A recent portrait of grey seal diet in the Gulf of St-Lawrence between 2015-22

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Abstract

In the last 30 years, Atlantic grey seal populations have increased from 7,300 to 434,300. Concurrently, cod, white hake, winter skate and other fish populations in the Gulf of St-Lawrence have declined, with no signs of recovery. These changes were a direct or indirect result of overfishing, but its persistence, despite severe reductions in fishing efforts, is thought to be due to other factors. One causal explanation is increased predation by grey seals. Understanding grey seal diet is hence an important step towards determining their role in the ecosystem and their impact on fish populations. We reconstructed the diet of grey seals from digestive tracts harvested at three different areas of the Gulf of St-Lawrence between 2015-22. We used GLMM, to explore differences in consumption and contribution of sandlance, cod, and capelin between different years, sexes, and age-groups. These species were selected because they were identified as major prey species in reconstructed diet. We found geographical and seasonal differences in the diet of grey seals. These differences correspond to differences in prey populations, representative of the generalist predation behaviour of grey seals. We also detected interannual and ontogenic variations using GLMM in cod and capelin consumption. Overall, our study provides an up-to-date assessment of the diet composition of grey seals. Trophic interactions however are complex, our study is hence simply a piece in the puzzle to understanding the role of grey seals in the Gulf of St. Lawrence.

Introduction

Since the passing of regulations to protect marine mammals (DFO, 1993; NOAA, 1972), grey seal populations, in Atlantic Canada, have exponentially increased from approximately 7,300 in 1960s to 434,300 individuals (DFO, 2016). When a species' abundance drastically increases, they exert pressure on their environment. This is true even if such increase happens within their historic range (Alverson et al., 1988; Carey et al., 2012; Meiners, 2007; Valéry et al., 2009a, 2009b). Such overabundant species can have drastic economic and ecosystem-wide consequences: monopolising resources, spreading infectious diseases and parasites, exerting increased predation pressure, changing species composition, and more (Demaster & Sisson, 1992; Garrott et al., 1993; Koons et al., 2014). The rapid increase in grey seal abundance has hence raised concerns of their impact on their prey, including stocks of commercial importance.

Concurrent to the rapid increase in grey seals, fish populations in the Gulf of St-Lawrence have declined. The Atlantic cod, once plentiful, collapsed to the point where a moratorium was declared on its fishing (Ransom et al., 2012; Swain et al., 2019a). White hake adult abundance in the Southern Gulf is estimated to have declined by 91%, over three generations (COSEWIC, 2013). In 2014, adult abundance of winter skate was estimated to be 3% of the average in the 1970s (DFO, 2017). As a consequence, the early 1990s were marked by a shift in fish composition caused by decrease in biomass of large demersal fish and increase in the biomass of small-bodied fish (DFO, 2011) in the Gulf of St-Lawrence. These changes were a direct or indirect result of overfishing, but its persistence, despite severe reductions in fishing efforts, is thought to be due to other factors. One possible explanation is increased predation by grey seals (COSEWIC, 2013; DFO, 2011, 2017; Hammill et al., 2014; Swain et al., 2019b). Understanding grey seal diet is hence an important step towards determining their role in the ecosystem and their impact on fish populations.

Multiple techniques have been used to categorise the diet of predators: hard-part analysis, stable isotope signatures, fatty acid composition and DNA-metabarcoding (Bowen & Iverson, 2012). All methods have strengths and weaknesses; hence no single method is perfect. While others are more recent, hard-part analysis has been the commonly used method in the past (Murie & Lavigne, 1985, 1992; Jobling, 1987; Bowen & Harrison, 1994; Benoit & Bowen, 1990; W. D. Bowen et al., 1993; Jobling & Breiby, 1986; Bowen et al., 1993; Jobling & Breiby, 1986; Benoit & Bowen, 1990; Bowen & Harrison, 1994; Jobling, 1987; Murie & Lavigne, 1992, 1985). It hence allows comparisons to be made with previous diet studies. This method involves the recovery, identification, and measurement of hard parts like sagittal otoliths, cephalopod beaks or invertebrate exoskeleton from scants at haul-out sites or from digestive tracts from harvested animals. Prey weight and length are estimated by applying regression equations to measured otolith length.

In our study, we will use hard-part analysis to reconstruct the diet of grey seals from digestive tracts harvested at three different areas of the Gulf of St-Lawrence (*Fig.1*) between 2015-22. As our study spans over multiple years and geographic areas, sampled grey seals have encountered different prey assemblages, hence would differ in their foraging behaviour and diet (Bowen & Harrison, 2013; Hammill et al., 2013). In addition, grey seals exhibit sexual dimorphism, where adult males are larger and heavier than females (DFO, 2019). Adults in general also weigh more than juveniles and yearlings (NOAA, 2022). This too could lead to differences in foraging behaviour and diet (Beck et al., 2003, 2007; Lundström et al., 2005). We hence use GLMM to

