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Winter diet of grey seals in Cabot Strait

Régime alimentaire hivernal des phoques gris dans le détroit de Cabot

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ABSTRACT

The winter diet of grey seals, in the Cabot Strait, was examined to determine if they feed extensively on overwintering southern Gulf of St. Lawrence (NAFO area 4T) Atlantic cod that concentrate in this area. The stomach and intestines of 100 grey seals, collected between Cape Breton and St. Paul's Islands, were examined. The majority of samples, of which 50% contained food, were from males. Numerical correction factors (NCF) were applied to the intestine contents to account for otolith loss, but no correction was applied to the stomach content data. The diet of males and females differed greatly. Using the stomachs, Atlantic cod (50%), herring (21%), and white hake (13%) accounted for 84% of the male diet. Females fed primarily on herring (72.6%), winter flounder (17.3%), white hake (3.5%), sandlance (3.0%), and capelin (2.5%). Large robust otoliths of cod appear to be retained in the stomach, leading to an overestimate of the contribution of cod to the diet. The average size of cod consumed was 43.2 cm which is larger than that observed in other studies. NCF increased the importance of species with small, fragile otoliths such as herring, capelin, and sandlance while reducing that of fish with robust otoliths such as Atlantic cod and flatfish. Based on numerically adjusted data from the intestines, the importance of the many prey species differed when compared to the results from the stomach contents. Flatfish (*Pleuronetidae* sp.) and herring were the most important prey species in both males (27.5% and 28.2% respectively) and females (27.8% and 21.2% respectively) while the percentage of cod in the diet of males and females was 16.24% and 2.55%, respectively. The proportion of Atlantic cod and *Gadus* sp. combined in males was lower in the intestines than in the stomachs (24% vs. 52.9%). Using data from the intestines, females had a lower reliance on herring (21.2% vs 72.6%) and winter flounder (2.3% vs. 17.3%) when compared to stomach data. Only 12 of the 100 stomachs contained Atlantic cod otoliths while only 6 tested positive for cod DNA. No DNA was found in stomachs that did not also contain Atlantic cod otoliths in their stomachs suggesting that if belly biting occurs, it is not common in this area. Although no method of diet analysis is unbiased, the potential retention of large cod otoliths and the lack of correction factors for the stomach data suggest that the diet based upon the intestine contents is likely a more realistic description grey seal feeding during this study.

RÉSUMÉ

On a étudié le régime alimentaire hivernal des phoques gris dans le détroit de Cabot afin de déterminer s'ils consomment beaucoup de morues qui hivernent en grandes concentrations dans le sud du golfe du Saint-Laurent (zone 4T de l'OPANO). On a ainsi examiné l'estomac et les intestins de 100 phoques gris prélevés entre les îles du cap Breton et Saint-Paul. La majorité des échantillons, dont 50 % contenaient de la nourriture, provenaient d'individus mâles. Des facteurs de correction numérique ont été appliqués aux contenus intestinaux pour tenir compte de la perte d'otolithes, mais aucune correction n'a été apportée aux données en matière du contenu stomacal. Le régime alimentaire des mâles différait beaucoup de celui des femelles. Selon l'analyse du contenu des estomacs, la morue (50 %), le hareng (21 %) et la merluche blanche (13 %) constituaient 84 % du régime alimentaire des mâles. Les femelles s'étaient nourries principalement de hareng (72,6 %), de plie rouge (17,3 %), de merluche blanche (3,5 %), de lançon (3,0 %) et de capelan (2,5 %). Les gros otolithes solides de la morue semblent d'être retenus dans l'estomac des phoques, ce qui se traduit par une surestimation de la proportion de morue dans le régime alimentaire. La taille moyenne des morues consommées était de 43,2 cm, une valeur plus élevé que celle observée dans d'autres études. Les facteurs de correction numérique amplifient l'importance des espèces ayant des otolithes petits et fragiles comme le hareng, le capelan et le lançon, tandis qu'ils réduisent celle des poissons ayant des otolithes solides, comme la morue et les poissons plats. Les données numériquement ajustées tirées des analyses du contenu intestinal et du contenu stomacal donnent des proportions différentes pour de nombreuses espèces proies. Les poissons plats (*Pleuronetidae* sp.) et le hareng étaient les espèces proies les plus importantes pour les phoques, autant chez les mâles (27,5 % et 28,2 % respectivement) que chez les femelles (27,8 % et 21,2 % respectivement), tandis que le pourcentage de morue dans le régime alimentaire des mâles et des femelles était de 16,24 % et de 2,55 % respectivement. Chez les mâles, la proportion combinée de morue et d'espèces du genre *Gadus* était moins élevée dans les intestins que dans l'estomac (24 % contre 52,9 %). Comparé aux donnés du contenu stomacal, les données du contenu intestinal indiquaient que les femelles avaient consommé moins de hareng (21,2 % contre 72,6 %) et de plie rouge (2,3 % contre 17,3 %). Seuls douze des 100 estomacs contenaient des otolithes de morue, et la recherche d'ADN de morue n'a donné des résultats positifs que pour six estomacs. Aucun ADN n'a été trouvé dans les estomacs qui ne contenaient pas également des otolithes de morue, ce qui suggère que si certains phoques ne consomment que la cavité abdominale des poissons, ce comportement n'est pas fréquent dans cette zone. Bien qu'il n'existe pas de méthode totalement objective pour analyser le régime alimentaire, la rétention potentielle des grands otolithes de la morue dans l'estomac des phoques et l'absence de facteurs de correction pour les données du contenu stomacal, suggèrent que l'analyse des contenus intestinaux donne probablement une description plus réaliste du régime alimentaire des phoques pendant cette étude.

INTRODUCTION

Since the mid 1980s, a number of the Canadian stocks of Atlantic cod (*Gadus morhua*) have declined significantly. Even though fishing pressure has been reduced significantly since the early 1990s, many of these stocks have shown no sign of recovery. The most recent assessment of cod in the southern Gulf of St. Lawrence (NAFO area 4T) concluded that this stock will continue to decline due to high natural mortality and low recruitment in recent years (Swain and Chouinard 2008, DFO 2009). The most important factor contributing to the current low productivity of the southern Gulf cod stock appears to be the elevated natural mortality (*M*), which is estimated to have increased since the 1980s (Chouinard *et al*. 2005, Swain and Chouinard 2008).

During this same period, the abundance of many cod predators have increased significantly and in the case of harp and grey seals, are now at, or near, the highest levels estimated (Hammill and Stenson 2009, Thomas et al. 2007, Hammill and Stenson 2010). Unlike harp seals, who are smaller and only seasonal inhabitants, grey seals can be found in the southern Gulf throughout the year. The Canadian grey seal population has increased from approximately 15,000 animals in the 1960s to over 300,000 in 2007 (Thomas et al. 2007). For these reasons, the impact of grey seals on the recovery of southern Gulf cod has been an issue of great debate and study.

