Diets of Gray *(Halichoerus grypus)* and Harp *(Pagophilus groenlandicus)* Seals in Newfoundland Waters using Hard-part and Molecular Analyses

By

© Jessica E. Foley

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Abstract

I examined the diet of gray (*Halichoerus grypus*) and harp (*Pagophilus groenlandicus*) seals using: hard-part analysis (HPA; subdivided into stomach and intestine) and a multiplex Polymerase Chain Reaction (PCR) technique. Through these methods, I looked at harp and gray seal diet in Newfoundland waters, investigated otolith passage rates within the digestive tract, and developed a multiplex PCR technique to compare with HPA to further investigate biases associated with diet reconstruction based on HPA.

Both techniques provided evidence for retention of large prey and faster passage of smaller pray. I conclude that otoliths of different sizes and thicknesses are affected by digestion differently; I suggest that HPA should be conducted using samples from both the stomach and intestine, and that it should be used in conjunction with other methods of diet analysis like PCR as this may give a better idea of the diet, and prey sizes consumed.

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ANOVA	Analysis of variance
AIC	Akaike Information Criterion
BLAST	Basic Local Alignment Search Tool (NCBI)
Bp	Base pairs
COI	Cytochrome oxidase 1
°C	Degrees Celsius
ddH_20	Distilled dionized water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
FAA	Fatty acid analysis
H_2O	Water
HPA	Hard-parts analysis
Min	Minutes
mg	Milligrams
mm	Millimetre
mM	Millimole
n	Number
NA	Numerical abundance
NADH	Nicotinamide adenine dinucleotide
NCBI	National Center for Biotechnology Information

ND1	NADH dehydrogenase one
ND2	NADH dehydrogenase two
ND 5	NADH dehydrogenase five
ng	Nanograms
FO	Frequency of occurrence
PCR	Polymerase Chain Reaction
qPCR	Quantitative PCR
sec	Seconds
SIA	Stable Isotope Analysis
µL	Microlitre
µM	Micromole

1 Chapter: Introduction

1.1 Background

Accurate methods for determining diets of pinnipeds are important for understanding the impacts that seals may have on prey populations (Tollit *et al*. 2009). Food consumption by predators can be estimated using bioenergetics models, which incorporate the number of individual predators of different ages, and their energy requirements, seasonal distribution, and diet (Hammill and Stenson 2000). Diet is often the most difficult component of the model to estimate accurately. Various approaches can be used to obtain diet estimates, such as direct observation, hard-parts analysis (HPA), polymerase chain reaction (PCR) analyses of gut contents, fatty acid analysis (FAA), or stable isotope analysis (SIA). Each method of estimating diet has its own inherent biases. As such, each provides a differing view of diet.

In this study, I examined the diet of two seal species, gray seal[1] (*Halichoerus grypus*) and harp seal (*Pagophilus groenlandicus*) using two methods: HPA (subdivided into stomach and intestine) and a multiplex PCR

[1]Common and scientific fish names follow FishBase (Froses and Pauly 2013) and the marine vertebrate code of the Northwest Atlantic Fisheries Centre (Akenhead and LeGrow 1981). Seal common and scientific names follow the Committee on Taxonomy, The Society for Marine Mammalogy. 2017. Invertebrate common and scientific names follow the marine invertebrate code of the Northwest Atlantic Fisheries Centre (Lilly 1982).

technique. By comparing these methods, I investigated otolith passage rates and otolith retention within the digestive tract, and improved understanding of biases associated with dietary analysis.

1.2 Gray Seal

Gray seals are a large seal. Male gray seals can reach a length of 2.3 m, and weigh on average 250 kg while females reach 2.0 m in length and have an average weigh of 188 kg (DFO 2017; 2011b: Hammill and Stenson 2000). I studied gray seals from the Northwest Atlantic population (the other populations are Northeast Atlantic and Baltic Sea; Figure 1.1), whose distribution ranges from the northeastern United States to northern Labrador (Figure1.2). The Northwest Atlantic population is subdivided into three sub-populations based on breeding location: the Sable Island herd, the Southern Gulf of St. Lawrence herd, and the coastal Nova Scotia herd (DFO 2010a).

In the Northwest Atlantic, gray seals inhabit inshore and offshore regions of the continental shelf (Mansfield and Beck 1977; Stobo *et al.* 1990). They breed from late December to mid February on Sable Island and on multiple islands and ice packs throughout the Gulf of St. Lawrence (Figure 1.2; Mansfield and Beck 1977). After breeding, seals travel offshore to the east and north and remain there until they return to land to moult in May-June; subsequently, they disperse further throughout eastern Canadian waters before returning to their breeding grounds in the winter (Harvey *et al.* 2008; Lavigueur and Hammill 1993; Stobo *et al.* 1990; Stobo and Zwanenburg 1990).

Observations of gray seals have increased off Cape Breton Island and the

western coast of Newfoundland (Bowen *et al.* 2009); abundance also has increased in southwestern Nova Scotia and in the United States. Distribution and diet of the species in Newfoundland are not well known (DFO 2010a); however, it is thought that gray seals may feed on winter aggregations of Atlantic cod (*Gadus morhua*) in the northern Gulf of St. Lawrence (primarily the Burgeo Bank; Harvey *et al.* 2012; Swain *et al.* 2001; 2015).

1.2.1 *Gray Seal Diving and Diet*

Gray seals are relatively shallow divers despite their large size; most dives are < 50 m deep, but dives > 400 m in depth have been recorded (Beck *et al.* 2003). Diving depth differs according to sex, age, and time of year. Males and females dive at similar depths in the summer months, but males dive more frequently and deeper than females in the winter. Furthermore, juveniles tend to dive deeper than adults (Harvey *et al.* 2008). Such variations likely affect diet since prey species and size vary with depth.

As with diving, gray seal diet also varies according to sex, age, and season. The winter diet of females from the Cabot Strait consists primarily of Atlantic herring (*Clupea harengus*; 70% based on the proportion of the total energy in all stomachs), whereas males fed more on Atlantic cod (~50% based on the proportion of the total energy in all stomachs) and Atlantic herring (21%; Stenson *et al.* 2013). Beck *et al.* (2007) also found sexual differences in diet; both sand lance (*Ammodytes* spp.) and redfish (*Sebastes* spp.) were abundant in the diet of both sexes but male diet was more diverse across all seasons. Those authors found no

sexual differences in the diet of young animals (Beck *et al.* 2007).

Gray seal diet also varies seasonally. In the northern Gulf of St. Lawrence, the spring diet consists primarily of capelin (*Mallotus villosus*), Atlantic mackerel (*Scomber scombrus*), wolffish *(Anarhichas* spp.), and lumpfish (*Cyclopterus lumpus*). In contrast, summer diet is dominated by Atlantic cod, sand lance and winter flounder (*Pseudopleuronectes americanus*) (Bowen *et al*. 2008; Hammill *et al.* 2007).

In addition, diet varies geographically. Diet in western Newfoundland is dominated by five taxa that constitute 80% of dietary mass: sand lance; Atlantic cod plus unidentified *Gadus* species; winter flounder; lumpfish; and Atlantic mackerel (Hammill *et al*. 2007). Off the eastern coast of Newfoundland, the main prey are capelin, winter flounder, and gadoids; a sample from southern Newfoundland contains predominantly Atlantic cod, capelin, unidentified *Gadus* species, pleuronectids, and Atlantic herring (Hammill *et al.* 2007). Finally, gray seals in the southern Gulf of St. Lawrence feed primarily on sand lance, Atlantic cod, cunner *(Tautogolabrus adspersus*), white hake (*Urophycis tenuis*), and Atlantic herring (Hammill *et al.* 2007). Not only do gray seals affect prey populations by consumption, they also affect prey by changing its behaviour (i.e., causing anti-predator behavior), aid in parasite transmission, and compete with some prey species for common food sources (DFO 2010a).

1.2.2 *Population Increase of the Gray Seal in the Northwest Atlantic*

By the mid-1800s, due to extensive hunting, the Northwest Atlantic gray

seal population was on the verge of extinction. The population began to increase in the 1960s (~15,000) and is estimated to be 424,300 (95% CI=263,600 to 578,300) in the 2016 assessment, (Bowen *et al*. 2008; 2010a; 2011b; 2017; Hammill *et al.* 2014; Thomas *et al.* 2007). One reason for the increase is believed to be improved breeding conditions due to increased ice retention around Sable Island; this is thought to have resulted in increased survival of pups. The construction of the Cape Breton causeway in the mid 1950s blocked ice from entering the Atlantic, creating more stable ice for breeding. Another possible reason for population growth is increased adult survival due to decreased predation and reduced human activity (e.g. seal hunting: culls and bounties; DFO 2010a).

Since the mid 1980s, many Atlantic cod stocks within Canada have declined and remained low despite reduced fishing efforts put in place in the early 1990s. Today, many of these stocks show little or no signs of recovery and, in some regions, such as the southern Gulf of St. Lawrence, are at risk of extirpation (Chouinard *et al.* 2005; Swain and Chouinard 2008; Swain *et al*. 2015). Recently, fishermen have reported increased numbers of gray seals along the southern and western coasts of Newfoundland; however, there are no estimates for the number of seals in these areas (Bowen *et al.* 2009). The perceived increase in numbers has led to concerns for recovery of Atlantic cod populations and increases in seal worm (*Pseudoterranova decipiens*) in cod fillets from the northern Gulf of St. Lawrence (Bowen *et al*. 2008; DFO 2009b; 2010a). Gray seals and Atlantic cod may overlap in western and southern Newfoundland, where Atlantic cod form some large winter aggregations (Bowen *et al*. 2008; Bowen *et al.* 2009; Harvey *et al.* 2012; Swain *et*

al. 2001). Direct (e.g. predation) or indirect (e.g. competition and parasite transmission) effects of gray seals may be contributing to high mortality of Atlantic cod (DFO 2010a).

1.3 Harp Seals

Harp seals are smaller than gray seals and the sexes are of similar size: adults average 1.6 m in body length and 130 kg in body mass (Sergean 1991; DFO 2012).

There are three main populations of harp seals in the North Atlantic: White Sea/Barents Sea, Greenland Sea, and Northwest Atlantic populations (Figure 1.3). The latter is the largest population 7.4 million in 2014 (95% CI=6.6 to 8.2 million; Hammill *et al*. 2014b).

The Northwest Atlantic harp seal is migratory. Most seals summer in the Canadian Arctic and Greenland, then migrate south along the Labrador coast for the winter. Seals reach the Strait of Belle Isle around late November. Approximately 70% of the seals remain in northern Newfoundland and southern Labrador, while the others continue to move to the Gulf of St. Lawrence. They accumulate fat stores during the winter (November to February), before they haul out on pack ice in large whelping aggregations in late February-March. The main pupping areas are the "Gulf" (Gulf of St. Lawrence), and the "Front" (off southern Labrador and northeastern Newfoundland). Following weaning, females mate and disperse to forage until they haul out on the ice to moult between mid-April and late May. Most adult seals then return to the Arctic for the summer (Figure 1.4, Chabot *et al.* 1996; DFO 2010b; Sergeant 1991).

1.3.1 *Harp Seal Diving and Diet*

Harp seals can dive to 700 m depth but most dives are shallower; dives > 300 m depth account for only ~12% of dives (Folkow *et al.* 2004). Dive patterns vary seasonally (dives in fall and winter tend to be deeper than at other times of the year) and with time of day (dives tend to be deeper in daylight hours; Folkow *et al.* 2004; Stenson and Sjare 1997).

Harp seal diet also varies seasonally and geographically. In offshore areas, harp seals have a diverse diet that consists mainly of capelin and shrimp in the winter and capelin, shrimp, and sand lance in the summer. The inshore winter and summer diets of harp seals consist primarily of Arctic cod (*Boreogadus saida),* Atlantic cod, Atlantic herring, and capelin (Stenson 2013). Harp seals in western Newfoundland have a diverse diet dominated by capelin, Atlantic herring, Atlantic cod, and redfish, while the south coast diet is dominated by Atlantic cod and redfish (Hammill and Stenson 2004; Lawson *et al*. 1995). Finally, amphipods are an important constituent of diet in some areas (Bowen *et al*. 2008).

Diet also varies with age. In the Northwest Atlantic, pups (< 6 months of age) primarily eat invertebrates (euphausiids and amphipods), capelin, and sand lance (Lawson and Stenson 1997). Immature seals (6 months to 4 years of age) feed mainly on capelin, Arctic cod, sand lance, and shrimp. Finally, adults feed primarily on shrimp, flatfish, capelin, sand lance, and larger prey items like cod (Lawson and Stenson 1997; Sergeant 1991).

Harp seals feed most heavily in October, November, and December, in

preparation for pupping, mating and moulting. They feed least between April-June (during moult; Chabot and Stenson 2002; DFO 2010b).

1.3.2 *Harp Seals and Prey Abundance*

Since harp seals are the most abundant marine mammals in the Northwest Atlantic, they have been blamed for the collapse or slow recovery of multiple fish stocks (Bowen *et al*. 2008). One such stock is the Atlantic cod in the northern Gulf of St. Lawrence (NAFO divisions 3Pn4RS). Historically, this was the second largest population in the western Atlantic (Bowen *et al*. 2008). In the 1990s, many Atlantic cod stocks collapsed, which led to the imposition of multiple fishing moratoria. Overfishing was a main contributing factor to the collapse (Myers *et al.* 1996,1997; Savenkoff *et al.* 2004). There is considerable debate about whether predation by harp seals plays a role in the recovery of cod, although it is known that the diet consists of ~5% Atlantic cod by mass (Hammill and Stenson 2004).

1.4 Methods of Diet Analysis

Direct visual observation is a common technique for estimating diet of terrestrial mammalian predators, but is not feasible for pinnipeds as they spend much of their life in water and travel large distances (Austin *et al*. 2006; Sheppard and Harwood 2005). For some pinniped species, cameras have been used to provide information about foraging behavior, hunting patterns, hunting success, and prey types. However, camera use is limited by recording time and underwater visibility, provides small sample sizes, and cannot be used for many species (Austin *et al.* 2006; Bowen *et al.* 2002; Hooker and Baird 2001; Sheppard and Harwood, 2005;

Tollit *et al*. 2006). Most importantly, the use of cameras requires that animals be live-captured for both deployment and recovery of equipment, which limits the situations in which cameras can be used. Since it is rarely possible to employ direct observation, many indirect methods for determining diet have been developed, such as HPA, molecular analysis (e.g. PCR, quantitative PCR), SIA, and FAA (Sheppard and Harwood 2005).

1.4.1 *Hard-part Analysis (HPA)*

HPA is the most common method of diet analysis for pinnipeds (e.g. Bowen *et al.* 1993; Frost and Lowry 1980; Olesiuk *et al*. 1990). It involves recovery and identification of hard parts such as beaks, bones, otoliths, scales, and carapaces of prey from stomachs, intestines, or feces (Bowen 2000; Bowen and Harrison 1994; Fitch and Brownell 1968; Hammond *et al.* 1994; Prime and Hammond 1987). Sagittal otoliths of bony fish are often the most common hard part recovered; the shape of these structures is often species-specific, so they are valuable for reconstructing diets of predators, such as pinnipeds. Otolith length and weight regressions can also be used to estimate the size and weight of ingested prey (Bowen *et al.* 1993; 2008).

There are advantages and disadvantages to diet reconstruction based on HPA. Prey species, size, age, weight, caloric value, and relative proportions of ingested prey items can all be calculated from hard-part measurements (Beck *et al.* 1993; Murie and Lavigne 1991, 1992). However, differences in otolith size and morphology across prey species and ages can result in differential digestion rates

and retention times in the digestive tract (e.g., large robust otoliths are retained longer in the digestive tract than small delicate otoliths; Deagle and Tollit 2007; Jarman *et al.* 2002; Tollit *et al*. 1997; 2003). Other biases associated with HPA include (a) under-representation of soft-bodied prey species that lack hard parts, (b) differences in those types of hard parts that are lost due to digestion or regurgitation, and (c) under-representation of prey species whose hard parts may not always be ingested (as when seals selectively eat some viscera, like the liver, and discard the rest of the body (i.e. belly biting; Bowen 2000; Fu *et al*. 2001; Gudmundson *et al*. 2006). The magnitude of these biases has been investigated in several studies. For example, recovery rates of hard parts in captive seals can vary up to tenfold across prey species (Tollit *et al*. 1997). Meal size also can affect diet estimates obtained from HPA (Sinclair *et al*. 2011), because large meals of small prey pass through the digestive tract faster than do small meals of larger prey (Marcus *et al.* 1998). The presence of secondary prey items, i.e. those that were consumed by fish that themselves were eaten by seals, can also affect estimates of diet (Perrin *et al*. 1973). Although secondary prey is a problem inherent to all types of diet analysis, it is thought to account for a small part of the diet (< 1% by weight; Hammond and Grellier 2006).

Studies on captive seals have provided estimates of passage times, hard-part retention, and otolith loss (Bowen 2000; Grellier and Hammond 2006; Hall-Aspland *et al.* 2011; Sinclair *et al*. 2011; Tollit *et al.* 1997). These studies have led to the establishment of correction factors for reducing biases. Species-specific and grade-specific digestion coefficients (DC) have been developed for certain seal

species to deal with different degrees of otolith erosion seen in scat (Grellier and Hammond 2006). Numerical correction factors (NCF) were developed to account for complete hard part loss by feeding seals known amounts of prey and then determining how much individual prey were accounted for in scat, based on hard-part counts after the meal had passed. Applying NCF and DCs is intended to increase accuracy of diet analysis in two ways: first by accounting for complete otolith loss during digestion (i.e. NCF) and second, by obtaining more accurate estimates of prey length and weight given different degrees of otolith erosion in scat (i.e. DCs; e.g. Bowen 2000; Grellier and Hammond 2006). Unfortunately, such correction factors are not available for prey items collected from stomachs (DFO 2010a).

Prey occurs in varied stages of digestion in stomach samples, from fully intact prey to prey that have been completely digested. Therefore, only non-eroded or slightly eroded otoliths are measured. Non-measurable otoliths are assigned a size based on averages of measurable hard parts; however, this can be problematic as non-measurable otoliths are assumed to represent prey of the same size rather than smaller prey, which may not be correct (Tollit *et al.* 1997).

Looking at both the stomach and intestine considers a longer foraging time and represents multiple meals, not just the most recent meal. Looking at both the stomach and the intestine also provides the opportunity to investigate differences in passage rates of otoliths across the digestive tract, which cannot be done in captive studies. HPA results may vary based on sampling method due to under- or over-representation of hard parts in different parts of the digestive tract. Discrepancy in

otolith distribution by size and erosion state throughout the digestive tract may provide information on whether or not prey of different sizes or ages are being expelled at different rates (Sinclair *et al.* 2011). By analyzing different parts of the digestive tract, we may be able to learn how to correct for such bias and thereby increase the accuracy of HPA.

1.4.2 *Molecular Methods of Dietary Analysis*

Genomic techniques provide a relatively new opportunity to identify prey which can be compared to more traditional methods. PCR is a molecular technique that exponentially increases the number of target DNA sequences by using a thermal cycler to repeat heating and cooling cycles (Saiki *et al.* 1988).

Prey items contain species-specific DNA which persists in the digestive tract, making it possible to use a PCR with species-specific primers to determine the prey that a carnivore has ingested (Dunshea 2009; Symondson 2002). Species-specific PCR has been tested on DNA extracted from both scat and stomach contents of pinnipeds with promising results; (Deagle and Tollit 2007; Deagle *et al.* 2005; Marshall *et al.* 2010). These molecular methods provide an important addition to traditional HPA because it can identify prey with morphologically similar hard parts (e.g. as in salmonids) and it can also identify prey in the absence of hard parts (Deagle *et al.* 2005; Fu *et al.* 2001; Marshall *et al.* 2010; Purcell *et al*. 2004; Tollit *et al*. 2006). It can also be used to investigate biases associated with HPA, such as otolith retention time (Marshall *et al.* 2010; Tollit *et al*. 2006). However, these specific-species DNA analyses are expensive and time consuming,

and generally test for only a single species at a time. It should be noted, that this technique only provides a presence or absence of prey and cannot estimate the number of prey consumed.

Harper *et al*. (2005) and King *et al*. (2010) designed techniques to perform simultaneous species-specific tests for multiple prey items of carabid beetles (Carabidae). They did this by incorporating a multiplex PCR method with fluorescent-labeled primers for determining diets of invertebrate predators. This method was more rapid, accurate, and cost-efficient than conventional PCR.

1.4.3 *Stable Isotope Analysis (SIA)*

SIA has been used in diet studies and does not reveal the identity of the prey species directly, but instead provides information on prey trophic level and location (i.e. depth in the ocean; see below) over a few days or years, depending on the tissue used (Bowen and Iverson 2012; Crawford *et al.* 2008; Davenport and Bax 2002; Sherwood and Rose 2005; Tucker *et al.* 2007; 2008).

SIA is performed by observing the ratios of $\delta^{15}N$ and $\delta^{13}C$ in tissue samples. $\delta^{15}N$ is used primarily to determine prey trophic level since it is greater in higher predators (Davenport and Bax 2002; Sherwood and Rose 2005). $\delta^{13}C$ is used primarily to look at the depth in the ocean from which a prey was taken (e.g. pelagic organisms tend to be lower in $\delta^{13}C$ than benthic organisms; Davenport and Bax 2002; Sherwood and Rose 2005).

The primary problems with SIA are that: (a) it does not provide details on a

specific prey species and (b) isotope turnover rates vary depending on the metabolic activity of the body region sampled. Regions with high metabolic activity show faster isotopic turnover rates than do less metabolically active tissues which will effect diet interpretations (Crawford *et al.* 2008; Davenport and Bax 2002; Kurle 2002; Sherwood and Rose 2005; Tucker *et al.* 2007, 2008, 2009). Bayesian mixing models have been developed to estimate the contribution of different prey to predator diets (Cherry *et al*. 2011; Clouquet *et al*. 2006; Lerner *et al.* 2018; Moore and Semmens 2008; Parnell *et al*.2010) and rely upon *a priori* assumptions about the diet.

1.4.4 *Fatty-acid Analysis (FAA)*

FAA uses the distinctive chemical signature of individual fatty acids from different prey types to estimate diet (Budge *et al*. 2006; Grahl-Nielsen *et al*. 1999; Iverson *et al*. 2004; Walton and Pomeroy 2003). Differences in fatty acid signatures from two individuals indicate different diets but identifying the specific prey types that contributed the fatty acids is more difficult. This method is thought to enable both qualitative and quantitative analyses of predator diet. Different prey types have specific fatty acid signatures, which are incorporated into the predator's body fat, so fatty acid profiles can be determined in predator fat stores and used to identify prey. FAA can reveal spatial differences in diet as well (Grahl-Nielsen *et al.* 1999; Iverson *et al.* 2004). Quantified fatty acid signature analysis (QFASA) uses an optimization model to estimate prey composition by matching fatty acid signatures from the predator's blubber with those of its prey (Iverson *et al*. 2004).

There are several problems and concerns when using FAA. First, there is a need for a library of fatty acid signatures of prey species. There is also the need for conversion of ingested FA into seal blubber, which requires captive studies on different seal species fed different prey species to estimate the conversion coefficients (CC). however, not many CC estimates are available (Bowen and Iverson 2012).

It is important to consider the source of the tissue being analyzed. The blubber layer is highly stratified, with the outer layer composition dependent on the seal's age. In contrast, the inner layer contains only a weak signal about the predator-prey relationship, and furthermore is a metabolically active region with high turnover of fatty acids (Grahl-Nielsen *et al*. 2011). Other potential biases are different turnover rates of lipids in different prey and seal species (Grahl-Nielsen *et al*. 1999; Nordstrom *et al*. 2008), reduced accuracy during pulse feeding events (Hoberecht 2006) and increased error when investigating dietary changes over time (Wang *et al.* 2010). Extensive research is being done to deal with some of these problems; for example, fatty acid calibration coefficients are being developed to account for differential deposition and synthesis of fatty acids during lipid metabolism (Iverson *et al*. 2004; Tollit *et al*. 2006).

1.5 Comparisons of Methods for Determining Diet

Despite the biases outlined above, it may be possible to minimize error in estimating diet by using several methods jointly. Marshall *et al*. (2010) found that using PCR in conjunction with traditional HPA provided the opportunity to see how prey with large robust otoliths may be overrepresented in the diet due to retention

time, while prey items with smaller, fragile otoliths may be underrepresented. Sinclair *et al.* (2011) showed that otoliths of walleye pollock (*Theragra chalcogramma*) in northern fur seals (*Callorhinus ursinus*) vary in size at different locations of the digestive tract. They suggested smaller prey pass through the digestive tract more quickly than larger prey items. Furthermore, looking at different sections of the digestive tract and comparing HPA with PCR results may provide insights on hard-part passage rates. For instance, positive PCR results with no otoliths in stomach is not informative about what happened to the otoliths; however, if a prey's otoliths are found in the intestine, one can conclude that prey hard parts passed quickly through the digestive tract.

1.6 Objectives

The primary objective of this study was to improve understanding of biases associated with different methods of analyzing diets and to apply this understanding to determine the diet of harp and gray seals in Newfoundland waters. Specific objectives were:

1. Obtain reliable data on diets of gray and harp seals around Newfoundland through HPA of the stomach and intestines and with multiplex PCR (Chapters 2,3,4).
2. Study the relationship of otolith presence and size to location in the digestive tract. This allows for determination of species, size, or extent of digestion along the digestive tract (Chapter 2).
3. Develop a multiplex PCR method to detect four prey items simultaneously, from stomach contents, using species-specific primers

(Chapter 4).

4. Compare HPA results with multiplex results (Chapter 4).

5. Through multiplex PCR, determine possible effects that different degrees of stomach content digestion have on DNA amplification (Chapter 4).

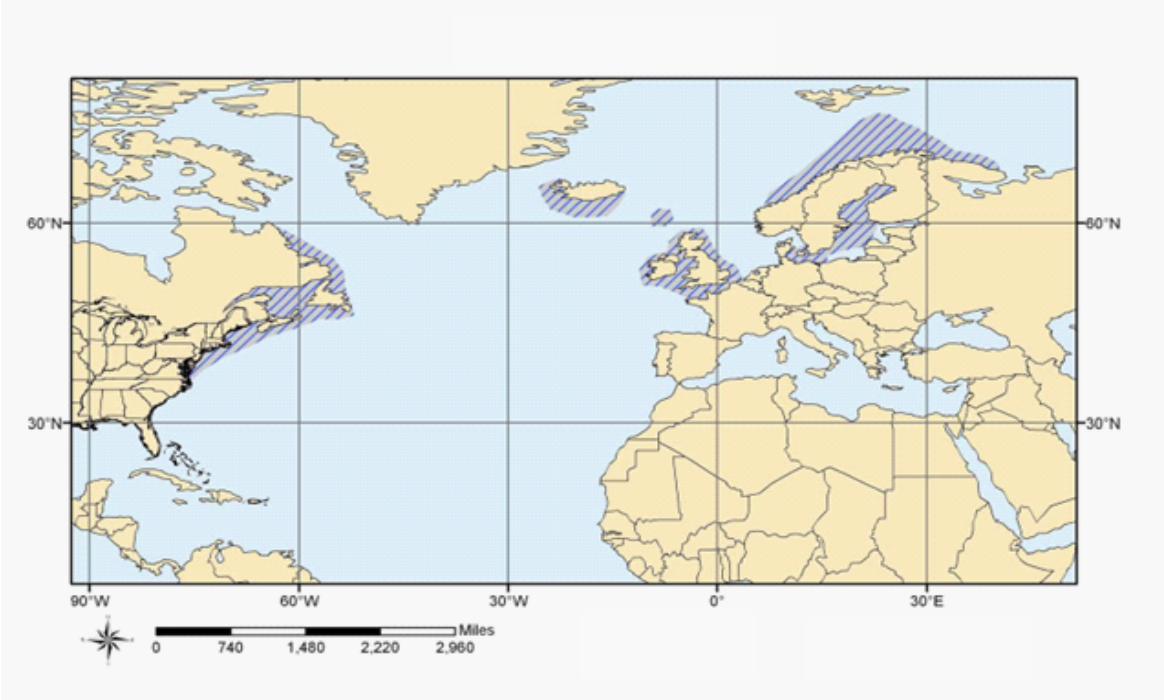


Figure 1-1: Distribution of gray seals (*Halichoerus grypus*) in the Northwest Atlantic, Northeast Atlantic and Baltic Sea outlined in gray (image obtained from NOAA, http://www.nmfs.noaa.gov/pr/species /mammals/pinnipeds/grayseal.htm)

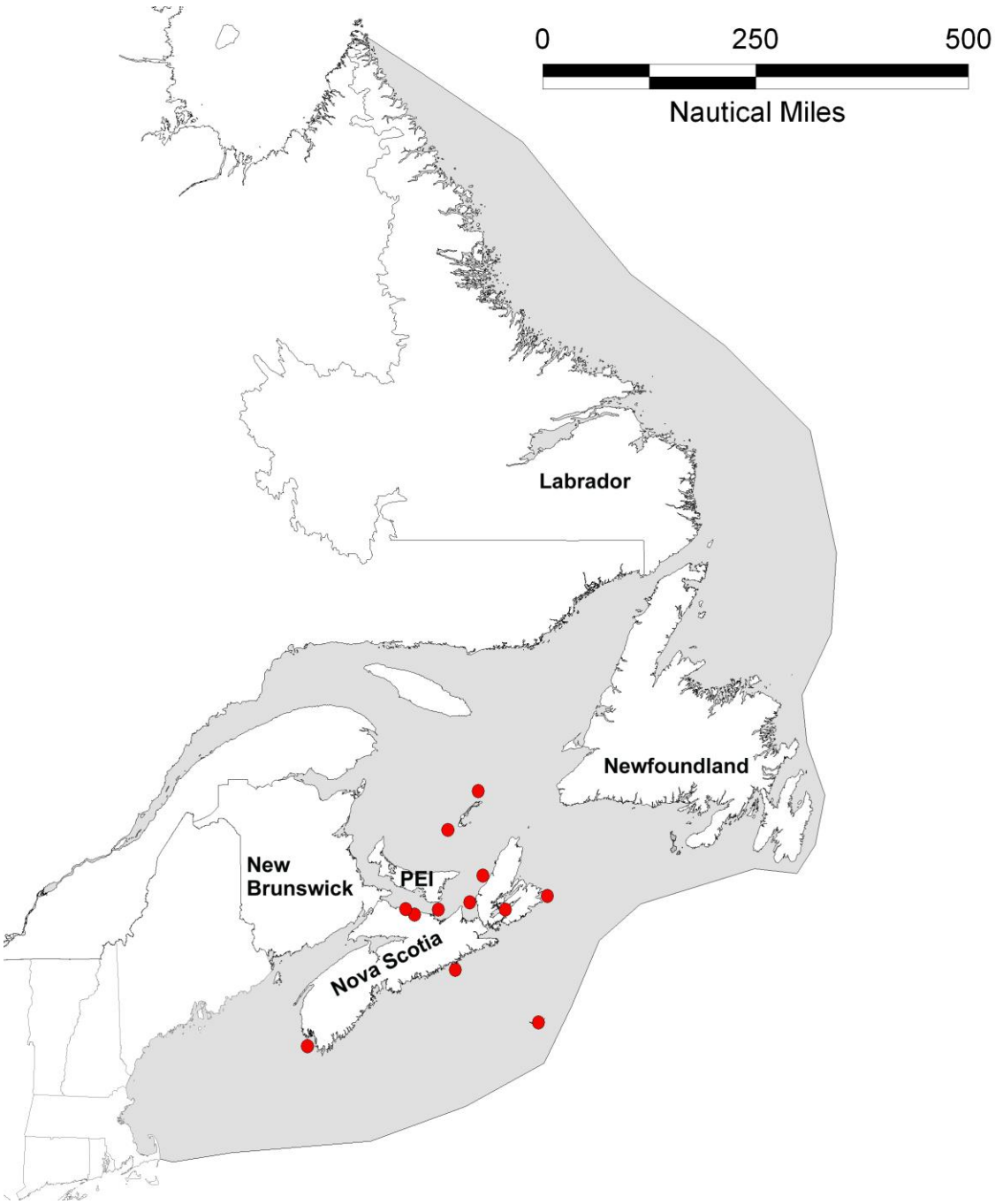


Figure 1-2: Gray seals (*Halichoerus grypus*) in the Northwest Atlantic are distributed from the northeastern United States to northern Labrador. Red dots represent main pupping colonies in Atlantic Canada (map developed by Lawson 2018; distribution data obtained from DFO 2011, NOAA).

