Differences in Adult and Chick Diet in Common Guillemots *Uria aalge* in the Baltic Sea

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Independent project • 15 credits
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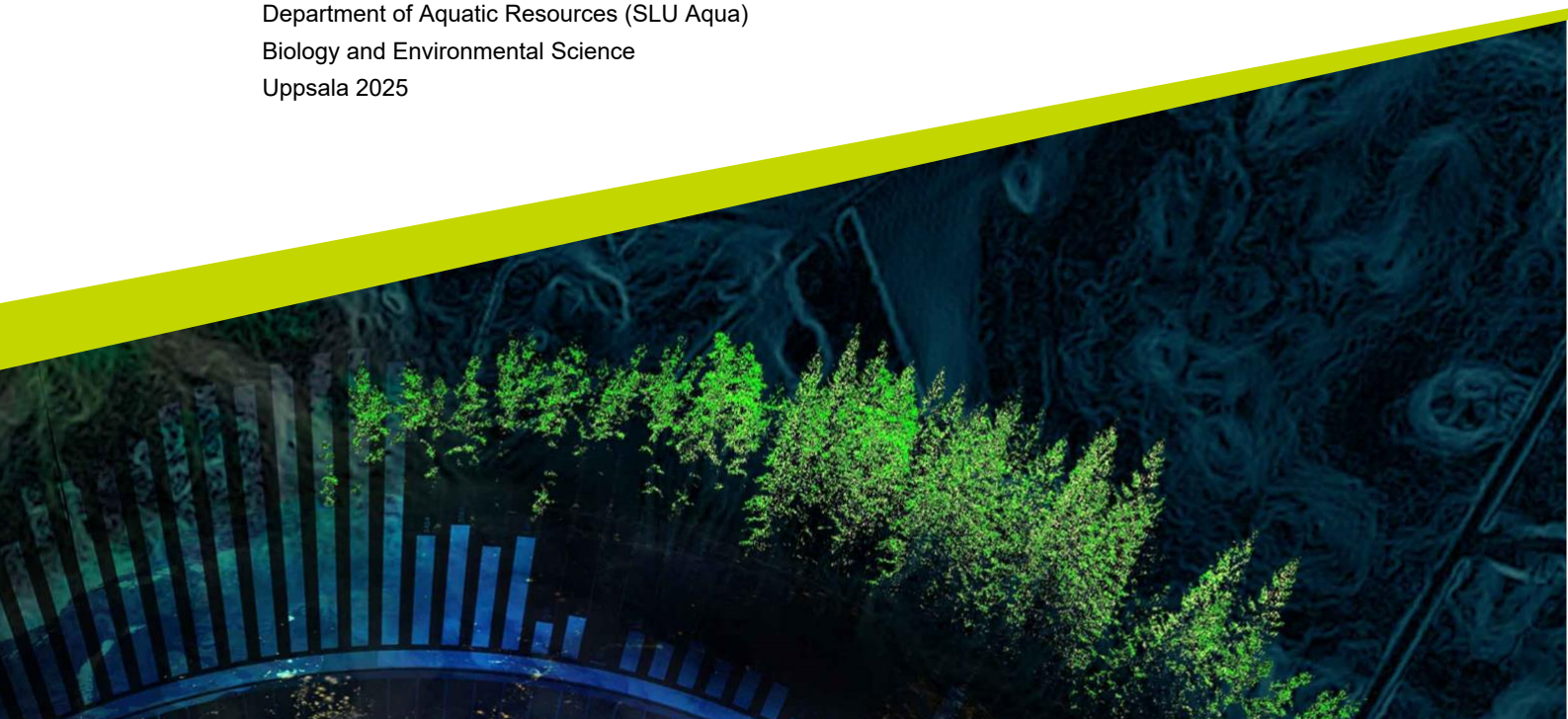
Biology and Environmental Science
Uppsala 2025



Differences in Adult and Chick Diet in Common Guillemots *Uria aalge* in the Baltic Sea.

Skillnader i dieten mellan vuxna och unga sillgrisslor i Östersjön.

Felicia Svensson Israelsson

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Credits:	15
Level:	First cycle, G2E
Course title:	Independent project in Biology
Course code:	EX0894
Programme/education:	Biology and Environmental Science
Course coordinating dept:	Department of Aquatic Resources (SLU Aqua)
Place of publication:	Uppsala
Year of publication:	2025
Copyright:	Example: All featured images are used with permission from the copyright owner.
Keywords:	Common Guillemot, diet, Stora Karlsö, eDNA, dPCR, seabird

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Abstract

Seabirds, including the Common Guillemot *Uria aalge*, are important bioindicators of marine ecosystems. Understanding their dietary habits provides critical insights into habitat status and changes in food availability. The aim of this study was to gain a deeper insight into the adult Common Guillemot diet in the Baltic Sea and investigate whether it differs from previously investigated diet of chicks. In this study, 50 adult faecal samples were collected at Stora Karlsö, the largest Common Guillemot colony in the Baltic Sea, prior to chick hatching in June 2024. DNA concentrations from three key prey species – sprat *Sprattus sprattus*, herring *Clupea harengus* and three-spined stickleback *Gasterosteus aculeatus* – were quantified using digital PCR (dPCR), a type of environmental DNA (eDNA) method. The results showed that the adult diet consists of a more diverse composition and other proportions than illustrated in previous adult diet research. This may be due to seasonal or time effects with differences in prey abundance. Furthermore, the result revealed a more diverse adult diet (45% sprat, 27% herring, 27% stickleback) compared to the chick diet (71% sprat, 23% herring, 6% stickleback), with a statistically significant difference confirmed by a Chi-square test of independence ($p < 0.00001$). These findings indicate that the adult birds self-feeding differs from their chick provisioning, likely reflecting differences in their nutritional requirements and foraging strategies. Since the Common Guillemot is a single-prey loader, it is probably more efficient for the adult to bring a large fish of high calorific value back to their chick. While the parents for self-feeding can make do with smaller, low-quality fish. The study emphasizes the value of eDNA analysis in faecal samples for seabird dietary research. It also highlights the need for further studies to deepen our understanding of the dietary differences between adult and chick Common Guillemots in the Baltic Sea. For instance, it would be interesting to monitor the same individuals over time to examine whether the adults consistently target the same prey when self-feeding, or if their diet varies from day to day.

