Master’s thesis

Dietary differences between Baltic ringed seals (*Phoca hispida botnica*) based on stable isotope and fatty acid analyses

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ABSTRACT

The Baltic ringed seal (*Phoca hispida botnica*) is an endangered and protected species in the Baltic Sea which underwent a significant decline in the 20th century mainly due to extensive hunting and environmental toxins. The species is currently increasing its numbers in the Bothnian Bay, where damage caused to fisheries by grey seals (*Halichoerus grypus*) and Baltic ringed seals has also increased. Seals not only reduce the catch, but also damage fish and break fish traps. Damage has been highest for whitefish (*Coregonus lavaretus* (L.), zander (*Sander lucioperca* (L.), and salmon (*Salmo salar* L.) fisheries. The aim of this thesis was to study the feeding and foraging behaviour of 45 individual Baltic ringed seals to determine species-specific diet using two biochemical methods in combination: stable isotope analysis and fatty acid analysis. Carbon and nitrogen isotopes (δ^{13}C and δ^{15}N) were analysed from muscle and liver tissues, while fatty acid composition was determined from blubber tissues. Short-term diet (within weeks) was determined from isotopic values of liver tissue, and was compared with analyses of stomach contents. Long-term diet (within months) was analysed from isotope values of muscle tissue and fatty acid composition of blubber tissues. The ringed seal diet was concentrated on pelagic and benthic fish species on the Swedish side of the Bothnian Bay. In spring adult and juvenile seals had similar diets, but in autumn adult seals concentrated slightly more on predator fish (36 %) than juveniles (34 %). According to biochemical methods, the most common prey items for seals were Baltic herring (*Clupea harengus membras* (L.) and three-spined stickleback (*Gasterosteus aculeatus* L.). There were differences between individuals. Benthic fish species such as eelpout (*Zoarces viviparous*) and fourhorn sculpin (*Myoxocephalus quadricornis*) were also abundant in seal diets. There was no evidence of isopods being used as prey. The dietary and foraging behaviour of ringed seals is perhaps not as straightforward as previously assumed. Therefore, further research on diets of this endangered species is needed to help solve problems with fisheries while maintaining sustainable management of seals.

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TIIVISTELMÄ

Itämerennorppa (*Phoca hispida botnica*) on uhanalainen ja suojeltu laji Itämerellä, sillä hyljekannat romahtivat 1900-luvulla laajan metsästyksen ja ympäristömyrkkyjen vuoksi. Perämerellä hyljekanta on tällä hetkellä kasvussa, ja harmaahylkeiden (*Halichoerus grypus*) ja itämerennorppien aiheuttamat vahingot ovat kasvaneet. Hylkeiden vuoksi kalastajat ovat menettäneet saalista. Hylkeet ovat myös vahingoittaneet kaloja ja rikkoneet pyydyksiä. Eniten tuhoa hylkeet ovat aiheuttaneet siika- (*Coregonus lavaretus* (L.), kuha- (*Sander lucioperca* (L.) ja lohi- (*Salmo salar* L.) kalastukselle. Tutkimuksen tavoitteena oli tutkia 45 itämerennorpan ravintoa kahden biokemiallisen menetelmän avulla: vakaat isotoopit ja rasvahappomääritys. Hiilen ja typen vakaita isotooppeja (δ^{13}C ja δ^{15}N) tutkittiin lihas- ja maksakudoksesta ja rasvahappokoostumus määritettiin traanista. Ravintoa tutkittiin maksakudoksesta, joka määrittää ravinnon muutaman viimeisten viikkojen ajalta ennen pyydystystä, ja tulosta verrattiin ravintomäärityksiin mahanäytteistä. Lihaskudoksen isotooppiarvoista ja traanin rasvahappokoostumuksesta arvioitiin norppien ravintoa viimeisten kuukausien ajalta ennen pyydystystä. Hylkeiden ravinto koostui pääasiassa pelagisista kalalajeista ja pohjakaloista. Keväällä nuoret ja aikuiset hylkeet söivät samankaltaista ravintoa, mutta syksyllä aikuiset söivät enemmän petokalaryhmän kalalajeja (36 %) verrattaessa nuoriin yksilöihin (34 %). Biokemialliset menetelmät määrittivät hylkeiden ravinnon koostuvan silakasta (*Clupea harengus membras* (L.) ja kolmipiikistä (*Gasterosteus aculeatus* L.). Pohjakalat kuten kivinilkka (*Zoarces viviparous*) ja härkäsimppu (*Myoxocephalus quadricornis*) olivat myös hylkeiden ravintoa. Äyriäisiä hylkeet eivät käyttäneet ravinnokseen. Hylkeiden ravinnon käyttö Perämerellä ei ole yksiselitteinen. Uhanalaisen hyljelajin ravinnonkäyttöä tulisi tutkia enemmän, jotta ongelmat kalatalouden kanssa voitaisiin ratkaista.