There are a number of hypotheses regarding the factors that could be limiting cod recovery by contributing to high *M*. In a review of the possible causes of this high mortality, Bowen et al. (2009) concluded that based on weight of evidence, grey seals could be limiting recovery in 4T cod. This conclusion was based primarily upon the spatial and temporal correlations between grey seal abundance and estimated cod *M*, and the lack of support for other hypotheses.

It is difficult to draw any definitive conclusion about the impact of grey seals on 4T cod, however, because of some apparent inconsistencies in the data. Most importantly, the increases in cod mortality have occurred in the older age groups while the available diet data suggests that seals feed primarily on juvenile cod. Chouinard et al (2005) suggest that possible biases in the available diet information for grey seals may underestimate the consumption of adult cod. They point out that there are significant gaps in the diet data for grey seals in the southern Gulf with most of it being collected from inshore areas during the summer. In contrast, very few data are available from the winter and from areas when cod are highly aggregated. Thus, seasonal and spatial biases in sampling may underestimate the proportion of cod in the diet as well as provide a biased representation of the age composition. They also suggest that predation on large cod may be underestimated if seals do not consume the heads of large cod (referred to as “belly-biting”) and therefore do not appear in traditional diet analyses that use hard parts.

Southern Gulf cod are highly migratory. From late April to early June, spawning occurs in the Shediac Valley and around the Magdalen Islands. During the summer, cod are widely distributed across the southern Gulf. During the fall, however, the cod concentrate off western Cape Breton as they migrate to the area off northern Cape Breton and Sydney Bight (4Vn) where they overwinter. During a study of the movements of grey seals using satellite transmitters, Harvey et al (2010) found that some grey seals overlap with wintering cod concentrations in the Cabot Strait area. Although data are available on the diets of grey seals in many areas throughout the Gulf (e.g. Benoit and Bowen 1990ab, Hammill and Stenson 2000,

Hammill et al. 2007, Hammill 2010), little is known about the diet during the winter period and particularly in these areas of overlap.

The objective of this study was to quantify the diet of grey seals that winter in the Cabot Strait to determine if they feed extensively on overwintering Atlantic cod. If cod are eaten, it is also important to determine the size range of individuals consumed.

MATERIALS AND METHODS

Sampling

Grey seal stomachs and intestines were obtained from a collector contracted by Fisheries and Oceans Canada (DFO). The sealer was requested to sample grey seals collected in an area bounded by 60.85°N 47.49°W, 59.93°N 47.48°W, 60.86°N 46.95°W, and 59.95°N 46.94°W between October and December 2008. Stomachs and intestines were removed in the field and stored at -20°C until analysis was performed. Jaws were also obtained and used for aging.

Hard Part Analyses

Stomach contents

Stomachs were weighed before and after dissection to obtain an overall food weight. Stomachs and prey were classified based on the state of digestion. The classification consisted of class 1 (no signs of digestion, fish fully intact and can therefore be measured and identified), class 2 (some digestion, skin is coming off, tail digested), class 3 (clumps of tissue still attached to bones), class 4 (floating tissue and bones present), class 5 (few bones and other hard parts present, may be small clumps of tissue present), and class 6 (no signs of tissue, primarily bones and otoliths present). Since there are often a number of different individual prey present, the percentage of overall contents in each class was estimated.

The stomach contents were emptied and rinsed through a series of three sieves of decreasing mesh size (4.75 mm, 2.0 mm, 1.0 mm). Small objects were caught in a tray underneath the sieves. Hard parts including otoliths, carapaces, beaks, and bones were retained, measured and used for identification to the lowest taxonomic level possible. Identification of hard parts was made using the methods outlined by Lawson et al. (1995) and Lawson and Stenson (1995, 1997). Fully intact fish or invertebrates were weighed and measured.

Otoliths were classified according to the degree of erosion present based on visual signs of degradation such as erosion on the sagittal otoliths margins and/or cracks. A rating of “NE” was no obvious signs of erosion, “SE” was slight erosion observed around the otolith margins, “PE” moderate erosion, and “ER” severe erosion. The number of unmeasured otoliths was recorded and each assigned a length based upon the average of measured otoliths from the same species in the stomach or in seals collected at the same time. Only otoliths showing no signs of erosion (NE) were measured. Otoliths were measured at the two longest points using either digital calipers (for larger otoliths) or an image analyses system (Photo Imager Pro). If a large number of otoliths of a single species were present, a subsample (~ 30 otoliths) was measured.

Original ingested prey lengths and weights were estimated from otolith measurements based upon regressions developed for individual fish species using local data whenever possible. The

total mass of invertebrates was estimated by multiplying the number of individuals by a mean mass for the species. Published estimates of energy content for each species were used to determine the total estimated amount of energy (kcal/g) that the seal obtained from its prey.

Intestine contents

Both large and small intestines were measured in length and then cut into shorter sections for analysis. Contents were then passed through a series of sieves and collected, identified and measured as described above.

Because of the overall higher level of erosion seen among otoliths in the intestine all otoliths, regardless of digestive state, were measured. ANOVA tests were performed for each species to determine if the lengths obtained from otoliths in different erosion states were comparable. If there was no significant difference between the lengths observed, they were combined. The length of fish ingested were obtained from either uneroded (NE) or uneroded and slightly eroded otoliths (NE and SE) depending upon species (Table 1); average lengths were assigned to fish with moderate or severe erosion. To estimate the number of individuals involved, it was assumed that otoliths of similar size and erosion were from the same fish. Unmatched otoliths were assumed to represent additional individuals. If left or right otoliths could be identified, the maximum number was used.

Data Analysis

The importance of individual prey species was based upon their contribution to the total energy ingested. The percentage of each prey species in the diet can be expressed based upon the total energy from each prey species in the sample (% $Energy_{Weight}$), i.e.

$$\% E_w = \frac{\text{Total estimated energy from a species found in all stomachs in the sample}}{\text{Total energy from all species in the sample}} \times 100\%$$

Alternatively, the importance of each prey species can be estimated as the average proportion within a stomach (%$Energy_{Proportion}$), i.e.

$$\% E_p = \text{Average of: } \frac{\text{Total estimated energy from a species in a stomach}}{\text{Total energy from all species in a stomach}} \times 100$$

To reduce the impact of stomachs containing only trace amounts of a single prey species, stomachs with less than 200 gm in total were not included in the analysis of average proportion.

