeret mellem årene 2014 og 2015 på Måkläppen. Sammenligninger mellem data fra Atlas for Danske Saltvandsfisk viste en præference for torsk i sældiæten, hvilket er i konflikt med de Danske Nationale landings data hvor torsk er den overvejende mest værdifulde art for fiskeriet.

Introduction

Aim

In this study the diet of grey seals (*Halichoerus grypus*) and variation in diet between seasons and years were assessed. This is relevant because of the increasing conflict between seals and fisheries in the Baltic Sea. The prey of grey seals was identified by analysing faecal samples collected over several seasons on two locations in the southern Baltic Sea. The aim is to investigate if any seasonal variation in diet occurs as well as geographical differences and compare these results with the commercial catch and the natural fish distribution and species composition. Molecular methods were performed by extracting DNA from scats, amplifying the prey DNA by Polymerase chain reaction (PCR) and sequencing the samples using Next Generation Sequencing. To investigate this topic it is important to understand the distribution, foraging behaviour and movements of the grey seals in the Baltic Sea.

Grey seal distribution and abundance

Grey seals are found on both sites of the North Atlantic and in the Baltic Sea in temperate and sub-Arctic waters. There are three distinct subpopulations that are morphologically and genetically different; the Baltic population, the Eastern Atlantic population and the Western Atlantic population (Härkönen et al. 2013; Klimova et al. 2014) (Figure 1). Two subspecies of the grey seal are recognized, the Baltic with the recently updated name *Halichoerus grypus grypus* and the Atlantic subspecies *Halichoerus grypus atlantica* (Olsen et al., 2016). The world total population size of grey seals is assessed to about 630 000 individuals and the populations are increasing (Bowen, 2016).

The Baltic grey seal population is estimated to have diverged from the Atlantic population between 10 000 and 4200 years ago. This is consistent with the time that a dispersal corridor was open between the Baltic and the Atlantic Sea about 15 000 to 8000 years ago (Fietz et al., 2016; Klimova et al., 2014). Archaeological discoveries confirm that the grey seal has been present in the Southern Baltic Sea for 6000 – 9000 years (Schmölcke, 2008; Fietz et al., 2016). The main difference between the Atlantic and the Baltic grey seal population is that the Atlantic population breeds in autumn while the Baltic population breeds in spring (Almkvist, 1982). The Baltic population shifted from autumn to spring breeding because they adapted to the periodically occurring ice in the Baltic Sea (Fietz et al., 2016).

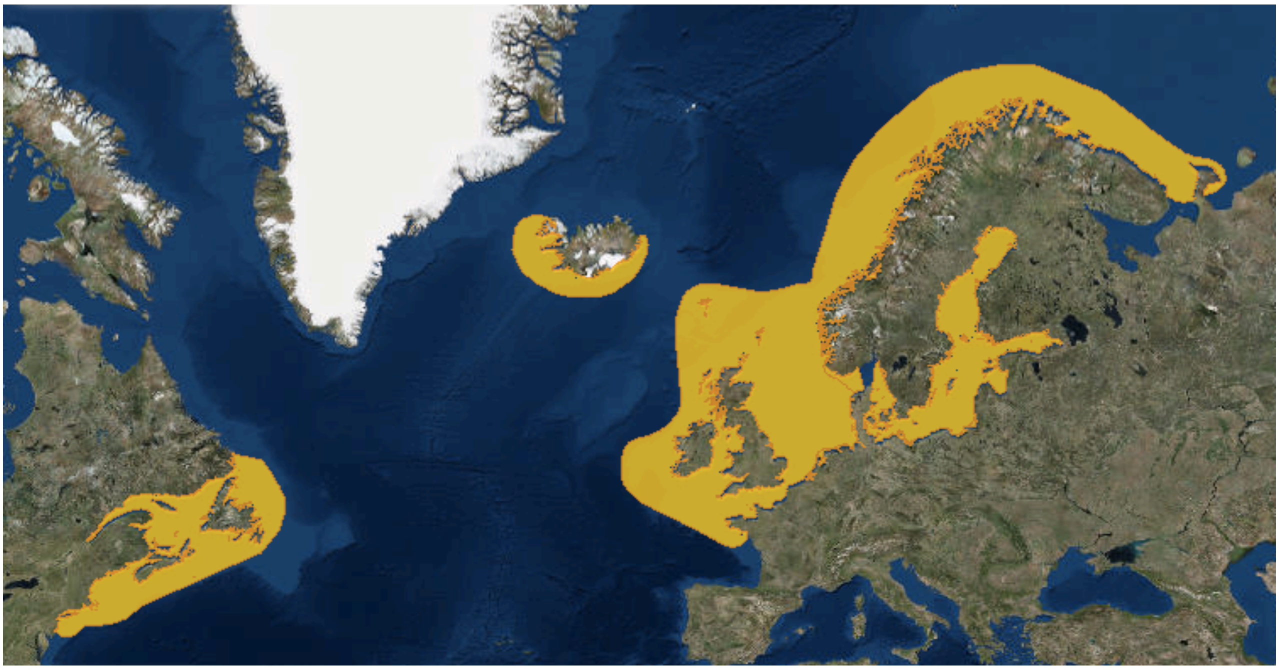

Figure 1 – The distribution of grey seals in the North Atlantic and Baltic Sea (Bowen, 2016). An observation of a grey seal from Greenland was made in 2010, so it does also occur here, although very rarely (Rosing-Asvid et al., 2010). Three populations are recognized – the Western Atlantic, the Eastern Atlantic and the Baltic.

The genetic distribution of grey seals was recently investigated by Fietz et al (2016), who set out to assess the subspecies boundaries in the Baltic Sea. Before the extinction of grey seals in the Danish waters, it was Baltic seals that occupied the South-west Baltic as well as the Kattegat. But with new genetic analyses it was discovered that the recolonizing seals that now occupy Kattegat are seals from the Atlantic population and admixture takes place at Rødsand in Southern Denmark. The seals from Øresund and areas around Bornholm are from the Baltic population, but it is possible that further admixture will take place in the future (Fietz et al., 2016).

The grey seal population in the Baltic Sea has increased to about 32 000 counted individuals in 2014, but since every individual seal cannot be detected, the actual population size is estimated to be higher (Figure 2) (helcom.fi) (Varjopuro, 2011). The largest abundance of grey seals is found in the Northern Baltic Proper between the Swedish and Finnish coast (Figure 3). In the Southern Baltic about 2500 seals have been counted in 2014 and it is assumed that about 400 of these haul out at Tat during the moulting season (helcom.fi). In 2015, about 800 grey seals were counted in a single day in the Danish Baltic. Though the occurrences of grey seal in the Danish waters are increasing, only 6 pups were registered on Danish ground in 2014-2015 (Galatius et al., 2016). The total number is assessed to about 45 000 individuals (Naturstyrelsen, 2016). The grey seal did not return to Danish waters until 2003 where pups were observed in the seal sanctuary of Rødsand (Jepsen, 2005; Galatius et al., 2016). Falsterbo (Måkläppen) is a haul-out site for a big seal colony of both harbour and grey seals, estimated to 50 – 500 animals.

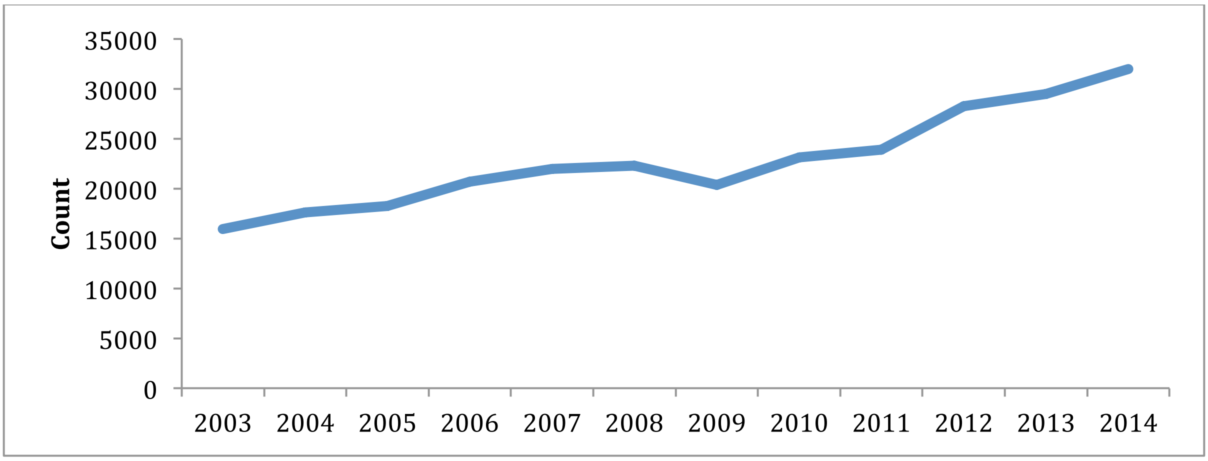

Figure 2 – The increase of the Baltic population of grey seals over the last 10 years. The seals are counted at haul-out sites in the moulting period, so the actual number of seals is higher, because the individuals at sea are not included in the count. The number has doubled in this period 2003 to 2014. (Data extracted from Helcom.fi)

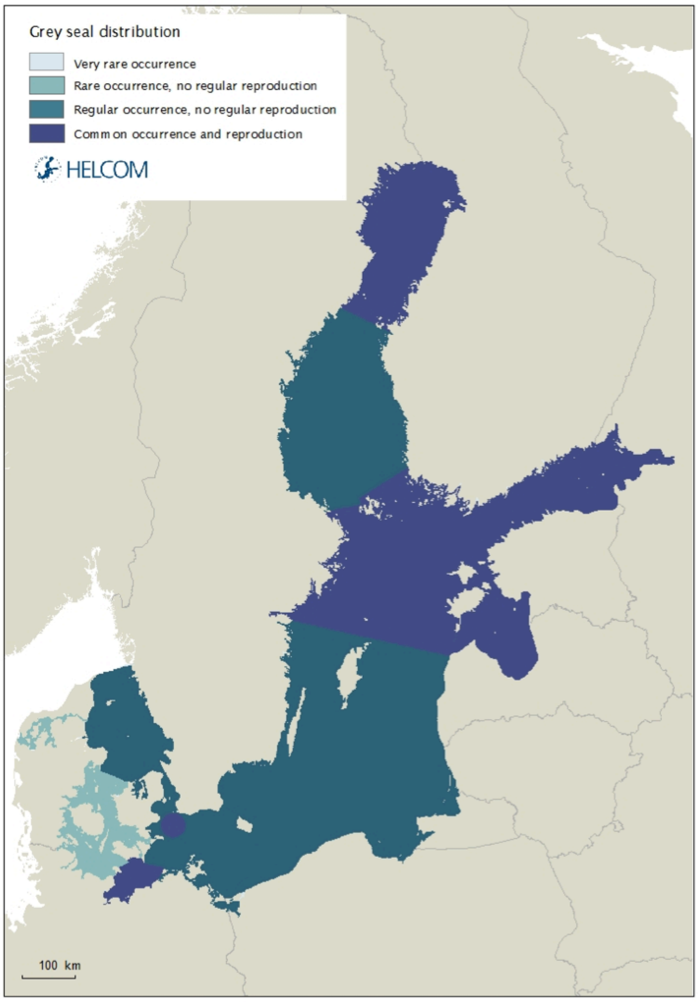

Figure 3 – The distribution of grey seals in the Baltic Sea. The largest abundance is found in the Baltic proper off the east coast of Sweden (Helcom.fi)

The history of the Baltic grey seal

Exploitation and hunting of grey seals

In the last centuries seals have been exploited for blubber, oil and meat and were very economically important. At the end of the 19th century seal hunting became less significant because seal oil used for production of e.g. leather was replaced by cheaper alternatives on the market (Harding & Härkönen, 1999). The grey seal was the most abundant seal in Southern Scandinavia in the 18th and 19th centuries and only after they were hunted to almost extinction did the harbour seal replace it as the most common (Olsen et al., 2010). The maximum grey seal number of the century in the Baltic Sea was in 1906 with a Baltic population size of 80 000 - 100 000 individuals (Harding & Härkönen, 1999). Around this time the seals became competitors instead of prey, causing the conflict with the fishermen to intensify (Bowen & Lidgard, 2013; Harding & Härkönen, 1999). Tauber (1882) stated in an article in the Danish Fishery journal (Fiskeritidende) "*When the last seal is killed, a new era in the history of fishery will begin*". He described the increasing problems with seals in the Danish waters and why it was necessary to begin a cull. At this time especially the grey seal was hunted because the pups are very vulnerable in the first few weeks. They are born with their embryonic fur, which makes them unable to go into the water until their first moult after 3-4 weeks in contrast to the harbour seal pups who moults while still in the womb, and are able to follow their mother in the water right away (Jepsen, 2005; Fietz et al., 2016). In 1889 Denmark introduced a bounty system for each seal killed, and 25 000 seals were shot in the following 20 years (Harding & Härkönen, 1999; Galatius et al., 2016). In 1909 the Danish fishermen asked the government for help to kill the last seals by increasing the bounty and optimizing gear. Furthermore they wanted all the Baltic countries to cooperate in the fight against the seals (Johansen et al., 1909). In 1927 the distribution of prizes stopped, but the hunt continued until the protection in 1967 in Denmark although to a lesser extent (Galatius et al., 2016). The Baltic grey seal population continued to decrease steadily and in 1975 the population consisted of 3600 seals while the minimum was reached in the beginning of 1980 with only 1500 seals remaining (Almkvist, 1982; Harding & Härkönen, 1999).

Environmental contaminants and PDV

In the beginning of the 1980s the Baltic Sea was seriously polluted by the persistent organic contaminants PCB and DDT (polychlorinated biphenyl is often used as dielectric, coolant fluids and in building materials while dichlorodiphenyltrichloroethane is an insecticide). This had a drastic effect on the seal reproduction and 50% of female grey seals were found to be sterile (Almkvist, 1982). Commonly occurring problems in the female seals were stenosis and occlusion of the uterus and they were correlated with higher PCB levels in tissues (Helle et al., 1976). Changes were introduced in environmental politics and the toxins were banned in 1977 (Helcom, 2007). This, along with the protection of seals in the Baltic Sea, made the grey seal populations increase rapidly (Klimova et al., 2014). The mean annual growth rate of the population was calculated to 7.5 % at the Swedish Baltic coast (Harding et al., 2013).

The harbour seal population in the Kattegat – Skagerak and the North Sea was afflicted with the Phocine Distemper Virus (PDV) in 1988 where more than 50 % of the seals perished. In 2002 the virus hit again causing the death of 30-50% of the population. Both times the outbreak started at Anholt in Kattegat and spread to most of the surrounding countries including Great Britain (Härkönen et al., 2006; Olsen et al., 2010; Dietz et al., 2013). The grey seals do not seem to be affected by the disease, but they could act as vectors between the Arctic and the North Atlantic. Harp seals (*Pagophilus groenlandicus*) has shown a high prevalence of antibodies to the virus and are highly migratory, so they might be the reason for the PDV outbreak in 1988 (Heide-Jørgensen et al., 1992). At Anholt the harbour seals, harp seals and grey seals live in close association and are therefore likely to experience cross-species transfer of the infection. (Härkönen et al., 2006; Olsen et al., 2010)

Management of the Baltic grey seal

The grey seal became protected in Denmark in 1967 and the harbour seal in 1976 (Miljø- og Fødevareministeriet, 2016). Both seals are included in the EU habitat directive and the Bern convention from 1979. In 1988 HELCOM recommended a prohibition of seal hunt in all of the Baltic Sea (Helcom, 2007; Jepsen, 2005; Harding & Härkönen, 1999). The Baltic Sea Action Plan of the Baltic Marine Environment Protection Commission (HELCOM BSAP) has agreed upon a strategy where seal populations are allowed to increase until they reach their carrying capacity. This may be in conflict with another management objective that they have proposed – that cod biomass should be recovered to the level where it can provide maximum sustainable yield (MacKenzie et al., 2011; Larsen et al., 2015; Helcom, 2007). The grey seal is listed as least concern on the IUCN HELCOM red list, but the management measures vary greatly for the different countries in the Baltic. In Finland, Sweden and Estonia, restricted hunting is allowed, while hunting is strictly prohibited in Russia, Poland and Germany (HELCOM Red List Marine Mammal Expert Group, 2013). This causes difficulties as the Baltic seals are one population, but vary greatly in their use of the waters of each country.

Movement of the grey seal

The grey seals have very large home ranges and strong dispersal capabilities, but they are philopatric and tend to concentrate their movements in relatively small areas near haul-out sites, especially in the open-water season (Oksanen et al., 2014; Klimova et al., 2014). They are known to regularly travel over large distances in a few days and then spend a longer period at a haul-out site (Sjöberg et al., 1995). Grey seals from the Rødsand seal sanctuary was found to have kernel home ranges of an average of 51 221 km^2 (ranging from 4160 to 119 583 km^2) which is 130 times larger than that of harbour seals from the same location (Dietz et al., 2003).

In the Southern Baltic there are only a few grey seal haul-out sites: Rødsand, Tat, Måkläppen and Utklippan. Around these locations the seal activity and foraging is expected to be high. Few studies have estimated the movement of the seals from the Southern Baltic Sea, but they provide very valuable data when assessing the foraging areas and migration. Dietz et al. (2003) investigated the movement of seals from Rødsand in Denmark. Grey seals were tagged and 5 out of 6 migrated to Sweden or Estonia. The sixth seal was a juvenile that drowned in a fish trap. In May 2016 tags were attached to 14 grey seals from Tat, Christiansø and 12 of them moved out of the Danish waters in a period of only a couple of months. Most of them relocated to other known haul-out sites in Sweden, Finland and Estonia, while only one of them moved west before also swimming to Sweden (Morten Tange Olsen pers. comm.) (Wildlifetracking.dk). These studies suggest that the Baltic grey seals have very large home ranges, and cannot be associated with a single location.

The conflict between seals and fisheries

In recent years the seal-fishery conflict has found its way to the media and has given rise to numerous articles in the newspapers. These articles cover the increasing number of fishermen who loose large parts of their catch to grey seals. Around Bornholm, the primary catch is cod (*Gadus morhua*) and the fishery on Christiansø has been abandoned because of losses due to seal interactions with the fishing gear. Apart from losing catch and experiencing damage to their gear, fishermen are also worried that the seals are a threat to the populations of cod and salmon (*Salmo salar*) in the waters around Bornholm (Larsen et al., 2015).

Seal-related damage to fisheries

In 1999 the damage caused by seals in Sweden was estimated to 3.5 million US$ (Westerberg et al 2000 in Lundström et al. 2007), and in 2006 it had increased to 7 million US$ counting both the direct catch losses and the indirect costs (Westerberg et al., 2006).

Harbour seals consume on average 4 kg of fish per day and grey seals about 5 kg (Bjørge et al., 2002; Hammill & Stenson, 2000). This makes the consumption each year extensive and it is a great concern for the fishermen what effect this has on the fish stocks in the Baltic Sea. The problem is most often regarded in four different ways in which seals can cause damage: 1) Predation on the fish stocks, which makes fewer fish available for the fisheries. The seals can also affect the behaviour of the fish by their presence and potentially chase away the fish from fishing gear. 2) Direct loss of catch when seals eat the fish from the fishing gear. 3) Damage to the fishing gear itself that occurs when seals catch the fish from the nets and 4) parasite infection in cod that causes a reduced health condition and value of the fish (Varjopuro, 2011; Hemmingsson et al., 2008; Buchmann & Kania, 2012).

Seals are known to use visual cues such as above-water buoys to locate fishing gear and damage both the fish and the gear (Fjälling et al., 2007). This along with other cues such as olfaction can make it easy for the seals to locate nets and the only solution will be to move the gear more often. In addition to damaged fish found in fishing gear, the hidden losses can be very large compared to the visible losses, though it is hard to estimate how seal presence affect the fish and how many fish are lost to seal consumption (Königson et al., 2009). Königson et al. 2009 found the hidden losses to be 44.2% in fleets where seals had raided while 13.5% of the fish were damaged. So an estimated 4 fish were lost to every 1 damaged in in this study from 2005. More studies about this could be useful, as it makes the losses even larger than previously recorded.

By-catch and hunting of seals

Seals can get stuck in the fishing gear, and it is most often juveniles and pups that are killed as by-catch, while adults are hunted because they are too close to the fishing gear (Lundström et al., 2007; Bjørge et al., 2002). Most of the by-catch in the Baltic Sea are caused by the coastal trap nets and gill nets (Vanhatalo et al., 2014). Seals have been observed with old fishing gear around the neck or other parts of the body and this often causes death by strangulation, starvation or infection (Allen et al., 2012). Westerberg et al. (2006) investigated the by-catch of grey seals in Sweden in 2001 and assumed that a minimum of 462 grey seals drowned in fishing gear. This number is probably higher as it only accounts for commercial fisheries. Most by-catches occur in traps for salmon and eel and gillnets for flatfish (Pleuronectidae) and cod. There was a lack of data from other Baltic countries, but a by-catch of approximately 1000 seals per year was proposed for the Baltic as a whole (Westerberg et al., 2006). In 2014 a similar study was made for the Northern Baltic Sea and the by-catch was estimated to approximately 1240-2860 seals. When taking the increasing seal population into account this means that the by-catch in this study was lower than that found in 2001. This is most likely because of the improvement of fishing gear, but there can also be problems with the reliability of the fishermen's reports on by-catch (Vanhatalo et al., 2014).

The hunting of grey seals does not occur very often anymore but various hypotheses of the benefits of hunting are still active. Especially protective hunting with the purpose of shooting rogue seals that are believed to cause the most damage, or by hunting close to fishing gear which make the seals avoid the area. It is though still unconfirmed if these methods are successful, and studies have shown differing results (Westerberg et al., 2006; Quick et al., 2004). In 2014 the Danish Nature Agency made new rules for the regulation of grey seals in Danish waters. Fishermen are now allowed to ask for dispensation to regulate seals in the period 16th of April until the 31st of January. The hunter is required to take a course about seal hunting before the regulation and only seals that are within 100 meters of the fishing gear can be shot (Naturstyrelsen, 2014). Because of these restrictions, a very limited number of seals are regulated each year. In 2015 10 dispensations were granted to shoot a total of 20 grey seals. No seals have subsequently been confirmed regulated (Naturstyrelsen, 2016). In Sweden the number of grey seals allowed regulated in 2015 is set to 350 individuals (Naturvardsverket.se).

Fish stocks in the Baltic Sea

The cod stock in the Baltic Sea is divided into two subpopulations – the Eastern stock located east of Bornholm and up to the Bothnian Bay and the Western stock located west of Bornholm and in the Belts (Bleil et al., 2009). The eastern stock has reached a record low in the 2000s and ICES advises the total catch of cod in 2017 to not exceed 26 994 tons (ICES, 2016). The two cod stocks differ in both maturation and spawning periods, but admixture does also occur especially if abiotic factors and limited food sources force the eastern population to migrate to the spawning areas normally used by the western population (Bleil et al., 2009). Oxygen is vital for the survival of eggs and larvae and salinity is important for the eggs to be able to float (Bagge et al., 1994). Therefore it is only possible for cod to spawn in areas with salinity levels above 11 g/kg. The salinity in the

Baltic Sea is dependent on inflow from the North Sea and changes according to the strength of the salinity in this inflow. The cod reproduction success is thus controlled by the abiotic conditions and in periods with both unfavourable conditions and overexploitation by fisheries the cod stock will be affected negatively (Vallin et al., 1999).