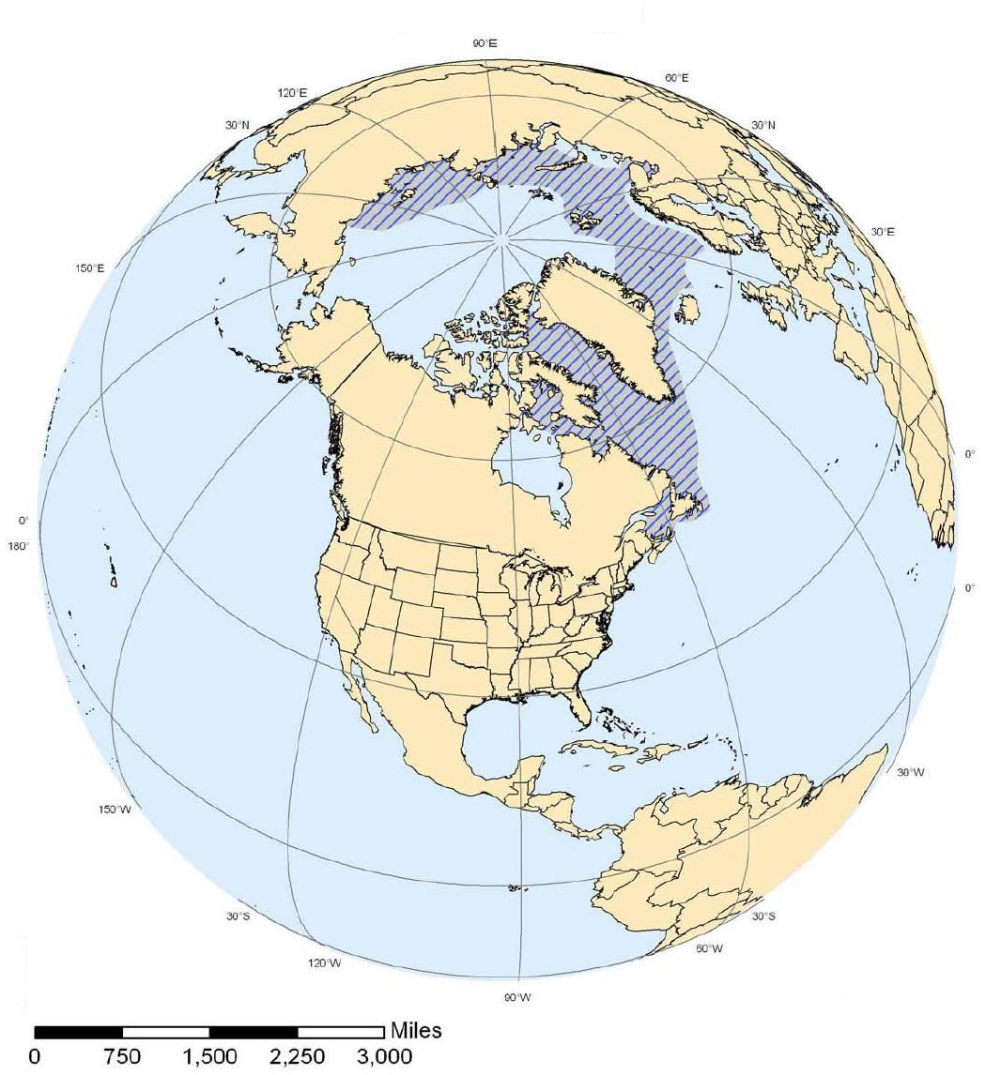


Figure 1-3: Range of harp seals *(Pagophilus groenlandicus*) in the White Sea/Barents Sea, Greenland Sea, and Northwest Atlantic outlined with gray hatching (image obtained from NOAA, http://www.nmfs.noaa.gov /pr/pdfs/rangemaps/harpseal.pdf**.**

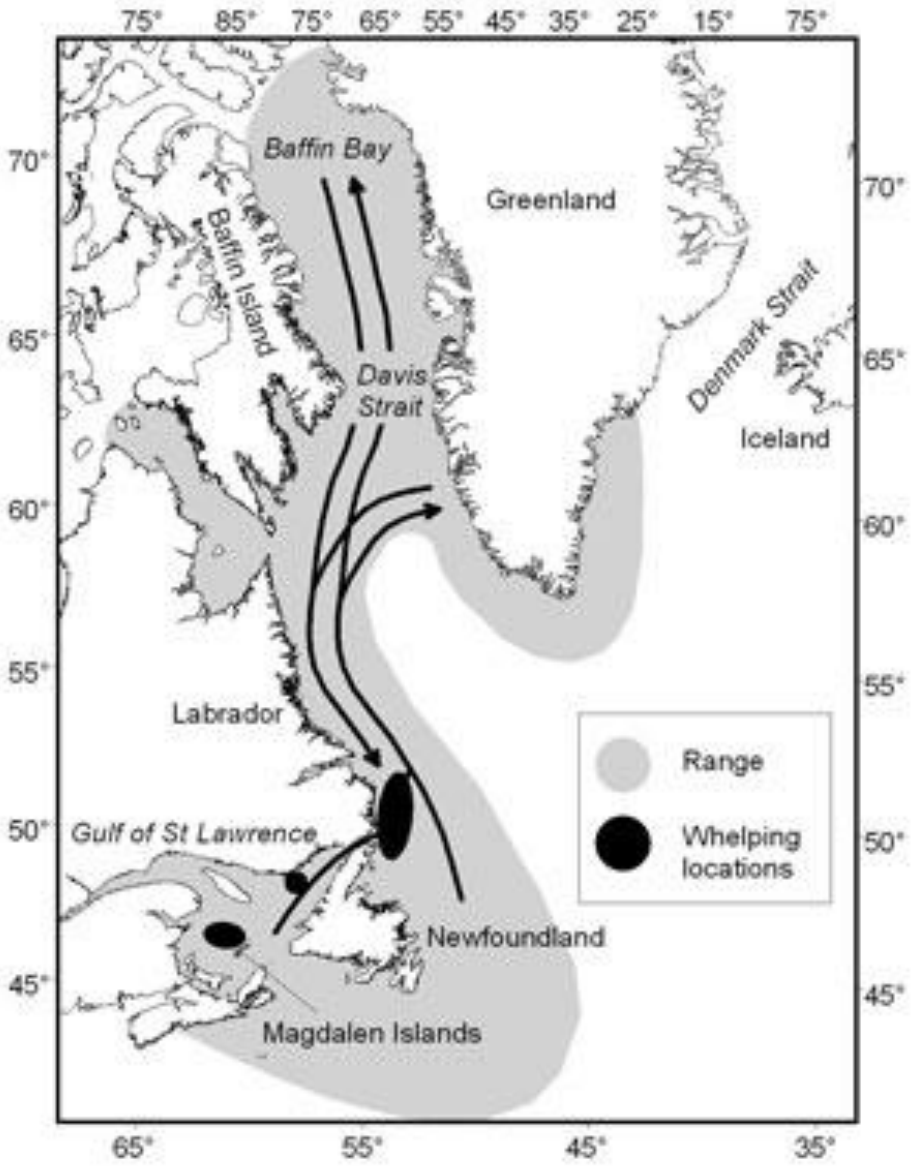


Figure 1-4: Distribution, migratory patterns, and breeding areas of harp seals *(Pagophilus groenlandicus*) in the Northwest Atlantic population (from http://www.dfo-mpo.gc.ca/species-especes/profiles-profils/harpseal-phoquegroenland-eng.html).

2 Chapter: The influence of otolith morphology, size, and location in the digestive tract on dietary reconstruction of seals

2.1 Abstract

In piscivorous predators, changes in otolith distribution and quality throughout the digestive tract may indicate if prey individuals of different size pass through the digestive tracts at different rates. I investigated how species-specific differences in otolith morphology and digestion time affect dietary estimates for harp seal (*Pagophilus groenlandicus*) and gray seal (*Halichoerus grypus*) using four fish species. Atlantic cod (*Gadus morhua*) has the largest and most robust otoliths, followed by Arctic cod (*Boreogadus saida*), sand lance (*Ammodytes* spp.), and capelin (*Mallotus villosus)* which have the smallest and most fragile. Otoliths were recovered from the digestive tracts (stomach and intestine) of 53 harp seals and 17 gray seals from the Northwest Atlantic Ocean. I found that much of the variability seen in otolith length down the digestive tract was due to variations between individual animals, however, some variability could be explained by otolith location and erosion state for some species. It was apparent that larger otoliths (e.g. Atlantic cod) tended to be retained preferentially; so obtaining prey size estimates from only the stomach or intestine can result in inflated or deflated size estimates respectively. State of erosion can also affect calculated prey size in a similar manner as seen in this study with Atlantic cod and sand lance. I conclude that otoliths of different size and thickness are affected by digestion differently, hence species-specific correction factors are needed for different stages of erosion and prey size class; in

addition, complete otolith loss must be accounted for.

2.2 Introduction

Many marine mammals are important predators with direct (e.g. predation) and indirect (e.g. competition and parasite transmission) impacts on other components of their ecosystems (Bowen 1997; Chouinard *et al*. 2005; DFO 2010a; Morissette *et al*. 2006; Savenkoff *et al*. 2004). The increases in populations of some seal species and the collapse of numerous fish populations in eastern Canadian waters over the past 50 years have ignited debate about effects of seals on natural mortality of fish (Bowen *et al*. 2008; 2009; DFO 2010a).

It is difficult to determine diet through direct observation since seals spend much of their life at sea (Bowen and Iverson 2012; Tollit *et al.* 2006). Indirect methods to determine seal diet include identification of hard parts in the digestive tract (Stomach to anus; HPA), molecular analysis (e.g. polymerase chain reaction (PCR); quantitative PCR), stable-isotope analysis (SIA), and fatty-acid analysis (FAA). Each of these methods may have different consequences on the results as they maybe measuring different aspects of diet and they also have their own limitations and biases which may result in different estimates of diet (Chapter 1; Bowen and Iverson 2012).

The most common method of diet analysis is HPA (Bowen *et al.* 1993; Frost and Lowry 1980; Olesiuk *et al*. 1990), which involves the recovery and identification of prey hard parts (e.g. beaks, bones, sagittal otoliths, scales, carapaces) from stomachs, intestines, or feces (Bowen 2000; Bowen and Harrison 1994; Hammond *et al*. 1994; Fitch and Brownell 1968; Prime and Hammond 1987). Although this can include vertebrae, scales and operculum plates, sagittal otoliths are frequently used, since their shape is

distinctive for different bony fish species, and otolith size can be used to estimate the length and weight of ingested prey (Bowen *et al.* 1993; 2008).

Stomach and intestine contents can be collected from dead animals, or from live animals by lavaging the stomach (Harvey and Antonelis 1994). Scat HPA is a non-invasive technique in which scat samples are collected at haul-out areas. However, scats usually cannot be assigned to individual seals, the number of scats varies greatly among individuals and one meal may be represented multiple times (Hammond and Rothery 1996; Tollet *et al*. 1997a,b; Reed *et al*. 1997). Prey species, size, age, weight, caloric value, and relative proportions of different prey items consumed can all be calculated from measurements of hard parts (Beck *et al.* 1993; Bowen and Iverson 2012; Murie and Lavigne 1991, 1992). However, there are several assumptions and limitations inherent in dietary reconstructions based on HPA. For example, otolith size and morphology vary among species leading to differential digestion rates and retention times in the digestive tract (e.g. large otoliths can be retained longer than small ones; Figure 2.1; Deagle and Tollit 2007; Jarman *et al.* 2002; Tollit *et al*. 1997, 2003). In addition, some prey species lack measurable hard parts, and hard parts may disappear or become unidentifiable due to digestion or regurgitation; furthermore, seals may preferentially consume soft parts of large prey (Bowen 2000; Da Silva and Nielson 1985; Fu *et al*. 2001; Gudmundson *et al*. 2006; Prime and Hammond 1987).

Captive studies have provided much information about passage times, hard-part retention, and otolith loss (e.g. Bowen 2000; Grellier and Hammond 2006; Hall-Aspland *et al.* 2011; Sinclair *et al*. 2011; Tollit *et al.* 1997). For example, digestion coefficients have been developed using feeding trials to address varied otolith digestion in scat

samples of gray seals (Grellier and Hammond 2006). To account for complete loss of otoliths during digestion, numerical correction factors (NCFs) were developed by feeding captive seals known amounts of prey and then determining how many individual preys were recovered in the scat (Bowen 2000). Applying such coefficients increases the accuracy of diet analysis by increasing the importance of fragile prey (NCF) or by enabling more accurate estimates of prey size (digestion coefficients; Bowen 2000; Grellier and Hammond 2006).

Correction factors for degree of erosion have not been developed for hard parts obtained from stomach (Bowen and Iverson 2012). Digestion occurs differently down the digestive tract. The stomach is the location were most erosion takes place since the stomach uses both chemical and mechanical means to break down prey (Bowen and Iverson 2013; Harvey 1987; Robbins 1983). The stomach is a highly acidic environment (pH ~2-4) which can digest hard parts and, in turn, negatively bias estimates of prey size, age, weight, and caloric value. (Bowen and Iverson 2012; Robbins 1983). NCF obtained for scat can be used for intestine HPA since further otolith erosion is thought to be limited (Guyton 1981; Harvey 1987; Stenson *et al*. 2013). Diet estimates obtained from stomach HPA are usually determined by measuring only otoliths with minimal erosion. Stenson *et al.* 2013 performed ANOVA tests to determine if the lengths obtained from otoliths in of erosion states 1 (not eroded) and 2 (slightly eroded) were comparable and found that in most cases lengths were not significantly different, however, in some cases they were. Heavily eroded otoliths are simply counted and assumed to be the same size as otoliths with minimal erosion which may or may not be correct (Tollit *et al.* 1997).

Differential movements of hard parts within the digestive tract are not well

known, but once food reaches the intestine, little chemical digestion occurs. Some items pass through the stomach very quickly, and empty stomachs are common in many diet studies (Hammill *et al* 2005; Prime and Hammond 1990; Rae 1968). Since some prey species can pass through the stomach quickly, analyzing prey remains throughout the gastrointestinal tract provides a longer window over which a seal has fed, and may represent multiple meals. It also provides the opportunity to investigate passage rates and the effect of erosion and mechanical breakdown on otoliths, since changes in otolith size, counts, distribution and quality throughout the digestive tract may indicate if prey of different size classes are passing through or being broken down in the digestive tract at different rates (Sinclair *et al.* 2011).

Harp seals (*Pagophilus groenlandicus*) and gray seals (*Halichoerus grypus*) are good models for investigating otolith erosion and passage rates, as both species are generalist predators that eat multiple species of bony fish, including capelin (*Mallotus villosus*), Atlantic herring (*Clupea harengus*), Atlantic cod (*Gadus morhua*), and sand lance (*Ammodytes* spp.; Bowen 2008, Hammill *et al.* 2007; Stenson *et al*. 2013; Hammill and Stenson 2000; Lawson and Stenson 1994). Historically gray seal diets from the Northwest Atlantic have been estimated mainly by analysis of hard parts in scat (e.g. Bowen and Harrison 1994, 2007; Bowen *et al.* 2011) as well as stomachs, and both stomach and intestines (e.g., Hammill *et al.* 2007; Stenson *et al*. 2013). Analysis of harp seal diet for the Northwest Atlantic has been based primarily on the study of hard parts in stomachs (e.g. Beck *et al.* 1993; Lawson *et al*. 1994;1995).

As discussed above, multiple factors affect estimates of seal diet based on HPA (Gudmundson *et al*. 2006; Harvey 1989; Marcus *et al.* 1998; Sinclair *et al.* 2011) and

examining both the stomach and intestine may affect diet reconstruction outcomes and shed light on biases associated with stomach and scat HPA. The objective of this study is to increase understanding of factors affecting the accuracy of diet estimates for harp and gray seals, to increase accuracy of estimates of prey consumption. I did this by comparing the distribution, otolith size and state of erosion throughout the digestive tract. The relationship between otolith location and size in the digestive tract for Atlantic cod, Arctic cod, capelin, and sand lance was examined to determine if there were differences in species, size, or digestive state in each portion of the digestive tract analyzed and the consequences the results have on diet studies were presented.

2.3 Methods

2.3.1 *Seal Digestive Tract Samples*

Stomachs and intestines were collected from 20 gray seals and 57 harp seals sampled around the island of Newfoundland from February 2008 to September 2011 (Appendix 2-A). All animals were collected under licenses issued by the Department of Fisheries and Oceans (DFO) and seals were killed using humane methods outlined in the marine mammal regulations of the Canadian Fisheries Act (http://laws-lois.justice.gc.ca/eng/regulations/SOR-93-56/). Digestive tracts (stomach to anus) were removed in the field and tied off at the oesophageal, pyloric, and anal sphincters to retain contents. They were then placed in labeled cloth bags and stored at -20°C until analysis was performed. Jaws from the same specimens were also retained for aging using cementum growth layers in the teeth (Hohn 2018).

2.3.2 *Hard-parts Analysis (HPA)*

After thawing stomachs, I cut them open and flushed out the contents with fresh water. Stomach contents, plus rinse water, were processed through a series of four sieves of decreasing mesh size (4.75, 2.0, 1.0, 0.8 mm). A Pyrex dish was placed below the sieves to ensure no items were lost. Intact fish or invertebrates were removed, measured, and weighed. All material retained in the sieves were rinsed with fresh water into a glass pan on a dark background and examined macroscopically. Otoliths, carapaces, beaks, and bones were retained for identification (Lawson *et al.* 1995).

To analyze intestinal contents, I first measured intestinal length to ± 1 cm. I measured the length of the small intestine (duodenum, jejunum, plus ileum) to ± 1 cm, then cut it into segments of equal length (determining the three functional anatomical regions externally was difficult). Finally, I measured the length of the colon to ± 1 cm. Intestinal contents were analyzed using the same method as the stomach contents.

2.3.3 *Otolith Erosion State Classification and Measurements*

Otoliths were identified to species when possible, using published identification keys and DFO otolith reference collections (Campana 2004; Hãrkönen 1986). The most common prey obtained from the analysis of the stomach and intestine for harp seals (Atlantic cod, Arctic cod, and capelin) or gray seals (sand lance; Figure 2.1) were identified. Following the procedures used to identify stomach contents, erosion of otoliths was assessed visually and scored on four classes: state 1, no erosion; state 2, minor erosion of margins; state 3, moderate erosion (otolith margins with deterioration); and

state 4, severe erosion, with extensive deterioration of margins, distorted shape and cracks (some otoliths in this class were not identifiable to species; Stenson *et al*. 2013). Stomachs often contained otoliths at different states of erosion. Some stomachs had full prey items, which were easily measured; they also contained partially digested prey, some with the prey's skulls still intact. Otoliths taken directly from the skull and otoliths present outside the skull that did not show visible signs of erosion were recorded as state 1 otoliths. I also measured state 2 otoliths. Previous work, I conducted looking at otolith erosion in gray seals showed that using otoliths of a greater erosion state would result in a significant decrease in otolith length, therefore I identified but did not measure otoliths in state 3 or 4 (Flight 2009 Unpublished; Stenson *et al.* 2013).

To estimate the number of individual bony fish represented, I measured only all left or all right otoliths from each sample, whichever were more numerous (Hammill *et al*. 2007; Lawson *et al*. 1995). I randomly subsampled 100 left or right otoliths from samples with large numbers (> 100) of individual prey. State 1 and 2 otoliths were measured to ± 0.01 mm using one of two methods. Otoliths < 4 mm in length were digitally photographed and measured using the program ImagePro Plus (Media Cybernetics, Inc.). Otoliths of ≥ 4 mm in length were measured using Vernier calipers. When left or right otoliths could not be determined, I assumed that otoliths of similar size (difference < 0.1 mm for otoliths < 5 mm in length; < 0.25 mm for otoliths ≥ 5 mm in length) and erosion were from the same fish. Unmatched otoliths were assumed to represent additional individuals.

2.3.4 *Data Analysis and Model Selection*

To examine otolith length and the proportions of state 1 and 2 otoliths along the digestive tract, I carried out two sets of analyses: (a) relationships of otolith length for each prey species in relation to erosion state and section of the digestive tract where sampled; and (b) relationships of the proportions of state 1 and 2 otoliths to section of the digestive tract where found and prey species. In all cases, I accounted for pseudo-replication, since I obtained multiple observations from each individual seal, by modeling the response variable as a generalized linear mixed model (GLMM), where I considered individual seal ('seal ID') as a random explanatory variable. Statistical analyses were performed in the R statistical language (R Core Team), and plots produced with the package ggplot2 (Wickham 2009).

2.3.4.1 Analysis 1: Otolith Length

To investigate otolith length down the digestive tract, I modeled otolith length as a general linear mixed model with a normal error, where 'seal ID' was considered as a random factor, to account for pseudo-replication. I included 'erosion state' (i.e. 1 or 2), 'section', and their interaction as explanatory variables. The full model was therefore of the form:

Otolith length ~ erosion state + section + erosion state * section + seal ID

2.3.4.2 Analysis 2: Proportions of Otoliths with limited erosion down the Digestive Tract

I modeled the mean proportions of otoliths with limited erosion (state 1 and 2) vs. moderately eroded (state 3 and 4) otoliths as a GLMM with a binomial error (logit link), where 'seal ID' was considered as a random factor, to account for pseudo-replication. For the harp seal samples, I included section and prey species (and their interaction) as explanatory variables because I wanted to evaluate the different proportions of otoliths

with limited erosion across prey species. The full model was:

Proportion of otoliths with limited erosion ~ section + prey species +section * prey species + seal ID.

As the only prey used to model the proportion of otoliths with limited erosion otoliths for gray seal was sand lance, prey species was not included in the model for these seals. Models were fit using the function glmer in package lme4 (Bates *et al.* 2013).

In all cases, model residuals were inspected visually to ensure that assumptions of homogeneity, normality, and independence were met (Breslow 1996; Cameron and Trivedi 1998; Dobson 2002; Hoffman 2004; Lindsey 1997). In the case of the proportion of otoliths with limited erosion otoliths, where the response variable is binomial, I calculated an approximate estimate of overdispersion. Overdispersion occurs when variation in the observed data is greater than predicted by the model (McCullagh and Nelder 1989). Overdispersion was calculated as the ratio between the sum of squared Pearson residuals and the residual degrees of freedom (Bates 2011). In all cases, the value of the dispersion parameter was close to 1, therefore I did not apply corrections for overdispersion.

For both sets of analyses I identified potential significant covariates and built all possible candidate models. I then ranked and selected the best models based on the Akaike information criterion (AIC), with the lowest AIC value being the model providing the best fit (Burnham and Anderson 2002). To determine a relative measure of empirical support for each model, I used Delta AIC (ΔAIC) and the evidence ratio (Ei; Anderson 2008). ΔAIC is the difference between the lowest AIC value and all other models. The evidence ratio is the ratio between the likelihood of the best model and the likelihood of

the alternative model (Anderson 2008, Burnham *et al*., 2011; Johnson and Omland 2004; Mazerolle *2013*). Models with ΔAIC of ≤ 2 were well supported and considered to be equally plausible (Burnham *et al*., 2011); ΔAIC between 4 and 7 were considered plausible while models with higher ΔAIC were disregarded as they have relatively little empirical support (Burnham and Anderson 2002, Anderson 2008). I did not present models with an Ei ≥ 10.

To assess goodness-of-fit of the models, I calculated the coefficient of determination (R^2). Traditionally R^2 is used as a summary statistic to look at the goodness-of-fit for fixed-effect models like analysis of variance (ANOVA) and generalized linear modes (GLMs). R^2 is a useful summary tool to evaluate model fit since it has no units, can objectively assess the fit of the model to the actual data, provides information on the variance explained by the model, and can be used to compare R^2 values from other studies (Nakagawa and Schielzeth 2013). However, several difficulties arise when calculating R^2 for GLMM. Calculating R^2 for mixed models is difficult because R^2 can be defined several ways and mixed models have multiple variance components, making them difficult to work with (Nakagawa and Schielzeth 2013, Snijders and Bosker 1999). Nakagawa and Schielzeth (2013) overcame some of these difficulties by using a marginal and conditional R^2: (1) marginal R^2 ($R^2_{GLMM(m)}$) is the variance explained by fixed factors; (2) conditional R^2 ($R^2_{GLMM(c)}$) is the variance explained by the total model (i.e. fixed and random factors), therefore the difference between these two measures of R^2 is the variance explained by the random factor (Nakagawa and Schielzeth 2013).

2.4 Results

Fifty-three harp seal digestive tracts (16 male and 37 female) and 17 gray seal digestive tracts (14 males, 2 females, and 1 unknown sex) contained at least one prey item. The most common prey identified by HPA were Atlantic cod, Arctic cod, capelin (harp seals), and sand lance (gray seal). These four prey species have otoliths with different shapes and sizes ranging from Atlantic cod (which have the largest most robust otoliths) to capelin (which have small delicate otoliths; Figure 2.1). Of the harp seal digestive tracts analyzed, 25 contained Atlantic cod otoliths, 30 had Arctic cod, and 27 had capelin otoliths. Of the gray seal digestive tracts analyzed, 13 had sand lance otoliths present.

I examined the proportions of otoliths with limited erosion through the digestive tract, for all seals (Table 2.1). I found that 57.4% of sand lance otoliths were of limited erosion, followed by 49.9 % of Arctic cod, 48.7% of Atlantic cod, and 38.1 % of capelin. Therefore, the percentage of otoliths with limited erosion varied with prey species and seal species. I examined 115 Atlantic cod, 259 Arctic cod, and 656 capelin state 1 and 2 otoliths from harp seals, and 715 state 1 and 2 sand lance otoliths from gray seals.

2.4.1 *Harp Seal: Analysis 1: Otolith Length*

Otolith length for Arctic cod and capelin did not vary down the digestive tract. There were no observed differences in mean otolith length for state 1 and 2 Arctic cod and capelin otoliths. It was found however, that the stomach had the greatest range of otolith sizes for both prey species (Figure 2.2). There was very little difference in length between erosion state 1 and 2 down the digestive tract for capelin and Arctic cod. No

Arctic cod otoliths of erosion state 2 were found in harp seal stomachs.

Otolith length in Atlantic cod got shorter down the digestive tract for both erosion states 1 and 2 otoliths. It was evident that state 2 otoliths had a smaller mean size down the digestive tract than state 1 otoliths, apart from the colon (Figure 2.2). Atlantic cod also exhibited a greater range of otolith sizes in the stomach than other areas of the digestive tract. Erosion state 1 otoliths had more variability than erosion state 2 otoliths in the stomach, lower small intestine and the colon, and in the upper small intestine, the variability between erosion state 1 and 2 were similar.

The best model to describe otolith length of Arctic cod and capelin otoliths down the digestive tract of harp seals included only 'seal ID'. For Arctic cod, the best model had approximately five and a half times the empirical support relative to the next best model (Table 2.2). The next best model included 'erosion state' as an explanatory variable and 'seal ID' as a random factor. For capelin, the best model had approximately seven times the empirical support relative to the next best model (Table 2.3). The next best model included 'erosion state' as an explanatory variable and 'seal ID' as a random factor. For Atlantic cod, the best model to describe otolith length across the digestive tract included 'section', 'erosion state', their interaction as explanatory variables and 'seal ID' as a random factor. This model had approximately six and a half times the empirical support relative to the next best model (Table 2.4) which included 'section' and 'erosion state' as explanatory variables and 'seal ID' as a random factor. Given that the best models for Arctic cod, capelin and Atlantic cod had more than 5-7 times the empirical support of the next best model, other models were not considered further.

The coefficients of determination for the best model were $R^2GLMM_{(c)}$=0.52 and

$R^2GLMM_{(m)}$ = 0.065. This indicates that the model explains 52% of the variability observed in otolith length. However, most of the explained variation (45.5%) is because of 'seal ID'.

Individual seals are of differing sex, age, size, likely have different foraging behaviors (i.e. different individual meal sizes and composition), have different activity levels after eating, and different time since consuming prey (Harvey 1989; Marcus *et al.* 1998; Sinclair *et al*. 2011). The analysis of Atlantic cod otolith lengths from the digestive tract of harp seals indicated that 'erosion state' and 'section' of the digestive tract are important variables for describing patterns in otolith length; however, much of the variation in length is due to variations between individual seals in the sample ('seal ID'). For state 2 otoliths the relationship between location in the digestive tract and otolith size was negative. The relationship between location and otolith size for state 1 otoliths was generally negative with the exception of the lower small intestine (Figure 2.3). The 95% confidence intervals indicated a high amount of uncertainty, likely due to the small sample size in some sections of the digestive tract.

2.4.2 *Gray seal Analysis 1: Otolith Length*

As I looked down the digestive tract, the average size of the state 1 otoliths was larger than the average size of state 2 otoliths. State 2 otoliths were smaller than state 1 otoliths in all regions (Figure 2.4). Erosion state 1 otoliths had a greater range of sizes in the stomach than erosion state 2; however, the range of otolith size was similar across the small intestines and the colon. In all cases the size range was lower in erosion state 2 otoliths.

The best model to describe sand lance otolith length down the digestive tract of gray seals included ‘erosion state’ as the explanatory variable and ‘seal ID’ as a random factor. This was the only possible model (Table 2.5).

The model indicates that there is a smaller mean otolith length for state 2 otoliths compared to state 1 otoliths. However, the model did not indicate that there would be a general increasing trend in the size of state 1 otoliths and decreasing trend of state 2 otoliths down the digestive tracts, which were seen in the box and whisker plot (Figure 2.4).

The $R^2GLMM_{(c)}$ was 0.2; therefore, only 20% of this result observed variance is explained by the model. The $R^2GLMM_{(m)}$ was 0.027, which means most of the explained variance comes from ‘seal ID’.

The analysis indicates ‘erosion state’ is an important variable when describing patterns in otolith length; however, much of the variation in length is due to variations between individual seals in the sample (‘seal ID’). This is likely due to differences in individual seal size, sex, behavior, etc. The effect of erosion state on otolith length was negative. Mean otolith length for state 1 otoliths was larger than that for state 2 otoliths regardless of the section of the digestive tract from which the otolith was obtained (Figure 2.5). The difference between state 1 and state 2 otoliths was 0.11 mm, which equates to a biological difference of 0.76 cm based on actual fish size using the equation FL= ((76.454*OL)-13.547))/10 (Lidster *et al.* 1994).

2.4.3 *Harp Seal Analysis 2: Proportion of Otoliths with Limited Erosion Down the Digestive Tract*

Proportions of otoliths with limited erosion down the digestive tract were different for Arctic cod, Atlantic cod, and capelin (Figure 2.6). For Arctic cod there was a small decrease in the mean proportion of otoliths with limited erosion as I looked down the digestive tract. Atlantic cod showed an increase in the mean proportion of otoliths with limited erosion as I looked down the digestive tract; for capelin, the proportion of otoliths with limited erosion was highest in the colon and lowest in the upper small intestine (Figure 2.6).

The best model to describe the proportion of otoliths with limited erosion down the digestive tract of harp seals includes 'section' (of the digestive tract), 'prey species', their interaction as explanatory variables, and 'seal ID' as a random factor. This was the only plausible model (Table 2.6).

For Arctic cod, the general trend was a decrease in the mean proportions of otoliths with limited erosion down the digestive tract of harp seals, as consistent with the data. For Atlantic cod and capelin, the general trend was an increase in the mean proportion of otoliths with limited erosion down the digestive tract of harp seals as consistent with the data (Figure 2.6, 2.7).

The calculated $R^2GLMM_{(c)}$ was 0.68 and The $R_2GLMM_{(m)}$ for the model was 0.6. This indicates that the model explains 68% of the variation observed in the proportion of otoliths with limited erosion evident in the data. Given the difference between the conditional and marginal R^2 values, most of the variation in the model can be attributed to the fixed effects; 'prey species' and 'section'.

The analysis indicated that 'prey species' and 'section' are important variables for describing patterns in the proportions of otoliths with limited erosion down the digestive

tract. Given the large difference in otolith morphology across species and that most of the digestion that happens to otoliths occurs in the stomach, the effect of 'species' and 'section' were expected. For Arctic cod, the predominant effect of 'section' on the proportion of otoliths with limited erosion was negative. For Atlantic cod and capelin, the effect was positive (Figure 2.7).

2.4.4 *Gray Seal Analysis 2: Proportions of Otoliths with Limited Erosion*

The mean proportions of sand lance otoliths with limited erosion down the digestive tract were different (Figure 2.8). Although there was no clear trend of decreasing or increasing mean proportions of otoliths with limited erosion down the digestive track, there was a pattern. The mean proportions of otoliths with limited erosion were highest in the lower small intestine and lowest in the stomach.

The best model to describe the proportion of otoliths with limited erosion down the digestive tract of gray seals included 'section' of the digestive tract as the explanatory variable, and 'seal ID' as a random factor. No other models were considered plausible (Table 2.7).

For sand lance, there is no clear trend of decreasing or increasing mean proportions of otoliths with limited erosion as I look down the digestive tract, which is consistent with the data; however, the mean proportion of otoliths with limited erosion was larger in the model than in the data (Figure 2.8, 2.9). Given the small sample size, a single large feeding event could greatly influence this result. Therefore, it is expected that 'seal ID' will explain much of the observed variation.