To determine the impact of otolith loss due to digestion, the number of otoliths present in the intestines were adjusted by applying a numerical correction factor for each species (Grellier and Hammond 2006, Tollit et al 2007, Hammill 2010, Bowen et al 2010, see Appendix 1).

Uncertainty in the diet estimates was quantified by bootstrapping (Resampling Stats, Arlington, VA, USA) the data using individual stomachs as the sampling unit. Samples were resampled 1000 times to determine the mean and standard error in the proportion contributed by each prey group.

Polymerase Chain Reaction (PCR) Analysis

A sample of digested stomach contents, or slurry, was collected from each stomach for DNA extraction. If the contents were highly digested, samples were obtained by running the contents through a sieve to minimize otolith loss, and then placed in marked, clean polypropylene collection tubes. If the slurry was thicker, it was stirred and then scooped into the clean collection tubes from different parts of the stomach to get a representative sample of the prey items. Approximately 30 mL of slurry was obtained per stomach. If tissue and/or full prey were present, approximately 1 cm^3 of the tissue was added to the slurry. Samples were stored at -20ºC until analyzed.

After thawing, the slurry samples were mixed vigorously and a 200 µL subsample taken for DNA extraction using the QIAamp DNA Mini Kit (Qiagen Inc., Missisauga, ON, Canada). To ensure that sufficient DNA was extracted, DNA concentration was determined by spectrophotometry (ND-1000 Spectrophotometer, NanoDrop Technologies Inc, Wilmington, DE, USA).

To determine if Atlantic cod DNA was present, PCR was performed on all stomachs using the primers described in Marshall et al. (2010). For each stomach, 2 µL of DNA extract from the stomach slurry was amplified using 2 µL from each primer of the primer pairs GmoF/R (Marshall et al. 2010) in a reaction containing 1X PCR Master mix (Promega, Madison, WI, USA). The total reaction volume was 25 µL and each primer was present in a concentration of 10 µM. The thermal cycling profile consisted of an initial denaturation of 94ºC for 5 minutes followed by 35 cycles of 94ºC for 30 seconds, 55ºC for 30 seconds, and 72ºC for 1 minute, and a final extension of 72ºC for 5 minutes. Thermal cycling was performed using the Applied Biosystems 9700 GeneAmp thermal cycler (850 Lincoln Centre Drive, Foster City, CA 94404, USA). Samples were then held at 4ºC. A 10 µL aliquot of each of the PCR products was analysed by gel electrophoresis using 1.5% agarose stained with EZ Vision (AMRESCO) in Tris/Borate/EDTA buffer (TBE) for approximately 60 min at 100V. Gels were photographed using the KodaK Gel Logic 200 gel imager (Admiral lace, Guelph, Ontario, Canada). A 100 base pair DNA ladder (BioShop Ontario, Can.) was used to determine molecular weight.

Each set of stomach DNA was amplified three times using the same method. A positive control, which contained the fish species in question, was run in the same PCR reaction, along with a negative control, which had no DNA. Any sample that was positive at least two times was considered to contain Atlantic cod DNA.

RESULTS

Sampling

Stomachs and intestines were obtained from a total of 100 grey seals caught between Port Hood, Cape Breton Island, and St. Paul's Island off the north coast of Cape Breton (Fig. 1). Samples were collected between 25 October and 29 December, 2008. The majority of seals (72) were male, while only 28 females were collected (Table 2). Of the 98 seals aged, ages ranged from 1 to 29 years. The majority of seals were adults with only 8 animals less than 4 years of age.

Hard Part Analyses

Stomach Contents

The diet of males and females differed greatly (Table 3). Based on the proportion of the total energy in all stomachs, Atlantic cod accounted for approximately one half of the diet of males. A further 2.2% of the energy could only be identified to *Gadus* sp. which may have included Atlantic cod. Herring contributed 21% of the energy, while white hake (13.0%) and flatfish (7.3%) were also important. In contrast, 72.6% of the energy females consumed came from herring while almost 17.3% was from winter flounder. Capelin, sandlance, and white hake were also present in measurable quantities.

Expressing the data as an average proportion in individual stomachs provides a slightly different view of the diet (Table 4). The apparent dominance of Atlantic cod in the diet of males is reduced (23.1%) with herring (20.6%) and white hake (23.2%) being similar in importance. The proportion of the energy obtained from flatfish was similar although the proportion that could only be identified as *Gadus* sp increase from 2% to 7%. In females, herring remained the most important prey. However, the importance of capelin (8.3%) and sandlance (12.8%) was substantially higher.

Cod lengths

A total of 28 cod otoliths were recovered from the 50 prey containing stomachs. Of these, 24 were measured. Based on these otoliths, the average size of cod consumed was 43.2 cm (SD = 13.3 cm) with the smallest being 11.2 cm and the largest 66.0 cm (Fig. 2).

Intestinal contents

Examination of the intestines suggests a more diverse diet than seen in the stomachs (Tables 5 and 6) with a number of species found in the intestines only. The importance of the various prey species also differs. Most importantly, flatfish (Pleuronetidae *sp*.) was identified as the most important prey species in both males and female based upon the intestinal contents. The proportion of Atlantic cod in the diet was much lower in males, although the proportion of fish identified only to the genus *Gadus* increased. Together they account for ~34% of the diet of males compared to 53% based upon the stomach data (Table 3). The female diet was also more diverse with a much lower reliance on herring and winter flounder. In contrast, there was evidence that females were feeding on Atlantic cod and *Gadus* sp. The proportion of sandlance in the diet of females was also higher.

Applying numerical correction factors increases our perception of the importance of species with small, fragile otoliths such as herring, capelin, and sandlance (Tables 7 and 8) while reducing the apparent importance of fish with robust otoliths such as Atlantic cod and flatfish. Based on numerically adjusted data from the intestines, the percentage of cod in the diet of males and females was 16.24 and 2.55%, respectively (Table 7).

PCR Analyses

Of the 100 seal stomachs observed, 12 had hard parts identified as Atlantic cod; an additional 4 had *Gadus* sp. which could have included Atlantic cod. Based upon the PCR analysis, however, only 6 of the 99 stomachs tested contained cod DNA (Table 9). All of these stomachs also had cod otoliths. Twenty seven of the 91 intestines containing food had Atlantic cod hard

parts, while 22 had otoliths that could only be identified as *Gadus* sp. Only 3 of the stomachs with a positive PCR result also had cod hard parts in the intestines. No DNA was found in stomachs that did not also contain Atlantic cod otoliths in their stomachs, even if cod or *Gadus* otoliths were identified in their intestine.