Keywords: Common Guillemot, diet, Stora Karlsö, eDNA, dPCR, seabird

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1. Introduction

Seabirds, including the Common Guillemot *Uria aalge*, have numerous attributes that makes them important bioindicators of marine ecosystems. For instance, they are top predators, easy to identify and monitor, they are affected by human activities and they react quickly to environmental changes (Parsons et al. 2008; Olsson & Hentati-Sundberg 2017; Rajpar et al. 2018). Hence, the seabirds can provide valuable knowledge about their habitat status and changes in food availability (Rajpar et al. 2018). For instance, changes in the Common Guillemot chick diet have been reported to be influenced by fishing impacts and changes in sea temperature (Anderson et al. 2014). Therefore, the diet of seabirds can be a valuable indicator of shifts in forage fish distribution and quantity.

One of the most researched marine ecosystems globally, with numerous studies covering various ecological and environmental parameters, is the Baltic Sea (Reusch et al. 2018). Not to mention the island of Stora Karlsö, which is the most significant breeding site for Common Guillemots and of great importance for scientific studies in the area. The first recorded mention of the species on Stora Karlsö was in 1741 by Carl von Linné. During the 19th century the population declined rapidly with only 20 individuals left by 1880. The underlying cause was presumably intensive egg collecting and hunting. However, shortly after a conservation organization purchased the island the population began to recover (Hedgren 1975). Today, the Swedish island host over half, possibly up to 70%, of the Common Guillemot population in the Baltic Sea (Olsson & Hentati-Sundberg 2017), with close to 25 000 breeding pairs (Hentati-Sundberg 2025).

The Common Guillemot is a colonial breeder, and the colonies are often located on high rocky islets or coastal cliffs. Each clutch consists of only one egg. The egg is incubated for 32 days and the chick is guarded by its parents for about three weeks, after which the chick leaves the ledge and sets of to sea, escorted by its male parent (Hedgren 1975). The Common Guillemots that live in the Baltic Sea spend the majority of their time in the open ocean and the only time the adults stay partly on land is during the breeding season (Hedgren 1976). Nearly all that breed in the Baltic Sea remains in this region during the year, only a few migrate (Olsson et al. 2000).

The Common Guillemot is a single-prey loader, which means that the adults only bring one prey at a time when they feed their chick (Bradstreet & Brown 1985, referenced in Kadin et al. 2012). The diet of the chicks in the Baltic Sea is quite well-documented and mainly consists of sprat *Sprattus sprattus* (Hedgren 1976; Österblom & Olsson 2002; Österblom et al. 2006; Kadin et al. 2016). In the 1970s

the chick diet at Stora Karlsö consisted of 92% sprat (Hedgren 1976). Similar result was reported in the 2000s where > 90% of the food fed to the chicks were clupeids, most likely sprat (Österblom & Olsson 2002; Kadin et al. 2016). However, the diet of adult Common Guillemots in the Baltic Sea remains much more unclear. The first and probably the only study of this topic was conducted in the 1990s and it examined the food choice of 64 offshore adults that had drowned in fishing nets around Christiansø in Denmark and south of Gotland. Their results also revealed a strong predominance of sprat (97%), which might imply that the adults specifically targeting them when self-feeding (Lyngs & Durinck 1998). On the one hand, the diet of chicks and adults appear to be similar and this might be a result of the calorific value in general being higher in sprat than in for instance young herrings (Hislop et al. 1991, referenced in Lyngs & Durinck 1998). On the other hand, there are no studies that have focused on analysing potential dietary differences in adult and chick Common Guillemots in the Baltic Sea. However, there are studies of Common Guillemots outside this area that have confirmed that the observed chick diet do not reflect the food choice of the adults (Mehlum 2001; Wilson et al. 2004; Sonntag & Hüppop 2005; Bugge et al. 2011). Due to that Common Guillemots only bring one fish at time to feed the chick, the best strategy should be to target a large prey item of high calorific value (Österblom et al. 2006). However, for self-feeding the more opportunistic adults can make do with smaller and lower-quality fish that would not be suitable to carry back to the colony (Sonntag & Hüppop 2005). This is aligned with the central-place foraging theory, which implies that adults aim to maximize the energy obtained for each unit of effort spent foraging, thus enhancing their chick's energy intake over time (Orians & Pearson 1979, referenced in Bugge et al. 2011). Additionally, variations in diet between adult and chick seabirds are often associated with that they have different nutritional needs, for instance the chicks have higher energetic demands to grow than the adults (Baird 1991).