explore differences in consumption and contribution of prey between different years, sexes, and age-groups.
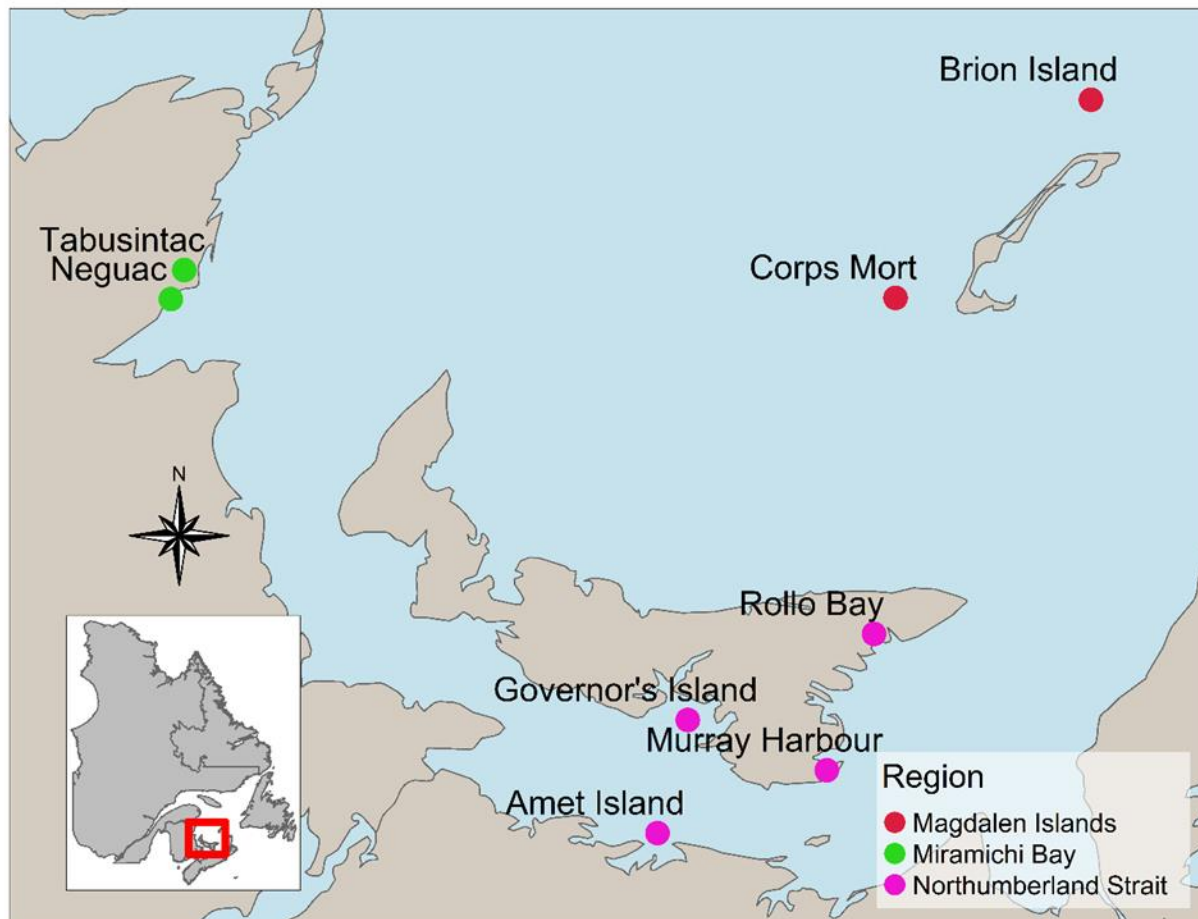

*Figure 1. **Sampling locations for our study.** 8 sites, representing 3 different areas of the Gulf of St-Lawrence, were sampled across seven years - 2015 to 2022.*

Methodology

Field

As part of the Department and Fisheries and Oceans Canada's program to monitor pinniped diet, 507 grey seals were obtained from commercial and scientific harvests conducted in the Gulf of St-Lawrence between 2015 and 2022. Grey seals were obtained from 8 different sites representing 3 different areas of the Gulf (*Fig.1*). Seals were sampled during the fall months of September and October in all areas, and also during summer months of June and July at Magdalen Islands. Sex of the seal was identified based on their reproductive organ. Two canine teeth were extracted to determine age using growth layers in tooth dentine (Cebuhar et al., 2021; Fletemeyer, 1977; Rust et al., 2019). Seals were assigned to one of three age-groups : yearling if age was less than one year, juvenile if age was between 1 and 5 years and adults if the age was above 5 years. Entire

digestive tract - stomach and intestines - were extracted and preserved at -20°C until further analysis.

Laboratory

Digestive tracts were opened along the external curve. When hard parts were absent, they were categorised as empty. Such empty digestive tracts accounted for 11% (56/507) of our sample. They were removed from subsequent analyses. When hard parts were present, they were separated from other contents using a 0.45 mm sieve. These hard parts were manually sorted based on structural similarities and conserved dry for identification. Invertebrates were identified to the order level, using cephalopod beaks or invertebrate exoskeleton. Fish were identified to the species level, whenever possible, using otoliths or undigested fish remains. Identification was done using reference collections (Fisheries and Oceans Canada, Mont-Joli, Québec) and identification guides (Campana, 2004; Härkönen, 1987). Hard parts that could not be identified to a species, because they were extremely degraded, were categorised as unidentified. For each sub-section of the digestive tract (stomach, small intestine, and large intestine), the prey species present was identified, and its hard parts counted. Based on the level of degradation, each otolith was assigned into one of three categories : D1 - perfectly conserved otolith with no signs of erosion of margins; D2 - near-perfect otolith with very few degradation marks and some signs of erosion on margins; D3 - degraded otolith with many degradation marks and highly eroded margins. When many otoliths of the same species were present within the same digestive subsection, a random subset of 30 otoliths were measured and assigned a degradation level. Altogether, this provided us information on the species present, the number of hard parts for each species, the subsection within the digestive tract where the hard parts were found, the level of degradation for each otolith and the length of each otolith.