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1. INTRODUCTION

The Baltic ringed seal (*Phoca hispida botnica*), is one of three seal species living in the Baltic Sea. The other two seal species are the grey seal (*Halichoerus grypus*) and the harbour seal (*Phoca vitulina*). Geographical and physical factors have made the Baltic Sea a vulnerable environment with extreme environmental conditions, such as variations of temperatures, low under 10 PSU salinity levels and low average depth, in need of special protection of environment and biodiversity (Hietala no date) . Furthermore, most species in the Baltic Sea live near the edge of their physiological tolerance range (Helle 1980). Marine mammals are predators at the top of the food web, and they are important indicator species in the ecosystem as their condition will reflect the state of the Baltic environment.

The Baltic ringed seal lives locally in the Baltic Sea, with populations in the Gulf of Riga, the Gulf of Finland, the Archipelago Sea and the Bothnian Bay (Bäcklin *et al.* 2013). The Baltic ringed seal is an endangered and protected species in the Baltic Sea due to the significant decline of the population in the 20th century (Helle 1980). At the beginning of the 1900s the species was heavily hunted and in the 1960s environmental toxins led to a high mortality and also caused reproductive disorders (Helle 1983). Today the population in the Bothnian Bay has been estimated to be around 6 500- 7 000 individuals, while the population in the Baltic Sea is around 10 000 individuals based on aerial surveys in 2011 (Anonymous 2013). Seal populations, both grey seals and Baltic ringed seals, have increased within recent years, which has raised conflicts between sustainable management of the populations and damage to the local fisheries. Damage caused by seals is highest for whitefish (*Coregonus lavaretus* (L.), zander (*Sander lucioperca* (L.) and salmon (*Salmo salar* L.) fisheries (Laanikari 2013). Compensation for losses and damage to professional fisheries has become too high, and other options to tackle the problem are being considered at the moment. To solve the problem with fisheries and sustainable management of Baltic ringed seals more species specific understanding on feeding ecology is needed.

Ringed seals are generalist feeders, and can switch from one food item to another; sometimes individuals concentrate either on pelagic or benthic prey (Thiemann *et al.* 2007, Sinisalo *et al.* 2008). Individual ringed seal communities may use a different habitat for feeding, as seals preferred pelagic fish such as vendace, (*Coregonus albula* (L.)) on the Swedish side of Bothnian Bay, while in territorial waters of Finland seals also fed on large benthic invertebrates such as *Saduria entomon* L. (Karlsson 2003, Sinisalo *et al.* 2008). Baltic ringed seals are considered to eat fewer prey species from the higher trophic levels in the food chain (such as salmon), as ringed seals concentrate more on pelagic fish (such as herring) and benthic isopods (Helle 1983, Käkelä *et al.* 1993, Mänttäri 2011). In general Baltic ringed seals are considered to be resident in the Bothnian Bay, but foraging migrations within the area are not really known (Helle 1983). Seals have extraordinarily fast digestion, which means that often the gut contents of sampled seals are empty (Parsons 1977). The remains of soft-bodied prey items are usually more or less digested, but hard parts such as otoliths can be found to determine of prey size and diet (Gudmundson *et al.* 2006, Lundström 2012a). The time for hard parts to dissolve is species-dependent and usually dietary studies based on stomach content need an extensive number of individual seals (Bowen & Iverson 2013). Long-term diet analysis is not feasible using such conventional analysis, so different biochemical methods have been developed.