Although sample sizes were small, the state of digestion appears to have some impact on the likelihood of getting a positive result. Approximately one-quarter of the stomachs that contained highly digested cod (100% Class 6) also contained cod DNA, while 40% of the stomachs with less digested cod tested positive for DNA (Table 10). Stomachs containing otoliths that were not considered eroded had positive PCR results 80% of the time, whereas only 12.5% of stomachs with highly eroded otoliths also had cod DNA.

DISCUSSION

Several methods have been developed to study diet composition in marine mammals. These include the reconstruction of diet composition based on recovery of hard parts from stomachs, intestines or faeces, the detection of DNA in faecal samples, analyses of fatty acid composition of blubber samples, and stable-isotope analyses of tissue from muscle, or some other tissue. All of these methods of diet analyses have individual strengths and weaknesses associated with them and our perceptions of the importance of a particular prey species in the diet can be influenced by the method used and how we present the data. For some methods, such as the analysis of scats, we have a good understanding of the general direction of the biases and how to reduce them. For example, because of differential digestion and passage rates, some authors correct otolith size and numbers obtained from scat samples to account for a negative bias in small, fragile otoliths (e.g. Bowen 2000, Grellier and Hammon 2006). Because diets are usually expressed as a proportion, an underestimate in one species will result in an over estimate of all others. To date it has been assumed that a simple multiplier will be sufficient, but some modelling has suggested the need for additional considerations (e.g. Arim and Naya 2003) and our results presented here, and in Hammill (2010), would suggest a more complicated relationship as well. Similar biases may also apply to stomach contents, but this has not been examined to the same extent. Some work on harp seals where animals were feeding on invertebrates and fish with small otoliths (e.g. capelin) showed that stomach contents can provide unbiased estimates of diet composition (Hammill et al. 2005), but this study has shown that grey seals feeding on prey with large robust otoliths retain these otoliths in the stomachs leading to an overestimate of the contribution of these species to the diet. Incorporating information of the degree of digestion of the stomach contents may further improve the accuracy of the estimates.

The use of total energy or weight has been the standard for most studies to describe population diet composition. The total energy/weight contribution of a prey in a sample is expressed as a proportion of the total prey mass in the sample. This approach is used to describe an average diet, but often the contribution of prey is due to the consumption of large amounts of certain species by a few individuals. Thus, it assumes that differences in the total mass of food in the stomachs reflect feeding behaviour of individuals in the area and time of sampling, but that the total sample is representative of the population. An alternative approach is to determine the contribution of each prey item to the diet composition of individuals, then to average these proportions across all individuals. This assumes that each individual seal is representative of the population. Some chemical methods (e.g. fatty acids, stable isotopes ratios) require that the prey species found in the diet of each individual be expressed as a proportion of the diet of each individual. Each individual is then weighted equally. If each seals were to eat the same amount

at a meal, the two methods should provide equivalent estimates, but optimal foraging theory would suggest that meal size will differ between prey patches. Presumably if each individual consumes a similar amount of food overall as other seals of the same size the results will be comparable, but how changes in individual meal size affects methods that provide 'snapshots' of individual meals is not clear. The exact manner in which the proportions of individual species are expressed in a stomach or intestine will depend upon the type of prey consumed and is affected by the behaviour of the prey and predator.

In this study we used PCR analysis to determine the presence or absence of cod in stomachs and compare it to hard part analyses. Only one half of the stomachs with cod otoliths also contained cod DNA. As expected, the likelihood of finding DNA appeared to be related to the degree of erosion. The lack of DNA in stomachs with otoliths may be due to rapid breakdown of DNA or the retention of otoliths within the stomach which would result in a positive bias in the estimates of cod in the diet. The lack of any sign of DNA in stomachs that did not contain cod otoliths suggests that, in this sample, there is no indication of seals feeding upon soft parts of cod without consuming the heads. The presence of otoliths from 65 cm cod also indicates that grey seals can consume at least some, large fish whole. We examined a relatively large sample of seals collected from an area where they overlap, and feed, on large cod. This suggests that if belly biting, which here also includes animals that may consume the entire body, but not the heads, occurs it does not appear to be common. However, if it occurs at a low frequency, it may be difficult to detect.

The size of the cod taken in our study are, on average, large than seen previously (e.g. Hammill et al. 2007). Only undigested otoliths were used to estimate size. Although there is likely to be biased associated with the assumption that smaller otoliths digest are the same rate as larger ones (likely to be false in at least some species), it still suggests that older fish are taken in this area. This may explain some of the high morality seen among larger fish (Chouinard et al. 2005, Swain and Chouinard 2008)

Currently there are multiple methods available to determine diet in marine mammals. All approaches are associated with several strengths and weaknesses, which limit our ability to determine the true diet composition. Ultimately, spatial-temporal sampling levels are likely the major challenge to understanding diet composition. Reconstruction of diet composition using hard part identification from digestive tract/faecal sampling provides information on diet composition within a narrow temporal (last three days) or spatial range (<60 km). Stomach contents provide a picture of feeding on the very short time frame while intestine data likely represent a slightly longer period. These data are useful when attempting to understand predator-prey interactions within small geographic areas or narrow windows in time. To overcome these challenges both the spatial and temporal period of sampling must be augmented. On the other hand, chemical methods provide information on assimilated diet accumulate over a timeframe of weeks or months. This provides information over a greater temporal and spatial scale, which is useful if animals have been foraging in the area of interest, but often it is difficult to assign diets to the individual regions the animals may have been foraging in. Which diet analysis method is the most appropriate to use depends upon the prey species involved and, to a great extent, upon the questions being asked. If the issue is one of predation in a localized area or time, for example, long term diet data may not be appropriate. If the questions relate to areas or times where sampling cannot be carried out, chemical methods may be more useful.

Another approach is to improve our understanding of the functional relationship of prey choice. This will involve a combination of diet sampling and trawling/acoustic surveys to understand

how seal might choose prey within the context of abundance of several potential prey (e.g. Lawson et al. 1998, Smout and Lundstrom 2007). However, this approach will also provide challenges in understanding the seasonal distribution of animals and the prey fields they encounter.

Given the apparent retention of large otoliths within the stomach and our lack of understanding of the retention times of smaller otoliths, the use of the intestine data appears to be more appropriate in this sample. However, it is important to correct these data for otoliths that may have been lost prior to sampling. Although the correction factors were developed for fecal samples, not intestinal data, they provide the best available correction for small prey and should be applied wherever possible. Appropriate correction factors for variable retention of prey in the stomachs are not currently available and need to be developed.