The reason the adult diet is less well-documented than the diet of the chicks, lies in the difficulties of finding an effective and ethical method of collecting field data. Firstly, it is difficult to observe what the adults eat because they capture and swallow their prey underwater (Sonntag & Hüppop 2005). Secondly, methods to be able to examine the stomach content requires either stomach flushing or killing the seabirds, which brings up ethical concerns (Barrett et al. 2007). However, genetic traces are left by organisms in their surroundings, including in soil, water and faeces, and are referred to as environmental DNA or eDNA (Good et al. 2024). A valuable method to study the diet that is non-invasive and enables large sample sizes is to analyse the faeces. Prey DNA found in animal faeces can serve as an important source of information for understanding dietary habits (Barrett et al. 2007), for instance dietary trends in seabirds (Deagle et al. 2007). DNA-metabarcoding is a quite new common technique in this field that has minimal

impact on the seabirds. By using universal primers with broad taxonomic reach, it is an effective method to obtain a comprehensive picture of the prey species composition of the diet. However, DNA-metabarcoding cannot be used to accurately quantify prey species abundance (de Leeuw et al. 2024). Quantitative PCR (qPCR) on the other hand, offers the possibility to quantitatively assess the abundance by using species-specific primers. That said, you need to design a separate assay for each target species you want to study (Murray et al. 2011), and to obtain absolute quantification, reference samples or standard curves are needed. Therefore, an even more effective method with increased sensitivity is digital PCR (dPCR), which do not need references or standard curves. The difference is that before amplification dPCR divides the sample into thousands of individual reactions, instead of qPCR that does bulk analysis. One of the advantages of this approach is that dPCR has a higher sensitivity for identifying low concentrations of DNA and small differences (*dPCR for Beginners* u.å.). In this study, the abundance of three key prey species, sprat *Sprattus sprattus*, herring *Clupea harengus* and three-spined stickleback *Gasterosteus aculeatus*, was examined in adult Common Guillemot faeces by using dPCR.

1.1 Aims and Objectives

The aim of this study is to gain a deeper insight into the adult Common Guillemot diet in the Baltic Sea and examine whether it differs from the chick diet. There are no previous studies that have analysed possible differences in adult and chick diet. Understanding the diet of the species is crucial for determining their habitat status and whether there is sufficient food available. While the diet of chicks is quite well-documented, the adult diet remains much more unclear due to the difficulties of finding an effective method of collecting field data. With the introduction of a quite new method, eDNA, we may finally be able to understand: How does the diet of adult and chick Common Guillemots in the Baltic Sea differ?

Based on existing research, I hypothesize that there are dietary differences between adult and chick Common Guillemots. The diet provided to chicks should not reflect what is optimal for the adult's self-feeding, due to that they are single-prey loaders. Furthermore, differences in the diet of adult and chick Common Guillemots have been reported by studies outside the Baltic Sea.

2. Materials and Methods

2.1 Study Site: Stora Karlsö

The island of Stora Karlsö (57°17'N, 18°58'E) has been a central key for both ornithological research and tourism for a long time. Stora Karlsö stands out as the most significant breeding site for Common Guillemots in the Baltic Sea, with close to 25 000 breeding pairs (Hentati-Sundberg 2025). Given the high level of public engagement and extensive research activity, efforts have long been directed towards developing low-impact methods that still enable detailed scientific study. Therefore, an artificial breeding site was constructed in the middle of the Common Guillemot colony in 2008 (Hentati-Sundberg et al. 2012). Today it is known as the Karlsö Auk Lab, and it consists of 35 breeding ledges with the capacity to hold at least 300 Common Guillemot breeding pairs (Hentati-Sundberg et al. 2025). It is made of a steel-frame structure with oak panel walls and the ledges are coated with limestone to replicate their natural cliff formations. Furthermore, the inside area, cameras and advanced technology enables researchers to closely monitor and study the seabirds. For instance, the technology can be used to study the Common Guillemot presence and behaviour (Olin et al. 2023).

2.1.1 Field Sampling

Fifty faecal samples from adult Common Guillemots were collected at the Auk Lab over a six-day period in late June 2024. The goal was to conduct the sampling before chick hatching began, ensuring that the faeces originated exclusively from adult birds. However, due to time constraints, a few of the final samples was collected when chicks had already hatched, but where it was certain that the faeces came from an adult. The samples were collected opportunistically, without a predetermined selection based on time of day, nesting site or specific nesting pairs.

Fresh faeces (i.e., not dried) was collected using a long spatula directly from the nesting site and the spatula was discarded after each use. One by one, the samples was placed into a labelled 50 ml Falcon tubes, which was then sealed and stored in an individual ziplock bag. The date, time, nesting site (ledge), and pair ID were recorded for all samples. The material was collected in batches and transported from the Auk Lab to the office. Each Falcon tube was then filled with 98% ethanol to preserve the sample, using a volume at least 15 times greater than the amount of faecal material. The ethanol preservation was conducted within 30 minutes after collection. Lastly, the tubes were sealed with Parafilm, returned to their respective ziplock bag and stored in -25 degrees C freezer until further

transport for analysis. Disposable gloves were worn during sampling and changed between each collection to prevent contamination.

2.2 Lab Analysis of Adult Faecal Samples

All samples were sent to SeAnalytics AB for dPCR analysis to obtain DNA concentrations of the three prey species, sprat *Sprattus sprattus*, herring *Clupea harengus* and three-spined stickleback *Gasterosteus aculeatus*. A more in-depth description of the methods used in the lab can be found in Appendix 1.

2.2.1 DNA Extraction

The DNA extraction was done with QIAGEN Blood and Tissue kit in accordance with the manufacture's protocol. However, with the modification of longer incubation time (1 hour) until the content had dissolved. After that the DNA concentration was measured with Qubit fluorometer and an "extraction blank" was used in each extraction round.