Biochemical methods such as stable isotope and fatty acid analyses are now widely used methods in dietary studies. The aim of this thesis was to use these two biochemical methods in combination to study the feeding and foraging behaviour of 45 individual ringed seals shot on the Swedish side of the Bothnian Bay. The main questions of this study were: did seals have seasonal variation in their diet, and is their diet concentrated on benthic isopods in spring and did the diet swift to more fatty fish species towards autumn. In particular, diet can vary significantly between individuals within a population. The methods are based on the fact that consumers need to be as energy efficient as possible, and thus modify isotopes and fatty acid composition as little as possible (S. Taipale unpublished information). Fatty acid and stable isotope analysis are independent; fatty acid analysis investigates lipid stores of marine mammal blubber, while stable isotope analysis investigates isotope ratios of carbon and nitrogen from multiple tissues (Tucker *et al.* 2008). Muscle and liver tissues of the seals are used for stable isotope analysis, when also temporal changes in diet can be investigated due to differences in metabolic activity within tissues (Tieszen *et al.* 1983). Both methods have been used separately to determine diets of Baltic ringed seals (Karlsson 2003, Sinisalo *et al.* 2008, Mänttäri 2011, Lundström 2012a, 2012b). However, in this thesis the methods were combined to obtain the most robust species-specific results for Baltic ringed seal diets.

2. BACKGROUND

2.1 Population and ecology of Baltic ringed seal

The Baltic ringed seal population in the early 1900s has been estimated to have been 45 000 to 200 000 individuals (Kokko *et al.* 1999). In the 1910s approximately 12 500 bounties were paid every year for killing Baltic ringed seals (Helle 1983). In the 1960s high levels of organochlorines, DDT and PCB compounds were enriched up the food web and accumulated with food into the fatty tissues of seals (Kokko *et al.* 1999). In the 1970s environmental toxins caused reproductive disorders and high levels of sterility of females, caused by uterine occlusions (Helle 1980). Due to the decline of the species, the Baltic ringed seal has been protected since 1986, and hunting has only been permitted with restricted licences granted by the Ministry of Agriculture and Forestry of Finland, approximately 30 licences per year (MMM 2007, Laanikari 2013). In Sweden management plan for ringed seal is in preparation. The Baltic ringed seal is classified as a near threatened species in the Red list of Finnish and Swedish species (Anonymous 2005, Rassi *et al.* 2010). The Baltic ringed seal lives in a brackish water area surrounded by several countries, and therefore active co-operation between these countries is key to sustainable management of the species (MMM 2007).

Ringed seal (*Phoca hispida*) is the smallest seal species in the world. Ringed seals are widespread in the northern hemisphere, and are the most abundant seal species in high arctic areas (Härkönen *et al.* 2008). In the Baltic Sea the species can be found from areas which are likely to freeze, as the majority (75 %) of the population resides in the Bothnian Bay (Anonymous 2013). The population in the Bothnian Bay has increased at a rate of 4.5 % yearly since 1988, while in the Gulf of Riga and the Archipelago Sea the populations have remained stable. In the Gulf of Finland the ringed seal population is currently decreasing (Bäcklin *et al.* 2013).

The Baltic ringed seal is one of the five subspecies of ringed seal. The closely related freshwater subspecies (*Pusa hispida saimensis* and *Pusa hispida ladogensis*) were landlocked in freshwater lakes during the last ice age and today these sub-species are genetically divergent (Helle 1983). Baltic ringed seal reaches sexual maturity at the age of 4 to 6 years, females earlier than males (Helle 1980). Baltic ringed seal gives birth on the ice, when it is thickest in February-March (Helle 1983). The pup will be born in a nest made in a snow drift, while the mother keeps an ice hole open with her webbed foot and nails during cold periods (Thiemann *et al.* 2007). The pup weighs on average five kilograms when born and nursing lasts 5–7 weeks (Anonymous 2013). During lactation females will move only a little, which will decrease the diversity of their diet in spring (Sinisalo *et al*. 2008). Overall, during the lactation and reproduction energy requirements of marine mammals are high (Kraft *et al.* 2006). After nursing ringed seals mate again and gestation lasts 11 months. Adult ringed seals have a length of 100–160 cm and weigh 50–120 kg.