This study has shown that Atlantic cod can be a major component of the grey seal diet particularly that of males and in areas where there is considerable overlap between aggregations of cod and grey seals (e.g. Harvey et al. 2010). It has also shown that the size of cod consumed is larger than has been generally observed in traditional shore based studies (e.g. Hammill 2010, Hammill et al. 2007). To assess the impact of grey seal predation on cod recovery, however, it is necessary to determine what proportion of the diet is accounted for by Atlantic cod. This is very difficult given the different values obtained using different sources and methods. By using different methods of determining diets, it may be possible to obtain a better understanding of what the true diet may be. They may also measure different aspects of feeding behaviour and understanding these differences may help explain why diets using different approaches may not be comparable. By recognizing the differences obtained using the various methods and identifying the factors that drive these differences, we make a first step in identifying the true diet.

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Table 1. Degree of erosion acceptable for estimation of length based upon otolith measurements for different species. NE indicates no visible sign of erosion while SE indicates only slight erosion observed.

Species	**NE**	**NE & SE**
Alosa sp.		X
Sandlance	X	
Atl. herring	X	
Sculpin		X
Daubed shanny		X
Atl. cod		X
Gadus sp.		X
Hake sp.		X
L. esmarki		X
Snake blenny	X	
Laparis sp.		X
Eelpout		X
Silver hake		X
Capelin	X	
O. mordax		X
Pleuronectidae	X	
Winter flounder		X
Scomberus sp.		X
Redfish		X
U. tenuis		X

Table 2. Grey seals sampled along Cape Breton and in the Cabot Strait, October through December 2008.

	N	Prey containing	
		Stomach	Intestine
Female	26	15	22
Male	74	35	69
Total	100	50	91

Table 3. Winter diet of grey seals collected in Cabot Strait expressed as the percentage of each species based upon the total energy in the entire sample of stomachs.

	All (N=50)		Male (N=35)		Female (N=15)	
	Average	SD	Average	SD	Average	SD
Atlantic cod	46.46	12.94	50.72	13.12		
Atlantic herring	26.09	9.90	21.00	9.69	72.63	18.93
Atlantic mackerel	1.18	0.87	1.29	1.00		
Barrelfish	0.03	0.03	0.03	0.04		
Brachyura (crab)	<0.01	<0.01	<0.01	<0.01		
Capelin	0.18	0.17			2.47	3.34
Cephalopoda	1.58	0.79	1.73	0.80		
Eelpout sp.	0.04	0.03	0.04	0.04		
Flatfish sp.	6.45	2.37	7.28	2.70	0.36	0.88
Gadus sp.	1.87	1.28	2.16	1.49		
Hyas sp. (crab)	<0.01	<0.01			0.01	0.01
Natantia (shrimp)	<0.01	<0.01	<0.01	<0.01		
Pollock	0.93	0.93	0.98	1.02		
Sandlance	0.67	0.49	0.48	0.49	2.97	3.23
Sculpin sp.	0.01	0.01	0.01	0.01		
Smelt	0.02	0.02			0.22	0.23
Unidentified fish	1.23	1.15	1.27	1.23	0.54	0.69
White hake	11.66	3.45	13.01	4.14	3.50	4.04
Winter flounder	1.60	1.63			17.31	17.03

Table 4. Winter diet (% energy) of grey seals collected in Cabot Strait expressed as the average proportion of each prey species in individual stomachs. Stomachs containing only trace amount of food (<200gm reconstructed) were not included.

	All (N=38)		Male (N=30)		Female (N=8)	
	Average	SD	Average	SD	Average	SD
Atlantic cod	18.93	5.17	23.11	6.03		
Atlantic herring	28.41	6.47	20.60	6.50	60.03	14.75
Atlantic mackerel	3.81	2.34	5.24	3.21		
Barrelfish	0.45	0.45	0.63	0.61		
Brachyura (crab)	<0.01	<0.01	<0.01	<0.01		
Capelin	1.73	1.46			8.28	6.74
Cephalopoda	5.22	2.68	6.51	3.51		
Eelpout sp.	0.01	0.01	0.01	0.01		
Flatfish sp.	4.94	2.08	6.28	2.59		
Gadus sp.	5.61	3.11	7.02	3.81		
Hyas sp. (crab)						
Natantia (shrimp)	<0.01	<0.01	<0.01	<0.01		
Pollock	0.63	0.62	0.78	0.76		
Sandlance	5.15	3.47	3.21	3.15	12.80	10.83
Sculpin sp.	<0.01	<0.01	0.01	<0.01		
Smelt	0.03	0.03			0.16	0.15
Unidentified fish	2.75	2.62	3.45	3.19	0.09	0.07
White hake	19.37	4.57	23.15	5.38	6.68	6.33
Winter flounder	2.65	2.52			12.05	11.45

Table 5. Winter diet of grey seals collected in Cabot Strait expressed as the percentage of each species based upon the total energy in the entire sample of intestines.

	All (N=91)		Male (N=69)		Female (N=22)	
	Average	SD	Average	SD	Average	SD
Alosa sp.	0.25	0.26	0.31	0.32		
Atlantic cod	20.21	4.33	22.77	4.83	4.49	3.24
Atlantic herring	14.96	4.51	14.73	4.96	14.73	8.63
Atlantic mackerel	0.07	0.04	0.08	0.05		
Bivalve	<0.01	<0.01			<0.01	<0.01
Brachyura (crab)	<0.01	<0.01	<0.01	<0.01	0.01	0.01
Capelin	0.97	0.51	0.67	0.49	3.08	1.90
Cephalopoda	0.06	0.04	0.08	0.06		
Daubed shanny	0.07	0.04	0.04	0.02	0.27	0.24
Eelpout sp.	1.52	1.24	1.84	1.41		
Esmark's eelpout	0.52	0.33	0.45	0.39	1.15	1.14
Flatfish sp.	33.83	5.46	32.77	6.13	41.12	10.01
Gadus sp.	11.33	1.89	10.90	1.86	13.31	7.03
Gastropoda	<0.01	<0.01			<0.01	0.01
Greenland halibut	0.08	0.09	0.10	0.10		
Hake sp.	10.17	2.60	11.60	2.96	1.36	0.91
Liparis sp.	0.03	0.01	0.03	0.01		
Redfish sp.	0.25	0.21	0.26	0.23	0.29	0.29
Sandlance	4.49	1.29	2.65	0.93	16.32	6.42
Sculpin sp.	0.32	0.10	0.25	0.10	0.77	0.34
Silver hake	0.10	0.10	0.12	0.12		
Smelt	0.03	0.03	0.03	0.03		
Snake blenny	0.02	0.01	0.02	0.01	0.03	0.02
Unidentified fish	0.02	0.01	0.01	0.01	0.02	0.01
Winter flounder	0.69	0.51	0.27	0.26	3.02	3.29

Table 6. Winter diet (% energy) of grey seals collected in Cabot Strait expressed as the average proportion of each prey species in individual intestines. Intestines containing only trace amount of food (<200gm reconstructed) were not included.