The calculated $R^2GLMM_{(c)}$ was 0.57 and the $R^2GLMM_{(m)}$ was 0.12. This

indicates that the model explains 57% of the variation in the proportion of otoliths with limited erosion seen in the data. Given the difference between the conditional and marginal R^2 values, very little variation can be explained by 'section'. This suggests that the random factor, 'seal ID', was the main factor behind the inconsistencies seen in the proportions of otoliths with limited erosion down the digestive tract (Figure 2.9). Given the small sample size of gray seals with sand lance in their digestive tract and the fact that individual seal effect accounts for most of the variability, the poor model fit is not surprising.

2.5 Discussion

Determining if changes in otolith distribution down the digestive tract are due to otolith size and morphology is difficult, particularly given how variable the data from individual seals was. There was evidence, however, that the otolith length of some prey species decreased in size down the digestive tract (i.e. Atlantic cod) and that otolith erosion state can is associated with otolith length (i.e. Atlantic cod, sand lance). I also found that the proportions of otoliths with limited erosion varied across fish species and down the digestive tract suggesting that different prey species pass through the digestive tract at different rates. These differences must be considered when attempting to estimate the diets of seals, particularly when using hard parts from different sources (e.g. stomach vs scats).

Chemical digestion and mechanical breakdown of prey, and their otoliths, occurs in the stomach. Once hard parts pass into the small intestine, chemical digestions ceases due to release of sodium bicarbonate which neutralizes acid in the small intestine but the otoliths are still impacted by mechanical actions (Guyton 1981; Harvey 1987). The time

prey spend in different sections of the digestive tract are dependent on several factors including prey size, number of prey, and seal activity level (Gudmundson *et al.* 2006; Harvey 1987; Helm 1984; Marcus *et al.*1998; Sinclair *et al.* 2011; Tollit *et al.* 1997).

The importance of 'seal ID' in all the models indicates the significance of individual variation in the size of prey consumed. Because of the large variability in prey otolith size consumed by individuals, many of the smaller differences observed in the data were difficult to detect. Variation in the size of a given prey species among individual seals may be due to a variety of factors including age, size, sex, location and prey availability, different foraging behavior, different activity levels after eating, etc. (Gudmundson *et al*. 2006; Harvey 1989; Marcus *et al.* 1998; Sinclair *et al.* 2011).

The best models to describe the data on otolith size of Arctic cod and capelin obtained from harp seals included the individual seal only (Table 2.8). Difference in erosion state and section of the digestive tract did not contribute to the model. This supports the assumption that both state 1 and 2 otoliths can be used to estimate prey size and that, since the colon contents are equivalent to scat contents, performing HPA on the stomach or scat will result in similar size estimates of Arctic cod and capelin. Also of interest is that there were no Arctic cod erosion state 2 otoliths present in the stomach. A possible explication for this is that Arctic cod is a schooling prey with medium sized robust otoliths. As suggested by Sinclear *et al*. 2011, schooling prey species consumed in large numbers move rapidly through the digestive tract; therefore, given their size and robustness erosion would be minimal. Also, individual seals that had recently fed could skew the data.

The best models for Atlantic cod (obtained from harp seal) and sand lance

(obtained from gray seal) included erosion state, in addition to 'seal ID' and section (Atlantic cod only). While the inclusion of erosion state explained a relatively small amount of the overall variability, including erosion state in the model indicates that the average length of state 2 otoliths may not be the same as state 1, even after accounting for variability among individual seals (Table 2.8). This means that using erosion state 2 otoliths for preforming size reconstructions for Atlantic cod and sand lance may result in smaller estimates of the size of prey ingested. For Atlantic cod, State 2 otoliths were smaller than state 1 in all sections of the digestive tract, although, the magnitude of the size difference between erosion state 1 and 2 otoliths was less further down the digestive tract. However, the small number of otoliths found in the lower parts of the digestive tract make any interpretation difficult. Erosion state 1 and 2 for sand lance was consistent down the digestive tract, therefore, measurements of erosion state 2 otoliths anywhere down the digestive tract may result in a similar bias, however, using state 1 otoliths from anywhere in the digestive tract would result in similar estimate of ingested prey. Although both sand lance and Atlantic cod have robust otoliths with a similar shape, they differed in their results because all sand lance otoliths were very small, making them less susceptible to retention in the stomach and more susceptible to mechanical breakdown in the intestine, which is likely why 'section' was not included in the best model for sand lance but it was for Atlantic cod.

I found that otoliths with limited erosion in the stomach where larger than those further down the digestive tract. This indicates that performing HPA on the stomach alone may result in a larger prey size estimates compared to intestine/scat HPA. This trend has been seen in other studies such as Sinclair *et al.* (2011). Gudmundson *et al.* (2006)

proposed that this trend could be a sign of otolith retention, complete digestion, or regurgitation of larger otoliths. Based on my seal sample, three seals had more than 20 Atlantic cod present but the proportions of otoliths with limited erosion were less than 35%, indicating that retention is likely occurring (Appendix 2-B).

Next, I looked at the proportions of otoliths with limited erosion present in different sections of the digestive tract. If retention of prey with larger robust otoliths is occurring in the stomach, the expectation is to see a low proportion of large otoliths with limited erosion and many eroded otoliths (Bowen and Iverson 2012). Conversely more otoliths with limited erosion further down the digestive tract would indicate fast passage.

The best model to describe any changes in the proportion of state 1 and 2 otoliths in harp seals included 'section', 'prey species' and 'seal ID'. In this model the fixed factors 'section' and 'prey species' accounted for most of the variability observed by the model, indicating that they were more important than the random factor, 'seal ID' (Table 2.8). These three species have very different otolith sizes and morphology, therefore comparing the three species may provide some insight as to how otolith size and shape are impacting diet outcomes (Figure 2.1).

Arctic cod had similar proportion of otoliths with limited erosion in all sections of the digestive tract sampled. This combined with the fact that Arctic cod had the same mean otolith length at all sections of the digestive tract indicates that there was no evidence of retention of larger otoliths in the stomach or fast passage of smaller prey. There were however, higher counts of Arctic cod otoliths in the stomach compared to the rest of the digestive tract. Since there are no other indications that otolith retention occurred, the difference may simply indicate that a number of the seals had recently fed

(Appendix 2-C, Table 2.1).

For capelin, the mean proportion of otoliths with limited erosion was highest in the colon when compared to the rest of the digestive tract (Figure 2.6). Since capelin mean otolith size was similar in all sections of the digestive tract, and the lower proportion of otoliths with limited erosion were found in the stomach and the upper small intestine, otolith retention was not occurring. The decrease in the proportion of eroded otoliths in the lower small intestine and the even larger decline seen in the colon are likely due to mechanical breakdown as otoliths moved along the digestive tract. (Table 2.1); This indicates that while the otolith size of state 1 and 2 otoliths is similar (and hence the size of prey consumed is consistent), the number of otoliths available for counting will be lower and the number of prey ingested will be under estimated, hence a large Numerical correction factor is used to account for total prey loss in scat analysis (7.87; Tollit *et al*. 2007). This NCF would need to be different if stomach contents are used instead of scats.

It was evident that Atlantic cod showed otolith retention in the stomach since I found there was a higher proportion of otoliths with limited erosion further down the digestive tract than in the stomach. However, this result may be influenced by a loss of taxonomic resolution of the otoliths as they travel down the digestive tract. Otoliths that were once identified as Atlantic cod in the lightly digested state may only be classified as *Gadus* spp. once they become more heavily erode and pass into the lower digestive tract, especially if they are also subjected to mechanical actions. There was evidence that more *Gadus* spp. was found in the intestine than in the stomach indicating that reduced taxonomic resolution was observed (Chapter 3). Fast passage of smaller otoliths may also be occurring since I observed a decrease in Atlantic cod otolith length as I moved down

the digestive tract. Retention of otoliths in the stomach results in a large proportion of eroded otoliths since both chemical and mechanical digestion occurs in the stomach (Bowen and Iverson 2013; Harvey 1987; Robbins 1983). Similar results have been observed for walleye pollock *(Theragra chalcogramma*) in northern fur seals (Sinclair *et al.* 2011).

The proportions of otoliths with limited erosion for sand lance was varied along the digestive tract of gray seals and was best described by a model that included 'section'. However, the importance of including section is not clear since it contributed relatively little to the overall variance in the data when compared to 'seal ID' (proportion of variance from fixed factors = 0.12; Table 2.8). The mean proportion of otoliths with limited erosion increases steadily until reaching the lower small intestine, and then dropped in the colon to about the same mean proportion as seen in the stomach. This suggests that this small prey may have a fast passage rate, mechanical destruction and/or that single feeding events are skewing the data. Sand lance showed no obvious increase or decrease in otolith size down the digestive tract, therefore there is no evidence of retention in the stomach (Appendix 2-E). The decrease in the proportion of eroded otoliths in the intestine are likely due to mechanical breakdown as otoliths moved along the digestive tract. Sand lance otoliths although small are moderately robust, and therefore the effect of mechanical destruction was not as great as seen with capelin. The NCF for sand lance is much lower than capelin but still accounts for a high loss of otoliths due to mechanical destruction seen here (2.86; Grellier and Hammond 2006). This NCF would have to be different for stomach contents are used instead of intestine/scats.

2.5.1 *Summary*

Much of the variation in otolith length down the digestive tract was due to variations between individual seals, however, some variability could be explained by otolith location as well as erosion state for some species. This supports the theory that otoliths of different sizes and morphologies are affected by digestion differently. It was apparent that for some species, (Arctic cod and capelin), using only stomach or scat analysis would result in similar prey size estimates. This assumption should not be carried across all species. Using only scat or stomach HPA could result in substantial difference in estimated prey size for others (i.e. Atlantic cod).

The proportions of otoliths with limited erosion were also very different across species and down the digestive tract. Given the large difference in size and morphology of otoliths for the species I examined, it was apparent that larger, more robust otoliths did exhibit signs of retention in the stomach and/or reduced taxonomic resolution further down the digestive tract. The magnitude of this effect will be dependent on a number of factors including individual seal, otolith size and otolith morphology.

This study also highlights the importance of using both the stomach and intestine from an individual seal to determine diet. Using the stomach and intestine provides a more comprehensive view of the most recent meal(s) and may lead to more accurate estimates of prey size and foraging patterns, as well as provides insight into some of the biases discussed above.

Table 2.1: Proportions of state 1 and 2 otoliths at different locations of the digestive tract, not separated by individual seal. Atlantic cod (*Gadus morhua*), Arctic cod (*Boreogadus saida*) and capelin (*Mallotus villosus*) were from harp seals (*Pagophilus groenlandicus*); sand lance (*Ammodytes* spp.) was from gray seals (*Halichoerus grypus*).

			Percent of Otoliths in Erosion State No.	
	No. of Seals	Total Prey Count [1]	1	2
Atlantic cod				
Stomach	17	165	39.9	1.8
Upper small intestine	13	33	30.3	39.4
Lower small intestine	15	28	35.7	28.6
Colon	6	10	50	30
Arctic cod				
Stomach	23	312	52.9	0
Upper small intestine	11	85	17.7	32.9
Lower small intestine	18	77	15.6	26
Colon	8	44	22.2	20
Capelin				
Stomach	20	632	38.9	0.8
Upper small intestine	16	287	17.8	9.4
Lower small intestine	17	759	21.2	17.5
Colon	16	43	48.8	27.9
Sand lance				
Stomach	7	563	42.3	11.2
Upper small intestine	6	182	24.7	44.5
Lower small intestine	10	197	31.5	37.6
Colon	11	304	32.6	17.4

[1] Includes all left or right otoliths (both measured and not measured) obtained from diet analysis.

Table 2.2: Model selection of general linear mixed models for the length of Arctic cod (*Boreogadus saida*) otoliths down the digestive tract of harp seals. ΔAICs are the differences in the Akaike's information criterion (AIC) scores between each model and the model with the lowest AIC score. The evidence ratio is the ratio between the likelihood of the best model and the likelihood of the alternative model.

Model	ΔAIC	Evidence ratio
1. length_mm ~ 1 + seal ID	0	1
2. length_mm ~ erosion_state + seal ID	3.41	5.51
3. length_mm ~ section + seal ID	6.98	32.73
4. length_mm ~ erosion_state + section + seal ID	9.82	136.3
5. length_mm ~ erosion_state + section + erosion_state * section + seal ID	*	*

*There were only erosion state 1 otoliths measured in the stomach for Arctic cod therefore the full model could not be assessed.

Table 2.3: Model selection of general linear mixed models for the length of state 1 and 2 capelin (*Millotus villosus*) otoliths down the digestive tract of harp seals. ΔAICs are the differences in the Akaike's information criterion (AIC) scores between each model and the model with the lowest AIC score. The evidence ratio is the ratio between the likelihood of the best model and the likelihood of the alternative model.

Model	ΔAIC	Evidence ratio
1. length_mm ~ 1 + seal ID	0	1
2. length_mm ~ erosion_state + seal ID	3.90	7.01
3. length_mm ~ section + seal ID	18.73	1.16×10^4
4. length_mm ~ erosion_state + section + seal ID	19.91	2.10×10^4
5. length_mm ~ erosion_state + section + erosion_state * section + seal ID	29.76	2.90×10^6

Table 2.4: Model selection of general linear mixed models for the length of state 1 and 2 Atlantic cod (*Gadus morhua*) otoliths down the digestive tract of harp seals. ΔAICs are the differences in the Akaike's information criterion (AIC) scores between each model and the model with the lowest AIC score. The evidence ratio is the ratio between the likelihood of the best model and the likelihood of the alternative model.

Model	ΔAIC	Evidence ratio
1. length_mm ~ erosion_state + section + erosion_state * section + seal ID	0	1
2. length_mm ~ erosion_state + section + seal ID	3.72	6.44
3. length_mm ~ erosion_state + seal ID	3.76	6.57
4. length_mm ~ section + seal ID	6.22	22.46
5. length_mm ~ 1 + seal ID	7.95	53.28

Table 2.5: Model selection of general linear mixed models for the length of state 1 and 2 sand lance (*Ammodytes* spp.) otoliths down the digestive tract of gray seals. ΔAICs are the differences in the Akaike's information criterion (AIC) scores between each model and the model with the lowest AIC score. The evidence ratio is the ratio between the likelihood of the best model and the likelihood of the alternative model.

Model	ΔAIC	Evidence Ratio
1.length_mm ~ erosion_state + seal ID	0.00	1.00E+00
2. length_mm ~ 1 + sealID	12.90	6.33E+02
3. length_mm ~ errosion_state + section +seal ID	18.31	9.47E+03
4. length_mm ~ section + seal ID	26.13	4.72E+05
5. length_mm ~ errosion_state + section +erosion_state * section + seal ID	29.06	2.05E+06

Table 2.6: Model selection of generalized linear mixed models for the proportion of state 1 and 2 (otoliths with limited erosion) Atlantic cod (*Gadus morhua*), Arctic cod (*Boreogadus saida*) and capelin (*Mallotus villosus*) otoliths down the digestive tract of harp seals (*Pagophilus groenlandicus*). ΔAICs are the differences in the Akaike's information criterion (AIC) scores between each model and the model with the lowest AIC. The evidence ratio is the ratio between the likelihood of the best model and the likelihood of the alternative model

Model	ΔAIC	Evidence Ratio
1. Proportion_of_otoliths_with_limited_erosion ~ section + prey species + section * prey species + seal ID	0.00	1
2. Proportion_of_otoliths_with_limited_erosion ~ section + prey species + seal ID	22.05	6.14E+04
3. Proportion_of_otoliths_with_limited_erosion ~ prey species + Seal ID	43.15	2.35E+09
4. Proportion_of_otoliths_with_limited_erosion ~ section + Seal ID	74.52	1.51E+16

Table 2.7: Model selection of generalized linear mixed models for the proportion of state 1 and 2 (otoliths with limited erosion) sand lance (*Ammodytes* spp.) otoliths down the digestive tract of gray seals (*Halichoerus grypus*). ΔAICs are the differences in the Akaike's information criterion (AIC) scores between each model and the model with the lowest AIC. The evidence ratio is the ratio between the likelihood of the best model and the likelihood of the alternative model

Model	ΔAIC	Evidence Ratio
1. Proportion_of_otoliths_with_limited_erosion ~ section + seal ID	0.00	1
2. Proportion_of_otoliths_with_limited_erosion ~ 1+ seal ID	47.48	2.04*1010

Table 2.8: Summary table showing the best models via AIC analysis for otoliths length and proportions of otoliths with limited erosion for Arctic cod (B*oreogadus saida*), capelin (*Mallotus villosus),* Atlantic cod *(Gadus morhua),* and sand lance *(Ammodyties spp.).* The table also summarizes the proportions of variability explained by the fixed effects of the models.

	Prey species	Analysis	Best model	Proportion of R2 that accounted for fixed effects
Harp	Arctic cod (B*oreogadus saida*)	Otolith length	length_mm~1 + sealID	no fixed factor
		Proportion of otoliths with limited erosion	Proportion_of_otoliths_with_limited_erosion ~section+prey_ species+section*prey_ species+sealID	0.6
	Capelin *(Mallotus villosus*)	Otolith length	length_mm~1 + sealID	no fixed factor
		Proportion of otoliths with limited erosion	Proportion_of_otoliths_with_limited_erosion ~section+prey_ species+section*prey_ species+sealID	0.6
	Atlantic cod (*Gadus morhua*)	Otolith length	length_mm~erosion_state*section+ sealID	0.065
		Proportion of otoliths with limited erosion	Proportion_of_otoliths_with_limited_erosion ~section+prey_ species+section*prey_ species+sealID	0.6
Grey	Sand lance (*Ammodytes* spp.)	Otolith length	length_mm~erosion_state+sealID	0.027
		Proportion of otoliths with limited erosion	Proportion_of_otoliths_with_limited_erosion ~section+sealID	0.12

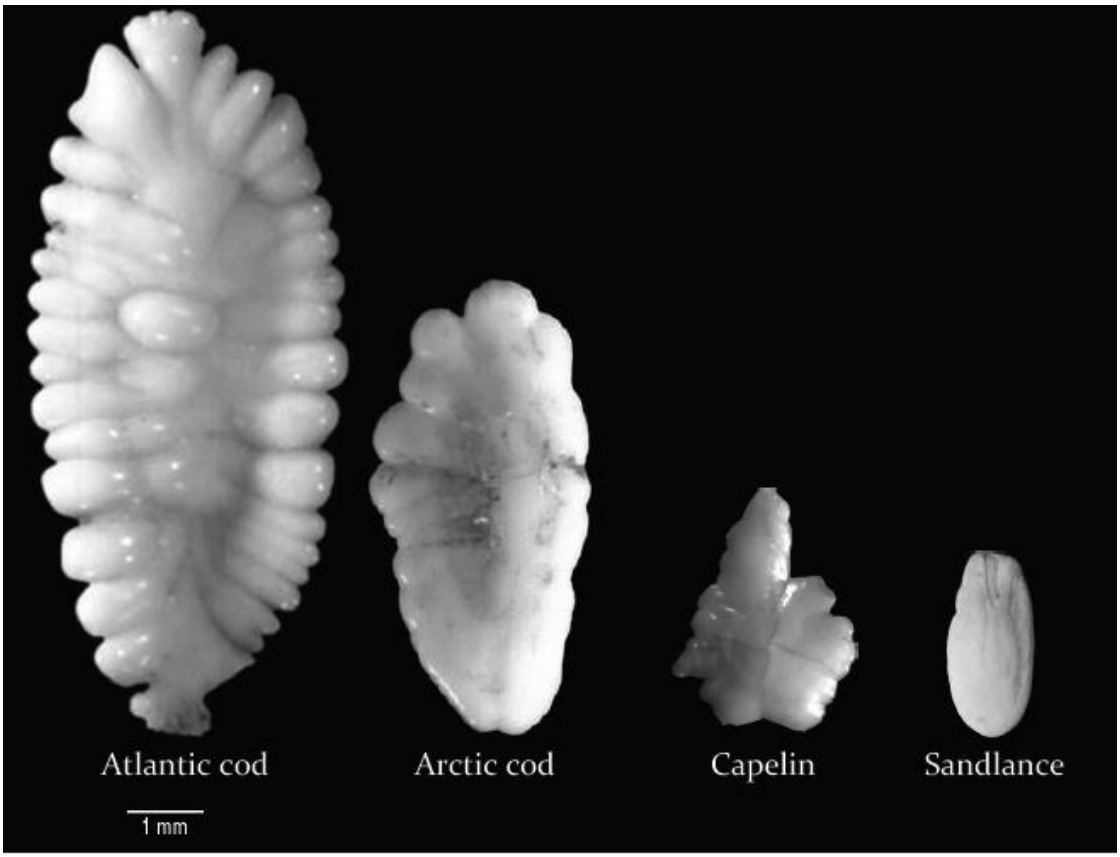


Figure 2-1: Photo of otoliths of Atlantic cod (*Gadus morhua*), Arctic cod (*Boreogadus saida*), capelin (*Mallotus villosus*) and sand lance (*Ammodytes* spp.), showing differences in shape and size across species. From Svetocheva *et al.*(2007)

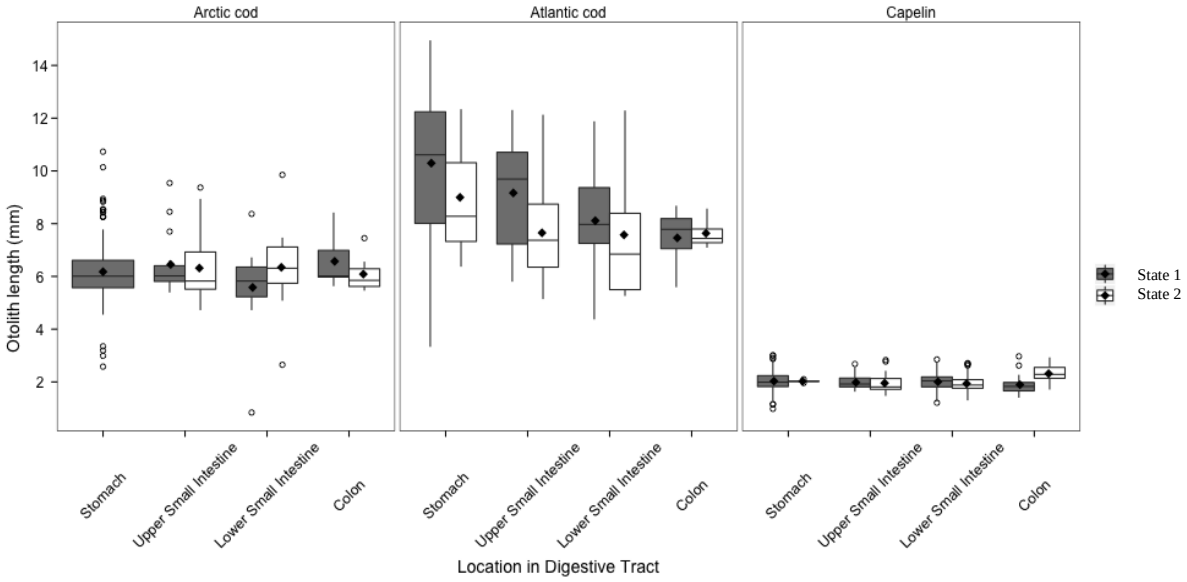


Figure 2-2: The lengths of state 1 and 2 Arctic cod *(Boreogadus saida*) and capelin (*Mallotus villosus*) otoliths showed no difference down the digestive tracts of 42 harp seal, however, a length decrease was observed down the digestive tracts for Atlantic cod (*Gadus morhua*) otoliths. Horizontal bar: median (50th percentile); the diamond (♦) is the mean value, box: interquartile range (IQR, 25th to 75th percentile); whiskers: last values within 1.5. IQR of the lower and higher quartile; circles above/below whiskers: otolith lengths that is much larger or smaller than the mean otolith size for the sample.

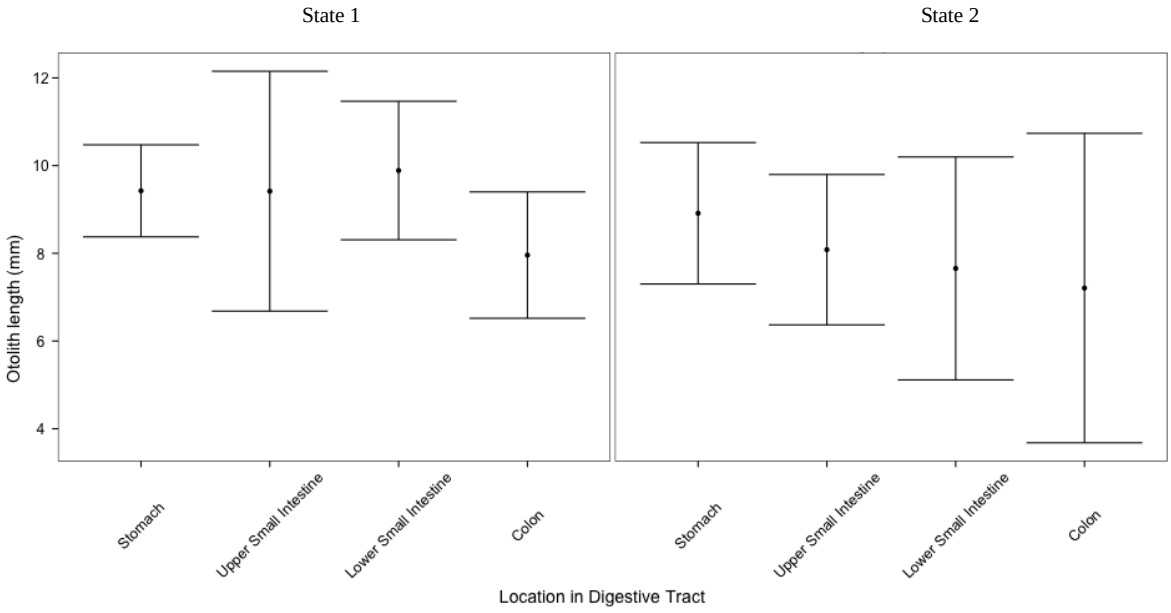


Figure 2-3: Effect display for the interaction of erosion state and digestive tract section in a general linear mixed model with a normal error fit to mean state 1 and 2 Atlantic cod (*Gadus morhua*) otolith length in the digestive tract of 25 harp seals (*Pagophilus groenlandicus*). The overall effect of 'erosion' and 'section' on otolith length was negative but the relationship between otolith location and size is more apparent for state 2 otoliths. A 95-percent confidence interval is drawn around the estimated mean effect.

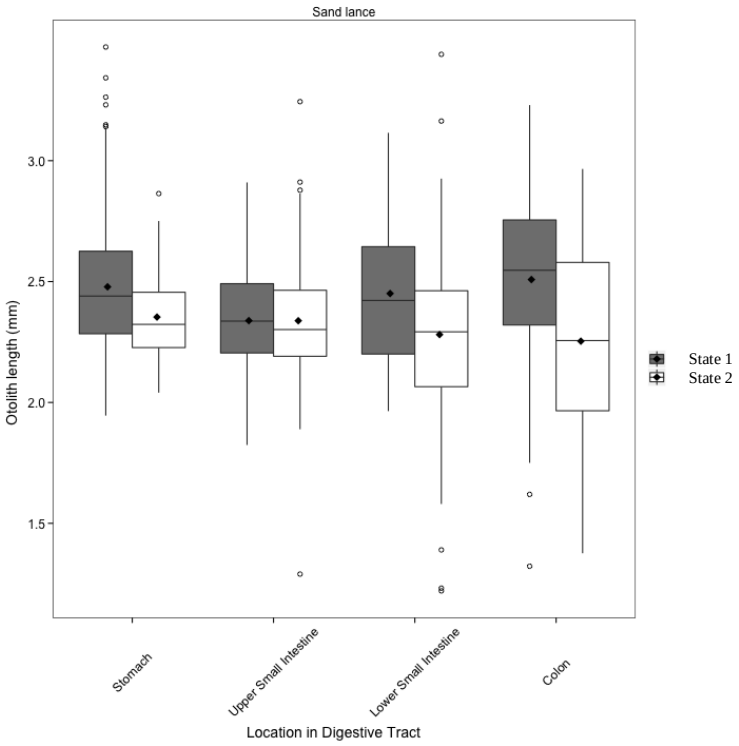


Figure 2-4: The length of state 1 sand lance (*Ammodytes* spp.) otoliths collected from 13 gray seals (*Halichoerus grypus*) showed an increase in otolith size down the digestive tract while state 2 otoliths collected from the same seals showed a decrease in size. Horizontal bar: median (50th percentile); diamond (♦): mean; box: interquartile range (IQR, 25th to 75th percentile); whiskers: last values within 1.5. IQR of the lower and higher quartile; circles above/below whiskers: otolith lengths that are much larger or smaller than the mean otolith size for the sample.

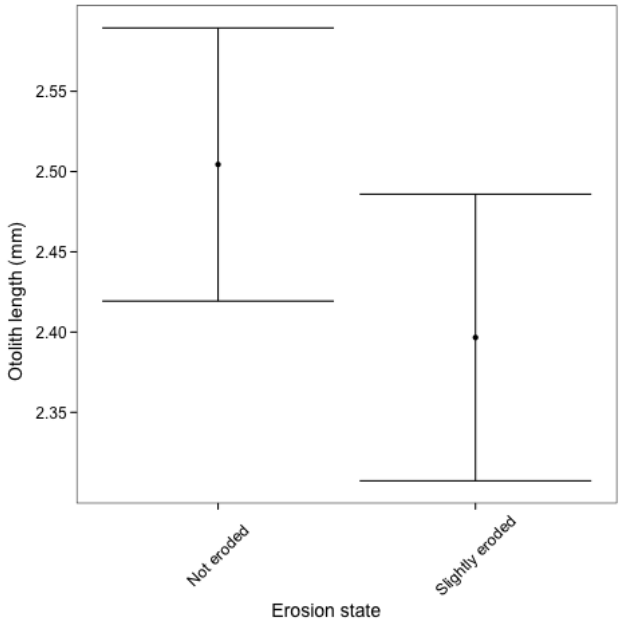


Figure 2-5: Effect display for the interaction of erosion state and otolith length. Individual seal mean otolith length (mm) for state 1 otoliths was larger than state 2 sand lance (*Ammodytes* spp.) otolith in the digestive tract of 13 gray seals (*Halichoerus grypus*). A 95-percent confidence interval is drawn around the estimated mean. There is no interaction present given that the mean otolith size is dependent on otolith erosion state.

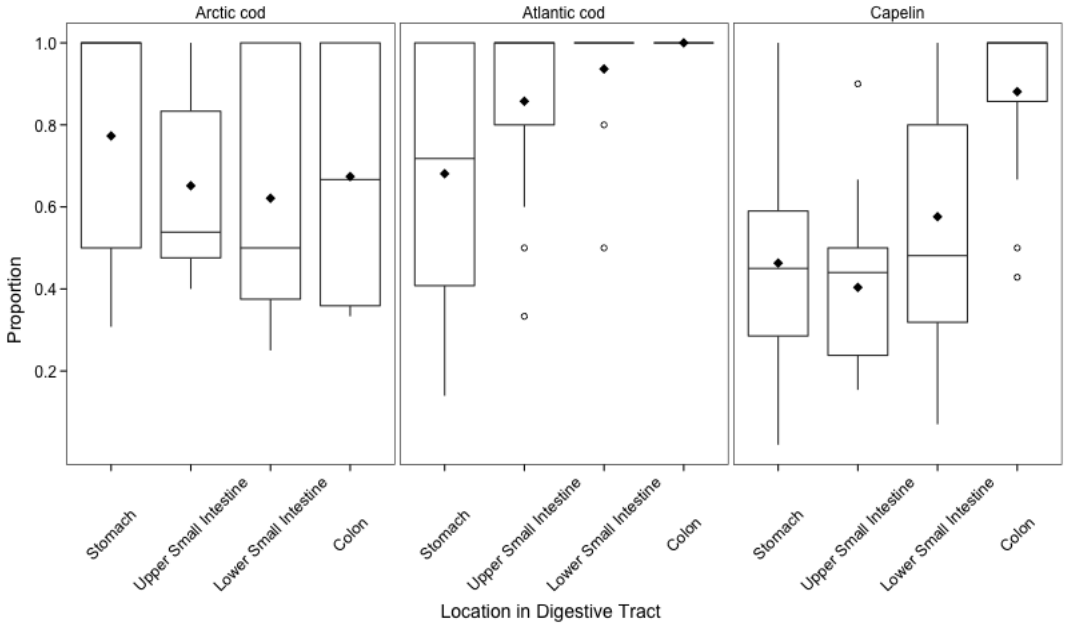


Figure 2-6: The mean proportion of state 1 and 2 Atlantic cod (*Gadus morhua*), Arctic cod (*Boreogadus saida*) and capelin (*Mallotus villosus*) otoliths down the digestive tract of 42 harp seals(*Pagophilus groenlandicus*); is different for all three prey species. Arctic cod shows a slight decrease in the proportion of otoliths with limited erosion down the digestive tract, while Atlantic cod and capelin has an increase in proportions of otoliths with limited erosion down the digestive tract. Horizontal bar: median (50th percentile); Diamond (♦): mean; box: interquartile range (IQR, 25th to 75th percentile); whiskers: last values within 1.5. IQR of the lower and higher quartile; circles above/below whiskers: the proportion of state 1 and 2 otoliths was much larger or smaller than the mean proportion of measurable otolith for the sample.