An adult Baltic ringed seal eats 2.5 – 3.5 kg prey per day; the amount and the species of the prey items vary between seasons (Helle 1983). Ringed seals go through annual changes in diet (Lundström 2012b). In general, ringed seals are opportunist feeders and diet is always dependent on available prey (Helle 1980). Ringed seals eat at least 12 different fish and crustacean species; usually the average prey size is around 10 cm (Anonymous 2013). Their teeth are ideal for eating crustaceans, as ringed seals can filter crustacean from dense schools (Helle 1983). Baltic ringed seals forage intensively in the late summer and autumn. The seals double their body weight from May to November, and some individuals can then exceed 100 kg weight (Helle 1983). The diving activity of Baltic ringed seals is greater during the daylight hours when feeding activity is also highest (Härkönen *et al*. 2008). Older individuals can dive deeper than younger individuals, as they have the ability to control circulation of blood to the organs with most need (Helle 1983). In general, young seals have limited ability to dive and capture prey; therefore their diet can be more restricted and less specialized (Lundström 2012b). Species with diurnal vertical migration such as Baltic herring (*Clupea harengus membras* (L.), could be a common prey item for Baltic ringed seals as seals are actively foraging in the bottom of sea floor during the daylight (Härkönen *et al.* 2008). Baltic herring is abundant throughout the year in the Baltic Sea, and is also the most common prey of seals (Härkönen *et al*. 2008). However, Baltic ringed seals have great seasonal variation in diet. In spring, between March and April, when the waters are free of ice seals have consumed isopods (*Saduria entomon* L.) (Helle 1983), while in the late summer seals have preferred more fatty prey items such as three-spined stickleback (*Gasterouteus aculeatus* L.) (Tormosov & Rezvov 1978, Käkelä *et al.*1993). Three-spined stickleback and Baltic herring are ideal prey items in the late summer as these species have high fat content and are therefore suitable for seals which are increasing their fat stores for winter (Tormosov & Rezvovin 1978, Käkelä *et al.* 1995, Rosen & Tollit 2012). There might be significant differences in diet between seal individuals, some seals might have been specialized for benthic isopods, while some can be concentrating on schooling fish (Sinisalo *et al.* 2006).

Reproductive disorders have decreased remarkably in recent years, with only 10. 8 % of over four years old females are infertile (Laanikari 2013). On the other hand, pregnancy rate was only 68 % between 2001 and 2011 (Bäcklin *et* al. 2013). There still are individuals suffering from uterine occlusions. According to the EU’s classification system the environmental status of the species is much below good (Bäcklin *et al.* 2013). However, protection of the species and environmental legislation has increased the population size in the past 30 years. Ringed seals do not have natural enemies in the Baltic Sea. However, there are other external threats such as by-catch losses, when individuals are trapped and died in fish traps, with young individuals particularly vulnerable (Anonymous 2013). Usually young individuals (age <1 year) are also trapped in fish nets during their foraging for small fishes and crustaceans from the bottom. Males are more often found trapped in several types of fishing nets. In Sweden every year approximately 50 individuals are lost due to by-catches (Lunneryd & Königson 2005). On the other hand, by-catches have not been seen as the major threat to the population size. Climate warming could cause serious problems to the survival of the species as Baltic ringed seal is highly dependent on snow cover and fast ice during reproduction (Rassi *et al.* 2010). To increase protection of threatened species a sanctuary for seals has been established on the Finnish side of the Bothnian Bay, in Möyly near Kemi, where 760 ha is protected areas (MMM 2007). On the Swedish side of the Bothnian Bay there is no such sanctuary for ringed seals (Anonymous 2005).

2.2 Damage to fisheries

Fishing is an important business in the Baltic Sea region (Lunneryd 2005). Fish farming is also an increasing industry, particularly in the Baltic proper area (Raitaniemi & Manninen 2013). Migratory fish species such as salmon declined markedly in the 1950s when construction of hydroelectric power plants on rivers started on a large scale in Finland. The Baltic salmon population has increased since the 1980s due to fish farming and restricted fishing (Raitaniemi & Manninen 2013). Increased seal population and the local fishery currently compete for the same prey items. In 2009 39 % of Finnish fishermen (600) had suffered damage caused by seals (Laanikari 2013). Damage has been highest for the whitefish, zander and salmon fishery, while Baltic herring is quantitatively (kg) the most valuable prey item for fisheries in the Baltic Sea (Laanikari 2013, Raitaniemi & Manninen 2013). Seals not only reduce the catch, but also damage fish and break fish traps (Lunneryd 2005). Traps used in professional fishing are being developed further, as today they are more seal resistant. On the other hand, protection of gill nets is impossible to implement (Anonymous 2013). In Sweden a pushup design trap for salmonids contains a large mesh at the end of the trap (Lunneryd 2005). Techniques to drive seals away from traps, for example by making painful signals with an Acoustic Harassment Devices (AHD) or feeding seals in non-fishing areas, are also being tested (Lunneryd 2005), but these methods have not been successful in the long run.

