Faculty of Biosciences, Fisheries and Economics

Department of Arctic and Marine Biology

Energetic consequences and implications for food consumption models when feeding on various sizes of cod in harp seals (*Phoca groenlandica*)

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Ann-Isabel Algera

BIO-3950 Master thesis in Biology

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Cover photo by Ann-Isabel Algera
Photo of a juvenile harp seal (*Phoca groenlandica*)

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This thesis is dedicated to my grandfather, Reidar A. Martinussen. Thank you for always believing in me.

- Ann-Isabel Algera

Abstract

The current population-based food consumption models assume that harp seals are swallowing fish whole. However, behaviours such as belly-biting and rejection of fishes' heads has been observed, possibly biasing diet analysis based on hard parts such as otoliths, and consequently underestimating the total food consumption of fish by harp seals. The purpose of this study was to examine whether individual fish are in fact being consumed in their entirety or if behaviours such as belly-biting and rejection of fish heads - where the seal actively seeks out the most energy rich part of its prey - is occurring under certain circumstances. Two female harp seals, maintained in human care, were fed live cod and saithe ranging from 90 g – 2710 g and 20 cm – 70 cm. The caloric energy content of the discarded piece(s) was quantified, estimating the energy wastage by the seals when they consume different sizes of prey. Fish that were too large to swallow were consequently torn into pieces before ingested. The adult harp seal was unable (or did not choose) to swallow whole those fish weighing above 750 g, whereas the juvenile showed the same trend at 380 g. Fish parts frequently rejected included the head, the head with parts of the back attached, and all but the abdomen of the fish. 81 % of the remains left by the adult contained otoliths, while this number was a staggering 100 % for the juvenile. 1 % to 40 % of the caloric content in the fish was wasted depending on the fish body mass, i.e. a larger body mass yielded a larger caloric wastage. Undigested fish parts were collected on seven occasions in the tanks, suggesting that regurgitation might be frequent when seals are feeding on bony fish species. The findings from this study indicate that harp seals may seek out the most energy-rich parts of a fish, subsequently leaving "lower-energy" remains behind, and that this "waste" of energy increases with the mass of its prey. As such, reassessment of the current estimations of food consumption may be valuable in terms of applying a suitable correction factor to account for this wastage.

Keywords: Harp seal, *Phoca groenlandica*, feeding behaviour, food consumption models, live fish, cod, saithe, belly-biting, selection, head rejection, energetic consequences, caloric waste, energy waste, energy content.

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List of Abbreviations

DCF = Digestion correction factors
DE = Digestive energy
EC = Energy content
EW = Energy wastage
FAA = Fatty acid analysis
FBM = Fish body mass
FE = Fecal energy
FID = Free induction decay
FM = Fasting metabolic rate
GEI = Gross energy intake
HIF = Heat increment of feeding
HPA = Hard part analysis
ISO = International Organization for Standardization
ME = Metabolizable energy
MGA = Molecular genetic analysis
NCF = Numerical correction factors
NE = Net energy
NMR = Nuclear magnetic resonance
PE = Production energy
RF = Radio frequency
SFC = Solid fat content
TEW = Total energy wastage
UE = Urinary energy
WR = Weight of fish remains

List of animals mentioned

Amphipods	*Parathemisto sp.*
	Gammarus sp.
Atlantic cod	*Gadus morhua*
Atlantic herring	*Culpea harengus*
California sea lion	*Zalophus californianus*
Capelin	*Mallotus villosus*
Crustaceans	*Themisto libellula*
	Thysanoessa spp.
Eelpouts	*Zoarces viviparus*
Grey seal	*Halichoerus grypus*
Harbour seal	*Phoca vitulina*
Harp seal	*Phoca groenlandica*
Hooded seal	*Cystophora cristata*
Pacific cod	*Gadus macrocephalus*
Pacific harbour seal	*Phoca vitulina richardii*
Pacific herring	*Culpea pallasii*
Polar cod	*Boreogadus saida*
Sandeels	*Ammodytes sp*.
Weddell seal	*Leptonychotes weddellii*

1. Introduction

1.1 The Harp seal (*Phoca groenlandica*)

The harp seal (*Phoca groenlandica*) is a top predator and the most abundant pinniped inhabiting the pack ice of the North Atlantic Ocean. There are three different stocks with separate management plans located in the Northwest Atlantic, the Greenland Sea, and the White Sea, each with different whelping areas (Sergeant, 1991). Current estimations for each of these populations are 7.400.000 (2013), 627.000 (2013), and 1.360.000 (2015) respectively *(Øigård et al., 2014, Hammill et al*., 2014, ICES, 2014). Whereas the Northwest Atlantic stock is stable (Hammill *et al*., 2014), the Greenland stock is at historically high numbers and continue to show a slow increase. However, it is possible that the population is reaching its carrying capacity due to the stabilization of pup production (Øigård *et al*., 2014). The White Sea stock is decreasing due to a decline in female reproductive success, with a subsequent reduction in pup production (ICES, 2011).

The two latter populations have been commercially exploited by Norwegian and Russian sealers in large numbers for centuries (Iversen, 1928, Sergeant, 1991) but sealing has since been regulated, and the hunting pressure is decreasing rapidly due to market pressures (and absence thereof), and the implementation of EU regulation (EC) No 1007/2009 prohibiting the trade of seal products (EP, 2009). Although the White Sea stock is decreasing, these two populations impose a considerable impact on the ecosystem and the commercial fisheries in the Norwegian waters due to their large numbers and the predation on commercially valuable fish stocks such as capelin (*Mallotus villosus*), herring (*Culpea harengus)* and cod (*Gadus morhua*). Periods of low food availability in the Barents Sea, associated with the collapse of capelin stocks during the mid 1980s, low numbers of herring, and an increasing population of harp seals, resulted in an extensive seal invasion of the coastal waters of North Norway in 1986 - 1988 (Haug *et al*., 1991, Nilssen *et al*., 1998). Such invasions caused massive implications for the Norwegian fishermen – tens of thousands harp seals were drowning in gillnets, gillnets were damaged, fish in nets were preyed upon, and the presence of seals affected fish behaviour and availability

(Nilssen *et al.*, 1992). The harp seal population has since increased further, and in combination with the fluctuating abundance of capelin (ICES, 2015), similar invasions are considered likely to occur in the future.

1.2 Energy flow

To survive and thrive, an animal must acquire sufficient energy for three main purposes: biosynthesis, maintenance, and generation of external work. This energy is gained from chemical energy in food, and assimilated through the chemical bonds of organic compounds therein. The cells in the various tissues then use the absorbed energy for physiological work (Hill, Wyse & Anderson, 2012). The energy flow in an animal describes how energy from food is transported in the body, and can be summarized as:

$$GEI = FE + UE + HIF + FM + PE \qquad \text{Equation 1}$$

Where: GEI is the gross energy intake, i.e the energy obtained from food, FE and UE is fecal and urine energy loss, HIF is the heat increment of feeding, FM is fasting metabolic rate which describes the energy used for biosynthesis and maintenance such as thermoregulation and basal metabolism, and PE is production energy, which represents the energy for generating work and the cost of growth and reproduction (Hammill *et al.*, 2010).

As described, not all consumed energy is immediately available for use. By subtracting the energy that is lost as faecal energy from GEI, digestive energy (DE) is obtained. When including energy lost through the urine, metabolizable energy (ME) is calculated. Additional energy is lost as heat due to the metabolism of food, and the remaining energy, called net energy (NE), is ready to be used by the animal (**Figure 1**) (Lavigne *et al.*, 1982). The efficiency of digestion is impacted by various factors such as prey type, food quality, size and time between meals, season, and the age, nutritional state and morphology of the digestive tract of the animal (Lawson *et al.*, 1997, Rosen and Trites, 2000).

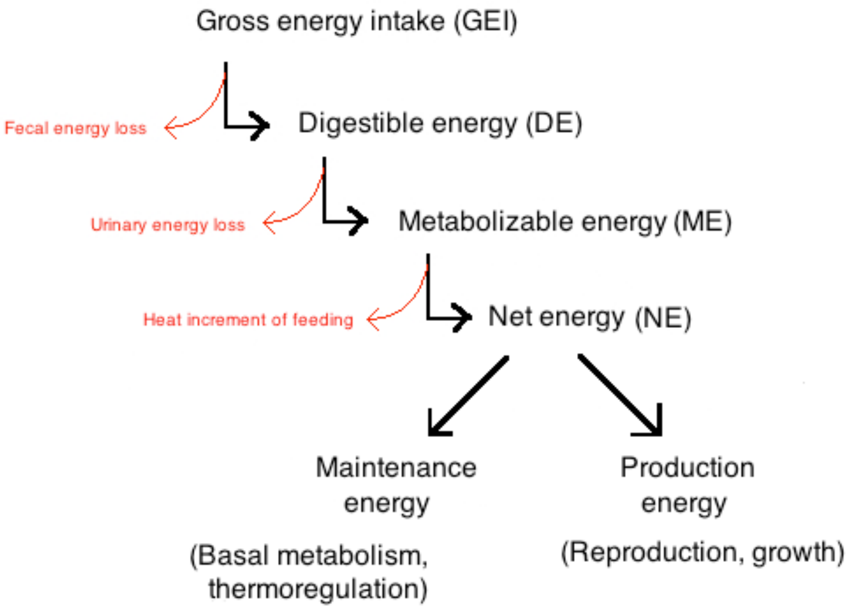

Figure 1. Energy flow in an animal.

The harp seal energy requirement is the foundation for estimating food consumption (Lavigne *et al.*, 1982), representing the amount of energy that each individual requires, i.e. its GEI, which depends on various factors such as age, sex, time of year, growth, locomotion, moult, reproduction etc. (Hammill *et al.*, 2010). For example, juvenile harp seals require a higher amount of energy for growth compared to adults (Nilssen *et al.*, 2000). In order to calculate GEI, these parameters have to be included, and will as such present a complex model.

The GEI based on models estimate an energy requirement of the Northwest Atlantic stock to range from 19.000 kJ/day to 31.300 kJ/day depending on which parameters were used (Hammill *et al.*, 2010), while the energy requirements of harp seals of the Greenland stock has been estimated, based on direct studies, to be a minimum of 25.600 kJ/day (Nordøy *et al.*, 1995). This yields an estimate of the mean daily food intake to be 3.3 % of their body mass (Nordøy *et al.*, 1995). Individual energy requirements of harp seals are then extrapolated to include the whole population. In order to convert the GEI to food intake i.e. the amount of prey of various species that each harp seal requires, digestibility of prey, and the seasonal changes in both diet composition and prey energy density must be known.

1.3 Prey energy density

The prey energy density should be calculated over a period of one year in order to document seasonal changes in protein and lipid content. It is usually measured using a bomb calorimeter, where the foodstuff is placed in a vessel containing pure oxygen, and ignited. The heat generated from the combustion is recorded via an increase in the temperature of the water that surrounds the vessel. This heat is an expression of the foodstuffs calorific value, and can be expressed as kilocalories (kcal) or kilojoules (kJ)(Hill, Wyse & Anderson, 2012).

It has been shown that the main prey items such as krill, capelin and herring in the Barents Sea undergo extensive seasonal changes in energy density. The energy content of gadoids, and cod particularly, is low compared to other species inhabiting the Barents Sea, and has an average of 4.9 $kJ \cdot g^{-1}$ (wet mass) throughout the year; ranging from 3.9 $kJ \cdot g^{-1}$ in August to 6.1 $kJ \cdot g^{-1}$ in April (Mårtensson *et al*., 1996). This average is currently being used in the present food consumption models (Nilssen *et al*., 2000). However, mainly immature cod were included in the study calculating energy content in cod, thus not accounting for the high lipid content in mature and pregnant female individuals during spawning (Worthy, 2001). Gadoids such as saithe have demonstrated seasonal differences in the energy density after reaching maturity (Pedersen and Hislop, 2001), displaying the importance of including mature cod in order to represent the whole population.

Variations in energy content of prey have to be accounted for in order to correctly estimate the food consumption of marine mammals. Low lipid content in prey species during the winter implies that the animals have to consume more prey during those months, and thus the food consumption estimates should be increased.

Another important factor is how much energy an animal receives when consuming various prey. In the case of harp seals, the obtained digestive energy of eating cod is 93.2 %, whereas the metabolizable energy is 84.3 % (Lawson *et al*., 1997); displaying a high efficiency of acquiring the energy in their food, despite the short transition time (<5 h) associated with pinnipeds and their digestion (Helm, 1984).

1.4 Diet analysis

In order to assess the potential impact the seals may have on prey dynamics, i.e. stock size, and to calculate annual food consumption, it is crucial to obtain accurate estimates of their diet. This is done by diet analysis. Knowledge of feeding behaviour and diet in harp seals, and marine mammals in general, has been challenging to acquire because the hunting and foraging of these species occur under water and often in remote locations, making it impossible to perform observational studies. Because of this, indirect methods determining the composition of marine mammal diets, with the supplement of time-depth recorders and satellite telemetry, have been used to gather information that links a predator and its prey spatially and temporally (Pierce and Boyle, 1991, Folkow *et al*., 2004, Nordøy *et al*., 2008, Kuhn *et al*., 2009). There are multiple methods of reconstructing the composition of an animals diet, performing various analyses on the seals such as stable isotope analysis, fatty acid analysis, molecular genetic analysis and the examination of its stomach, intestines and faecal samples or regurgitations. The latter identifies prey remains such as hard parts including fish otoliths, bones, exoskeletons and cephalopod beaks, and is most widely used. This method, also called hard part analysis (HPA), is the method of choice for harp seals, which is based on the contents of their stomachs and intestines. Species of fish can be determined from their otoliths due to its specific form and structure, and otolith lengths also demonstrate an allometric relationship with the initial length and weight of the fish (Härkönen, 1986). Using this allometric relationship, the quantity of a certain prey species in the predator's diet can be estimated, which provides an important picture of the predator's effect on the prey. In order to estimate the total number of each fish species, the number of intact skulls together with fresh specimens, and the count of the free otoliths divided by two, is added together (Nilssen *et al*., 1995b). To calculate the total prey consumption, multiple estimations of harp seal diet compositions are combined to account for the spatio-temporal variations these pinnipeds display (Nilssen *et al*., 2000).

1.5 Harp seal diet

From stomach and intestine analysis, the harp seal diet has been found to vary in time and space due to the seasonal migration of the species, prey availability and ecosystem productivity (Nordøy *et al*., 2008). This is manifested in their highly variable body mass and food intake, eating almost double the amount during the summer months (June, July, August and September) compared to other months, and maintaining good condition from September to February, before displaying a decrease in blubber thickness and body condition after breeding and moulting (Nilssen, 1995, Nilssen *et al*., 2000, Hammill *et al*., 2010). This feeding frenzy during the summer months allows them to reach weights of up to 200 kg, and lengths of 1.9 metres (Haug and Bjørge, 2010).

Harp seal food intake during breeding and moulting is reduced, since they eat little and less intensively, mainly on crustaceans (Nilssen *et al*., 1995a). For the White Sea stock, breeding occurs in late February, and moulting takes place in April-May. They do, however, engage in feeding in between these two periods, and during this time, the females of the White Sea stock feed off the coast of Kola Peninsula and Finnmark, Norway, where they predate on the spawning capelin stock (Gjøsæter, 1995, Nordøy *et al*., 2008). The juveniles and males stay in the White Sea, and feed on a variety of prey such as pacific herring (*Culpea pallasii*), sandeels (*Ammodytes* sp.), eelpouts (*Zoarces viviparus*), and small crustaceans until moulting (Nordøy *et al*., 2008). The Greenland stock breeds in late March, and stays in the West Ice to feed in order to prepare for the moult at the end of May *(*Folkow *et al*., 2004). Here they prey upon species such as amphipods (*Parathemisto sp*. and *Gammarus sp*), krill (*Thysanoessa sp.),* and polar cod (*Boreogadus saida*) (Potelev *et al*., 2000, Folkow *et al*., 2004).

The species' energy reserves are low after moulting, and in order to replenish their energy stores, they migrate northwards and engage in extensive summer feedings, foraging on prey with high-energy content. Prey such as crustaceans (*Themisto libellula* and *Thysanoessa spp*) and polar cod dominate their diet during summer and autumn when the seals are located in the northern Barents Sea (Lindstrøm *et al*., 2013). When the ice cover expands during winter, the seals migrate further south and switch to a diet consisting of fish, including polar cod, capelin and herring before returning to their breeding grounds (Nilssen *et al*., 1995b).

1.6 Uncertainties associated with hard part analysis

As previously stated, methods relying on hard parts for analysis represents the most common means of calculating diet composition in pinnipeds, yet they face biases and may misrepresent the results either by underestimating or overestimating the biomass of prey species consumed due to variables such as unidentifiable fish in the stomach, size of prey, degradation of otoliths, and prey lacking otoliths (Tollit *et al*., 1997, 2003, 2007, Jobling and Breiby, 1986, Murie and Lavigne, 1986, Jobling, 1987). This method does also not account for the otoliths already consumed by the fish itself prior to being ingested by the predator, and will only take the last feeding bouts of the seal into consideration, with most otoliths passing through the gastrointestinal system within 24 hours of ingestion (Jobling and Breiby, 1986, Harvey, 1989). Additionally, behaviours such as regurgitation of hard parts including skulls with intact otoliths, otoliths themselves, and bones, may also present errors when such structures are used to determine the biomass of prey and the diet composition. Finally, since otoliths are located in the head of the fish, the head must be consumed otherwise a bias on the number of fish consumed may be introduced despite the fact that the otoliths may be recovered from the stomach, intestinal tract or scat.

Whereas the accuracy of the identification of species in hard part analysis can be affected by behaviours such as head rejection and belly-biting, in addition unidentifiable fish, and the different degrees of digestion and retention of otoliths (Tollit *et al*., 1997, 2007, Grellier and Hammond, 2006), methods such as fatty acid analysis (FAA) and molecular genetic analysis (MGA) are other methods used in diet composition studies. Both are able to identify fish to species level, and such methods can therefore be used to complement HPA, improving the information gained from examining stomach and faecal contents (Marshall *et al*., 2010). However, they lack the power to identify specific individuals consumed, thus operating with presence/no-presence only, and thereby cannot conclude or reject the theory that harp seals only consume part of its prey.