Correcting for digestion

When passing through the gastrointestinal tract of seals, otoliths may be partially or even completely digested. This would lead to serious underestimations of otolith length and otolith count (W. D. Bowen, 2000; Cottrell et al., 1996; Dellinger & Trillmich, 1988; Murie & Lavigne, 1986a). As we use otolith length to estimate fish wet weight and size, this would create a bias in favour of smaller prey. To correct for such bias, we only used perfect or near perfect otoliths (D1-D2) to estimate fish metrics (*Supp.1*). We used mean wet weight, estimated from measured otoliths, as wet weight for fish whose otoliths were extremely degraded (D3) or not measured, using hierarchical extrapolation (*Fig.2*). To correct for underestimation of prey count, we used numerical correction factors (NCFs) (W. D. Bowen, 1991; Grellier & Hammond, 2006; Murie & Lavigne, 1986b; Phillips & Harvey, 2009; Tollit et al., 2007; Wilson et al., 2017). NCF is the ratio of the number of otoliths excreted to the number of otoliths ingested. The more fragile and smaller the otolith, the higher the possibility of complete digestion, hence the bigger the numerical correction factor. We multiplied species-specific NCFs (*Tab.1*) to otolith counts of the intestines. We did not apply NCF to the otolith recovered from the stomach because NCFs for the stomach do not exist. A previous study on hard seals, however, has shown that this does not bias estimation (Hammill et al., 2005). As each fish has two sagittal otoliths, pairing otoliths provided us with fish counts.
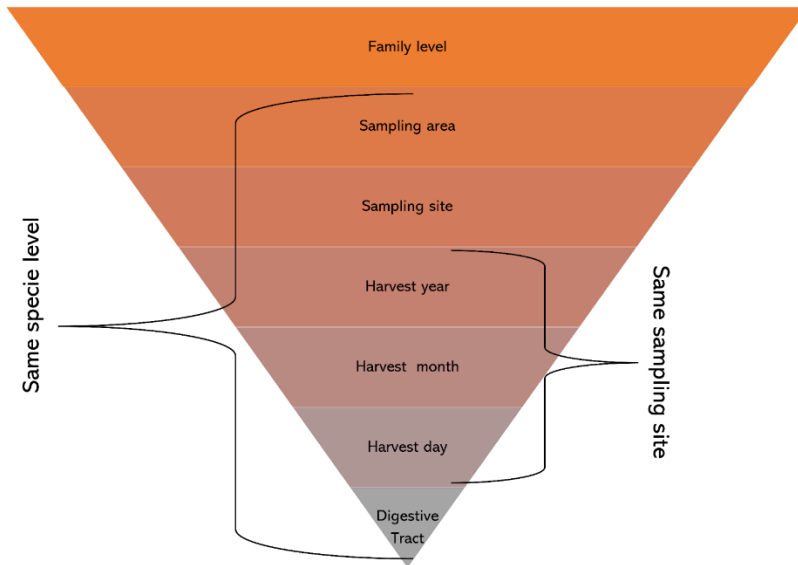
Figure 2. **Illustration of hierarchical extrapolation used to estimate the wet weight of fish whose otoliths were not measured or were extremely degraded.** We used the mean weight weight calculated from measured otoliths of a species, as wet weight for fish whose otoliths were not measured or were extremely degraded (D3), found within the same digestive tract, same day, month, and so forth.

Correction factors and regression equations are species-specific and can not be applied to unidentified fish. In addition, they have not been calculated for invertebrates. In light of this limitation of hard-part analysis, we excluded invertebrates and unidentified fish from subsequent analyses. Invertebrates accounted for 0.002% (134/63,950) of all identified prey prior to correction. Unidentified fish accounted for 0.0001% (9/63,825) of all fish prior to correction. The following invertebrates were excluded from our analyses: *Buccinum sp., Ilex sp., Cancer irroratus, Caridae sp., Chionoecetes opilio, Crangon sp., Penaeidae sp, Gammaridae sp., Homarus americanus, Hyas sp., Illex illecebrosus, Isopode sp., Nereis sp., Pandalus montaguii, Pangarus sp, Priapulus caudatus.*

Table 1. **Species-specific numerical correction factors.** (Bowen, 1991; Grellier & Hammond, 2006; Phillips & Harvey, 2009; Wilson et al., 2017; Hammill et al., 2014b).