Development of equipment to drive seals away has reduced damage but the problem is not totally eliminated. In 2013 the Ministry of Agriculture and Forestry of Finland proposed restricting hunting permits to 100 individual Baltic ringed seals. The proposal assumed that licences would be permitted according to damage to fisheries by seals; the proposal was not carried further (Laanikari 2013). Hunting permits were supposed to be for the Bothnian Bay, where seal populations have slowly increased.

2.3 Biochemical methods in dietary studies

2.3.1 Stable isotope analysis

Stomach content analysis has been widely used in dietary studies of marine mammals (Bowen & Iverson 2013). The most common method to determine diet is based on stomach content, intestines and faeces, where hard parts of prey items are identified. However, seals have extraordinarily fast digestion which means that often the gut contents of sampled seals are empty. Moreover, long-term diet analysis is not feasible using such conventional analyses, and therefore various biochemical methods have been developed.

Isotopes are based on the number of neutrons in the nucleus of elements. Isotopes of carbon, nitrogen, oxygen, sulphur and hydrogen have been used to study migration, physiology, habitat use, diet, community structure, and trophic level among and within individual species (Phillips & Cregg 2003, Newsome *et al.* 2010, Querouil *et al.* 2013). Carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopes are most widely used in dietary studies as they will provide two dimensional data of the individual diet of consumers (Tucker *et al.* 2008). Isotopic ratios are expressed in δ notation with units of per mill, (‰). The delta value (δ) tells the isotopic ratio compared to international standards (for carbon PDB limestone and for nitrogen atmospheric nitrogen; Fry 2006). The standards are used to establish a value of zero for studied elements, and the larger the delta value (δ) of a studied material the more of the heavier isotope is present compared to the standard.

Isotopic ratios of the food items consumed by seals are reflected in tissues of the animal. The amount assimilated for each prey species depends on the separation of heavier isotopes in the digestion and assimilation processes (Phillips & Eldridge 2006). Sources of prey have different $\delta^{13}C$ and $\delta^{15}N$ values, heavier isotopes are more energy consuming to utilize and therefore it will rich the diet in food web upwards (Fry 2006). Difference between consumed diet and tissue will differ between species and tissue analyzed (Hobson *et al.* 1996). Fractionation is a small modification occurring during formation of heavy and light isotopes, and is the most important issue to take into account in isotope analysis (Fry 2006). Fractionation factors for tissues are determined experimentally by feeding a constant diet for animals to determine how isotopes fractionate between the tissue and the diet (Bowen & Iverson 2013). Naturally occurring nitrogen isotopes ^{15}N and ^{14}N have greater fractionation compared to carbon ^{13}C and ^{12}C (Hobson *et al.* 1996).

In dietary studies the nitrogen value ($\delta^{15}N$) can indicate the level of the food web of a consumer (the trophic level of a species), while the carbon value ($\delta^{13}C$) can indicate differences in prey items in the diet (Vander Zanden *et al*. 1997, Sinisalo *et al.* 2008, Cipro *et al.* 2012). Stable isotope analysis can provide dietary data from days to months due to differences in metabolic rate of tissues (Hobson *et al*. 1996, Sinisalo *et al.* 2008). Isotopic turnover rate of tissue is affected by metabolic rate of protein (Kurle & Worthy 2002, Cipro *et al.* 2012). Recent diet composition can be determined from liver tissue, which has intermediate turnover rate and reflects diet over weeks. Long-term diet composition can be determined from muscle and blubber tissue with slow turnover rate which will reflect average diet over months. Blood and plasma have the fastest rate and will demonstrate the most recent diet (Lesage *et al.* 2002, Kurle & Worthy 2002, Sinisalo *et al.* 2008). By analysing different tissues diet can be determined for different time periods.