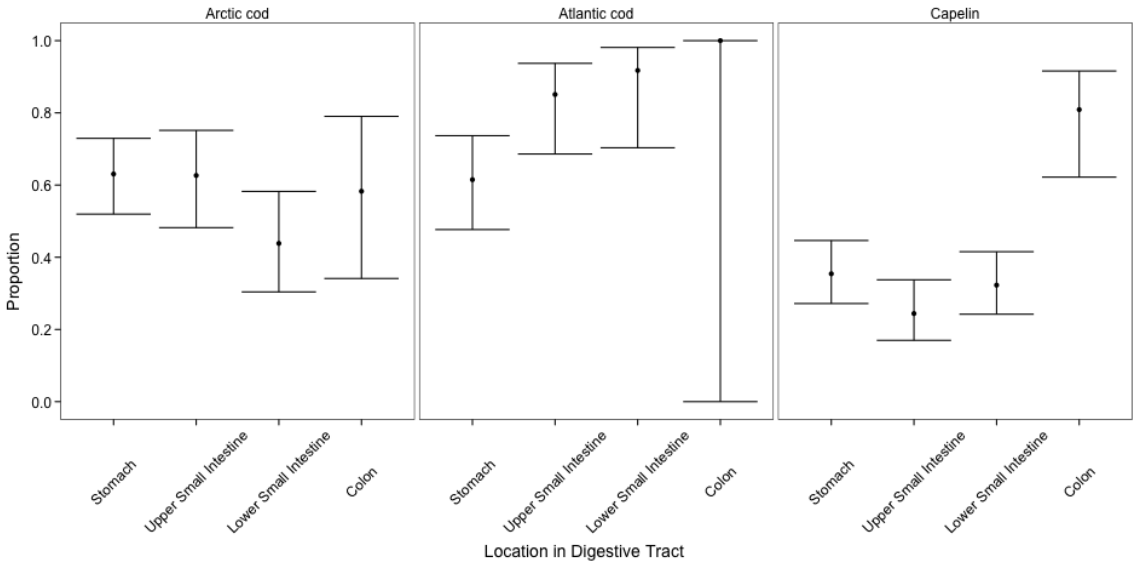


Figure 2-7: Effect plot showing mean proportion of state 1 and 2 otoliths in the digestive tract of 42 harp seals (*Pagophilus groenlandicus*) given location in the digestive tract and prey species (Atlantic cod (*Gadus morhua*), Arctic cod (*Boreogadus saida*) and capelin (*Mallotus villosus*) . There is an interaction present given that the mean proportion of state 1 and 2 otoliths down the digestive tract depends on prey species. Arctic cod shows a slight decrease in the proportion measurable down the digestive tract, while Atlantic cod and capelin has an increase in proportions measurable down the digestive tract. A 95-percent confidence interval is drawn around the estimated mean.

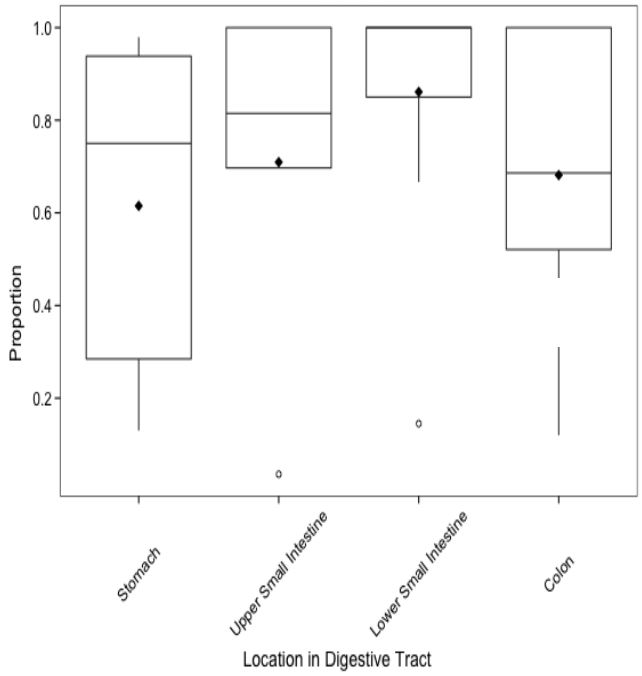


Figure 2-8: The mean proportion of state 1 and 2 sand lance (*Ammodytes* spp.) otoliths down the digestive tract of 13 gray seals (*Halichoerus grypus*) was highest in the lower small intestine and lowest in the stomach. Horizontal bar: median (50th percentile); Diamond (♦): mean; box: interquartile range (IQR, 25th to 75th percentile); whiskers: last values within 1.5. IQR of the lower and higher quartile; circles above/below whiskers: the proportion of state 1 and 2 otoliths was much larger or smaller than the mean proportion of measurable otolith for the sample.

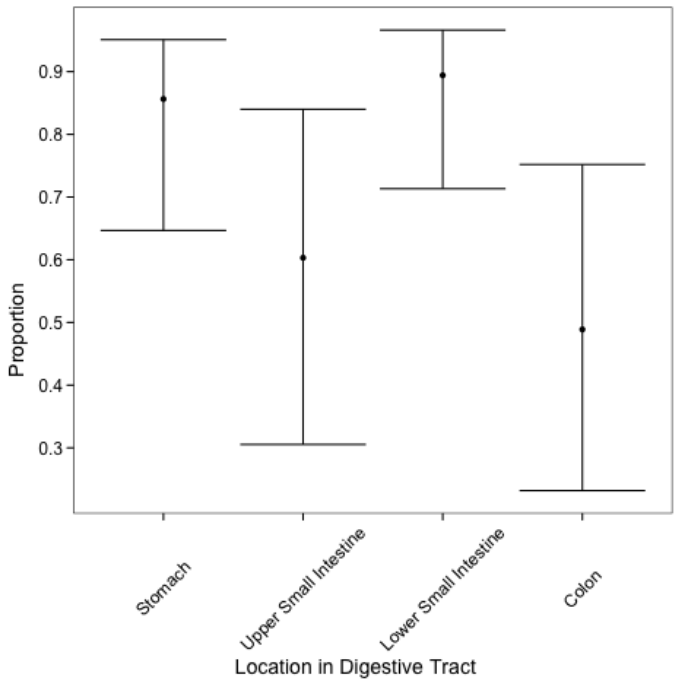


Figure 2-9: Effect plot of the mean proportion of state 1 and 2 sand lance (*Ammodytes* spp.) otoliths down the digestive tract of 13 gray seals (*Halichoerus grypus*). The mean proportion of state 1 and 2 otoliths was highest in the stomach and lowest in the colon. A 95-percent confidence interval is drawn around the estimated mean.

Appendix 2-A: Harp seals (*Pagophilus groenlandicus*); gray seals (*Halichoerus grypus*) collected from Newfoundland with entire intestines obtained from 2008- 2012.

			Length (m)		
Sex	Seal ID	Species	Upper small intestine	Lower small intestine	Colon
U	20100158	Gray	10.15	10.15	0.47
M	20100159	Gray	14.5	14.5	0.96
M	20100160	Gray	14.9	14.9	0.76
M	20100161	Gray	12.7	12.7	
M	20110001	Gray	19.46	19.46	1.7
F	20110082	Gray	14.95	14.95	0.99
M	20110104	Gray	17.2	17.2	1.05
M	20110105	Gray	11.7	11.7	0.56
M	20110106	Gray	7.55	7.55	0.51
F	20110107	Gray	10.1	10.1	0.44
M	20110108	Gray	17.3	17.3	1.31
M	20120005	Gray	12.42	12.42	0.39
M	20120007	Gray	12.83	12.83	0.25
M	20120008	Gray	18.5	18.5	0.35
M	20120009	Gray	12.9	12.9	0.49
F	20120011	Gray	10.65	10.65	0.65
M	20120012	Gray	10.71	10.71	0.75
M	20120013	Gray	11	11	0.75
F	20120014	Gray	10.7	10.7	1
F	20120016	Gray	10.4	10.4	
M	20082055	Harp	12.27	12.27	0.35
F	20082057	Harp	9.5	9.5	0.45
F	20082061	Harp	10.3	10.3	0.63
F	20082677	Harp	8.67	8.67	0.34
F	20082681	Harp	8.31	8.31	0.22
M	20090099	Harp	7.6	7.6	0.425
M	20091693	Harp	9.74	9.74	0.29
M	20091695	Harp	11.33	11.33	0.29
M	20091697	Harp	10.29	10.29	0.41
F	20091802	Harp	9.55	9.55	0.33
F	20091809	Harp	9.37	9.37	0.26
F	20091931	Harp	8.45	8.45	0.4
F	20091947	Harp	10.75	10.75	0.53
F	20091951	Harp	11.25	11.25	0.71
F	20091957	Harp	13.35	13.35	0.62

Length (m)

Sex	Seal ID	Species	Upper small intestine	Lower small intestine	Colon
F	20091960	Harp	12.3	12.3	0.69
F	20091964	Harp	13.16	13.16	0.6
M	20091978	Harp	9.9	9.9	0.41
F	20091988	Harp	9.93	9.93	0.11
M	20091990	Harp	12.4	12.4	0.41
F	20100107	Harp	10.06	10.06	0.49
F	20100108	Harp	10.9	10.9	0.5
M	20100109	Harp	7.25	7.25	0.28
F	20100110	Harp	8.51	8.51	0.51
F	20100111	Harp	7.88	7.88	0.43
M	20100112	Harp	7.57	7.57	0.28
F	20102952	Harp	9.58	9.58	0.39
F	20102957	Harp	11.31	11.31	0.39
F	20102962	Harp	10.94	10.94	0.47
F	20102968	Harp	8.52	8.52	0.36
M	20110185	Harp	11.02	11.02	0.53
F	20110186	Harp	9.5	9.5	0.28
F	20111128	Harp	10.97	10.97	0.61
F	20111131	Harp	12.4	12.4	0.61
F	20111132	Harp	10.37	10.37	0.73
M	20111133	Harp	15.79	15.79	0.69
M	20111134	Harp	10.73	10.73	0.4
F	20111139	Harp	9.95	9.95	0.79
F	20111317	Harp	11.27	11.27	0.33
F	20111318	Harp	11.36	11.36	0.59
F	20111320	Harp	10.72	10.72	0.54
F	20111324	Harp	12.55	12.55	0.48
F	20111325	Harp	14.38	14.38	0.46
M	20111326	Harp	10.68	10.68	0.39
F	20111328	Harp	9.16	9.16	0.7
F	20111329	Harp	12.1	12.1	0.41
F	20111331	Harp	10.96	10.96	0.5
F	20111332	Harp	16.01	16.01	0.76
F	20111333	Harp	11.2	11.2	0.72
F	20111334	Harp	13.15	13.15	0.7
F	20111335	Harp	9.93	9.93	0.64
F	20111336	Harp	11.46	11.46	0.64
M	20111338	Harp	10.33	10.33	0.51
M	20111339	Harp	12.15	12.15	0.72

Length (m)

Sex	Seal ID	Species	Upper small intestine	Lower small intestine	Colon
M	20113346	Harp	10.15	10.15	0.54
F	20113371	Harp	11.5	11.5	0.68
F	20113393	Harp	9.43	9.43	0.92

Appendix 2-B: The number of Atlantic cod (*Gadus morhus*) otoliths present (n), for each individual seal and the percentage of erosion state 1 and 2 otoliths separated by stomach, upper small intestine, lower small intestine and colon.

	Stomach		Upper sm. Int.		Lower sm. Int.		Colon	
Seal ID	% measurable	n	% measurable	n	% measurable	n	% measurable	n
20082055	100.00	1	100.00	1				
20082057	66.67	6			100.00	2		
20091697	100.00	1						
20091931	100.00	1	100.00	1	0.00	2		
20091947	20.00	5	60.00	10	80.00	5	100.00	1
20091951	50.00	10	100.00	6	50.00	2		
20091960	17.86	28						
20091964	13.95	43	100.00	1				
20091978					0.00	2	0.00	1
20091990					100.00	1		
20100107	100.00	2			100.00	2		
20102957	42.85	7	50.00	2	0.00	3	0.00	1
20110185	34.62	26						
20111128							100.00	1
20111139	0.00	2	100.00	2	100.00	1		
20111318			100.00	1	100.00	2		
20111320	76.92	13						
20111325					100.00	1		
20111326					100.00	1		
20111328	66.67	6	33.33	3	100.00	1		
20111332			100.00	1	0.00	1		
20111333	100.00	3	100.00	1	100.00	2		
20111335							100.00	5
20111338	100.00	4	0.00	2				
20111339	100.00	7	100.00	2			100.00	1

Appendix 2-C: The number of Arctic cod (Boreogadus saida) otoliths present (n), for each individual seal and the percentage of erosion state 1 and 2 otoliths separated by stomach, upper small intestine, lower small intestine and colon.

	Stomach		Upper sm. Int.		Lower sm. Int.		Colon	
Seal ID	% measurable	n	% measurable	n	% measurable	n	% measurable	n
20082057	50.00	2					0.00	2
20082061	100.00	1						
20091809	100.00	1						
20091947	48.00	50	45.16	31				
20091951	100.00	1	40.00	5	25.00	4		
20091960	0.00	4						
20100107	100.00	1			100.00	1		
20102957	30.77	26	50.00	2				
20102962	100.00	1			33.33	3		
20102968	100.00	2						
20111131							38.46	26
20111132	100.00	1			50.00	6		
20111133							33.33	6
20111134					100.00	1		
20111139	50.00	4			50.00	2		
20111318	100.00	17	66.67	3	66.67	3		
20111320	100.00	1						
20111324			100.00	1	50.00	2	100.00	1
20111325	62.50	16	53.85	13	25.00	4		
20111326	100.00	2			60.00	5		
20111328	0.00	2			50.00	2		
20111329	100.00	1	100.00	1	100.00	1		
20111331	100.00	1	100.00	1				
20111332	41.55	77	44.44	18	28.57	21	33.33	3
20111333	45.45	22	50.00	4	29.41	17	100.00	2
20111334					100.00	1		
20111335							100.00	1
20111336	61.84	76	66.67	6	100.00	1	66.67	3
20111339	33.33	3			100.00	1		
20113346					50.00	2		

Appendix 2-D: The number of capelin (Mallotus villosus) otoliths present (n), for each individual seal and the percentage of erosion state 1 and 2 otoliths separated by stomach, upper small intestine, lower small intestine and colon.

	Stomach		Upper sm. Int.		Lower sm. Int.		Colon	
Seal ID	% measurable	n	% measurable	n	% measurable	n	% measurable	n
20082055	56.00	50	90.00	10	100.00	2	100.00	2
20082057	100.00	1						
20082061	0.00	4						
20091693	35.29	17	50.00	2	72.73	11		
20091695	60.00	20						
20091697	50.00	8			80.00	5	42.86	7
20091802	23.08	26	66.67	3	66.67	3		
20091809	40.00	5			75.00	8	100.00	3
20091931	2.00	*50	0.00	36	7.00	100	66.67	3
20091947							0.00	1
20091951			50.00	2	100.00	2		
20100107	62.00	*50	25.00	44	40.96	166	100.00	4
20111128							100.00	1
20111139					25.00	4	0.00	1
20111318	48.00	*50	44.00	25	46.31	149		
20111324	58.00	*50	23.81	21	31.87	91	100.00	4
20111325	20.00	*50	30.77	13	100.00	2		
20111326	68.00	*50	15.91	44	28.13	32	0.00	1
20111328	15.63	32	50.00	2			100.00	1
20111329	100.00	2						
20111331	12.00	*50	17.65	17	22.22	27		
20111333	45.00	20	15.38	13	80.00	10		
20111334	34.00	*50	45.65	46	48.87	133	85.71	7
20111335							100.00	1
20111336			0.00	2				
20111339	44.68	47	0.00	7	42.86	14	50.00	2
20113346							100.00	3

*proportions based on subsamples of 100 otoliths (i.e. 50 individuals collected)

Appendix 2-E: The number of sand lance (Ammodytes spp.) otoliths present (n), for each individual seal and the percentage of erosion state 1 and 2 otoliths separated by stomach, upper small intestine, lower small intestine and colon.

	Stomach		Upper sm. Int.		Lower sm. Int.		Colon	
Seal ID	% measurable	n	% measurable	n	% measurable	n	% measurable	n
20100158	13.01	123	0.00	4	80.00	5		
20100160	33.33	9	100.00	3	100.00	6	24.00	50
20110104	75.00	4					12.00	50
20110105	23.60	178	0.04	28	14.49	69	0.00	6
20110108					66.67	3	68.00	50
20110306							0.00	1
20120004	96.97	66	69.70	66	100.00	4	100.00	6
20120005	97.94	97	85.19	27	100.00	26	50.00	4
20120006	74.42	86	100.00	54	100.00	43	100.00	11
20120008					100.00	28	69.23	65
20120012							100.00	1
20120013					100.00	2		
20120014					100.00	11	58.33	60

3 Chapter: Gray (*Halichoerus grypus*) and Harp Seal (*Pagophilus groenlandicus*) Diets in Newfoundland Waters based on Hard-Part Analyses of the Digestive Tract

3.1 Abstract

I investigated the diet of gray (*Halichoerus grypus*) and harp (*Pagophilus groenlandicus)* seals in Newfoundland waters. The stomach or digestive tract (stomachs, small intestines, and large intestines) of 35 gray and 145 harp seals were examined and prey remains identified to the lowest possible taxonomic group. I accounted for complete otolith loss by applying Numerical Correction Factors (NCF) to the intestine only. For gray seals, 16 prey taxa were found. In the stomach, sand lance (*Ammodytes* spp.) and Atlantic herring (*Clupea harengus*) accounted for ~80% of the diet by reconstructed wet mass (RWM) and reconstructed energy (RE). In the intestine, sand lance accounted for 94.5% and 93.5% of the diet by corrected RWM and RE, respectively. From the larger sample of harp seals, 52 prey taxa were identified. Arctic cod (*Boreogadus saida*), Atlantic cod (*Gadus morhua*), and capelin (*Mallotus villosus*) were common prey items in both the stomach and intestines, accounting for > 76% of the diet by RWM and RE in each region. The addition of NCF amplified the importance of prey with small, fragile otoliths like capelin and sand lance and, decreased the importance of prey with large robust otoliths like Atlantic cod.

Size trends of otoliths from Arctic cod, capelin and sand lance were similar throughout the digestive tract whereas Atlantic cod averaged larger in the stomach (25.7 ±

0.74 cm (SE) in body length) than in the intestine (20.6 ± 1.49 cm). This may be due to different evacuation times of different sized prey, hard part retention and/or differences in the prey composition of successive meals.

3.2 Introduction

Little published information on the diets of gray seals (*Halichoerus grypus*) in Newfoundland is available in the literature; in contrast, the diet of harp seals (*Pagophilus groenlandicus*) in Newfoundland is well known (e.g. Hammill and Stenson 2000; Hammill *et al*. 2007; Lawson and Stenson 1997; Lawson *et al.* 1994;1995). Gray seals and harp seals often inhabit similar areas in Newfoundland waters; therefore, comparing their diets will provide insight as to how they utilize marine resources.

In the 1990s many fish stocks collapsed, resulting in multiple fishing moratoria. Overfishing was found to be one of the main contributing factors to the collapse (Halliday and Fanning 2006; Myers *et al.* 1996;1997; Savenkoff *et al.* 2004), although other environmental factors may also have been important. Seals did not appear to contribute significantly to the decline. There is considerable debate as to what effect, if any, the seal populations may be having on prey stock recovery (Bowen *1997;* Hammill and Stenson 2004; DFO 2010a,b; DFO 2012).

3.2.1 *Gray Seal Biology*

The Northwest Atlantic gray seal population has increased in size from ~15,000 to 424,300 (95% CI=263,600 to 578,300) animals over the past 50 years (Bowen *et tal*. 2008; DFO 2010a; 2017; Hammill *et al.* 2014; Thomas *et al*. 2011). Recently, fishermen have reported an increase in the number of gray seal sightings along the southern and

western coasts of Newfoundland. Although gray seals have historically been present in the Newfoundland area, many fishers believe they have extended their range (G. B. Stenson, personal communication 2013). However, scientific documentation of increased numbers or expanded distribution is lacking (Bowen *et al.* 2009; DFO 2010a).The perceived increase in abundance has led to concerns regarding the recovery of many fish stocks including Atlantic cod (*Gadus morhua*), white hake (*Urophycis tenuis*), and winter skate (*Laucoraja ocellata*; Bowen *et al*. 2008; 2009; DFO 2010a).

3.2.2 *Harp Seal Biology*

The Northwest Atlantic harp seal is the most abundant pinniped in the Northwest Atlantic, with an estimated population of 7.4 million in 2014 (95% CI=6.6 to 8.2 million; Hammill *et al*. 2014b). However, they are not resident, and most individuals summer in the Canadian Arctic and Greenland. By late November and December, most are found further south along the Labrador coast and into the Gulf of St. Lawrence (DFO 2010b; DFO 2012; Lavigne and Kovacs 1988; Sergeant 1991). The large population size of harp seals has led to concerns regarding the recovery of fish stocks as well (Hammill and Stenson 2004; DFO 2010b).

3.2.3 *Diet Analysis*

The most commonly used method of diet analysis is hard-part analysis (HPA). Consumed prey items often have hard parts such as bones, otoliths, cephalopod beaks, or carapaces that are retained in the digestive tract for some time after consumption (Bowen 2000; Bowen and Harrison 1994; Fitch and Brownell 1968; Frost and Lowry 1980;

Olesiuk *et al*. 1990; Prime and Hammond 1987; 1990). These hard parts can be used to identify prey, sometimes to the species level (Arim and Naya 2003). Sagittal otoliths of bony fishes are species-specific and are the most commonly examined hard part in piscivorous predators; in addition, sagittal otoliths increase with body size and so can be used to estimate size and energy content of prey (Lawson *et al*. 1995; Lidster *et al.* 1994; Murie and Lavigne 1991, 1992).

Dietary reconstruction requires some assumptions and has limitations. For example, in HPA retention time in the digestive tract varies with otolith size (e.g. length and thickness), which in turn vary interspecifically and with prey body size (Chapter 2; Deagle and Tollit, 2007; Jarman *et al*. 2002; Tollit *et al*. 1997; 2003). The manner in which prey are consumed can also affect the composition of digestive tract contents; predators may not eat the head or hard parts may be regurgitated (Fu *et al*. 2001; Gudmundson *et al*. 2006). Also, ingested prey themselves may contain prey items (Perrin *et al*. 1973). Studies of the diet in captive pinnipeds have illuminated some of the difficulties associated with the use of HPA.

Captive studies have documented recovery rates of hard parts in feces; these vary up to tenfold across prey species due to differences in otolith size (Tollit *et al*. 1997). To address this problem, numerical correction factors (NCFs) have been developed to account for complete digestion of hard parts (Bowen 2000). These are based on the numbers of hard parts fed to, and recovered from, captive pinnipeds (Tollit *et al*. 1997). NCFs have only been developed for scat analysis, therefore, their applicability to stomach data is unknown (Chapter 2; DFO 2010b). Applying NCFs to scats reduces the importance of fish with robust otoliths and increases the importance of prey with small

fragile otoliths.

Diet indices such as Frequency of Occurrence (FO), Reconstructed Wet Mass (RWM), Reconstructed Energy (RE) are often used to help interpret diet data. FO expresses how many individual seals consume the same prey types and documents rare foods which may be missed by other measures (Tollit *et al*. 2007; G. B. Stenson, personal communication 2013). Reconstructed wet mass gives the weight contribution of each prey item, while the RE looks at how much energy each prey type contributes to the diet (Hammill *et al.* 2007; Lawson *et al*. 1994; 1995; Stenson *et al*. 2013).

My objectives were to identify prey in stomachs and intestines of gray and harp seals from Newfoundland via HPA. I compared species composition from different parts of the digestive tract by looking at the frequency of occurrence (FO), reconstructed wet mass (RWM) and reconstructed energy (RE) contributions of different prey types to the diet. Finally, I applied NCFs to look at what effect they had on the interpretation of prey results.

3.3 Methods

3.3.1 *Seal Digestive Tract Samples*

Seals were collected under permits issued by Fisheries and Oceans Canada and killed using the legally prescribed methods described in the Marine Mammal Regulations in the Fisheries Act. Digestive tracts (stomach to anus) were removed in the field and tied off at the oesophageal, pyloric, and anal sphincters to retain contents. They were then placed in labeled cloth bags and stored at -20°C until analysis (see Figs. 3.1 and 3.2; locations and dates are in Appendix 3B). Teeth from the same animals were also obtained

for aging (Hohn 2018).

3.3.2 *Stomach Sampling and Analysis*

Stomachs were weighed both full and empty to obtain an overall estimate of prey mass. Contents were emptied into and rinsed through a series of four sieves of decreasing mesh size (4.75, 2.0, 1.0, 0.8 mm). Hard parts smaller than 0.8 mm were captured in a dish at the base of the sieves. Once placed in the sieve, water was run over the contents to remove unwanted materials such as unidentifiable tissue and skin. Prey such as fully intact fish or invertebrates were counted, weighed, and measured. Each sieve was rinsed with water into a glass pan and placed on a dark background so hard parts could be identified. Hard parts (otoliths, carapaces, beaks, bones, etc.) were retained and used for species identification to the lowest taxonomic level possible (Lawson *et al.* 1995).

3.3.3 *Intestine Sampling and Analysis*

The length of the small intestine (duodenum, jejunum, and ileum together) was measured to the nearest cm and cut into two pieces of equal length (determining the three regions externally is difficult). Finally, colon length was measured. The contents of each of the three sections were collected as described above.

3.3.4 *Otolith Erosion and Prey Measurements*

I assessed the degree of erosion of otoliths from all samples based on signs of degradation of the otolith margins as follows: state 1: no signs of erosion; state 2: slight

erosion around margins; state 3: otolith margins showing signs of deterioration and cracks sometimes present; and state 4: major deterioration of margins or shape distorted or cracks present (sometimes state 4 otoliths were not identifiable). Stomachs often contained prey at many different stages of digestion. Otoliths of intact fish skulls were recorded as state 1. I measured only otoliths in states 1 or 2 (Chapter 2; Bowen 2000; Hammill *et al.* 2007; Hammond and Rothery 1996).

When otoliths were numerous (> 100 individuals or > 200 otoliths of a single species), I subsampled 100 otoliths (i.e. 50 individual prey) at random. Otoliths were identified to species when possible, following Hãrkönen (1986), Campana (2004), and reference collections at Fisheries and Oceans Canada, St. John's, Newfoundland and Labrador. To estimate the number of individual prey, all left or all right otoliths were measured from a sample (the maximum count was used: Hammill *et al*. 2007). Otoliths were measured to ± 0.01 mm: otoliths < 4 mm in length were digitally photographed and measured using the program ImagePro Plus (Media Cybernetics, Inc. 401 N. Washington Street, Suite 350, Rockville, MD 20850 USA); otoliths ≥ 4 mm in length were measured with Vernier calipers. When left or right side could not be determined, I assumed that otoliths of similar length (difference < 0.1 mm for otoliths < 5 mm long; difference < 0.25 mm for otoliths ≥ 5 mm long) and stage of erosion were from the same fish. Unmatched otoliths were assumed to represent additional individuals (Stenson *et al.* 2013).

Unmeasured otoliths were counted and identified; it was assumed that their lengths were the mean size of measured otoliths obtained from the same seal. When otoliths were not available for averaging, from the same seal, averages were obtained

from seals collected from the same sampling area and time following; Lawson *et al.* 1995. Otolith lengths were then used to estimate prey mass and length using known regression equations (Table 3.1).

Fully intact invertebrate prey were counted, weighted, and measured. Damaged invertebrate prey were counted based on the number of hard parts (e.g. beaks, carapaces) and recorded. An average biomass of undigested individuals was applied to partially digested invertebrate prey to estimate the total biomass consumed.

3.3.5 *Numerical Correction Factors*

Available taxon-specific NCFs were used for intestine samples (Table 3.2; Bowen 2000; Grellier and Hammond 2005; Hammond and Grellier 2006; Lundström *et al.* 2010). NCFs were only applied to intestine data since NCFs are derived for scat analysis, and therefore may not be appropriate for stomach analysis (Harvey 1987).

3.3.6 *Statistical Methods*

The representation of different prey species in the diet was quantified in three ways: frequency of occurrence (FO), reconstructed wet mass (RWM) and reconstructed energy (RE; Hammill *et al.* 2007; Lawson *et al*. 1994; 1995; Stenson *et al*. 2013). FO is an assessment of the presence or absence of prey and provides an indication of how often certain prey items appear in the diet. It was computed as $FO= [S_i/S_t]\bullet 100$, where S_i = number of stomachs containing prey *i* and S_t = total number of stomachs containing prey. RWM also quantifies the importance of different prey species and was computed as $RWM = (M_a/M_s)\bullet 100$, where M_a= reconstructed wet mass of all individuals of prey

species *i* and M_s = total reconstructed wet mass of all prey. Mass was reconstructed using the regression equations in Table 3.1.

The energy contribution of prey to the seals' energy income is important. I estimated energy in the diet by different prey species as RE = (E_a/E_s) •100 where E_a = reconstructed energy of all individuals of prey species *i* and E_s = total reconstructed energy of all individuals of all prey species (energy measures were calculated by multiplying the reconstructed prey mass by the caloric values listed in Table 3.1).

3.4 Results

3.4.1 *Sampling*

I analyzed the diet of 35 gray seals 20 of which had both stomach and full intestines present. Twenty-seven gray seal stomachs and 17 intestines contained prey. Sixteen prey taxa were found (Table 3.3). In two gray seal digestive tracts, prey were present only in the stomach in four others they were only in the intestine.

I analyzed stomach contents from 145 harp seals (57 with intestines). Harp seal samples containing prey consisted of 109 stomachs (36 male; 73 female) and 53 intestines (16 male; 37 female). I identified 52 prey taxa (Table 3.4). One harp seal digestive tract contained food only in the stomach and four others had prey only in the intestine.

3.4.2 *Diet Composition*

3.4.2.1 Gray Seal Digestive Tract Analysis

I identified 11 prey taxa in the stomachs and 11 in the intestines of gray seals. Based on the criteria that 10% FO, RWM, or RE constituted common prey, there were

four prey taxa that could be considered common in the stomach and five in the intestine (Table 3.3). Sand lance was a prime contributor to the diet by all three measures. This species had the highest FO and RWM in both stomach and intestine, the greatest RE in the intestine, and the second-highest RE in the stomach (Table 3.3). FO estimates suggest that Atlantic herring and unidentified fish prey were common in the stomach and intestine. Atlantic herring had the highest RE and the second highest RWM in the stomach, but it was not common in the intestine. FO was > 10% for unidentified cephalopods in the stomach, but not in the intestine. In the intestine, *Gadus* spp. and flatfish were considered to be common prey (FO >10%). Although *Gadus* spp. and winter flounder where present in the stomach they were not considered to be common.

The RE computed for 27 gray seal stomachs was 1905 ± 330 kcal (SD; range 1.1 – 5659), per seal. RE for 17 gray seal intestines was 3305 ± 1662 kcal (SD; range 1.15 – 28,853), per seal. These figures also show that RE estimates were more variable for intestine contents than stomach contents.

The reconstructed fish lengths for sand lance were similar throughout the digestive tract, with an average reconstructed fish length of ~17 cm in all regions (Figure 3.3).

3.4.2.2 Corrected Prey Weight and Energy for Gray Seal Intestines

NCFs applied to intestine data for gray seals resulted in an increase in the estimate of prey species, such as sand lance, that have small otoliths (Table 3.3). For example, RWM and RE for sand lance increased by 6.1% and 5.9%, respectively, when NCFs were applied. Conversely, estimates for fish species with robust otoliths such as *Gadus* spp. and flatfish were reduced (decreases in estimates of -2.7% and -2.4% respectively; Table

3.3).

3.4.2.3 Harp Seal Digestive Tract Analysis

I identified 48 prey taxa in the stomach and 31 in the intestine. Based on the criterion that 10% FO, RWM, or RE constituted common prey, there were seven common prey taxa in the stomach and 10 in the intestine (Table 3.4).