1.7 Population-based food consumption models

Using the estimates of the diet composition, energy density of prey, population size, seasonal changes in distribution, and the population demography of the seals, it is possible to make realistic estimates of their total food consumption, and the consumption of different prey groups. The resulting estimation of annual food consumption of harp seals in the Barents Sea in 1998 - based on 2.22 million animals (with 800.000 animals more than the latest stock size estimate) - ranged between 3.35 – 5.05 million tonnes depending on prey choice. Provided that capelin was abundant, cod accounted for 100.500 tonnes. The consumption of cod could increase to 296.300 – 515.700 tonnes if the capelin stock was small, and would probably increase additionally due to undetermined gadoids in the group “other fish” (Nilssen *et al*., 2000).

Hammill and Stenson (2000) estimated a consumption of 90.924 tonnes cod for the Northwest Atlantic harp seal stock in 1996, comprising of 5.2 million animals (2.2 million animals less than today), which accounts for 50 % of the cod consumed by all the seal species that are distributed in Atlantic Canada.

The current food consumption estimates are based on the assumption that harp seals ingest whole fish due to the type and size distribution of otoliths found during previous diet analyses, and one would therefore assume that all fish presented in this thesis would be consumed as a whole, not leaving any remains behind. Harp seals have in fact been observed swallowing 35 cm to 60 cm long Atlantic cod whole (Stenson, G 2016, *pers. comm*., 6 April). However, a lack of data on harp seal feeding behaviour means one cannot be sure as to what degree they ingest whole fish every time, or if there are circumstances at which they only consume their prey partially. Approaches such as stomach temperature sensors, mandibular sensors and acceleration data loggers (Liebsch *et al*., 2007, Suzuki *et al*., 2009) could to some extent be used to investigate prey size and species, however, up to date, their objectives has been to identify prey capture events. An effective and valuable method to observe free-living pinnipeds and their feeding behaviour is animal-borne imaging systems. Such approaches have been successful in observing prey species selection in seals such as the Pacific harbour seal (*Phoca vitulina richardii*) and the Weddell seal (*Leptonychotes weddellii*) (Davis *et al*., 1999, Bowen *et al*., 2002).

However, these types of studies have not been performed on harp seals yet, and the prey handling (pursuit, capture, and consumption) of larger sized prey in particular, has not been examined.

There are several studies confirming that prey is not always consumed in its entirety or swallowed whole. Larger prey being torn up into pieces has been observed in multiple seal species, including California sea lions (*Zalophus californianus*), Weddell seals, and the Pacific harbour seal (Davis *et al*., 1999, Phillips and Harvey, 2009, Sweeney and Harvey, 2011). The latter left heads of various fish uneaten, which is evidence that some pinnipeds do not always consume the otoliths essential to the HPA method. Belly-biting is another behaviour where the seals selectively seek out the soft body parts with high energy content of their prey (such as the lipid-rich liver and other abdominal organs) without consuming the muscle or head. This behaviour has also been observed in wild harp seals (Fu *et al*., 2001, Lilly and Murphy, 2004).

By using the HPA method in diet studies, one excludes the possibility of harp seals consuming only parts of their prey, subsequently not accounting for behaviours such as belly-biting or the consumption of fish that has been torn up prior to ingestion, possibly lacking specific body parts (e.g. the head). This lack of knowledge regarding to what degree such behaviours occur in harp seals could possibly underestimate prey consumption. The subsequent energy waste associated with not eating the whole fish is unknown, and might have some impact in regard to the seals' total consumption. Studies on the energetic consequences of feeding on various sized fish has not been performed before, and our knowledge of the subject is limited; hence such energy wastage is not accounted for in the models estimating food consumption. If behaviours such as belly-biting or rejection of fish heads are frequent, marine mammals must consume higher amounts of prey in order to ingest the same number of calories and fulfil their energy requirements, and these seemingly small adjustments can yield large ecological implications.

With a population size of nearly 2 million animals, the harp seal stocks that are found in the Barents Sea have the potential to impact the structure of fish communities. It is therefore important that both food consumption models and knowledge of diet composition are accurate. Understanding harp seal consumption of prey and what might be left behind in terms of body parts is an important factor in predicting their total food consumption. New knowledge may in turn be used to improve the current energy-based population consumption models by applying suitable correction factors, accounting for the energy wastage when only parts of the fish is consumed. It is envisaged that the estimates calculated in this study will improve the accuracy of food consumption models.

1.8 Aim of study

To our knowledge, this is the first experimental study to focus on the relationship between a predators feeding behaviour, in this case the harp seal, and the size of its prey and the proportion of food consumed when the prey reaches a certain size. In this study we principally aimed at investigating whether harp seals always consume fish prey in its entirety or if there are conditions under which the seals consume only part of their prey. A further aim was to assess the possible energetic impacts of harp seal consumption of different sizes of fish, and therefore quantify how much energy these pinnipeds might lose if they would show preferences for certain parts of the fish when it becomes a particular size. This can in turn provide data for an improvement of the estimates of food intake by these animals. In addition, the study wanted to offer insight into which parts of the fish are left behind if the seals selectively chose to eat only specific parts, in order to improve our understanding of the harp seal feeding behaviour of larger sized fish.

2. Materials and methods

This study was part of the research project "COEXIST – Condition and energy expenditure estimates from free-ranging marine mammals" approved by the Norwegian Animal Research Authority (Fots ID: 6093). The Danish Ministry of foreign affairs approved the expedition in Greenlandic waters, and the Norwegian Directorate of fisheries and Greenlandic Ministry of Fisheries, Hunting and Agriculture granted permission to capture the harp seals used in this study. All personnel involved had FELASA category C course, allowing the planning and conduction of the animal experiment.

2.1 Animals

2.1.1 Seals

This study is based on the behavioural observations of two harp seals (*Phoca groenlandica;* one adult - approx. 7 years, one juvenile - 1 ½ years) maintained in human care at the Institute of Arctic and Marine Biology, UiT - The Arctic University of Norway. These seals were among the six females (three adults of undetermined age and three new-born whitecoats) captured in March 2014 on the whelping patches on the drift ice off the eastern coast of Greenland, and transported to Tromsø by the University's RV «Helmer Hanssen» (71°01'N - 71°02'N, 17°36'W - 17°48'W). After capture, the seals were held in two separate indoor seawater tanks (42.000 litre each, 5.6 x 5.8 x 1.6 m), the adults in one and the young in the other, with a continuous supply of fresh seawater (60 l/min) and access to a dry haulout platform and freshwater. The tanks apply two types of filtration systems, one belt filter removing crude particles, and two sand filters removing finer particles. For upkeeping, the seals had been hand-fed at least twice a day a combination of high-quality thawed, fresh-frozen capelin and herring, supplied by Pelagia AS, integrated with multivitamin supplement (Sea Tabs® MA, Pacific Research Laboratories, Inc., San Diego, USA) prior to this experiment. The diet was individually tuned, aiming at keeping an optimal weight in relation to the age and the physiological status of the individual seals, which was 3 kg a day for both seals during the time of the study. The adult received enough food in order to oscillate yearly around its optimal weight, whereas the juvenile was provided with food to let it grow naturally.

2.1.2 Fishes

A total of 162 live fishes were used in this study; 141 of them were cod, and 21 were saithe. Cod was the main species used in this study in order to calculate the energetic consequences harp seals have of feeding on fish of various sizes, and the subsequent caloric loss they experience of not consuming their prey whole. This is due to its commercial value, but also the accessibility, and its variations in size.

Saithe was also used on occasion in the juvenile seal's experiments in order to examine whether there were any variations between the two fish species. The adult seal did not receive saithe due to its small size (saithe obtained did not exceed 440 g), which would not present any remains, and thus not contribute with data to calculate energy waste. Previous diet studies were contemplated in order to provide the harp seals with the various size distributions of cod similar to what they would feed upon in the wild. The size of the fishes utilized in this study ranged from 20 cm - 70 cm and 90 g - 2710 g, with the average weight and length being 39 ± 1.5 cm and 595 ± 70 g respectively, representing a large range of what the seals would encounter in the wild, and those sizes they might feed upon (**Table 1**). Live fish was used to mimic the conditions at sea, and to stimulate the seals hunting instinct, thereby making them catch it, in order to provide an opportunity of recording their feeding behaviour up close.

Table 1. Parameters of the fishes captured.

	Length (cm)	Weight (g)
Average	38.9	595.3
Median	37	465
Mode	32	310
Max	70	2710
Min	20	90
Range	50	2620
Standard deviation	9.8	455.7
Sample size	162	162
Confidence coefficient	1.96	1.96
Margin of error	1.5	70.2
Upper bound	40.4	665.5
Lower bound	37.4	525.1

2.1.3 Food restriction protocol

The experimental procedures were conducted over a time period of 9 weeks from 25.08.2015 – 30.10.2015, with 21 individual experiments including 162 live fish feeding attempts (141 cod, 21 saithe). Throughout the study, seawater temperatures in the tanks ranged from 7.0°C - 10.2 °C, with an average osmolality of 831 mOsmol (**Appendix I and II**), and a simulated 70°N photoperiod. The harp seals utilized in this study displayed good appetites but in order to improve their food motivation, food restriction with partial fasting was initiated. The experimental study was performed every Monday, Wednesday, and Friday such that the animals were fasted every other day (Tuesday, Thursday, Sunday) and were fed normally on one day of the week (Saturday) as shown in **table 2**. Both harp seals were involved in other projects, necessitating continued training. To minimize interference during this study, and to maintain their acquired behaviours, reinforcement during training consisted of gelatine blocks and occasionally 100-200 g herring during fasting days, and when the animals were being weighed.

The seals were weighed via a voluntary based training method throughout the study, and the weights are presented in **Appendix III**. To ensure that the seals received adequate nourishment, the live fish ration was integrated with thawed fish up to the weight of their individual daily rations, presented during a training session at the end of each live-fish feeding experiment.

Table 2. Timeline of the weekly schedule describing each day of the week.

Mondays	Tuesdays	Wednesdays	Thursdays	Fridays	Saturdays	Sundays
Experiment	Fasting	Experiment	Fasting	Experiment	Normal feeding	Fasting
Live fish	Gelatine blocks	Live fish	Gelatine blocks	Live fish		Gelatine blocks
Rest of daily ration		Rest of daily ration		Rest of daily ration		

2.2 Study area and fieldwork

The study area for this experiment covered two separate locations: 1) Henrikvika; where the live cod and saithe was caught, and 2) the Institute for Arctic and Marine Biology; the research animal facilities at the Arctic Biology building (hereafter called AAB). Cod and saithe were presented to the seals at the latter, in which the harp seal feeding behaviour was observed, and the discarded fish parts were collected (**Table 6 and 7**).

2.2.1 Henrikvika, Kaldfjord

In Henrikvika (69°41’N, 18°39’E), located 30 minutes outside Tromsø, cod and saithe were caught from either a boat or floating jetty, using a recreational rod and nylon line attached to 8 - 18 g fishhooks depending on which size of fish was needed. The fish was then temporarily placed in a modified fish cage moored to the floating jetty, awaiting transport and use in the experiment. Due to safety regulations, two people were required to be present in the boat at all times during fishing. Fish was caught from the boat once a week (Mondays) for the duration of the experiment and used for experiments on Mondays, Wednesdays and Fridays of the same week. If the number of fish caught on Mondays was insufficient, additional days were used to catch fish from the floating jetty. On Wednesdays and Fridays, fish were collected from the fish cage and transported to AAB.

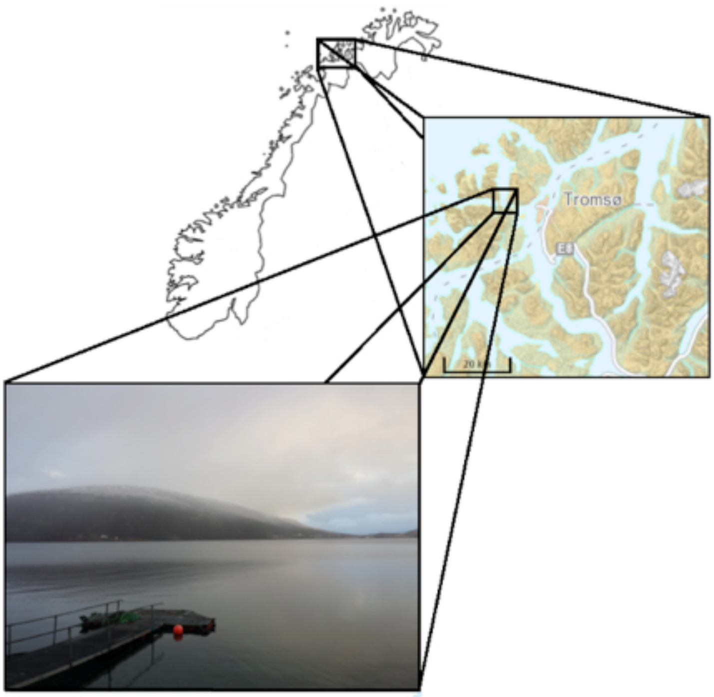

Figure 2. Map of the study area, mainland Norway (upper), parts of Troms (middle), and Henrikvika (lower) where the fishing took place.

A satisfactory number of cod (no greater than 10) was fished, and handled carefully as to avoid damage. They were maintained in an on-board transport bucket (75 l), employed with fresh seawater during each procedure before they were unloaded into the fish cage. This cage was constructed of a plastic-coated wire mesh on a wooden frame (117 cm x 77 cm x 82 cm) and was suspended in the water, via attachment to the floating jetty, for the entire experimental period. An opening was made in the top of the cage to allow for the addition/removal of fish. Buckets were used for the transfer of fish in each procedure. The fishing continued until 30 fishes were caught, with an average of eight fish collected from Henrikvika prior to each experiment, consisting of a range of different sizes (20 cm to 70 cm; 90 g to 2710 g). They were safely transported to the research facility in a securely fastened 90 l lidded bucket.

Figure 3. Sea cage in which the fish were held.

The regulations for the transport of aquaculture animals were followed, and the density of the fishes during transport did not exceed five kg/100 l. The duration of the transfer was estimated to be broken down as follows:

- Loading the fish into the 90 l bucket (five minutes).
- Driving from Henrikvika to AAB (30 minutes).
- Loading the fish into the oxygenated tank for acclimation at AAB (five minutes).

2.2.2. Arctic Animal Biology building, Tromsø

The tanks in which the seals were kept contained 42.000 l, and measured 5.6 x 5.8 x 1.6 m. For the live fish feeding experiment the adult seal was alone in one tank while the juvenile shared a tank with another juvenile, but the two were separated by a plywood barrier dividing it in two equal-size sections. However, since the mesh size in the juveniles' pool was too large to prevent fish swimming from one side to the other, a plastic-coated wire mesh with a smaller mesh size was attached with cable ties. Part of this divide is removable (hereafter called metal enclosure), so that the seals can move between sides of the tank in between experiments.

The oxygenated holding tank (>200 l) was filled in advance of fishing on Mondays and before the retrieval of fish on Wednesdays and Fridays. This tank was provided with a continuous flow of saltwater from the same source supplying the tanks of the seals. It was also continuously supplied with oxygen with the help of a compression motor and tubes positioned on the base of the tank. The fishes were unloaded into the oxygenated holding tank, and had a minimum of 15 minutes to acclimate before the trials started.

2.3 Experimental protocol

2.3.1 Presenting the fish

All fish were given a number, and the species, length and weight was recorded prior to presenting it to the seal. The fish in the holding tank were captured using a landing net, before being placed gently on a measuring board with the tip of the mouth touching the 90° plastic edge. The total length was measured to the closest 0.5 cm from the nose to the end of the caudal fin. The fish was then weighed on a digital fish scale (Berkley max 23 kg) hanging from the wall, by placing the fish in a plastic bag and weighing it to the closest 10 grams. All the fish were alive and acclimated when transferred to the pools, during which times the seals where not disturbed.

Before presenting a fish, the metal enclosure was put in place to separate the two juveniles sharing the tank. After the process of weighing and measuring the length of each fish, they were carried in the plastic bag to the tank, and carefully released from either the side (juvenile seal) or from the platform (adult seal). The fish were not chosen at random but rather according to the seal that was to receive it. The adult harp seal received bigger fish (200 g – 2710 g), while the juvenile seal received smaller fish (90 g - 1350 g). The first fish that was fed to the adult seal was chosen at random when it came to size. Hence, some trials started with a big fish (>1.0 kg), and others with smaller fish (<1.0 kg). The experimental trials were designed to include two to eleven fish depending on the fish's size; the adult receiving on average five fish, and the juvenile three. Each prey item was given separately and consecutively in order to collect the remaining parts of the fish. The experiments were recorded for documentation using a GoPro® Hero 4 camera placed in a waterproof casing on the side of the pool.

Some parameters were set to ensure the same conditions during the experiment. Each fish was left in the tank for 30 minutes, and the remains of the fish were left in the tank additionally 30 minutes or until the seal ceased to show interest for it. If the fish was consumed before 30 minutes had passed, another fish was weighed, measured and presented to the seal. All fish remains were collected from the tanks to be analysed. If the seal did not show any interest, i.e. ignored the fish, it was removed once the 30 minutes had passed. If the seal still showed interest after 30 minutes (eating, playing etc.) the fish was left in the tank for an additional 30 minutes. If the seal continued to eat the fish after 60 minutes, it was left in there for a further 30-minute period. The fish was removed after one and a half hours regardless of the seal's interest and attention at this point. If the fish had not been killed during the encounter with the seal, it was humanely euthanized. This was done by the use of blunt force trauma, followed by the destruction of the brain.

2.3.2 Fish remains

The remains from each fish were removed from the pools using a metal pole with a net attached to the end, and were taken pictures of using a digital camera. They were then inspected for otoliths, weighed, placed in a labelled plastic bag (to prevent desiccation), and stored frozen at -20°C. Samples were kept in the freezer until all experiments were complete, then, they were homogenized and sent to ALS Laboratory Group, Cambridgeshire, England, for chemical analysis. Cod that were not used in the experiment were labelled as "extra" fish, and placed in the same freezer for further use. This "extra" fish (hereby called reference fish) was also sent to ALS where they were analysed for fat, protein, water, ash, and energy content, which was later used to create a regression. The results of the analysis and regression are presented in **table 4 and figure 5**.