	All (N=74)		Male (N=56)		Female (N=18)	
	Average	SD	Average	SD	Average	SD
Alosa sp.	0.20	0.19	0.25	0.24		
Atlantic cod	14.02	2.98	17.27	3.65	4.56	3.73
Atlantic herring	16.54	3.51	16.11	3.94	17.64	7.30
Atlantic mackerel	0.08	0.05	0.10	0.07		
Bivalve	<0.01	<0.01			<0.01	<0.01
Brachyura (crab)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Capelin	2.03	1.03	1.07	0.85	5.36	3.48
Cephalopoda	0.02	0.02	0.03	0.02		
Daubed shanny	0.15	0.08	0.09	0.08	0.33	0.25
Eelpout sp.	1.27	0.85	1.71	1.13		
Esmark's eelpout	0.36	0.24	0.19	0.15	0.81	0.81
Flatfish sp.	30.61	3.82	30.29	4.27	32.00	9.36
Gadus sp.	11.82	2.10	12.13	2.08	10.41	5.66
Gastropoda						
Greenland halibut						
Hake sp.	10.42	2.30	13.27	2.95	1.25	0.87
Liparis sp.	0.03	0.01	0.04	0.02		
Redfish sp.	0.77	0.65	0.87	0.80	0.28	0.28
Sandlance	9.29	2.58	4.95	1.77	22.68	8.83
Sculpin sp.	0.40	0.11	0.35	0.12	0.52	0.32
Silver hake	0.15	0.15	0.20	0.20		
Smelt	0.07	0.07	0.10	0.10		
Snake blenny	0.03	0.02	0.04	0.03	0.02	0.02
Unidentified fish	0.02	0.01	0.02	0.01	0.02	0.02
Winter flounder	1.70	1.24	0.90	0.91	4.11	3.88

Table 7. Winter diet of grey seals collected in Cabot Strait expressed as the percentage of each species based upon the total energy in the entire sample of intestines after correcting for otolith digestion by applying numerical correction factors listed in Appendix 1.

	All (N=91)		Male (N=69)		Female (N=22)	
	Average	SD	Average	SD	Average	SD
Alosa sp.	0.46	0.45	0.62	0.57		
Atlantic cod	13.79	3.25	16.24	4.09	2.55	1.88
Atlantic herring	26.78	6.51	28.21	7.45	21.23	10.91
Atlantic mackerel	0.06	0.04	0.08	0.05		
Bivalve	<0.01	<0.01			<0.01	<0.01
Brachyura (crab)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Capelin	4.87	2.30	3.28	2.45	12.08	6.74
Cephalopoda	0.04	0.03	0.05	0.04		
Daubed shanny	0.06	0.03	0.04	0.02	0.18	0.16
Eelpout sp.	1.17	0.94	1.40	1.16		
Esmark's eelpout	0.41	0.26	0.33	0.28	0.72	0.72
Flatfish sp.	27.75	5.25	27.49	5.97	27.85	9.53
Gadus sp.	7.81	1.47	7.82	1.54	7.82	4.62
Gastropoda	<0.01	<0.01			<0.01	<0.01
Greenland halibut	0.07	0.07	0.08	0.09		
Hake sp.	7.02	1.99	8.39	2.42	0.74	0.52
Liparis sp.	0.02	0.01	0.02	0.01		
Redfish sp.	0.17	0.13	0.17	0.16	0.16	0.17
Sandlance	8.20	2.23	4.92	1.69	23.20	8.35
Sculpin sp.	0.58	0.18	0.46	0.18	1.15	0.52
Silver hake	0.07	0.07	0.09	0.09		
Smelt	0.07	0.07	0.08	0.08		
Snake blenny	0.02	0.01	0.02	0.01	0.02	0.01
Unidentified fish	0.01	<0.01	0.01	<0.01	0.01	0.01
Winter flounder	0.55	0.40	0.21	0.22	2.27	2.27

Table 8. Winter diet (% energy) of grey seals collected in Cabot Strait expressed as the average proportion of each prey species in individual intestines, after correcting for otolith digestion by applying numerical correction factors listed in Appendix 1.

	All (N=77)		Male (N=58)		Female (N=19)	
	Average	SD	Average	SD	Average	SD
Alosa sp.	0.19	0.18	0.25	0.24		
Atlantic cod	12.52	2.71	15.49	3.49	4.33	3.48
Atlantic herring	18.03	3.53	18.92	4.20	14.71	6.34
Atlantic mackerel	0.22	0.18	0.29	0.23		
Bivalve	<0.01	<0.01			<0.01	<0.01
Brachyura (crab)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Capelin	5.94	2.31	3.67	2.01	12.71	6.72
Cephalopoda	0.02	0.01	0.03	0.02		
Daubed shanny	0.12	0.07	0.08	0.06	0.23	0.19
Eelpout sp.	1.13	0.73	1.42	0.96		
Esmark's eelpout	0.31	0.20	0.18	0.14	0.74	0.69
Flatfish sp.	29.06	3.84	28.68	4.18	29.11	8.63
Gadus sp.	9.39	1.82	9.75	1.73	9.00	5.34
Gastropoda						
Greenland halibut						
Hake sp.	8.39	1.98	10.82	2.52	0.83	0.56
Liparis sp.	0.02	0.01	0.03	0.01		
Redfish sp.	0.64	0.53	0.79	0.73	0.20	0.20
Sandlance	11.26	2.75	7.44	2.12	23.80	8.65
Sculpin sp.	0.71	0.18	0.69	0.21	0.77	0.36
Silver hake	0.13	0.12	0.18	0.17		
Smelt	0.21	0.21	0.26	0.27		
Snake blenny	0.03	0.02	0.03	0.02	0.02	0.02
Unidentified fish	0.02	0.01	0.02	0.01	0.02	0.01
Winter flounder	1.66	1.11	0.98	0.98	3.51	3.55

Table 9: Comparison of the presence of Atlantic cod (++) and Gadus sp. (+) in the stomachs and intestines of grey seals collected in Cabot Strait using Hard Part Analysis (HPA) and Polymerase chain reaction (PCR) of stomach contents.