Based on both stomach and intestine, Arctic cod, Atlantic cod, capelin, Atlantic herring, and shrimp/prawns were important contributors to the diet by at least one measure. Atlantic cod was the most common prey in the stomach by all three measures; capelin had the highest RWM and RE, as well as the second highest FO in the intestine (Table 3.4). In the stomach, *Pandalus* spp. and shorthorn sculpin (*Myoxocephalus scorpius*) had FO > 10%, but in the intestine were not common by any measure. Daubed shanny (*Leptoclinus maculatus*), fourline snakeblenny (*Eumesogrammus praecisus*), and *Liparis* spp. were identified as important prey in the intestine but not in the stomach (Table 3.4).

The average RE estimates for the 109 harp seal stomachs were 2010 ± 313 kcal (SE; range: 0.35 – 17,722), per seal. The average RE for the 53 harp seal intestines was 1280 ± 287 kcal (range: 0.35 -12,007) per seal. Thus, RE was higher and more variable for stomach samples.

Atlantic cod tended to be longer in stomach samples (25.7 cm) than the intestine (20.6 cm). The size range in stomach also was greater than in the intestine (Figure 3.4). The mean length of Arctic cod in the diet was ~15 cm (for both stomach and intestine), and that of capelin was ~12 cm (for both stomach and intestine; Figures 3.5, 3.6).

3.4.2.4 Corrected Prey Weight and Energy for Harp Seal Intestines

NCF estimates revealed a similar pattern to gray seals. Species with small, fragile otoliths such as capelin increased in importance (NFC for capelin is 7.87, Tollit *et al*. 2007; Table 3.4). RWM and RE for capelin increased by 34.8% and 34.5%, respectively when NCFs were applied. Conversely, estimates of importance declined for fish species with robust otoliths such as Atlantic cod and Arctic cod (estimates declined by -9.0% and -8.9% respectively; Table 3.4).

3.5 Discussion

I investigated HPA results from stomachs and intestines and found that they give different views of diet. Prey taxa found in intestines primarily had large robust otoliths that are more likely to withstand digestion and often showed high levels of erosion representative of older meals. Prey taxa found in the stomach had a much broader range of erosion levels and prey types including both vertebrate and invertebrate prey and prey with a wider range of otolith morphologies. I also found that the diet estimates obtained for gray and harp seals differed indicating that these seal species occupy their own niche in Newfoundland waters.

3.5.1 *Diet Composition for Gray Seals*

In this study, there were only six females out of 35 seals collected. Given that gray seals are sexually dimorphic, and males and females seasonally have different diets, the number of males and females will likely affect diet estimates (Beck *et al.*2007; Harvey *et al*. 2008; Stenson *et al.* 2013). Previous telemetry research of gray seals in the Northwest Atlantic showed that females tend to feed in the more southern areas where prey patches

were close to their haul-out sites and tend to stay in shallower waters, therefore, it was not surprising to see primarily male gray seals in this study area (Breed *et al* 2009;Boyd 1998; Harvey *et al.* 2008; 2012). This is an important consideration when looking at the effect gray seals have on Newfoundland fish stocks. Diet analyses that considers both males and females in nearby areas should not be consistent with diet findings in Newfoundland waters comprised primarily of males.

Gray seals are generalist predators and feed on many different prey taxa, although they are mainly piscivorous (Benoît and Bowen 1990a, b; Bowen *et al*.1993; Hammill *et al.* 2007; Murie and Lavigne 1992; Stenson *et al*. 2013). Sixteen prey taxa were identified, and sand lance was the main prey consumed by gray seals. Sand lance is a small schooling prey, with small but robust otoliths (Morrow 1979; Scott 1973). For this reason, sand lance otoliths pass quickly through the digestive tract, and are better able to withstand digestion than more fragile otoliths.

Atlantic herring, another important prey, was found to have the highest RE in the stomach. Atlantic herring has a higher caloric value than sand lance (2.24 vs.1.05; Table 3.1), which is why Atlantic herring contributed the most energy. Atlantic herring hard parts were found in lesser amounts in the intestine than in the stomach, likely due to their small size and fragile structure, which makes them more susceptible to mechanical damage and gives them a high surface area to volume ratio; this may explain why I detected fewer herring otoliths in the intestine (Da Silva and Nilson 1985; Morrow, 1979; Tollit *et al*. 2003). Passage time may also affect the likelihood of finding herring otoliths; however, passage time can vary with several factors including seal activity level and meal size (Helm 1984, Marcus *et al.*1998, Tollit *et al.* 1997). Both Atlantic herring and sand

lance are schooling species that tend to be found in waters less than 200 m in depth (sand lance 0-108 m; Coad and Reist, 2004: Atlantic herring 0-200 m; Scott and Scott 1988), making them highly accessible to gray seals, which can dive more than 400 m (Beck *et al.* 2003). One cannot determine from my findings whether these species are preferred or are eaten in proportion to their abundance and accessibility.

My findings are similar to the only other study of gray seal diets in Newfoundland. Hammill *et al.* (2007) found that gray seals on Newfoundland's west coast had a diet consisting primarily of Atlantic cod, *Gadus* spp., winter flounder, sand lance, lumpfish, Atlantic herring, and Atlantic mackerel, in agreement with my study. Hammill *et al*. (2007) also found prey species I did not encounter (e.g. smelt (*Osmerus mordax*), lumpfish (*Cyclopterus lumpus*), and shrimp), differences which could reflect my small sample sizes, seasonal differences between studies, and geographic location.

FO estimates found unidentified cephalopods to be a common prey in the stomach, but not in the intestine. Beaks are often retained in the stomach long after soft parts have been digested; therefore, overestimation of cephalopod prey in the stomach is likely (Pitcher 1980; Bigg and Fawcett 1985). FO estimates found *Gadus* spp. and flatfish (and unidentified fish) to be common in the intestine. Since these taxa have large robust otoliths, it is likely that they pass through the digestive tract slowly and are subject to high amounts of mechanical breakdown. This results in reduced taxonomic resolution, which may cause numerous species to be clumped together into a higher taxonomic grouping, in turn increasing their abundance in intestine samples.

RE estimations were substantially higher for the intestine than the stomach. This finding can be explained by the brief passage time of small prey items like sand lance

(more than twice as many of this species were found in the intestine than the stomach). Small fish may spend less than 6 hr in the stomach and intestines can contain prey 12-90 hr after feeding (Murie and Lavigne 1986; Tollit *et al.* 2003). Sand lance was of similar size throughout the digestive tract, a finding that supports this interpretation (Figure 3.3).

3.5.2 *Diet Composition for Harp Seals*

Harp seals are generalist predators. Their primary prey species range from pelagic forms like capelin and Arctic cod, to benthic crustaceans and fish (Bowen *et al*. 2008; Lawson and Stenson 1997; Stenson 2012; Sergeant 1991; 1973). In my study, Arctic cod, Atlantic cod, and capelin were common prey items in both stomach and intestine, accounting for > 76% of diet by RWM and RE estimates. These findings are like previous studies in Newfoundland which found capelin, Arctic cod, herring, Atlantic cod, redfish, sculpin, and amphipods to be important prey species (e.g. Bowen *et al*. 2008; Hammill and Stenson 2000; Lawson and Stenson 1997; Lawson *et al.* 1995).

Both body size and otolith robustness affect detectability in different parts of the digestive tract, as noted above. For example, Atlantic cod exhibited a greater range in body size and tended to be larger in the stomach than in the intestine, suggesting that small individuals had a shorter evacuation time and large prey were retained longer in the stomach (Gudmundson *et al.* 2006; Harvey 1987; Sinclair *et al.* 2011). This has also been observed for walleye pollock (*Theragra chalcogramma*) in northern fur seals (*Callorhinus ursinus*; Sinclair *et al.* 2011). This means retention of larger prey in the stomach is likely for many prey species and can result in the overestimation of prey with larger otoliths in the diet based on stomach analysis alone, whereas scat analysis will

likely result in the underestimation of larger prey items in the diet. This knowledge is important as it can bias diet studies if not considered.

Shrimp was a common prey using FO estimates, based on stomach but not intestine samples. This can be explained by rapid digestion of the exoskeleton in the stomach.

By studying both the stomach and intestine, the overrepresentation of prey with larger state 1 and 2 otoliths in the stomach and the overrepresentation of prey with smaller otoliths in the intestine may even out, providing a more accurate idea of the most recent meal(s) and prey sizes consumed.

3.5.3 *Stomach vs. Intestine Diet Reconstruction*

Stomachs and intestines give different views of an animal's diet. Intestines represent prey ingested over a longer time course and greater geographic area than do stomach samples (Hammill *et al*. 2005). Intestine contents also are less affected by differential rates of digestion than stomach contents, given that stomachs have prey at very different stages of digestion and are subject to both mechanical and chemical digestion (Bowen and Iverson 2013; Harvey 1987; Robbins 1983). Furthermore, there are species-specific differences in the amount of time it takes for hard parts to pass through the stomach. This bias is not present in the intestine (Bowen 2000; Hammond and Prime 1990). However, in animals that feed on both vertebrate and invertebrate prey, there is a greater likelihood of obtaining undigested prey in the stomach than in the intestine. The intestine is thought to underestimate invertebrate prey and may miss prey when hard parts are not consumed (Hammill *et al.* 2005).

In this study, stomach and intestine samples differed within both seal species. Prey taxa not found in intestines primarily had small, fragile otoliths (i.e. Atlantic mackerel (*Scomber scombrus*), capelin), uncalcified bones (e.g. skate), had low numbers present in the stomach or decreased taxonomic resolution in the intestine when compared to the stomach (i.e. winter flounder in the stomach vs. flatfish in the intestine). Prey identified in the intestine primarily had large robust otoliths that are more likely to withstand digestion (i.e. redfish) and often showed high levels of erosion representative of older meals. Harp seals had greater prey diversity in the stomach than in the intestine. This can be explained by the cumulative effects of digestion during passage, rendering some prey types as unidentifiable by the time they appear in the intestine (Bowen 2000; Hammill *et al.* 2005).

3.5.4 *The Effect of NCFs and Diet Indices*

No chemical digestion occurs in the intestine (Harvey 1987), so intestine samples can be treated as though they are scats. NCF estimates were therefore applied. NCF estimates reflected the increased importance of species with small, fragile otoliths, which in turn affected the reconstructed weight and energy contributions. Many of the calculations were based on proportions, so an increase in the proportion of a fragile otolith caused a decrease in the relative importance of prey with large robust otoliths. This was seen clearly in the harp seal samples; capelin counts increased from 1134 individuals to 8925 individuals, which reduced the importance of large prey with robust otoiths (e.g. Atlantic cod).

Different diet indices have their own limitations and biases when it comes to

reconstruction of diet, which may result in different estimates of diet. They also answer different questions about diet. FO is a useful measure since it expresses how many individual seals consume the same prey types and documents rare foods which may be missed by other measures (Klare *et al.* 2011). However, FO does not reflect how much prey is consumed, or prey size or energy density (Klare *et al.* 2011; Tollit *et al*. 2007). RWM and RE provide better measures of diet since they include such information. I estimated FO for completeness and comparison with other studies, but base most of my discussion on my estimates of RWM and RE.

3.5.5 *Dietary Differences Between Gray and Harp Seals in Newfoundland Waters*

In this study, harp seals were not selected specifically to compare their diet with gray seal. The majority of gray seals used in this study were from the southern and western coasts of Newfoundland (e.g. Burgeo, Cape Ray, Trout River; NAFO divisions 4R 3Pn, 3Ps Figure 3.1; Appendix 3A, 3B) whereas most of the harp seals were from northeastern and eastern Newfoundland (e.g. St. Anthony, Brighton, Princeton; NAFO divisions 3K, 3L; Figure 3.2; Appendix 3A,3B). The time of year in which the samples were collected also varied (Appendix 3B). These sample differences may account for some of the dietary differences that I observed. I also reviewed harp seal diet literature from the same locations as our gray seal samples, so diets were more comparable.

By comparing other studies of harp seal diet from the southern and western coasts with my gray seal diet findings and previous gray seal studies, it becomes apparent that gray and harp seals occupy their own niche when feeding around Newfoundland. Gray seals occupy Newfoundland waters throughout the summer and winter months when my

samples were obtained (Appendix 3B). Most harp seals summer in the Canadian Arctic and Greenland, and then migrate south along the Labrador coast in the winter months (DFO 2010b; Sergeant 1991). My samples reflected these biological differences between the two species.

When gray and harp seals are in Newfoundland, their diets are different. Although there is overlap in prey species consumed, gray seals show a tendency to feed on more demersal species such as sand lance, gadoids, flatfish, and skate. Skate was a substantial component of gray seal diet, (FO = 6.8 for stomach samples) but was not detected in harp seals. There was very little crustacean prey in gray seal samples, whereas harp seals show a greater tendency to feed on crustaceans plus pelagic fish species such as capelin and Arctic cod. Arctic cod was a main prey for harp seals, (FO = 34.9 for stomach samples) but was not detected in gray seal. Furthermore, harp seals have a more diverse diet (this study; Hammill *et al*. 2007; Lawson *et al*. 1994; Stenson *et al.* 2013).

Prey size also differed between gray and harp seals. Gray seals fed on larger individual fish than harp seals: Atlantic cod averaged 44.7 cm in length for gray seal stomach samples, but only 25.7 cm in harp seal stomach samples (Figure 3.4). My sample of gray seals was small, but my estimate of length agrees with estimates by Stenson *et al*. (2013). Since gray seals are substantially larger than harp seals it makes since that they consumed larger prey. Harp seals from the southern and western coast of Newfoundland (4RS, 4R, 3Pn; Appendix 3A) feed on smaller cod than was seen in gray seals (90% less than 31 cm, 95% < 40 cm; Hammill and Stenson 2000; 2004).

Table 3.1: Regression equations used for calculating prey length and weight based on otolith length and caloric values for prey taxa identified from stomachs and intestines of gray seals (*Halichoerus grypus*) and harp seals (*Pagophilus groenlandicus*) analyzed in this study.

Common name	Scientific name	Prey length equation (cm)[a]	Source[b]	Prey weight equation (g)[a]	Source[b]	Caloric value	Source[b]
Sand lance	*Ammodytes spp.*	((76.454*OL) – 13.547))/10	1	0.371*(OL^3.89)	13	1.0512	12
Atlantic herring (Gulf)	*Clupea harengus*	(5.6553*OL) + 0.4795	2	0.00509*(FL^3.16138)	2	2.24	12
Atlantic herring (NL)	*Clupea harengus*	(15.627 + (57.86*OL))/10	1	1.48*(OL^3.08)	6	2.24	12
Sculpin	Cottida*e*	(27*OL)/10	3	1.565*OL	3	1.29	3
Arctic cod	*Boreogadus saida*	(19.433 + (18.612*OL) + 0.546* (OL^2))/10	1	0.2*(OL^2.64)	6	1.15	3
Atlantic cod (NL)	*Gadus morhua*	4.4986 + 0.1184 * (OL) + 0.1997 * (OL)^ 2	4	(10^ (-5.2106 + 3.0879 * LOG10 (FL))) * 1000	4	1.01	12
Atlantic cod (Gulf)	*Gadus morhua*	6.152 + (0.7341*OL) + (0.1323* (OL^2))	5	0.0032*(FL^3.2644)	5	1.01	12
Rock cod	*Gadus ogac*	0.1001*OL^2 + 0.9985*OL +2.6473	3	0.0101*OL^4.0995	3	1.01	3
Snailfish	*Liparis spp.*	5.7414*OL^1.3634	3	0.0065*FL^3.1802	3	1.08	3
Capelin (NL)	*Mallotus villosus*	((215.741*OL) - (176.657*(OL^2)) + (71.062*OL^3) - (9.449*OL^4) - 23.151)/10	1	e^((LN(FL)*3.808) - 7.63	11	2.01	12
Capelin (Gulf)	*Mallotus* villosus	5.2997*(OL^1.01921)	2	1.31383*(OL^2.46456)	2	2.01	12
Smelt	*Osmerus mordax*	2.8571*OL^1.131	3	0.0026*(FL^3.3001)	3	1.65	3
American plaice	*Hipploglossoides platessoides*	4.0964*OL^1.1816	3	0.0044*FL^3.1983	3	1.02	3
Flatfishes	*Pleuronectidae*	4.0964*OL^1.1816	3	0.0044*FL^3.1983	3	1.02	3

Table 3.1 continued

Common name	Scientific name	Prey length equation (cm)[a]	Source[b]	Prey weight equation (g)[b]	Source[b]	Caloric value	Source[c]
Redfish (NL)	*Sebastes spp.*	(0.12*(OL^2)) + 9.82	6	0.13*(OL^3.12)	6	1.32	3
Snakeblenny	*Lumpenus lumpretaeformis*	9.2666*OL^0.6212	3	0.0009*FL^3.4609	3	1.14	3
Daubed shanny	*Lumpenus maculatus*	9.2666*OL^0.6212	3	0.0009*FL^3.4609	3	1.14	3
Esmark's eelpout	*Lycodes esmarki*	2.6478*OL^1.5958	3	0.0049*FL^2.9696	3	1.51	3
Arctic eelpout	*Lycodes reticulatus*	2.5241*OL^1.6747	3	0.004*FL^3.0911	3	1.51	3
Eelpout	*Lycodes sp.*	2.6478*OL^1.5958	3	0.0049*FL^2.9696	3	1.51	3
Atlantic mackerel	*Scomber scombrus*	(7.33*OL) + 0.37	2	1.094*(OL^4.039)	2	1.15	3
Winter flounder	*Pseudopleuronectes americanus*	(8.389*OL) - 8.559	7	0.0079*(FL^3.12)	7	1.02	3
Hookear sculpin	*Artediellus atlanticus*	-0.1261*OL^2 + 2.9693*OL	3	0.2345*FL^2 - 0.9733*FL	3	1.29	3
Mailed sculpin	*Triglops sp.*	0.5445*OL^2 + 1.1569*OL + 2.9606	3	0.0019*FL^3.5581	3	1.29	3
Shorthorn sculpin	*Myoxocephalus scorpius*	0.2024*OL + 2.574*OL	3	0.0069*FL^3.2435	3	1.29	2
Atlantic spiny lumpsucker	*Eumicrotremus spinosus*	0.5661*e^2.3303*OL	3	0.0065*FL^3.1802	3	1.44	3
Salmon	*Salmo salar*	(8.84*OL) - 4.51	8	16.78*(OL^2.45)	8	1.58	3
Longhorn sculpin	*Myoxocephalus octodecemspinosus*	2.7269*OL^1.1626	3	0.5962*FL – 9.9759*FL + 52.168	3	1.29	3

Table 3.1 continued

Common name	Scientific name	Prey length equation (cm)[a]	Ref	Prey weight equation (g)[b]	Source[c]	Caloric value	Source[c]
Threebeard rockling	*Gaidropsarus ensis*	4.42241*OL^1.4878	3	0.0017*FL^3.4352	3	1.3	3
White hake	*Urophysis tenuis*	1.52504*(OL^1.1456)	9	0.003998*(FL^3.1718)	9	1.44	3
Lanternfishes	*Myctophidae*	0.3437*OL^2 + 0.6088*OL + 3.6332	3	0.1538*FL^2 -1.1205*FL +3.3602	3	1.08	3
Winter flounder	*Pseudopleuronectes americanus*	(8.389*OL) - 8.559	7	0.0079*(FL^3.12)	7	1.02	3
Threespine stickleback	*Gasterosteus aculeatus*	-2.58 + 14.84*OL	10	(2.02*OL)^4.28	10	1.08	3
Greenland halibut	*Reinhardtius hippoglossoides*	0.0009*OL^2 + 4.962*OL	3	0.0025*FL^3.3399	3	1.33	12

[a] OL = Otolith length; FL = fish length

[b] 1) Lidster *et al*. 1994; 2) Proust 1996, 3) Obtained from proximal content analyses performed at the Canadian Department of Fisheries and Oceans, Inspection Section laboratories in St. John's, Newfoundland. 4) Healey 2000; 5) Hammill 2000; 6) Ross 1992; 7) Bowen and Harrison 1996; 8) Harkonen 1986; 9) Clay and Clay 1991; 10) Leopold *et al*. 2001; 11) Carscadden and Frank. 2002; 12) Lawson *et al.* 1998; 13) Bowen *et al.* 1993

Table 3.2: Numerical correction factors (NCF) applied to gray seal (*Halichoerus grypus*) and harp seal (*Pagophilus groenlandicus*) prey obtained from intestinal contents to account for complete digestion of prey otoliths. Table is from Bowen *et al.* (2011). The value for fourline snakeblenny was used for daubed shanny and three-spine stickleback; the value for lumpfish was used for spiny lump sucker; and the value for sculpin was used for *Liparis* spp**.**

Common Name	Scientific Name	Length (cm)	NCF	Rounded NCF	Species	Ref
Atlantic herring	*Clupea harengus*	20.2–29.3	2.867	2.9	Gray seal	1
Atlantic mackerel	*Scomber scombrus*	26.6–33.0	1.391	1.4	Gray seal	1
Sand lance (sandeel)	*Ammodytes marinus*	13.2–22.4	2.861	2.9	Gray seal	1
Atlantic cod	*Gadus morhua*	15.8–51.7	1.06	1.1	Gray seal	1
large gadoids		10.0–51.7	1.069	1.1	Gray seal	1
American plaice	*Hippoglossoides platessoides*	13.8–34.3	1.294	1.3	Gray seal	1
All flatfish		13.8–34.3	1.241	1.2	Gray seal	1
Squid	*Loligo forbesii*	13.5–337.0	1.064	1.1	Gray seal	1
Capelin	*Mallotus villosus*	14.3-14.8	7.87	7.9	Steller sea lion	2
Sculpin	*Cottidae*			2.9		3
Eel pout	*Lycodes spp.*			1.2		3
Lumpfish	*Cyclopterus lumpus*			2.9		3
Winter flounder	*Psuedopleuronectes americanius*			1.3		3
Redfish	*Sebastes spp.*			1.1		3
Ocean pout	*Zoarces americanus*			2.9		3
American plaice	*Hippogloossides platessoides*			1.3		3
Fourline snakeblenny	*Eumesogrammus praecisus*			1.3		3

1. Grellier and Hammond 2006, 2. Tollit *et al*. 2007, 3. Bowen *et al.* 2011; assumed values based on otolith size and robustness of similar species from Campana (2004) and Härkönen (1986).

Table 3.3: Compilation of prey species and quantitative indices for 27 stomachs and 17 intestines of gray seals (*Halichoerus grypus*) that contained prey. Seals were collected in western and southern Newfoundland in 2010-2011. Intestines show quantitative indices before and after correcting for otolith digestion by applying NCFs (Table 3.2). Stomachs and intestines containing trace amounts of food (< 200 gm reconstructed) were excluded. NCFs from table 3.2 were applied.

Prey	Scientific name	Stomach				Intestine					
		Count	FO[1]	RWM[2]	RE[3]	Count	FO[1]	RWM[2]	RE[3]	Corrected with NCF RWM[2]	Corrected with NCF RE[3]
Fish											
Atlantic cod	*Gadus morhua*	2	4.5	7.4	5.3	1	5.9	0.6	0.6	0.3	0.3
Gadus spp.	*Gadus* spp.	1	2.3	2.9	2.1	5	29.4	4.5	4.3	1.8	1.8
Capelin	*Mallotus villosus*	1	2.3	0.3	0.4						
Sand lance	*Ammodytes* spp.	12	27.7	51	37.9	12	70.6	88.4	87.6	94.5	93.5
Atlantic herring	*Clupea harengus*	8	18.2	28.9	45.8	3	17.7	0.9	1.8	0.9	1.9
Atlantic mackerel	*Scomber scombrus*	3	6.8	1	0.8						
Redfish spp.	*Sebastes* spp.					1	5.9	1.2	1.5	0.5	0.6
Arctic staghorn	*Gymnocanthus tricuspis*					1	5.9	<0.1	<0.1	<0.1	<0.1
Sculpin spp.	Cottid*ae* spp.					1	5.9	<0.1	<0.1	<0.1	<0.1

[1]FO: frequency of occurrence, [2]RWM: reconstructed wet mass, [3]RE: reconstructed energy

Table 3.3 continued

Prey	Scientific name	Stomach				Intestine					
										Corrected with NCF	
		Count	FO[1]	RWM[2]	RE[3]	Count	FO[1]	RWM[2]	RE[3]	RWM[2]	RE[3]
Winter flounder	*Psuedopleuronectes americanius*	1	2.3	1.9	1.4						
Flatfish	*Pleuronectidae*					4	23.5	4.4	4.2	2	1.9
Skate	*Raja* spp.	3	6.8	3.4	3.1						
Unidentified fish		4	9.1	<0.1	<0.1	3	17.7	<0.1	<0.1	<0.1	<0.1
Invertebrate											
Unidentified cephalopod		8	18.2	2.8	2.8	1	5.9	0.1	0.1	<0.1	<0.1
Unidentified shrimp						1	5.9	<0.1	<0.1		
Unidentified		1	2.3	0.4	0.4						
Total		44		100	100	33		100	100	100	100

[1]FO: frequency of occurrence, [2]RWM: reconstructed wet mass, [3]RE: reconstructed energy

Table 3.4: Compilation of prey species and quantitative indices for 109 stomachs and 53 intestines of harp seals (*Pagophilus groenlandicus*) that contained prey. Seals were collected from northern and eastern Newfoundland in 2007-2011. Intestines show quantitative indices before and after correcting for otolith digestion by applying NCFs (Table 3.2). Stomachs and intestines containing only trace amounts of food (< 200 gm reconstructed) were excluded.

Prey	Scientific name	Stomach				Intestine					
				RWM[2]	RE[3]			RWM[2]	RE[3]	Corrected with NCF	
		Count	FO[1]			Count	FO[1]			RWM[2]	RE[3]
Fish											
Arctic cod	*Boreogadus saida*	38	35	7.2	6.5	26	49.1	14	10.5	5.1	3.3
Atlantic cod	*Gadus morhus*	48	44	67.1	56.8	21	39.6	14.2	9.3	5.2	2.9
Gadus spp.	*Gadus spp.*	3	2.8	0.8	0.7	7	13.2	2.1	1.4	0.8	0.4
Rock cod	*Gadus ogac*	2	1.8	0.8	0.7	1	1.9	1.5	1	0.6	0.3
Rockling	*Gaidropsarus ensis*					1	1.9	<0.1	<0.1	<0.1	<0.1
White hake	*Urophysis tenuis*	2	1.8	<0.1	<0.1						
Capelin	*Mallotus villosus*	44	40	7.6	12.8	24	45.3	21.3	27.8	56.1	62.3
Atlantic herring	*Clupea harengus*	18	17	5.3	9.9	12	22.6	16	23.2	15.3	18.9
Sand lance	*Ammodytes spp.*	6	5.5	<0.1	<0.1	2	3.8	<0.1	<0.1	<0.1	<0.1
Atlantic manefish	*Caristius groenlandicus*	1	0.9	0.2	0.2						
Smelt	*Osmerus mordax*	3	2.8	1.3	1.9						
Salmonid.	*Salmonidae*	1	0.9	0.1	0.1						

Table 3.4 Continued

Prey	*Scientific name*	Stomach				Intestine					
				RWM[2]	RE[3]			RWM[2]	RE[3]	Corrected with NCF	
		Count	FO[1]			Count	FO[1]			RWM[2]	RE[3]
Threespine stickleback	*Gasterosteus aculeatus*	2	1.8	0.4	0.4	4	7.6	0.5	0.4	0.2	0.2
Lantern fish spp.	*Myctophidae*	1	0.9	<0.1	<0.1						
Daubed shanny	*Lumpenus maculatus*	9	8.3	0.1	0.1	7	13.2	0.8	0.6	0.3	0.2
Snake blenny	*Lumpenus lumpretaeformis*	4	3.7	0.1	0.1	8	15.9	2	1.5	0.9	0.6
Liparis spp.	*Liparis spp.*	8	7.3	0.2	0.2	8	15.1	8	5.6	7.8	4.6
Esmark's eelpout	*Lycodes esmarki*	2	1.8	0.2	0.2						
Arctic eelpout	*Lycodes reticulatus*	3	2.8	2.3	2.9	3	5.6	12.9	12.7	5.2	4.3
Eelpout sp.	*Lycodes spp.*	3	2.8	0.1	0.1	1	1.9	4.6	4.5	4.6	1.5
Hookear sculpin	*Artediellus atlanticus*	3	2.8	<0.1	<0.1	2	3.8	<0.1	<0.1	<0.1	<0.1
Arctic deep-sea sculpin	*Myoxocephalus scorpioides*	2	1.8	0.2	0.2						
Arctic staghorn sculpin	*Gymnocanthus tricuspis*					1	1.9	<0.1	<0.1	<0.1	<0.1
Shorthorn sculpin	*Myoxocephalus scorpius*	13	12	0.9	1	2	3.8	0.1	0.1	0.1	<0.1

[1]FO: frequency of occurrence, [2]RWM: reconstructed wet mass, [3]RE: reconstructed energy

Table 3.4 Continued

		Stomach				Intestine					
				RWM[2]	RE[3]			RWM[2]	RE[3]	Corrected with NCF	
Prey	*Scientific name*	Count	FO[1]			Count	FO[1]			RWM[2]	RE[3]
Longhorn sculpin	*Myoxocephalus octodecemspinosus*	1	0.9	<0.1	<0.1						
Mailed sculpin	*Triglops sp.*	2	1.8	<0.1	<0.1	2	3.8	<0.1	<0.1	<0.1	<0.1
Redfish sp.	*Sebastes spp*	2	1.8	1.2	1.3						
Sculpin sp.	Cottidae spp.	7	6.4	<0.1	<0.1	8	15.9	0.2	0.2	0.2	0.1
Spinny lump sucker	*Eumicrotremus spinosus*					2	3.8	<0.1	<0.1	<0.1	<0.1
Greenland halibut	*Reinhardtius hippoglossoides*	1	0.9	0.1	0.1						
Winter flounder	*Pseudopleuronectes americanus*	1	0.9	0.1	0.1	1	1.9	0.5	0.1	0.5	0.1
Flatfish sp.	*Pleuronectidae*	8	7.3	0.2	0.1	5	9.4	0.2	0.1	0.2	<0.1
Unidentified fish		11	10	<0.1	<0.1	9	17	<0.1	0.3	<0.1	<0.1
Cephalapoda											
Teuthoidea		5	4.6	<0.1	0.1						
Sepiolidea		1	0.9	<0.1	<0.1						
Octopoda spp.		1	0.9	<0.1	<0.1						
Unidentified spp.		2	1.8	<0.1	<0.1	2	3.8	0.1	0.1	0.1	<0.1

[1]FO: frequency of occurrence, [2]RWM: reconstructed wet mass, [3]RE: reconstructed energy

Table 3.4 Continued

		Stomach				Intestine					
				RWM[2]	RE[3]			RWM[2]	RE[3]	Corrected with NCF	
Prey	*Scientific name*	Count	FO[1]			Count	FO[1]			RWM[2]	RE[3]
Shrimp and prawns		3	2.8	<0.1	<0.1						
Argis dentata	*Argis dentata*										
Eualus gaimardii	*Eualus gaimardii*	2	1.8	<0.1	<0.1						
Lebbeus groenlandicus	*Lebbeus groenlandicus*	1	0.9	<0.1	<0.1						
northern shrimp	*Pandalus borealis*	1	0.9	<0.1	<0.1						
Pandalus spp.	*Pandalus spp.*	15	14	1.5	1.5	3	5.7	<0.1	0.1		
pink shrimp	*Pandalus montagui*	5	4.6	0.9	0.9						
Unidentified shrimp\prawn		17	16	0.1	0.11	7	13.2	0.5	0.4		
Pasiphaea sp.	*Pasiphaea sp.*	2	1.8	0.6	0.6	1	1.9	0.1	0.4		
Hyperiidae	*Hyperiidae*	11	10	0.3	0.3	4	7.6	<0.1	<0.1		
Euphausiacea (Euphausiid)	*Euphausiacea*	4	3.7	0.1	0.1	3	5.7	0.1	0.1		
Mysidae	*Mysidae*	2	1.8	<0.1	<0.1	1	1.9	0.1	0.1		
Other invertebrates											
Brachyura	*Brachyura*	1	0.9	<0.1	<0.1						
Blue mussel	*Mytilus edulis*	1	0.9	<0.1	<0.1						
Bivalve	*Bivalvia*					2	3.8	<0.1	<0.1		
Birds											
Sea bird		1	0.9	<0.1	<0.1						

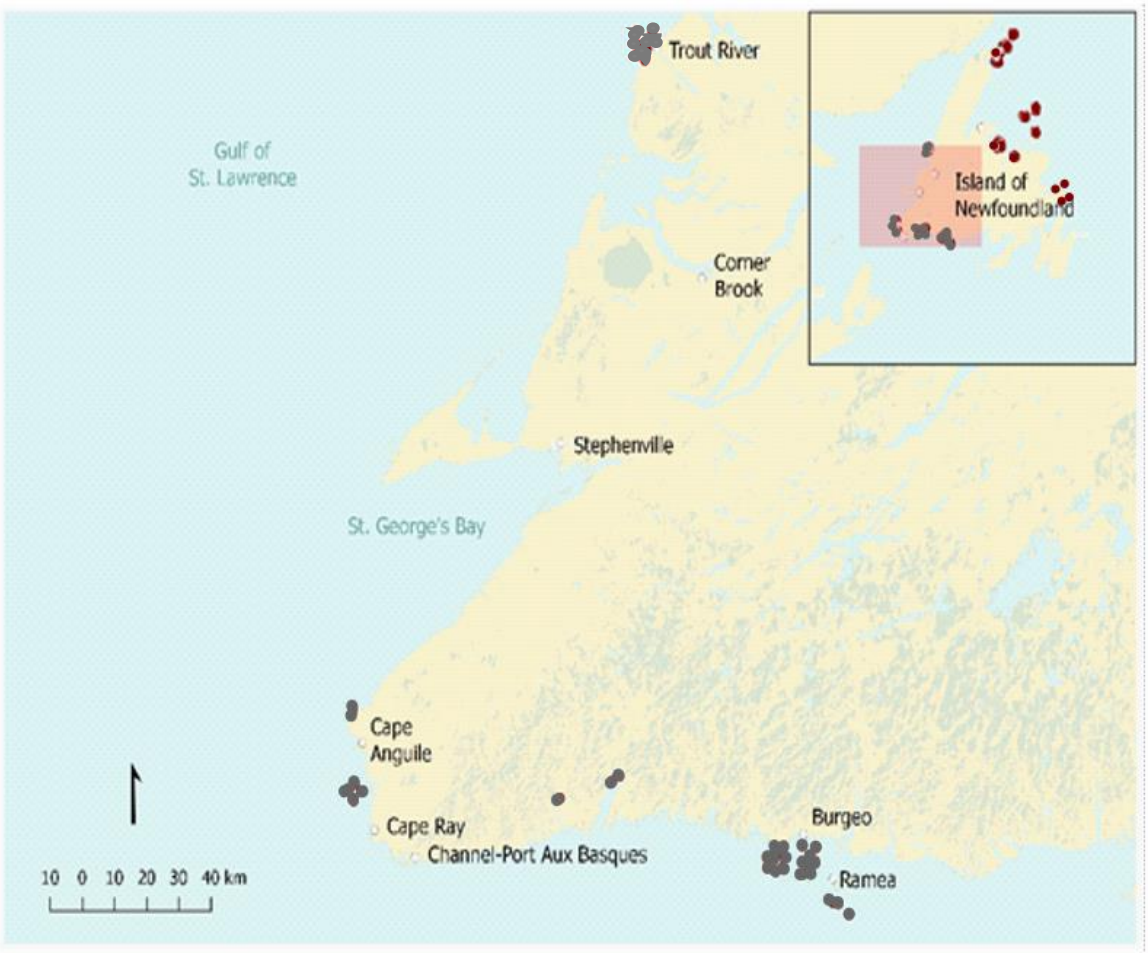


Figure 3-1: Gray dots show the locations of 35 gray seals (*Halichoerus grypus*) sampled for research purposes between 2010 and 2011 around the island of Newfoundland.