Prey species	NCF
Ammodytes sp. (Sand lance)	2.9
Anarhichas lupus (Wolffish)	2.9
Artediellus sp. (Sculpins of genus Artediellus)	2.9
Clupea harengus (Atlantic herring)	2.9
Clupeidae sp. (Herring)	2.9
Cottidae sp. (Sculpin)	2.9
Cryptacanthodes maculatus (Wrymouth)	1.8
Enchelyopus cimbrius (Fourbeard rockling)	1.3
Eumesogrammes praecisus (Fourline snakeblenny)	1.3

Gadus sp. (Cod)	1.1
Hemithipterus americanus (Sea raven)	2.9
Hippoglossoides platessoides (American plaice)	1.2
Leptoclinus maculatus (Daubed shanny)	1.3
Limanda ferruginea (Yellowtail flounder)	1.3
Lumpenus lampretaeformis (Snakeblenny)	1.3
Lycodes sp. (Eeelpout)	1.2
Mallotus villosus (Capelin)	4.3
Melanostigma atlanticum (Atlantic soft pout)	2.1
Myoxocephalus octodecemspinosus (Longhorn sculpin)	2.8
Myoxocephalus quadricornis (Fourhorn sculpin)	2.8
Myoxocepahlus scorpius (Shorthorn sculpin)	2.8
Myoxocephalus sp. (Horned sculpins)	2.8
Osmerus mordax (Smelt)	1.9
Pleuronectidae sp. (Righteye flounder)	1.2
Pseudopleuronectes americanus (Winter flounder)	1.3
Sebastes sp. (Red fish)	1.1
Scomber scombrus (Atlantic mackerel)	1.4
Scophthalmus aquosus (Windowpane)	1.3
Stichaeus punctatus (Arctic shanny)	1.3
Tautogolabrus adspersus (Cunner)	2.9
Urophycis tenuis (White hake)	1.1
Zoarces americanus (Ocean Pout)	2.9

Diet metrics

We used frequency of occurrence (FO), numerical abundance (NA), and wet weight proportion (WW) to express diet composition. Frequency of occurrence is the number of digestive tracts where a species was present divided by the total number of digestive tracts (excluding empty digestive tracts). It describes population-wide food habits by showing what percentage of the population feeds on what prey species (*Tab.2*). Numerical abundance is the total number of individuals of a species divided by the total number of all prey, present within the same digestive tracts as the species of interest. It describes feeding behaviour of individuals by showing the number of prey ingested (*Tab.2*). Wet weight proportion is the average contribution of a species in weight divided by the total contribution of all prey in weight. It provides information on the impact on prey population. Using simulations, Ahlbeck et al. (2012) showed that wet weight proportion is the most representative of true diet. We will hence primarily use wet weight proportion to express grey seal diet. By using wet weight proportion instead of wet weight alone, we also correct for biases due to differences in sizes between different age-groups and sexes (DFO, 2019; NOAA, 2022).

Statistical analyses

      A. Estimating consumption for different sampling areas and seasons using bootstrap simulations

The foraging behaviour and diet of grey seals shows temporal and geographic variation reflecting differences in prey assemblages (W. Bowen & Harrison, 2013; M. Hammill et al., 2013). We hence

estimated consumption for each sampling area and season. In addition, grey seals also show high interindividual variability (Amanda & Sean, 2015; Badger et al., 2021; Galatius et al., 2022), leading to very high variance estimates. To hence improve the robustness of our consumption estimates, we use bootstrap simulations to resample our data. During each iteration, 60% of the data was resampled. Each digestive tract was treated as a sampling unit. Each iteration provided the mean wet weight proportions for all prey species present. This process was repeated a thousand times and the average of the mean wet weight proportions along with their standard deviation was calculated (*Tab.3*). To eliminate the effect of subsetting on the diet estimation, we re-estimated the diet using different subsets (30%, 45%, 75%, and 90%) of our data (*Tab.3*).

B. Exploring variation in diet between different years, sexes, and age-groups

In addition to temporal variation, intrinsic properties like sex and age can also affect the foraging behaviour and diet of grey seals (Beck et al., 2003, 2007; Lundström et al., 2005). To hence explore the effect of year, age-group, and sex on the presence and proportion of major prey species - sandlance, cod, and capelin - identified in previous bootstrap analysis, we used a two-part Hurdle model. To avoid the confounding effect of geography, we limited our analysis to data obtained from Magdalen Islands during Summer harvest. We chose this subset as it had the highest sample size of 240 as compared to other subsets. To model variation in presence, we used a binomial generalised linear mixed model. We used a mixed model with harvest date as our random variable, because seals harvested on the same day and location had a higher probability of having similar diet as they encountered similar prey assemblages as compared to seals harvested a few weeks apart over different harvesting events. Our model satisfied the assumptions of binomial GLMM because it was not over-dispersed and the residues for our random variable were normally distributed (*Supp.2*). To model variation in proportion, we used a beta-distributed generalised linear mixed model. The rationale for using a mixed model is the same as that for presence data. The beta-distribution is restricted between 0 and 1 and does not include 0 or 1. However as our data had seals that had only preyed on one species, hence with a proportion value of 1, we used a weighted average transformation to restrict our data between 0 and 1. Our model satisfied the assumption of beta-distributed GLMM because the residues of our random variable were normally distributed (*Supp.2*). Both GLMM models were created using the glmmTMB package in R. Among all possible model subsets, we selected models with the least covariates (i.e. most parsimonious) that had a ΔAIC value inferior to 2. When a null model had a ΔAIC value inferior to 2, we selected the null model.