The videos of the seal's behaviour were analysed at the end of each experiment (how interested the seal seemed before the fish was presented and during the time it was in the tank; if it swam directly towards the fish, if it played with it; if it managed to swallow the fish whole etc.).

2.4 Sampling

2.4.1 Water, temperature and organic material

Different parameters were measured prior to each experiment. Water samples were taken from the tank, labelled, and kept in the freezer ready for further analysis (**See section 2.4.2**). The seawater temperature was measured with a digital thermometer (Fluke 54 II) and a mercury thermometer (**Appendix I**). A metal pole with a net attached to the end or 15 x 15 cm buckets were used to collect samples of scat and regurgitations at the end of each live-fish feeding experiment.

2.4.2 Osmolality measurements

An Osmomat® 030 from Gonotec, Berlin, Germany (range: 0 – 3000 mOsmol/kg, reproducibility of < ±0.5 % or < ±2 digits) was used to measure the osmolality of the water samples using a BIOVIT proline pipette (20 - 200 µl) and 100 µl tip cones by freezing point osmometry. This was done to make sure the conditions in the pool were normal (approx. 1000 mOsmol) on the day of the experiment, so as not to confound the experiment in any way by affecting either the fish or the seals. The apparatus was calibrated with 50 µl distilled water with an osmolality of 0 mOsmol kg $^{-1}$, and was used to obtain the zero value. It was also calibrated with 50 µl calibration fluid (Gonotec, Berlin, Germany) of 850 mOsmol kg^{-1} NaCl/H_2O in order to calibrate the machine to the osmolality. A sample of the calibration solution was used to confirm the same reading of 0.850, and the osmolality of distilled water was checked twice to make sure that the same reading was achieved and that the calibration was successful.

All 21 bottles of seawater from the pools were thawed over night and mixed prior to pipetting 50 µl of each sample into a measuring vessel. The liquid was undercooled, and its freezing point was recorded and converted into an osmolality value. Three parallels were performed for each sample, all with new measuring vessels to avoid contamination, and the average was calculated (**Appendix II**).

2.4.3 Homogenizing fish samples

The remains of the fish were categorized into four different groups depending on which parts that were not consumed. These were so named:

1) Head of cod.
2) Head of saithe.
3) Head of cod with parts of the back and backbone attached.
4) Cod lacking its abdomen.

Out of the 38 samples, 20 were chosen for further analysis, including five reference fish, seven heads, four head with parts of the back attached, and four cod lacking their abdomens. Three samples (6-15, 10-15, 11-15) had two to three parts of similar size (same body part) homogenized together to ensure that the sample weight was above 250 g (requirement from ALS Laboratory Group).

Table 3. Samples sent to ALS Laboratory Group. The samples were assigned to four different groups based on the leftover parts: 1) reference fish, 2) head, 3) head with parts of the back attached, 4) Cod lacking its abdomen.

Reference fish	Heads	Head with parts of the back attached	Body lacking the abdomen
1-15	6-15	13-15	17-15
2-15	7-15	14-15	18-15
3-15	8-15	15-15	19-15
4-15	9-15	16-15	20-15
5-15	10-15		
	11-15		
	12-15		

The samples were prepared in a frozen state to make the cutting and mixing easier. The fish were roughly chopped with a cutting machine (ATOM, SM 280, Cardano al Campo, Italy), and minced in a meat grinder (Sirman, TC-model, Curtarolo, Italy). The fish paste was gathered in a bowl, and thoroughly mixed by hand to homogenize the sample. Glass jars from ALS were labelled and filled with the homogenized 250 g samples, placed in a cooling bag and sent to ALS the following day. The mincing machine and bowl were cleaned between each mixing event. A parallel sample was taken and kept at AAB when the fish remains exceeded 250 g. The mincing machine had some leftovers in it after mincing, which was collected on five occasions so that an average could be calculated in order to see how much of the sample was typically excluded (**Appendix IV**).

2.5 Methods performed by ALS

The analyses of the homogenized fish samples were performed by ALS Laboratory Group in their laboratory in Medcalfe way, Chatteris, Cambridgeshire, PE16 6QZ, England.

2.5.1 Water

Water content was calculated by the official methods of analysis of AOAC International, 16th edition. The 2 - 4 g sample was covered with a partially closed lid and dried at 102°C ± 2°C over a time frame of 16 hours. The sample was then transferred to a desiccator to cool after drying. The calculation to measure moisture was performed using the following equation.

$$\text{Moisture (\%)} = \frac{(W1 - W2) \times 100}{W1}$$

Equation 2

Where: W1 = weight (g) of sample before drying.

W2 = weight (g) of sample after drying.

2.5.2 Ash

Ash was calculated using the BS 4401 Part 1 1998 Commission Regulation (EC) 152/2009 method. To remove the organic matter, a homogenized sample of 5 g was placed in a crucible (container of metal) that had previously been heated to 550 °C, and subsequently cooled. The crucible was then placed on a calibrated muffle-furnace and gradually heated to make the sample carbonize. The temperature was kept at 550 °C until white, light grey or reddish ash was obtained, before placed in a desiccator where it was cooled and weighed. The content of ash was calculated by the use of these equations:

$$\% \text{ Ash (wet)} = \frac{Crucible\ and\ ash\ -\ crucible}{Crucible\ and\ sample\ -\ crucible} \times 100$$

Equation 3

$$\% \text{ Ash (dry)} = \frac{\%\ Ash\ (wet)}{(100\ -\ \%\ moisture)} \times 100$$

Equation 4

2.5.3 Fibre

Fibre was calculated using the official procedure of AOAC 985.29, where three dried samples underwent sequential enzymatic digestion by α-Amylase, protease, and amyloglucosidase. The samples were precipitated and filtered, and the residues were washed with alcohol and acetone before being dried and weighed. The soluble and insoluble fibre residues were collected in three crucibles, two of which underwent further processing, determining ash and protein. The total dietary fibre was calculated by subtracting the weight of the residue from the weight of the collected ash and protein.

Total dietary fibre = Weight (residue) – Weight (ash + protein) Equation 5

2.5.4 Fat

Total lipid content was measured by oven drying and pulsed Nuclear Magnetic Resonance (NMR) based on the method ISO 8292-1 published by International Organization for Standardization (ISO). An MQC23 NMR analyser from Oxford Instruments, Oxfordshire, UK was used to determine the solid fat content (SFC) of the samples. This method measures the direct ratio (or signals) between the solid and liquid parts of the sample from the NMR Free Induction Decay (FID), which is the signal after the sample has been exposed to NMR. The radio intensity is then plotted as a function of time (**Figure 4**). This signal is generated due to the excitation of the hydrogen in the fat, and is sent out when hydrogen relaxes and goes back into equilibrium state (PNA, 2015). Solid signals decay faster than liquid signals, and it's therefore possible to distinguish the two, and get two points on the FID. The ratio can be found by the use of this equation:

$$\% \text{ SFC} = \frac{(f * S - L)}{f * S} \times 100 \qquad \text{Equation 6}$$

Where: f = a correction factor to correct for the fact that it’s not possible to take a measurement immediately after the radio frequency pulse due to “dead-time” of the sample probe.

S = the total solid plus liquid signal.

L = the liquid signal only.

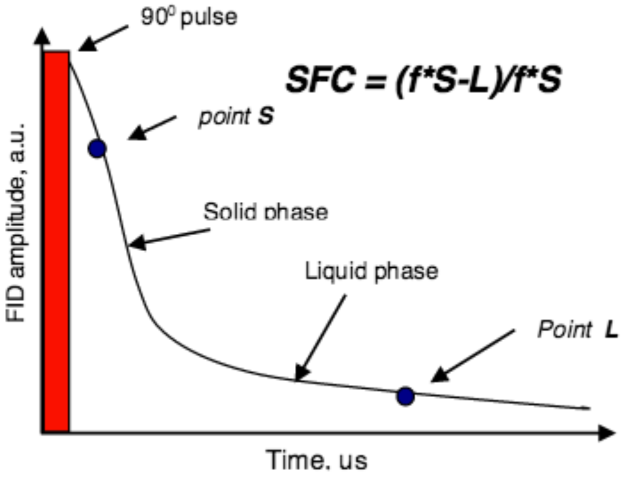

Figure 4. FID based calculations (PNA, 2015).

A dried sample was put in a 50°C heating block for at least 30 minutes to stabilize the crystal structures before being subjected to pulsed NMR. A short and powerful burst of 90° radio frequency (RF) energy was sent onto the sample whilst in a magnetic field, and the two signals were recorded. The resonance of the sample was compared against a two-point calibration curve, and the resulting lipid content was determined automatically. The instrument used was calibrated against olive oil (with known weight and 100 % oil), and the appropriate correction factor was used if necessary.

2.5.5 Protein

Protein was measured by the Dumas combustion method using a FP628 machine (LECO Corporation, St. Joseph, Michigan, USA), determining the total nitrogen content, and converting it to protein by the use of a correction factor. This is called crude protein (nitrogen*correction factor) (Simonne *et al.*, 1996). The sample was homogenized, weighed and heated to 1000°C in a furnace in the presence of pure oxygen. Substances such as gaseous nitrogen oxides, carbon dioxide and water were produced from the sample (Velp Scientifica, 2016).

$$Sample + O_2 \rightarrow NO_x + H_2O + CO_2 + O_2 \qquad \text{Reaction 1}$$

The mixture of gases then passed through a chamber with copper (catalyst heater, 650 °C), helping to reduce it into N_2. Water and carbon dioxide were removed by different traps (Velp Scientifica, 2016).

$$NO_x + H_2O + CO_2 + O_2 + Cu \rightarrow H_2O + CO_2 + N_2 \rightarrow N_2 \qquad \text{Reaction 2}$$

With helium as the reference and carrier gas, the sample was taken and analysed, and quantified by a thermal conductivity cell. This result was processed by a computer, which in turn calculated the nitrogen content of the sample, and converted it into protein with the help of a correction factor of 6.25.

$$\text{Protein} = 6.25 * \text{N-total} \qquad \text{Equation 7}$$

2.5.6 Carbohydrate

The carbohydrate content was calculated by these equations:

$$\%\ \text{Total Carbohydrate} = 100 - (\%\ \text{Moisture} + \%\ \text{Protein} + \%\ \text{Fat} + \%\ \text{Ash}) \qquad \text{Equation 8}$$

$$\%\ \text{Available Carbohydrate (by difference)} = \qquad \text{Equation 9}$$
$$100 - (\%\text{Moisture} + \%\text{Protein} + \%\text{Fat} + \%\text{Ash} + \%\text{Dietary Fibre})$$

2.5.7 Energy

Energy was calculated from the previous analytical results of protein, fat, available carbohydrate and dietary fibre. The method is based on EC 1169/2011 Food Information to Consumers Regulation (Human Food) and Animal Feeding Stuffs Regulations 2005.

The formula to measure energy is:

$$\text{Energy (Kcal)} = (\text{Protein} \times 5{,}7\ \text{kcal per g}) + (\text{Fat} \times 9{,}5\ \text{kcal per g}) + (\text{Carbohydrate} \times 4{,}0\ \text{kcal per g}) + (\text{Fibre} \times 2) \qquad \text{Equation 10}$$

2.6 Statistical analysis

All graphs, tables and regressions (**Equations 11 – 15**) were made in Microsoft® Excel® for Mac 2011, version 14.1.0.

3. Results

3.1 Body mass

Body mass was measured every week (variable from week to week) to assess the condition of the animals, and to assess how the study may have affected them. It was concluded that the seals were food motivated and in good shape if they showed a positive energy balance, i.e. an increase in body mass. The initial body mass of the adult seal was 94.0 kg (18. August), increasing to 99.6 kg by the end of the experiment, with an average daily increase of 0.08 kg/day. The juvenile had an initial body mass of 56.8 kg (18. August), which increased to 61.4 kg by the end of the experiment, gaining on average 0.07 kg/day (**Appendix III**). In the case of the adult seal, the diet consisted of 24 % cod, and 76 % herring and capelin during the study, whereas the juvenile seal received 7 % and 93 % respectively. The seals increase in body mass indicated that their energy requirements were met throughout the study.

3.2 Results from ALS

3.2.1 Energy content of reference fish

Five cod ranging from 320 g to 1060 g were selected in order to make a regression line. These fish were sent to ALS Laboratory Group for analysis (**Table 4**). A regression line was constructed based on the fish body mass and energy content of each fish (**Figure 5).** The equation is given as follows:

$$EC = 1.1857FBM - 79.4 \qquad \text{Equation 11}$$

Where: EC is energy content of the fish (kcal).

FBM is fish body mass (g).

This equation was in turn used to estimate the energy content of the all consumed fish. The average energy content of the reference fish per 100 g was calculated to be 102.4 kcal or 4.3 kJ • g^{-1} (wet mass).

Table 4. Output of the analysis (performed by ALS) of five reference fish. The weight of each fish is displayed, as well as ash, water, protein, fat, carbohydrate, and energy content.

Fish body mass (g)	Ash g/100g	Water g/100g	Protein g/100g	Fat g/100g	Carbohydrate g/100g	Energy Kcal/100g
320	2,70	80,2	16,2	0,7	0,2	99,8
370	3,10	80	16,8	0,6	<0,1	101,5
470	2,7	79,5	17,1	0,9	<0,1	106,0
590	3,2	82	15,4	0,4	<0,1	91,6
1060	3,3	77	17,4	0,9	1,4	113,3

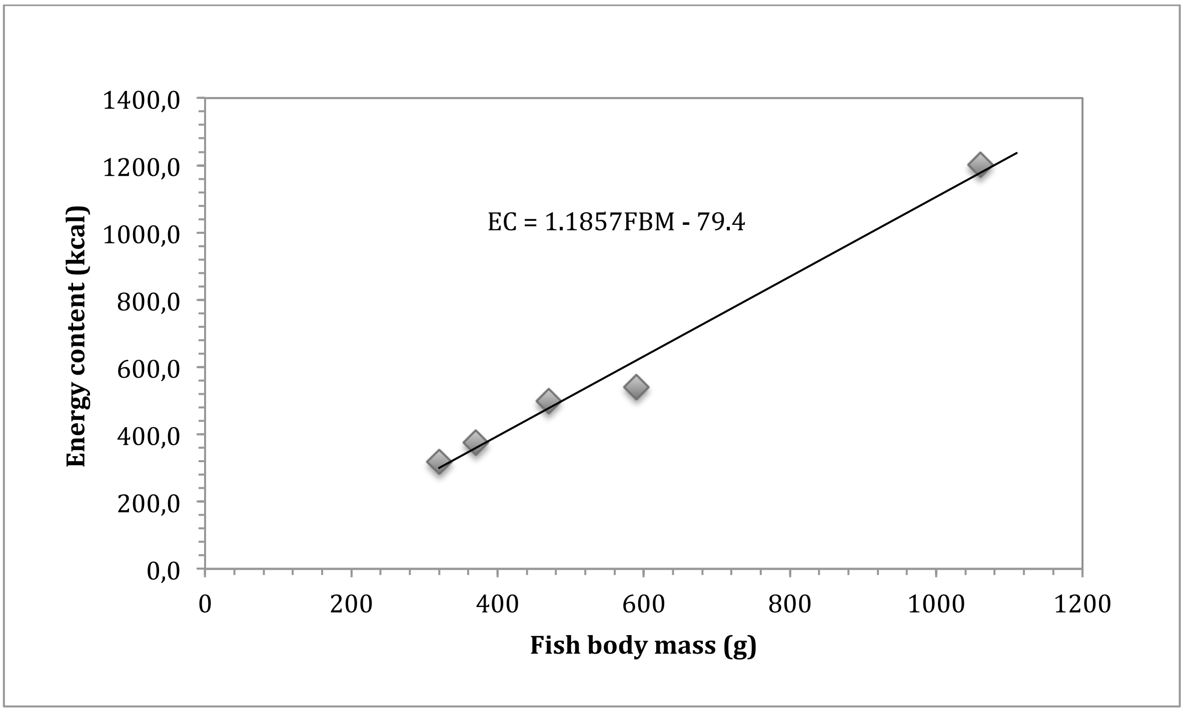

Figure 5. Energy content (kcal) in relation to fish body mass (g) of five whole reference fish. A linear regression line has been added, and is described as EC = 1.1857FBM - 79.4, where EC is energy content and FBM is fish body mass.

3.2.2 Energy content of fish remains

The two harp seals in this study left fish remains in 38 cases - 16 from the adult seal and 22 from the juvenile seal - which were all categorized in four groups: 1) head of cod, 2) head of saithe 3) head of cod with parts of its back attached, and 4) cod lacking its abdomen. Samples from these groups were selected for analysis in order to calculate energy, protein, fat, carbohydrate, water, and ash content (**Table 5**). The average kcal/100 g for each group was calculated, and applied to the fish remains within the same category, in order to estimate their energy content. These results are presented in **table 6** for the adult seal and **table 7** for the juvenile seal. The energy content of cod heads ranged from 72.2 - 88.6 kcal/100 g, with an average of 83.7 kcal/100 g; whilst the saithe heads ranged from 80 – 80.6 kcal/100 g with an average of 80.3 kcal/100 g. The group consisting of head of cod with parts of its back attached ranged from 84.4 - 93.3 kcal/100 g, with an average of 89.1 kcal per 100 g. Cod lacking their abdomen had an energy content ranging from 88 - 102.2 kcal/100 g. This gave an average of 95 kcal/100 g (**Appendix V**).

Table 5. Samples sent for analysis performed by ALS, displaying fish body mass (initial and remains), protein, fat, carbohydrate, energy kcal/100g, and energy content in the remains categorized in four different groups (H: head of cod, HS: head of saithe, HB: head of cod with parts of its back attached, A: cod lacking its abdomen). Energy content of each leftover is estimated based on the average kcal/100g for each group.