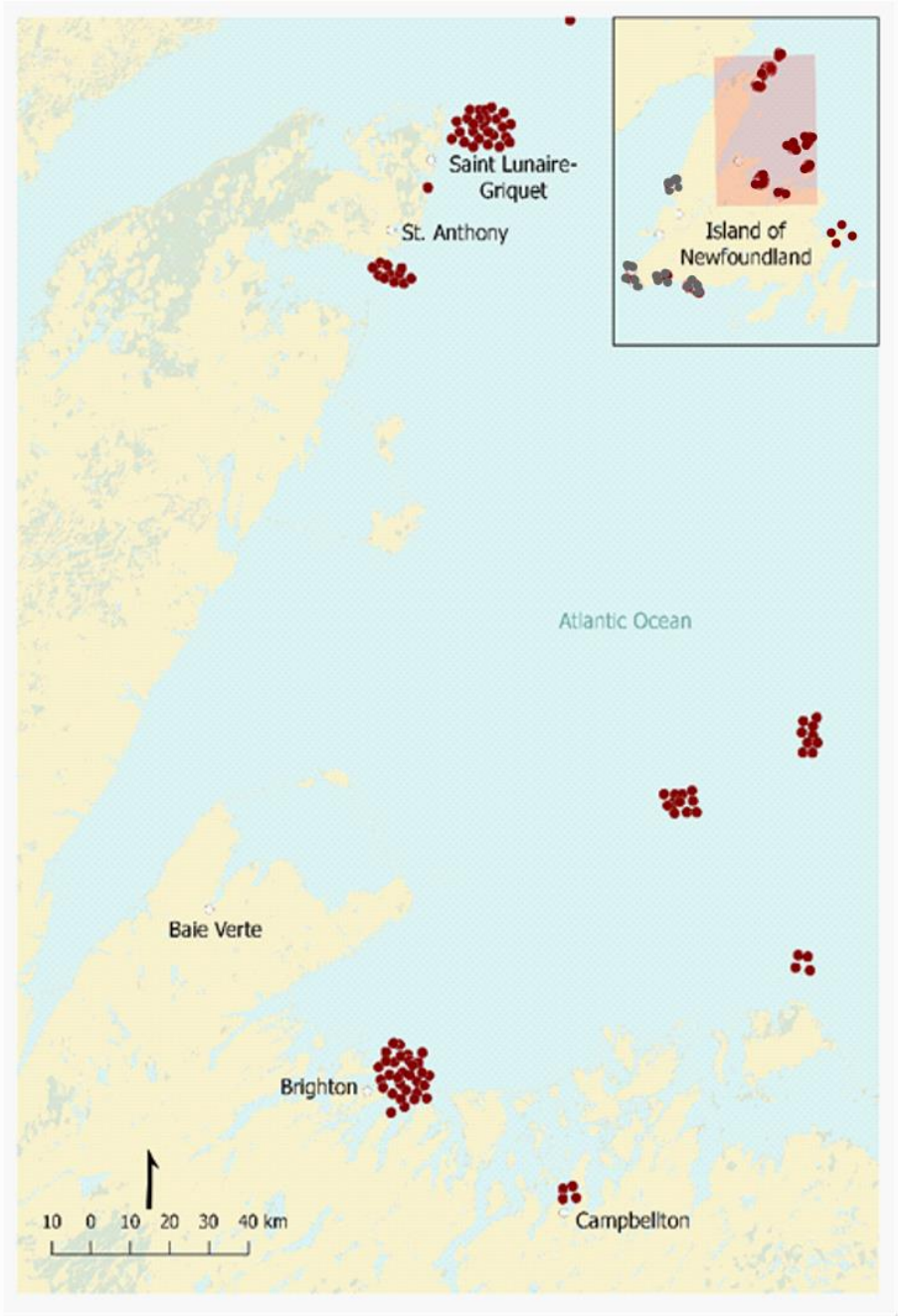


Figure 3-2: Red dots show the locations of harp seals (*Pagophilus groenlandicus*) sampled for research purposes from 2007 to 2011 around the island of Newfoundland. (Map by K. Morrissey 2018).

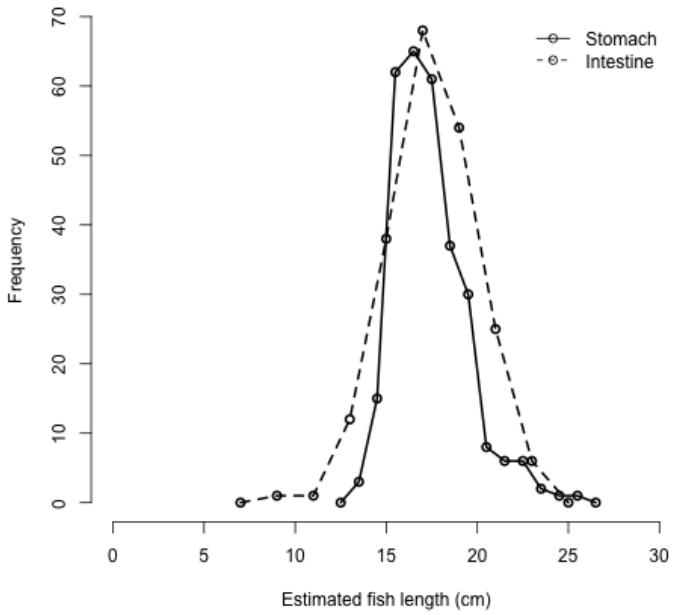


Figure 3-3: Frequency distributions of estimated fork length of sand lance (*Ammodytes spp*.) obtained from regression equations (Table 3.1) of state 1 otoliths, based on samples from gray seal (*Halichoerus grypus*) stomachs (234) and intestines (205) using a bin size of 2 cm. The average fish length was 17 cm in both regions.

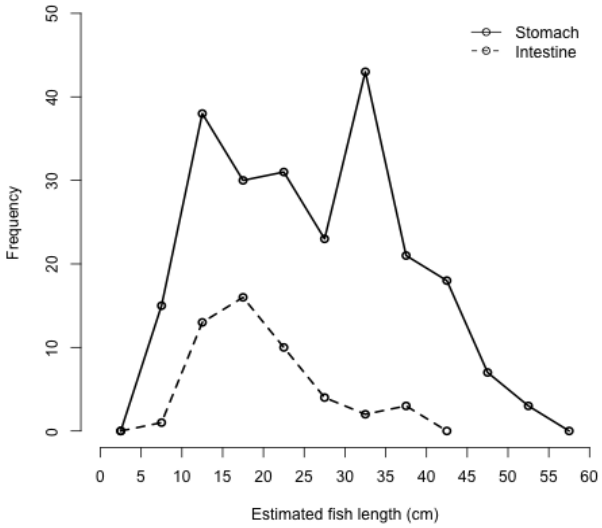


Figure 3-4: Frequency distribution of estimated fork length of Atlantic cod (*Gadus morhua*) obtained from regression equations (Table 3.1) of state 1 otoliths, obtained from harp seal (*Pagophilus groenlandicus*) stomachs (229) and intestines (25). Using a bin size of 5 cm. The average fish length was 25.69 cm in the stomach and 20.61 cm in the intestine.

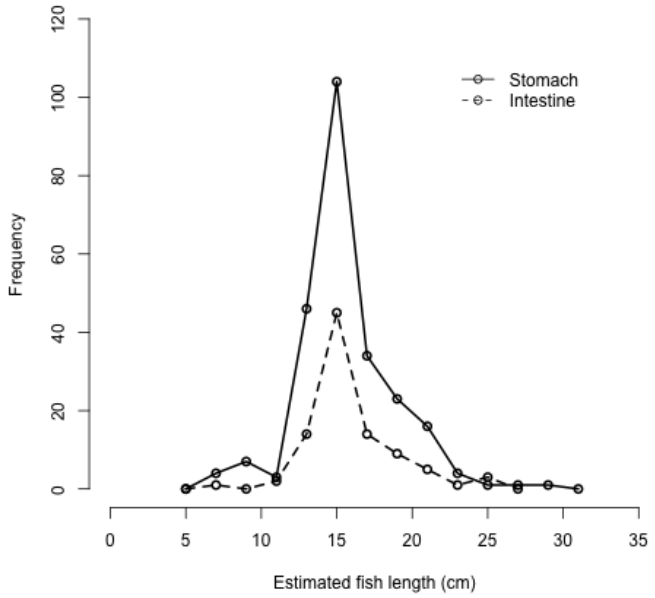


Figure 3-5: Frequency distributions of estimated fork length of Arctic cod (*Boreogadus saida*) obtained from regression equations (Table 3.1) of state 1 and state 2 otoliths, obtained from harp seal (*Pagophilus groenlandicus*) stomachs (244) and intestines (94) using a bin size of 2 cm. The average fish length was ~15 cm in both the stomach and intestine.

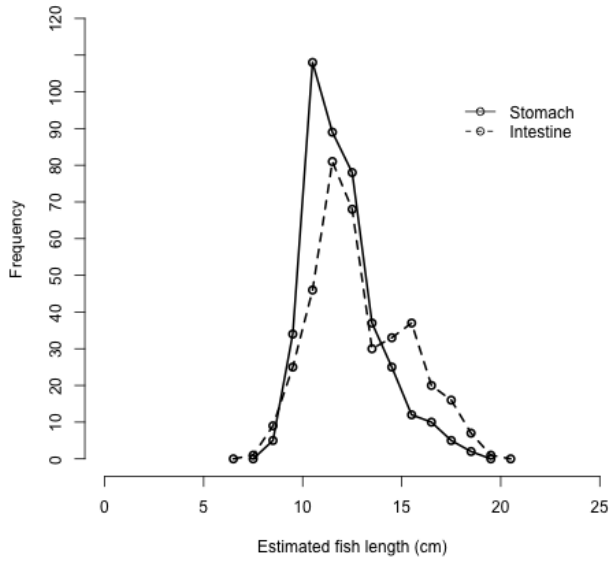
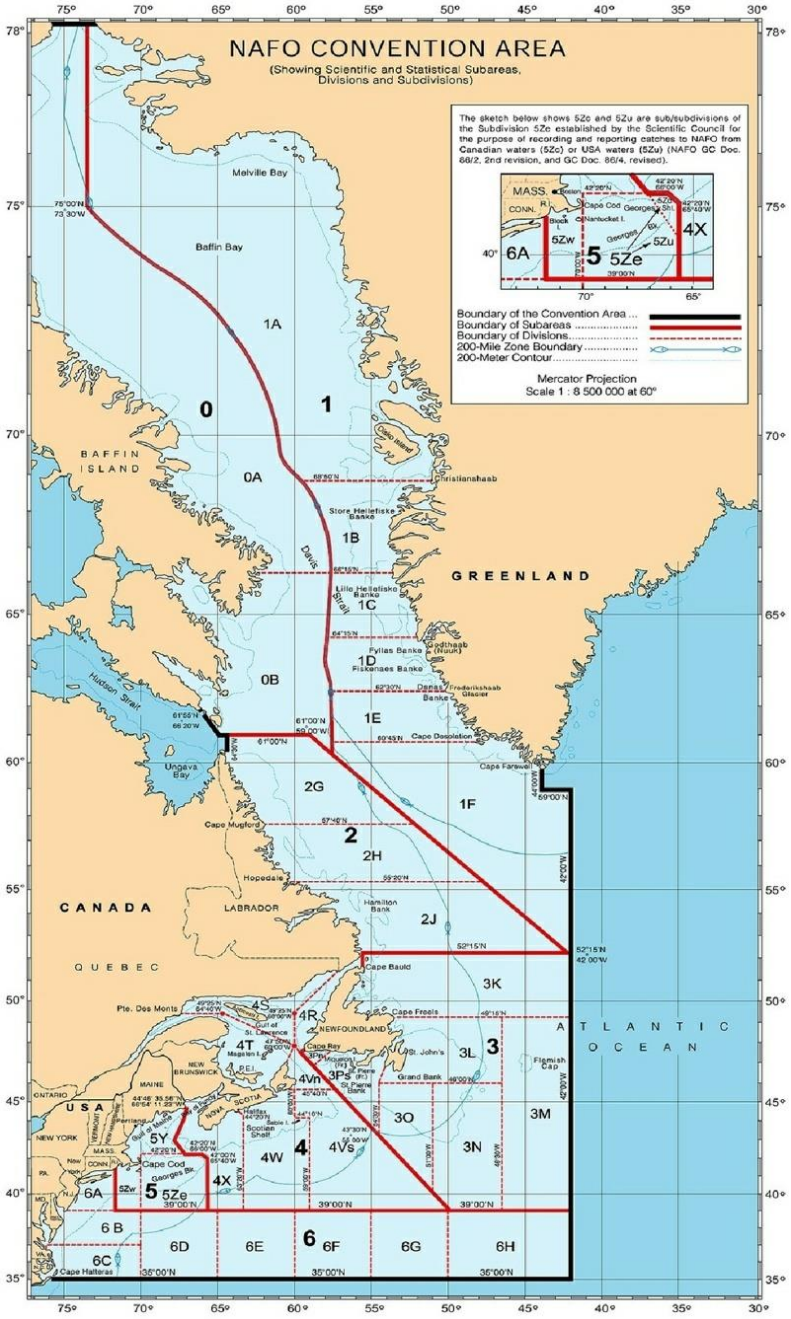


Figure 3-6: Frequency distributions of estimated fork length of Capelin (*Mallotus villosus*) obtained from regression equations (Table 3.1) of state 1 and 2 otoliths, obtained from harp seal (*Pagophilus groenlandicus*) stomachs (374) and intestines (405) using a bin size of 1 cm. The average fish length was ~12 cm in both the stomach and intestine.

Appendix 3-A: NAFO map showing fishery management divisions of the Northwest Atlantic (image from http://www.dfo-mpo.gc.ca/international/media/images/NAFOmap-carteOPANOlg-eng.jpg



Appendix 3-B: Summary of location and date of capture for gray seals (*Halichoerus grypus*) and harp seals (*Pagophilus groenlandicus*) obtained by collectors from the island of Newfoundland.

Sex		ID#	Year	Month	Day	Community	Area	Stomach	Intestine
Gray	Harp								
U		20100158	2010	8	16	Burgeo	302	1	1
M		20100159	2010	8	16	Burgeo	302	1	1
M		20100160	2010	8	16	Burgeo	303	1	1
M		20100161	2010	8	16	Shoal Cove West	401	1	
M		20110001	2010	10	19	Cape Ray	404	1	1
F		20110082	2011	2	6	Cape Ray	404	1	1
M		20110086	2010	12	2	Trout River	402	1	
M		20110088	2010	12	2	Trout River	402	1	
M		20110095	2010	12	7	Trout River	402	1	
M		20110099	2010	12	8	Trout River	402	1	
M		20110100	2010	12	17	Trout River	402	1	
M		20110102	2010	12	20	Trout River	402	1	
M		20110103	2010	12	20	Trout River	402	1	
M		20110104	2011	2	24	Burgeo	302	1	1
M		20110105	2011	2	24	Burgeo	302	1	1
M		20110106	2011	2	24	Burgeo	302	1	1
F		20110107	2011	2	24	Burgeo	302	1	1
M		20110108	2011	2	24	Burgeo	302	1	1
M		20110305	2011	8	20	Cape Ray	404	1	
M		20110306	2011	8	13	Cape Ray	404	1	
F		20120001	2011	9	19	Burgeo	301	1	
M		20120002	2011	9	19	Burgeo	301	1	
M		20120003	2011	9	19	Burgeo	301	1	
M		20120004	2011	9	19	Burgeo	301	1	1
M		20120005	2011	9	19	Burgeo	301	1	1
M		20120006	2011	9	19	Burgeo	301	1	1
M		20120007	2011	9	19	Burgeo	301	1	1
M		20120008	2011	9	19	Burgeo	301	1	1
M		20120009	2011	9	19	Burgeo	301	1	1
M		20120010	2011	9	19	Burgeo	301	1	
F		20120011	2011	9	19	Port aux Basques	404	1	1
M		20120012	2011	9	19	Port aux Basques	404	1	1
M		20120013	2011	9	19	Port aux Basques	404	1	1
F		20120014	2011	9	13	Codroy	404	1	1

Appendix 3B continued

Sex		ID#	Year	Month	Day	Community	Area	Stomach	Intestine
Gray	Harp								
F		20120016	2011	9	13	Codroy	404	1	
	M	20070171	2007	1	16	Cambellton	339	1	
	M	20070174	2007	1	16	Cambellton	339	1	
	M	20070179	2007	1	16	Cambellton	339	1	
	M	20070180	2007	1	16	Cambellton	339	1	
	F	20072347	2007	2	3	Brighton	340	1	
	F	20072348	2007	2	11	Brighton	340	1	
	F	20072356	2007	1	22	Princeton	338	1	
	M	20072357	2007	1	22	Princeton	338	1	
	F	20072358	2007	1	22	Princeton	338	1	
	F	20072711	2006	12	26	St. Anthony	342	1	
	M	20072729	2007	1	26	St. Anthony	341	1	
	F	20072745	2007	2	17	Griquet	342	1	
	F	20072757	2006	12	1	Griquet	342	1	
	F	20072771	2007	2	20	Offshore, Northeast NF	344	1	
	M	20072872	2007	2	17	Northeast NF	342	1	
	M	20072873	2007	2	17	Northeast NF	342	1	
	M	20072890	2007	2	22	St. Anthony	341	1	
	F	20072893	2006	12	26	St. Anthony	342	1	
	F	20072895	2006	12	26	St. Anthony	342	1	
	M	20072899	2007	1	18	St. Anthony	341	1	
	F	20073138	2007	5	28	Northeast NF	342	1	
	F	20081652	2008	2	23	Northeast NF	339	1	
	M	20081653	2008	2	23	Northeast NF	339	1	
	F	20081660	2008	2	23	Northeast NF	339	1	
	M	20082055	2008	2	26	Brighton	340	1	1
	F	20082057	2008	2	26	Brighton	340	1	1
	F	20082061	2008	2	26	Brighton	340	1	1
	F	20082063	2008	2	26	Brighton	340	1	
	M	20082064	2008	2	26	Brighton	340	1	
	M	20082067	2008	2	26	Brighton	340	1	
	F	20082078	2008	2	23	Brighton	340	1	
	F	20082093	2008	2	6	Brighton	340	1	
	M	20082363	2007	12	20	St. Anthony	342	1	
	M	20082677	2008	2	15	Offshore, Northeast NF	345	1	1
	M	20082680	2008	2	15	Offshore, Northeast NF	345	1	
	F	20082681	2008	2	15	Offshore, Northeast NF	345	1	1
	F	20082684	2008	2	15	Offshore, Northeast NF	345	1	
	M	20082685	2008	2	15	Offshore, Northeast NF	345	1	

Appendix 3B continued

Sex		ID#	Year	Month	Day	Community	Area	Stomach	Intestine
Gray	Harp								
	F	20082686	2008	2	15	Offshore, Northeast NF	345	1	
	M	20082688	2008	2	15	Offshore, Northeast NF	345	1	
	M	20090099	2009	5	5	Smith Sound	337	1	1
	F	20090102	2008	12	28	Smith Sound	337	1	
	F	20090103	2009	5	5	Smith Sound	337	1	
	M	20090104	2009	3	4	Smith Sound	337	1	
	F	20090105	2009	5	7	Smith Sound	337	1	
	M	20091691	2009	2	12	Offshore, Northeast NF	338	1	
	M	20091693	2009	2	14	Offshore, Northeast NF	339	1	1
	M	20091695	2009	2	15	Offshore, Northeast NF	344	1	1
	M	20091697	2009	2	16	Offshore, Northeast NF	344	1	1
	F	20091698	2009	2	16	Offshore, Northeast NF	344	1	
	F	20091700	2009	2	17	Offshore, Northeast NF	344	1	
	F	20091802	2009	2	17	Offshore, Northeast NF	344	1	1
	M	20091803	2009	2	17	Offshore, Northeast NF	344	1	
	F	20091809	2009	2	17	Offshore, Northeast NF	344	1	1
	F	20091811	2009	2	17	Offshore, Northeast NF	344	1	
	F	20091812	2009	2	17	Offshore, Northeast NF	344	1	
	M	20091814	2009	2	17	Offshore, Northeast NF	344	1	
	M	20091815	2009	1	12	Griquet	342	1	
	F	20091816	2009	1	12	Griquet	342	1	
	F	20091817	2009	1	12	Griquet	342	1	
	M	20091818	2009	1	9	Griquet	342	1	
	M	20091819	2009	1	7	Griquet	342	1	
	M	20091820	2009	1	7	Griquet	342	1	
	M	20091821	2009	1	7	Griquet	342	1	
	M	20091822	2009	1	7	Griquet	342	1	
	F	20091823	2009	1	7	Griquet	342	1	
	F	20091824	2009	1	7	Griquet	342	1	
	F	20091825	2009	1	17	Griquet	342	1	
	F	20091826	2009	1	17	Griquet	342	1	
	F	20091827	2009	1	17	Griquet	342	1	
	M	20091828	2009	1	17	Griquet	342	1	
	F	20091829	2009	1	17	Griquet	342	1	
	F	20091830	2009	1	12	Griquet	342	1	
	F	20091930	2009	2	23	Brighton	340	1	
	F	20091931	2009	2	17	Brighton	340	1	1

Appendix 3B continued

Sex		ID#	Year	Month	Day	Community	Area	Stomach	Intestine
Gray	Harp								
	F	20091932	2009	2	17	Brighton	340	1	
	F	20091933	2009	2	14	Brighton	340	1	
	F	20091934	2009	2	17	Brighton	340	1	
	F	20091936	2009	2	12	Brighton	340	1	
	F	20091938	2009	2	5	Brighton	340	1	
	F	20091939	2009	2	17	Brighton	340	1	
	F	20091941	2009	2	14	Brighton	340	1	
	F	20091944	2009	2	17	Brighton	340	1	
	F	20091946	2009	2	17	Brighton	340	1	
	F	20091947	2009	1	31	Brighton	340	1	1
	F	20091948	2009	2	17	Brighton	340	1	
	F	20091950	2009	2	11	Brighton	340	1	
	F	20091951	2009	2	14	Brighton	340	1	1
	F	20091952	2009	1	8	Brighton	340	1	
	M	20091954	2008	12	30	Brighton	340	1	
	M	20091956	2009	1	2	Brighton	340	1	
	F	20091957	2008	12	30	Brighton	340	1	1
	F	20091959	2009	2	12	Brighton	340	1	
	F	20091960	2009	1	31	Brighton	340	1	1
	F	20091962	2009	2	5	Brighton	340	1	
	F	20091964	2009	1	9	Brighton	340	1	1
	F	20091974	2008	12	29	Brighton	340	1	
	F	20091976	2008	12	30	Brighton	340	1	
	M	20091978	2008	12	29	Brighton	340	1	1
	M	20091984	2008	12	30	Brighton	340	1	
	M	20091986	2009	2	16	Brighton	340	1	
	F	20091988	2009	1	8	Brighton	340	1	1
	F	20091989	2009	1	24	Brighton	340	1	
	M	20091990	2008	12	27	Brighton	340	1	1
	M	20091991	2008	12	30	Brighton	340	1	
	F	20100107	2010	2	8	Trinity Bay	337	1	1
	F	20100108	2010	2	10	Trinity Bay	337	1	1
	M	20100109	2010	5	11	Trinity Bay	337	1	1
	F	20100110	2010	5	13	Trinity Bay	337	1	1
	F	20100111	2010	5	13	Trinity Bay	337	1	1
	M	20100112	2010	5	24	Trinity Bay	337	1	1
	F	20102952	2010	2	15	Brighton	340	1	1
	F	20102953	2010	2	6	Brighton	340	1	
	F	20102954	2010	1	23	Brighton	340	1	

Appendix 3B continued

Sex		ID#	Year	Month	Day	Community	Area	Stomach	Intestine
Gray	Harp								
	F	20102957	2010	1	21	Brighton	340	1	1
	F	20102962	2010	2	4	Brighton	340	1	1
	F	20102968	2010	2	15	Brighton	340	1	1
	F	20102977	2010	2	4	Brighton	340	1	
	M	20110185	2011	1	4	Smith Sound	337	1	1
	F	20110186	2011	2	22	Smith Sound	337	1	1
	F	20111128	2011	2	12	Griquet	342	1	1
	F	20111131	2011	1	31	Princeton	338	1	1
	F	20111132	2011	2	10	Princeton	338	1	1
	M	20111133	2011	2	10	Princeton	338	1	1
	M	20111134	2011	1	30	Princeton	338	1	1
	F	20111139	2011	1	30	Princeton	338	1	1
	F	20111317	2011	2	24	Brighton	340	1	1
	F	20111318	2011	2	8	Brighton	340	1	1
	F	20111320	2011	1	29	Brighton	340	1	1
	F	20111324	2011	2	8	Brighton	340	1	1
	F	20111325	2011	2	5	Brighton	340	1	1
	M	20111326	2011	1	29	Brighton	340	1	1
	F	20111328	2011	2	6	Brighton	340	1	1
	F	20111329	2011	1	29	Brighton	340	1	1
	F	20111331	2011	1	29	Brighton	340	1	1
	F	20111332	2011	1	31	Brighton	340	1	1
	F	20111333	2011	2	8	Brighton	340	1	1
	F	20111334	2011	1	31	Brighton	340	1	1
	F	20111335	2011	2	21	Brighton	340	1	1
	F	20111336	2011	1	31	Brighton	340	1	1
	M	20111338	2011	1	15	Brighton	340	1	1
	M	20111339	2011	1	15	Brighton	340	1	1
	M	20113346	2011	1	7	Brighton	341	1	1
	F	20113371	2011	2	3	Griquet	342	1	1
	F	20113393	2011	2	3	Griquet	342	1	1

4 Chapter: A Multiplex PCR Method for Identifying Multiple Prey Items in Stomach Contents of Gray (*Halichoerus grypus*) and Harp (*Pagophilus groenlandicus*) Seals

4.1 Abstract

Molecular methods such as polymerase chain reaction (PCR) have proven useful for identifying prey of many different species of predator and may provide insights on the differential retention of hard parts. Unfortunately, such methods are often labour-intensive and costly. Here I describe a rapid, sensitive, and practical approach to PCR detection of prey using multiplex PCR with species-specific oligonucleotides (19-22 bp). I tested for the presence of Atlantic cod (*Gadus morhua*), Arctic cod *(Boreogadus saida*), capelin (*Mallotus villosus*), and sand lance (*Ammodytes spp.*) in stomach samples of 29 gray (*Halichoerus grypus*) and 110 harp (*Pagophilus groenlandicus*) seals from the Northwest Atlantic Ocean. Amplicons of expected sizes were obtained for all four primer pairs in a single reaction.

For some stomachs containing hard parts of one or more of the four prey species I tested for, no DNA amplification occurred; this was most marked for Atlantic cod, suggesting that large otoliths may be retained longer in the gut. Conversely, DNA amplification sometimes occurred in the absence of hard parts. This may be due to the breakdown or rapid digestion of fragile hard parts, the regurgitation of hard parts, or the ingestion of viscera only ("belly biting"). Digestion state had an impact on the likelihood of DNA amplification.

The molecular identification of multiple prey items simultaneously is possible and can be a useful tool when used together with hard-part analysis (HPA). Using dilution curves, quantitative measures of template DNA were obtained; however, results were highly variable, and the precision may be too low to make this technique useful.

4.2 Introduction

Accurate estimates of the diet of predators are necessary for understanding how marine carnivores like seals affect prey populations (Beck *et al*. 2007; Deagle *et al*. 2007). This is particularly important in situations where predator populations are large, as is the case for gray seals (*Halichoerus grypus*) and harp seals (*Pagophilus groenlandicus*) in the Northwest Atlantic (Bowen *et al*. 2008; DFO 2010a; Hammill *et al*. 2007; Thomas *et al*. 2007). Both species are generalist predators that feed on a variety of fish and invertebrates including capelin (*Mallotus villosus*), sand lance (*Ammodytes* spp.), Atlantic herring (*Clupea harengus*), Atlantic cod (*Gadus morhua*), flatfish (Pleuronectidae), and squid (Bowen and Harrison 1994; Bowen *et al*. 2008; Hammill and Stenson 2004; Hammill *et al*. 2005; 2007; Stenson *et a*l. 2013).

Food consumption by predators can be estimated using bioenergetics models that incorporate the number of individual predators in different age classes and their energy requirements, seasonal distribution, and diet (Hammill and Stenson 2000). Diets are difficult to estimate accurately, but are an important component of such models, so many methods for diet assessment have been developed including direct observation, hard-part analysis (HPA), polymerase chain reaction (PCR) -based methods, fatty acid analysis (FAA), and stable isotope analysis (SIA). Each of these methods has advantages and disadvantages (Chapter 1; Bowen and Iverson 2012).

Prey items contain species-specific DNA that persists in the digestive tract, making it possible to use a PCR with species-specific primers to determine which prey species have been ingested (Dunshea 2009; Symondson 2002). DNA from stomach contents is often sheared (≤ 800 bp; Marshall *et al.* 2010), so using small regions for PCR amplification increases the chances of successful prey identification, assuming the fragments used are unique to the prey species. It is also important for primers to be of similar size, because the sensitivity of PCR depends on amplicon size (i.e. smaller amplicons are detected more often; Marshall *et al.* 2010). Species-specific PCR has been tested on DNA extracted from both scat and stomach contents of pinnipeds; however, to date the technique has only been used to identify a single prey item at a time (Deagle and Tollit 2007; Deagle *et al.* 2005; Marshall *et al*. 2010).

Harper *et al*. (2005) and King *et al*. (2010) used multiplex PCR and fluorescent-labeled primers for determining diets of carabid beetles (*Pterostichus melanarius*) and other invertebrate predators with techniques allowing for the identification of multiple prey items with each test. Testing simultaneously for multiple prey items is less time-consuming, more cost-efficient, and more accurate than traditional PCR analysis, given that fluorometry works on a finer scale than gel electrophoresis (Hellberg *et al.* 2010).