Overexploitation is most likely the cause for the decrease in cod stock, but MacKenzie et al. (2011) accounted for other aspects such as seal predation and abiotic factors. They found that abiotic factors such as salinity, temperature and eutrophication, could have a much larger effect on the cod population than the impact of seals. Thus in models where overexploitation and decreased salinity were present, seal predation were not a major factor affecting the recovery of cod in the Baltic Sea. It was assumed that the proportion of cod in seal diet decline when cod are rare because of the seals' opportunistic foraging nature (MacKenzie et al., 2011). In a study from Scotland it is suggested that seals may hunt parts of the cod stock that is not available to the fisheries and it is unlikely that seal predation is the reason for decline in the cod stock, although they might be impairing the recovery of the stock (Cook et al., 2015). A study of the herring (*Clupea harengus*) stock in the Bothnian Sea showed that the predation by grey seals is within the measurement errors in stock assessment data. This means that grey seal consumption of herring had a low impact on the biomass of herring relative to errors from measurement uncertainties (Gårdmark et al., 2012).

Increasing occurrences of parasites in the Baltic cod

A relatively new problem in the Baltic Sea is the increasing occurrence of the parasite, seal worm or cod worm (*Pseudoterranova decipiens*), in cod flesh and liver. The parasite has a wide distribution, but has only been discovered extremely rarely in the Baltic, probably because of the low number of seals (Buchmann & Kania, 2012). In the early 20th century the parasite were frequently found in cod liver, but as the seal population decreased, so did the abundance of seal worm. As the seal numbers in the Baltic have started to increase again, the occurrences of seal worms in cod has followed this trend (Haarder et al., 2014). An analysis was made of cod in the waters east of Bornholm infected with seal worm in 1982-1983 compared to 2011. No cod was infected in the 1980s but a markedly increase in parasite occurrence was found in 2011 (Buchmann & Kania, 2012). A study by Mehrdana et al. (2014) confirmed an increasing occurrence of seal worms in the Baltic with a recorded prevalence of infection of more than 50% in larger cod and up to 20% in smaller cod. Liver worms (*Contracaecum osculatum*) were found in 100 % of cods with a prevalence of up to

230 worms per fish. These worms, that are also transferred by seals, can affect the fitness of the cod, especially if they are infected by large numbers of parasites (Mehrdana et al., 2014).

Seals are the final host of the seal worm while crustaceans and fish are the 1st and 2nd hosts. The factors that affect parasite abundance has been determined to be salinity, seal density and fish length (Lunneryd et al., 2015). With lower salinity in the north of the Baltic a decrease in nematode occurrence is observed. This is most likely because of a lower abundance of the intermediate hosts in these areas (Lunneryd et al., 2015). The seal worm has zoonotic potential as undercooked fish can infect humans (Haarder et al., 2014; Buchmann & Kania, 2012). Moreover the value of the cod is lessened and parasites have to be removed from fillets which is very time consuming (Buchmann & Kania, 2012). It is unknown how many cods are destroyed or sold for a lower price due to the presence of parasites. It might be a considerable number, and it is likely that the problem grows as the seal population increases (Lunneryd et al., 2015).

Diet of the grey seal

Seals are opportunistic feeders, but their diets are often dominated by a few key species. The prey species can vary geographically and seasonally relative to which species are most abundant (Andersen et al., 2007; Olsen et al., 2010; Lundström et al., 2010). Seasonal variations in the seal diet can reflect the migration and spawning patterns of the prey species. Changes can also be recorded in geographic region, age group of seals, gender of seal and conditions under which the samples were collected. Lundström et al. (2010) found the main factors influencing the diet to be geographic region, sampling condition and age group. In a study from 2007, Baltic herring was the dominating prey species in the diet of grey seals in the Baltic Proper and Gulf of Bothnia, followed by common whitefish (*Coregonus lavaretus*), cyprinids (Cyprinidae), European flounder (*Platichthys flesus*) and European sprat. Cod only consisted of a small part of the diet. Differences between areas were found, and it is most likely because of different prey availability in the areas (Lundström et al., 2007). In a study covering the entire Baltic Sea, Atlantic herring was found to be the most common prey species targeted by grey seals followed by European sprat in the south and common whitefish in the north. There was not found any seasonal variation (Lundström et al., 2010). In these studies, cod did not contribute much to the grey seal diet, but in a DNA study by Pittman et al. (in prep.), the results were quite different. Of all grey seal scat samples collected near Christiansø more than 90% contained DNA from the cod-family, about 50% from Clupeidae and about 20% from Belonidae. Grey seals from Rødsand also preferred codfishes but consumed less codfish than the seals at Christiansø and the second most consumed fish family was Clupeidae.

Diet analyses

Morphological identification of hard parts

Numerous studies have investigated the diet of seals by extracting otoliths and other hard parts from stomach and scat contents (Lundström et al., 2007; Härkönen, 1987; Lundström et al., 2010; Bjørge et al., 2002). This method has its benefits but also several drawbacks. Underestimation is very likely from counting otoliths because of erosion in the stomach, which especially affects small and fragile otoliths (Härkönen, 1987; Lundström et al., 2010). Secondary ingestion can also be a problem as the prey species themselves have hard parts from prey in their stomach, and these will not be possible to differentiate from prey consumed by the seal (Pierce et al., 1991). Large species such as salmon can be hard to detect because seals often discard the head or only eat the soft parts of the fish (Suuronen & Lehtonen, 2012; Pierce et al., 1991; Lundström et al., 2010). When analysing hard parts from stomach and intestine it can sometimes be difficult to avoid non-random as most dead seals are found either in nets, on the shore or shot close to fishing gear. This can bias the results as seals have often consumed species in the nets or nothing at all if they have died of starvation (Lundström et al., 2010). Numerical correction factors (NCF) are a method to compensate for the loss of hard parts due to digestion in the stomach. This also comes with a lot of bias, but ignoring it would probably be worse (Bowen, 2000; Lundström et al., 2007). It is important to take into account that the same correction factors cannot be used for hard parts taken from the stomach or intestines and those from faecal samples. The hard parts from scats are more eroded than those from stomachs and therefore different correction factors must be applied for each type (Bowen, 2000).

Molecular analysis

Next Generation Sequencing methods have been used extensively in diet studies because they make it possible to identify numerous species from a large amount of samples using sequencing of DNA barcodes (Pompanon et al., 2012). This method is also known as metabarcoding where DNA from environmental samples such as faeces, blood, saliva, pollen etc. are extracted and sequencing is performed on amplicons with tagged universal primers (Bohmann et al., 2014). The DNA present in such environmental samples is often degraded and therefore of short length, and the NGS approach has proven very useful for obtaining results (Hibert et al., 2013). The problem when looking at the diet of carnivores is the amplification of the predators DNA along with the prey DNA (Shehzad et al., 2012). This can be rectified by using either species or group-specific primers (Deagle, Kirkwood, and Jarman 2009).

The 16S mitochondrial gene is the most commonly used, as multiple copies are present in each cell and makes it ideal for low-quality samples such as faeces (Parsons et al., 2005). The method is relatively easy and non-invasive, which is an advantage especially when dealing with vulnerable or elusive species. Furthermore it can provide information about both the identity and population genetics of the predator as well as the prey (Parsons et al., 2005). It is especially useful if samples lack hard parts or if the species targeted is known to leave few hard parts or some that are difficult to differentiate from other species. It has proven possible to find species even though very low concentrations of DNA was present in the scat (Matejusová et al., 2008). The major drawback of this method is that it is difficult to accurately relate the number of prey DNA molecules identified in dietary studies to the relative biomass of different prey items consumed (Matejusová et al., 2008; Deagle et al., 2010). A problem that also occurs in the morphological identification method is the inability to differentiate secondary prey from the actual prey of the predator (Pinol et al., 2014). This can be a serious bias, as it is not possible to make corrections for the diet of the prey. Other uncertainties include the need for a good reference database to be able to correctly identify prey, and the loss of species identification if the universal primers do not target all types of prey.

Stable isotopes

Analysis of stable isotope ratios in animal tissues is a widely used technique for determining the trophic level in which a predator forages as well as the most important prey in the diet. Carbon and nitrogen isotope ratios vary depending on location, trophic level and diet, and the values vary between organisms. This type of analysis represents the diet over the last weeks to months of diet (Burns et al., 1998). It is useful if it is the overall trends in diet that is the aim of the investigation, but stable isotope analyses gives poor taxonomic resolution and can be confused with species with similar isotope signatures (Deagle et al. 2010).

Fatty acid signatures

Fatty acids are released from ingested lipid molecules and are taken up by the predator’s tissue. They are either used as an energy source, or re-estified and stored in adipose tissue. Some of the fatty acids are metabolized inside the predator, but the rest is deposited in adipose tissue with little modification. Fatty acids are very diverse and their composition can be maintained through the food web, from zooplankton to whales. Fatty acids are mostly used qualitatively to understand trophic levels and spatial and temporal differences in diets. They can also be used for quantitative estimates of predator diet. Calibration coefficients are needed to account for the metabolism of fatty acids in predators and the variance in how the acid is laid down. Species with a higher fat content will contribute more to the predator's signature than low-fat species (Iverson et al., 2004). The innermost blubber layer is the one that resembles the prey fatty acid signatures more closely, because it is the most metabolically active layer. Dietary lipids likely influence this layer most directly (Andersen et al., 2004). Where the analysis of hard parts reflects the last couple of meals of a seal, the method of applying fatty acid signatures is useful for a longer period diet analysis of an animal (Beck et al., 2007).

Critter cams

Not much is known about how seals hunt, as it occurs under the surface. A method of investigating the foraging tactics of pinnipeds is by using critter cams that are glued to the back of the animal. These cameras are very useful to get an idea of the actual hunting techniques of seals by analysing recordings. Bowen et al. (2002) investigated the foraging techniques of harbour seals in Canada. They used critter cams on adult males and took a blubber sample from each one to compare the fatty acid signature of the blubber to fatty acid profiles of potential prey species. A combination of data from the critter cams and the blubber samples gave a similar picture of the prey species consumed by the seals (Bowen et al., 2002).

Materials and methods

Sampling location

A total sample size of 248 scats was used in this study. The scats were collected at Måkläppen (Falsterbo), Sweden (N = 163) in 2014-2015 (Figure 4 and 5) and Tat (Christiansø), Denmark (N = 85) in 2015-2016 (Figure 5 and 6). Scats were collected every month except December, so months were combined according to season. Winter (December, January, February), spring (March, April, May), summer (June, July, August) and autumn (September, October, November). Scats were collected on the beach or rock of the location and placed in individual plastic bags stored at -20°C until further processing. Måkläppen is a haul-out site for a large colony of grey seals and harbour seals. Tat, near Christiansø, is a resting place for grey seals coming in from other places in the Baltic, such as Rødsand, Utklippan and Måkläppen.

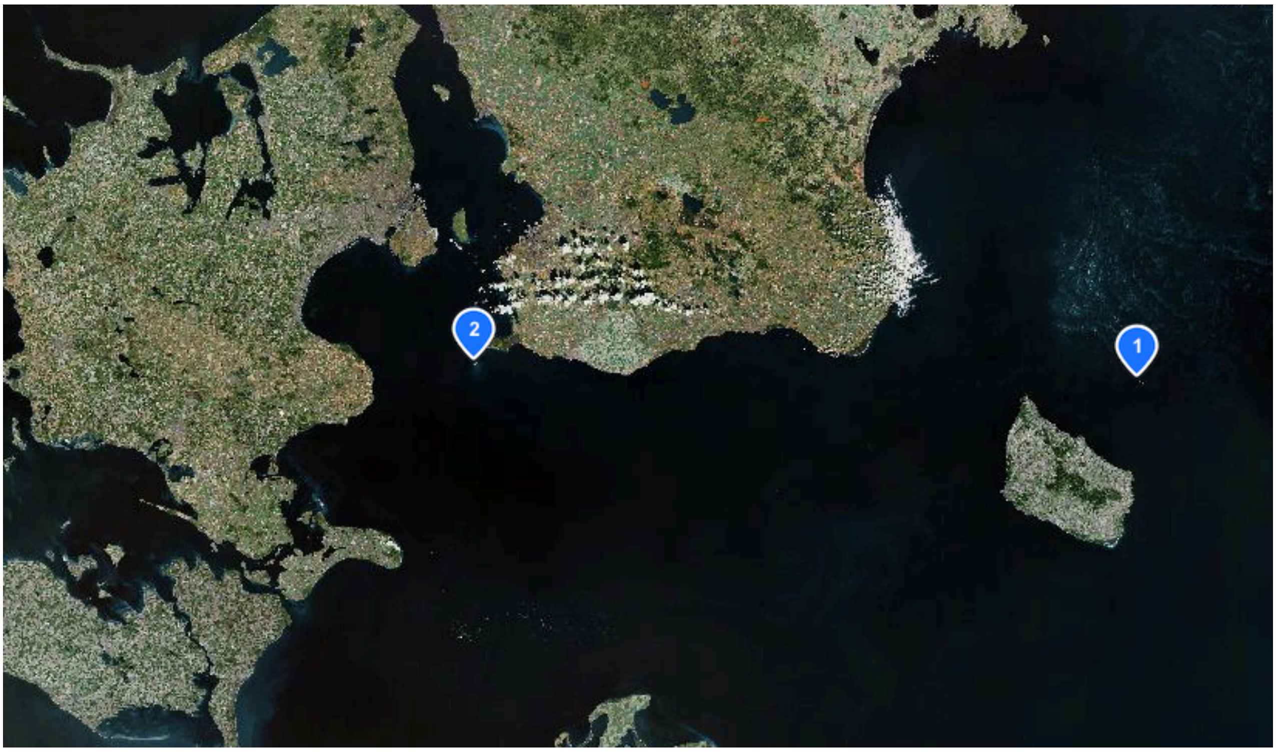

Figure 4 – Sample locations: 1: Tat (Christiansø), Denmark, 2: Måkläppen (Falsterbo), Sweden in the Southern Baltic Sea.

Figure 5 – Grey seals (left), harbour seals (middle) and cormorants (right) at Måkläppen in Sweden. Photo: Jeppe Dalgaard Balle

Figure 6 – Grey seals on Tat in the Danish Baltic Sea. Photo: Morten Tange Olsen

DNA extraction

The scats were left to thaw for at least four hours before a small piece from the middle of the scat was taken out and put in a 1.5 ml eppendorf tube. Some scats were entirely covered in sand, making it impossible to avoid sand in the sample. Only one sample was taken from each scat because previous studies have shown an even distribution of DNA throughout the scat, and thus no need for sub-sampling (Matejusová et al. 2008; Pittman et al. in prep.). A plastic knife washed in 70 % ethanol (and for sample 210 to 274 washed in 5 % bleach followed by 70 % ethanol) was used to take out the piece of scat. Knife and gloves were changed after each sample. Samples were stored in a box at -18°C until DNA extraction.

Extraction was done in a flow hood cleaned with 5 % bleach and 70 % ethanol. A small piece (about 20 mg) of sample was taken out of the tube with a metal spoon, a sterile spatula or a pipette tip and put in a new tube. The spoon was rinsed in bleach and ethanol and sterilized in an open flame before and between each sample. Extraction of the samples was done using the Thermo Scientific KingFisher Cell and Tissue DNA Kit following the manufacturer's protocol. Incubation time was at a minimum of 8 hours and a maximum of 16 hours. A maximum of 24 samples was extracted at any one time including 2 negative controls. The quality of the DNA extractions was estimated on a 1% agarose gel using electrophoresis (130 volt, 350 amps, 40 minutes with a 1 kb ladder). The samples were stored at -18°C until further analysis.

Seal species determination

Samples were amplified for the mitochondrial control region using the primer pair: HG001F (5' - CACCACCAGCACCCAAAG - 3') and HG001R (5' - TCATAGCTGAGTGATACCG - 3') to determine seal species origin of each scat. The primers bind to the control region on the mitochondrial DNA targeting seals. Polymerase Chain Reaction (PCR) was performed on an Eppendorf Mastercycler gradient PCR machine, using an amplification master mix containing 2.5 µl 10x Taq Gold buffer, 2.0 µl$MgCl_2$, 1 µl Bovine Serum Albumin (BSA), 0.25 µl dNTP, 0.2 µl AmpliTaq $Gold_{TM}$ polymerase and 1 µl of each primer (10 µM stock). 1 µl of the extracted DNA was used and 16.05 µl dH_2O to reach 25 µl in total. Cycling conditions were as follows: initial denaturation at 95°C for 5 minutes, 40 cycles of denaturation at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds and elongation at 72°C for 30 seconds followed by a final elongation step at 72°C for 7 minutes. After the final step the samples were cooled down to 4°C. Each PCR run included a PCR control. To determine the amplification results and lengths, 3-5 µl of each sample with loading buffer was run on a 2% agarose gel using electrophoresis (130 volt, 350 amps and 40 minutes with a 100 bp ladder). The samples were sent to Macrogen Europe (the Netherlands) for Sanger sequencing. The sequences were assembled using Geneious 9.0.4 followed by BLASTn (Basic Local Alignment Search Tool for nucleotides) in Genbank.

Diet determination

Samples were amplified using fusion primers with an adapter, FluPad and index incorporated to make each primer unique. The primers were group-specific and used to amplify short regions of the 16S mtDNA gene, targeting fish. 10 forward primers and 20 reverse primers made enough combinations for 200 samples. The template specific forward primer had the sequence (5' - GACCCTATGGAGCTTTAGAC - 3') and the reverse primer (5' - CGCTGTTATCCCTADRGTAACT - 3'). Polymerase Chain Reaction was performed in 25 µl reactions containing 2.5 µl 10x Taq $Gold_{TM}$ buffer, 2.5 µl 25mM $MgCl_2$, 0.25 mM dNTPs, 0.25 Taq Gold polymerase, 1 µl 10 µM 16SFish F primer, 1 µl 10 µM 16SFish R primer and 2 µl extracted DNA. Some samples did not work with this mixture so they were re-amplified with 5 µl extracted DNA, 3 µl $MgCl_2$ and accordingly less dH_2O. Cycling conditions were as follows: initial denaturation at 95°C 5 min followed by 35 cycles of denaturation at 95°C for 30 seconds, primer annealing at 52°C for 30 seconds end extension at 72°C for 45 seconds followed by a final extension at 72°C for 10 minutes. After PCR amplification, 3 µl of each product with loading buffer was run on a 2% agarose gel for 40 minutes at 130 volt and 350 amps alongside a 100 bp ladder. The lengths as determined from the gel were around 350 basepairs. PCR products were divided into two groups based on the clearness of the bands. All successful samples were pooled together in 2 tubes with each half of the samples and 4 controls. 1 µl was taken from samples that had shown a clear band on the gel, and 2 µl of samples that had shown a weaker band. Qiaquick was used for purification of the PCR products according to the manufacturer's protocol. Qubit was used for determination of DNA concentration of each tube. Tube one had a concentration of 3.68 µg/mL and tube two 5.58 µg/mL. The concentration is recommended to be between 1 and 10 µg/mL so no dilution was needed. Another PCR Purification Process Procedure was performed, followed by Qubit. The concentrations were 2.88 ng/µl for pool 1 and 4.5 ng/µl for pool 2. Afterwards the libraries were run on a Tape Station and the concentration was determined to 2280 pg/µl for pool 1 and 1720 pg/µl for pool 2. The two libraries were pooled together according to the concentration from the Tape Station, 15 µl of pool 1 and 19.74 µl of pool 2. The library was sequenced on an Illumina MiSeq (300bp V2 Nano) using single-end sequencing.

Sequence data processing

The sequences were identified and sorted in Geneious R9 (Version 9.0.5) using the unique tag combination. In the first step, the primers were trimmed from the sequences in both 5' and 3' end. Then reads were separated by barcode in both ends and placed in folders specific for each sample. Lastly the barcodes were trimmed, making the reads clean 16S fish sequences.

Sequences were aligned with a reference database containing all known species found in the Baltic Sea. All species were furthermore queried against GenBank using BLASTn. Only sequences with an identity match of 99 or 100 % to a species were used. Of 8 controls sequenced, 1 had a presence of fish DNA. The control K7 had a total of 5 sequences from the family Pleuronectidae. Because of this contamination the limit of sequences for a species to be considered present in a sample was set to 10. Two species had only one occurrence of 5 sequences each (*Scomber scombrus* and *Taurulus bubalis*) and was discarded.

Data analysis

The number of sequences of each fish species in a sample was recorded in Excel. Frequency of Occurrence (FO_i) was calculated as the number of seal scats containing the fish species (N_i) in relation to the total number of seal scats containing prey (N_t).

$$(FO_i) = (N_i/N_t) \cdot 100$$

Data was treated overall with the two locations combined, to examine the total number of species and divided to recognize the variation between areas. The data was also grouped into seasons for each location to determine any variation over the course of the year. Yearly variation was investigated between the spring 2014 and 2015 at Måkläppen. Lastly, the Frequency of Occurrence (FO_s) was calculated for the number of sequences. This was done by dividing the number of sequences for each fish species with the total number of sequences. This FO_s was correlated with the FO_i from the samples to assess if it was possible to estimate the fish species quantity from the number of sequences.

Fish distribution

The prey found in this study was compared to the fish distribution and abundance in the Baltic Sea from data extracted from the Atlas of Marine Fishes of Denmark (unpublished database). This database consists of records from scientific studies, commercial fisheries and recreational fishermen. Every fishing trip is recorded along with date, month and year and the number and species of fish. The comparison was done to ensure that the identified species geographic distribution matched that of the assumed foraging areas of the grey seals from Denmark and Sweden. It also makes it possible to investigate if the grey seals are purely opportunistic feeders or have preferences for specific species. The data was limited to the years 2000-2016 and all recorded species were noted. The fish species were treated as present or absent on a given day, so no matter how many of the species were found in a single day, only one record were made. The total fishing events were calculated by counting all different dates recorded. The Frequency of Occurrence (FO_f) could then be calculated by dividing the fishing event presence of a species with the total number of fishing events. Finding the FO_f of the fish species in the Baltic Sea makes it possible to compare it with the Frequency of Occurrence of the fish found in the seal diet (FO_i). The comparison should illustrate if the most abundant species in the areas are also the most abundant in the seal diet.

Commercial catch and value

Data from the Danish National landings database was extracted to estimate the value of different species in comparison to the grey seal diet. Data from the years 2012 to 2015 was used. The data was sorted to match the fish atlas database with the Eastern Zealand landings and the Bornholm landings separated. The average of each species per year for the 4 years were calculated and the tons and DKK of each species were used.

Statistical analysis

To investigate any significant differences in diet between the locations, seasons and years, Fisher's exact test for count data was performed. The number of occurrences for the two variables tested for significant difference of each species could then be compared for significance. RStudio version 1.0.44 (2009-2016) was used for the statistical analyses. The variation between localities and the seasonal variation for each location was tested for each prey species. To find any yearly variation, the years 2014 and 2015 for Måkläppen were tested against each other. The difference between fish species distribution in the area was tested against the abundance of the fish species in the seal diet. The significance level was set at $P = 0.05$.

Results

Sequence data

A total of 980 350 sequences were produced from the MiSeq run with a %GC content of 42.6 and sequence length of 325 basepairs. 164 489 sequences did not match any of the primers so 815 861 sequences were successfully applied to both primer tags. The final lengths of the sequences after trimming was 189-240 basepairs and up to 26323 sequences were identified for each species present in a sample.

Sample quality

Of the 248 scats collected, only 154 showed DNA concentration high enough for sequencing. 8 controls were sent to sequencing as well, to test for contamination. Of the 154 samples, 9 did not contain enough sequences to be included in the analysis. The final number of working samples was thus 145 in total, 64 from Tat, Denmark and 81 from Måkläppen, Sweden (Table 1).