	HPA		PCR	Erosion/Digestion	
	Stom	Int.		Otolith	Stomach
20090044	-	-	-		
20090045	-	-	-		
20090046	-	-	-		
20090047	-	+	-		
20090048	++	-	+	PE[1]	20%5 ; 80%6
20090049	-	-	-		
20090050	-	-	-		
20090051	+	+	-	ER[2]	100%6
20090052	+	+	-	ER	100%6
20090053	-	-	-		
20090054	+	-	-	ER	100%6
20090055	-	-	-		
20090056	-	+	-		
20090057	-	-	-		
20090058	-	-	-		
20090059	-	+	-		
20090060	-	-	-		
20096000	-	+	-		
20096001	-	-	-		
20096002	++	+	-	ER	100%6
20096003	-	-	-		
20096004	-	-	-		
20096005	-	-	-		
20096006	-	+	-		
20096007	-	-	-		
20096008	-	+	-		
20096009	-	-	-		
20096010	-	++	-		
20096011	-	-	-		
20096012	++	++	+	NE[3]	100%6
20096013	-	+	-		
20096014	-	-	-		
20096015	-	-	-		
20096016	++	++	-	ER	30%2;20%3;50%6
20096017	-	++	-		
20096018	-	++	-		
20096019	-	++	-		
20096020	-	-	-		
20096021	-	+	-		
20096022	-	-	-		

	HPA		PCR	Erosion/Digestion	
	Stom	Int.		Otolith	Stomach
20096023	-	-	-		
20096024	-	+	-		
20096025	-	-	-		
20096026	-	++	-		
20096027	-	++	-		
20096028	-	-	-		
20096029	-	-	-		
20096030	-	-	-		
20096031	++	++	+	NE	100%6
20096032	-	++	-		
20096033	++	+	-	SE	100%6
20096034	-	-	-		
20096035	++	++	-	ER	100%6
20096036	-	-	-		
20096037	-	+	-		
20096038	-	++	-		
20096039	-	++	-		
20096040	NA	++	NA		
20096041	-	+	-		
20096042	++	-	+	NE	50%3;50%4
20096043	-	-	-		
20096044	++	++	+	ER	100%6
20096045	-	++	-		
20096046	++	++	-	NE	40%5; 60%6
20096047	++	-	+	NE	40%4;30%5;30%6
20096048	-	-	-		
20096049	-	++	-		
20096050	-	-	-		
20096051	-	-	-		
20096052	-	++	-		
20096053	-	-	-		
20096054	-	++	-		
20096055	-	++	-		
20096056	-	-	-		
20096057	-	-	-		
20096058	-	+	-		
20096059	+	++	-	ER	100%6
20096060	-	-	-		
20096061	-	-	-		
20096062	-	-	-		
20096063	-	++	-		
20096064	-	-	-		
20096065	-	++	NA		

	HPA		PCR	Erosion/Digestion	
	Stom	Int.		Otolith	Stomach
20096066	-	-	-		
20096067	-	-	-		
20096068	-	+	-		
20096069	-	-	-		
20096070	-	++	-		
20096071	-	+	-		
20096072	-	+	-		
20096073	-	+	-		
20096074	-	+	-		
20096075	-	-	-		
20096076	-	+	-		
20096077	-	-	-		
20096078	-	++	-		
20096079	++	-	-	PE	100%6
20096080	-	++	-		
20096081	-	-	-		
20096082	-	-	-		
G. morhua	12	27	6		
Gadus sp.	4	21			

[1] PE = Partial or moderate erosion
[2] ER = Severe erosion
[3] NR = No obvious signs of erosion

Table 10: Relationship between stomach digestive state and otolith erosion on the occurrence of positive and negative PCR results.

	Negative Stomach	Positive Digestion	% Positive
100% Class 6	8	3	27.3
less than 100% Class 6	2	3	40.0
	Otolith Erosion		
Not eroded	1	4	80.0
Slight erosion	1	0	0.0
Partial erosion	1	1	50.0
Significantly eroded	7	1	12.5

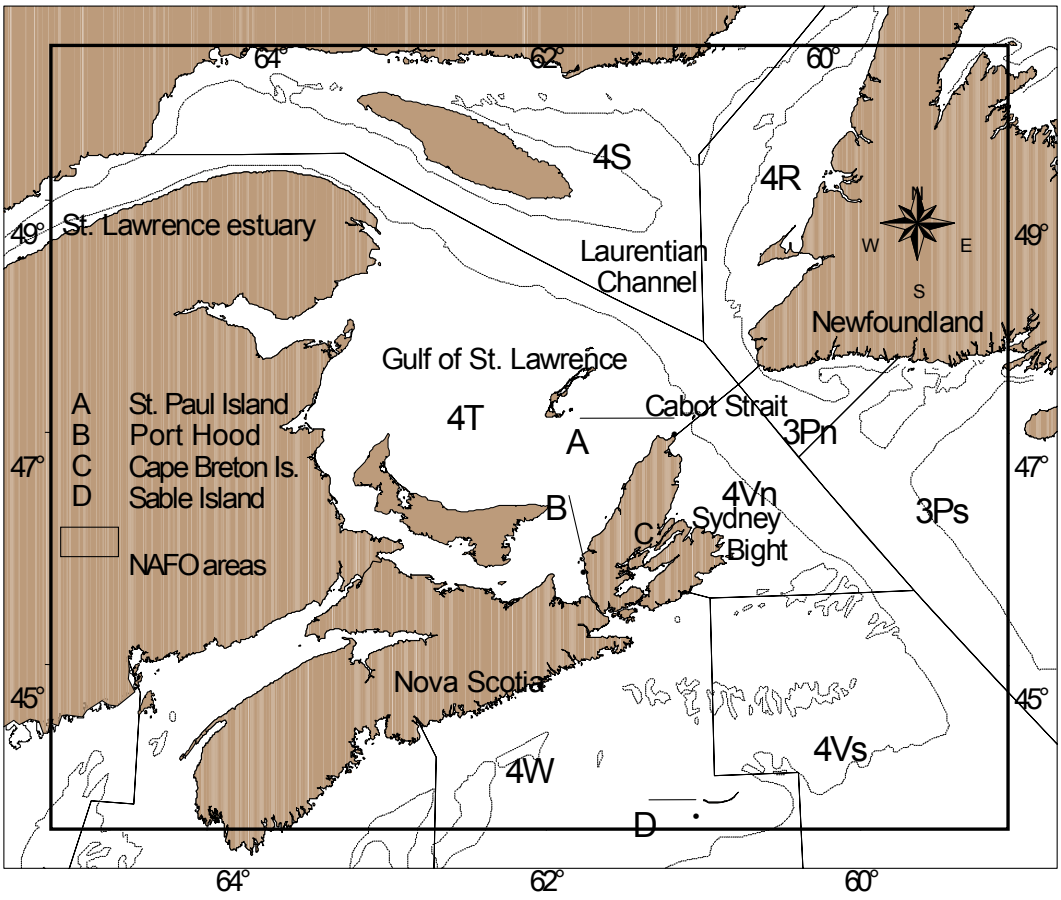

Figure 1. Southern Gulf of St. Lawrence.