The objectives of this study were as follows: to develop a multiplex PCR method to detect multiple prey items simultaneously using species-specific primers; to detect the presence of Atlantic cod, Arctic cod, capelin, and sand lance in stomach contents of gray and harp seals using multiplex PCR with species-specific oligonucleotides; to compare the multiplex PCR results with the occurrence of hard parts from the same stomachs; to determine the relationship of DNA amplification to state of digestion; and finally to

determine if multiplex PCR could allow for a semi-quantitative analysis of template DNA.

4.3 Materials and Methods

4.3.1 *Stomach-Contents Samples*

Thirty-four gray seals and 131 harp seals collected from around the island of Newfoundland between 2007 and 2011 were examined. All seals were collected under permits issued by Fisheries and Oceans Canada and killed using methods outlined in the Marine Mammal Regulations. Digestive tracts (stomach to anus) were removed in the field and tied off at the oesophageal, pyloric, and anal sphincters to retain contents. They were then placed in labeled cloth bags and stored at -20°C until analyses was performed. Teeth from the same specimens were also obtained for aging (Hohn 2018).

4.3.2 *Hard-Parts Analysis*

After weighing, stomachs were cut open longitudinally and the contents were emptied and rinsed through a series of four sieves of decreasing mesh size (4.75, 2.0, 1.0, and 0.8 mm); otoliths that passed through the finest filter were also collected and retained in a dish placed at the bottom of the sieves. Unwanted material such as unidentifiable tissue and skin were removed. Fully intact fish or invertebrates were counted, weighed, and measured. Each sieve was rinsed with water into a glass pan and placed on a dark background, so all otoliths or invertebrates could be counted and removed. Hard parts including otoliths, carapaces, beaks, and bones were retained and later used for species identification to the lowest taxonomic level possible, following Lawson e*t al.* (1995). The

state of digestion of fish prey items was scored into classes (1 to 6) based on how extensively digested the material was (Table 4.1; Stenson *et al*., 2013). Multiple prey types or multiple individuals of the same prey species often were present, so I characterized each stomach by the percentage of prey in each class for each prey type.

Approximately 30 ml of slurry (mixed stomach contents) was obtained from each stomach before rinsing and stored at -20ºC for DNA extraction; if tissue or full prey were present, approximately 1 cm^3 of the tissue was added to the slurry (Marshall *et al.* 2010).

4.3.3 *DNA Extraction*

I extracted DNA from ~25 mg frozen muscle tissue samples from seals and from selected species of important fish prey (see below) for primer testing. Slurry samples were thawed completely, mixed vigorously and a 200 µl subsample was taken for DNA extraction. I extracted DNA with QIAamp DNA Mini Kit (Qiagen Inc., Missisauga, ON, Canada), according to the manufacturer's tissue protocol, with a final elution of DNA in 200 µl of AE buffer. I evaluated the samples for concentration and purity with a NanoDrop 1000 Spectrophotometer (NanoDrop Inc., Delaware, USA), and the fragment size by agarose gel electrophoresis. I did not analyze stomach DNA extractions with total concentrations < 2.5 ng/µl.

4.3.4 *Primers for Multiplex PCR Amplification*

I used primer sequences designed for four important prey types (Atlantic cod, Arctic cod, capelin, and sand lance) (Table 4.2). Expected amplicon sizes were 120-175 base pairs (bp) long. All primers are for mitochondrial genes: Gmo6F2 and Gmo6R2 in

the NADH dehydrogenase subunit (ND) 5 gene; Bsa3F and Bsa3R in the ND1 and ND2 genes respectively; Mvi3F and Mvi2R in the cytochrome c oxidase I gene and Aam1F and Aam1R in the 16S rRNA gene.

Primers were previously tested with harp seal diet samples, but not gray seals. To ensure that primers would not amplify gray seal DNA, I looked for potential annealing sites using the program BLAST (Basic Local Alignment Search Tool; National Library of Medicine (NCBI), which searches for the most similar DNA sequences from the DNA database and identifies possible matches. I then aligned the eight primer sequences along the gray seal mitochondrial genome (GenBank Accession No. X72004) using Sequencher version 4.7 (GeneCodes Corporation) and determined that no potential annealing sites were present. Primer interactions were assessed by the Multiple Primer Analyzer (Thermo Fisher Scientific inc. 2011) to ensure these primers could be used in a multiplex PCR, without interaction between them. This program checks for complementary regions, especially at the 3' end, between the primers by lining them up next to each other.

4.3.5 *Multiplex PCR Amplification and Electrophoretic Analysis*

The four species-specific amplicons were co-amplified in a single multiplex reaction containing species-specific primers for the four fish species (Table 4.2). The forward primers were fluorescently labeled at the 5'–end with NED, VIC, 6-FAM, or PET (Applied Bio systems), and amplification was performed with a Qiagen Multiplex kit (Qiagen Inc.). Each reaction contained 12.5 µl Master mix, 2.5 µl Q solution, 2.5 µl primer mix (primer concentrations were 0.1 µM for Atlantic cod and capelin, and 0.075 µM for Arctic cod and sand lance), 5.5 µl distilled water, and 2 µl template DNA.

Positive controls, containing DNA from each fish species, and negative comtrols (no-template DNA and seal species), were included with each set of samples. The thermal cycling profile consisted of an initial denaturation of 95°C for 15 minutes, followed by 35 cycles of 94°C for 30s, 63.2°C for 30s, and 72°C for 90 s, followed by a final extension at 72°C for 10 min. Thermal cycling was performed with the Applied Biosystems 9700 GeneAmp thermal cycler (850 Lincoln Centre Drive, Foster City, CA 94404, USA).

A 1 µl aliquot of each multiplex PCR product was mixed with 8.8 µl Hi Di Formamide and 0.2 µl Liz 500 standard and analyzed by electrophoresis using an ABI 3730 or 3130 DNA Analyzer. Electropherograms were viewed with PeakScanner version 1.0 (Applied Biosystems 2011), and each stomach sample was subjected to the amplification procedure twice (three times if results were inconclusive). Any peak ≥ 100 fluorescent units, at the expected product size, was designated as positive (Harper *et al*. 2005). The expected product size was determined by looking at the range (in bp) observed in the positive controls (Table 4.3). A stomach was considered positive for a prey item if it was identified in at least two of the amplifications. If peaks appeared in the no-template control, or if the expected product for a different species occurred in one of the positive controls, the results for that set of samples were inconclusive and the entire procedure was repeated.

To test the efficiency of the multiplex PCR protocol, when all four prey items were present, DNA from all four prey species was combined in three ways and subjected to PCR: 1) 0.5 µl of the stock DNA extractions from each of the four species combined (i.e. mixed concentrations to simulate stomach like conditions; different amounts for each prey); 2) 100 ng of DNA from each species; and 3) 50 ng of DNA from each species

(Table 4.4). The 100 ng and 50 ng amounts were chosen as they provided sufficient DNA for the reaction to occur. Also, these higher amounts of DNA in the reaction could provide evidence of one primer pair outcompeting other primers for reagents within the reaction by showing positive results for only some of the prey species.

4.3.6 *Serial Dilution of Prey DNA*

To construct standard curves for quantification of prey DNA, I prepared serial dilutions consisting of 100 ng, 50 ng, 25 ng, 10 ng, and 5 ng of template for each prey species and subjected them to the multiplex PCR procedure described above. Standard curves for both peak height and peak area were constructed with known amounts of template. Regressions were used to determine if the standard curves could be used to quantify DNA from the concentrations tested.

4.4 Results

4.4.1 *Hard-Part Analysis*

In this study, stomachs from 34 gray and 131 harp seals were used. Atlantic cod otoliths were found in 37 harp seals and one gray seal, Arctic cod was present in 17 harp seals, capelin was present in 32 harp seals and one gray seal, and sand lance was found in 12 gray seals and seven harp seals. Thirty-seven seals contained no hard parts (Table 4.5 and Appendix 4A).

4.4.2 *DNA Recovery from Harp and Gray Seal Stomachs*

DNA concentrations obtained ranged from 0 – 327 ng/µl (Appendix 4B), with 29

gray seal stomachs and 110 harp seal stomachs containing a concentration ≥ 2.5 ng/µl (mean 31.35 ng/µl); these 139 samples were retained for multiplex PCR analysis.

4.4.3 *Tests of Primer Specificity*

Generally, DNA isolated from stomach contents was sheared to low molecular mass fragments (Marshall *et al*. 2010); however, I was able to amplify 125-175 bp PCR products. In non-multiplex PCR tests, each species-specific primer pair amplified only the DNA of target prey species. To test the efficiency of the multiplex PCR protocol, I subjected the three combinations of DNA from each of the four prey species (0.5 µl of stock DNA from each prey species, 100 ng of DNA from each prey, and 50 ng of DNA from each prey), to multiplex PCR. In all three tests, I detected expected amplicons for all four prey items (Table 4.4). Fluorescent peaks were highest in the third test, where the lowest amount of template DNA was used (50 ng of each of the four prey) except for capelin, for which the peaks stayed relatively constant. PCR product lengths were slightly longer than expected, likely due to the addition of the fluorescent label (Table 4.3).

4.4.4 *Multiplex PCR Amplification of Stomach DNA from Harp and Gray Seals*

Based on the criterion that two positive PCR results are required to conclude the prey species is present in a stomach, 85 of the 139 stomachs contained no prey while 54 stomachs contained at least one of the four prey items (Table 4.5, Appendix 4 A). Of these, 40 contained only one prey species' DNA, 12 contained DNA from two prey species, and two contained DNA from three prey species. Capelin DNA was found in 30 samples, Atlantic cod in 16, Arctic cod in 12, and sand lance in 13 samples. No amplicons

of the expected sizes were observed in the negative control (PCR reagents with no template DNA) for any of the four prey-specific primer pairs; the species-specific controls showed only the expected PCR product.

Stomach samples containing robust otoliths seemed to be less likely to show DNA detection by multiplex PCR, when compared with samples containing small fragile otoliths. Atlantic cod otoliths are the most robust and capelin otoliths the smallest and most fragile (Table 4.5). Based on HPA, Atlantic cod was present in 37 seals (36 harp seals; one gray seal). Of these, Atlantic cod DNA amplification was observed in 14 samples (PCR detection rate of 37.8%). Two other samples showed positive PCR amplification for Atlantic cod; however, hard parts were absent. Arctic cod hard parts were present in 17 harp seal samples and successful PCR amplification results were obtained for ten of these (58.8% PCR detection rate based on hard parts). A further two positive PCR results were obtained in the absence of hard parts. Capelin otoliths were recovered from 33 samples (32 harp seals; one gray seal), of which 22 showed successful amplification (66.7% PCR detection rate). DNA amplification of capelin occurred in eight samples from which hard parts were not recovered. Sand lance hard parts were found in 19 samples (12 gray seals; seven harp seals) of which 11 showed successful PCR amplification (57.9% PCR detection rate) and a further two samples had positive PCR results in the absence of hard parts.

4.4.5 *Digestive State*

Trends suggest that the digestive state of stomach contents affected the likelihood of getting positive species-specific PCR results, though results were not statistically

significant (Table 4.6). The likelihood of PCR detection was highest for least-digested prey items, especially for Arctic cod, capelin, and sand lance. For example, capelin, sand lance, and Arctic cod combined were detected 11 out of 11 times (100% CI=72%-100%) by PCR for lower classes of digestion (All <100% class 5; Table 4.6).

4.4.6 *Standard Curve Quantification of Template DNA*

Serial dilutions were performed to construct standard curves for quantifying starting concentrations of prey DNA, and to investigate if amplification of prey species at lower concentrations of template DNA was occurring. All five DNA concentrations were detectable using multiplex PCR followed by fluorescence detection for all four species (Appendix 4C). Sand lance showed a clear linear decrease of fluorescence with decrease in concentration (Figure 4.1). I therefore used sand lance as a model to determine if height or area can be used to calculate the starting concentration for sand lance DNA from seal stomach samples. This was done using the equations shown in Figure 4.1. Seven seals showed DNA concentrations of ≤ 5 ng using both peak height and peak area equations. Peak area gave a higher starting concentration than peak height in all cases. The largest difference was observed in sample G136 (peak area =86.6 ng and peak height =77.8 ng). In both cases, G136 had the highest starting concentration of sand lance (Table 4.7). It became obvious there is too much variability in between PCR duplicates and for this reason, precision may be too low to make this technique useful.

Standard curves were also made for Atlantic cod, Arctic cod, and capelin. All five concentrations were detectable, but these curves were not used for interpolating starting template concentration given the precision issue discussed above (Appendix 4C-1 to 4C-

3).

4.5 Discussion

The results demonstrate that multiplex PCR analysis can be used to identify multiple prey items simultaneously, offering numerous benefits over single species-specific PCRs or agarose gel electrophoresis. Polymerase chain reaction was successful in identifying prey from fecal and stomach samples of pinnipeds, but current methods only test for one prey species at a time (e.g. Deagle *et al*. 2005; Jarman *et al*. 2004; Marshall *et al.* 2010). Given that gray seals and harp seals are generalist predators, testing one prey at a time is both costly and time consuming. The approach described here uses a four species multiplex PCR, which allows for the identification of multiple prey items in one reaction. This greatly cuts down on the number of runs performed when conducting this type of diet analysis. The use of fluorescent markers also helps to improve sensitivity over single species-specific PCR given that fluorometry works on a finer scale than gel electrophoresis (Harper *et al.* 2005).

4.5.1 *HPA Versus Non-quantitative Multiplex PCR*

When one or all four prey types were present in the DNA sample, each tested primer pair amplified species-specific DNA in seal stomach samples using multiplex reaction (Table 4.4). There was a difference however in how well each primer pair worked (see below). Stomachs containing Atlantic cod, Arctic cod, capelin, or sand lance hard parts did not always show DNA amplification. My findings suggest that this resulted from the extent of digestion of prey remains (e.g. Deagle and Tollit 2007; Tollit *et al.* 1997, 2003): DNA amplification was related to the state of digestion of prey remains.

Small prey (i.e. Arctic cod, capelin, and sand lance) that were highly digested showed a lower detection rate than those that were digested little (Table 4.6).

Atlantic cod was detected less than other prey types, possibly due to retention of its large hard parts in the stomach after the DNA had degraded. When assigning stomach digestive states, it is not always possible to differentiate digestive state for every prey species in the stomach, therefore, it is also possible that the tissue in the stomach that has not been digested may not be Atlantic cod tissue. Alternatively, the primer parameters I used may not have been optimal for this species. Primers have different melting points and annealing points depending on length, base composition (A, T, G and C), concentration, and ionic reaction environment (Qiagen 2011). If the melting point is too high, the product will have low primer-template binding, resulting in a low product yield. If the temperature is too low, non-specific binding is more likely (PRIMER Biosoft, 2013). This makes it difficult to find optimal cycle conditions for primer pairs of four different species. Another common problem with multiplex PCR that could explain my findings is that some primers bind with greater affinity and therefore can utilize the reagents in the PCR reaction, leaving low amounts for primers that react more slowly; however, in cases where only Atlantic cod was present this would not be the case (Qiagen 2011). Finally, the amplicons targeted for each prey species in this study were of slightly different size (125 bp to 175 bp) to avoid the phenomenon of "pull up", which occurs when there is a very strong signal from amplification, which causes multiple colors from the fluorescent labels to be expressed at that amplicon size (i.e. primers cannot be scored reliably when several of the same size are present; Harper *et al.* 2005). These size differences may also account for the differences seen in the results across species.

Differences in prey size and otolith morphology affect occurrence of hard parts. When compared to many other prey species, Atlantic cod have large robust otoliths and can reach larger sizes, so they should be detectable in stomach contents. Paradoxically, I recorded positive PCR results in the absence of hard parts for two stomach samples. One of the samples contained otoliths of rock cod (*Gadus ogac*). Rock cod and Atlantic cod are closely related, so the primer pair for Atlantic cod may not be specific enough to differentiate between the two species. "Belly biting" or regurgitation are other possible explanations for my finding (Gudmundson et *al*. 2006; Fu *et al.* 2001). Unfortunately, for samples that resulted in positive PCR in the absence of hard parts, no corresponding intestinal samples were analyzed. Future research should include intestines to assess the passage of hard parts.

Arctic cod, capelin, and sand lance are smaller fish species on average than Atlantic cod. Arctic cod have a medium sized robust otoliths, capelin are small fish with small fragile otoliths (Scott and Scott 1988; Harvey *et al*. 2000) and sand lance are a small fish with small robust otoliths (Lilly and Simpson 2000; Scott and Scott 1988). There were two instances where Arctic cod PCR was positive in the absence of hard parts, eight instances where capelin PCR was positive in the absence of hard parts and two cases where sand lance PCR was positive in the absence of hard parts. The schooling behavior of these fish and their smaller size may result in either brief passage time or quick deterioration of otoliths. Sinclair *et al.* (2011) demonstrated that the smaller otoliths present in large volumes in the digestive tract (e.g. schooling prey) were of high quality, indicating brief passage times. When few prey items were consumed however, they passed slowly through the digestive tract, often resulting in otolith breakdown.

Unfortunately, the specimens for which there was positive PCR detection of Arctic cod, capelin and sand lance in the absence of hard parts lacked corresponding intestinal samples. Therefore, it is difficult to conclude whether fast passage time or otolith degradation led to these results. The small size and fragile nature of these otoliths also may result in otoliths being lost or missed during HPA (Marshall *et al.* 2010).

There was no indication that primers for Arctic cod, capelin, or sand lance were identifying other prey species. For example, no other prey species were found consistently in the stomachs with positive PCR in the absence of hard parts. Also, no hard parts from closely related prey species were found in the absence of the targeted prey hard parts. Of the eight stomachs containing capelin DNA and lacking hard parts, three contained no visible hard parts. Therefore, it seems small fragments of DNA remained in the stomach longer than some of the smaller otoliths, which are passed quickly to the rest of the digestive tract.

4.5.2 *Standard Curve Quantification of Template DNA*

All four prey types were successfully amplified at five dilution concentrations, showing that prey can be identified at both high and low concentrations of DNA (Figure 4.1, Appendix 4C). All prey, except for capelin, showed a decrease in relative fluorescence units as DNA concentration decreased. The strong expression of capelin at low concentrations suggests that this primer pair worked effectively within my reaction conditions. Due to the high level of florescence observed with capelin at low concentrations makes it virtually impossible to determine starting template concentrations from seal stomach samples.

The fluorescent peaks of Atlantic cod, Arctic cod and sand lance showed decreases in height and area as concentrations decreased (Figure 4.1, Appendix 4C). The height and area fluorescent peaks of sand lance were used to calculate starting template DNA. Positive multiplex results varied across replicates, therefore the precision may be too low to make this technique useful. Numerous factors can affect peak height, peak area, and DNA concentration, such as pipetting errors and the quality of the DNA sample. In this study, I found that peak height and peak area showed some relation to initial DNA concentrations and template quality. However, in all cases, peak area had calculated higher starting template concentration for sand lance (Table 4.7).

4.5.3 *The Effect of Increasing the Positive Threshold for Multiplex PCR Results*

Scoring products with florescent units below 100 florescent units was difficult due to background signals in the electropherograms. Background is signal that is not caused by DNA. It is caused by several factors such as instrument noise, inconsistencies in the CE buffer composition, air bubbles and other contaminants (Kok *et al*.1998). I considered any result having a florescent unit > 100 to be positive, following Harper *et al.* (2005). Increasing the threshold to 150 had no effect on the incidence of positive Arctic cod PCR results, but Atlantic cod, capelin, and sand lance all had fewer positive results. Increasing the threshold for Atlantic cod, capelin and sand lance resulted in a reduced number of positive PCR results, however, the samples that were removed had hard parts present via HPA. Increasing the threshold to 200 and 500 resulted in a further loss of positives PCR results in samples that had hard parts present. The 100 fluorescent units' threshold was the best threshold of those tested (Figure 4.2).

4.6 Conclusions

Several important findings emerged from this study. First, optimization of the multiplex cycle conditions for all four fish species appears to remain the biggest problem I encountered. Secondly, highly digested materials were less likely to show positive DNA results, which need to be considered in further studies. Finally, for all species-specific primer sets, peak height and area varied between runs for the same stomach. The precision of this technique may be too low for reliable standard curve quantification of template DNA.

Despite the observations listed above, using multiplex PCR along with HPA may provide insight into passage rates of hard parts and DNA degradation. Since this multiplex PCR technique allows for the screening of multiple prey items simultaneously, it reduces cost and time required to perform this type of study and it also offers increased sensitivity compared with traditional PCR methods (Harper *et al*. 2005). Molecular identification of multiple prey items simultaneously is possible and a useful tool when used in conjunction with HPA of stomach and intestine contents. This is especially true for prey species of which HPA is lacking i.e. invertebrate prey or prey that lack identifiable hard parts.

Table 4.1: Coding scheme for quantifying extent of digestion of prey in the stomachs of gray (*Halichoerus grypus*) and harp (*Pagophilus groenlandicus*) seals from Newfoundland. Degree of digestion ranged from class 1 (fully intact prey items with no signs of digestion) to class 6 (complete digestion, only hard parts are present).

Digestion Class	Description
1	No signs of digestion; fish fully intact
2	Some digestion; skin coming off, tail digested
3	Clumps of tissue still attached to bones
4	Floating tissue and bones present
5	Small clumps of soft tissue but mostly bone present
6	No signs of soft tissue; only bones or otoliths present

Table 4.2: Product length and sequences of species-specific primer pairs, with fluorescent labels, used in the detection of Atlantic cod (*Gadus morhua*), Arctic cod (*Boreogadus saida*), capelin (*Mallotus villosus*), and sand lance (*Ammodytes* spp.) in stomach contents of (*Halichoerus grypus*) and harp (*Pagophilus groenlandicus*) seals from Newfoundland. (Primers designed by Greg Dale)

		Target Amplicon		
Primer	Sequence	Length(bp)	Gene	Species
Gmo6F2	CTCTACATCTTTAGGGTTCGTC-VIC	135	ND5	Atlantic cod
Gmo6R2	GCAATAGCTTTGGGACCAG			
Mvi3F	ACCTTGCGGGTATCTCCTCT-6-FAM	175	COI	Capelin
Mvi2R	AAGCATTGTAATTCCAGCGG			
Aam1F	GCATAACGAGGGCTTAGCTG-PET	166	16S	Sand lance
Aam1R	CAGGTACCATTTGGTTTGGG			
Bsa3F	TACCCCGAACATGTTGGTTCG-NED	125	ND1&2	Arctic cod
Bsa3R	AGGCTAATAGCCAGTGGGAAC			

Table 4.3: Range of base pair (bp) size used to determine positive results for the four species specific primer pairs, based on positive control results.

	Atlantic cod *(Gadus morhua)*	Arctic cod (*Boreogadus saida)*	Capelin (*Mallotus villosus)*	Sand lance (*Ammodytes* spp.)
Expected size (bp)	135	125	175	166
Positive min	138.5	129.5	182.5	173
Positive max	140	131.0	184	174.5

Table 4.4: In all three tests, with different starting fish DNA concentrations, Atlantic cod (*Gadus morhua*), Arctic cod (*Boreogadus saida*), capelin (*Mallotus villosus*), and sand lance (*Ammodytes* spp.) were amplified, within the same reaction. The peak fluorescence height, area and length in base pairs (bp) were recorded.

Three Prey DNA Mixture	Atlantic cod			Arctic cod			Capelin			Sand lance		
	Peak Height	Peak Area	Length (bp)	Peak Height	Peak Area	Length (bp)	Peak Height	Peak Area	Length (bp)	Peak Height	Peak Area	Length (bp)
1[a]	7498	4295	139.6	30658	15999	130.3	32130	24900	1835	448	260	174.0
2[b]	11500	6684	139.7	27317	14145	130.4	27938	13522	1837	417	216	174.1
3[c]	20558	11843	139.7	32206	20266	130.3	31902	24728	1835	603	305	174.0

[a]Mixed concentrations: Capelin, 200.9 ng; Arctic cod, 91.0 ng; sand lance, 130.9 ng; Atlantic cod, 61.5 ng.

[b]All samples had 100 ng.

[c]All samples contained 50 ng.

Table 4.5: Comparison of HPA and species-specific PCR results for each seal stomach sample. The percent PCR detection of prey based on hard part results show that Atlantic cod *(Gadus morhus)* had the lowest detection rate, followed by sand lance (*Ammodytes* spp.) and Arctic cod (*Boreogadus saida*). Capelin (*Mallotus villosus*) had the highest detection rate. Capelin also had the greatest number of positive PCR amplifications in the absence of hard parts.)

			HPA detection		
	Prey		YES	NO	% PCR detection vs. HPA*
DNA detection	Atlantic cod	YES	14	2	37.8
		NO	23		
	Arctic cod	YES	10	2	58.8
		NO	7		
	Capelin	YES	22	8	66.7
		NO	11		
	Sand lance	YES	11	2	57.9
		NO	8		

*Stomach samples showed positive amplification in the absence of hard parts. % PCR detection does not include those PCR samples.

Table 4.6: Detectability of Atlantic cod (*Gadus morhua*), Arctic cod (*Boreogadus saida*), capelin (*Mallotus villosus*), and sand lance (*Ammodytes* spp.) via PCR analysis. Detectability of prey species decreased as digestion progressed. For example, for the least digested samples (all samples <100% class 5 combined; see Table 4.1), PCR detected small species in all 11 samples (i.e. 100%, vs. 53% for more digested samples), and Atlantic cod in three of seven samples (43% vs. 31% for more digested samples).

	Atlantic cod		Arctic cod		Capelin		Sand lance	
Stomach digestion	+/- [1]	% Positive	+/- [1]	% Positive	+/- [1]	% Positive	+/- [1]	% Positive
100% class 6	4/12	25.0	5/4	55.6	9/8	52.9	2/6	25.0
100% class 5 to 99% class 6	4/8	33.3	3/3	50.0	7/2	77. 8	1/1	50.0
All <100% class 5	3/4	42.9	2/0	100	4/0	100	5/0	100
Digestive class unknown	2/0	100		NA	2/1	66.7	2/1	67.0

[1] Count of positive PCR results over PCR negative results

Table 4.7: Starting concentrations of sand lance (*Ammodytes* spp.), obtained from the average peak height and average peak area, for 13 seal gray seals (*Halichoerus grypus*) samples that showed positive results for sand lance. Starting concentrations were calculated using the sand lance peak height and peak area regression equations (Peak Height $y = 164x + 860$ $R^2 = 0.99$ and Peak Area ($y = 86.5x + 441$ $R^2 = 0.99$). Both average peak height and area yielded similar concentrations.

Seal ID	Average sand lance peak area with SE	Calculated starting concentration (ng)	Average Sand lance peak height ± SE	Calculated starting concentration (ng)
G100	64.3 ± 4.40 (n=2)	<5	109 ± 1.00 (n=2)	<5
G107	366 ± 180 (n=2)	<5	662 ± 344 (n=2)	<5
G123	885 ± 331 (n=2)	5.13	1655 ± 574 (n=2)	<5
G131	348 ± 73.8 (n=3)	<5	625 ± 126 (n=3)	<5
G132	3775 ± 88.4 (n=3)	38.5	6939 ± 283 (n=3)	37.0
G133	2562 ± 612 (n=3)	24.5	4643 ± 1200 (n=3)	23.0
G135	2583 ± 572 (n=3)	24.8	4633 ± 1017 (n=3)	23.0
G136	7929 ± 920 (n=3)	86.6	13628 ± 622 (n=3)	77.8
G137	2954 ± 261 (n=3)	29.1	5301 ± 387 (n=3)	2
148**	661 ± 251 (n=3)	<5	1083 ± 819 (n=3)	<5
162**	535 ± 348 (n=2)	<5	1002 ± 666 (n=2)	<5
163**	494 ± 325 (n=2)	<5	839 ± 532 (n=2)	<5
164**	5017 ± 3182 (n=2)	52.9	9034 ± 5845 (n=2)	49.8

** Stomach DNA extractions performed by G. Dale

n = sample size

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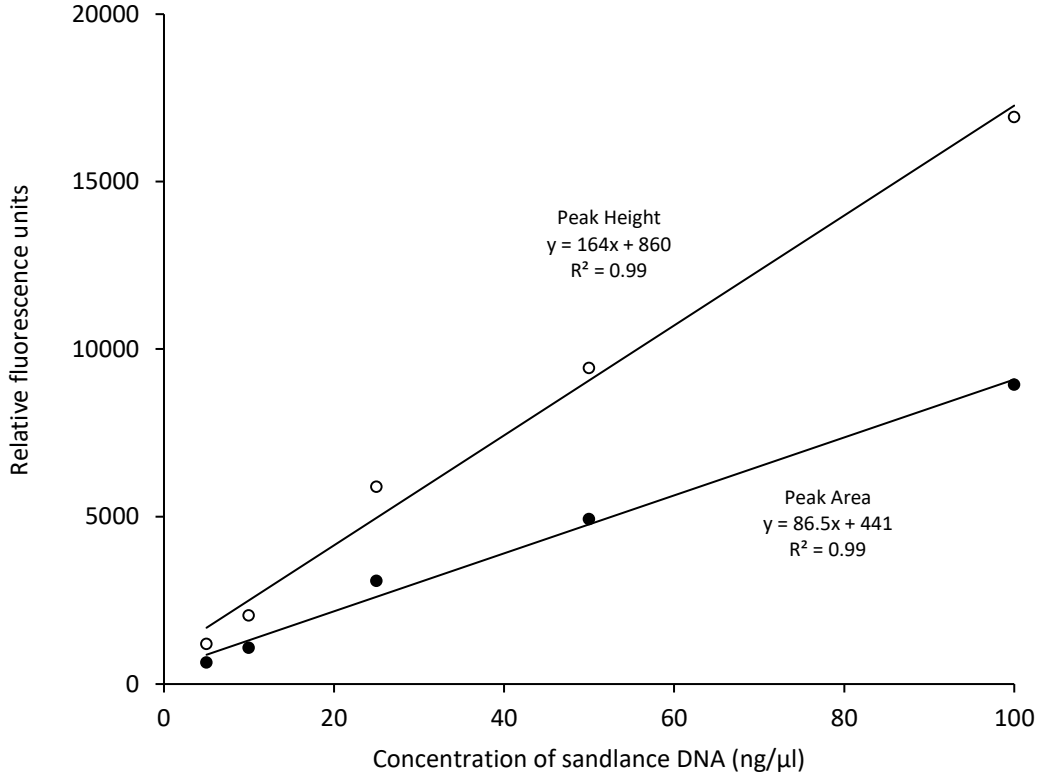


Figure 4-1: Serial dilution of sand lance (*Ammodytes* spp.) DNA showed that as DNA concentration increased, the maximal peak height and peak area, based on fluorescence, also increased. Dilution concentrations were 5 ng, 10 ng, 25 ng, 50 ng, and 100 ng.

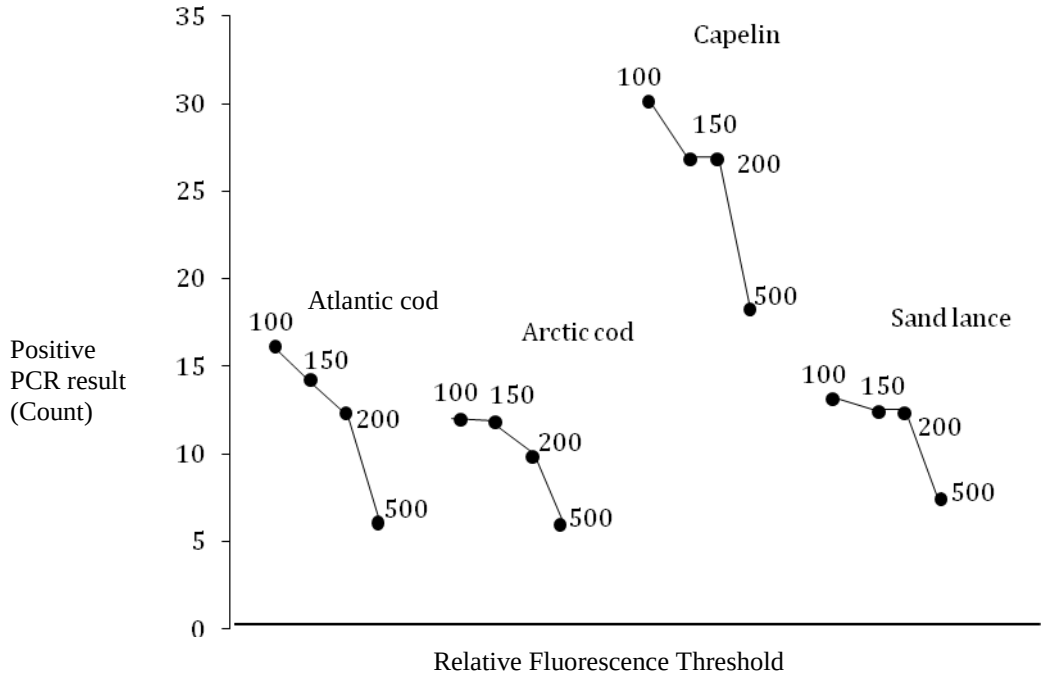


Figure 4-2: The threshold of acceptable PCR positives did not lead to an increase in PCR efficiency. There was a consistent decrease in the number of positive PCR results obtained for all four prey species, Atlantic cod (*Gadus morhua*), Arctic cod (*Boreogadus saida*), capelin (*Mallotus villosus*), and sand lance (*Ammodytes* spp.), as the set threshold increased. Thresholds were set at 100, 150, 200 and 500 relative fluorescence units.