The quality of the scat was noted (e.g. dry or sandy) and all information written during the collection. All of the scats from Tat were noted as fresh and of the 85 scats, 64 worked. Three scats were green and plant-like and did not yield any DNA, so it is possible that these were not actual seal scats. Three categories were noted from Måkläppen. 48 were noted as days to weeks old by the collectors and of these 24 worked and 24 did not work. 37 scats were noted as probably more than a day old when subsampling especially because of dryness. Of these 17 worked, and 20 did not work. 78 samples were noted as fresh by the collectors and of these 40 worked and 38 did not. As there was no statistical significance between the categories from Måkläppen, they were pooled into one group and compared statistically with the samples from Tat. A significant difference was found between the two locations (P = 0.00013). Furthermore there were 52 samples across the groups mentioned previously from Måkläppen noted as "sandy" or "very sandy" and of these only 21 were sequenced successfully.

Table 1 – The number of successful samples from each season and location. The numbers in brackets are the total number of scats. 248 samples were collected in total, and of these, 145 yielded DNA of high enough quality to be used. Samples from Måkläppen in spring are greatly overrepresented compared to the other seasons. The samples from Tat are more evenly distributed over the seasons.

Location	Winter	Spring	Summer	Autumn	Total
Måkläppen 2014		24(48)	5(8)	9(20)	38(76)
Måkläppen 2015		42(83)	1(4)		43(87)
Tat 2015		4(5)	13(20)	17(18)	34(43)
Tat 2016	21(28)	9(14)			30(42)
Total	21(28)	79(150)	19(32)	26(38)	145(248)

Seal species determination

The first 23 scats from Christiansø were determined to grey seal by Sanger sequencing. The rest were assumed grey seals as well, because this is the only species present in the area around Christiansø. All scats from Måkläppen were confirmed as grey seals from Sanger sequencing.

Overall diet

A total of 23 fish taxa were found in the 145 samples. The family Pleuronectidae could not be differentiated to species because of lack of variation in the targeted mitochondrial 16S region. Cod was the most common prey with a FO_i of 65 % followed by garfish with 39 %, herring with 37 % and sprat with 34 %. The family Pleuronectidae was found in 19 % of the samples.

Table 2 – Family, species and common name of the 23 fish taxa found in this study. Count is the number of scats containing the species, FO_i is the number of samples containing the species divided by total number of samples containing prey. Reads are the total number of sequences for each species and FO_s is the sequences per species divided by the total number of sequences for all species. Sorted by abundance in total samples.

Family	Species	Common name	Count	FO_i	Reads	FO_s
Gadidae	*Gadus morhua*	Atlantic cod	94	64.83	438179	55.54
Belonidae	*Belone belone*	Garfish	56	38.62	140654	17.83
Clupeidae	*Clupea harengus*	Atlantic herring	54	37.24	47939	6.08
Clupeidae	*Sprattus sprattus*	European sprat	49	33.79	54647	6.93
Pleuronectidae	*Pleuronectes/Platichthys*	Flatfishes	27	18.62	20962	2.66
Ammodytidae	*Hyperoplus lanceolatus*	Great sand eel	16	11.03	45017	5.71
Zoarcidae	*Zoarces viviparus*	Viviparous eelpout	11	7.59	16090	2.04
Lotidae	*Enchelyopus cimbrius*	Fourbeard rockling	8	5.52	462	0.06
Gadidae	*Merlangius merlangus*	Whiting	6	4.14	4395	0.56
Cyclopteridae	*Cyclopterus lumpus*	Lumpsucker	5	3.45	1694	0.21
Ammodytidae	*Ammodytes tobianus*	Lesser sand eel	5	3.45	2745	0.35
Scophthalmidae	*Scophthalmus rhombus*	Brill	3	2.07	128	0.02
Anguillidae	*Anguilla anguilla*	European eel	3	2.07	262	0.03
Salmonidae	*Salmo salar*	Atlantic salmon	3	2.07	2428	0.31
Salmonidae	*Salmo trutta*	Brown trout	2	1.38	1694	0.21
Gasterosteidae	*Gasterosteus aculeatus*	Three-spined stickleback	2	1.38	49	0.01
Gobiidae	*Pomatoschistus minutus*	Sand goby	2	1.38	28	0.00
Gadidae	*Pollachius virens*	Saithe	1	0.69	1051	0.13
Gobiidae	*Neogobius melanostomus*	Round goby	1	0.69	168	0.02
Esocidae	*Esox lucius*	Northern Pike	1	0.69	9711	1.23
Percidae	*Perca fluviatilis*	European Perch	1	0.69	603	0.08
Gobiidae	*Gobiusculus flavescens*	Two-spotted goby	1	0.69	55	0.01
Gobiidae	*Gobius niger*	Black goby	1	0.69	13	0.00

Location differences

The seals from Måkläppen had consumed a total of 20 species and one family. The most common species was garfish with a FO_i of 63 % (Figure 7). The second most abundant species was cod with a FO_i of 42 % followed by herring with 22 % and great sand eel with 20 %. In contrast, the seals from Tat, Denmark had consumed a total of 10 species and 1 family with the most common species being cod with a FO_i of 92 % (Figure 8). Then followed sprat (72 %), herring (56 %) and flatfishes with 36 % FO_i. There was a significant difference ($P < 0.023$) between the locations for all species that contributed to the overall diet with more than 5 % (Supplementary Table 3).

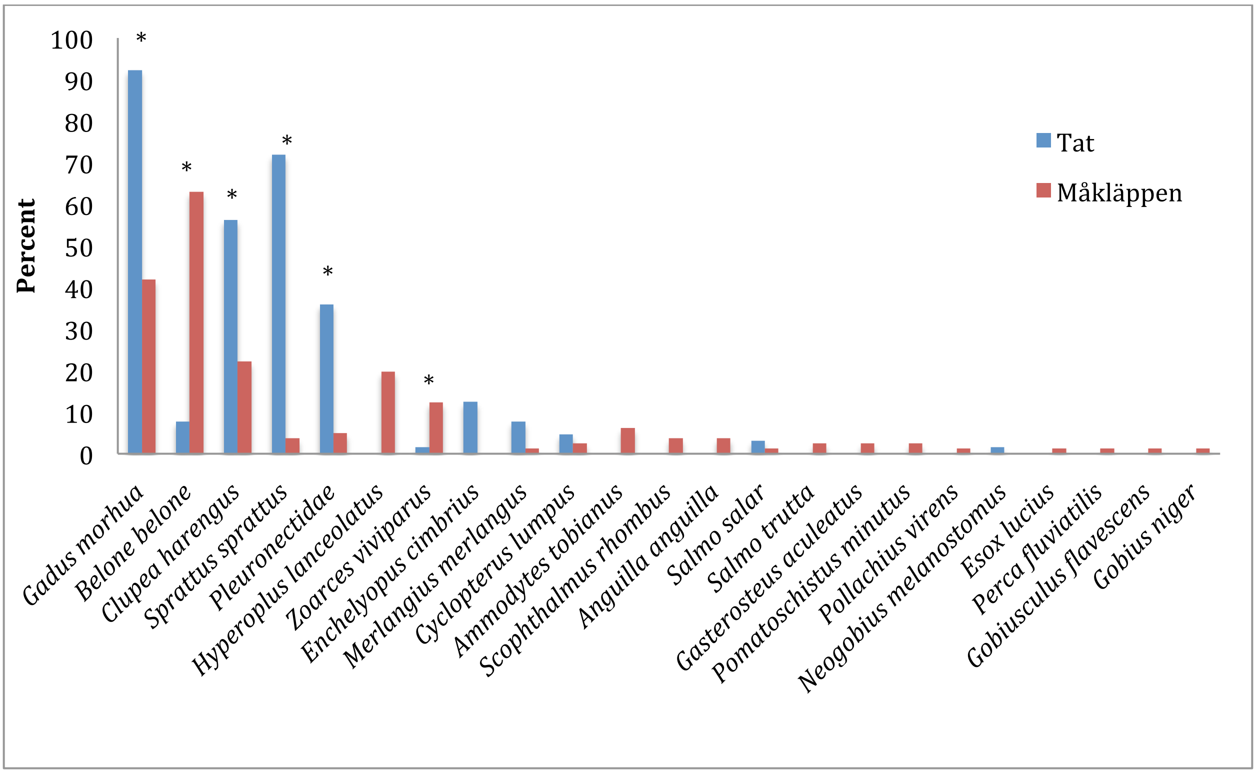

Figure 7 – A total of 23 fish taxa were found in a total of 145 samples. 21 of these taxa were found from the samples from Måkläppen and 11 from Tat. The data is calculated as the Frequency of Occurrence (FO_i) of each fish species. Significant difference was found for all species contributing with more than 5 % FO_i to the overall diet (marked with a star).

Table 3 – Distribution of prey species across years and locations. The data is Frequency of Occurrence calculated by dividing the number of scats with the fish species present with the total number of scats from that season.

	Måkläppen						**Tat**					
	2014			**2015**			**2015**				**2016**	
	Spring	**Summer**	**Autumn**	**Spring**	**Summer**	**Total FO**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Total FO**
Number of samples	24	5	9	42	1	**81**	4	13	17	21	9	**64**
Gadus morhua	41.67	80.00	88.89	28.57		**41.98**	100.00	92.31	94.12	85.71	100.00	**92.19**
Belone belone	66.67		22.22	78.57		**62.96**		15.38	5.88	9.52		**7.81**
Clupea harengus	16.67	20.00	22.22	26.19		**22.22**	75.00	61.54	64.71	38.10	66.67	**56.25**
Sprattus sprattus				7.14		**3.70**	75.00	76.92	82.35	52.38	88.89	**71.88**
Pleuronectidae	8.33		11.11		100.00	**4.94**		7.69	23.53	66.67	44.44	**35.94**
Hyperoplus lanceolatus	33.33	20.00		16.67		**19.75**						
Zoarces viviparus	12.50	40.00	11.11	9.52		**12.35**				4.76		**1.56**
Enchelyopus cimbrius							25.00		29.41	4.76	11.11	**12.50**
Merlangius merlangus				2.38		**1.23**			17.65	9.52		**7.81**
Cyclopterus lumpus	4.17			2.38		**2.47**			5.88	9.52		**4.69**
Ammodytes tobianus	12.50			4.76		**6.17**						
Scophthalmus rhombus	8.33		11.11			**3.70**						
Anguilla anguilla		20.00	11.11	2.38		**3.70**						
Salmo salar	4.17					**1.23**			5.88	4.76		**3.13**
Salmo trutta			11.11	2.38		**2.47**						
Gasterosteus aculeatus	4.17			2.38		**2.47**						
Pomatoschistus minutus	8.33					**2.47**						
Pollachius virens	4.17					**1.23**						
Neogobius melanostomus										4.76		**1.56**
Esox lucius				2.38		**1.23**						
Perca fluviatilis				2.38		**1.23**						

Seasonal variations

To estimate the variation between seasons, the FO_i was calculated for the seasons available for each location. The season with the largest number of species found was at Måkläppen in spring were all 20 species and 1 family were consumed. The seasons with the fewest species present was spring and summer at Tat where only 5 fish species were consumed. The maximum species consumed by one seal was 5 and the minimum was 1 species.

Table 4 – Number of fish species found in the different seasons are shown in the first row. The second row displays average number of species consumed by the seals. The third row shows the maximum number of species consumed by an individual seal. The minimum number of species consumed was 1. The sequences are the total number of reads calculated for each season and location. The maximum number of sequences present in a sample is shown in the last row.

	Species	Average/seal	Max/seal	Sequences	Average/seal	Max/seal
Tat	11	2.95	5	501378	15427.02	29160
Winter	11	2.9	5	96406	8764.18	17994
Spring	5	3	4	55375	7910.71	16670
Summer	5	2.54	4	140049	20007.00	29160
Autumn	9	3.29	5	209548	23283.11	21842
Måkläppen	21	1.99	5	287588	3550.47	25141
Spring	21	2.03	5	270637	4100.56	25141
Summer	6	1.67	3	8305	2372.86	3813
Autumn	8	1.89	4	8646	1729.20	2522

At Måkläppen the most common prey was garfish with an overall FO_i of 63 %. It is largely because of the dominance in spring diet where garfish contribute with a FO_i of 75 %. Cod contributes with 33 % in spring but increases to 67 % in summer and 89 % in autumn (Figure 8). Herring consumption is relatively similar over the seasons with a FO_i of about 20%. The consumption of eelpout (*Zoarces viviparous*) increases in summer with a FO_i of 33 % and contributes with about 10 % in spring and autumn. Great sand eel (*Hyperoplus lanceolatus*) is as important as herring in spring and summer, but does not occur in the autumn diet. The lesser sand eel (*Ammodytes tobianus*) occurs only in spring with a FO_i of 8 %. Lastly Pleuronectidae is consumed all seasons, but with the highest occurrences in summer with a FO_i of 17 %.

There was no significant difference between cod in spring and summer (P = 0.18), and summer and autumn (P = 0.53), but a significant difference between spring and autumn (P = 0.002). For garfish there was no difference between summer and autumn, but a significant difference between both spring and summer (P = 0.0007) and spring and autumn (P = 0.0038). There was no difference between the seasons when testing herring, eelpout and great sand eel (Supplementary Table 4).

At Tat, cod was consumed all year with a maximum of 100 % FO_i in spring and a minimum of 86 % in winter (Figure 9). Sprat and herring were also consumed all year round, with a FO_i of about 80 % and 65 % respectively, but with a decrease in winter. Pleuronectidae was the second most dominant prey in winter with a FO_i of 67 %. The fourbeard rockling (*Enchelyopus cimbrius*) was most important in autumn where it increased to a FO of 29 %. Garfish is of minor importance most of the year, with no occurrences in spring and a FO of 15 % in summer. There was no significant difference between seasons for cod, sprat, herring or fourbeard rockling (Supplementary Table 5), but there was a significant difference in the amount of Pleuronectidae consumed between winter and summer (P = 0.001) and winter and autumn (P = 0.01).

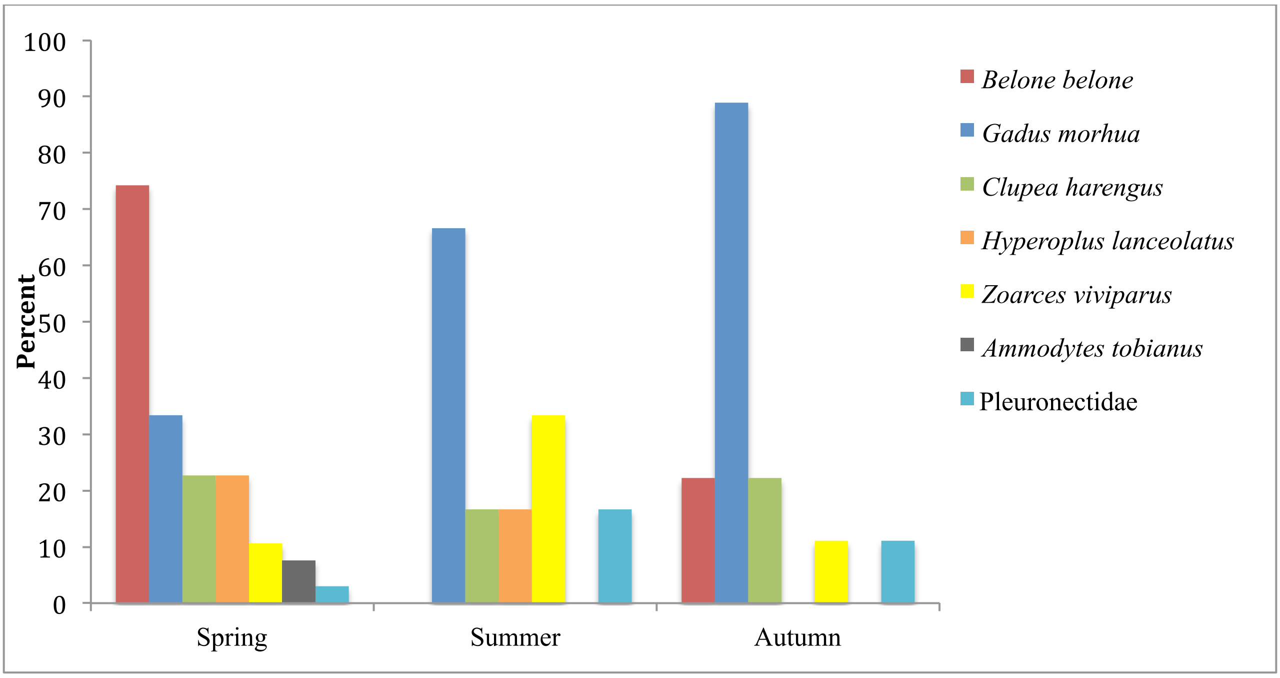

Figure 8 – Seasonal variation of the 7 most common species from Måkläppen (Falsterbo). A total of 81 samples distributed over 3 seasons (spring: 66 samples, summer: 6 samples and autumn: 9 samples). Only the species contributing to the diet with more than 10 % FO_i where tested statistically. Garfish (*Belone belone*) shows a significant difference between spring and summer (P<0.001), and spring and autumn (P=0.004), and cod (*Gadus morhua*) between spring and autumn (P=0.002).

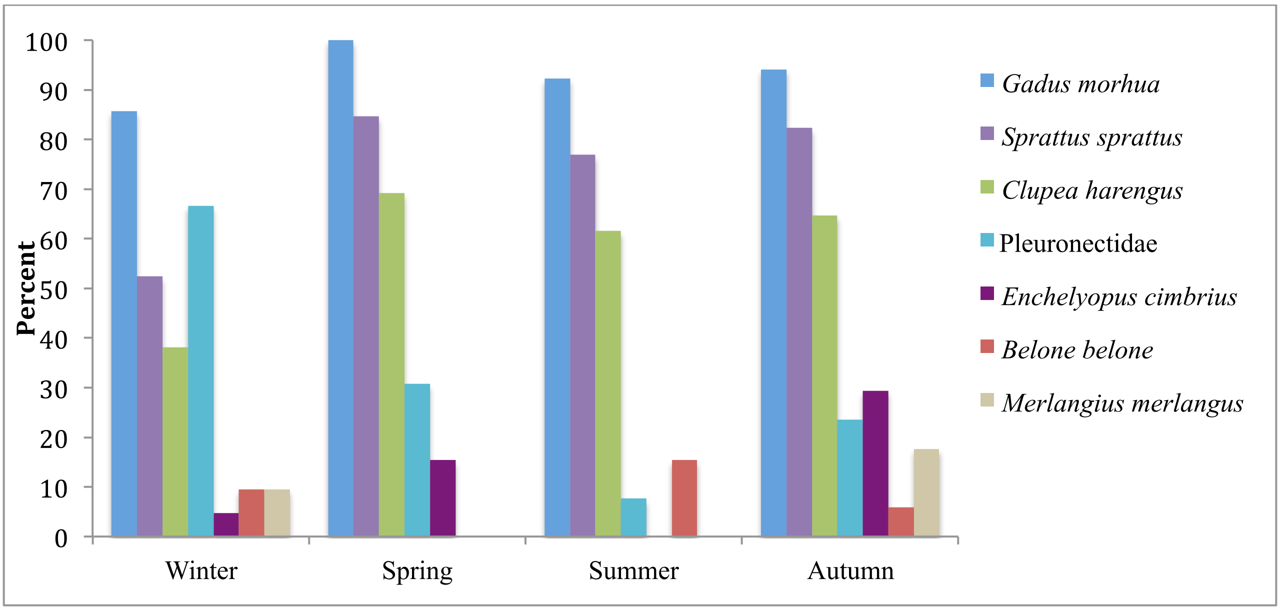

Figure 9 – The seasonal variation of the 7 most common species from Tat. A total number of 64 samples distributed over 4 seasons (winter: 21 samples, spring: 13 samples, summer: 13 samples and autumn: 17 samples). Only species contributing with more than 10% FO_i were tested statistically. No significant difference was found between any of the species, except Pleuronectidae that showed a significant difference between winter and summer (P=0.001) and winter and autumn (P=0.01).

Yearly variation

The collection of samples used in this study took place over a little more than a year, so the samples from two springs were available. At Måkläppen a relatively large number of samples were collected each spring, making it possible to compare the two years. The 6 most dominant species are the same over the two years (Figure 10). Of the remaining 15 species only two are found both years while 13 are different between the two years. No significant difference was found between the two years for any of the 6 most abundant species (P > 0.14) (Supplementary Table 6).

The total number of spring samples was much smaller on Tat with only 4 samples from 2015 and 9 samples from 2016. Because of this small sample size, no comparison was made between years at Tat.

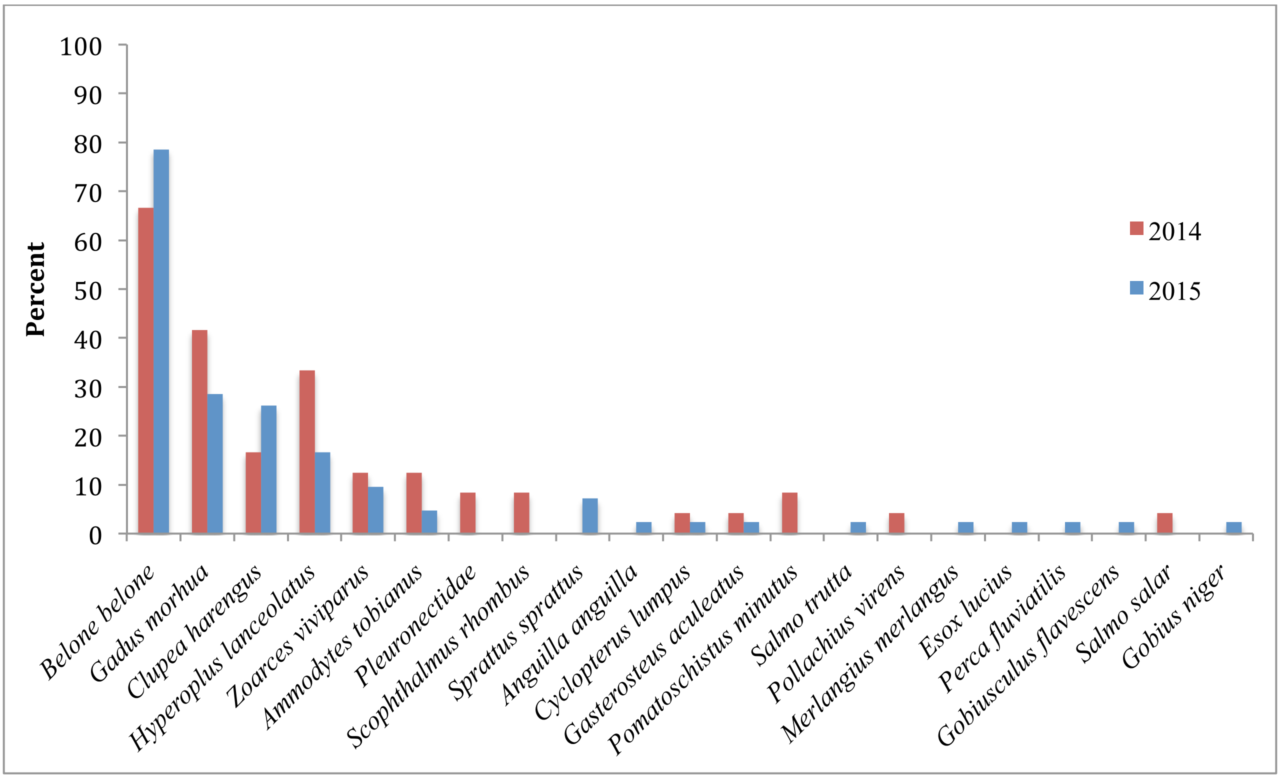

Figure 10 – The variation between the spring of 2014 and 2015 at Måkläppen. A total number of 24 samples from 2014 and 42 samples from 2015 were used. No significant difference was found between the two years for any of the 6 most abundant species.