2.2.2 Assay Development

The assays consist of primers that amplify a short fragment of the mitochondrial COI gene in the target species and a probe that is complementary to the PCR product. The dPCR assays of the two prey species herring and sprat had already been developed before by SeAnalytics AB. In previous tests, both COI assays were 100% specific. The assay of the three-spined stickleback was developed by the lab for this project. The probes for the assays were modified with different dyes.

2.2.3 Digital PCR Analysis

The concentration of DNA from the three target species was analysed with dPCR in the lab. This method divides each sample into thousands of individual reactions before amplification, which allows dPCR to obtain absolute quantification without any reference samples or standard curves (*dPCR for Beginners* u.å.). After division, all the partitions hold none, one or a few target molecules. Each microreaction is then amplified individually by the dPCR machine and the presence or absence of the target molecule in all the partitions is then determined with the fluorescent probes. A partition that contains the target DNA will emit a fluorescent signal (on) and a partition without the target will remain dark (off). This on/ off nature is the reason behind the name "digital" PCR, since it reminds of computers with information encoded with ones and zeros. The dPCR machine is only required to identify whether a partition is on or off and analyse the number of each. Lastly, Poisson statistics is used to calculate the absolute number of target DNA (*Fundamentals of digital PCR* u.å.). All 50 sample was analysed two times

to receive more accurate DNA concentrations (copies/uL). In addition, two positive controls (DNA of the three target species) and two negative controls (no template control to monitor for contamination) were used in the analysis.

2.3 Data Analysis

Firstly, a mean of the DNA concentrations (copies/uL) for each adult faecal sample was calculated, due to that all 50 samples had been analysed two times in the lab. The proportions of each prey species were then calculated in all the 50 samples individually using the mean DNA concentrations (copies/uL). In addition, mean proportions of the three prey species in the adult diet were calculated. Furthermore, mean proportions of the three prey species in the chick diet were calculated with data that was collected by PA Berglund (Baltic Seabird Project, unpublished data) during a six-year period (2019-2024).

2.3.1 Statistical Analysis

To examine if there was a statistically significant difference in the proportions of the three prey species between the adult and chick diet, a Chi-square test of independence was performed.

3. Results

3.1 Adult Diet

The mean proportion of prey species in the 50 adult Common Guillemot faecal samples was 45% sprat *Sprattus sprattus*, 27% herring *Clupea harengus* and 27% three-spined stickleback *Gasterosteus aculeatus*. An overview of the distribution of the three prey species in the samples can be found in Figure 1. While some samples contained only one prey species and a few others nearly so, most of the samples were composed of a mixture of all three species.

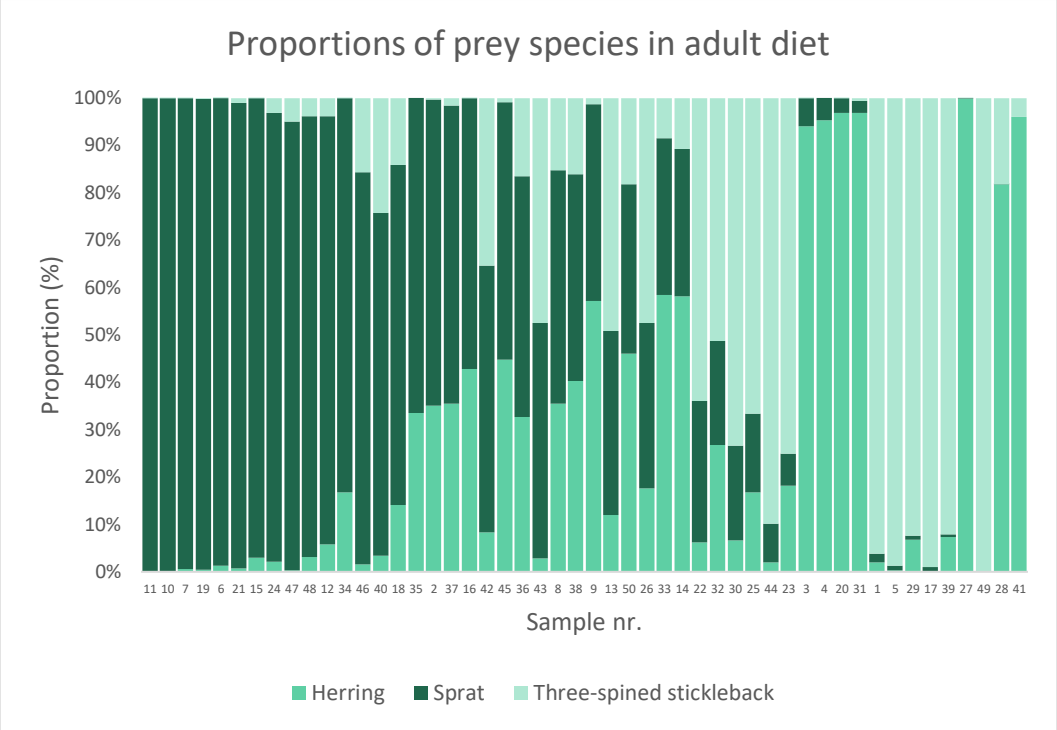

Figure 1. Proportion of sprat, herring and three-spined stickleback in 50 adult Common Guillemot faecal samples. The samples are sorted from the highest to the lowest proportion of sprat.

In Figure 2, the proportion of sprat in adult Common Guillemot diet is presented. Two samples consisted of solely sprat and three more samples was close with 99%. Furthermore, four samples did not contain sprat at all (0%). Overall, the graph presented a relatively even decrease in proportion of sprat in the 50 samples.