Results

*Table 2. **Overall Frequency of Occurrence and Numerical Abundance for different prey species.** Numbers in brackets represent the number of digestive tracts for FO and the number of prey individuals for NA.*

Prey species	Frequency of Occurrence	Numerical Abundance
Ammodytes sp. (Sand lance)	67.4 (265)	89.3 (77191)
Gadus sp. (Cod)	35.9 (141)	5.3 (1745)
Mallotus villosus (Capelin)	25.7 (101)	67.6 (17013)
Pleuronectidae sp. (Righteye flounder)	21.6 (85)	2.2 (469)

Pseudopleuronectes americanus (Winter flounder)	14 (55)	8.2 (838)
Urophycis tenuis (White hake)	11.2 (44)	1.5 (139)
Clupea harengus (Atlantic herring)	9.4 (37)	8.3 (391)
Hippoglossoides platessoides (American plaice)	5.9 (23)	4.5 (216)
Scophthalmus aquosus (Windowpane)	5.1 (20)	2.1 (110)
Zoarces americanus (Ocean Pout)	5.1 (20)	2.5 (71)
Cottidae sp. (Sculpin)	4.3 (17)	5 (95)
Tautogolabrus adspersus (Cunner)	3.1 (12)	5.4 (168)
Myoxocephalus sp. (Horned sculpins)	2.8 (11)	10.3 (94)
Anarhichas lupus (Wolffish)	2.3 (9)	0.9 (17)
Enchelyopus cimbrius (Fourbeard rockling)	2 (8)	3.2 (52)
Limanda ferruginea (Yellowtail flounder)	2 (8)	1.6 (17)
Lumpenus lampretaeformis (Snakeblenny)	2 (8)	3.6 (83)
Sebastes sp. (Red fish)	2 (8)	4.3 (45)
Eumesogrammes praecisus (Fourline snakeblenny)	1 (4)	0.4 (6)
Myoxocepahlus scorpius (Shorthorn sculpin)	0.8 (3)	0.6 (5)
Leptoclinus maculatus (Daubed shanny)	0.5 (2)	4.7 (4)
Osmerus mordax (Smelt)	0.5 (2)	2.7 (23)
Scomber scombrus (Atlantic mackerel)	0.5 (2)	0.3 (2)
Stichaeus punctatus (Arctic shanny)	0.5 (2)	0.6 (4)
Clupeidae sp. (Herring)	0.3 (1)	0.3 (3)
Cryptacanthodes maculatus (Wrymouth)	0.3 (1)	21.7 (39)
Hemithipterus americanus (Sea raven)	0.3 (1)	0.7 (6)
Lycodes sp. (Eeelpout)	0.3 (1)	7.6 (5)
Melanostigma atlanticum (Atlantic soft pout)	0.3 (1)	0.6 (3)
Myoxocephalus octodecemspinosus (Longhorn sculpin)	0.3 (1)	29.6 (40)
Myoxocephalus quadricornis (Fourhorn sculpin)	0.3 (1)	3.9 (2)

Grey seals consume a variety of prey species. Our study identified 16 invertebrate species and 31 fish species. However, despite the large number of invertebrates recovered from digestive tracts, they accounted for 0.002% of the diet estimated in frequency of occurrence, prior to correction for partial digestion. Among fish species, sandlance, cod, capelin and righteye flounder were commonly ingested species, present in more than 20% of digestive tracts. Their contribution in numerical abundance however varied drastically. Sandlance and capelin were consumed in large numbers, when consumed they accounted for more than 60% of all prey ingested. Cod and righteye flounder, on the other hand, when consumed accounted for less than 6% of all prey ingested.

Table 3. **Pooled wet weight contribution (± standard deviation) for different sampling areas and seasons estimated using bootstrap simulation of 60% data over 1000 iterations.** *The range*

Prey species	Magdalen summer	Magdalen fall	Miramichi fall	Northumberland fall
Ammodytes sp. (Sand lance)	49 ± 4.2 (49 - 50)	23 ± 5.3 (23 - 23.6)	84.9 ± 4.4 (83.9 - 85.2)	5.6 ± 3.7 (5.3 - 6)
Mallotus villosus (Capelin)	13.9 ± 2.8 (13.7 - 13.9)	0.1 ± 0.1 (0.1 - 0.1)		
Gadus sp. (Cod)	29.7 ± 4.6 (28.7 - 29.8)	61 ± 6.4 (60 - 61)	0.1 ± 0 (0.1 - 0.1)	0.7 ± 0.3 (0.7 - 0.9)
Clupea harengus (Atlantic herring)	0 ± 0 (0 - 0)	0.3 ± 0.1 (0.3 - 0.3)	2.5 ± 1.7 (2.4 - 2.5)	11 ± 4.7 (10.3 - 12.7)
Pleuronectidae sp. (Righteye flounder)	2 ± 0.4 (2 - 2.1)	1.9 ± 0.4 (1.9 - 2)	7.8 ± 3.3 (7.5 - 8.7)	4.3 ± 1.8 (4 - 5)
Pseudopleuronectes americanus (Winter flounder)	0.9 ± 0.5 (0.9 - 0.9)	0.1 ± 0 (0.1 - 0.1)	1 ± 0.3 (1 - 1.1)	22.3 ± 7.1 (21.4 - 23.7)
Tautogolabrus adspersus (Cunner)	0.1 ± 0.1 (0.1 - 0.1)		1.9 ± 1.6 (1.7 - 2)	30.8 ± 17.9 (24.6 - 33.8)
Limanda ferruginea (Yellowtail flounder)	0.3 ± 0.1 (0.3 - 0.3)	0 ± 0 (0 - 0)	0.5 ± 0.4 (0.5 - 0.6)	
Scomber scombrus (Atlantic mackerel)		0 ± 0 (0 - 0)		0 ± 0 (0 - 0)
Sebastes sp. (Red fish)	0 ± 0 (0 - 0)			0.9 ± 0.6 (0.9 - 1.1)
Scophthalmus aquosus (Windowpane)		0 ± 0 (0 - 0)	0.7 ± 0.3 (0.6 - 0.7)	1.2 ± 0.5 (1.2 - 1.3)
Urophycis tenuis (White hake)	1 ± 0.6 (1 - 1)	0.2 ± 0.1 (0.2 - 0.2)	0.1 ± 0.1 (0.1 - 0.1)	3.1 ± 1.4 (2.9 - 3.5)