	Fish body mass (g)	Weight of discarded part (g)	Protein g/100g	Fat g/100g	Carbohydrate g/100g	Energy Kcal/100g	Total kcal in leftover
H	560	230	12	0.4	<0.1	72.2	166
	960	180	14.6	0.5	<0.1	88.0	158
	1000	240	14.6	0.5	<0.1	88.0	211
	1170	380	13.9	0.5	<0.1	84.0	319
	1300	400	14.8	0.4	0.1	88.6	354
	1610	550	14	0.6	0	85.5	470
HS	220	70	13.3	0.5	<0.1	80.6	56
	280	110	13.3	0.5	<0.1	80.6	89
	390	120	13.3	0.5	<0.1	80.6	97
	390	140	13.2	0.5	<0.1	80.0	112
	440	120	13.2	0.5	<0.1	80.0	96
HB	850	280	13.2	0.5	1.6	86.4	242
	1020	450	15.5	0.4	<0.1	92.2	415
	1510	690	14.3	0.3	<0.1	84.4	582
	1850	870	15.7	0.4	<0.1	93.3	812
A	270	240	14.6	0.5	<0.1	88.0	211
	340	320	14.8	0.5	0	89.1	285
	380	350	17.1	0.5	<0.1	102.2	358
	460	420	17	0.4	<0.1	100.7	423

3.3 Consumed fish

3.3.1 Adult seal

In total, 96 fish (95 cod and 1 saithe) were presented to the adult seal. Out of 96 fish, 59 (61.4%) were consumed whole, while 16 of the fish remains were collected from the tank after the time period given in the materials and methods section (**See section 2.3.1**). The adult seal received on average five fish ranging between 200 g – 2710 g, and consumed on average 1625 g per experiment.

The fish remains of the adult seal were placed in two categories; 1) head, and 2) head with parts of the back attached, consisting of ten and six pieces respectively (**Table 6; Figure 6**). The estimated energy content of the remains was based on the average energy content of the samples from the same category that was sent for analysis (**Appendix V**). Otoliths were absent from three of the sixteen remains: two were from the first fish of the day, while one was from the second fish. This could indicate that the first fish in the experiment was consumed to a larger degree than that of the last fish, however, the adult seal displayed a similar incentive to hunt both first and last fish.

Figure 6. The two types of fish remains frequently discarded by the adult seal. Fish head (upper), and head of cod with parts of back attached (lower).

Table 6. All remains from the adult seal displaying fish body mass (g), the weight of the discarded parts (g), type of body part (H: head of cod, HB: head with parts of the back attached), and energy content (kcal) of the remains.

Fish body mass (g)	Weight of discarded parts (g)	Body part	Energy content (kcal)
750	10*	H	8
820	150	H	125
830	190*	H	159
860	150	H	125
960	180	H	151
1000	240	H	201
1170	380	H	318
1240	300*	H	251
1300	400	H	335
1610	550	H	460
850	280	HB	249
910	410	HB	365
1020	450	HB	401
1510	690	HB	615
1780	770	HB	686
1850	870	HB	775

* Indicates the absence of otoliths

3.3.1.1 Fish sizes and calculating the weight of the remains

Of the 59 fishes that were eaten, fish less than or equal to 750 g were swallowed whole, while fish larger than 750 g were torn up by the seal before being ingested. Remains were collected from all the fishes that were torn apart; the sizes of which ranged from 43 cm to 61 cm. Fish with a mass larger than 2400 g were ignored. The mass of the remains increased linearly with the starting fish mass (**Figure 7**), and a linear regression based on all fish that had remains was made; estimating the weight of the remains in relation to the initial size of the fish. The equation is given as follows:

WR = 0.6187 FBM – 337 Equation 12

Where: WR is the weight of the fish remains (g).
FBM is fish body mass (g).

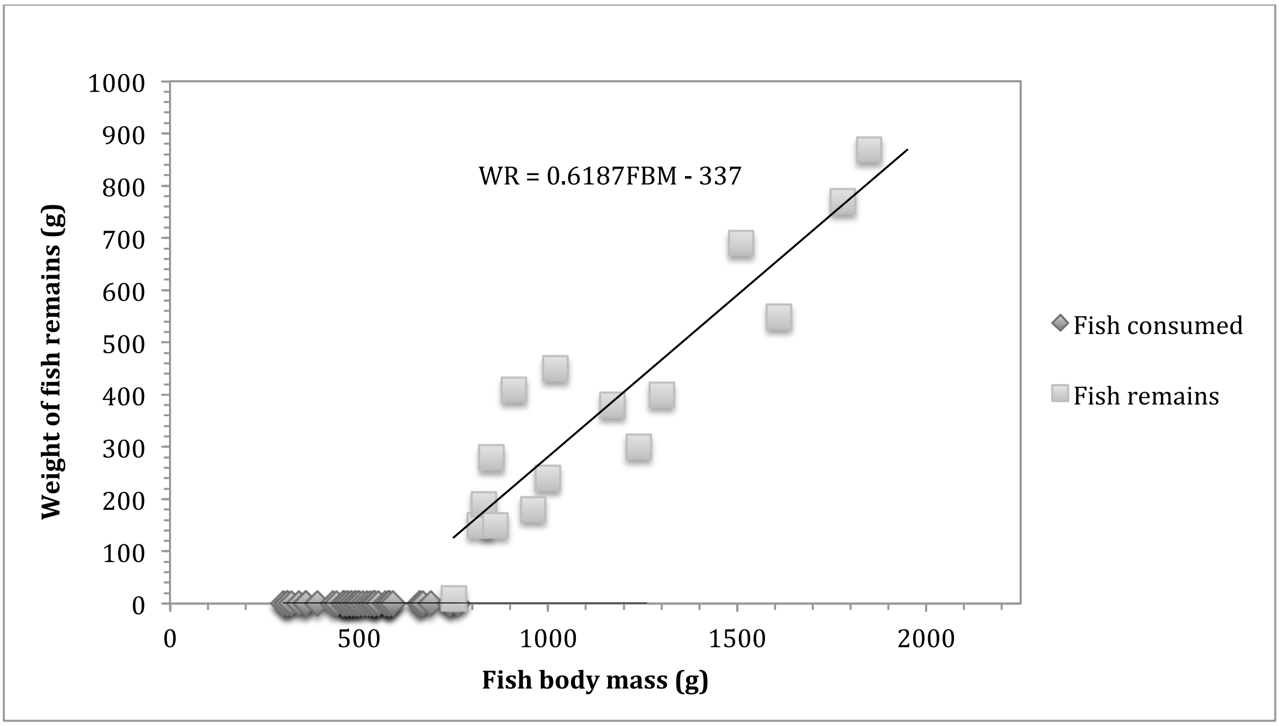

Figure 7. Scatterplot demonstrating the fish body weight (g) of all consumed fish by the adult seal in relation to the weight of their remains (g). A linear regression line has been added, and is described as WR = 0.6187FBM - 337, where WR is the weight of remains and FBM is fish body mass.

For example, assuming an initial weight of 1400 g, remains of cod left by the seal would be approximately 500 g when using this equation.

3.3.1.2 Calculating energy waste

There was a positive correlation between the mass of the fish and the total energy wastage (i.e. the energy content of the fish remains measured in calories; **Figure 8**). As body mass of the fish increases, so does the energy wastage. The energy waste when not consuming the whole cod for the adult seal can be estimated with **equation 13**, and allows for the approximation of food wastage in terms of calories to be estimated, which in turn can be used to account for the part of the fish that is excluded.

$$TEW = 0.548FBM - 306$$ Equation 13

Where: TEW is total energy wastage (kcal).
FBM is fish body mass (g).

The adult seal left approximately 800 g of remains when presented with a fish weighing 1850 g. Considering the fish initially consisted of approximately 2100 kcal, this means the seal wasted approximately 700 kcal – above 30 % of the fish's caloric content – a substantial amount of energy wastage.

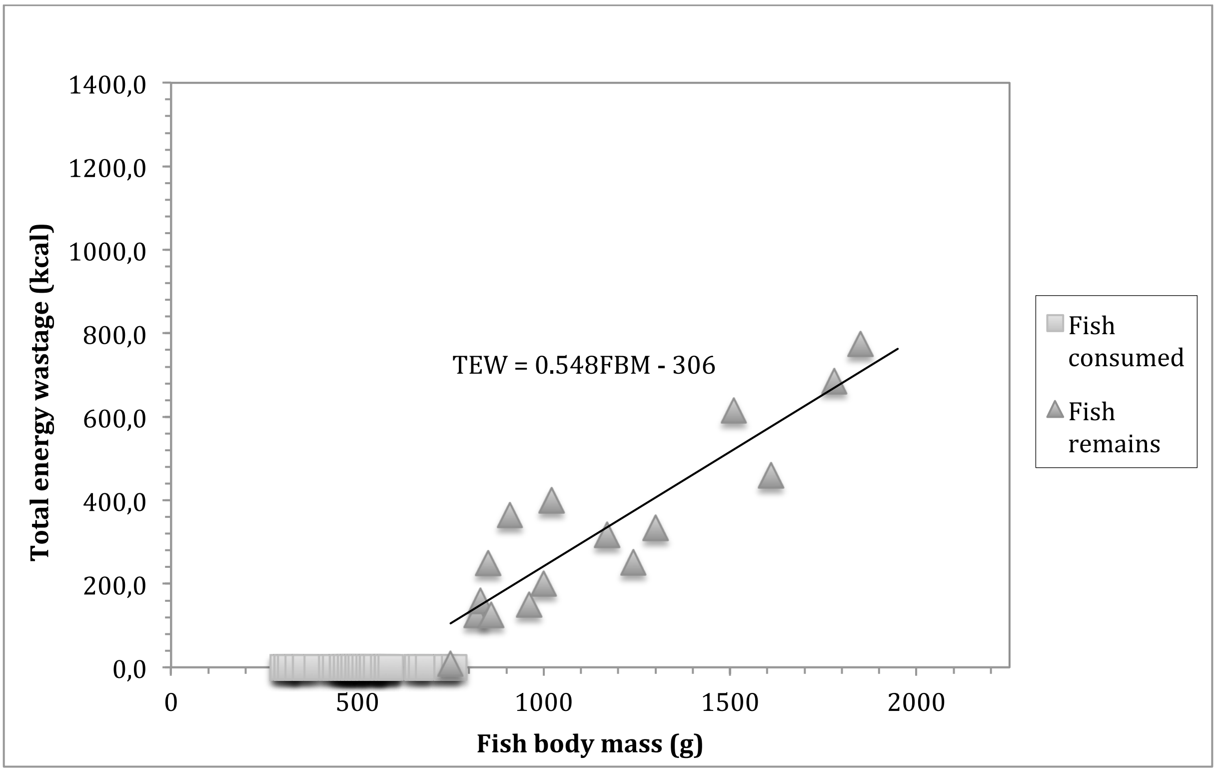

Figure 8. Scatterplot showing total energy wastage (kcal) in relation to fish body mass (g) for the 58 codfish consumed by the adult seal (saithe is not included). A linear regression line is fitted to the values, where TEW is total energy wastage and FBM is fish body mass.

The energy content of all consumed fish were calculated by the regression from the reference fish (**Equation 11**) in addition to the estimation of the energy content of the discarded piece from the same fish. The percentage of energy wastage (i.e. the energy content of the fish remains measured in calories) in relation to the fish's body mass (g) for each fish consumed was then calculated, and is shown in **figure 9.** As the fish body mass increases, so does the energy wastage displayed as a percentage (%). This energy waste from the adult harp seal ranged from 1 – 37 % depending on the fish's body mass. The regression line described can be used to estimate the energy wastage (%) of a fish of a given size when presented to a 90-100 kg adult harp seal. As an example: when an adult harp seal captures a cod weighing approximately 1200 g, over 20 % of the fish's energy will be subsequently lost to the seal's environment.

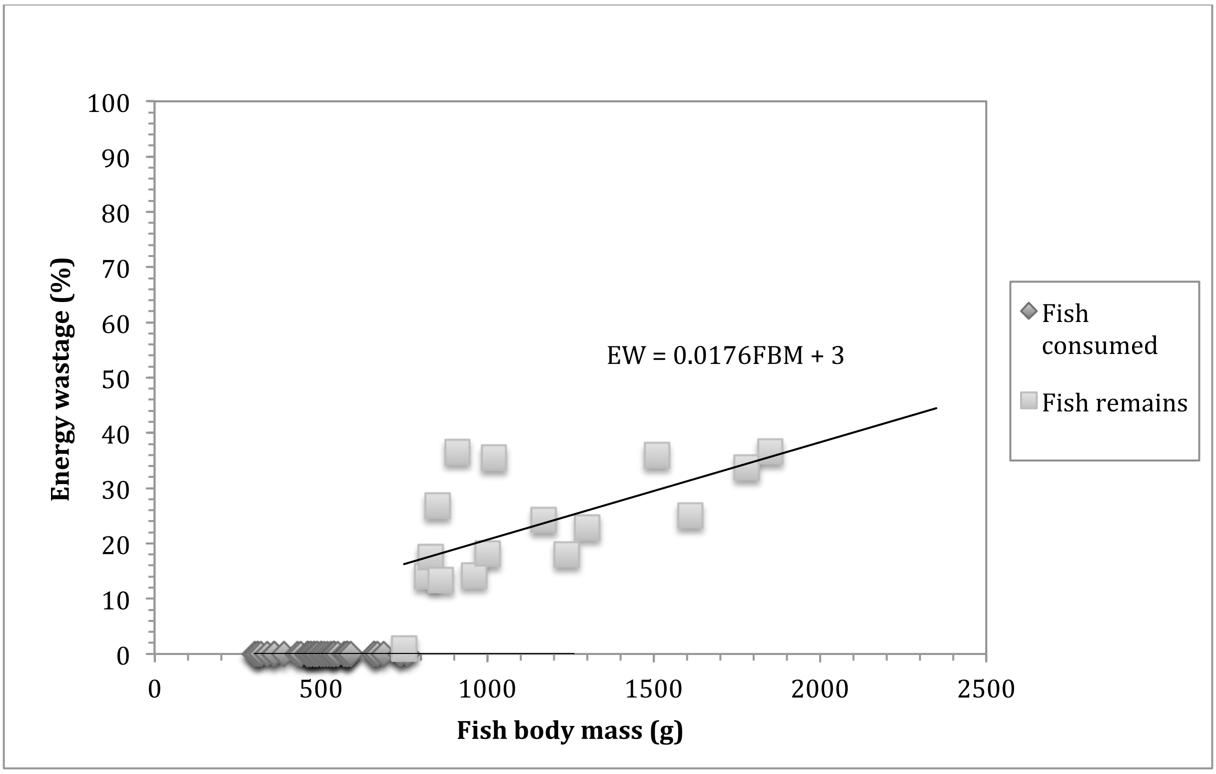

Figure 9. Scatterplot showing the percentage of energy wastage in relation to fish body mass (g) for each of the 58 codfish consumed by the adult seal. A linear regression is included, where EW is energy wastage and FBM is fish body mass

3.3.2 Juvenile seal

The juvenile was presented with 59 fishes, consisting of 39 cod and 20 saithe. Out of the 59 fishes, 51 (86.4%; 32 cod, 19 saithe) were eaten either whole (29 fishes) or partially (22 fishes). The juvenile seal received on average three fish ranging between 90 g – 1350 g, and consumed on average 424 g per experiment.

The juvenile seal consistently left two body-parts, namely the head (in seven cases, both cod and saithe) and cod lacking its abdomen (in 15 cases; **Table 7**, **Figure 10**). The leftovers were categorized by body part and the energy content (kcal) was calculated using the average energy content from the same category produced by ALS (**Table 5**). The average energy content of the groups are listed in **Appendix V**. All remains contained intact otoliths.

Figure 10. The fish remains frequently discarded by the juvenile seal. Head of cod (upper) and cod lacking its abdomen (lower).

Table 7. All remains from the juvenile seal displaying fish body mass (g), the weight of the discarded parts (g), type of body part (HS: head of saithe, H: head of cod, A: cod lacking its abdomen), and energy content (kcal) of the remains.

Fish body mass (g)	Weight of discarded parts (g)	Body part	Energy content (kcal)
220	70	HS	56
280	110	HS	88
390	140	HS	112
390	120	HS	96
440	120	HS	96
560	230	H	192
650	325	H	272
120	120	A	114
220	210	A	200
250	240	A	228
260	230	A	219
270	240	A	228
300	270	A	257
310	280	A	266
310	280	A	266
340	320	A	304
360	360	A	342
370	350	A	333
380	350	A	333
400	390	A	371
440	420	A	399
460	420	A	399

3.3.2.1 Fish sizes and calculating the weight of the remains

Whereas the adult seal displayed a clear trend in how much remains that would be left behind depending on the fish body mass, the juvenile seal showed less of such a tendency. Of the 51 fishes that were eaten, fishes less than or equal to 380 g were either swallowed whole or torn up in pieces, while fishes greater than 380 g was always torn up by the seal before being ingested possibly due to the limitation of its jaw size (**Figure 11**). In the cases when remains were present, heads from the fishes were not consumed, and all otoliths were still in the discarded fish pieces. The juvenile seal showed a consistent belly-biting behaviour, where it only consumed the soft parts of the body (abdomen with liver and other abdominal organs). This was performed in fifteen cases, and only involved cod (**Figure 11**), which may be due to several factors including that the seal was not particularly food motivated, i.e. low appetite; that there were too many bones in the cod; that the seal had developed a preference for herring, etc. This behaviour was not exhibited when saithe was presented, which is further discussed in **section 4.2.2**. The juvenile seal showed a clear preference to saithe since 19 out of 20 were eaten; five of which were collected as discarded heads (**Figure 12**).

The remaining weight of the cod not consumed in relation to the fish body mass gave this regression, and can be estimated by:

$$WR = 1.2143FBM - 458.5 \qquad \text{Equation 14}$$

Where: WR is the weight of the fish remains (g).
FBM is fish body mass (g).

Using this equation, approximately 150 g of the fish would be discarded if a juvenile seal were offered a cod weighing 500 g.