Available fish species

The data from the Atlas of Marine Fishes of Denmark was used as a measure for the distribution and abundance of the fish species in the Baltic Sea. The Frequency of Occurrence (FO_f) was calculated for the Belts area and the Bornholm area with data from 2000 – 2016 combined, and the most abundant species from the two areas are shown (Figure 11 and 12). In the Belts area, flatfishes were the most common species with a FO_f of 61 %. The second most abundant fish was cod (FO_f = 54 %) followed by trout (FO_f = 43 %), eelpout (FO_f = 40 %) and eel (FO_f = 33 %). In the Bornholm area flatfishes were also the most abundant species with a FO_f of 69 % closely followed by cod (FO_f = 69 %). Turbot (*Scophthalmus maximus*) was the third most abundant species (FO_f = 31 %) followed by herring (FO_f = 31 %), whiting (*Merlangius merlangus*) (FO_f = 29 %) and sprat with a FO_f of 28 %.

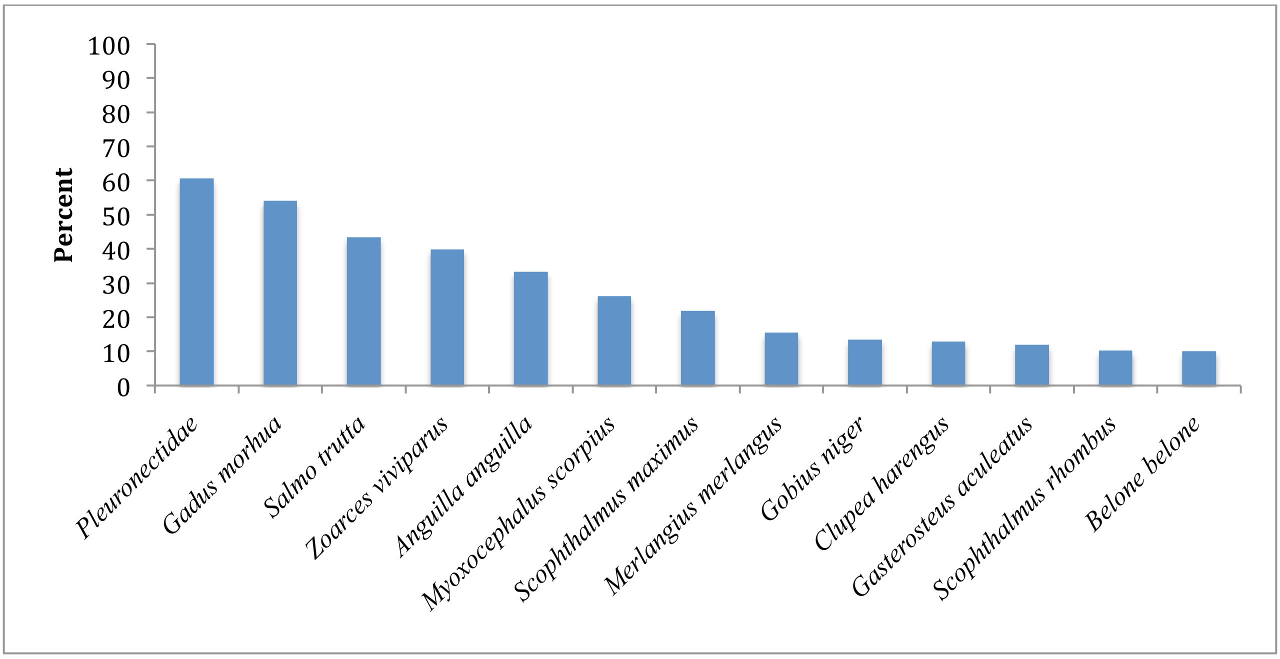

Figure 11 – The Frequency of Occurrence of the fish species in the Belts area as assessed from the Atlas of Marine Fishes of Denmark. Shown on the histogram are only fish species with a FO_f of more than 10 %. Flatfishes (Pleuronectidae) are the most abundant species (FO_f = 60.58 %) followed by cod (*Gadus morhua*) (FO_f = 54 %) and trout (*Salmo trutta*) (FO_f = 43.49 %).

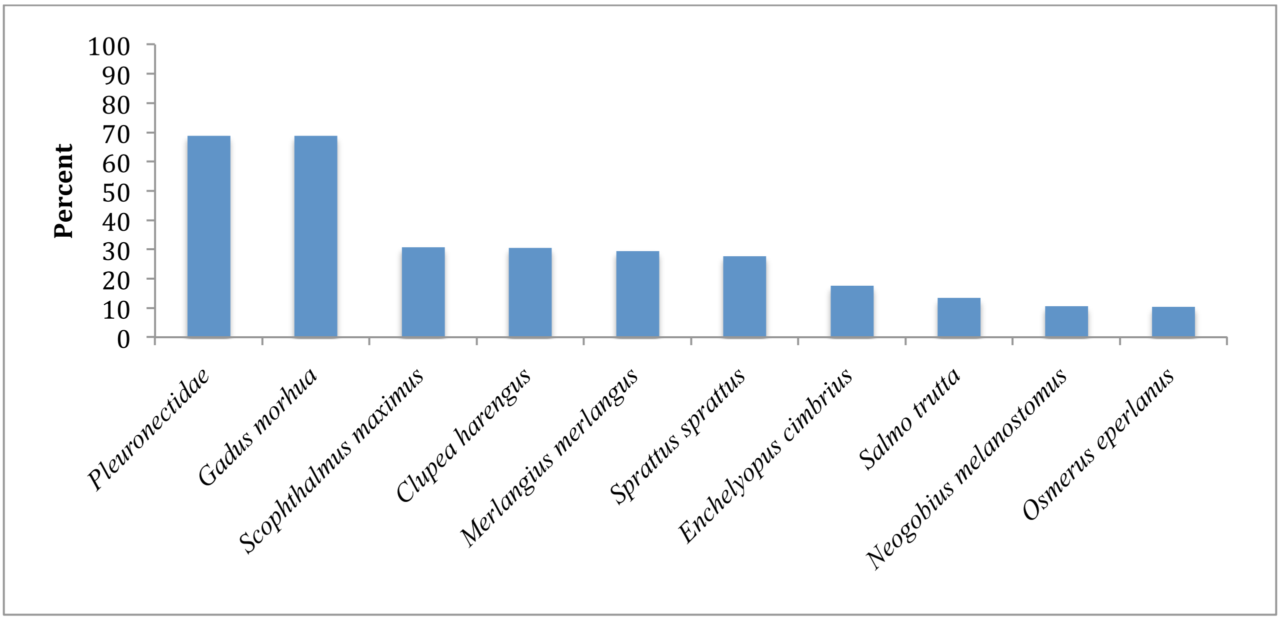

Figure 12 – The Frequency of Occurrence of the fish species in the Bornholm area as assessed from the Atlas of Marine Fishes of Denmark. Shown on the histogram are only species with a FO_f of more than 10 %. Flatfishes are the most abundant species with a FO_f of 68.8 % followed by cod (FO_f = 68.7 %), turbot (*Scophthalmus maximus*) (FO_f = 30.77 %) and herring (FO_f = 30.56 %).

Comparison between fish abundance and seal diet

The seal diet was compared with the available fish species to assess if the most abundant species are also the ones consumed by the seals. The samples from Måkläppen in Sweden were compared with the Belts area data as we assume that this is the seals main foraging area (Figure 13). There was a significant difference between the fish data and the seal diet for garfish, cod, herring, great sand eel, eelpout, flatfishes, eel, three-spined stickleback, trout, whiting, perch and black goby ($P < 0.03$). No significant difference were found between fish data and seal diet for the species lesser sand eel ($P = 0.24$), brill ($P = 0.06$), sprat ($P = 0.38$), lumpsucker ($P = 0.33$), sand goby ($P = 0.09$), saithe ($P = 0.34$), pike ($P = 0.06$), two-spotted goby ($P = 0.09$) and salmon ($P = 1$) (Supplementary Table 7).

The samples from Tat in Denmark were compared with the data from the Bornholm area because we assume that this is the seals main foraging area (Figure 14). A statistical difference was found between the fish data and the seal diet for the species garfish, cod, herring, flatfishes, sprat, whiting and round goby ($P<0.038$). No statistical difference was found between the fish data and the seal diet for the species eelpout ($P = 0.05$), lumpsucker ($P = 0.19$), salmon ($P = 0.77$) and fourbeard rockling ($P = 0.39$) (Supplementary Table 8).

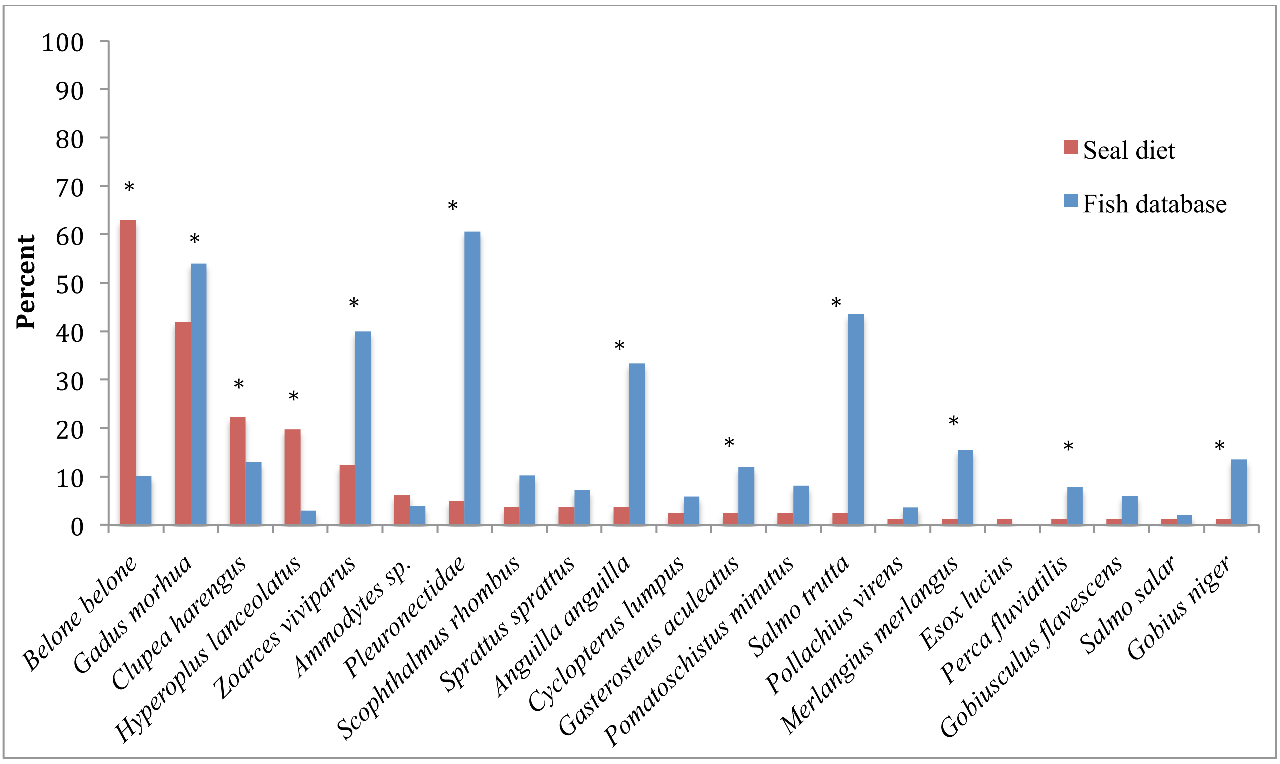

Figure 13 – Comparison of the FO_f of the fish atlas from the Belts and the FO_i of the seal diet from Måkläppen. Significant differences between the two datasets are marked with a star.

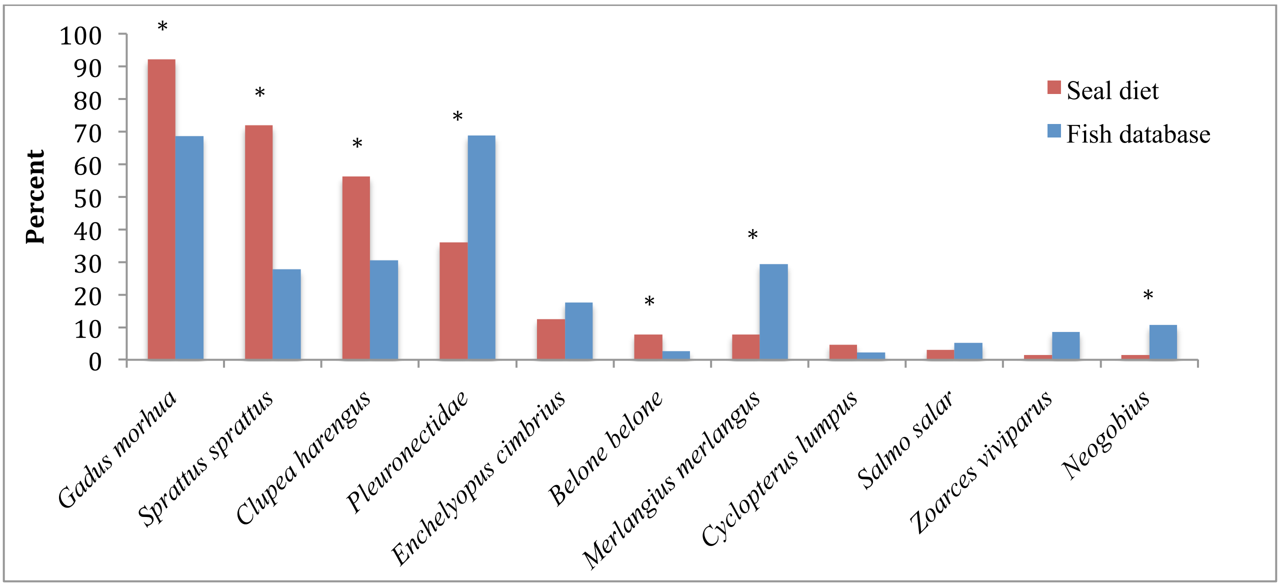

Figure 14 – Comparison of The FO of the fish atlas from Bornholm area and the FO of seal diet on Tat. Significant differences between the two datasets are marked with a star.

Seasonal variation in fish abundance in relation to seal consumption

The fish abundance was calculated for the spring in the Belts in comparison with the seal diet from Måkläppen because of the dominance of garfish (Figure 15). Garfish is still greatly overrepresented in the seal diet with a P value < 0.001. Herring, great sand eel, eelpout, flatfishes, eel, lumpsucker, trout and whiting were also significantly different between the fish data and seal diet (P< 0.04). No significant difference was found in cod (P = 0.53), lesser sand eel (P = 0.59), brill (P = 0.62), sprat (P = 0.62), three-spined stickleback (P = 0.056), sand goby (P = 0.58), saithe (P = 1), pike (P = 0.055), perch (P = 0.18), two-spotted goby (P = 0.71), salmon (P = 1) and black goby (P = 0.055) (Supplementary Table 9). Summer and autumn were not compared between seal diet and fish availability because of the small sample sizes from the seal diet these seasons.

The fish abundance was also calculated for the Bornholm area compared with the seal diet from Tat. In the winter period (Figure 16) no significant difference was found between the fish data and seal diet in cod (P = 0.21), herring (P = 0.08), fourbeard rockling (P = 0.77), whiting (P = 0.12), lumpsucker (P = 0.25) and salmon (P = 0.19). Sprat and flatfishes showed a significant difference between the two datasets (P < 0.001) and garfish as this species only was found in the seal diet (Supplementary Table 10). In spring (Figure 17) there was found significant difference between cod (P = 0.024), sprat (P = 0.0005), herring (P = 0.021) and flatfishes (P = 0.012). No significant difference was found between the seal diet and the fish database for fourbeard rockling (P = 0.53)

(Supplementary table 11). In summer (Figure 18) there was a significant difference between cod (P = 0.0007), sprat (P < 0.00001), herring (P = 0.00002) and flatfishes (P = 0.0002) and no significant difference was found between the fish database and the seal diet for garfish (P = 0.136) (Supplementary Table 12). In the autumn (Figure 19) significant differences were found between sprat (P = 0.0008) and flatfishes (P = 0.0001) but the remaining species consumed by the seals did not show any difference from the fish data available (Supplementary Table 13).

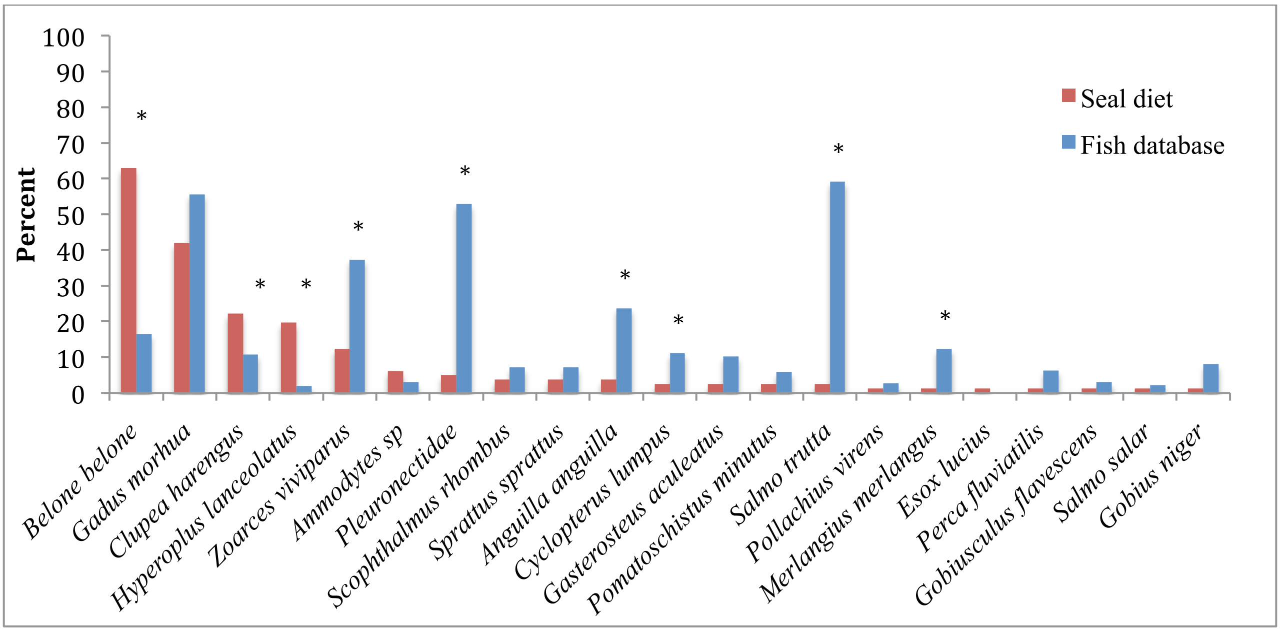

Figure 15 – Comparison of The FO of the fish atlas from the Belts area and the FO of seal diet on Måkläppen in spring. The significant differences between the two datasets are marked with a star.

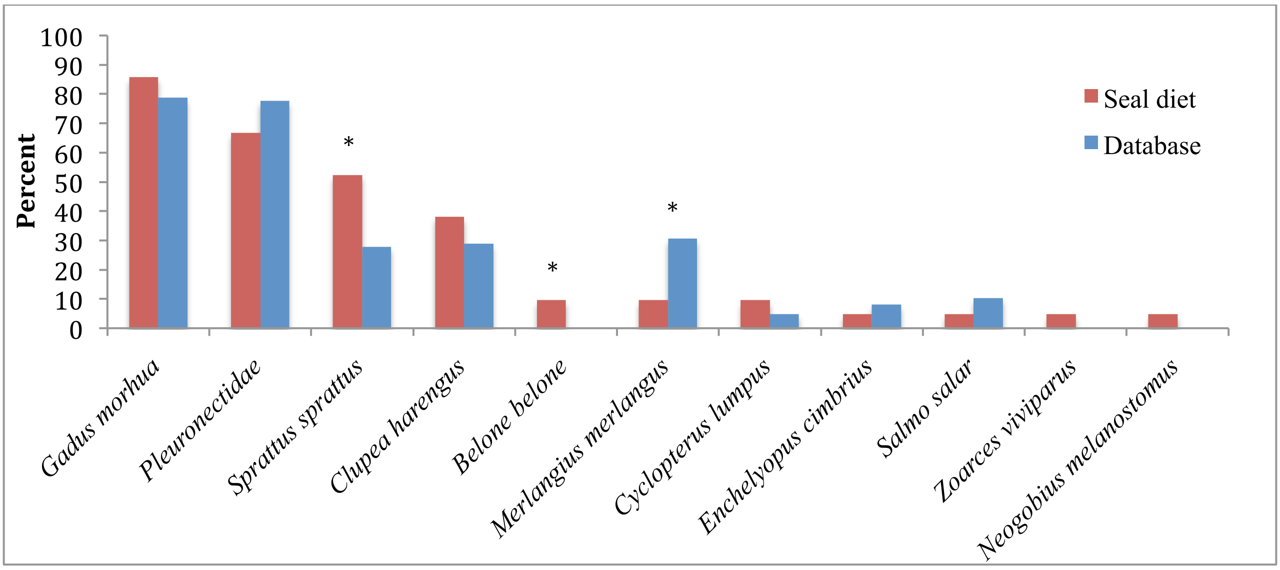

Figure 16 – Comparison of The FO of the fish atlas from Bornholm area and the FO of seal diet on Tat in winter. The significant differences between the two datasets are marked with a star.

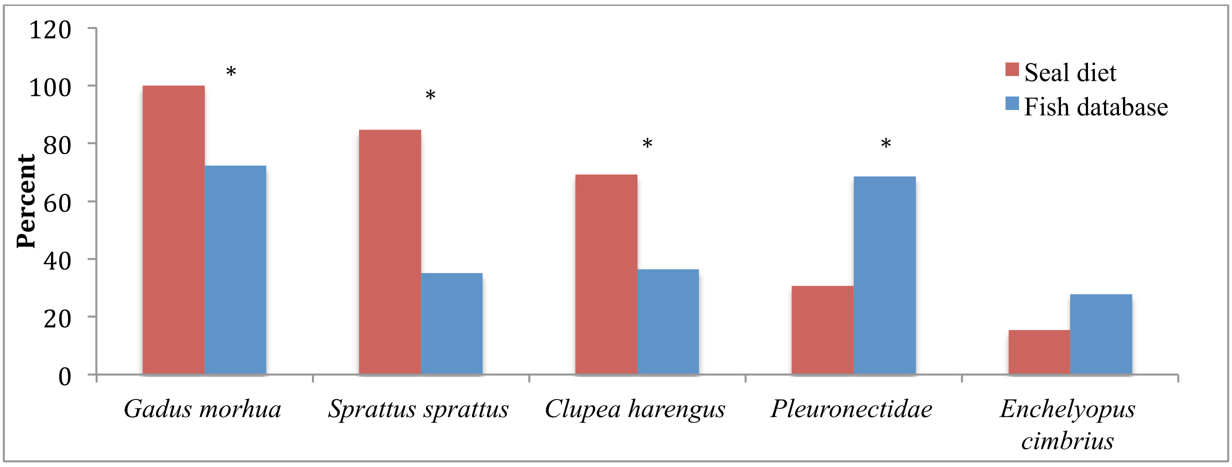

Figure 17 – Comparison of The FO of the fish atlas from Bornholm area and the FO of seal diet on Tat in spring. The significant differences between the two datasets are marked with a star.