Appendix 4-A: Comparison of results from HPA and species–specific multiplex PCR for gray (*Halichoerus grypus*) and harp (*Pagophilus groenlandicus*) seal samples.

	Atlantic cod (*Gadus morhua*)		Arctic cod (*Boreogadus saida*)		Capelin (*Mallotus villosus*)		Sand lance (*Ammodytes* spp.)	
Sample ID	HP	PCR	HP	PCR	HP	PCR	HP	PCR
2	-	-	-	-	+	+	-	-
9	-	-	-	-	+	+	-	-
10	-	-	-	-	+	-	-	-
12	-	-	-	-	+	-	-	-
13	+	+	-	-	-	-	-	-
14	+	-	-	-	+	-	-	-
15	-	-	+	-	+	+	-	-
16	+	-	+	+	+	-	-	-
17	-	-	-	-	-	-	+	-
18	+	+	+	-	-	-	-	-
19	+	-	+	-	-	-	-	-
21	+	+	-	-	-	-	-	-
23	+	-	-	-	-	-	+	-
24	+	-	-	-	-	-	-	-
25	+	-	-	-	-	-	-	-
27	+	-	-	-	-	-	-	-
29	-	-	-	-	+	+	-	-
31	+	+	-	-	-	-	-	-
33	+	-	-	-	+	+	-	-
34	+	-	-	-	+	+	-	-
35	+	-	-	-	-	-	-	-
36	+	+	+	-	-	-	-	-
37	-	-	+	+	+	+	-	-
39	+	+	-	-	-	-	-	-
40	+	-	+	-	+	-	-	-
48	-	-	-	-	+	-	-	-
52	+	-	-	-	-	-	-	-
57	-	-	-	-	+	+	-	-
58	-	-	-	-	-	-	+	-

*Stomach samples showed positive amplification in the absence of hard parts.
** Stomach DNA extractions performed by G Dale

Appendix 4A continued

	Atlantic cod		Arctic cod		Capelin		Sand lance	
Sample ID	HP	PCR	HP	PCR	HP	PCR	HP	PCR
61	+	+	-	-	-	-	-	-
62	+	-	-	-	+	+	-	-
65	+	-	-	-	-	-	-	-
67	+	+	-	-	-	-	-	-
73	+	-	+	+	+	-	+	-
74	-	-	-	-	+	-	-	-
75	+	+	-	-	-	-	-	-
76	+	-	-	-	+	+	-	-
77	+	+	-	-	+	-	-	-
78	-	-	+	+	-	-	-	-
81	+	+	-	-	-	-	-	-
82	-	-	-	-	+	+	-	-
83	+	-	-	-	-	-	-	-
85	+	-	-	-	-	-	-	-
86	-	-	-	-	+	+	-	-
G 90	-	-	-	-	+	+	-	-
91	+	-	+	-	-	-	-	-
G 99	-	-	-	-	-	-	-	-
G 100	-	-	-	-	-	-	+	+
G 107	-	-	-	-	-	-	+	+
108	-	-	+	+	+	+	-	-
G111	-	-	-	-	-	-	+	-
119	-	-	+	-	-	-	-	-
G123	-	-	-	-	-	-	+	+
G128	-	-	-	-	-	-	+	-
G131	-	-	-	-	-	-	+	+
G132	-	-	-	-	-	-	+	+
G133	+	+	-	-	-	-	+	+
G134	-	-	-	-	-	-	+	-
G135	-	-	-	-	-	-	+	+
G136	-	-	-	-	-	-	+	+
G137	-	-	-	-	-	-	+	+
142	-	-	-	-	-	+*	-	-
145**	-	-	-	+*	+	+	+	-
146**	-	-	+	+	+	+	-	-

*Stomach samples showed positive amplification in the absence of hard-parts.
** Stomach DNA extractions performed by Greg Dale

Appendix 4-A continued

	Atlantic cod		Arctic cod		Capelin		Sand lance	
Sample ID	HP	PCR	HP	PCR	HP	PCR	HP	PCR
147**	-	-	-	-	-	+*	-	-
148**	-	-	-	-	+	-	+	+
149**	+	-	-	-	+	+	-	-
151**	+	+	-	-	-	+*	-	-
154**	rock cod	+*	-	+*	-	+*	-	-
155**	-	-	-	-	-	+*	-	-
156**	+	-	+	+	+	+	-	-
157**	+	-	+	+	+	+	-	-
158**	+	+	+	+	-	+*	-	-
159**	-	-	+	+	+	+	-	-
161**	-	-	-	-	-	+*	-	-
162**	-	-	-	-	+	+	-	+*
163**	-	-	-	-	-	+*	-	+*
164**	+	-	-	-	+	+	+	+
166**	-	+*	-	-	+	-	-	-
Total	37	16	17	12	33	30	19	13

*Stomach samples showed positive amplification in the absence of hard-parts.

** Stomach DNA extractions performed by G Dale

Appendix 4-B: Sample concentration and purity obtained from absorbance of all molecules in the sample that absorb at the wavelength of 260 nm using nanodrop 1000 spectrophotometer (Thermo Fisher Scientific) of DNA extracted from prey remains in the stomachs of gray (*Halichoerus grypus*) and harp (*Pagophilus groenlandicus*) seals. 260/280 ratio assesses the purity of DNA (ratio of ~1.8 is generally accepted as "pure"). The 260/230 ratio tells nucleic acid purity (Expected values are 2.0-2.2).

Sample ID	Concentration (ng/ul)	260/280	260/230
G100	4.25	1.13	0.21
G105	6.16	1.04	1.14
G106	11.9	0.98	0.71
G107	11.0	1.42	1.05
G109	20.5	2.01	1.37
G110	36.2	2.01	1.82
G111	15.3	2.04	2.87
G113	2.50	2.46	-2.28
G121	2.78	1.55	0.73
G123	14.1	2.2	1.35
G124	-1.26	6.77	0.37
G125	12.1	2.05	1.64
G87	1.25	1.64	0.23
G88	1.69	1.41	1.84
G89	5.31	1.64	-271
G90	13.0	0.82	1.25
G96	4.99	1.41	-991
G99	13.3	1.19	0.54
H1	9.79	1.50	1.37
H10	11.4	1.14	0.69
H101	2.48	2.61	0.37
H102	0.05	-0.25	-0.01
H103	30.9	0.72	0.87
H104	48.2	0.85	0.91
H108	18.1	1.90	0.91
H11	1.23	1.39	0.28
H112	73.2	0.81	0.99
H114	18.1	1.25	1.05
H115	65.0	0.83	0.96
H116	11.2	1.6	0.59
H117	12.2	1.49	0.34
H118	9.68	1.77	0.77
H119	9.79	2.88	0.90

Appendix 4B continued

Sample ID	ng/ul	260/280	260/230
H12	3.26	1.23	0.32
H120	20.1	1.61	0.95
H122	17.3	1.04	0.85
H13	35.0	0.93	0.67
H14	14.5	0.71	0.86
H15	26.1	0.95	0.41
H16	20.6	0.74	0.82
H17	5.64	0.66	0.46
H18	3.59	0.66	0.54
H19	13.2	0.84	0.71
H2	40.1	0.76	0.92
H20	-0.92	1.42	-0.16
H21	6.16	0.81	1.06
H22	60.9	1.88	1.97
H23	9.01	0.84	0.66
H24	9.03	0.73	0.99
H25	37.0	0.89	0.66
H26	15.8	0.78	1.03
H27	18.7	0.81	0.75
H28	-0.24	0.16	-0.12
H29	14.0	0.65	1.07
H3	2.48	4.67	-27.0
H30	0.90	0.76	0.33
H31	4.38	1.16	0.48
H32	0.14	0.19	1.10
H33	25.9	0.76	0.92
H34	18.7	0.70	0.98
H35	7.58	0.62	1.08
H36	7.15	1.64	0.63
H37	36.9	0.84	0.93
H38	0.41	0.65	0.11
H39	15.1	0.82	0.74
H4	20.2	1.86	1.04
H40	3.11	0.65	0.40
H41	76.3	2.00	1.89
H42	81.8	2.15	2.21
H43	11.5	2.27	1.98

Appendix 4B Continued

Sample ID	ng/ul	260/280	260/230
H44	44.2	0.83	0.74
H45	1.48	2.54	1.52
H46	-0.77	0.42	0.72
H47	1.57	1.99	0.2
H48	3.21	1.33	0.65
H49	17.5	1.85	1.25
H5	23.4	1.66	1.12
H50	80.2	1.86	1.23
H51	19.3	2.00	1.14
H52	17.1	1.09	0.50
H53	28.0	1.05	0.85
H54	95.4	2.05	1.75
H55	5.60	2.21	0.56
H56	27.1	1.12	0.90
H57	36.2	0.91	0.74
H58	4.18	1.83	1.34
H59	46.8	0.88	1.08
H6	-2.22	1.17	0.93
H60	7.14	2.23	0.48
H61	47.7	0.87	0.86
H62	43.3	0.89	0.98
H63	14.5	1.24	0.57
H64	7.85	2.06	0.66
H65	11.7	1.25	0.67
H66	-0.08	0.04	-1.12
H67	6.19	0.78	0.88
H68	1.38	11.0	0.20
H69	-1.65	0.64	0.40
H7	-1.24	1.10	-2.45
H70	0.53	-0.30	0.07
H71	-1.10	0.48	0.21
H72	-1.07	0.59	0.32
H73	43.2	0.89	1.00
H74	2.5	17.22	1.21
H75	3.18	0.85	7.48
H76	17.9	0.77	1.07
H77	15.7	0.99	0.72

Appendix 4B continued

Sample ID	ng/ul	260/280	260/230
H78	35.0	0.83	0.8
H79	23.5	0.79	0.93
H8	-1.91	2.00	-4.7
H80	0.84	0.84	0.24
H81	6.60	0.85	0.63
H82	43.0	0.80	0.98
H83	13.3	0.92	0.71
H84	18.9	0.82	0.89
H85	5.57	1.01	4.28
H86	5.18	0.83	0.57
H89	6.34	1.11	0.55
H9	10.9	1.10	0.58
H91	3.59	0.80	0.66
H92	7.71	0.81	1.11
H93	102	0.79	1.02
H94	79.5	0.79	1.07
H95	35.9	0.76	1.06
H97	61.3	0.84	0.96
G126	2.83	1.78	0.75
G127	141	2.08	2.10
G128	181	2.03	2.25
G129	2.36	0.94	0.48
G130	2.50	0.74	0.13
G131	16.7	1.23	0.41
G132	24.4	1.89	1.71
G133	9.76	1.35	1.35
G134	5.79	1.48	1.25
G135	32.3	1.40	0.64
G136	38.8	1.80	1.21
G137	17.0	1.71	1.09
G138	8.89	1.66	1.40
G139	1.22	3.72	0.36
G140	116	1.81	1.83
G141	60.4	1.42	0.71
H142	7.69	1.34	0.56
143**	79.5	2.10	2.08
144**	43.1	1.95	1.43

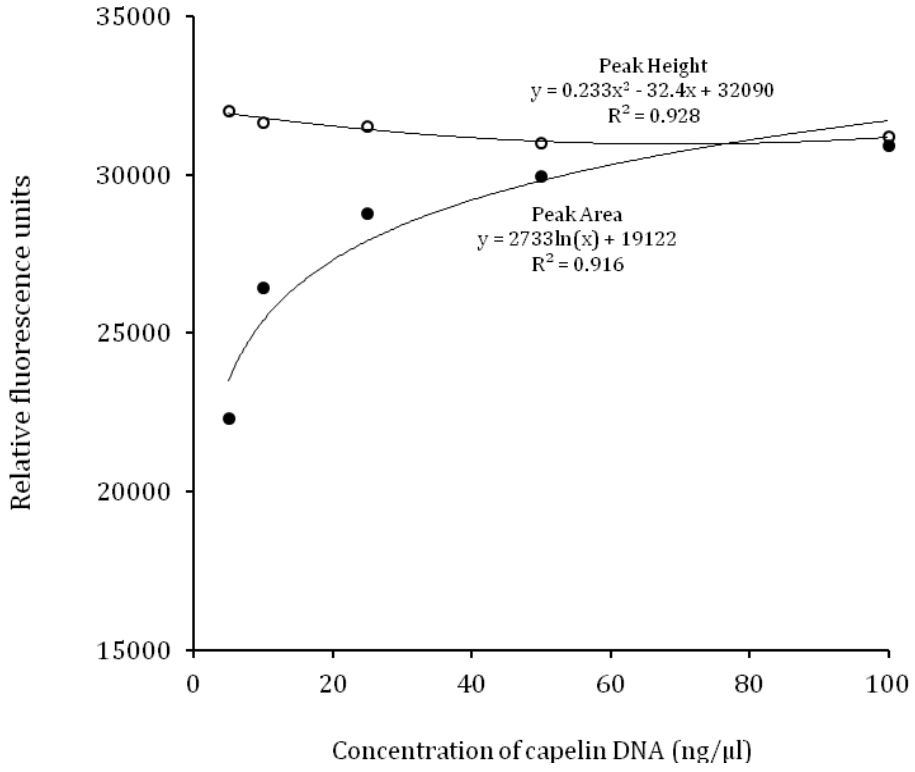
** Stomach DNA extractions performed by G. Dale

Appendix 4B continued

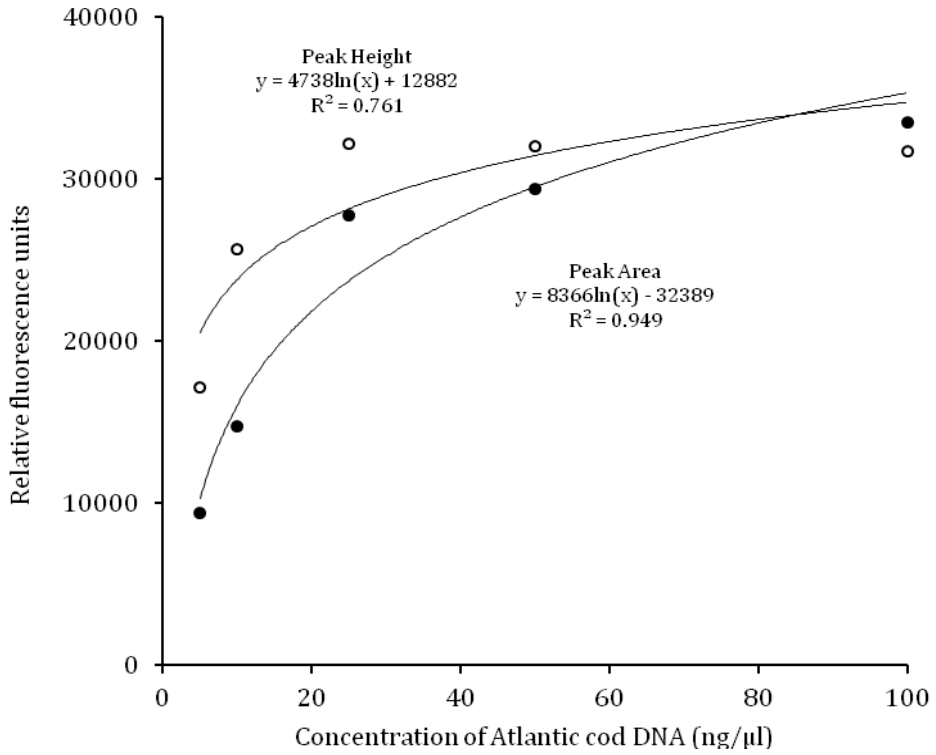
Sample ID	ng/ul	260/280	260/230
145**	54.8	0.87	1.08
146**	18.6	1.27	1.35
147**	35.6	1.15	1.03
148**	24.3	2.00	1.77
149**	29.1	0.84	1.02
150**	28.8	0.90	1.12
151**	23.3	1.26	1.16
152**	327	2.07	2.18
153**	177	1.95	2.06
154**	44.6	1.16	0.33
155**	25.7	1.99	1.37
156**	56.6	0.90	0.85
157**	49.8	0.86	1.04
158**	44.2	1.66	1.51
159**	13.5	1.47	0.47
160**	34.0	1.46	0.83
161**	46.0	2.07	2.09
162**	13.2	1.59	1.02
163**	34.7	0.99	0.59
164**	56.3	0.86	1.05
165**	8.16	1.54	1.55
166**	69.7	1.76	1.29

** Stomach DNA extractions performed by G Dale

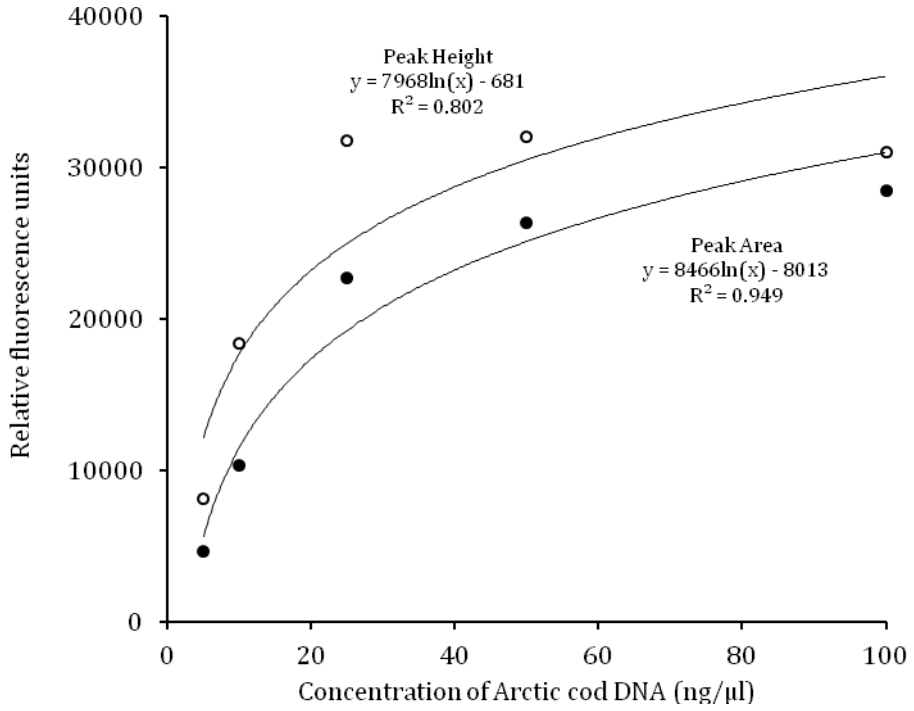
Appendix 4-C: Serial dilution curves for capelin (Mallotus villosus) (A), Atlantic cod (Gadus morhua) (B) and Arctic cod (Boreogadus saida) (C). Dilution concentrations were 5 ng, 10 ng, 25 ng, 50 ng and 100 ng.



A: As concentration of capelin DNA decreases, peak height remains relatively consent and peak area fluorescence decreases.



B: As concentration of Atlantic cod DNA decreases, so does peak height and peak area fluorescence.



C: As concentration of Arctic cod decreases, so does peak height and peak area fluorescence.

5 Chapter: Summary

For this project, I used hard-part analysis (HPA) of stomachs, HPA of the intestine, and multiplex polymerase chain reaction (PCR) to investigate seal diets. Because of the increases in populations of some seal species and the collapse of many fish populations in eastern Canadian waters, it is important to have accurate methods for determining diets of pinnipeds to understand the impacts that seals may have on prey populations (Bowen *et al*. 2008; 2009; DFO, 2010a; Tollit *et al*. 2009). The primary objective of this study was to improve our understanding of biases associated with different methods of analyzing diets and then apply this understanding to determine the diet of gray seals (*Halichoerus grypus*) and harp seals (*Pagophilus groenlandicus*) in Newfoundland waters where gray and harp seals reside. The gray seals I sampled were primarily from the southwest coast and harp seal samples were primarily obtained from north coast of Newfoundland.

5.1 Diet of gray and harp seal: summary

I found that both gray and harp seals are generalist predators that occupy different niches in Newfoundland waters. Of the gray seals sampled, 16 prey species were identified in their diet, compared to 52 in harp seals. I found that gray seals are more piscivorous and tend to feed on more demersal fish than do harp seals (Chapter 3).

5.1.1 *Gray seal diet*

Gray seals are a sexually dimorphic seal, with males being substantially larger than females, a fact that has implications for diet (Breed *et al*. 2009; Bowen and Harrison 2006; DFO 2017). In my study, there were six female and 29 male specimens. Previous telemetric research on gray seals found that, in the northwest Atlantic, females tend to feed closer to haul out areas and tend to make shorter foraging trips than males (Breed *et al.* 2009; Harvey *et al.* 2008; 2012). This may explain the large number of males in my sample. Distributional differences between female and male gray seals in Newfoundland waters is important to consider when investigating the effects of gray seals on fish stocks since males and females feed differently regardless of area (Beck *et al.* 2007; Breed *et al*. 2009; Bowen and Harrison 2006; Bowen *et al.* 2008; 2011;Hammill *et al.* 2007; Harvey *et al.* 2008; Stenson *et al.* 2013), therefore, the diet in Newfoundland needs to be examined.

In my study, gray seals fed primarily on sand lance (*Ammodytes* spp.), Atlantic herring (*Clupea harengus*), skate (*Raja* spp.), flatfish (pleuronectids), and gadoids. I also found squid in several stomachs. I identified some of the same prey species as found by Hammill *et al*. (2007) which examined earlier samples from the west coast of Newfoundland, however some prey species were not observed in my study (i.e. Smelt (*Osmerus mordax),* lumpfish (*Cyclopterus lumpus*) and shrimp. The differences between that study and my study may be due to interannual, seasonal and geographic distribution and my smaller sample size.

5.1.2 *Harp seal diet*

Harp seals are smaller than gray seals, and males and females are of similar size (Sergean 1991). Both male and female harp seal migrate to Newfoundland waters in the fall and winter months, after which most adult seals return to the Arctic for the summer (Chabot *et al.* 1996; DFO 2010b; Sergeant 1991).

Harp seals from my study area fed primarily on capelin (*Mallotus villosus*), Arctic cod (*Boreogadus saida*), Atlantic cod (*Gadus morhua*), herring, sculpins (Cottida), and invertebrate prey like shrimp. These findings are similar to previous studies in Newfoundland, which found capelin, Arctic cod, herring, Atlantic cod, redfish, sculpin, and amphipods to be important prey species (e.g., Bowen *et al.* 2008; Hammill and Stenson 2000; Lawson and Stenson 1997; Lawson *et al.* 1995).

5.2 Dietary analysis using stomach contents vs intestinal HPA

When possible, I performed dietary analysis on the stomach and the intestine, which provided differences in reconstructed diet and estimates of prey size. The breakdown of prey occurs differently in stomach and intestines, which contributed to some of the observed differences. The stomach uses both chemical and mechanical means to break down prey. Prey that is kept in the stomach for long periods of time show signs of chemical and mechanical erosion due to the high acidity and peristaltic movements of the stomach (Guyton 1981; Harvey 1987). Prey that passes quickly from the stomach, will not be subjected to much chemical digestion, but will be mechanically broken down which occurs in the intestine (Harvey 1987). In my study, prey taxa identified in the intestines tended

to have robust hard parts (e.g. parts of flatfish, gadoids, and sand lance). In addition, I identified fewer species in the intestine for harp seals, likely due to the reduced taxonomic resolution of prey (e.g., prey remains in the stomach could have been identified as the species Atlantic cod, but in the intestines only to the level of the genus *Gadus*). The time prey spends in different sections of the digestive tract depend on several factors including prey size, the number of prey, and activity level of the seal itself (Gudmundson *et al.* 2006; Harvey 1987; Helm 1984; Marcus *et al.*1998; Sinclair *et al.* 2011; Tollit *et al.* 1997a).

The only prey items that I found in large numbers in stomach and intestines were Atlantic cod, Arctic cod, and capelin (from harp seals), and sand lance (from gray seals). I used these prey species to examine otolith passage rates and otolith retention within the stomach and intestine to improve understanding of biases associated with the dietary analysis.

5.3 Importance of individual seal on diet

I looked at otolith lengths down the digestive tract for four prey species and each species showed different results. Much of the variation in otolith length is due to variations between individual seals in the sample (Chapter 2). Variation in prey sizes among individual seals are due to a variety of factors including seal species, life stage, sex, size, prey availability, or foraging behaviours (Gudmundson *et al*. 2006; Harvey 1989; Marcus *et al.* 1998; Sinclair *et al.* 2011). Otolith location in the digestive tract and erosion state could also explain some variability of prey sizes within the digestive tract for some prey species (Chapter 2). Differences in otolith size and distribution down the digestive tract for four

different prey species supports the notion that otoliths of different sizes and morphologies pass through the digestive tract at different rates and, therefore, have different exposures to chemical erosion (stomach) and mechanical breakdown (stomach and intestine).

5.4 HPA and PCR analysis

I employed two different dietary techniques -- HPA and multiplex PCR analysis -- to investigate otolith identification and passage rates. HPA involves the recovery and identification of prey hard parts (e.g., beaks, bones, sagittal otoliths, scales, carapaces) from stomachs, intestines, or feces (Bowen 2000; Bowen and Harrison 1994; Hammond *et al*. 1994; Fitch and Brownell 1968; Prime and Hammond 1987). PCR is a molecular technique that exponentially increases the number of target DNA sequences by using a thermal cycler to repeat heating and cooling cycles (Saiki *et al.* 1988). I developed a multiplex PCR technique that could identify four prey species simultaneously. If species-specific DNA from any of the target prey species was present, it would show up on the electropherograms as either present or absent.

Both techniques provided evidence that retention of prey with large hard parts was present (e.g., Atlantic cod). They also provided evidence indicating that some smaller prey may pass faster through the digestive tract and may experience high amounts of mechanical breakdown (e.g., capelin, sand lance). Additionally, I found that prey with medium-sized but robust otoliths (e.g., Arctic cod) were not as affected by erosion or mechanical breakdown as were otoliths from other prey species.

5.4.1 *HPA vs. PCR analysis for Atlantic cod prey*

By examining otoliths from the stomach and the intestines, I found evidence that Atlantic cod, a prey species with large, robust otoliths, had a greater range of sizes in the stomach and that the average size was larger in the stomach than in the intestines. Estimates of prey size between non-eroded and slightly eroded otoliths indicated that state of erosion can negatively bias the estimate of prey size. Furthermore, I found that the proportion of non-eroded and slightly eroded otoliths increased past the stomach, suggesting that retention in the stomach is occurring (Chapter 2). Evidence supporting these interpretations was provided by PCR findings, which showed that Atlantic cod had 23 cases out of 37 where hard parts were present, but no DNA was amplified (Chapter 4). The lack of DNA present in the stomach indicates that hard parts are retained in the stomach longer than prey DNA and suggests that Atlantic cod estimates made with HPA analysis are inflated due to increased retention time (Marshall *et al.* 2011). Furthermore, two stomach samples had a positive PCR result for Atlantic cod but contained no hard parts. Possible explanations for this is smaller individual prey size or larger meals which could result in faster passage times of prey, the seal eating only the viscera or the seal regurgitating hard parts (Bowen 2000; Fu *et al*. 2001; Gudmundson *et al*. 2006). Alternatively, one of these seal stomach samples did have rock cod (*Gadus ogac*) identified as a hard part so in this case, it is possible that the otoliths were either identified incorrectly or that the species-specific primer pairs for Atlantic cod were not specific enough to

differentiate between rock and Atlantic cod.

5.4.2 *HPA vs. PCR analysis for Capelin prey*

Capelin is a small prey species with small, fragile otoliths. These passed through the stomach and intestines very quickly and substantial mechanical breakdown and erosion were noted; for example, there was a sharp drop in the number of otoliths found in the colon (Chapter 2).

Capelin is a schooling species, so large numbers can be consumed in a single feeding event, which could result in a few seals with high otolith counts skewing the prey counts and size consumed (Scott and Scott 1988; Sinclear *et al.* 2011). My PCR results provided further evidence of rapid passage and otolith digestion since there were eight cases where capelin DNA was amplified in the absence of hard parts. Capelin also had the highest rate of PCR detection among the species I identified (Chapter 4).

5.4.3 *HPA vs. PCR for Arctic cod prey*

Arctic cod has medium sized robust otoliths, which did not change in size or state of erosion from stomach to colon (Chapter 2). Therefore, of the three prey species analyzed for harp seal, dietary estimates for Arctic cod were influenced the least by erosion due to retention or mechanical breakdown. Detection of Arctic cod by PCR was higher than for Atlantic cod but was slightly less than for capelin. There were also two cases where PCR results were positive in the absence of hard parts, presumably due to hard parts not being consumed, or regurgitation (Bowen

2000; Fu *et al*. 2001; Gudmundson *et al*. 2006). However, given the small size of Arctic cod and its schooling behaviour, rapid passage of prey remains may be possible, resulting in DNA still being present in the stomach even after hard parts have passed.

5.4.4 *HPA vs. PCR for sand lance prey*

Sand lance was the only prey found in my gray seal sample in high numbers for stomach and intestines. Sand lance has small, robust otoliths, which did not change in size from stomach to colon; I interpret this to indicate that retention was not occurring. Estimates of prey size between uneroded and slightly eroded otoliths differed, indicating that combining erosion states could lead to a slight underestimate of prey size. The incidence of slightly eroded otoliths increased between the stomach and the lower small intestine, and then declined in the colon. This suggests that small prey may have a fast passage rate or are prone to mechanical destruction, or that single feeding events skewed the data. Sand lance had similar PCR results as Arctic cod. Given their small size and robust nature, they likely pass quickly through the digestive tract; however, they are more susceptible to mechanical erosion in the digestive tract due to their small size.

5.5 Numerical correction factors

Total counts of individual prey obtained by HPA are often lower than the number consumed due to complete digestion of hard parts. One approach to account for complete loss of otoliths during digestion is numerical correction

factors (NCF). NCFs have been developed using the recovery rates of known prey hard parts in feces of captive seals (Tollit *et al*. 1997a). NCFs have only been developed for scat analysis; therefore, their applicability to stomach data is unknown (DFO 2010b). I did not apply NCF to stomach HPA since I found there were large difference in prey counts down the digestive tract and that in some cases there was a substantial reduction in prey size estimates from the stomach to the colon (e.g., Atlantic cod). Prey obtained from the stomach are affected greatly by the state of digestion of stomach contents and are therefore not equivalent to scat. I applied NCFs to intestinal contents. Since diet calculations are based on proportions, an increase in the proportion of a fragile otolith caused a decrease in the relative importance of prey with large robust otoliths. I found that this procedure reduced the importance of fish with robust otoliths (e.g., Atlantic cod) and increased the importance of prey with small, fragile otoliths (e.g., capelin) (Chapter 3). Given the retention of otoliths in the stomach observed for Atlantic cod and the observed loss of prey counts for prey with more fragile otoliths (e.g., capelin). Applying NCF will help to decrease biases in prey count from scat or intestinal analysis. There were differences in otolith counts and lengths across the digestive tract (Chapter 2), however, so I believe using NCF for the small intestine would not be comparable to scat and therefore NCF should only be applied to the colon if at all.

With more analysis of complete stomachs and intestines, development of some type of correction factor for lost or eroded otoliths from the stomachs may be possible, however, they would have to consider different stages of digestion,

from recent feeding events that contain non-digested prey to stomach samples that contain only bones and otoliths.

5.6 Summary

Otoliths are invaluable in dietary reconstruction for piscivorous marine predators. Using these prey species as a model, we may be able to conclude that otoliths of similar size and morphology will react in similar ways within the digestive tract of seals. I observed different rates of digestion across the digestive tract for four different prey species, therefore, it is important to consider that diet analysis performed solely on the stomach will likely lead to larger prey size estimates and identify more prey, while analyzing only intestine/scat could result in an underestimate of; fish prey with large, robust hard parts; invertebrate prey; fragile prey and reconstructed prey size. Also, scats/intestine often have highly degraded hard parts that makes identification to the species level difficult. The consequences of using different diet analysis techniques must be considered and using multiple approaches may result in a more comprehensive estimate of diet. Further work should be done to develop correction factors for stomach analysis that account for different stages of stomach digestion. In addition, multiplex PCR protocols should be developed for a wider variety of prey species, especially invertebrate prey, to compare with HPA results. More PCR testing would provide a better understanding of some seal foraging behaviors such as ‘belly biting’ of larger prey items and could provide better identification of invertebrate prey that is quickly broken down in the stomach.

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