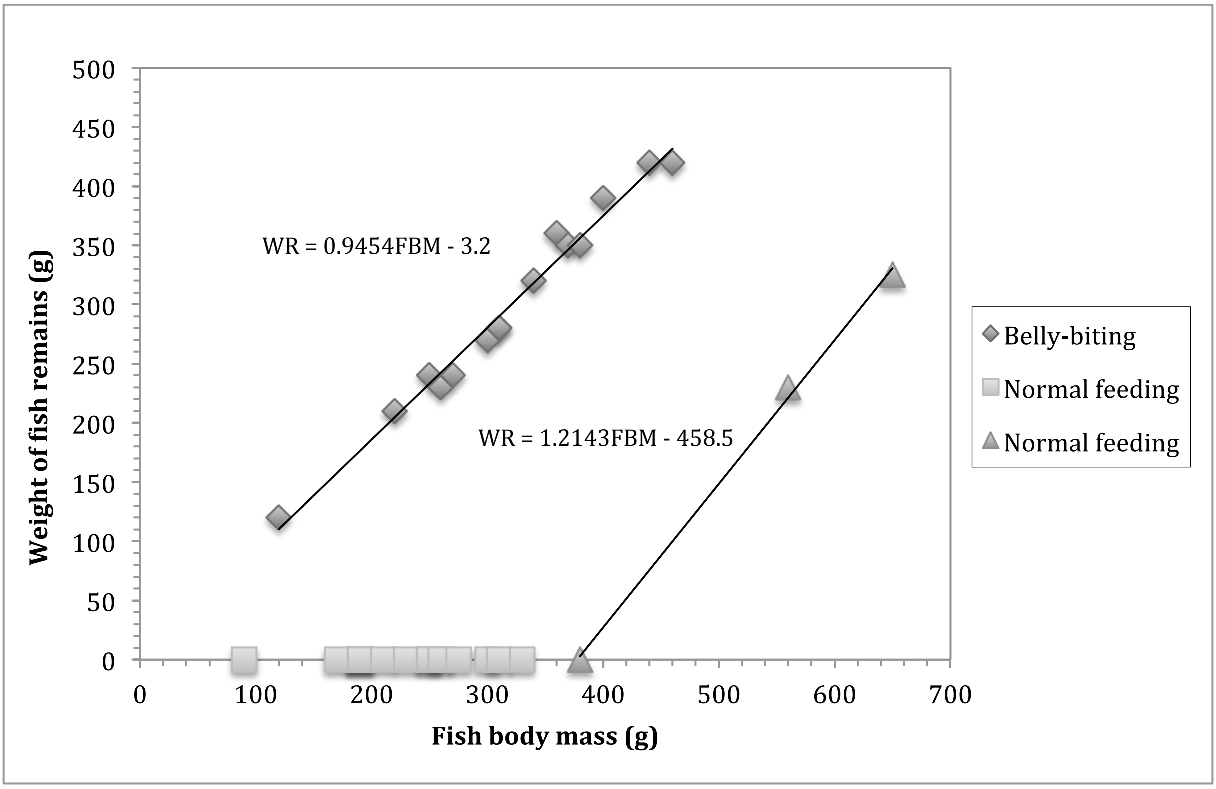

Figure 11. Scatterplot demonstrating the weight of fish remains (g) in relation to the fish body mass (g) of the 32 cod that were presented and consumed by the juvenile seal. Black diamonds represent the behaviour belly-biting, grey squares and triangles represent "normal" feeding. A regression line has been fitted to both, where WR is the weight of the fish remains, and FBM is fish body mass.

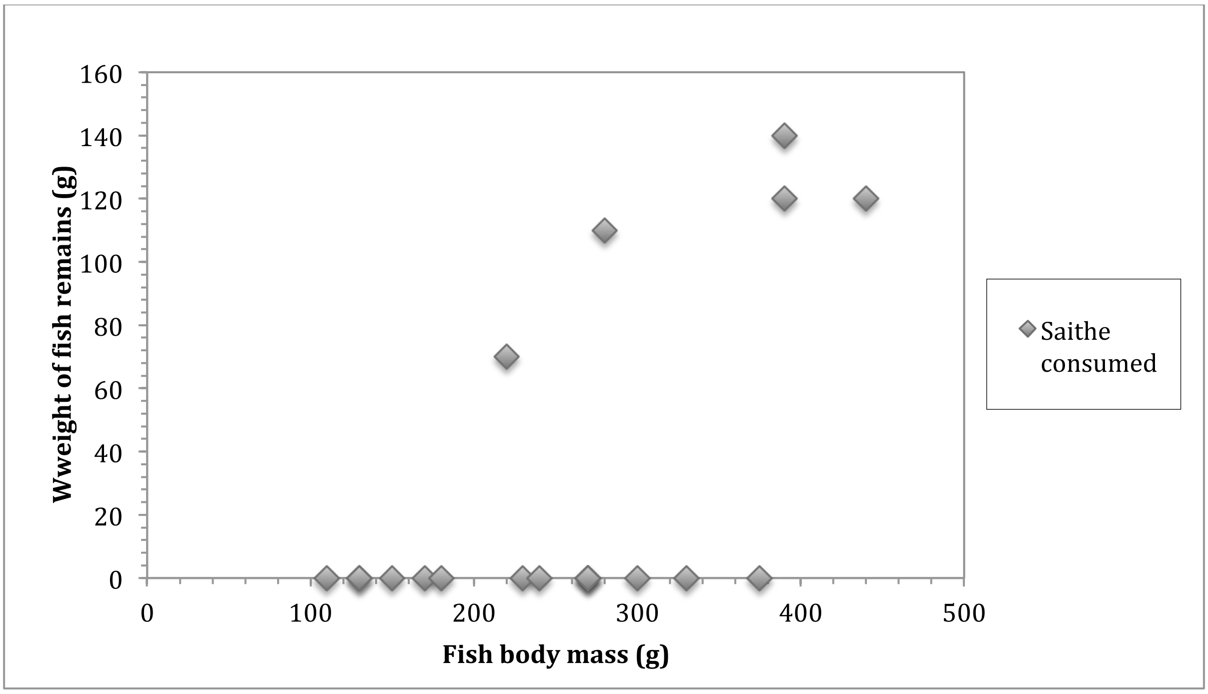

Figure 12. Scatterplot demonstrating the weight of fish remains (g) in relation to the fish body mass (g) for all 19 consumed saithe presented to the juvenile seal. Remains were collected from five saithe.

3.3.2.2 Calculating energy waste

As with the adult seal, the juvenile also showed evidence of increased energy wastage with the increasing weight of the fish. However, the juvenile displayed a more increasing trend, consequently losing more energy than that of the adult seal. The total energy wastage of all codfish eaten "normally" is shown in **figure 13**, and the energetic wastage of not consuming whole cod can be estimated by this regression:

$$TEW = 1.016FBM - 384 \qquad \text{Equation 15}$$

Where: TEW is total energy wastage (kcal).
FBM is fish body mass (g).

The total energy wastage would amount to approximately 330 kcal when feeding a juvenile harp seal a cod weighing 700 g.

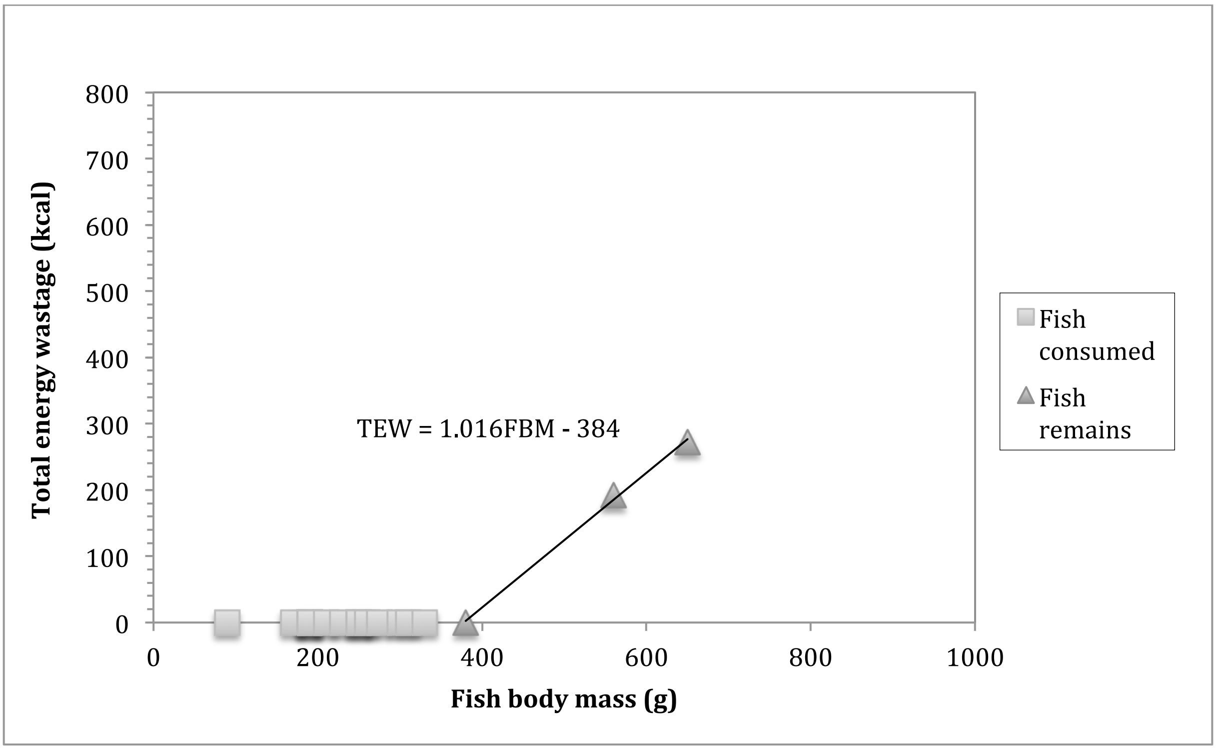

Figure 13. Scatterplot demonstrating total energy wastage (kcal) in relation to fish body mass (g) for the 17 codfish consumed by "normal" feeding by the juvenile seal. A linear regression line has been fitted to the values, where TEW is total energy wastage and FBM is fish body mass.

Total energy wastage after belly-biting is presented in **figure 14**. The amount of energy waste increased with the increasing fish body mass. This can be explained by the amount of mass ingested being far less than that of the mass left behind when performing the behaviour known as belly-biting, and is further discussed in **section 4.2.2**.

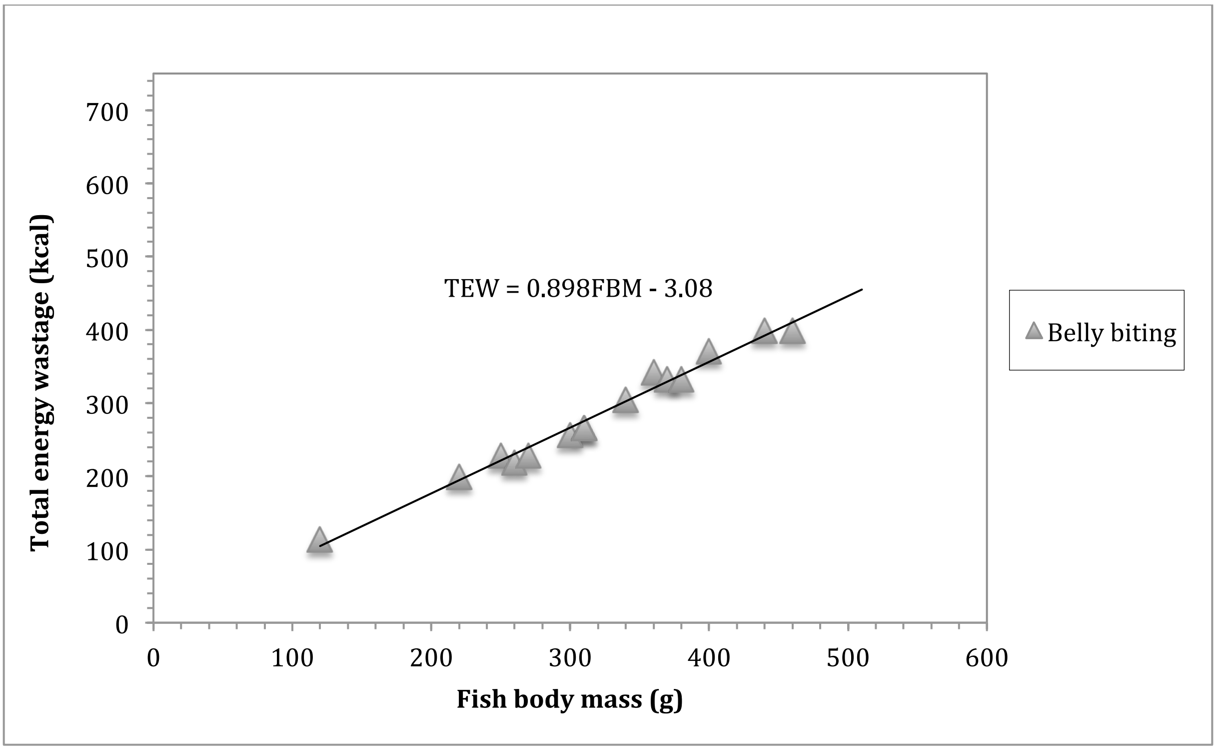

Figure 14. Scatterplot demonstrating total energy wastage (kcal) in relation to fish body mass (g) shown for the 15 codfish consumed by belly-biting by the juvenile seal, with a linear regression line fitted and described. TEW is total energy wastage and FBM is fish body mass.

The energy wastage a juvenile harp seal is experiencing when partially consuming a cod is displayed in **figure 15** as a percentage. This value increases linearly with the increasing fish body mass (g). A juvenile harp seal consuming a 650 g cod would lose around 40 % of the fish's energy content.

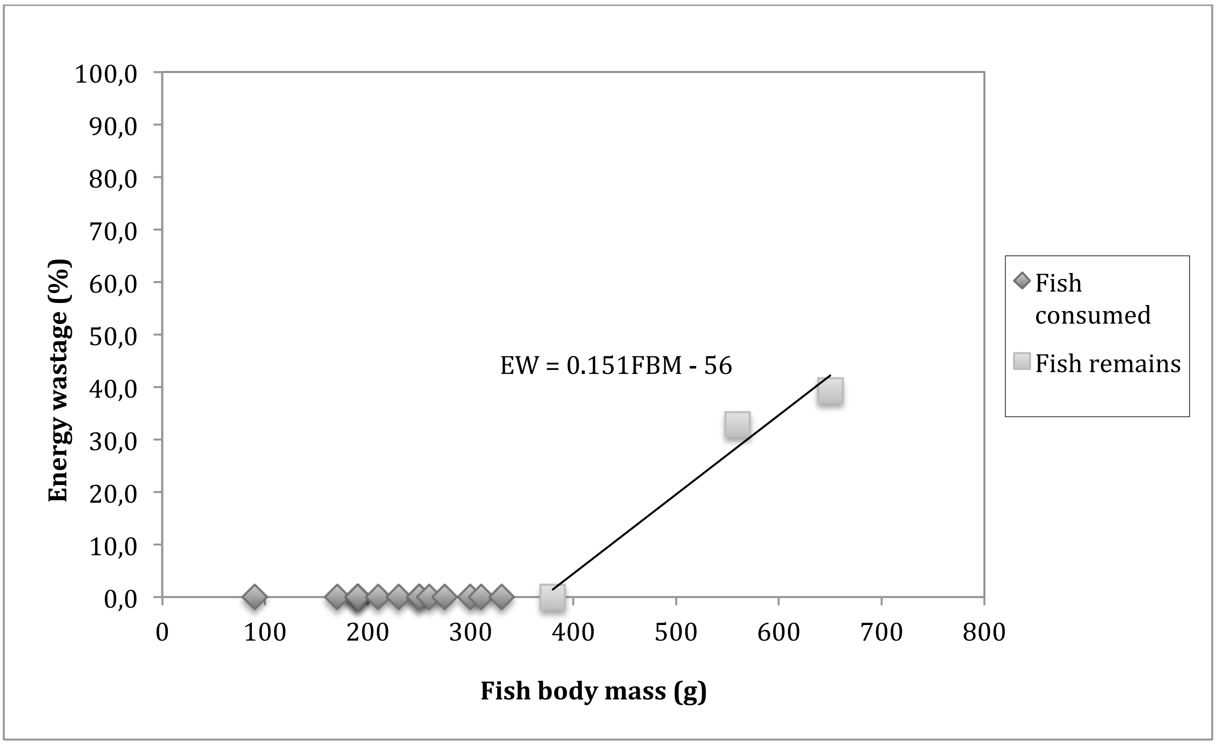

Figure 15. Scatterplot showing the percentage of energy wastage in relation to fish body mass (g) for each of the 17 codfish consumed by the juvenile seal (saithe or remains without abdomen are not included). A linear regression is fitted, where EW is energy wastage and FBM is fish body mass.

3.4 Collected scat and regurgitations

Fish bones and undigested parts were found on seven occasions during the study (7th, 9th, 14th, 16th, 19th, 27th, 28th of October). They were retrieved from the tank of both the adult and the juvenile seal, and collected with a net no more than a couple of hours after the experiments had finished. Remains from the 27th October were collected when the tank was drained. Fish bones were found in the adult's tank on all occasions except for the 28th of October, while bones were collected from the juvenile's tank on two days only (19th and 28th). Vertebral columns (intact or in pieces) were found on all occasions, and were therefore the most frequent part recovered (**Figure 16**). During the 27th and 28th of October, undigested parts including otoliths, stomachs, gills, pyloric caeca, jaws, and skin were found. The undigested state of these remains suggests that they had been regurgitated. This raises the question of how many times these parts were not included in the weighing of the fish remains, and may therefore present some uncertainty as to how much the remains actually weighed but the remains were impossible to relate to the exact fish, and were therefore impossible to include in the calculation of energy wastage.

Interestingly, skulls containing otoliths were also found on three occasions.