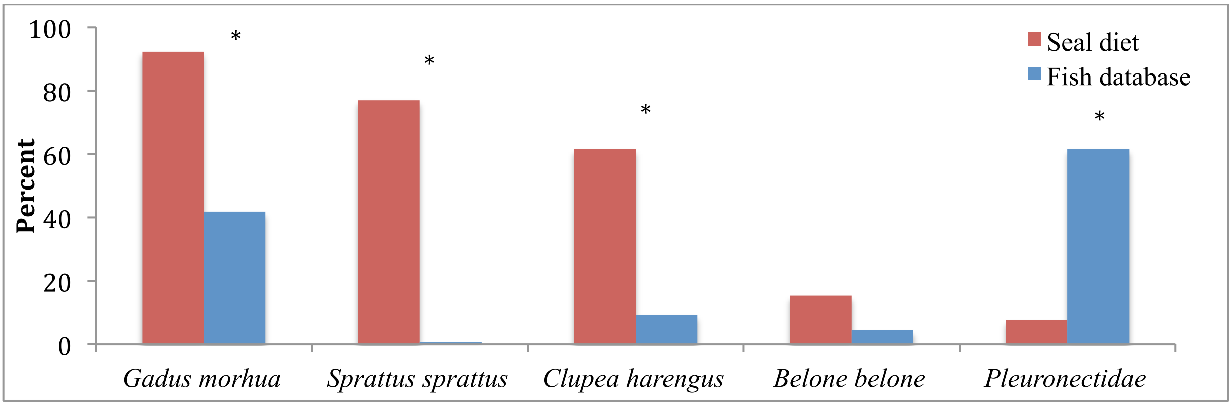

Figure 18 – Comparison of The FO of the fish atlas from Bornholm area and the FO of seal diet on Tat in summer. The significant differences between the two datasets are marked with a star.

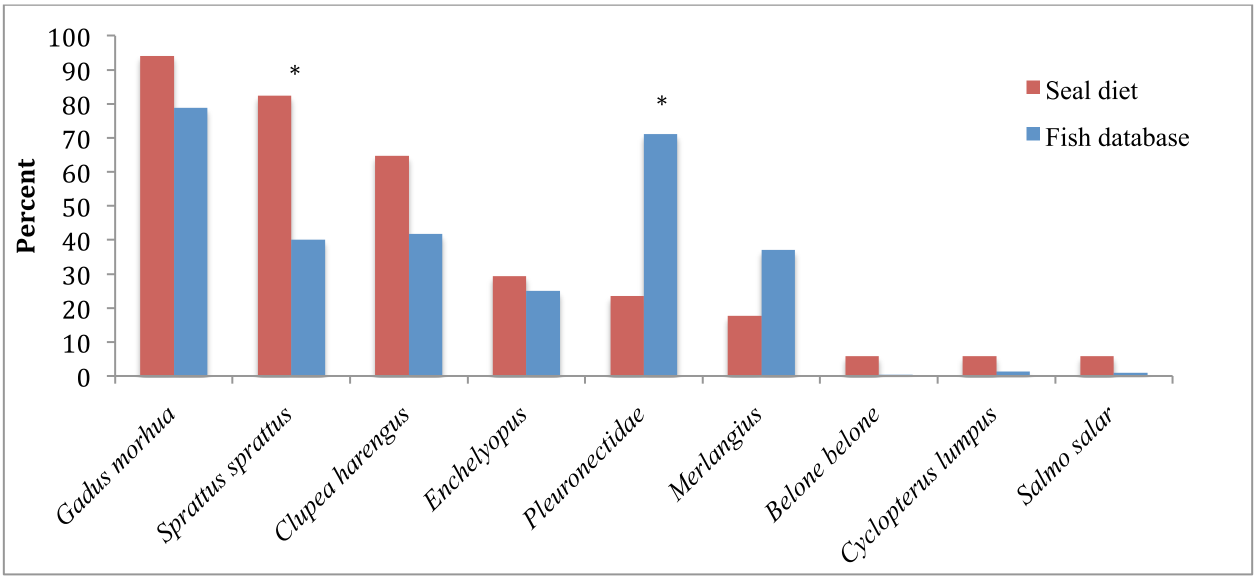

Figure 19 – Comparison of The FO of the fish atlas from Bornholm area and the FO of seal diet on Tat in autumn. The significant differences between the two datasets are marked with a star.

Landings data

Data from the landings in 2012 – 2015 was extracted from the Danish National landings database to determine the most important fish species targeted by the fisheries. The data was divided into landings on the eastern coast of Zealand and landings on Bornholm. The average of each species per year for the 4 years was calculated (Figure 20 and 21). At the eastern coast of Zealand, herring contributed with the largest biomass of 2581.7 tons and a value of 955 381 DKK. Cod contributed with 2506.5 tons and a much larger value of 25 741 096 DKK. Sprat represented a biomass of 556.7 tons and a value of 772 233 DKK. Flatfishes contributed with 443.4 tons and have a value of 10 412 600 DKK and garfish contributed with 120.2 tons and a value of 983 968 DKK.

The data from Bornholm (Figure 22 and 23) had cod as the most important species with a biomass of 6214.4 tons and a value of 40 564 015 DKK. Sprat contributes with a biomass of 2735.6 tons and a value of 5 305 640 DKK and flatfishes represents a biomass of 516.1 tons and a value of 2 092 362 DKK.

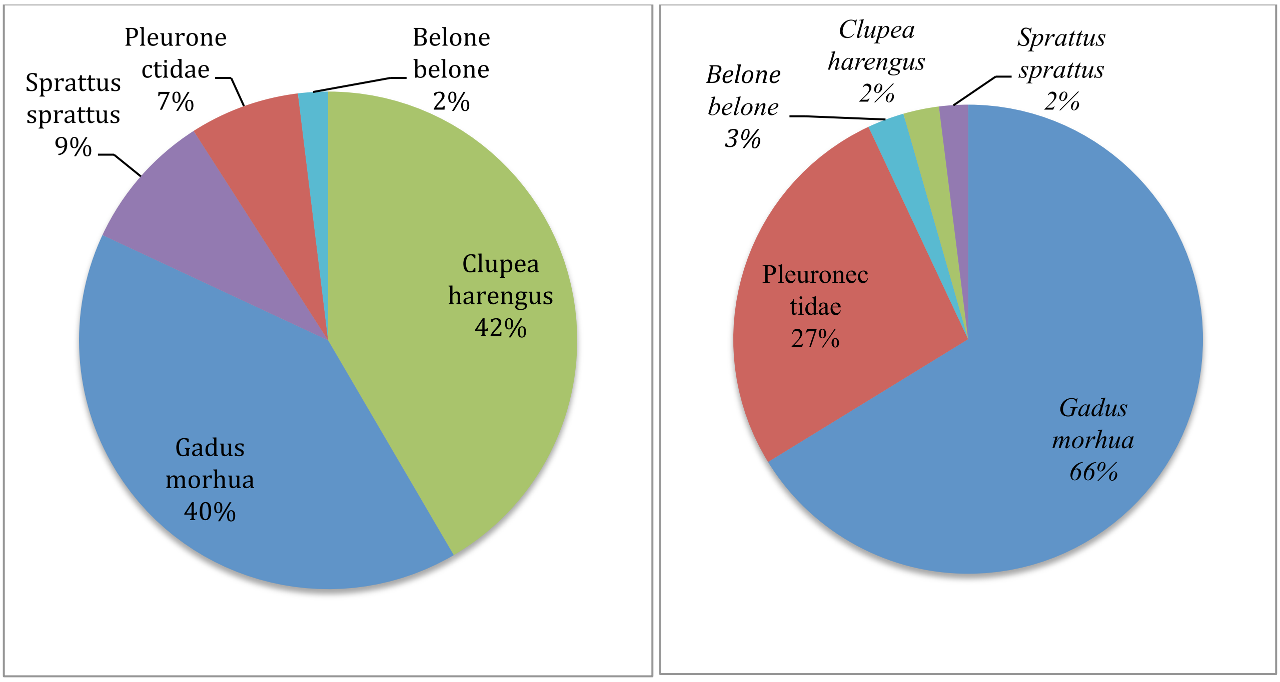

Figure 20 and 21 – The most landed species in the Belts area according to biomass (left) and value (right). Herring contributes with 2581.7 tons, cod with 2506.5 tons, sprat with 556.7 tons, flatfishes with 443.4 tons and garfish with 120.2 tons. Cod contributes with a value of 25 741 096 DKK, flatfishes with 10 412 600 DKK, garfish with 983 968 DKK, herring with 955 381 and sprat with 772 233 DKK

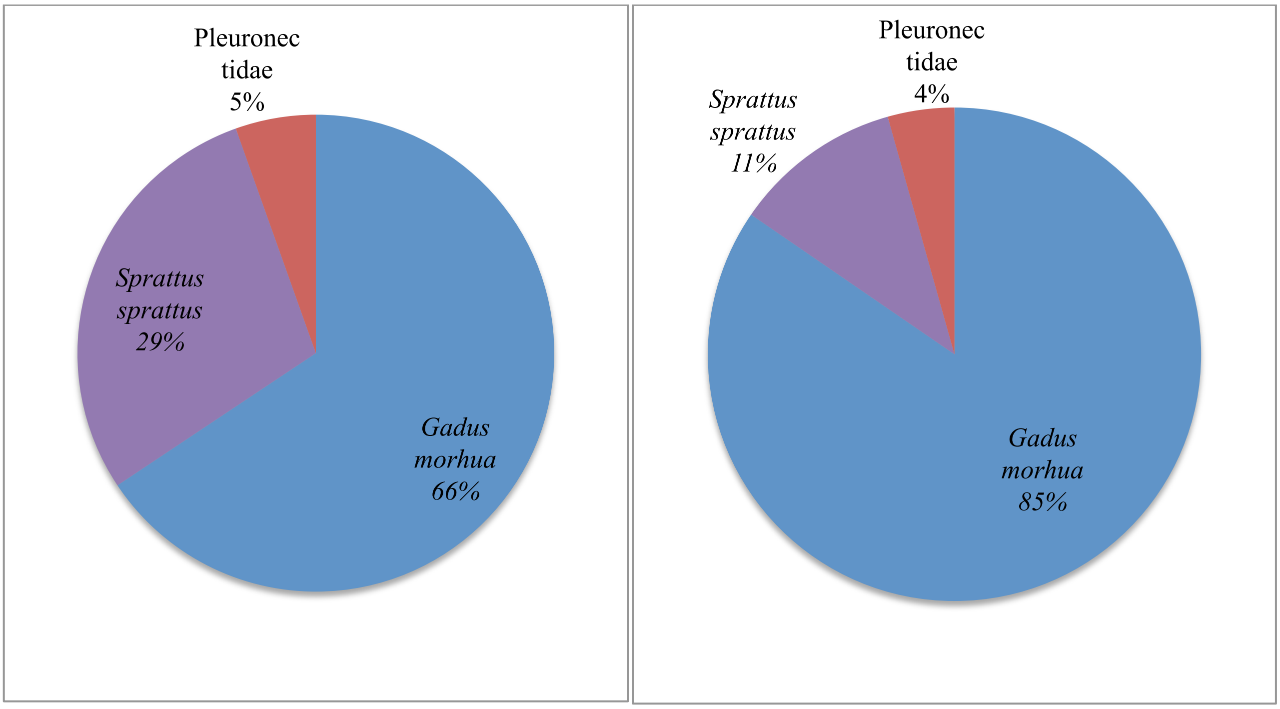

Figure 22 and 23 – The most landed species in the Bornholm area according to biomass (left) and value (right). Cod contributes with a biomass of 6214.4 tons, sprat with 2735.6 tons and flatfishes with 516.1 tons. Cod contributes with a value of 40 564 015 DKK, sprat with 5 305 640 DKK and flatfishes with 2 092 362 DKK.

Quantification of prey

The Frequency of Occurrence of the number of samples containing the species (FO_i) was correlated with the Frequency of Occurrence of number of sequences for each species (FO_s). Only cod showed a relative similarity in the two FOs with a difference of 10 %. The FOs underestimated garfish, which was half the value of the FO_i. Sprat and herring were both very underrepresented in the comparison and their FO_s were less than a third of the FO_i. The Frequency of Occurrences did not match with the rank of species, as herring contributed with fewer sequences than sprat while the FO_i was higher for herring. The same is the case with the fewer sequences for flatfishes compared to sand eel.

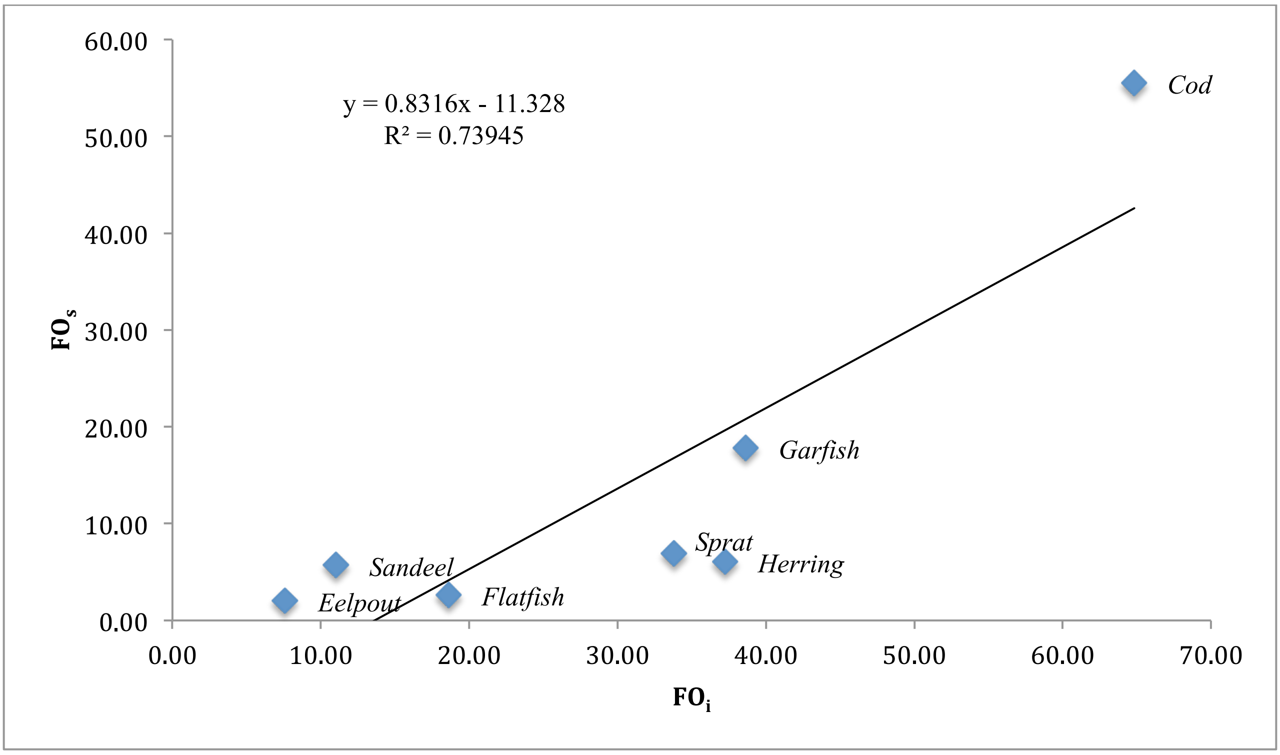

Figure 24 – Correlation between the frequencies of occurrences of number of samples containing the species (FO_i) versus the number of sequences of the species (FO_s). The 7 most correlated species are included. Cod shows a fairly good similarity with only 10 % difference between the frequencies. The rest of the species does not really match the Frequency of Occurrence of the number of sequences.

Discussion

The diet of grey seals

Overall findings

The overall findings in this study demonstrate a relatively varying diet of grey seals in the Southern Baltic Sea with 23 different taxa consumed. As expected the most common prey species was cod, most likely because it is an abundant fish in the area around Bornholm. The second most common species was garfish, which was surprising as they are not a dominant species in the Baltic Sea. The species fourbeard rockling, saithe and brill has to our knowledge not been found in the diet of grey seals from the Baltic before (Pittman et al. in prep.; Scharff-Olsen in prep.). Garfish has not been found to be a big part of the diet in previous studies either (Lundström et al., 2010, 2007).

Location differences

The seals from Måkläppen tended towards a more diverse diet with 20 species and one family compared to 10 species and one family consumed by the seals on Tat. The comparison between the two locations showed a significant difference in all the species with a FO of more than 10% (Supplementary Table 3). This suggests that the seals from the two locations consume different prey and therefore most likely also forages in different areas. The most noticeable difference is the much larger share of garfish in the diet from Måkläppen compared to a minor importance on Tat. A reason for this difference is most likely the distribution of samples across the year, where Tat has a much more even distribution compared to Måkläppen where 80% of the samples were collected during spring. This makes the data from Måkläppen biased because the summer and autumn samples were relatively few.

The variation between locations may be influenced by site-specific differences. At Tat the seals seem to come in mostly from north (wildlifetracking.dk) and forage around Bornholm where cod is a common species. It could also be expected that the seals migrate to the Bornholm area because of the high abundance of cod and therefore use Tat as a resting place. Måkläppen is a commonly used haul-out site and seals might tend to be more resident. The greater amount of garfish in their diet suggests that they forage closer to Øresund and in the Western Baltic than the seals from Tat. Grey seals from Måkläppen and Rødsand were tagged in a study by (Galatius et al. in prep) and showed a concentration in seal activity in the area between Denmark and Sweden. They did not swim north of Amager but had a distribution from Falster to Sweden with less activity closer to Bornholm. This suggests that the seals from Måkläppen and the seals from Tat do forage in different areas, which reflect the difference in species found in their diets.

Seasonal variation

The seasonal variation gives an indication of the migration and abundance of the fish species in the areas where the seals are foraging. Grey seals have been reported to consume locally and seasonally abundant prey and taking advantage of the available species, but some studies have found no variation between seasons when accounting for all factors (Lundström et al., 2010; Hammond et al., 1994; Brown et al., 2012). Differences have also been found between males and females between seasons, which can be explained by the larger energy requirements for females in the pupping season (Beck et al., 2007).

At Måkläppen the seals consumed significantly more garfish in the spring than in summer and autumn. This correlates with the garfish migration into Øresund in the spring. The seals as opportunistic feeders are apparent as they switch to a diet with a much larger share of garfish when these become more abundant in the area. It also suggests that garfish are relatively easy for the seals to catch. The seals at Måkläppen consume more cod in the autumn than in the spring, which suggests a lesser abundance of garfish in the autumn and a relatively stable occurrence of cod during the seasons.

The seals at Tat consume fewer species than the ones from Måkläppen. There is no significant difference in the seasonal variation except for the flatfishes, which are consumed in larger amounts in winter. This suggests a shift in the availability of the species during winter, or the flatfishes might be easier to catch, while cod and herring becomes less available. It is also possible that seals target flatfishes more in the winter because this is their spawning season (Pers. Comm. Peter Rask Møller). Spawning fish might be easier to catch and are also more energy rich when they are filled with eggs. The fourbeard rockling is most common in the diet in spring and autumn while the whiting is only present in autumn and winter samples. This also suggests a shift in the availability of these species over the course of the year.

Yearly variation

The collection of samples took place over more than a year, so spring samples were available for two years and therefore a comparison was performed. At Måkläppen an overrepresentation of samples was collected in spring and a relatively large sample size was available for each year. There was no significant difference between any of the most common species found in the two years, which is to be expected. Other studies have found no differences between years and it is more likely to find a difference between decades than years (Lundström et al., 2010; Berg et al., 2002; Härkönen, 1987). At Tat the sample sizes for the two years were very small and therefore no comparison was included.

Comparison between fish abundance and seal diet

As can be seen on figure 11 and 12, the most dominant species in the two areas are quite different. In the Belts area the most abundant species was the European flounder (*Platichthys flesus*) and because of the inability to differentiate the 16S gene in the Pleuronectidae family to species, the three species flounder, plaice (*Pleuronectes platessa*) and common dab (*Limanda limanda*) were combined. Of the most common species in the Belts area, only the species *Myoxocephalus scorpius* and *Scophthalmus maximus* did not occur in the seal diet. The most consumed species was garfish, which is not very common in the area, suggesting that the seals target this type of prey when they are migrating in the spring. Cod is significantly more common in the area than in the seal diet, which could be because of the large amount of garfish consumed in the spring. Because of the overrepresentation of spring samples from Måkläppen it is not possible to conclude anything about the diet over the year. The seals consumed significantly more herring and great sand eel than found in the area. The most significant deviations in the compared FOs are eelpout, flatfishes, eel, trout and black goby, which were very common in the area, but contributed relatively little to the seal diet. 12 of the fish species showed a significant difference between the fish abundance and the seal diet. Only one species found in the seal diet was not found in the belts area – the pike, which is a freshwater fish, and therefore the seal that consumed this species was probably close to a river or an estuary. It seems that the seals do not target flatfishes, trout and eel as much as would be expected because of their abundance in the area, and this is beneficial for the fisheries as these are valuable species.

The most abundant fish species from the Bornholm area were the Pleuronectidae family, cod, turbot (*Scophthalmus maximus*), herring, whiting, sprat and fourbeard rockling. All these species except turbot were also found in the seal diet. 7 species show significant differences between the seal diet and the fish abundance in the area, especially cod, sprat and herring are targeted by seals compared to their abundance in the area. Pleuronectidae and whiting have a significantly higher abundance in the area than the FO_i of the seal diet. Garfish has a very low abundance in the area, but are significantly more common in the seal diet. Lastly the invasive round goby occurs significantly more in the area than in the seal diet. It would be very beneficial if the seals targeted this species because it is invasive, and it is possible that as it becomes more common, the encounter rate will grow and therefore more consumption will occur. The Pleuronectidae are more common in the area than in the seal diet and this suggests that they are not targeted as much by seals as cod, sprat and herring.

The way the data from the Atlas of Marine fishes of Denmark was calculated did not take into account the number of fish of the species that was caught in a single event. Therefore fish from trawling or fish caught in large amounts in a single catch might be underrepresented. Reports from recreational fishermen are also available in the database and this might be a reason why trout occurs on such a large part of the fishing events.

Seasonal difference between fish abundance and seal diet

The samples from Måkläppen were extremely biased towards spring as about 80 % were collected during this season. Therefore the fish data FO_f was calculated for spring to make it possible to more accurately compare the data. The most common species in the area were trout with a FO_f of 59.1 %, cod with 55.6 % and Pleuronectidae with 52.9 %. Garfish increased in abundance to 16.6 %, but was still significantly lower than the occurrence in the seal diet. Cod did not show a significant difference in the comparison, but otherwise there were no major differences from the overall data.

The Bornholm area was divided into all four seasons as the Tat samples were relatively evenly distributed over the year. The seasons were compared between the seal diet and the fish atlas because all contained a relatively large number of samples. The most common species in the area during winter was cod with a FO_f of 78.7 % closely followed by Pleuronectidae with 77.6 %. Turbot, whiting, herring and sprat were the next in line with turbot being the only species not found in the seal diet. Cod, flatfishes and herring showed no significant difference between the seal diet and the fish database in contrast to the overall data findings. Sprat, garfish and whiting were the only species showing a significant difference between the datasets, which is 4 less than in the overall findings for Bornholm. Garfish, eelpout and round goby were consumed by the seals but did not occur in the fish database in winter. In spring the seals consumed significantly more cod, sprat and herring than were available in the fish database. Significantly more flatfishes were available in the area, but were not consumed by the seals. Fourbeard rockling did not show a significant difference between the seal diet and the fish database. The third most common species found in the fish database in spring was whiting, but this species did not occur in the seal diet. In summer cod, sprat, herring and flatfishes varied significantly across the fish database and the seal diet, yet no difference was found for garfish. Much less cod, sprat and herring were available this season, but the seals seemed to target them largely while not consuming the much more abundant flatfishes. In autumn only sprat and flatfishes were significantly different between the fish database and the seal diet. The seals consumed a larger amount of sprat than was available in the area, and less flatfishes. Otherwise this season shows a fairly similar picture between the seal diet and the fish database, which suggests that the seals target the fish species that are common in the area. No whiting or turbot is consumed, even though they are relatively abundant in this season.