Despite the large diversity in prey, only five species - sandlance, cod, capelin, cunner, winter flounder, and herring - were identified as major prey species - they contributed more than 10% to the diet estimated in wet weight. Moreover, the contribution of these major species varied

drastically between different areas and between seasons at the same sampling area. At Magdalen Islands, during the summer, sandlance, cod and capelin were the major prey species. Together, they accounted for 92.6% of the total diet. Whereas in the winter at the same sampling area, cod and sandlance alone were major prey species. They accounted for 84% of the total diet. In contrast to the summer, cod became the most consumed prey estimated in wet weight percentage in the fall (61% in the fall compared to only 29.7% in the summer) replacing sandlance. The contribution of capelin to the diet decreased from 13.9% during the summer to 0.1% during fall. Different areas showed differences in major prey species. While cunner, winter flounder and herring were major prey species in the Northumberland Strait, their pooled contribution to the diet in other areas was always less than 5%. Such geographic and seasonal differences are representative of the generalist predation behaviour of grey seals.
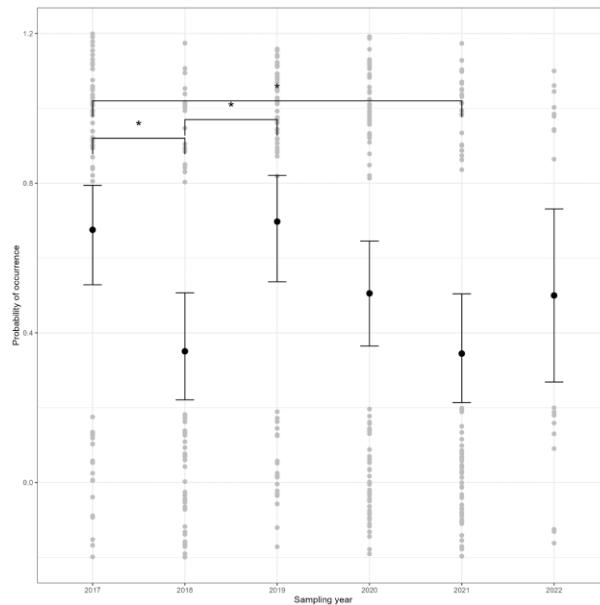

Figure 3. **Inter-annual variation in the consumption (presence) of sandlance estimated using binomial GLMM.** *Sample size of 177. Error bars represent 95% confidence intervals.*