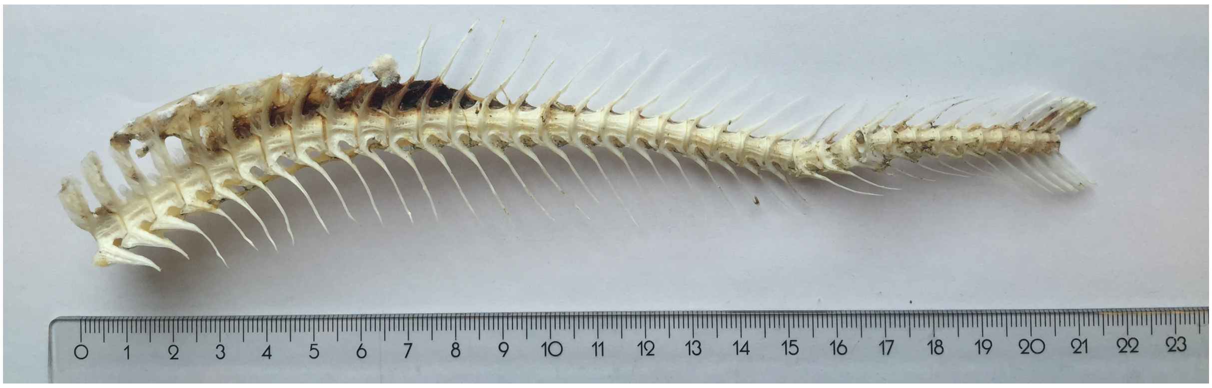

Figure 16. Undigested vertebral column collected from the adult harp seal tank 9th of October.

3.5 Feeding behaviour

3.5.1 Adult seal

The adult seal swallowed fish whole and head first that were of a size less than or equal to 750 g. This was performed under water. The adult attempted to swallow larger cod (>750 g) whole when presented with them, however, all attempts were unsuccessful. It would secure its prey with a sudden movement, and bite on the fish neck in order to kill it, before it was brought to the surface. At the surface, the seal would use its claws to rip the flesh apart. The neck and abdomen were consumed first, before the head was separated from the body of the fish. The body would then be consumed whole if small enough, or the seal would continue to tear the fish into smaller pieces. The head would be unguarded and left alone until the body was fully consumed, and only occasionally revisited after ingestion of the body. Only part of the head was consumed during such incidents, and most was left as discarded pieces.

3.5.2 Juvenile

The juvenile seal showed a more playful behaviour when presented with the fish, where it would bite, play, and push the fish before killing it. The juvenile would also occasionally remove the intestines of the fish, and play with them. It displayed the same technique as the adult when consuming fish larger than 380 g. The abdomen was consumed first, followed by the separation of the fish head. The body was always ingested prior to the head of the fish.

4. Discussion

Captive feeding studies on marine mammals can provide insight into several aspects such as uncertainties associated with diet analysis, and feeding behaviour and habits, which in turn can lead to the improvement of current population-level consumption models and estimates. The challenges in execution and consequent lack of observational studies in the wild on these deep-diving, remote living marine mammal species makes captive feeding studies a valuable source of information, enabling a better understanding of the behaviour of marine mammals such as pinnipeds despite the approximation to free living behaviour that these represent.

The diet of harp seals has been studied in detail, confirming the theory that these animals are generalists, displaying highly variable diets depending on their seasonal distributions (Folkow *et al*., 2004, Nordøy *et al*., 2008). Crustaceans with high-energy content dominate in the summer and autumn, while fish such as polar cod, capelin and herring represent their main prey during the winter (Nilssen *et al*., 1995b, Lindstrøm *et al*., 2013). To what extent they consume larger fish such as cod, and how they do it, remains to be described. Since studies quantifying behaviours such as belly-biting and rejection of fish heads (with the subsequent proportion of food left behind) are scarce, food consumption estimates are based on the assumption that fish are consumed whole by harp seals, not accounting for the caloric energy waste the seals might experience when feeding on only parts of its prey. If behaviours such as belly-biting, where the seals seek out the most energy rich parts of a fish, i.e. the liver, and rejection of the fish head, which might not contribute much to the total energy gain, are frequently used by pinnipeds when feeding on larger sized prey, the importance of these species as prey can be underestimated, thereby causing the calculations of food consumption models to be imprecise.

The energetic waste of partially consuming the prey, in terms of the caloric value in the discarded piece(s) of the fish (not accounting for the energy the seal acquires for its metabolism) is an interesting aspect of harp seal feeding behaviour. The ability to account for this energy wastage in harp seals is of great interest since it would improve population-based food consumption models as well as allowing the interaction between harp seals and commercial fisheries to be re-evaluated.

This thesis is the first of its kind to provide a description of the caloric energy wastage occurring when seals are fed experimentally on larger sized fish. In this study, we examined the consequences of feeding on various sizes of fish, i.e. cod and saithe, in two female harp seals, and we estimated the energy wastage that occurred when pieces of fish were discarded by the seals.

The results of this study indicate that in this particular experimental setup:

1. Harp seals show preferences for specific parts of their prey when prey reaches a certain size.
2. There is an apparent selection based on energy content.
3. The waste of energy increases linearly with increasing size of prey.

4.1 Technical aspects

4.1.1 Environment

In order to collect the remains of the fish, and to maintain consistent conditions during all experiments, studies like this must be executed in confined settings that display similar conditions over long periods of time. Such settings would be difficult to achieve in the wild, and are thus best performed in captivity. Unfortunately, the non-natural environments generate artificial situations that may impact the seals, thereby complicating the interpretation of the results. These confounding effects are discussed in the following sections. Parameters such as water osmolality and seawater temperature were monitored throughout the study to ensure similar conditions to that of the natural environment of both seals and fishes.

4.1.2 Seals

Two female seals (one juvenile, one adult) were used in this study. A large sample size is difficult to obtain when working with captive animals, and due to the restricted size of the study area it would not have been possible to preform this on more animals. A high number of fish were utilized in order to account for this small sample size. One can argue that the captivity and well-established daily feeding and training routines (that existed 1 ½ years prior to this experiment), might have impacted the seals' incentive to hunt live fish. However, both seals showed interest in pursuing and consuming prey in an apparently natural manner.

The seal's motivation for catching and eating fish is an important aspect of this study. It is quite possible that the seals would lose motivation or become satiated, and cease to pursuit the last fish in the experiment, displaying a higher incentive to prey on the fish presented to the seals at an early stage of each experiment. The sizes of fish presented to the seals were random, i.e. some trials would start with large fish, and some with small fish. However, all fish in the study were treated as individuals, not reflecting the number they represented, which may have had an impact on the analysis. In order to increase the motivation of the seals, food restriction with partial fasting was initiated every other day, which was assumed to stimulate their appetite. The daily routine could possibly reinforce this aspect, since the seals were accustomed to feed a minimum of twice a day, thereby increasing their motivation to feed once fish was available. Additionally, feeding the seals with live fish was performed to trigger the seal's innate hunting instinct and inherent natural feeding behaviour. Lastly, the months in which this study was performed represents the period during which harp seals gorge themselves in food in order to build up energy resources after the periods of breeding and moulting.

4.1.3 Fishes

In order to make sure that the seals would be provided with the size distribution of fish they would feed upon in the wild, previous diet studies were contemplated. The Barents Sea harp seal population is estimated to consume 100.000 tonnes of cod annually, with higher rates if the capelin stock is small (Nilssen *et al.*, 2000). Gadoids also dominated the seals diet during the invasions of the Norwegian coast in the late 1986-1988, and again in 1995 (Haug *et al.*, 1991, Nilssen *et al.*, 1992, 1998). Lawson *et al* (1995) estimated the mean length of Atlantic cod consumed by harp seals in near-shore waters in Canada to range from 18.3 cm – 25.3 cm between years. Offshore areas averaged at 35.8 ±10.49 cm, with the occasionally larger cod consumed (max 50.5 cm) (Lawson and Stenson, 1997). During the seal invasion on the Norwegian coast, the majority of cod length ranged from 10 cm – 30 cm (Nilssen *et al*., 1992). The fishes utilized in this study ranged between 20 cm – 70 cm, and 90 g – 2710 g (**Appendix VII**), representing a large range of what harp seals would encounter in the wild.

Since each fish was presented sequentially and in a tank without natural elements, neither the natural landscapes, nor saithe's natural schooling setup were met. The fish presented would probably not react the same way as they would have in the wild, e.g. by hiding in kelp or having protection in numbers, and this setup could be seen as facilitated feeding. However, the fish were gently placed in the tanks after being acclimated, and not handed to the seals in order to try to account for the non-natural setup and to assure, in any way possible, that the fish were behaving as they would in the wild.

4.2 Fish remains

Current food consumption model are based on hard parts such as fish bones, otoliths, exoskeletons, cephalopod beaks, and intact specimens. Prior diet studies have provided knowledge on the lengths of cod consumed from the collected otoliths, indicating that the majority of cod ingested by harp seals range between 10 cm and 35 cm, with larger fish consumed occasionally (Nilssen *et al*., 1992, Lawson and Stenson, 1997, Hammill and Stenson, 2002). It is generally assumed that otoliths and cephalopod beaks found in the stomach of the seals display the correct picture when it comes to size distribution of prey and the seals diet composition. Since otoliths are the main indicator when estimating the importance of cod in the harp seal diet, it is assumed that the fish are consumed whole due to the location of such structures, which lies in the head directly behind the brain – a part of the fish that is ingested when predators swallow their prey whole. However, the type and size distribution of the otoliths may not be representative of the actual ratio of each species due to partial feeding, such as in the case of rejection of the head or belly-biting, and regurgitation of hard parts.

4.2.1 Selective rejection of heads

The assumption that cod is consumed whole implies, as previously mentioned, that no remains are left behind, and that no energy is wasted. In light of the results of this study, it appears that fish that are small enough to be swallowed whole are consumed as such, while larger fish are not (i.e. **Figure 7**). There are multiple studies suggesting that seals selectively reject the fish heads of larger fish (Pitcher, 1980, Phillips and Harvey, 2009, Sweeney and Harvey, 2011), and bring the prey to the surface before ripping it apart with their claws (Brown and Mate, 1983, Roffe and Mate, 1984, Davis *et al*., 1999). This is confirmed by the adult and juvenile seal in this study, whom were displaying a foraging behaviour where fish above 750 g and 380 g respectively, were torn up prior to ingestion, leaving remains such as pieces of heads, heads with parts of the back and backbone attached, and fish lacking their abdomens (**Table 6 and 7**).

There will undoubtedly be individual variations in how large of a fish an adult harp seal is able to swallow in one go, however, the results of this study show that the adult seal was not able (or did not choose) to swallow fish whole above 750 g (**Figure 7**). In the case of the adult seal, we were able to collect the remains of all fish that were torn up prior to ingestion. Due to the limitations of the study situation, in which the seals were fed on a daily basis prior to the experiment, the individuals' anticipation or knowledge that they would get fed eventually (and therefore not spend a lot of time on the torn off heads) could potentially bias the results. This potential error is difficult to account for when using captive animals. However, all remains were left in the pool for 30 minutes before removed in order to give the seal enough time to consume the fish *ad libitum*, and in the case of the adult seal, the fallen pieces were not consumed or returned to, contradicting the statements of Pemberton *et al* (1994), which suggests that all fragments are recovered by captive harp seals. It might also be suggested that the harp seals in this study could afford to be selective in the sense that they were expected to be fed, if not the same day, the day after. This may result in a smaller amount of food being consumed, since both harp seals were provided with the remaining daily ration of sliced fish after each experiment. However, a pause lasting 1 - 4 hours was carried out (depending on the day) before the seals were fed the rest of their daily ration to minimize this aspect. Additionally, the consumption of larger prey might have affected the seals, demanding further handling time. This can be applied to seals in the wild, determining if they chose to consume prey of smaller size in order to minimize handling time, and rapid switch to another prey item. The setup of the experimental design where each harp seal was given fish sequentially may not be representative of how they encounter their prey in the wild. This does not mean that cod is not consumed, and that they would not use time to handle larger fish. It is, however, unknown whether harp seals consume what is available or specifically target smaller prey, in this case cod. Harp seals have been found to show neutrally prey preference to Atlantic cod (i.e. exploiting cod randomly), which is confirmed by their low intake off the coast of Newfoundland (Lawson *et al*., 1998), and positively selective (i.e. preference for cod) towards cod in the Barents Sea (Lindstrøm *et al*., 1998). However, small and large cod were not distinguished, and the abundance of Atlantic cod in the waters off Newfoundland were low at the time of calculation. Similarly, the positive selection of cod in the Barents Sea could be due to a possible underestimation of cod in that particular area. Later investigations have shown harp seals to either avoid or randomly exploit gadoids (Lindstrøm *et al*., 2013) Similar studies on grey seals concluded that cod was not selected based upon its size (Bowen and Harrison, 1994), therefore it is possible that harp seals behave in the same way. In any case, harp seals are known to feed on a variety of cod sizes, consuming prey up to 65 cm in size (Nilssen *et al*., 1992).

The juvenile seal did not consume fish above 380 g without tearing it apart (**Figure 11**), and all of the remains that were collected included the head of the fish, and thus its otoliths. The collected remains also included the heads of smaller sized fish, fish that could have been swallowed whole (i.e. saithe, initial body mass 220g). With an average of three fish, both cod and saithe, during each experiment, the reason for why it would leave the heads but still consume its daily ration after the experiment is unclear. While the anticipation of additional fish may have influenced this behaviour, due to the small amount of fish received, this is speculative. The fish was left in the tank until the seal ceased to display interest to it in order to allow for remaining parts to be revisited.

4.2.2 Belly-biting

Whereas the theory that harp seals do reject the head of the fish when it reaches a certain size is strengthened by this work, the occurrence and frequency of belly-biting in the wild cannot be easily confirmed based on captive animal experiments due to several technical aspects. Belly-biting has been observed by several seal species, especially harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) in connection with commercial fishing nets and fish farms, where they prey on trapped fish through the meshes of the nets (Cosgrove *et al*., 2013, Northridge *et al*., 2013). Depredation of cod by harp seals in Norway has also been described, particularly during the so-called “seal invasions” where commercial gillnet fisheries reported damaged fish. In this instance, large fish (3 - 4 kg) entangled in nets were missing their ventral soft parts (Nilssen *et al*., 1992). There is still some uncertainty regarding to what extent this behaviour occurs in the wild with free-swimming large fish, where food items are less dense/abundant. It seems unlikely that predators such as harp seals only consume the soft parts of their prey if food is scarce. As a consequence it should be safe to assume that belly-biting does occur occasionally in the wild due to the numerous observations and detailed reports of seals feeding on fish bellies, with additional sightings from divers that have found dead cod with their abdomens missing (Fu *et al*., 2001, McLaren *et al*., 2001, Lilly and Murphy, 2004, Chouinard *et al*., 2005, O’Boyle and Sinclair, 2012). However, other studies reject the theory based on sightings done on-board offshore trawlers, where all Atlantic cod were swallowed whole and head first with a mean length of 49 cm (Pemberton *et al*., 1994). In this study, the juvenile harp seal performed belly-biting on 15 cod (**Figure 11 and 14**), whereas the adult did not, indicating that this behaviour may be more common in younger harp seals. When exhibiting such behaviour, the weight of fish parts ingested is far lower than that of the total weight of the fish. Indeed, this could be performed to enhance predatory skills, or for the purposes of playing/socializing, or teaching purposes. It is possible that the juvenile seal only consumed the abdomen of the cod due to the anticipation of receiving additional fish, selecting the energy-rich parts in spite of the food restriction and partial fasting. This could be explained by the fact that belly-biting is thought to occur when the abundance of prey is high such as in the case of depredation or when the fish reaches a certain size. In contrast, the juvenile did not perform this behaviour on similar sized saithe, indicating that there is a disparity between the two species. Since cod contains larger and more robust bones, saithe is more similar to herring, to which the seals were accustomed, and therefore the juvenile seal might be more inclined to consume the latter.

The use of juvenile seals captured during their post-weaning fast in captive feeding studies could also have influenced the results since their ability to feed as a seal raised in the wild cannot be easily assessed. The juvenile harp seal utilized in this study was accustomed to receiving pieces of dead herring and capelin from the trainers without having to tear the fish in bite size parts by itself. Whereas the adult seal did not have any problems with the handling of the fish, the juvenile showed some evidence of difficulties whilst attempting to consume larger fish in the form of head thrusting and sound, and this might be the reason for it to perform belly-biting, though it is difficult to confirm this impression. However, prior to the experiments in this thesis, the juvenile was kept in a sea pen for a couple of weeks for another study, during which it was observed preying on different fish species naturally present in the enclosure, demonstrating its innate hunting instinct.

4.2.3 Selection based on energy content

The average energy content of the reference fish in this study was calculated to be 102.4 kcal/100g ± 7.03, with five values ranging from 91.6 kcal to 113.3 kcal (**Table 4**). This yields 4.3 kJ • g^{-1} (wet mass), which is slightly lower than other studies performed on cod during the same period (Mårtensson *et al*., 1996).

Since the harp seals in this study did not consume the heads in the majority of the experiments, one explanation can be derived: body parts such as a cod's head consists mainly of bones, and it might be disadvantageous to consume a part that requires extensive handling, prolonged digestion and lower caloric intake. Within this context, selection of parts in which the majority of energy lies might be favourable in order to gain maximum value of its prey, and minimizing the handling time. Whereas the mean energy content of the whole fish is 102.4 kcal/100g ± 7.03, the mean energy content of the remains that only included the head of cod yields 84.4 kcal/100g ± 4.97. When including the remains of cod consisting of head with parts of its back attached, the energy content increases to 86.2 kcal/100g ± 3.59. This shows that the head represents lower energy yields, and therefore, it might be beneficial in a typical situation to select the parts of the fish that are easy to consume and contains more energy by rejecting the parts with lower energy of the fish such as the head. By excluding the head, and not filling its stomach with low energy material, the seal has the possibility to profit from more energy dense parts of the fish' body.

Another type of selection, when the seal is consuming only the abdomen and intestinal organs, could be based on energy content. The gadoids typically store their energy as lipids in the liver, which may account for up to 9 % of the body mass in mature Northeast Arctic cod (Yaragina and Marshall, 2000). These reserves are used when the metabolic demands are not sustained by food intake, either during overwintering, migration or reproduction/spawning when the food intake is low and/or they engage in fasting (Holdway and Beamish, 1984). The total lipid content of cod exhibits seasonal fluctuations, mainly due to the variation of the lipid composition in the liver, which reaches a maximum lipid content just prior to spawning from February to April (Jørgensen and Fiksen, 2006). Thus the possibility that harp seals engage in belly-biting to gain access to the lipid-rich liver could be advantageous when the abundance of cod is high.