2.3.2 Fatty acid analysis

Fatty acids are the largest constituent of lipids, and there are over 70 different fatty acids found in marine ecosystems. Blubber is a specialized adipose tissue, which functions to store energy and is the main thermoregulatory tissue of seals (Strandberg 2012). Fatty acids found from marine mammals contain carbon chain length of 14 to 24, and zero to six double bonds developing a terminal methyl group and an acid group opposite ends (Table 1) (Smith *et al.* 1997,Iverson *et al.* 2004, Lundström 2012b). Fatty acids usually are reported as mol percent of total fatty acid (Budge *et al.* 2006). Fatty acid containing zero or one double bond is known as saturated (SAFA) and monounsaturated (MUFA) fatty acids, while two or more double bonds are known as polyunsaturated (PUFA) fatty acids. The structure of the fatty acids used in the study is illustrated in Table 1. In dietary studies fatty acids can be used as trophic biomarkers of food quality through the trophic levels, and consumers diet can be determined (Dalsgaard *et al.* 2003). Fatty acids with ω-6 or ω-3 double ponds and fatty acids such as arachidonic acid (ARA) 20:4ω6, eicosapentaenoic acid (EPA) 20:5ω3 and docosahexaenoic acid (DHA) 22:6ω3 are indicator fatty acids and dietary markers in dietary studies as they are related to diet (Karlsson 2003, Iverson *et al.* 2004, Strandberg 2012). Baltic ringed seals are unable to synthesize DHA and therefore it is only related to diet (Karlsson 2003). Seals have been showed to consume a diet of five percent or more fat, which increases the content of long-chain fatty acids (Käkelä &

Hyvärinen, 1996). Short branched-chain fatty acids are not related to diet as they are oxidized straight after consumption (Budge *et al.* 2006).

Table 1. Classification of fatty acids based on type, structure and abbreviation (Brett *et al.* 2006). The first number (14- 24) indicates carbon chain length with zero to six double bonds and the last number expresses double bond on the methyl end (designated with ω) (Smith *et al.* 1997).

Type	Structure	Abbreviation
Saturated fatty acids	14:0, 15:0, 16:0, 17:0, 18:0, 20:0	SAFA
Monounsaturated fatty acids	16:1ω9, 16:1ω7, 18:1ω7, 18:1ω9, 20:1ω9	MUFA
α- Linolenic acid	18:3ω3	ALA
Stearidonic acid	18:4ω3	SDA
Eicosapentaenoic acid	20:5ω3	EPA
Docosahexaenoic acid	22:6ω3	DHA
Linoleic acid	18:2ω6	LIN
ɣ- Linolenic acid	18:3ω6	GLA
Arachidonic acid	20:4ω6	ARA
Polyunsaturated fatty acids	ALA, SDA, LIN, GLA, EPA, DHA & ARA	PUFA
Highly unsaturated fatty acids	EPA, DHA, ARA	HUFA
C18 ω3 PUFAs	primarily GLA and SDA	
C18 ω6 PUFAs	primarily LIN and GLA	
ω3 PUFAs	ALA, SDA, EPA and DHA	
ω6 PUFAs	LIN, GLA and ARA	
Branched fatty acids	i-14:0, i-15:0, a-15:0, i-17:0, a-17:0	Bact Fas

As fatty acids used as biomarkers will reflect diet, the method is useful in food web studies (Iverson *et al.* 2004). The best biomarkers are those which represent only one food item. However concentrations are low if only one food item is implemented (Smith *et al.* 1997, Strandberg *et al.* 2008). Using fatty acids as biomarkers in dietary studies of seals relies on background data for fatty acid signatures of prey. In addition, long-chain fatty acids of prey are the diet signatures, which will transfer into the adipose tissue of seals directly or with little modification (Bugde *et al.* 2006, Thiemann *et al.* 2007, Strandberg *et al.* 2011, Rosen & Tollit 2012,). As there can be intra-specific differences in fatty acid composition within the Baltic Sea (Tucker *et al.* 2008, Lundström 2012c,), prey items analysed should preferably originate from the same area as the studied seals. Common prey species for Baltic ringed seals and their fatty acids which are used as biomarkers in dietary studies are illustrated in Table 2. In addition, highly unsaturated fatty acids EPA and DHA are abundant in fatty fish species.

Table 2. Biomarker fatty acids of benthic and pelagic systems, and common prey items for seals in the Bothnian Bay (Käkelä *et al.* 1993, 2005, Kirsch *et al.* 1998, Budge *et al.* 2002).