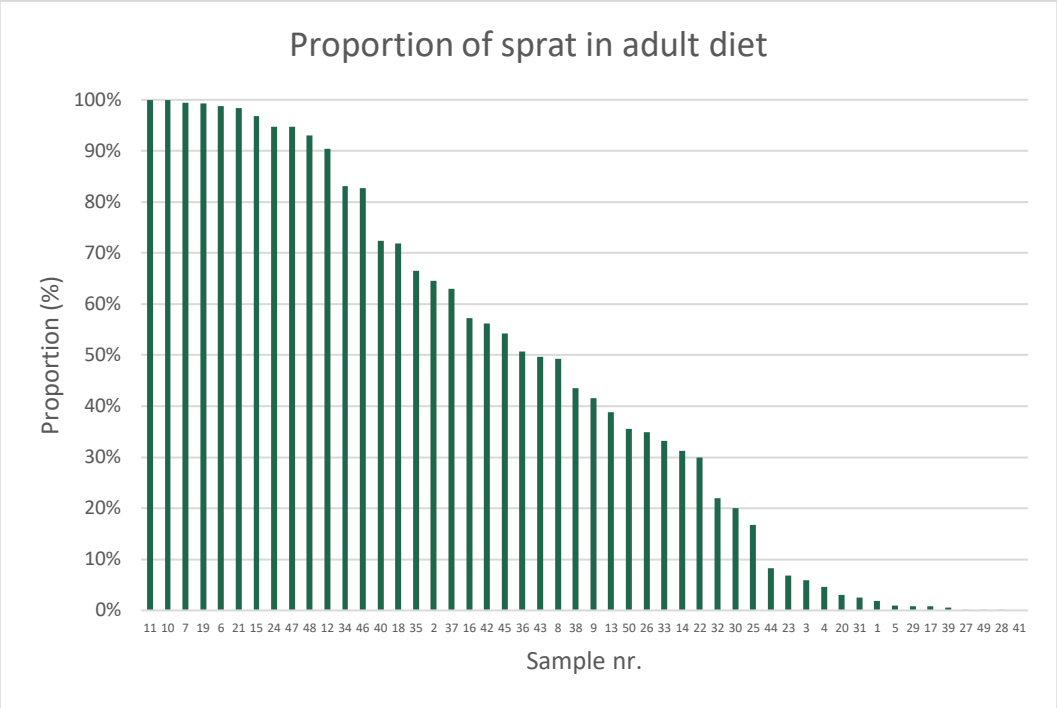

Figure 2. Proportion of sprat in 50 adult Common Guillemot faecal samples. The samples are sorted from the highest to the lowest proportion of sprat.

The proportion of herring in adult Common Guillemot diet is illustrated in Figure 3. There was only one sample that contained herring exclusively. Additionally, herring was absent (0%) in 7 samples. This graph showed a more dramatic decrease in proportion of herring than the graph of proportion of sprat did.

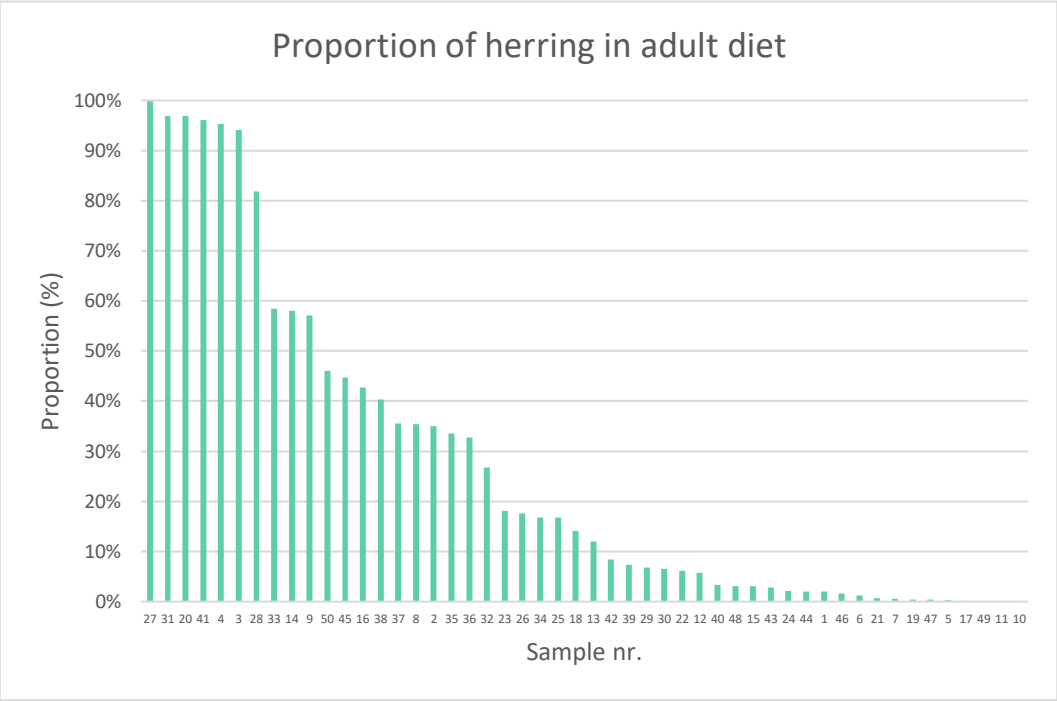

Figure 3. Proportion of herring in 50 adult Common Guillemot faecal samples. The samples are sorted from the highest to the lowest proportion of herring.

In Figure 4, the proportion of three-spined stickleback in adult Common Guillemot diet is featured. One sample contained only this prey species and two more samples nearly so with 99%. Moreover, there were 14 samples that did not contain three-spined stickleback at all (0%). This graph also showed a more rapid decrease in proportion of three-spined stickleback than the graph of proportion of sprat did.