4.2.4 Calculating energy waste

As the results indicate, when a part of the fish is not consumed, some energy is consequently lost to the seal. The two equations presented in the results (**Equations 13 and 15**), convey the total energy wastage (measured in calories) of only consuming cod partially for both the adult and juvenile harp seal in this study. When harp seals selectively choose the high-energy parts of the fish, energy in terms of calories, will be lost in the discarded part to the seals environment. They will, however, have the possibility to continue to feed on additional energy-rich parts of other fish rather than being satiated by “lower-energy” fish parts. The seals in this study lost from 1 % to 40 %, of the energy from the fish by not consuming it whole. This was directly related to the fish body mass, and the energy waste increased with the size of the fish. The energy that the seals “lose” has to be obtained by consuming additional prey, which will subsequently result in more prey being consumed. If such results also apply to the harp seals in the wild, the amount of cod removed from the ecosystem by harp seals might be underestimated and should therefore be re-evaluated.

4.3 Implications for food consumption models

4.3.1 The absence of otoliths

As previously mentioned in the introduction, otoliths serve as an identification tool when classifying different species by hard parts from stomach, intestine and faecal samples. However, there are several challenges with the identification and use of otoliths. Firstly, the recovery of otoliths is positively correlated with their length, width, and robustness, which vary between species (Tollit *et al*., 1997, 2007). They erode at different rates, with gadoid otoliths being robust and eroding slowly, whilst herring otoliths may be fully eroded after six or seven hours (Jobling and Breiby, 1986, Jobling, 1987). This will give a bias toward the isolation of larger otoliths that persist longer, whilst fragile ones may be missed. Prey without hard parts, or soft-bodied prey, may be overlooked, and thus may not be included in the estimate of dietary composition. Lastly, otoliths erode when they travel through the gastrointestinal tract, making the initial size of the prey difficult to determine. Size may often be underestimated, especially if the fish is of larger size, due to the higher proportion of partial erosion than in otoliths from smaller fish (Tollit *et al*., 1997). In order to account for such problems, the application of numerical correction factors (NCF’s) can help to improve the estimates of number of prey consumed, while digestion correction factors (DCF’s) will take the erosion of otoliths into account and thus improve the estimate of the initial size of the fish. Incorporating the two will improve the accuracy of size estimation and quantity of prey (Tollit *et al*., 1997, Grellier and Hammond, 2006, Phillips and Harvey, 2009, Wilson *et al*., 2013). There are, however, no correction factors that account for missing otoliths.

In addition to the obstacles of digestion and species-specific otoliths, the absence of them in marine mammal stomachs, i.e. harp seals, resulting from seals either rejecting the head of a given fish or engaging in behaviours such as belly-biting, serve as further implications for accurate food consumption estimates. In this study, the majority of all remains contained otoliths (35/38; 92%), which were consequently lacking from the seals' stomachs. This frequent rejection of the head of large cod (43 cm - 61 cm; **see section 3.3.1**) in the case of the adult seal creates complications when describing the harp seal diet based on the recovery of otoliths from the stomach or intestines if only fish below a certain mass are fully consumed. Fish equal to this length range (43 cm – 61 cm) are generally between four to six years of age (Berg and Albert, 2003), and not collecting otoliths from that size range of cod would impact the estimates of diet studies, underestimating the number of cod consumed (especially older cod) and thus its relative importance in harp seal diets. Furthermore, the ratio at which ingestion of head and otoliths versus parts of the body may differ between species, where the heads of larger species (i.e. cod) would be more often discarded, whereas the heads of smaller species would not. This would impact the diet estimates additionally (Brown and Mate, 1983, Jobling and Breiby, 1986).

Harp seals do, however, consume the head of larger sized cod (>43 cm), indicated by the otoliths retrieved from their stomachs (Lawson and Stenson, 1997). Although the results of this study indicate that otoliths from fish larger than 1240 g (54 cm) would be absent from the stomach contents of the two harp seals, such sizes have been recovered in seal stomachs in the wild suggesting that the head is not always rejected.

4.3.2 Regurgitations of fish parts

The undigested bones, skulls with intact otoliths, and other parts of the fish that were collected on seven occasions during the study indicate that regurgitation might be frequent when feeding on bony fish species. This has been observed in different seal species, both in water and on land (Kirkman *et al*., 2000, Bowen *et al*., 2002). Such regurgitations were recovered when Steller sea lions (*Eumetopias jubatus*) had meals that consisted of large fish (Tollit *et al*., 2007). Regurgitation may be performed to remove large bones, or help with further digestion if such items are re-ingested after regurgitation. Bowen *et al* (2002) discussed the possibility that the regurgitations were to remove sand and saltwater, whereas Tollit *et al* (2007) suggested that larger sized fish, or specific species of prey might be regurgitated due to their size or species. The latter study showed that 87.5 % of Pacific cod otoliths (*Gadus macrocephalus)* had been regurgitated. In either case, regurgitations of parts that include bones and otoliths would further compromise estimations of diet composition of cod when using estimates based on HPA methods, and would underestimate species that are regurgitated more frequently. Current food consumption models might be biased considering that hard parts from these prey species may be absent from the stomach, intestine and faecal samples.

On the other hand, gastroliths (pebbles or stones in stomach) have been found in a harp seal that was caught in a gillnet during the seal invasion along the coast of North Norway, indicating the assistance of the digestion of hard fish bones and flesh (Nordøy, 1995), which can reduce the importance/frequency of such regurgitations.

5. Conclusions and future research

This thesis provides insight into how a harp seal consume large fish such as cod. It also calculates the percentage of energy wastage when the seal does not consume the whole fish. Consequently, it reveals important parameters that might be advantageous to incorporate into population-based food consumption models. It appears that the harp seals in this study will reject fish heads as the fishes reach a certain size, and that most of these heads still contain otoliths. As such, it may be valuable to reassess current estimations of food consumption in harp seals, and to apply a correction factor so that the wasted energy associated with only partially consumed fish is accounted for. This can in turn provide new information on harp seal feeding behaviour and food consumption. Although the results indicate that belly-biting occur in juvenile harp seals, more knowledge is required before assumptions can be made regarding whether or not this behaviour is frequent in the wild.

It further suggests that models that use otoliths as a quantitative measure to estimate prey consumption may not give reliable indications of predator-prey interactions, and should therefore be used with caution since these results indicate that otoliths may be absent from stomach, intestine and faecal contents.

Considering this is the first study to calculate the energy wastage when harp seals feed partially on cod, these results should be considered as relative results, not absolute, and further investigations should be carried out in order to improve and/or validate the calculations done in this study. Due to the restricted sample size, additional individuals should be utilized to confirm the theories hypothesised. The supplementary data should corroborate the data found in this study before being used as an input for energy-based population consumption models. Future studies might consider incorporating more species of prey to assess which is preferred, and which is left behind. One option is to offer several fish of various sizes simultaneously, since this experimental setup might be more representative of the seals' natural environment. In addition, females and males of different age-classes should be included to determine if there are any differences between sexes. The season at which the study is executed should also be paid particular attention. By incorporating the study field (large sea pen, 90 m x 30 m) and instruments (acceleration and camera data loggers) of ‘COEXIST’, feeding behaviour can be studied in detail in a more natural environment, revealing additional information regarding the diet composition and preferences of the harp seal as well as detailed prey capture behaviour.

In combination with additional studies confirming the results of this thesis, it would be interesting to conduct similar co-studies on different species in captivity (i.e. hooded seals; *Cystophora cristata*) in order to examine whether they display the same behaviours.

6. References

BERG, E. & ALBERT, O. T. 2003. Cod in fjords and coastal waters of North Norway: distribution and variation in length and maturity at age. *Ices Journal of Marine Science,* 60**,** 787-797.

BOWEN, W. D. & HARRISON, G. D. 1994. Offshore diet of grey seals *Halichoerus grypus* near Sable Island, Canada. *Marine ecology progress series,* 112**,** 1-11.

BOWEN, W. D., TULLY, D., BONESS, D. J., BULHEIER, B. M. & MARSHALL, G. J. 2002. Prey-dependent foraging tactics and prey profitability in a marine mammal. *Marine Ecology Progress Series,* 244**,** 235-245.

BROWN, R. F. & MATE, B. R. 1983. Abundance, movements, and feeding habits of harbor seals, *Phoca vitulina*, at Netarts and Tillamook bays, Oregon. *Fishery Bulletin,* 81**,** 291-301.

CHOUINARD, G. A., SWAIN, D. P., HAMMILL, M. O. & POIRIER, G. A. 2005. Covariation between grey seal (*Halichoerus grypus*) abundance and natural mortality of cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. *Canadian journal of fisheries and aquatic sciences,* 62**,** 1991-2000.

COSGROVE, R., CRONIN, M., REID, D., GOSCH, M., SHERIDAN, M., CHOPIN, N. & JESSOPP, M. 2013. Seal depredation and bycatch in set net fisheries in Irish waters. *Fisheries Research Series,* 10.

DAVIS, R. W., FUIMAN, L. A., WILLIAMS, T. M., COLLIER, S. O., HAGEY, W. P., KANATOUS, S. B., KOHIN, S. & HORNING, M. 1999. Hunting behavior of a marine mammal beneath the Antarctic fast ice. *Science,* 283**,** 993-996.

EP (EUROPEAN PARLIAMENT). 2009. *Regulation (EC) No 1007/2009 Of The European Parliament And Of The Council of 16 September 2009 on trade in seal products* [Online]. Brussels, Belgium, European Parliament. Available: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32009R1007 [Accessed 24 April 2016].

FOLKOW, L. P., NORDØY, E. S. & BLIX, A. S. 2004. Distribution and diving behaviour of harp seals (*Pagophilus groenlandicus*) from the Greenland Sea stock. *Polar Biology,* 27**,** 281-298.

FU, C., MOHN, R. & FANNING, L. P. 2001. Why the Atlantic cod (*Gadus morhu*a) stock off eastern Nova Scotia has not recovered. *Canadian Journal of Fisheries and Aquatic Sciences,* 58**,** 1613-1623.

GJØSÆTER, H. 1995. Pelagic fish and the ecological impact of the modern fishing industry in the Barents Sea. *Arctic,* 48**,** 267-278.

GRELLIER, K. & HAMMOND, P. S. 2006. Robust digestion and passage rate estimates for hard parts of grey seal (*Halichoerus grypus*) prey. *Canadian Journal of Fisheries and Aquatic Sciences,* 63**,** 1982-1998.

HAMMILL, M. O., RYG, M. S. & CHABOT, D. 2010. Seasonal changes in energy requirements of harp seals. *Journal of Northwest Atlantic Fishery Science,* 42**,** 135-152. doi:10.2960/J.v42.m660.

HAMMILL, M. O. & STENSON, G. B. 2000. Estimated prey consumption by Harp seals (*Phoca groenlandica*), Hooded seals (*Cystophora cristata*), Grey seals (*Halichoerus grypus*) and Harbour seals (*Phoca vitulina*) in Atlantic Canada. *Journal of Northwest Atlantic Fishery Science, 26, 1-23.*

HAMMILL, M. O. & STENSON, G. B. 2002. Estimated consumption of Atlantic cod (*Gadus morhua*) and some other prey by grey seals (*Halichoerus grypus*) and harp seals (*Phoca groenlandica*) in the southern Gulf of St. Lawrence (NAFO zone 4T). *Can. Sci. Advis. Sec. Res. Doc 2002/054*.

HAMMILL, M. O., STENSON, G. B., MOSNIER, A. & DONIOL-VALCROZE, T. 2014. Abundance Estimates of Northwest Atlantic Harp seals and Management advice for 2014. *DFO Can. Sci. Advis. Sec. Res. Doc. 2014/022***,** v + 33 p.

HARVEY, J. T. 1989. Assessment of errors associated with harbour seal (*Phoca vitulina*) faecal sampling. *Journal of Zoology,* 219**,** 101-111.

HAUG, T. & BJØRGE, A. 2010. Harp seal. *In:* BJØRGE, A., FAGERHEIM, K.-A., LYDERSEN, C., SKERN-MAURITZEN, M. & WIIG, Ø. (eds.) *Sjøens pattedyr 2010 [Marine Mammals]. Fisken og havet, special edition 2-2010, 36-37.*

HAUG, T., KRØYER, A. B., NILSSEN, K. T., UGLAND, K. I. & ASPHOLM, P. E. 1991. Harp seal (*Phoca groenlandica*) invasions in Norwegian coastal waters: age composition and feeding habits. *ICES Journal of Marine Science,* 48**,** 363-371.

HELM, R. C. 1984. Rate of digestion in three species of pinnipeds. *Canadian Journal of Zoology,* 62**,** 1751-1756.

HILL, R. W., WYSE, G. A. & ANDERSON, M. 2012. Chapter 7, Energy metabolism. *Animal Physiology, Third Edition.* Sunderland, Massachusetts: Sinauer Associates, Inc. Publishers.

HOLDWAY, D. A. & BEAMISH, F. W. H. 1984. Specific growth rate and proximate body composition of Atlantic cod (*Gadus morhua L.*). *Journal of Experimental Marine Biology and Ecology,* 81**,** 147-170.

HÄRKÖNEN, T. 1986. *Guide to the otoliths of the bony fishes of the northeast Atlantic,* Hellerup, Denmark, Danbiu ApS.

ICES 2011. Report of the Working Group on Harp and Hooded Seals (WGHARP). 15-19 August 2011, St. Andrews, Scotland, UK: ICES CM 2011/ACOM:22. 78 pp.

ICES 2014. Report of the ICES/NAFO Working Group on Harp and Hooded Seals (WGHARP). 17-21 November 2014, Quebec City, Quebec, Canada: ICES CM 2014/ACOM:20. 62 pp.

ICES 2015. Report of the Arctic Fisheries Working Group (AFWG), 23-29 April 2015. Hamburg, Germany. ICES CM 2015/ACOM:05. 639 pp.

IVERSEN, T. 1928. Årsberetning vedkommende Norges fiskerier, 1927. 1-84. In Norwegian.

JOBLING, M. 1987. Marine mammal faeces samples as indicators of prey importance - A source of error in bioenergetics studies. *Sarsia,* 72**,** 255-260.

JOBLING, M. & BREIBY, A. 1986. The use and abuse of fish otoliths in studies of feeding habits of marine piscivores. *Sarsia,* 71**,** 265-274.

JØRGENSEN, C. & FIKSEN, Ø. 2006. State-dependent energy allocation in cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences,* 63**,** 186-199.

KIRKMAN, S. P., WILSON, W., KLAGES, N. T. W., BESTER, M. N. & ISAKSEN, K. 2000. Diet and estimated food consumption of Antarctic fur seals at Bouvetøya during summer. *Polar Biology,* 23**,** 745-752.

KUHN, C. E., CROCKER, D. E., TREMBLAY, Y. & COSTA, D. P. 2009. Time to eat: measurements of feeding behaviour in a large marine predator, the northern elephant seal *Mirounga angustirostris*. *Journal of Animal Ecology,* 78**,** 513-523.

LAVIGNE, D. M., BARCHARD, W., INNES, S. & ØRITSLAND, N. A. 1982. Pinniped bioenergetics. *FAO Fish. Ser. No. 5. Vol IV. pp. 191-235.*

LAWSON, J. W., ANDERSON, J. T., DALLEY, E. L. & STENSON, G. B. 1998. Selective foraging by harp seals *Phoca groenlandica* in nearshore and offshore waters of Newfoundland, 1993 and 1994. *Marine Ecology Progress Series,* 163**,** 1-10.

LAWSON, J. W., MILLER, E. H. & NOSEWORTHY, E. 1997. Variation in assimilation efficiency and digestive efficiency of captive harp seals (*Phoca groenlandica*) on different diets. *Canadian Journal of Zoology,* 75**,** 1285-1291.

LAWSON, J. W. & STENSON, G. B. 1997. Diet of northwest Atlantic harp seals (*Phoca groenlandica*) in offshore areas. *Canadian Journal of Zoology,* 75**,** 2095-2106.

LAWSON, J. W., STENSON, G. B. & MCKINNON, D. G. 1995. Diet of harp seals (*Phoca groenlandica*) in nearshore waters of the northwest Atlantic during 1990 – 1993. *Canadian Journal of Zoology,* 73**,** 1805-1818.

LIEBSCH, N., WILSON, R. P., BORNEMANN, H., ADELUNG, D. & PLÖTZ, J. 2007. Mouthing off about fish capture: Jaw movement in pinnipeds reveals the real secrets of ingestion. *Deep Sea Research Part II: Topical Studies in Oceanography,* 54**,** 256-269.

LILLY, G. R. & MURPHY, E. F. 2004. Biology, fishery and status of the 2GH and 2J3KL (northern) cod stocks: information supporting an assessment of allowable harm under the Species at Risk Act for the COSEWIC-defined Newfoundland and Labrador population of Atlantic cod (*Gadus morhua*). *DFO Can. Sci. Advis. Sec. Res. Doc. 2004/102*.

LINDSTRØM, U., HARBITZ, A., HAUG, T. & NILSSEN, K. T. 1998. Do harp seals *Phoca groenlandica* exhibit particular prey preferences? *Ices Journal of Marine Science,* 55**,** 941-953.

LINDSTRØM, U., NILSSEN, K. T., PETTERSEN, L. M. S. & HAUG, T. 2013. Harp seal foraging behaviour during summer around Svalbard in the northern Barents Sea: diet composition and the selection of prey. *Polar Biology,* 36**,** 305-320.

MARSHALL, H. D., HART, K. A., YASKOWIAK, E. S., STENSON, G. B., MCKINNON, D. & PERRY, E. A. 2010. Molecular identification of prey in the stomach contents of Harp Seals (*Pagophilus groenlandicus*) using species-specific oligonucleotides. *Molecular Ecology Resources,* 10**,** 181-189.