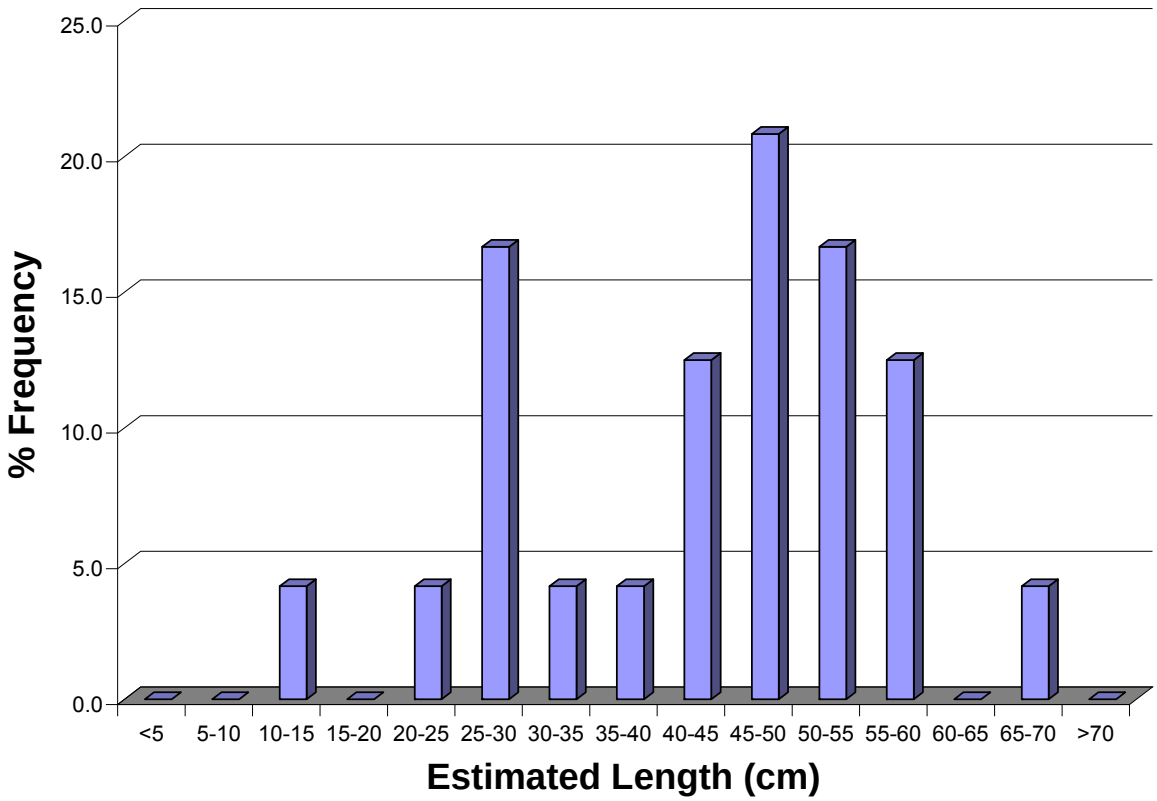

Figure 2. Estimated lengths of cod consumed by grey seals in Cabot Strait area between October and December 2008. The total number of otoliths measured = 24.

Appendix 1. Numerical correction factors (NCF) applied to grey seal prey obtained from intestinal contents to account for complete digestion of prey otoliths. Taken from Bowen et al (2010). Value for fourline snake blenny was used for daubed shanney and Atlantic herring applied to Alosa sp.

Common name	Scientific name	Size (cm)*	NFC	Rounded NCF	Species	Source
Atlantic herring	*Clupea harengus*	20.2–29.3	2.867	2.9	Grey seal	1
Atlantic mackerel Sandeel	*Scomber scombrus*	26.6–33.0	1.391	1.4		1
	Ammodytes marinus	13.2–22.4	2.861	2.9		1
Atlantic cod	*Gadus morhua*	15.8–51.7	1.060	1.1		1
Haddock	*Melanogrammus aeglefinus*	13.5–37.9	1.113	1.1		1
European hake	*Merluccius merluccius*	16.5–40.2	1.081	1.1		1
Whiting	*Merlangius merlangus*	10.0–35.0	1.027	1.0		1
All large gadoids		10.0–51.7	1.069	1.1		
Common dab	*Limanda limanda*	14.8–29.3	1.226	1.2		1
Flounder	*Platichthys flesus*	23.1–32.5	1.418	1.4		1
Lemon sole	*Microstomus kitt*	14.9–32.1	1.539	1.5		1
Long rough dab	*Hippoglossoides platessoides*	14.0–23.9	1.163	1.2		1
European plaice	*Pleuronectes platessa*	13.8–34.3	1.190	1.2		1
Witch flounder	*Glyptocephalus cynoglossus*	24.7–32.0	1.037	1.0		1
Flounder–plaice		13.8–34.3	1.294	1.3		1
All flatfish		13.8–34.3	1.241	1.2		
Squid	*Loligo forbesii*	13.5–337.0	1.064	1.1		1
Capelin	*Mallotus villous*	14.3-14.8	7.87	7.9	Steller seal lion	2
Surf Smelt	*Hypomesus pretiosus*	16.7	4.33	4.3	Harbour seal	3
Wolffish	*Anarhichas lupus*			***2.9***		
Sculpin	Cottidae			***2.9***		
Lumpfish	*Cyclopterus lumpus*			***2.9***		
Eel pout	*Lycodes* sp			***1.2***		
Winter flounder	*Psuedopleuronectes americanius*			***1.3***		
Redfish	*Sebastes* sp			***1.1***		
White Hake	*Urophysis tenuis*			***1.1***		
Ocean pout	*Zoarces americanus*			***2.9***		
American Plaice	*Hippogloossides platessoides*			***1.3***		
Yellowtail flounder	*Limanda feruginea*			***1.3***		
Windowpane flounder	*Scophthalmus aquosus*			***1.3***		
Cunner	*Tautagolabrus adspersus*			***2.9***		
Fourline snake blenny	*Eumesogrammus praecisus*			***1.3***		
Butterfish	*Perprilus triacanthus*					
Silver hake	*Merluccius bilinearis*			***1.1***		
Pollock	*Pollachius virens*			***1.1***		

[1] Grellier and Hammond 2006
[2] Tollit et al. 2007
[3] Cottrell et al. 1996