It thus seems that in winter and autumn the seals consume more of the available species than they do in spring and summer. This could be because of a change in the availability of the species, as some demersal fish overwinter and some might migrate to other areas during autumn and winter.

The molecular diet determination method

Sample quality

Of the 248 samples in all, only 145 succeeded in yielding DNA enough to go through all the steps leading up to the data analysis. Of these, 64 of 85 came from Tat and 81 of 175 from Måkläppen. It is a large number that did not work and significantly more from Måkläppen where about half of the samples did not work. This means that new and better methods will be useful, as large amounts of work is spent on samples that end up not providing a usable result. A reason that more than half of the samples from Måkläppen were unsuccessful could be due to the time period in which they were exposed to degradation on the beach; thus most of the samples used in this study were further noted as days to weeks old. Additionally, samples from Måkläppen contained high amounts of sand. Some samples were almost entirely made up of sand and should probably be left out in future studies. Of the 52 samples noted as "sandy" only 21 yielded high enough DNA concentration to be included in the results. At Tat the scats do not lie exposed for as long, because the tide floods the skerry regularly and therefore it is only fresh scats that remains for collection. This might be the reason for a larger per cent of samples from Tat working compared to the samples from Måkläppen.

DNA extraction

For the DNA extraction the ThermoScientific Kingfisher robot was used because it yielded good quality DNA and had a relatively fast extraction time. Half an hour was needed for 12 samples and while the robot was running it was possible to prepare the next 12 samples. Using a robot might also make contamination less likely because of less pipetting and therefore fewer risks of mistakes. To compare the robot with the more commonly used QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA) 5 samples were extracted with both methods. On the electrophoresis gel the samples extracted using QIAamp showed weaker bonds than the samples extracted on the robot. Potentially, this might be caused by a larger dilution in the QIAamp manual, yet the robot was considered to be evenly good or better than the Stool kit and also time effective.

This study shows a high taxonomic resolution where only one family was impossible to identify to species. The database used for alignment of the sequences was useful and could be used for future studies of fish species from the Southern Baltic Sea. Three species were added (perch, pike and mackerel) so the reference database should cover most seal diet relevant species found in the Baltic Sea.

Regarding the DNA extraction only one sample was taken from each scat instead of subsamples. Studies have reported that the distribution of DNA within a scat was not distributed evenly, but can occur in pulses according to the prey consumed by the seal (Deagle et al., 2005). Other studies have shown a relatively homogenous distribution, so subsamples were considered unnecessary (Matejusová et al. 2008; Pittman et al. in prep.).

Bias, errors and uncertainty

The metabarcoding method is good for this type of study, as it gives a good picture of the species, but a large sample size is needed, as many samples must be expected to fail in yielding high quality DNA. It is unlikely that any species were overlooked as they were both aligned to the database and run in BLASTn in Genbank. Biases might have occurred if the primers did not work equally well on every type of fish or if the DNA of some fish species are degraded faster than others. The primers successfully targeted the 16S mitochondrial DNA gene in fish, but the gene is identical in the family Pleuronectidae and it was thus not possible to differentiate to species of flatfishes.

One control (K7) contained 5 sequences of Pleuronectidae, which means it has been contaminated at one of the steps in the extraction process. The samples run in the same group as the K7 control did not contain any Pleuronectidae sequences, so it was considered unlikely that the samples were contaminated. Precautions were made based on this error though, so the limit for the presence or absence of a species was set to 10 sequences. It is unlikely that any of the other samples were contaminated as most of them contained many more sequences than 10.

Frequency of occurrence was used for the data analysis and this is useful as a relative measure of the importance of prey species, but it is not very useful if the quantification of prey biomass is the purpose of the study. The importance of minor prey items is often exaggerated in occurrence studies and small numbers of contamination or secondary predation can have a large effect on the diet estimates (Thomas et al., 2014). A combination with another dietary analysis method could be useful in minimizing the biases for each method.

Secondary predation

The diet of the prey can be observed amongst that of the predator's, which is a problem both with morphological and molecular methods. Some prey can originate from the prey of the consumed fish and should therefore not count as the seals prey. Cod is a predator and because such a large amount of the diet consists of this species it is possible that cod rather than the seal consume some of the smaller fish (sprat, herring, sticklebacks, gobies, sand eels etc.). Garfish is also a predator and the same small fishes, especially clupeids are major components in the diet of this species (fishbase.org). It is possible that secondary prey DNA would be more degraded than the primary prey DNA, because of the double exposure through the intestines of the predators, but biases can still occur. Unresolved taxa could also be attributed to secondary consumption because it was too degraded to be recognized to species.

Quantification of prey

Estimating the quantity of the prey species is not easy. It was originally assumed that the number of sequences generated would be an estimation of the biomass consumed, but it has proven to be more complicated than that (Symondson & Harwood, 2014). Even so it is likely that large-bodied or frequently eaten prey items contribute more DNA to the sample than rare species (Deagle, Kirkwood, and Jarman 2009). Some studies have concluded that a semi-quantitative estimate of diet was reasonably accurate (Deagle et al., 2005; Bowles et al., 2011), but others found no relations between per cent mass contribution of different taxa to diet, and FO of the DNA remains in scats (Casper et al., 2007; Deagle & Tollit, 2007). A reason why metabarcoding is difficult to use for quantification of prey in the diet is that prey species may differ in amount of DNA present per unit biomass and tissue digestibility. The amount of mtDNA per gram of tissue varies between fish species and there is a difference in the survival abilities of tissue during the process of digestion (Deagle et al., 2010, 2005; Thomas et al., 2014). It could be possible to develop correction factors for each species based on their DNA survival during digestion and amount of DNA present, but they will not necessarily be sufficient to account for the bias (Deagle & Tollit, 2007). Thomas et al. (2014) developed correction factors for tissue differentiation in harbour seal diet to lessen the bias for the variation in mtDNA densities in prey fish. They found the protein rich mackerel to be overrepresented in the analysis, which suggests that muscle density could be a measure for mtDNA density. Correction factors for digestion bias was also developed, but did not seem to impact the proportional diet estimates as much, even though these have been assumed more important in other studies (Thomas et al., 2014; Deagle et al., 2010). Most of these correction factors are developed for captive animals, because these are the only studies where known diet can be used. To use any of the same factors for wild animals causes problems as it is likely that prey digestibility differ between species and individuals and exercise likely affects the digestive efficiency in the seal (Deagle et al., 2010; Bowen, 2000).

A comparison between the frequency of occurrence (the number of samples containing a certain prey divided by the total number of samples) and the number of sequences per species divided by the total number of sequences were made. If these two frequencies were similar it would be possible to approximately quantify the abundance of the prey species in the seal diet. The only species where the frequencies were relatively close to each other was cod with only 9% difference. The other species did not show the same similarity in frequencies and they did not even have the same ranking. This either means that there is no correlation between the number of sequences and number of occurrences in the samples, or it suggests that cod contributed much more to the diet than any of the other species. It is reasonable that cod contribute more biomass to the diet than the other species, as it is the largest species and therefore may be preferred by the seals as it provides more energy compared to foraging and handling time (the total time taken for a predator to pursue, capture and consume a prey) than do smaller prey (Bowen et al., 2002). It is still problematic to quantify prey species because it is likely that there are prey-specific differences in tissue DNA density (Deagle et al., 2010) and it is not possible to relate this to the probability of cod contributing with more biomass to the seal samples.

Seal interactions with fisheries

Commercial value of prey species

Some of the prey species found in the seal diet was also commercially valuable. Especially cod is assessed as in decline and are very valuable for the fisheries. Data on fish landings in Eastern Zealand and Bornholm were extracted from the Danish National databases and the most valuable species were assessed for mass and value. In the Belts area the most landed species were herring with 42 % of the total mass in ton followed by cod with 40 %, sprat with 9 % and flatfishes with 7 %. In relation to the value of the landing, cod contributed with 66 % of the total DKK compared to herring with 2 % and the flatfishes with 27 % of the value. Garfish contributed with a minor value. From Bornholm the most landed fish was cod with 66 % of the total mass followed by sprat with 29 % and flatfishes with 5 %. Cod contributed with 85 % of the value in DKK while sprat contributed with 11 % and flatfishes with 4 %. These data shows that the seals and fisheries do overlap greatly in relation to the most targeted species. Especially the very high value of cod on Bornholm is in conflict with the very high Frequency of Occurrence of cod found in the seal diet. Flatfishes do not seem to be a problem of the same size, because the occurrence of these species in the seal diet was of minor importance except on Tat during the winter.

Cod stock in the Baltic Sea

The EU commission proposed new quotas for the Baltic cod stock in 2017. A reduction on 88 % for the western Baltic cod stock and 39 % on the eastern stock was proposed, but the Council for the European Union negotiated the quotas to the lesser reduction of 56 % for the western stock and 25 % for the eastern. The quotas for catches of herring, salmon, plaice and sprat have been increased (Council for the European Union, 2016). The new quotas have also affected the recreational fishermen, as they can now only catch 5 cod per day. (Fiskeavisen.dk). The large reductions of the cod quotas were proposed based on the advice from the biologists in ICES, to help the stocks increase from their current low levels. Reactions to the negotiations have suggested that the aversion to meet the proposals from ICES can cause the cod stocks in the Baltic Sea to collapse (the Danish Society for Nature Conservation) (Balticeye.org). This is a real threat and a continuous overexploitation can have devastating consequences for the Baltic cod. Eero et al. (2015) investigated the health condition of Eastern Baltic cod. The abundance in biomass of the Eastern Baltic cod has increased since the record low 2000s, but there is a continuously decline in the nutritional condition of cods. The proportion of cod with very low conditions has increased to 20 % in recent years and it is apparent in all of the central Baltic. The reasons for this decline can be due to low availability of prey, low oxygen in larger areas, increased infestation with parasites or size selectivity in the commercial fisheries. The absence of large sized cod is confirmed by the fisheries and is a significant stock development that in worst case scenario can lead to stock collapse (Eero et al., 2015).

In regard to the seal-fishery conflict the reduced quotas could be a benefit, as the fishing time would be lower and therefore possibly the interactions between the seals and the fishing gear. If the cod becomes rarer in the Baltic Sea, it is probable that the seals will shift to a more abundant prey such as herring, as they will encounter cod less often and therefore foraging time would be too long if they were to forage exclusively for cod.

New rules for the hunting of seals in Denmark

In 2016 the Nature Agency in Denmark made a new action plan for the fisheries around Bornholm because of the complaints from fishermen who experienced damage on catch caused by grey seals. The new action involves a seal hunting corps who is permitted to shoot 40 seals if they are within 500 meters of fishing gear. This number of 40 seals is estimated from the 800 seals present in the Danish Baltic (Naturstyrelsen, 2016). It is very uncertain if this arrangement will benefit the fisheries, as the seals in the Danish Baltic most likely are 'visitors' who forage over very large areas in most of the Baltic Sea. Hence, seals occupying Danish waters cannot be determined as a Danish population, as seals from Sweden, Estonia, Finland and Russia also visit the same areas. It is thus not certain, which seals are damaging catch in the Bornholm area.

Culling and the predators effects on prey

Culling of a predator that is in conflict with humans has happened numerous times in the past. It is assumed that predator control will increase the prey populations to the benefit of humans, but this is not always the case (Bowen & Lidgard, 2013). It is possible that the predator eats the prey that otherwise would have perished naturally or there can be unexpected consequences of the removal of a top predator that cannot be foreseen (Bowen & Lidgard, 2013). Other predators are likely to take the place of the top predator, or immigration of seals from another colony could occur and thereby the problem would be unchanged (Bosetti & Pearce, 2003). A study by Morissette et al. (2012) used ecosystem models to show the effect of culling of marine mammals in different parts of the world. The results did not show any great effect on fisheries, but suggested that marine mammals have an important indirect effect on trophic structure, rather than a direct prey-predator relationship. The results also showed that there would be no big changes in prey availability if all marine mammals were extirpated (Morissette et al., 2012). It is controversial to suggest a culling of a marine mammal as they are popular among the public and have so recently recovered from almost extinction. Scientific evidence of the effects of culling is highly uncertain and it should therefore be investigated thoroughly before any actions are taken (Bowen & Lidgard, 2013). The Baltic grey seal population is still increasing and it will probably not make any difference if problem seals are regulated. The greatest concern for the fisheries is the raiding of nets, and it is difficult to find a solution to this problem.

Prey preference and profitability

The grey seal is considered an opportunistic predator and will prey on fish with different traits (Brown et al., 2012;). This study confirms this proposal as the seals consumed many different species with different behaviours and ranges in the water column. The most common species consumed by the seals could be grouped into either demersal, benthopelagic or pelagic. The demersal species include flatfishes, sand eels, eelpout, fourbeard rockling, brill, eel, saithe and the gobies. The benthopelagic species include cod, herring, lumpsucker, whiting, salmon and three-spined stickleback. The pelagic species include garfish, sprat and trout (fishbase.org). The two freshwater/brackish fish found in this study was perch and pike and they are not expected to move far from rivers and estuaries. The seals experience much different anti-predator behaviour from their prey forcing them to alter hunting tactics depending on target species (Bowen et al., 2002). Many demersal fish are cryptic to blend into their environments and schooling species use the effect of the many to confuse predators. Bowen et al (2002) examined the hunting techniques of harbour seals using critter cams and found that specific prey was hunted in different ways. Flatfishes and sand lances were hunted at the bottom where they were hiding until the seal thrusted its head forward to capture the prey. For schooling fish the seals most often separated small groups or individual fish from the group and were then easily able to capture them. The study found indications that prey differ in profitability, which might be a contributing factor when seals decide on a prey along with encounter rates (Bowen et al., 2002). It is probable that cod is a profitable prey because of a large size and a relatively high encounter rate. The large amount of garfish consumed in the Belts area suggests that this species is very profitable in spring and therefore replace cod as the most commonly consumed prey.

Economic value of seals

The economic value of seals in Denmark has not been investigated yet, but studies from other countries have tried to calculate the worth of seals. Bosetti and Pearce (2003) found that the economic value of seals is higher than the loss suffered by the fishing industry in Southern England. A seal Sanctuary in Gweek had a turnover of 1 million pounds per year and seal watching was also popular especially for families with children. There seems to be a willingness to pay for the knowledge that seals are conserved in the wild. This money could be used to compensate the fishermen for the fish lost to seals (Bosetti & Pearce, 2003).

There is an increasing interest in seal watching in Denmark and in 2015 there were 18 companies offering seal watching activities (Christian Riisager-Pedersen Pers. Comm.). These activities can make the seals more popular in the eyes of the public, and cause an increased economic value of the seals. If the protected status of the seals is terminated it will most likely cause a change of their behaviour, as they will become shy and thereby harder to watch from a close distance. No seal watching are present at Christiansø, but it is possible that it could happen in the future, if the public becomes aware of the possibility.

Some important measures have to be taken before allowing seal watching either in boat or by foot. It can be a challenge to balance the desire from visitors for close encounters with the wildlife while minimizing human disturbance on animals. Stress is a factor caused by disturbance and this might reduce the fitness of the animals in regard to foraging time and maternal care (Granquist & Nilsson, 2013). Osinga et al. (2012) found that humans at a distance of less than 50 meters always led to disturbance of harbour seals in the Dutch Wadden Sea. This had the potential to create panic so mother and pup were separated, which can have negative effects on the pups survival. Code of conducts are often created when managing wildlife, and are important for the reduction of disturbance of tourists on wild animal populations (Granquist & Sigurjonsdottir, 2014).

Possible solutions

There is a need to adapt to the new situation that is the presence of seals in the Baltic Sea, and not viewing it as a problem that can be solved (Varjopuro, 2011). It will probably not be possible to find a solution that satisfies every part of the conflict. It is certain that it will not be possible to cull the seals because of ethical rules and the dismay of the public, but it is unlikely that the fishermen will allow the problem to continue unchanged. So some kind of compromise has to be made. The most beneficial approach will probably be by enhancing the nets so the seals are not able to destroy them. The Pontoon net has proven useful and easy to handle, but even though good results are seen in the beginning, the seals rapidly adapt to new methods of exploiting the net to their advantage like waiting for the fish in the opening of the net (Westerberg et al., 2006). DTU Aqua is continuously developing new technologies and methods of fishing and more mobile fishing and fishing gear might be a good approach.

Acoustic methods, such as acoustic harassment devises (AHD), can be used to keep away marine mammals, but the problem with seals is their rapid ability to habituate to new situations. Sometimes the sounds might act opposite their purpose and attract seals instead of repel them. The device has to be extremely unpleasant, and if the seal is hungry enough it might still be insufficient. It is also unknown to what extent the devices affect other animals in the area (Westerberg et al., 2006; Quick et al., 2004). Pingers have been used to keep away porpoises (*Phocoena phocoena*) from fishing gear, but not with very great success as the animals habituate to the sounds very quickly (Teilmann et al., 2006).

A controversial way to investigate how much of an impact the grey seals on Tat have on the fisheries around Bornholm, would be to chase away the seals over a longer period, so that they give up the skerries as a resting place. As the seals do not breed here, it would probably not have a devastating impact on the population because the seals could move to other haul-out sites instead. If there were no remarkable difference in the losses due to seals, it would be clear that a culling would not make any difference. If there were a difference, it would have to be decided if the gain from a decrease in losses for the fishermen would be worth the loss of seals in the Danish waters. Even if the seals moved to another haul-out site, it would not mean that they would also forage in another area.

The increasing problem with seal parasites (*Pseudoterranova decipiens*) can affect the value of cod significantly and could possibly be as big of a problem as seals damage on fish and gear. A possible solution could be to treat seals with anti-helminthic to kill the parasites once and for all (Buchmann & Kania, 2012). This would be extremely comprehensive, but could also give amazing results.

Concluding remarks

In conclusion this study shows that grey seals from Tat in the Southern Baltic Sea consume a substantial amount of cod and that this is also the far most valuable species for the fisheries in this area. The seals from Måkläppen in Sweden mostly consume garfish, but the results have to be interpreted with caution, as the data is considerably skewed towards the spring period where garfish is more common. Cod also consists of a large part of the diet for the seals from Måkläppen and more data from the summer, winter and autumn are needed to provide a more complete picture. The diet of the seals from the two locations was significantly different which suggests a difference in the foraging areas. No yearly variation was observed between the spring period of 2014 and 2015 at Måkläppen and a relatively small seasonal variation was found at Tat.

Further studies

This study has focused on molecular determination of seal diet. It was not possible to look at otoliths because of time restrictions, but it will hopefully be possible in the future. Otolith identification may confirm some of the fish species found by the molecular method and likely also add some. It will also be possible to estimate the size of the fish ingested and the number in each scat. This can shed light on the size range of the fish consumed by the seals, which could be important in relation to the fisheries, as the seals prey size preference might overlap with the fisheries. Scats are continuously collected at both Måkläppen and Tat, so more data can be analysed in the future with e.g. differences between years as a main purpose.

A feather was found in a sample from Tat, so it would be interesting to analyse the samples for bird DNA as well as other potential prey like cephalopods and crustaceans. Bird remains have been found in grey seal diet from other areas of the Baltic Sea (Karl Lundström pers. comm.) As these species are not as commercially important as fish, it would be relevant to investigate how large a part they contribute to the seal diet. If a more comprehensive examination of the diet of grey seals were performed, all possible prey items would be essential to include. It would be relevant to apply molecular methods to such studies, as it is relatively hard to find and determine cephalopod beaks and other hard parts.

Parasites are, as mentioned before, an increasing problem in cod in the Baltic Sea, and studies could further illuminate the size of this problem. A parasite was found in one scat, though no identification was performed. The samples used for this study could be used to find DNA from parasites and could be of great interest to the fisheries. The micro biome of organisms has proved to be very informative of the evolution and adaptability of the host, and this would also be relevant to study for seals. Faecal samples are a way to investigate this. Lastly, investigations of the toxicity of phytoplankton can be performed on faecal samples of marine mammals, and this could be very interesting, as the toxins can impair the reproductive success of the animals.

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References

Allen, R., Jarvis, D., Sayer, S. & Mills, C. (2012). Entanglement of grey seals Halichoerus grypus at a haul out site in Cornwall, UK. *Marine Pollution Bulletin*. [Online]. 64 (12). p.pp. 2815–2819. Available from: http://dx.doi.org/10.1016/j.marpolbul.2012.09.005.

Almkvist, L. (1982). Baltic Marine mammals - A status report. *International Council for the Exploration of the Sea*.

Andersen, S.M., Lydersen, C., Grahl-Nielsen, O. & Kovacs, K.M. (2004). Autumn diet of harbour seal (Phoca vitulina) at Prins Karls Forland, Svalbard, assessed via scat and fatty-acid analyses. *Canadian Journal of Zoology*. 82 (8). p.pp. 1230–1245.

Andersen, S.M., Teilmann, J., Harders, P.B., Hansen, E.H. & Hjøllund, D. (2007). Diet of harbour seals and great cormorants in Limfjord, Denmark: Interspecific competition and interaction with fishery. *ICES Journal of Marine Science*. 64 (6). p.pp. 1235–1245.

Bagge, O., Thurow, F., Steffensen, E. & Bay, J. (1994). The Baltic cod. *Dana*. [Online]. 10 (Figure 2). p.pp. 1–28. Available from: http://agris.fao.org/agris-search/search/display.do?f=1995/DK/DK95002.xml;DK9521335.

Beck, C.A., Iverson, S.J., Bowen, W.D. & Blanchard, W. (2007). Sex differences in grey seal diet reflect seasonal variation in foraging behaviour and reproductive expenditure: Evidence from quantitative fatty acid signature analysis. *Journal of Animal Ecology*. 76 (3). p.pp. 490–502.

Berg, I., Haug, T. & Nilssen, K.T. (2002). Harbour seal (Phoca vitulina) diet in Vesterålen, North Norway. *Sarsia: North Atlantic Marine Science*. 87 (6). p.pp. 451–461.

Bjørge, A., Bekkby, T., Bakkestuen, V. & Framstad, E. (2002). Interactions between harbour seals , Phoca vitulina , and fisheries in complex coastal waters explored by combined Geographic Information System (GIS) and energetics modelling. *ICES Journal of Marine Science*. 59 (1). p.pp. 29–42.

Bleil, M., Oeberst, R. & Urrutia, P. (2009). Seasonal maturity development of Baltic cod in different spawning areas: importance of the Arkona Sea for the summer spawning stock. *Journal of Applied Ichthyology*. 25 (1). p.pp. 10–17.

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W. & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution*. [Online]. 29 (6). p.pp. 358–367. Available from: http://dx.doi.org/10.1016/j.tree.2014.04.003.

Bosetti, V. & Pearce, D. (2003). A study of environmental conflict: the economic value of Grey

Seals in southwest England. *Biodiversity and Conservation*. 12 (12). p.pp. 2361–2392.

Bowen, D. (2016). *Halichoerus grypus. The IUCN Red List of Threatened Species 2016*.

Bowen, W.D. (2000). Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. *Canadian Journal of Fisheries and Aquatic Sciences*. 57 (5). p.pp. 898–905.

Bowen, W.D. & Lidgard, D. (2013). Marine mammal culling programs: Review of effects on predator and prey populations. *Mammal Review*. 43 (3). p.pp. 207–220.

Bowen, W.D., Tully, D., Boness, D.J., Bulheier, B.M. & Marshall, G.J. (2002). Prey-dependent foraging tactics and prey profitability in a marine mammal. *Marine Ecology Progress Series*. 244 (November). p.pp. 235–245.

Bowles, E., Schulte, P.M., Tollit, D.J., Deagle, B.E. & Trites, A.W. (2011). Proportion of prey consumed can be determined from faecal DNA using real-time PCR. *Molecular Ecology Resources*. 11 (3). p.pp. 530–540.

Brown, S.L., Bearhop, S., Harrod, C. & McDonald, R. a. (2012). A review of spatial and temporal variation in grey and common seal diet in the United Kingdom and Ireland. *Journal of the Marine Biological Association of the United Kingdom*. 92 (8). p.pp. 1711–1722.