MCLAREN, I., BRAULT, S., HARWOOD, J. & VARDY, D. 2001. Report of the eminent panel on seal management. *In:* DEPARTMENT OF FISHERIES AND OCEANS. OTTAWA, C. (ed.).

MURIE, D. J. & LAVIGNE, D. M. 1986. Interpretation of otoliths in stomach content analyses of phocid seals: quantifying fish consumption. *Canadian Journal of Zoology,* 64**,** 1152-1157.

MÅRTENSSON, P. E., LAGER GOTAAS, A. R., NORDØY, E. S. & BLIX, A. S. 1996. Seasonal changes in energy density of prey of northeast Atlantic seals and whales. *Marine Mammal Science,* 12**,** 635-640.

NILSSEN, K. T. 1995. Seasonal distribution, condition and feeding habits of Barents Sea harp seals (*Phoca groenlandica*). *In:* ARNOLDUS SCHYTTE BLIX, L. W. & ØYVIND, U. (eds.) *Developments in Marine Biology 4: Whales, seals, fish and man.* Elsevier Science.

NILSSEN, K. T., GROTNES, P. E. & HAUG, T. 1992. The effect of invading harp seals (*Phoca groenlandica*) on local coastal fish stocks of North Norway. *Fisheries Research,* 13**,** 25-37.

NILSSEN, K. T., HAUG, T., POTELEV, V., STASENKOV, V. A. & TIMOSHENKO, Y. K. 1995a. Food habits of harp seals (*Phoca groenlandica*) during lactation and moult in March-May in the southern Barents Sea and White Sea. *Ices Journal of Marine Science,* 52**,** 33-41.

NILSSEN, K. T., HAUG, T., POTELOV, V. & TIMOSHENKO, Y. K. 1995b. Feeding habits of harp seals (*Phoca groenlandica*) during early summer and autumn in the northern Barents Sea. *Polar Biology,* 15**,** 485-493.

NILSSEN, K. T., HAUG, T., ØRITSLAND, T., LINDBLOM, L. & KJELLQWIST, S. A. 1998. Invasions of harp seals *Phoca groenlandic*a ERXLEBEN to coastal waters of Norway in 1995: ecological and demographic implications. *Sarsia,* 83**,** 337-345.

NILSSEN, K. T., PEDERSEN, O.-P., FOLKOW, L. P. & HAUG, T. 2000. Food consumption estimates of Barents Sea harp seals. *NAMMCO Scientific Publications,* 2**,** 9-27.

NORDØY, E. S. 1995. Gastroliths in the harp seal *Phoca groenlandica*. *Polar Research,* 14**,** 335-338.

NORDØY, E. S., FOLKOW, L. P., POTELOV, V., PRISCHEMIKHIN, V. & BLIX, A. S. 2008. Seasonal distribution and dive behaviour of harp seals (*Pagophilus groenlandicus*) of the White Sea–Barents Sea stock. *Polar Biology,* 31**,** 1119-1135.

NORDØY, E. S., MÅRTENSSON, P. E., LAGER, A. R., FOLKOW, L. P. & BLIX, A. S. 1995. Food consumption of the Northeast Atlantic stock of harp seals. *In:* ARNOLDUS SCHYTTE BLIX, L. W. & ØYVIND, U. (eds.) *Developments in Marine Biology.* Elsevier Science.

NORTHRIDGE, S., CORAM, A. & GORDON, J. 2013. Investigations on seal depredation at Scottish fish farms. Edinburgh: Scottish Government

O'BOYLE, R. & SINCLAIR, M. 2012. Seal–cod interactions on the Eastern Scotian Shelf: Reconsideration of modelling assumptions. *Fisheries Research,* 115–116**,** 1-13.

PEDERSEN, J. & HISLOP, J. R. G. 2001. Seasonal variations in the energy density of fishes in the North Sea. *Journal of Fish Biology,* 59**,** 380-389.

PEMBERTON, D., MERDSOY, B., GALES, R. & RENOUF, D. 1994. The interaction between offshore cod trawlers and harp *Phoca groenlandica* and hooded *Cystophora cristata* seals off Newfoundland, Canada. *Biological Conservation,* 68**,** 123-127.

PHILLIPS, E. M. & HARVEY, J. T. 2009. A captive feeding study with the Pacific harbor seal (*Phoca vitulina richardii*): Implications for scat analysis. *Marine Mammal Science,* 25**,** 373-391.

PIERCE, G. J. & BOYLE, P. R. 1991. A review of methods for diet analysis in piscivorous marine mammals. *Oceanogr Mar Biol,* 29**,** 409-486.

PITCHER, K. W. 1980. Stomach contents and faeces as indicators of harbor seal, *Phoca vitulina*, diets in the Gulf of Alaska. *Fishery Bulletin,* 78**,** 797-798.

PNA (Process NMR Associates). 2015. Solid Fat Content by Time-Domain NMR *Resonance systems.* http://www.process-nmr.com/SFC.htm. [Accessed 20 February 2016].

POTELEV, V., NILSSEN, K. T., SVETOCHEV, V. & HAUG, T. 2000. Feeding habits of harp (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*) during late winter, spring and early summer in the Greenland Sea. NAMMCO Sci. Publ. 2**:** 40-49.

ROFFE, T. J. & MATE, B. R. 1984. Abundances and feeding habits of pinnipeds in the Rouge river, Oregon. *The Journal of Wildlife Management,* 48**,** 1262-1274.

ROSEN, D. A. S. & TRITES, A. W. 2000. Digestive efficiency and dry-matter digestibility in Steller sea lions fed herring, pollock, squid, and salmon. *Canadian Journal of Zoology,* 78**,** 234-239.

SERGEANT, D. E. 1991. Harp seals, man and ice. *Canadian journal of fisheries and aquatic sciences,* 114**,** 1-153.

SIMONNE, A. H., SIMONNE, E. H., EITENMILLER, R. R., MILLS, H. A. & CRESMAN, C. P. 1996. Could the dumas method replace the kjeldahl digestion for nitrogen and crude protein determinations in foods? *Journal of the Science of Food and Agriculture,* 73.

SUZUKI, I., NAITO, Y., FOLKOW, L. P., MIYAZAKI, N. & BLIX, A. S. 2009. Validation of a device for accurate timing of feeding events in marine animals. *Polar Biology,* 32**,** 667-671.

SWEENEY, J. M. & HARVEY, J. T. 2011. Diet estimation in California sea lions, *Zalophus californianus*. *Marine Mammal Science,* 27**,** 279-301.

TOLLIT, D. J., HEASLIP, S. G., BARRICK, R. L. & TRITES, A. W. 2007. Impact of diet-index selection and the digestion of prey hard remains on determining the diet of the Steller sea lion (*Eumetopias jubatus*). *Canadian Journal of Zoology,* 85**,** 1-15.

TOLLIT, D. J., STEWARD, M. J., THOMPSON, P. M., PIERCE, G. J., SANTOS, M. B. & HUGES, S. 1997. Species and size differences in the digestion of otoliths and beaks: implications for estimates of pinniped diet composition. *Canadian journal of fisheries and aquatic sciences,* 54**,** 105-119.

TOLLIT, D. J., WONG, M., WINSHIP, A. J., ROSEN, D. A. S. & TRITES, A. W. 2003. Quantifying errors associated with using prey skeletal structures from fecal samples to determine the diet of Steller's sea lion (*Eumetopias Jubatus*). *Marine Mammal Science,* 19**,** 724-744.

VELP SCIENTIFICA. 2016. *Dumas nitrogen and total nitrogen content* [Online]. http://www.velp.com/en/service_support/dumas_nitrogen: VELP Scientifica. [Accessed 10 February 2016].

WILSON, L. J., GRELLIER, K. & HAMMOND, P. S. 2013. Improved estimates of digestion correction factors and passage rates for harbour seal (*Phoca vitulina*) prey. Sea Mammal Research Unit, Scottish Oceans Institute, University of St. Andrews.

WORTHY, G. A. J. 2001. Chapter 36 Nutrition and Energetics. *In:* DIERAUF, L. & GULLAND, F. M. D. (eds.) *CRC Handbook of Marine Mammal Medicine: Health, Disease, and Rehabilitation. Second edition.* CRC Press, Taylor & Francis Group, Boca Raton, pp. 791-817.

YARAGINA, N. A. & MARSHALL, C. T. 2000. Trophic influences on interannual and seasonal variation in the liver condition index of Northeast Arctic cod (*Gadus morhua*). *ICES Journal of Marine Science,* 57**,** 42-55.

ØIGÅRD, T. A., HAUG, T. & NILSSEN, K. T. 2014. From pup production to quotas: current status of harp seals in the Greenland Sea. *Ices Journal of Marine Science,* 71**,** 537-545.

7. Appendix

Appendix I

Water temperature in the tanks

Table I. The water temperature in the tanks throughout the experimental period.

Date	Temperature (°C)
25.aug	9,9
14.sep	9,8
16.sep	9,9
18.sep	10,1
21.sep	10,2
23.sep	10,1
28.sep	10,1
30.sep	10,2
02.okt	9,6
05.okt	9,2
07.okt	9
09.okt	8,8
12.okt	8,5
14.okt	8,3
16.okt	8,4
19.okt	8,5
21.okt	8
23.okt	7,5
26.okt	7
28.okt	7
30.okt	7

Appendix II

Osmolality of water samples

Table II. The osmolality of each water sample collected from the tanks during the study and the average of the three repeats presented in mOsmol.

Date	1	2	3	Average
25. Aug.	0,956	0,958	0,955	0,956
14. Sept.	0,821	0,824	0,821	0,822
16. Sept.	0,945	0,961	0,975	0,960
18. Sept.	0,966	0,955	0,973	0,965
21. Sept.	0,963	0,959	0,964	0,962
23. Sept.	0,494	0,514	0,518	0,509
28. Sept.	0,956	0,962	0,984	0,967
30. Sept.	0,981	0,983	0,986	0,983
02. Oct.	0,847	0,854	0,851	0,851
05. Oct.	0,744	0,742	0,747	0,744
07. Oct.	0,951	0,965	0,959	0,958
09. Oct.	0,752	0,751	0,751	0,751
12. Oct.	0,707	0,705	0,701	0,704
14. Oct	0,650	0,649	0,683	0,661
16. Oct.	0,452	0,454	0,458	0,455
19. Oct.	0,954	0,958	0,958	0,957
21. Oct.	0,960	0,955	0,955	0,957
23. Oct.	0,987	0,962	0,955	0,968
26. Oct.	0,955	0,963	0,973	0,964
28. Oct.	0,928	0,930	0,930	0,929
30. Oct.	0,421	0,419	0,419	0,420

Appendix III

Weight of the seals

Table III. The weight of both the adult and juvenile harp seal during the study. All measurements are given in kilograms (kg).

Date	Weight (kg)	
	Juvenile	Adult
18. Aug.	56,8	94
31. Aug.	58,6	96,2
05. Sept.	59,4	96,8
11. Sept.	61,0	99,0
17. Sept.	61,3	-
21. Sept.	-	99,0
28. Sept.	60,8	
30. Sept.	-	102,0
06. Oct.	61,4	101,2
15. Oct.	61,8	-
16. Oct.	-	99,8
21. Oct.	60,6	-
23. Oct.	-	99,0
02. Nov.	61,4	99,6
At end	61,4	99,6
At start	56,8	94
Increase (kg)	4,6	5,6
Per day	0,07	0,08

Appendix IV

Chemical composition of the samples sent to ALS

Table IV. Sample number and weight sent to ALS, with its corresponding output on ash, water, protein, fat, carbohydrate, and energy content. An average of leftover fish remains in the mincing machine was also calculated.

Sample #	Grams	Grams left in machine	Ash g/100g	Water g/100g	Protein g/100g	Fat g/100g	Carbohydrate g/100g	Energy Kcal/100g
1-15	251		2,70	80,2	16,2	0,7	0,2	99,8
2-15	253		3,10	80	16,8	0,6	<0,1	101,5
3-15	253		2,7	79,5	17,1	0,9	<0,1	106,0
4-15	254	48	3,2	82	15,4	0,4	<0,1	91,6
5-15	252		3,3	77	17,4	0,9	1,4	113,3
6-15	250		6,3	80,1	14,6	0,5	<0,1	88,0
7-15	255	48	6,4	78,3	14,8	0,4	0,1	88,6
8-15	260		5,1	81,6	13,9	0,5	<0,1	84,0
9-15	253		5,4	80	14	0,6	0	85,5
10-15	206		4	82,4	13,2	0,5	<0,1	80,0
11-15	225		3,7	82,6	13,3	0,5	<0,1	80,6
12-15	170	36	4,4	83,3	12	0,4	<0,1	72,2
13-15	252	59	5	81,4	14,3	0,3	<0,1	84,4
14-15	252	41	3,8	80,8	15,5	0,4	<0,1	92,2
15-15	254		4,3	80,6	15,7	0,4	<0,1	93,3
16-15	214		3,6	81,1	13,2	0,5	1,6	86,4
17-15	240		2,7	82	14,8	0,5	0	89,1
18-15	170		2,9	82,6	14,6	0,5	<0,1	88,0
19-15	264		3,3	79,4	17,1	0,5	<0,1	102,2
20-15	252		2,7	80,9	17	0,4	<0,1	100,7
Average:		46,4						

Appendix V

Average energy content of the fish remains

Table V. Average energy content for all categorized groups used to estimate energy contents of the remaining parts of fish collected from the tanks of the adult and juvenile seal.

Body part	Samples kcal/100g	Average energy content kcal/100g
Head	72,2 88 88,6 84 85,5	83,7
Head + back	84,4 92,2 93,3 86,4	89,1
Cod lacking its abdomen	89,1 88 102,2 100,7	95
Head saithe	80 80,6	80,3

Appendix VI

Information on the number of fish and fish consumption from each experiment

Table VI. Number of fish presented to each harp seal during the experimental period, with information on how many fish they received and consumed, including the weight of that in grams.

No. of experiment	No. of fish	Adult fish consumption	Adult total consumption (g)	Juvenile fish consumption	Juvenile total consumption (g)
1	4	1	200	3	975
2	6	1	0	0	0
3	4	1	430	3	220
4	11	6	2520	3	220
5	9	5	1030	3	340
6	6	4	1360	2	90
7	7	5	1520	2	570
8	2	2	890	0	0
9	10	5	1830	5	280
10	7	4	1900	3	390
11	7	4	340	3	750
12	6	5	1980	1	240
13	9	5	3030	4	500
14	8	5	2560	3	590
15	8	6	2080	2	60
16	9	5	1620	4	510
17	9	5	1490	4	670
18	8	5	1230	3	610
19	11	8	3760	3	300
20	11	7	2320	4	540
21	11	7	2040	4	1050

Appendix VII

Fish utilized in the study

Table VII. List of all fish utilized in the study, provided with information on species, length and weight.

Species	Length (cm)	Weight (g)
Saithe	32	375
Saithe	20	200
Cod	25	275
Cod	40	650
Cod	32	360
Cod	31	290
Cod	32	350
Cod	44	590
Cod	34	350
Cod	26,5	160
Cod	34	310
Cod	27	190
Cod	35	360
Cod	36	430
Cod	33	260
Cod	30	190
Cod	37	460
Cod	39	530
Cod	35	360
Cod	40	590
Cod	32	320
Cod	40	580
Cod	28	190
Cod	35	370
Cod	29	220
Cod	49	830
Cod	54	1350
Cod	23	120
Cod	39	500
Cod	32	340
Cod	35	390
Cod	33	360
Saithe	34	440
Cod	39	570
Cod	35,5	320
Cod	61,5	1740
Cod	24	120

Cod	41	470
Cod	23	90
Cod	44	750
Cod	46	960
Saithe	32	300
Saithe	30,5	270
Cod	37	510
Cod	34	450
Cod	31	210
Cod	41	580
Cod	35	310
Cod	38	580
Cod	54	1240
Saithe	24	110
Saithe	31,5	280
Cod	40	480
Cod	43	690
Saithe	26	170
Cod	29	170
Cod	32	280
Cod	31	310
Cod	40	540
Cod	34	300
Cod	56	1610
Saithe	34	390
Saithe	24	130
Cod	30	220
Cod	49	860
Saithe	28	220
Cod	66	2430
Saithe	32	330
Cod	33	340
Saithe	35	390
Cod	45	710
Cod	39	570
Cod	50	1170
Cod	47	820
Cod	41	520
Saithe	30	240
Cod	49	1090
Cod	43	670
Cod	53	1300
Cod	40	540

Cod	37	460
Cod	43	820
Saithe	31	270
Saithe	29	230
Cod	31	280
Cod	37	460
Cod	30	260
Cod	48	860
Cod	58	1510
Saithe	31	270
Cod	40	540
Cod	37	490
Cod	32	310
Cod	31	250
Cod	59	1630
Cod	70	2710
Cod	32	270
Cod	44	740
Cod	48	910
Cod	32	300
Cod	39	480
Cod	36	360
Cod	54	1410
Cod	40	500
Cod	48	1020
Saithe	25	170
Saithe	24	130
Saithe	25,5	180
Cod	63	1710
Cod	32	310
Cod	41	550
Cod	41,5	620
Cod	37	510
Cod	61	1850
Cod	31	190
Saithe	24	150
Cod	43	560
Cod	46	880
Cod	49	880
Cod	50	970
Cod	56	1260
Cod	56	1570
Cod	27	170

Cod	31	230
Cod	30	210
Cod	43	660
Cod	45	850
Cod	57	1360
Cod	46	860
Cod	58	1780
Cod	31	250
Cod	35	380
Cod	46	690
Cod	46	760
Cod	38	440
Cod	41	660
Cod	38	490
Cod	48	960
Cod	38	470
Cod	37	440
Cod	40	480
Cod	49	1000
Cod	34	370
Cod	40	540
Cod	32	250
Cod	31	260
Cod	40	580
Cod	44	600
Cod	38	400
Cod	39	500
Cod	42	670
Cod	62	1700
Cod	33	300
Cod	32	330
Cod	50	760
Cod	54	1240
Cod	35	380
Cod	36	460
Cod	44	740
Cod	42	540
Cod	45	860
Cod	50	1350