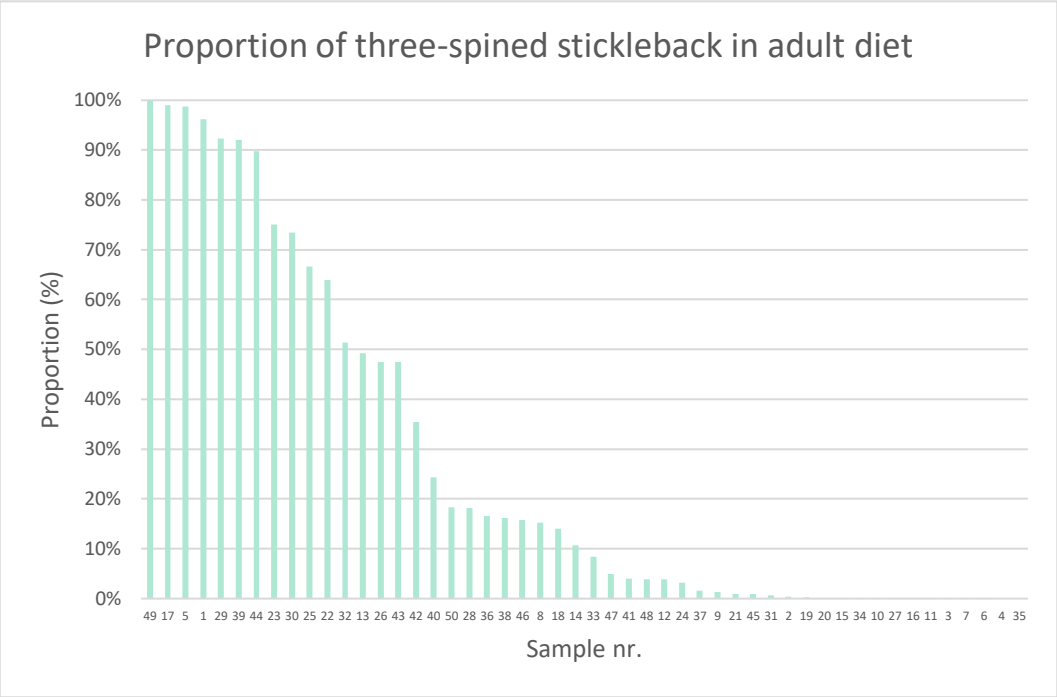

Figure 4. Proportion of three-spined stickleback in 50 adult Common Guillemot faecal samples. The samples are sorted from the highest to the lowest proportion of three-spined stickleback.

3.2 Difference in Adult and Chick Diet

The dietary differences between adult and chick Common Guillemots are illustrated in Figure 5. The adult diet composition consisted of 45% sprat compared to 71% in the chick diet. Furthermore, three-spined stickleback accounted for 27% of the adult diet, in contrast to only 6% in the chick diet. However, the proportion of herring was quite similar between the adult and chick diet, with 27% and 23% respectively. The largest differences between the diets were that the proportion of three-spined stickleback was larger in the adult diet and that sprat was less dominant than in the chick diet.

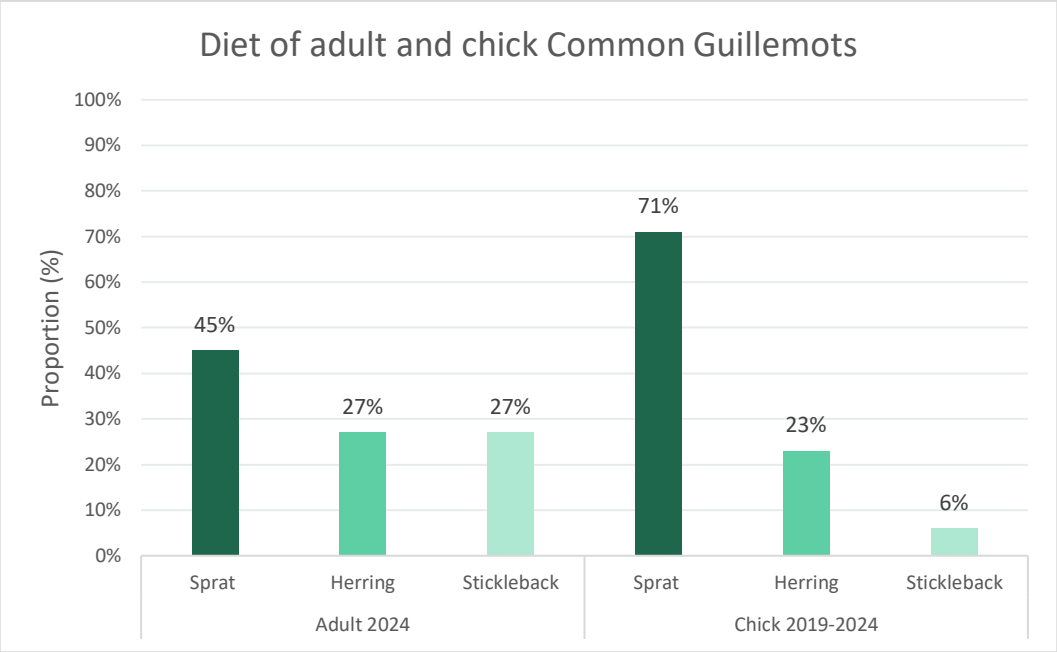

Figure 5. Proportion of sprat, herring and three-spined stickleback in adult diet 2024 and chick diet 2019-2024 in the Baltic Sea.