When comparing variation in prey consumption (presence) for the three major species - sandlance, cod, and capelin - at Magdalen Island during the summer, we found there to be no effect of sex, age or year for cod and capelin. The consumption of sandlance however, varied inter-annually, ranging overall between 34.4% in 2021 to 69.7% in 2019. We did not find any effect of age or sex on sandlance consumption.
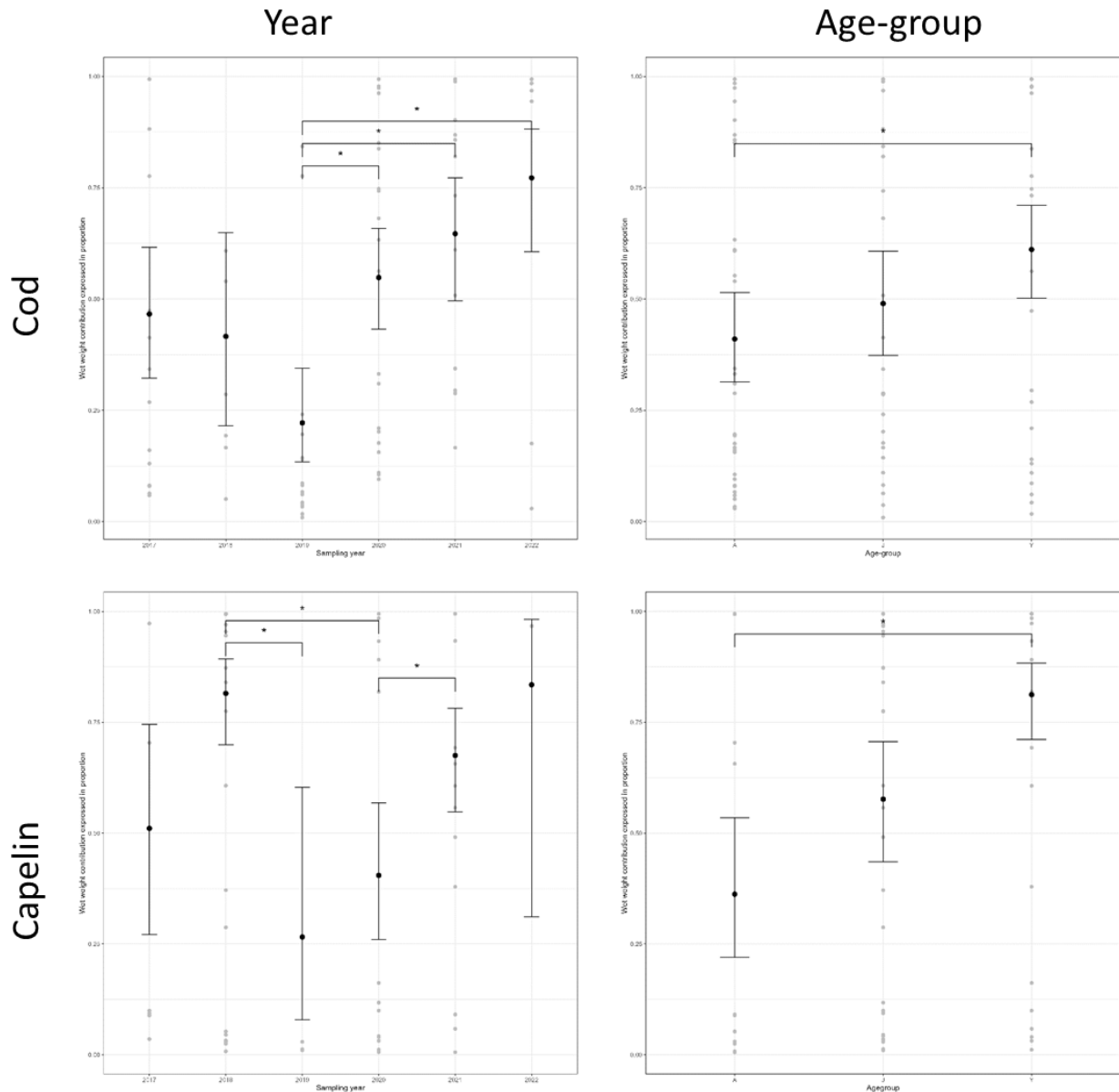
*Figure 4. **Inter-annual and ontogenic variation in the contribution of cod (upper panels) and capelin (lower panels) estimated using beta distributed GLMM.** Sample size of 83 for cod and 94 for capelin. Error bars represent 95% confidence intervals.*

When comparing variation in the contribution of prey to the diet in wet weight proportion for the three major species - sandlance, cod, and capelin - at Magdalen Island during the summer, we found there to be no effect of sex, age or year for sandlance. The contribution of cod and capelin however varied inter-annually and between different age-groups. The contribution of cod to the diet of grey seals showed a steady increase from 22.2% in 2019 to 77.2% in 2022. This difference however was not significant. The contribution of both, cod and capelin, decreased with increase in age. Yearlings consumed significantly more cod and capelin, 61.1% and 81.2% respectively - estimated in wet weight percentage -, as compared to adults, 41.0% and 36.2% respectively. The

contribution of both prey in juveniles however was in-between and did not differ significantly from yearlings or adults. We did not find any effect of sex on cod or capelin contribution.

Discussion

During the mid-1980s, prior to the collapse of cod in the Gulf of St-Lawrence, Benoit and Bowen (1990) estimated the frequency of occurrence of cod to be 13.5% in the southern Gulf region. Another study done in late 1990s and early 2000s, estimated the frequency of occurrence to be similar at 16.7% (Hammill et al., 2007). A decade later, our results suggest that the frequency of occurrence of cod has increased two folds to 35.9%, making cod a more common prey than before. However, such differences could also be due to differences in sampling area and season. We ourselves have shown that the grey seal diet shows higher geographic and seasonal variation (*Fig.5*). Hence, caution must be taken while interpreting and comparing results between different studies.
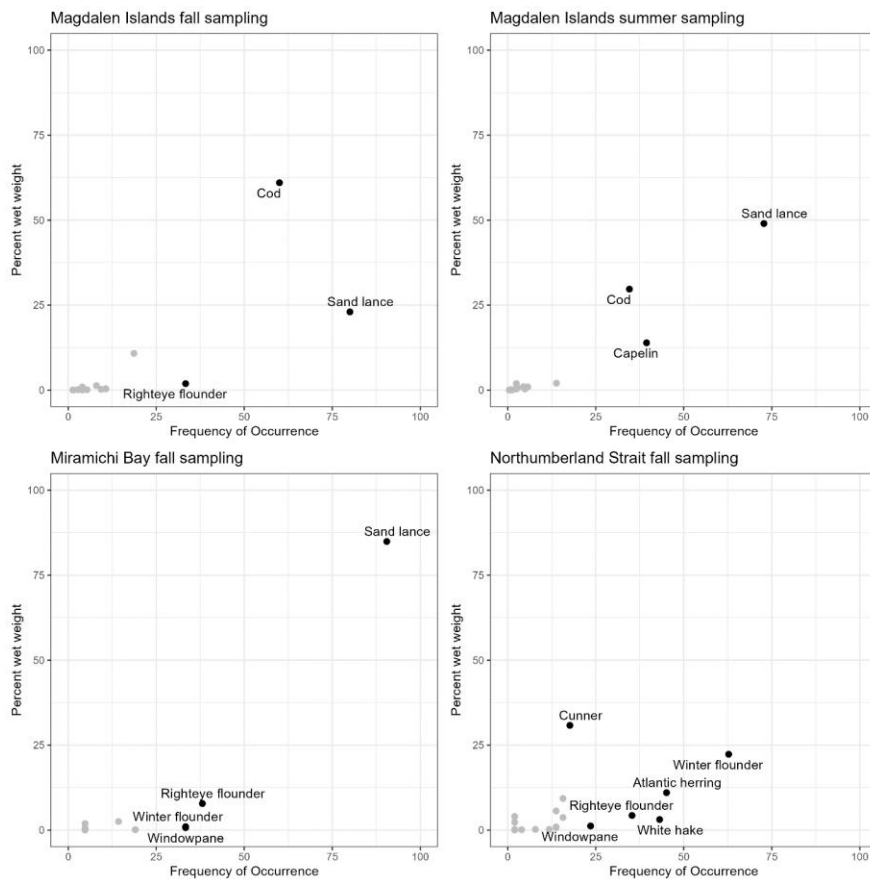