Buchmann, K. & Kania, P. (2012). Emerging Pseudoterranova decipiens (Krabbe, 1878) problems in Baltic cod, Gadus morhua L., associated with grey seal colonization of spawning grounds. *Journal of Fish Diseases*. 35 (11). p.pp. 861–866.

Burns, J.M., Trumble, S.J., Castellini, M.A. & Testa, J.W. (1998). The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. *Polar Biology*. 19 (4). p.pp. 272–282.

Casper, R.M., Jarman, S.N., Deagle, B.E., Gales, N.J. & Hindell, M.A. (2007). Detecting prey from DNA in predator scats: A comparison with morphological analysis, using Arctocephalus seals fed a known diet. *Journal of Experimental Marine Biology and Ecology*. 347 (1–2). p.pp. 144–154.

Cook, R.M., Holmes, S.J. & Fryer, R.J. (2015). Grey seal predation impairs recovery of an over-exploited fish stock. *Journal of Applied Ecology*. 52 (4). p.pp. 969–979.

Council for the European Union (2016). *Outcome of the Council Meeting*. Luxembourg.

Deagle, B.E., Chiaradia, A., McInnes, J. & Jarman, S.N. (2010). Pyrosequencing faecal DNA to determine diet of little penguins: Is what goes in what comes out? *Conservation Genetics*. 11 (5). p.pp. 2039–2048.

Deagle, B.E., Kirkwood, R. & Jarman, S.N. (2009). Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology*. 18 (9). p.pp. 2022–2038.

Deagle, B.E. & Tollit, D.J. (2007). Quantitative analysis of prey DNA in pinniped faeces: Potential to estimate diet composition? *Conservation Genetics*. 8 (3). p.pp. 743–747.

Deagle, B.E., Tollit, D.J., Jarman, S.N., Hindell, M.A., Trites, A.W. & Gales, N.J. (2005). Molecular scatology as a tool to study diet: Analysis of prey DNA in scats from captive Steller sea lions. *Molecular Ecology*. 14 (6). p.pp. 1831–1842.

Dietz, R., Teilmann, J. & Henriksen, O. (2003). Movements of seals from Rødsand seal sanctuary. *National Environmental Research Institute.* 429

Dietz, R., Teilmann, J., Andersen, S.M., Rigét, F. & Olsen, M.T. (2013). Movements and site fidelity of harbour seals (Phoca vitulina) in Kattegat, Denmark, with the implications for the epidermiology of the phocine distemper virus. *ICES Journal of Marine Science*. 70 (October). p.pp. 1439–1450.

Eero, M., Hjelm, J., Behrens, J., Buchmann, K., Cardinale, M., Casini, M., Gasyukov, P., Holmgren, N., Horbowy, J., Hussy, K., Kirkegaard, E., Kornilovs, G., Krumme, U., Koster, F.W., Oeberst, R., Plikshs, M., Radtke, K., Raid, T., Schmidt, J., Tomczak, M.T., Vinther, M., Zimmermann, C. & Storr-Paulsen, M. (2015). Eastern Baltic cod in distress: Biological changes and challenges for stock assessment. *ICES Journal of Marine Science*. 72 (8). p.pp. 2180–2186.

Fietz, K., Galatius, A., Teilmann, J., Dietz, R., Frie, A.K., Klimova, A., Palsbøll, P.J., Jensen, L.F., Graves, J.A., Hoffman, J.I. & Olsen, M.T. (2016). Shift of grey seal subspecies boundaries in response to climate, culling and conservation. *Molecular Ecology*. [Online]. 25 (17). p.pp. 4097–4112. Available from: http://doi.wiley.com/10.1111/mec.13748.

Fjälling, A., Kleiner, J. & Beszcynska, M. (2007). Evidence that grey seals (Halichoerus grypus) use above-water vision to locate baited buoys. *NAMMCO Scientific Publications*. 6. p.pp. 215–227.

Galatius, A., Olsen, M.T. & Teilmann, J. (2016). Konflikter mellem sæler og fiskeri i danmark – baggrund og studier i sælers biologi og interaktioner med fiskeriet. *Nationalt Center for Miljø og Energi.* nr. 184

Galatius, A., Teilmann, J., Olsen, M.T. & Salling, A. (in prep.). Overlap of seals and fisheries in the southwestern Baltic.

Granquist, S.M. & Nilsson, P.-Å. (2013). Who’s watching whom? - an interdisciplinary approach to the study of seal-watching tourism in Iceland. *Journal of Cleaner Production*. [Online]. 111. p.pp. 471–478. Available from: http://dx.doi.org/10.1016/j.jclepro.2014.11.060.

Granquist, S.M. & Sigurjonsdottir, H. (2014). The effect of land based seal watching tourism on the haul-out behaviour of harbour seals (Phoca vitulina) in Iceland. *Applied Animal Behaviour Science*. [Online]. 156. p.pp. 85–93. Available from: http://dx.doi.org/10.1016/j.applanim.2014.04.004.

Gårdmark, A., Östman, Ö., Nielsen, A., Lundström, K., Karlsson, O., Pönni, J. & Aho, T. (2012). Does predation by grey seals (Halichoerus grypus) affect Bothnian Sea herring stock estimates? *ICES Journal of Marine Science*. 70 (4). p.pp. 883–891.

Hammill, M.O. & Stenson, G.B. (2000). Estimated prey consumption by harp seals (Phoca groenlandica), hooded seals (Cystophora cristata), grey seals (Halichoerus grypus) and harbour seals (Phoca vitulina) in Atlantic Canada. *Journal of Northwest Atlantic Fishery Science*. 26. p.pp. 1–23.

Hammond, P.S., Hall, A.J. & Prime, J.H. (1994). The Diet of Grey Seals in the Inner and Outer Hebrides. *Journal of Applied Ecology*. 31 (4). p.pp. 737–746.

Harding, K.C., Härkönen, T., Helander, B. & Karlsson, O. (2013). Status of Baltic grey seals: Population assessment and extinction risk. *NAMMCO Scientific Publications*. [Online]. 6. p.pp. 33–56. Available from: http://septentrio.uit.no/index.php/NAMMCOSP/article/view/2720.

Harding, K.C. & Härkönen, T.J. (1999). Development in the Baltic Grey Seal (Halichoerus grypus) and Ringed Seal (Phoca hispida) Populations during the 20th Century. *Ambio*. [Online]. 28 (7). p.pp. 619–627. Available from: http://www.jstor.org/stable/4314968.

Heide-Jørgensen, M.P., Härkönen, T., Dietz, R. & Thompson, P.M. (1992). Retrospective of the 1988 European seal epizootic. *Diseases of Aquatic Organisms*. 13 (1). p.pp. 37–62.

Helcom (2007). *HELCOM Baltic Sea Action Plan.* Krakow.

HELCOM Red List Marine Mammal Expert Group (2013). Halichoerus grypus distribution and status in the Baltic Sea region. [Online]. *ICES* 2012. p.p. 7. Available from: www.helcom.fi.

Helle, E., Olsson, M. & Jensen, S. (1976). PCB Levels Correlated with Pathological Changes in Seal Uteri. *Ambio*. 5 (5). p.pp. 261–262.

Hemmingsson, M., Fjälling, A. & Lunneryd, S.G. (2008). The pontoon trap: Description and function of a seal-safe trap-net. *Fisheries Research*. 93 (3). p.pp. 357–359.

Hibert, F., Taberlet, P., Chave, J., Scotti-Saintagne, C., Sabatier, D. & Richard-Hansen, C. (2013).

Unveiling the Diet of Elusive Rainforest Herbivores in Next Generation Sequencing Era? The Tapir as a Case Study. *PLoS ONE*. 8 (4).

Härkönen, T. (1987). Seasonal and regional variations in the feeding habits of the harbour seal, Phoca vitulina, in the Skagerrak and the Kattegat. *Journal of Zoology*. [Online]. 213. p.pp. 535–543. Available from: http://onlinelibrary.wiley.com/doi/10.1111/j.1469-7998.1987.tb03724.x/abstract.

Härkönen, T., Dietz, R., Reijnders, P., Teilmann, J., Harding, K., Hall, A., Brasseur, S., Siebert, U., Goodman, S.J., Jepson, P.D., Rasmussen, T.D. & Thompson, P. (2006). The 1988 and 2002 phocine distemper virus epidemics in European harbour seals. *Diseases of Aquatic Organisms*. 68 (2). p.pp. 115–130.

Härkönen, T., Galatius, A., Bräeger, S., Karlsson, O. & Ahola, M. (2013). Population growth rate, abundance and distribution of marine mammals. *HELCOM* p.1-34

Haarder, S., Kania, P.W., Galatius, A. & Buchmann, K. (2014). Increased Contracaecum osculatum infection in Baltic cod (Gadus morhua) livers (1982-2012) associated with increasing grey seal (Halichoerus grypus) populations. *Journal of Wildlife Diseases*. [Online]. 50 (3). p.pp. 537–543. Available from: http://www.bioone.org/doi/10.7589/2012-12-320.

ICES (2016). Cod (Gadus morhua) in subdivisions 24-32 (eastern Baltic stock) (Eastern Baltic Sea). *ICES* 32 (May 2015). p.pp. 1–8.

Iverson, S.J., Field, C., Bowen, W.D.O.N. & Blanchard, W. (2004). Quantitative Fatty Acid Signature Analysis : A New Method of Estimating Predator Diets. *Ecological Monographs*. 74 (2). p.pp. 211–235.

Jepsen, P.U. (2005). Forvaltningsplan for spættet sæl (Phoca vitulina) og gråsæl (Halichoerus grypus) i Danmark. *Miljøministeriet - Skov - og Naturstyelsen*. P 1-36

Johansen, H., Hansen, C., Jensen, N.S., Lauritsen, J.P. & Bøving-Petersen, J.O. (1909). Saltvandsfiskeriets skadedyr og kampen imod dem. *Dansk fiskeriforenings Udvalg til bekæmpelse af fiskeriets skadedyr.*

Klimova, A., Phillips, C.D., Fietz, K., Olsen, M.T., Harwood, J., Amos, W. & Hoffman, J.I. (2014). Global population structure and demographic history of the grey seal. *Molecular Ecology*. 23 (16). p.pp. 3999–4017.

Könighson, S., Lunneryd, S.G., Stridh, H. & Sundqvist, F. (2009). Grey seal predation in cod gillnet fisheries in the central Baltic sea. *Journal of Northwest Atlantic Fishery Science*. 42. p.pp. 41–47.

Larsen, F., Krog, C., Klaustrup, M. & Buchmann, K. (2015). Kortlægning af sælskader i dansk fiskeri. *DTU aqua-rapport* nr. 299-2015

Lundström, K., Hjerne, O., Lunneryd, S. & Karlsson, O. (2010). Understanding the diet composition of marine mammals : grey seals (Halichoerus grypus) in the Baltic Sea. *ICES Journal of Marine Science* 67 p.pp. 1230–1239.

Lundström, K., Hjerne, O., Alexandersson, K. & Karlsson, O. (2007). Estimation of grey seal (Halichoerus grypus) diet composition in the Baltic Sea. *NAMMCO Scientific Publications*. [Online]. 6 (0). p.p. 177. Available from: https://www.ub.uit.no/baser/septentrio/index.php/NAMMCOSP/article/view/2733.

Lunneryd, S.-G., Boström, M.K. & Aspholm, P.E. (2015). Sealworm (*Pseudoterranova decipiens*) infection in grey seals (*Halichoerus grypus*), cod (*Gadus morhua*) and shorthorn sculpin (*Myoxocephalus scorpius*) in the Baltic Sea. *Parasitology Research*. [Online]. 114 (1). p.pp. 257–264. Available from: http://link.springer.com/10.1007/s00436-014-4187-z.

MacKenzie, B.R., Eero, M. & Ojaveer, H. (2011). Could seals prevent cod recovery in the Baltic Sea? *PLoS ONE*. 6 (5).

Matejusová, I., Doig, F., Middlemas, S.J., Mackay, S., Douglas, A., Armstrong, J.D., Cunningham, C.O. & Snow, M. (2008). Using quantitative real-time PCR to detect salmonid prey in scats of grey Halichoerus grypus and harbour Phoca vitulina seals in Scotland - an experimental and field study. *Journal of Applied Ecology*. 45. p.pp. 632–640.

Mehrdana, F., Bahlool, Q.Z.M., Skov, J., Marana, M.H., Sindberg, D., Mundeling, M., Overgaard, B.C., Korbut, R., Strøm, S.B., Kania, P.W. & Buchmann, K. (2014). Occurrence of zoonotic nematodes Pseudoterranova decipiens, Contracaecum osculatum and Anisakis simplex in cod (Gadus morhua) from the Baltic Sea. *Veterinary Parasitology*. [Online]. 205 (3–4). p.pp. 581–587. Available from: http://dx.doi.org/10.1016/j.vetpar.2014.08.027.

Miljø- og Fødevareministeriet (2016). Bekendtgørelse nr. 188 af 26. februar 2016 om udpegning og administration af internationale naturbeskyttelsesområder samt beskyttelse af visse arter. *Miljø- og Fødevareministeriet* [Online]. Available from: https://www.retsinformation.dk/Forms/R0710.aspx?id=177832.

Morissette, L., Christensen, V. & Pauly, D. (2012). Marine Mammal Impacts in Exploited Ecosystems: Would Large Scale Culling Benefit Fisheries? *PLoS ONE*. 7 (9).

Naturstyrelsen (2014). Forslag om midlertidig ordning til regulering af gråsæl. *Miljøministeriet* p. 1-6

Naturstyrelsen (2016). Sæljægerkorps skal hjælpe trængte fiskere. *Miljøministeriet*

Oksanen, S.M., Ahola, M.P., Lehtonen, E. & Kunnasranta, M. (2014). Using movement data of Baltic grey seals to examine foraging-sit fidelity: implications for seal-fishery conflict mitigation. *Marine Ecology Progress Series*. 507.

Olsen, M.T., Galatius, A., Biard, V., Gregersen, K. & Kinze, C.C. (2016). The forgotten type specimen of the grey seal [Halichoerus grypus (Fabricius, 1791)] from the island of Amager, Denmark. *Zoological Journal of the Linnean Society*. (April).

Olsen, M.T., Andersen, S.M., Teilmann, J., Dietz, R., Edrén, S.M.C., Linnet, A. & Härkönen, T. (2010). Status of the harbour seal (phoca vitulina) in Southern Scandinavia. *NAMMCO Scientific Publications*. 8. p.pp. 77–94.

Osinga, N., Nussbaum, S.B., Brakefield, P.M. & Udo de Haes, H.A. (2012). Response of common seals (Phoca vitulina) to human disturbances in the Dollard estuary of the Wadden Sea. *Mammalian Biology*. [Online]. 77 (4). p.pp. 281–287. Available from: http://dx.doi.org/10.1016/j.mambio.2012.02.005.

Parsons, K.M., Piertney, S.B., Middlemas, S.J., Hammond, P.S. & Armstrong, J.D. (2005). DNA-based identification of salmonid prey species in seal faeces. *Journal of Zoology London*. [Online]. 266 (3). p.pp. 275–281. Available from: http://doi.wiley.com/10.1017/S0952836905006904.

Pierce, G.J., Thompson, P.M., Miller, A., Diack, J.S.W., Miller, D. & Boyle, P.R. (1991). Seasonal variation in the diet of common seals (Phoca vitulina) in the Moray Firth area of Scotland. *Journal of Zoology London*. 223 (August). p.pp. 641–652.

Pinol, J., San Andrés, V., Clare, E.L., Mir, G. & Symondson, W.O.C. (2014). A pragmatic approach to the analysis of diets of generalist predators: The use of next-generation sequencing with no blocking probes. *Molecular Ecology Resources*. 14 (1). p.pp. 18–26.

Pittman, A., Galatius, A., Teilmann, J., Dietz, R., Jarnit, S., Andersen, S.M. & Olsen, M.T. (in prep.). When protected becomes pest: Molecular diet analysis of grey seals (Halichoerus grypus) and harbour seals (Phoca vitulina) in Denmark in light og the seal-fisheries conflict. *University of Copenhagen*

Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N. & Taberlet, P. (2012). Who is eating what: Diet assessment using next generation sequencing. *Molecular Ecology*. 21 (8). p.pp. 1931–1950.

Quick, N.J., Middlemas, S.J. & Armstrong, J.D. (2004). A survey of antipredator controls at marine salmon farms in Scotland. *Aquaculture*. [Online]. 230 (1–4). p.pp. 169–180. Available from: http://dx.doi.org/10.1016/S0044-8486(03)00428-9.

Rosing-Asvid, A., Teilmann, J., Dietz, R. & Olsen, M.T. (2010). First confirmed record of grey seals in Greenland. *Arctic*. 63 (4). p.pp. 471–473.

Scharff-Olsen, C.H. (2015). Geographical variation in the diet within and between the harbour seals (Phoca vitulina), grey seals (Halichoerus grypus) and Baltic ringed seals (Pusa hispida) inhabiting the greater Baltic Sea. *University of Copenhagen.*

Schmölcke, U. (2008). Holocene environmental changes and the seal (Phocidae) fauna of the Baltic Sea: Coming, going and staying. *Mammal Review*. 38 (4). p.pp. 231–246.

Shehzad, W., Riaz, T., Nawaz, M.A., Miquel, C., Poillot, C., Shah, S.A., Pompanon, F., Coissac, E. & Taberlet, P. (2012). Carnivore diet analysis based on next-generation sequencing: Application to the leopard cat (Prionailurus bengalensis) in Pakistan. *Molecular Ecology*. 21 (8). p.pp. 1951–1965.

Sjöberg, M., Fedak, M.A. & McConnell, B.J. (1995). Movements and diurnal behaviour patterns in a Baltic grey seal (Halichoerus grypus). *Polar Biology*. 15 (8). p.pp. 593–595.

Suuronen, P. & Lehtonen, E. (2012). The role of salmonids in the diet of grey and ringed seals in the Bothnian Bay, northern Baltic Sea. *Fisheries Research*. [Online]. 125–126. p.pp. 283–288. Available from: http://dx.doi.org/10.1016/j.fishres.2012.03.007.

Symondson, W.O.C. & Harwood, J.D. (2014). Special issue on molecular detection of trophic interactions: Unpicking the tangled bank. *Molecular Ecology*. 23 (15). p.pp. 3601–3604.

Tauber, P. (1882). Om sælhundene. *Fiskeritidende*.

Teilmann, J., Tougaard, J., Miller, L.A., Kirketerp, T., Hansen, K. & Brando, S. (2006). Reactions of captive harbor porpoises (Phocoena phocoena) to pinger-like sounds. *Marine Mammal Science*. 22 (2). p.pp. 240–260.

Thomas, A.C., Jarman, S.N., Haman, K.H., Trites, A.W. & Deagle, B.E. (2014). Improving accuracy of DNA diet estimates using food tissue control materials and an evaluation of proxies for digestion bias. *Molecular Ecology*. 23 (15). p.pp. 3706–3718.

Vallin, L., Nissling, A. & Westin, L. (1999). Potential factors influencing reproductive success of Baltic cod, Gadus morhua: A review. *Ambio, 28, 92-99.* 28 (1). p.pp. 92–99.

Vanhatalo, J., Vetemaa, M., Herrero, A., Aho, T. & Tiilikainen, R. (2014). By-catch of grey seals (Halichoerus grypus) in Baltic fisheries - A Bayesian analysis of interview survey. *PLoS ONE*. 9 (11). p.pp. 1–16.

Varjopuro, R. (2011). Co-existence of seals and fisheries? Adaptation of a coastal fishery for recovery of the Baltic grey seal. *Marine Policy*. [Online]. 35 (4). p.pp. 450–456. Available from: http://dx.doi.org/10.1016/j.marpol.2010.10.023.

Westerberg, H., Lunneryd, S.G., Fjalling, A. & Wahlberg, M. (2006). Reconciling fisheries activities with the conservation of seals throughout the development of new fishing gear: A case study from the Baltic fishery-gray seal conflict. *Reconciling Fisheries with Conservation, Vols I and Ii*. 49. p.pp. 1281–1291.

http://www.iucnredlist.org/details/9660/0

http://www.helcom.fi/baltic-sea-trends/data-maps/biodiversity/seals

http://www.fiskeavisen.dk/nyhed/torskekvoter-max-5-hjemtagne-torsk-pr-dag-lystfiskere-i-oestersoen

http://balticeye.org/en/fisheries/eu-ministers-decision-on-the-cod-quota-is-about-the-future-of-fishing/

http://www.dn.dk/Default.aspx?ID=48228

http://www.fishbase.org/search.php

http://www.naturvardsverket.se/Var-natur/Jakt/Jakt-pa-sal/

Supplementary

Table 1 – The barcode combinations of the fusion primers for each sample.