The Chi-square test of independence showed that there was a statistically significant difference in the proportion of the three prey species in adult and chick diet ($x^2 = 24.6$, $df = 2$, $p = 4.52 \times 10^{-6}$).

4. Discussion

This study examined the diet of adult Common Guillemots in the Baltic Sea and how it differs from the chick diet. I found that the adult diet, consisting of 45% sprat *Sprattus sprattus*, 27% herring *Clupea harengus* and 27% three-spined stickleback *Gasterosteus aculeatus*, contrasted sharply with previous research of Common Guillemot adult diet in the Baltic Sea. The first and probably the only study of adult diet reported 97% sprat dominance (Lyngs & Durinck 1998), which is a substantially larger proportion compared to the result this study presented. Their conclusion to the strong predominance of sprat was that the adults might specifically target sprat when self-feeding. According to my results, some of the adults consumed solely sprat, which could mean that those individuals target sprat when self-feeding. However, most of the samples were composed of a mixture of the three prey species, which do not indicate that the adult birds specifically target solely sprat as thought in the previous study. For instance, there were adults that exclusively fed on herring and three-spined stickleback in my study. On the other hand, the proportion of sprat was more even in all the sample and not as absent compared to the other two prey species. This might imply that sprat still is a more common prey species among the adult Common Guillemots in the Baltic Sea than herring and three-spined stickleback. However, the adult diet seems to consist of a more diverse composition and other proportions than previously reported in the Baltic Sea. These differences in the adult diet could be due to seasonal effects. Previous research examined stomach contents from birds mainly caught in fishing nets during September-November (Lyngs & Durinck 1998) unlike my study where the sample collection was carried out in June. One possible explanation to the lower sprat proportion found in this study is that sprat is less available in the spring/summer than in the fall/winter. A non-mutually exclusive, alternative explanation would be that the difference in the diet is linked to the time aspect, since previous research was carried out during 1990-1996. During the late 1980s sprat increased significantly in the Baltic Sea (Alheit et al. 2005). It is possible that sprat was predominant in the adult diet due to that the sprat was abundant when the study was conducted in the beginning of the 1990s. However, since the beginning of the 2000s there has been a large increase in three-spined stickleback in the Baltic Sea (Olsson et al. 2019; Olin et al. 2022) and that may be the reason why the proportion of stickleback is larger in the adult diet today compared to previously reported adult diet. Furthermore, sprat has decreased in the Baltic Sea since the 1990s (ICES 2024) and it is possible that the Common Guillemots has taken three-spined stickleback and herring as complements to the sprat. Moreover, my sampling was conducted at Stora Karlsö, while previous study examined seabirds outside Christiansø in Denmark and south of Gotland. Even if both

studies took place in the Baltic Sea, there is still possible that different prey abundances in the areas may have influenced the adult diet.

I also found that there is a dietary difference between adult and chick Common Guillemots in the Baltic Sea. The adult diet composition consisted of less sprat compared to the chick diet, whereas the proportion of three-spined stickleback was larger. On the other hand, the proportion of herring was quite similar between the two groups, but slightly larger in the adult diet. The composition of the chick diet has consisted of a predominance of sprat (>90%) in previous studies at Stora Karlsö (Hedgren 1976; Österblom & Olsson 2002). However, given the much less well-documented adult diet and the lack of studies investigating potential dietary differences between adult and chick Common Guillemots in the Baltic Sea, it has maybe been assumed that their diets are the same. On the other hand, studies of Common Guillemots outside the Baltic Sea have reported the opposite, showing that the chick diet does not reflect the food choice of the adults (Mehlum 2001; Wilson et al. 2004; Sonntag & Hüppop 2005; Bugge et al. 2011), which corresponds with my result. These dietary differences may be associated with the central-place foraging theory. Since the Common Guillemot is a single prey-loader there is probably more efficient for the adult to bring a larger fish of high calorific value, such as sprat, back to their chick. While the parents for self-feeding can make do with smaller, low-quality fish that is not suitable to bring back to their offspring (Sonntag & Hüppop 2005). Moreover, these differences in the adult and chick diet are not only observed in Common Guillemots, but also in studies of other single prey-loaders for instance Black Guillemots (Ewins 1986), Thick-billed Murres (Ito et al. 2010), Little Terns (Catry et al. 2006) and Crested Terns (McLeay et al. 2009). This may indicate that the dietary differences between seabird adults and chicks are influenced by their foraging strategy. In addition, dietary differences between seabird adults and chicks are often a result of their different nutritional requirements, for instance the chicks have higher energetic needs to grow than the adults (Baird 1991). Therefore, the dietary differences observed between adult and chick Common Guillemots may reflect that they have different nutritional needs to meet.

4.1 Limitations

Analysing seabird faeces using eDNA has proven to be an effective method for understanding their dietary habits. Furthermore, the method is non-invasive and has little to no impact on the seabirds, especially when compared to more intrusive techniques such as stomach flushing or killing the birds – methods that raise ethical concerns (Barrett et al. 2007). However, when analysing faeces, it is important to consider that no breeding site is sterile, is contains layers of older

faeces that may be included in the samples during collection. In addition, despite that gloves and tools were changed between the collection of different samples, contamination can still occur. Moreover, DNA from different prey species may degrade at varying rates during digestion, introducing prey-specific biases. Still, these biases appear to be less significant than those usually found in traditional dietary analyses (Deagle & Tollit 2007).