*Figure 5. **Summary of results highlighting important prey species identified by their frequency of occurrence and/or wet weight percentage.** Only prey species that contribute more than 20% in FO or WW have been annotated.*

During fall months, Atlantic cod in the southern Gulf are known to move from coastal to offshore areas (Comeau et al., 2001). In addition, overwinter cod populations in the eastern region of the Gulf pass by Magdalen Islands before arriving at their wintering ground in Cabot Strait (Sinclair & Currie, 1994). As a result, the abundance of cod increases near Magdalen Islands during the fall

months. This would hence explain the increase in consumption of cod observed during the fall months as compared to the winter months at Magdalen Islands (*Fig.5*).

Ontogenic and interannual differences in prey consumption detected in our models have also been detected in the previous studies (Beck et al., 2003, 2007; Lundström et al., 2005; Bowen & Harrison, 2013; Hammill et al., 2013). Our models however suffer from low fit, which can be visualised by the number of raw data points outside the 95% confidence intervals for beta distributed GLMM (*Fig.4*). This represents the high interindividual variation among grey seals (Amanda & Sean, 2015; Badger et al., 2021; Galatius et al., 2022). Our models did not detect an effect of sex. This could be because sexual dimorphism in grey seals is prevalent in adults, and not in juveniles and yearlings. As a result, the lack of interaction term in our model, leads to a dilution of such effect. We did not use interaction terms in our model as this led to sub-groups with extremely small sample sizes.

Major limitations of hard-part analysis include loss of otoliths due to partial digestion and lack of correction (NCF) and conversion (regression equations) factors for all prey species. To correct for numerical bias, we used NCF calculated using control experiments on grey seals whenever possible (Bowen, 1991; Grellier & Hammond, 2006). When NCF were not available for grey seals, we used NCF obtained from harbour seals (Phillips & Harvey, 2009; Wilson et al., 2017) as they are both phocids. When NCF were not available for either of these two species, we extrapolated NCF from other prey species that had similar otolith shape and belonged to the same family. As NCF depends on the shape and robustness of the otolith, this technique has also been used in other hard-part analyses (Hammill et al., 2014b). We did not use NCFs calculated from otariids, despite their phylogenetic similarity, because a previous study by Tollit has shown that digestion varies drastically between phocids and otariids (Tollit et al., 2007). NCFs also do not exist for invertebrates species, however, previous studies have shown that grey seals are migratorily piscivores (Benoît & Bowen, 1990; M. O. Hammill et al., 2007, 2014b). A finding also confirmed by our study - invertebrates accounted for 0.002% of diet estimated as frequency of occurrence prior to correction.

Caution must be practised while making inferences from our study. Samples in our study were harvested in nearshore areas. In addition, prey consumed from digestive tracts represent feeding that occurred at most 64 hours prior to harvest. Hence, the diet and its variation observed in our study might not be well-representative of an offshore diet. In addition, the diet estimated in our study does not represent one feeding event but multiple. Limiting our dataset to otoliths obtained from the stomach alone would be more representative of a single feeding event. However, due to the rapid passage time for stomachs, such analyses would warrant a much larger sample size as most stomachs would be empty. This was hence not undertaken in our analysis.

Through our analysis, we portray a recent picture of grey seal diet in the Gulf of St-Lawrence. The diet of grey seals shows large variations among the different sampling areas in the Gulf, but also seasonal variation between Summer and Fall months at Magdalen Islands. Our results also suggest that cod and capelin contribute more to the diet of yearling than adult grey seals. We provide an up-to-date compilation of resources like NCF and regression equations that can be used to re-perform hard-part analyses in the future. Future research should focus on integrating information obtained from other diet analyses like stable isotope signatures, fatty acid composition and DNA-metabarcoding to complement hard-part analysis.

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