Sample	Forward	Reverse	F Index	R index
1T	F1	R3	AGGAAT	ACGTCATG
2T	F2	R4	TCATAG	TCATGTCG
3T	F3	R5	TACTATG	TAGCGTCG
4T	F4	R6	TGATGAC	TCTACTCG
7T	F5	R7	TAGCGAC	ATGACTCG
9T	F1	R4	AGGAAT	TCATGTCG
10T	F2	R5	TCATAG	TAGCGTCG
11T	F3	R6	TACTATG	TCTACTCG
12T	F4	R7	TGATGAC	ATGACTCG
13T	F5	R8	TAGCGAC	ATCTATCG
14T	F1	R5	AGGAAT	TAGCGTCG
19T	F2	R6	TCATAG	TCTACTCG
20T	F3	R7	TACTATG	ATGACTCG
22T	F4	R8	TGATGAC	ATCTATCG
23T	F1	R6	AGGAAT	TCTACTCG
24T	F2	R7	TCATAG	ATGACTCG
25T	F3	R8	TACTATG	ATCTATCG
26T	F1	R7	AGGAAT	ATGACTCG
27T	F2	R8	TCATAG	ATCTATCG
28T	F9	R1	ACTACTC	TATCGATG
29T	F9	R2	ACTACTC	ATGCGATG
30T	F9	R3	ACTACTC	ACGTCATG
35M	F7	R6	TCGACTC	TCTACTCG
36M	F9	R5	ACTACTC	TAGCGTCG
38M	F9	R6	ACTACTC	TCTACTCG
40M	F4	R2	TGATGAC	ATGCGATG
41M	F5	R3	TAGCGAC	ACGTCATG
43M	F6	R4	ACACGAC	TCATGTCG
48M	F4	R1	TGATGAC	TATCGATG
49M	F5	R2	TAGCGAC	ATGCGATG
50M	F9	R7	ACTACTC	ATGACTCG
52M	F7	R4	TCGACTC	TCATGTCG
54M	F9	R8	ACTACTC	ATCTATCG
57M	F6	R2	ACACGAC	ATGCGATG
62M	F7	R2	TCGACTC	ATGCGATG
63M	F8	R3	ATGACTC	ACGTCATG
64M	F7	R1	TCGACTC	TATCGATG

65M	F8	R2	ATGACTC	ATGCGATG
66M	F8	R1	ATGACTC	TATCGATG
67M	F9	R9	ACTACTC	ACAGATCG
68M	F2	R20	TCATAG	TACATACG
69M	F9	R11	ACTACTC	AGATACTC
70M	F3	R20	TACTATG	TACATACG
71M	F9	R13	ACTACTC	AGACGCTC
72M	F9	R14	ACTACTC	TCGCGCTC
73M	F10	R1	AGCTACTG	TATCGATG
76M	F10	R4	AGCTACTG	TCATGTCG
78M	F10	R6	AGCTACTG	TCTACTCG
79M	F10	R7	AGCTACTG	ATGACTCG
80M	F10	R8	AGCTACTG	ATCTATCG
81M	F10	R9	AGCTACTG	ACAGATCG
83M	F10	R11	AGCTACTG	AGATACTC
84M	F10	R12	AGCTACTG	TGCTACTC
85M	F10	R13	AGCTACTG	AGACGCTC
86M	F1	R9	AGGAAT	ACAGATCG
87M	F1	R10	AGGAAT	ATACTGCG
92M	F1	R15	AGGAAT	ATGCGCTC
94M	F1	R16	AGGAAT	TCTCGACG
98M	F2	R12	TCATAG	TGCTACTC
99M	F2	R13	TCATAG	AGACGCTC
102M	F2	R14	TCATAG	TCGCGCTC
103M	F2	R15	TCATAG	ATGCGCTC
107M	F3	R9	TACTATG	ACAGATCG
108M	F3	R10	TACTATG	ATACTGCG
109M	F3	R11	TACTATG	AGATACTC
110M	F3	R12	TACTATG	TGCTACTC
111M	F3	R13	TACTATG	AGACGCTC
112M	F3	R14	TACTATG	TCGCGCTC
113M	F3	R2	TACTATG	ATGCGATG
115M	F3	R16	TACTATG	TCTCGACG
120M	F4	R12	TGATGAC	TGCTACTC
118M	F8	R8	ATGACTC	ATCTATCG
121M	F4	R13	TGATGAC	AGACGCTC
123M	F4	R15	TGATGAC	ATGCGCTC
127M	F5	R12	TAGCGAC	TGCTACTC
129M	F5	R4	TAGCGAC	TCATGTCG
130M	F6	R7	ACACGAC	ATGACTCG
132M	F5	R13	TAGCGAC	AGACGCTC
133M	F5	R14	TAGCGAC	TCGCGCTC
134M	F5	R15	TAGCGAC	ATGCGCTC

140M	F3	R3	TACTATG	ACGTCATG
141M	F6	R15	ACACGAC	ATGCGCTC
147M	F4	R5	TGATGAC	TAGCGTCG
149M	F7	R15	TCGACTC	ATGCGCTC
155M	F5	R10	TAGCGAC	ATACTGCG
159M	F7	R12	TCGACTC	TGCTACTC
160M	F8	R13	ATGACTC	AGACGCTC
161M	F8	R14	ATGACTC	TCGCGCTC
163M	F7	R7	TCGACTC	ATGACTCG
164M	F5	R9	TAGCGAC	ACAGATCG
166M	F6	R6	ACACGAC	TCTACTCG
167M	F6	R10	ACACGAC	ATACTGCG
169M	F7	R11	TCGACTC	AGATACTC
171M	F6	R9	ACACGAC	ACAGATCG
173M	F7	R10	TCGACTC	ATACTGCG
174M	F8	R11	ATGACTC	AGATACTC
175M	F7	R9	TCGACTC	ACAGATCG
176M	F8	R10	ATGACTC	ATACTGCG
177M	F8	R9	ATGACTC	ACAGATCG
179M	F9	R16	ACTACTC	TCTCGACG
180M	F10	R15	AGCTACTG	ATGCGCTC
181M	F10	R16	AGCTACTG	TCTCGACG
183M	F2	R18	TCATAG	TCGTGACG
184M	F3	R19	TACTATG	ATGTGACG
196M	F1	R2	AGGAAT	ATGCGATG
198M	F5	R6	TAGCGAC	TCTACTCG
199M	F7	R8	TCGACTC	ATCTATCG
200M	F4	R17	TGATGAC	ACATGACG
201M	F5	R18	TAGCGAC	TCGTGACG
206M	F7	R20	TCGACTC	TACATACG
210T	F1	R1	AGGAAT	TATCGATG
211T	F2	R1	TCATAG	TATCGATG
212T	F2	R2	TCATAG	ATGCGATG
213T	F3	R1	TACTATG	TATCGATG
214T	F3	R4	TACTATG	TCATGTCG
215T	F4	R3	TGATGAC	ACGTCATG
216T	F4	R4	TGATGAC	TCATGTCG
217T	F5	R1	TAGCGAC	TATCGATG
218T	F5	R5	TAGCGAC	TAGCGTCG
220T	F6	R3	ACACGAC	ACGTCATG
221T	F1	R17	AGGAAT	ACATGACG
222T	F1	R18	AGGAAT	TCGTGACG
228T	F6	R5	ACACGAC	TAGCGTCG

229T	F7	R3	TCGACTC	ACGTCATG
230T	F7	R5	TCGACTC	TAGCGTCG
231T	F8	R4	ATGACTC	TCATGTCG
232T	F8	R5	ATGACTC	TAGCGTCG
233T	F8	R6	ATGACTC	TCTACTCG
234T	F8	R7	ATGACTC	ATGACTCG
235T	F9	R4	ACTACTC	TCATGTCG
236T	F10	R2	AGCTACTG	ATGCGATG
239T	F1	R19	AGGAAT	ATGTGACG
240T	F1	R20	AGGAAT	TACATACG
241T	F2	R17	TCATAG	ACATGACG
242T	F2	R19	TCATAG	ATGTGACG
246T	F1	R11	AGGAAT	AGATACTC
247T	F1	R14	AGGAAT	TCGCGCTC
248T	F2	R9	TCATAG	ACAGATCG
250T	F6	R20	ACACGAC	TACATACG
251T	F7	R17	TCGACTC	ACATGACG
253T	F9	R20	ACTACTC	TACATACG
255T	F4	R11	TGATGAC	AGATACTC
257T	F3	R17	TACTATG	ACATGACG
258T	F3	R18	TACTATG	TCGTGACG
265T	F5	R19	TAGCGAC	ATGTGACG
266T	F5	R20	TAGCGAC	TACATACG
267T	F6	R17	ACACGAC	ACATGACG
268T	F5	R16	TAGCGAC	TCTCGACG
270T	F6	R12	ACACGAC	TGCTACTC
272T	F6	R14	ACACGAC	TCGCGCTC
273T	F6	R16	ACACGAC	TCTCGACG
275T	F4	R18	TGATGAC	TCGTGACG
276T	F4	R19	TGATGAC	ATGTGACG
277T	F4	R20	TGATGAC	TACATACG
K3	F6	R18	ACACGAC	TCGTGACG
K5	F8	R19	ATGACTC	ATGTGACG
K7	F7	R19	TCGACTC	ATGTGACG
K9	F9	R19	ACTACTC	ATGTGACG
K18	F10	R18	AGCTACTG	TCGTGACG
K20	F10	R20	AGCTACTG	TACATACG
K21	F6	R8	ACACGAC	ATCTATCG
K25	F8	R16	ATGACTC	TCTCGACG

Table 1 – Info and number of sequences for each sample. Shown are the species with a Frequency of Occurrence of more than 5 %.

Sample	**Month**	**Year**	**Location**	***Cod***	***Garfish***	***Herring***	***Sprat***	***Flatfish***	***Great sandeel***	***Eelpout***	***Fourbeard Rockling***
1T	5	2015	Tat	8621		578	68				82
2T	5	2015	Tat	4191							
3T	5	2015	Tat	2451		119	367				
4T	5	2015	Tat	404		186	234				
7T	6	2015	Tat	17427		11	541				
9T	6	2015	Tat	416			6741				
10T	6	2015	Tat		75	327	2467				
11T	6	2015	Tat	12386							
12T	6	2015	Tat	4967							
13T	6	2015	Tat	33		10065	445				
14T	6	2015	Tat	26323			2837				
19T	8	2015	Tat	2236	158	49	1789				
20T	8	2015	Tat	7903			121				
22T	8	2015	Tat	22280		427	82				
23T	8	2015	Tat	12433		123					
24T	8	2015	Tat	5993		14	20				
25T	8	2015	Tat	130		123	424	683			
26T	9	2015	Tat	5490		45					
27T	9	2015	Tat	17928		2516	19				99
28T	9	2015	Tat	18639		178	436	49			
29T	9	2015	Tat				5609				
30T	9	2015	Tat	10143		4532	20				49
35M	4	2014	Mak	47	1670						
36M	4	2014	Mak	17	273					85	
38M	4	2014	Mak		328						
40M	4	2014	Mak		431						
41M	4	2014	Mak		1498						
43M	4	2014	Mak		30				1081		
48M	4	2014	Mak							2019	
49M	4	2014	Mak	17	1451						
50M	4	2014	Mak	187				680			
52M	5	2014	Mak		1080						
57M	5	2014	Mak		1602						
62M	5	2014	Mak		3714						
63M	5	2014	Mak	611	42				12		
64M	5	2014	Mak		15				17045		
65M	5	2014	Mak		502						
66M	5	2014	Mak						641		

68M	5	2014	Mak			93			7907		
70M	5	2014	Mak	13810		589			543		
71M	5	2014	Mak	6540					142		
72M	5	2014	Mak	31	2171						
73M	5	2014	Mak	173	3686	90					
79M	5	2014	Mak	33		29		29		160	
80M	5	2014	Mak						1686		
81M	5	2014	Mak		5883						
83M	7	2014	Mak							2947	
84M	7	2014	Mak	380							
85M	7	2014	Mak	15						292	
86M	7	2014	Mak	489							
87M	7	2014	Mak	270		62			21		
92M	9	2014	Mak	39	2452					31	
94M	9	2014	Mak	11	1462						
98M	11	2014	Mak			466					
99M	11	2014	Mak	172							
102M	11	2014	Mak	374							
107M	11	2014	Mak	838				10			
108M	11	2014	Mak	12							
109M	11	2014	Mak	240							
110M	11	2014	Mak	282		2108					
111M	4	2015	Mak		1072						
112M	4	2015	Mak						2925		
113M	4	2015	Mak		1692						
115M	4	2015	Mak	11	132				1476		
118M	4	2015	Mak	1540		384					
120M	4	2015	Mak		2126	13					
121M	4	2015	Mak		5000						
123M	4	2015	Mak		403						
127M	4	2015	Mak	121	22	62					
129M	4	2015	Mak		3728						
130M	4	2015	Mak		14695	88					
132M	4	2015	Mak		1753						
133M	4	2015	Mak		495						
134M	4	2015	Mak	4890					15	12	
140M	4	2015	Mak		3263						
141M	4	2015	Mak		2528						
147M	4	2015	Mak		3237						
149M	4	2015	Mak		3765	11					
155M	4	2015	Mak	422	698	3613	58				
159M	4	2015	Mak	5120						2784	
160M	4	2015	Mak		5249						

161M	4	2015	Mak		1750						
164M	4	2015	Mak		5222						
166M	4	2015	Mak	8		201			1293		
167M	4	2015	Mak		980						
169M	4	2015	Mak	204		1124					
171M	4	2015	Mak		4699						
173M	4	2015	Mak		4016						
174M	4	2015	Mak		2728						
175M	4	2015	Mak	6196		36				7564	
176M	5	2015	Mak	7385					17		
177M	5	2015	Mak	3929		3066	249			95	
179M	5	2015	Mak		7672						
180M	5	2015	Mak		11555						
181M	5	2015	Mak		1328						
183M	5	2015	Mak		5423						
184M	5	2015	Mak		5328						
196M	5	2015	Mak		47				10138		
198M	5	2015	Mak	229	669				75		
199M	5	2015	Mak		4847						
200M	5	2015	Mak		2482						
201M	5	2015	Mak	827	94	236	13				
206M	6	2015	Mak					3813			
210T	10	2015	Tat	7818				684			
211T	10	2015	Tat	13934		2440	1723				
212T	10	2015	Tat	361			3492				
213T	10	2015	Tat	9561		107	8034	1251			
214T	10	2015	Tat	5115		263	1232				42
215T	10	2015	Tat	11162		36	7549	148			
216T	10	2015	Tat	3436	2660		165				
217T	10	2015	Tat	19372			88				57
218T	10	2015	Tat	4047			2291				
220T	10	2015	Tat	9386		6952	1123				
221T	10	2015	Tat	3352		59					
222T	10	2015	Tat	7625		74	1774				92
228T	1	2016	Tat	5727		20	144	155			24
229T	1	2016	Tat	5349			542	1945			
230T	1	2016	Tat			131		324			
231T	1	2016	Tat	3920	302			1232			
232T	1	2016	Tat	16386		369	280	637			
233T	1	2016	Tat	468				1110			
234T	1	2016	Tat	5323			267	816			
235T	1	2016	Tat	2245		45		335			
236T	1	2016	Tat	851	471					101	

239T	1	2016	Tat	4839		20	359	28			
240T	1	2016	Tat	351							
241T	1	2016	Tat					2562			
242T	1	2016	Tat	172				1259			
246T	2	2016	Tat	5367		4856	49	19			
247T	2	2016	Tat					3021			
248T	2	2016	Tat	5144			228				
250T	2	2016	Tat	3238			18				
251T	2	2016	Tat	4470		12	49	57			
253T	2	2016	Tat	1909							
255T	2	2016	Tat	765			212				
257T	2	2016	Tat	6074		46	39				
265T	3	2016	Tat	1003			63	12			
266T	3	2016	Tat	4560		10	95				17
267T	3	2016	Tat	358		67	834	38			
268T	3	2016	Tat	245		686					
270T	3	2016	Tat	8581			10				
272T	3	2016	Tat	15352		85	1233				
275T	3	2016	Tat	4054			184				
276T	3	2016	Tat	357		17	18	19			
277T	3	2016	Tat	38		80	12	46			

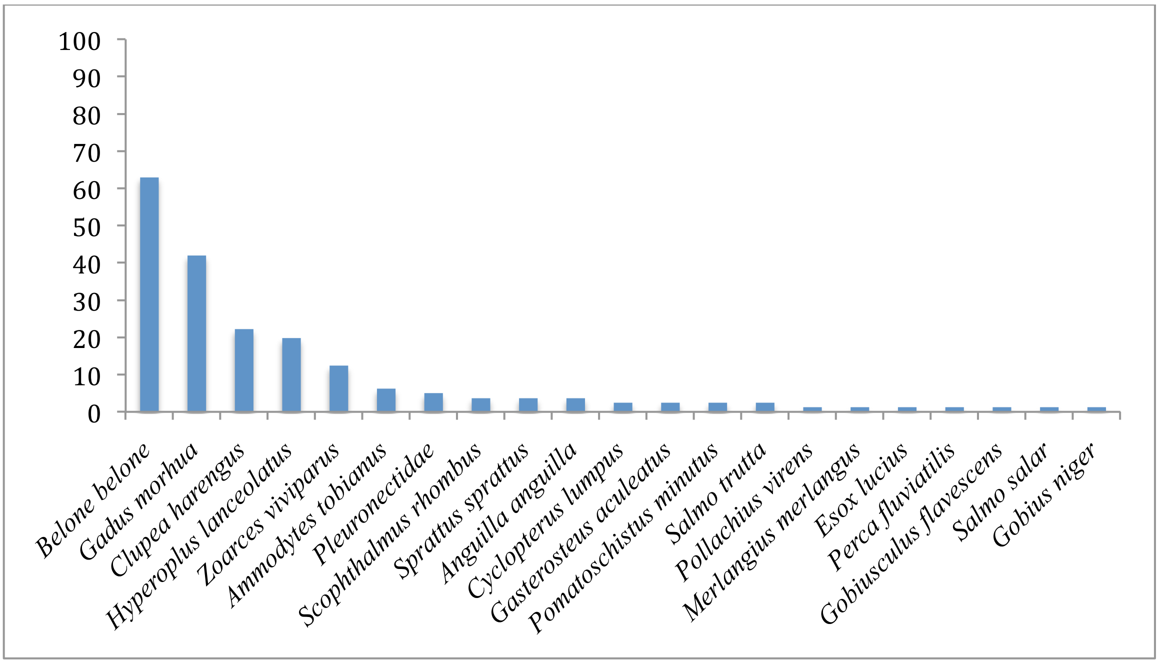

Figure 1 – 20 fish taxa were found in a total of 81 samples from Måkläppen. The data is calculated as the Frequency of Occurrence (FO_i) of each fish species. The most common species was garfish with a frequency of occurrence of 62.96%.

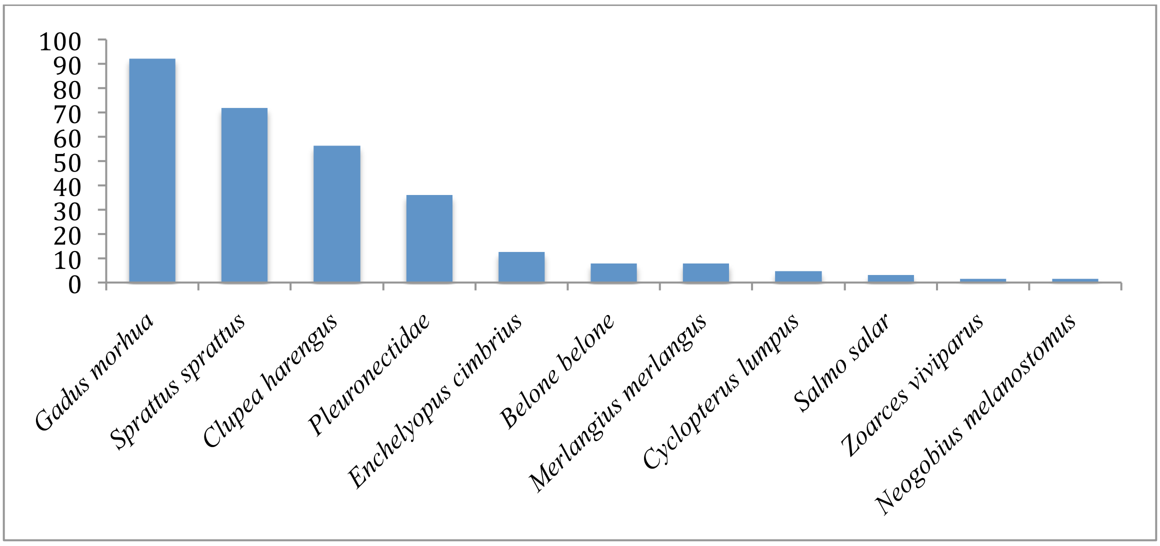

Figure 2 – 10 fish species and 1 family were found in a total of 64 scat samples from Tat in the southern Baltic Sea. The most common species was cod with a frequency of occurrence of 62.19%.

Table 3 – Statistical P values for the comparison between the two locations. The shown species contribute to the overall diet with more than 5 % FO_i.

Prey	**P value**
Gadus morhua	<0.00001
Belone belone	<0.00001
Clupea harengus	<0.00001
Sprattus sprattus	<0.00001
Pleuronectidae	<0.00001
Hyperoplus lanceulatus	<0.00001
Zoarces viviparus	0.023
Enchelyopus cimbrius	0.001

Table 4 – Statistical P values for the comparison between seasons from Måkläppen. The shown species contribute to the overall diet with more than 10 % FO_i.

	P value		
Prey	**Spring-Summer**	**Spring-Autumn**	**Summer-Autumn**
Belone belone	0.0007	0.004	0.49
Gadus morhua	0.18	0.002	0.53
Clupea harengus	1	1	1
Hyperoplus laneolatus	1		
Zoarces viviparus	0.16	1	0.52

Table 5 – Statistical P values for the comparison between seasons from Tat. The shown species contribute to the overall diet with more than 10 % FO_i.

	P value					
Prey	**Winter-Spring**	**Winter-Summer**	**Winter-Autumn**	**Spring-Summer**	**Spring-Autumn**	**Summer-Autumn**
Gadus morhua	0.27	1	0.61	1	1	1
Sprattus sprattus	0.08	0.28	0.009	1	1	1
Clupea harengus	0.16	0.28	0.19	1	1	1
Pleuronetidae	0.08	0.001	0.01	0.32	0.7	0.35
Enchelyopus cimbrius	0.54		0.07		0.43	

Table 6 – Statistical P values for the comparison between the years 2014 spring and 2015 spring from Måkläppen. The shown species are the most abundant found in both the years.

Prey	**P value**
Belone belone	0.38
Gadus morhua	0.29
Clupea harengus	0.54
Hyperoplus lanceolatus	0.14
Zoarces viviparus	0.7
Ammodytes tobianus	0.34

Table 7 – Statistical P values for the comparison between the fish atlas data from the Belts and the seal diet data from Måkläppen.

Prey	**P value**
Belone belone	<0.00001
Gadus morhua	0.033
Clupea harengus	0.02
Hyperoplus lanceolatus	<0.00001
Zoarces viviparus	<0.00001
Ammodytes tobianus / sp.	0.24
Pleuronectidae	<0.00001
Scophthalmus rhombus	0.06
Sprattus sprattus	0.38
Anguilla anguilla	<0.00001
Cyclopterus lumpus	0.33
Gasterosterus aculeatus	0.005
Pomatoschistus minutus	0.09
Salmo trutta	<0.00001
Pollachius virens	0.37
Merlangius merlangus	0.00003
Esox lucius	0.06
Perca fluviatilis	0.02
Gobiusculus flavescens	0.09
Salmo salar	1
Gobius niger	0.00019

Table 8 – Statistical P values for the comparison between the fish atlas data from the Bornholm area and the seal diet data from Tat.

Prey	**P value**
Gadus morhua	0.000016
Sprattus sprattus	<0.00001
Clupea harengus	0.000045
Pleuronectidae	<0.00001
Enchelyopus cimbrius	0.392
Belone belone	0.038
Merlangius merlangus	0.000073
Cyclopterus lumpus	0.195
Salmo salar	0.765
Zoarces viviparus	0.054
Neogobius melanostomus	0.016

Table 9 – Statistical P values for the comparison between the fish atlas data from the Belts area and the seal diet data from Måkläppen in spring.

Prey	**P value**
Belone belone	<0.00001
Gadus morhua	0.527
Clupea harengus	0.00029
Hyperoplus lanceolatus	<0.00001
Zoarces viviparus	0.00018
Ammodytes tobianus / sp.	0.59
Pleuronectidae	<0.00001
Scophthalmus rhombus	0.618
Sprattus sprattus	0.619
Anguilla anguilla	0.00006
Cyclopterus lumpus	0.038
Gasterosterus aculeatus	0.056
Pomatoschistus minutus	0.58
Salmo trutta	<0.0001
Pollachius virens	1
Merlangius merlangus	0.0047
Esox lucius	0.055
Perca fluviatilis	0.176
Gobiusculus flavescens	0.71
Salmo salar	1
Gobius niger	0.055

Table 10 – Statistical P values for the comparison between the fish atlas data from the Bornholm area and the seal diet data from Tat in winter.

Prey	**P value**
Gadus morhua	0.577
Pleuronectidae	0.28
Sprattus sprattus	0.026
Clupea harengus	0.45
Belone belone	0.01
Merlangius merlangus	0.044
Cyclopterus lumpus	0.315
Enchelyopus cimbrius	1
Salmo salar	0.7
Zoarces viviparus	0.1
Neogobius melanostomus	0.1

Table 11 – Statistical P values for the comparison between the fish atlas data from the Bornholm area and the seal diet data from Tat in spring.

Prey	P value
Gadus morhua	0.024
Sprattus sprattus	0.00049
Clupea harengus	0.021
Pleuronectidae	0.012
Enchelypous cimbrius	0.53

Table 12 – Statistical P values for the comparison between the fish atlas data from the Bornholm area and the seal diet data from Tat in summer.

Prey	P value
Gadus morhua	0.00066
Sprattus sprattus	<0.00001
Clupea harengus	0.00002
Belone belone	0.136
Pleuronectidae	0.00016

Table 13 – Statistical P values for the comparison between the fish atlas data from the Bornholm area and the seal diet data from Tat in autumn.

Prey	P value
Gadus morhua	0.21
Sprattus sprattus	0.00079
Clupea harengus	0.079
Enchelyopus cimbrius	0.77
Pleuronectidae	0.00013
Merlangius merlangus	0.12
Belone belone	0.13
Cyclopterus lumpus	0.248
Salmo salar	0.19