4.2 Conclusions and Future Recommendations

In conclusion, this study demonstrates that there is a clear difference between the diets of adult and chick Common Guillemots in the Baltic Sea. This knowledge is important as seabirds function as bioindicators, providing key insights into habitat conditions and prey availability. Given the limited research on the diet of adult Common Guillemots in the Baltic Sea, there is a clear need for further studies of this topic to deepen our understanding of the dietary differences between adults and chicks. An advantage of analysing faeces using eDNA is that it is effective and enables large sample sizes. Therefore, it would be an interesting monitoring method to use for future studies with even larger sample sizes. In this study, the sampling period for adult Common Guillemot faeces was limited to six days to be able to collect the samples before the chicks hatched. Therefore, no consideration of time of day, nesting site or breeding pairs was taken. These are factors that would be interesting to implement and analyse in future research. For instance, it would be interesting to monitor the same individuals over time to examine whether they show preferences and consistently target the same prey when self-feeding, or if their diet varies from day to day. Moreover, it would be interesting to include other prey species in the analysis of the adult diet. For instance, sand lance *Ammodytidae*, which has been observed in previous research both in the adult diet (Lyngs & Durinck 1998) and in the chick diet data collected by PA Berglund (Baltic Seabird Project, unpublished data).

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Appendix 1

METODER

1. DNA extraktioner
DNA extraktioner gjordes med QIAGEN Blood and Tissue kit enligt tillverkarens protokoll med en modifikation att proverna inkuberades i lysisbufferten lite längre (1 timme), tills allt innehåll löstes upp.

DNA koncentrationer mättes med Qubit fluorometer. I varje extraktionsomgång inkluderades en negativ kontroll (”extraktionsblank”) – ett Eppendorf rör fyllt med lysisbufferten som inkuberades och processades tillsammans med proverna.

Totala DNA koncentrationer i proverna varierade mellan < 0,2 och 10 ng/ µl (alla DNA koncentrationer finns i en tabell). Inget DNA uppmättes i negativa extraktionskontroller.

2. Assay utveckling
dPCR assayer för sill (Clupea harengus) och skarpsill (Sprattus sprattus) har tidigare utvecklats av SeAnalytics AB. Assayer består av primrar som amplifierar en kort (133 bp.) fragment av mitokondriell COI-genen i respektive art och en prob som är komplementär till PCR produkten (Tabell 1). I tidigare tester med DNA extraherat från vävnaden av de två arterna visade sig bägge COI assayerna att vara 100 % specifika.

Assayen för storspigg (Gasterosteus aculeatus) utvecklades för detta projekt. COI sekvenser för svenska spiggar (storspigg, småspigg och tångspigg) laddades ner från BOLD databasen och visade 15-18 % divergens mellan arterna. Primrar och prob för storspigg valdes enligt QIAGEN dPCR rekommendationer och med så många nukleotidskillnader till två övriga arter som möjligt.

Prober för de assayerna är modifierade med olika färgämnen (FAM, CY5 och HEX) bundna i 5’ änden och BHQ (”Black-hole quencher”)-modifikationen av 3’ änden (quenchern skuggar färgämnet i den intakta proben men släpper det när proben sätter sig på PCR produkten).

Tabell 1. Primer och prob sekvenser, PCR produkt längd i baspar och beräknad annealing temperatur.

Assay 1: Ch_COI (sill, mtDNA, 133 bp, Ta=54°)

Forward primer	TGCAGGAGCATCAGTTG
Reverse primer	AGGCGTTTGGTATTGTGA
Prob	FAM – TATGAAACCACCCGCAATC – BHQ1
Assay 2: COI_Ss (skarpsill, mtDNA, 133 bp, Ta=58°)	
Forward primer	CACGCGGGGGCATCG
Reverse primer	CAGGGGTGTTTGGTATTGTGA
Prob	Cy5 - AATATGAAGCCGCCCTCAATT - BHQ2
Assay 3: GG_COI (storspigg, mtDNA, 113 bp, Ta=58°)	
Forward primer	TTATCCACCCCTCTCTGGG
Reverse primer	TAATGAAGTTGATTGCCCCCAG
Prob	HEX – CCTCGCCCATGCAGGTGCT – BHQ1

3. dPCR analyser

Koncentration av DNA från målarterna strömming och skarpsill i proverna analyserades med hjälp av digital PCR (dPCR). Metoden beskrivs i på QiaGens webbplats: https://www.qiagen.com/us/applications/digital-pcr/beginners

Proverna analyserades i 26K-nanoplattor där varje platta rymmer 24 dPCR reaktioner och varje reaktion delas i ca 26 tusen partitioner. Varje prov analyserades i duplikat för att få mer exakta mål-DNA koncentrationer. Varje platta inkluderade 10 prover (x2 duplikat), en positiv kontroll (blandning av sill och skarpsill DNA extraherat från vävnad och utspätt till ca 0.05 ng/ul) och en negativ kontroll (”NTC” – no template kontroll), kontrollerna också i duplikat.

PCR utfördes i 40 µl reaktionsvolym innehållande 10 µl Qiagen x4 Probe mix, 1 µl av varje primer-prob assay (slutliga primer:prob koncentrationer 600:300 nm), 10 µl av DNA och 18 µl vatten, till 40 µl totalvolym. PCR cykling parametrar finns i Tabell 2. Imaging gjordes med default-parametrar för de olika färgkanalerna.

Tabell 2. PCR cykling parametrar

Cykler	Temperatur	Tid
1x	95° C	2 min
40 x	95° C	30 sec
	56° C	1 min

Analys av dPCR resultat: efter körningen kontrollerades dPCR kvalitet för varje platta genom antal valida partitioner för varje prov (bör ligga kring 25 000); stark signal i positiva kontroller; ingen signal i non-templat kontroller; och jämn fördelning av positiva partitioner över provytan på 1D-plot.

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