Fish species /system	Biomarker fatty acids	Abbreviation
Benthic	17:0, 18:1ω7, 20:4ω6, 22:6ω3	SAFA, MUFA, ARA , DHA
Pelagic	14:0, 22:1ω11, 20:1ω9, 18:2ω6, 18:3ω3, 18:4ω3, 18:1ω9	SAFA, MUFAs, LIN, ALA, SDA
Baltic herring	18:1ω9	MUFA
Sea trout	18:3ω3	ALA
Vendace	18:3ω3	ALA
Baltic cod	22:6ω3	DHA
Salmon	20:5ω3, 22:6ω3	EPA, DHA

Estimation of predator diet can be determined by applying a statistical model, fatty acid signature analysis (FASA). Fatty acid signature will reflect temporal and spatial changes in diet, but also quantitative appraisal of composition of prey species (Budge *et al.*

2006). Quantitative fatty acid signature analysis (QFASA) has been developed to detect abundance of specific prey species (Iverson *et al.* 2004, Rosen & Tollit 2012). QFASA is a relatively new method which is based on assumptions verified with experimental feeding and biochemical knowledge (Bowen & Iverson 2013). FASA can be misleading if calibration coefficients (CCs), also known as “fractionation factors” for each consumer and diets are not corrected sufficiently (Thiemann *et al.* 2007, Rosen & Tollit 2012). Overall, fatty acid signature analysis is a powerful tool in dietary studies due to different biosynthetic pathways.

In blubber fatty acids are usually stored as triacylglycerols (TAG); a TAG molecule includes three fatty acids included in a glycerol backbone (Strandberg 2012). TAG is an important reservoir for seals due to their limited ability to store carbohydrates (Iverson *et al.* 2004, Budge *et al.* 2006). Seal blubber consists almost entirely of TAG, without wax esters or short-chain fatty acids (Käkelä & Hyvärinen 1996). Seal blubber is not homogenous through its depth (Karlsson 2003, Thiemann *et al.* 2004) and consists of three layers. A structural layer near the muscle reflects recent diet (Strandberd *et al.* 2008). The blubber layer near the skin, superficial blubber, is metabolically active (Budge *et al.* 2006). The middle layer of blubber is a storage site, which expands with food availability; the layer is considered present if the blubber thickness is more than 3 cm (Strandberg *et al.* 2008). For example in thin animals (blubber $\leq$ 3 cm), the middle layer is usually absent which increases the metabolic cost of maintaining thermal balance (Strandberg *et al.* 2008).

3. MATERIAL AND METHODS

3.1. Seal samples

A total of 45 Baltic ringed seals were examined, all from the Swedish side of the Bothnian Bay, the Northern part of the Baltic Sea, (65°N 21-23°E) (Table 3). The ringed seals were shot in 2007 (n=2), 2008 (n=36) and 2009 (n=7). The majority of the seals (n= 36) were shot in spring or in early summer. Muscle and liver samples were dissected by Charlotta Moraeus and the stomach contents analysis was done by Annika Strömberg from the Swedish Museum of Natural History, Department of Environmental Research & Monitoring (Appendix 5). The blubber samples were collected by Sven-Gunnar Lunneryd and Karl Lundström of the Swedish Agricultural University (SLU) Department of Aquatic Resources. Samples were stored in a freezer (-20 ^{o}C) before the analysis. Carbon and nitrogen stable isotope values (δ^{13}C and δ^{15}N) were analyzed from liver and muscle tissues, while fatty acid (FA) analysis was determined from blubber tissues. The stable isotope values and fatty acid signatures for prey items are from Mänttäri (2011) and Lundström (2012c), respectively.

Table 3. Age, sex (female [F], male [M]), month, coordinates, length and weight of 45 Baltic ringed seals from the Bothnian Bay.

Seal No.	Seal.	Age (yr)	Sex	Month	Coordinates	Length (m)	Weight (kg)
1	5298	1	F	2008 June	N 65° 45’ E 22° 43’	1.1	26.0
2	5336	5	F	2008 May	N 65° 38’ E 23° 06’	1.3	44.3
3	5334	1	M	2008 June	N 65° 42’ E 23° 01’	1.0	31.5
4	5300	1	F	2008 May	N 65° 40’ E 23° 03’	1.1	34.3
5	5046	1	M	2008 June	N 65° 45’ E 22° 43’	1.1	23.4
6	5017	3	F	2008 May	N 65° 43’ E 22° 35’	1.1	24.1

7	5009	2	M	2008 May	N 65° 42’ E 23° 01’	1.1	30.0
8	5052	3	M	2008 June	N 65° 40’ E 23° 03’	1.1	33.0
9	5004	1	M	2008 May	N 65° 45’ E 22° 43’	1.0	25.2
10	5023	1	F	2008 June	N 65° 26’ E 22° 24’	1.1	27.5
11	5323	7	M	2008 May	N 65° 20’ E 21° 54’	1.2	36.8
12	8900	0-1	F	2008 June	N 65° 43’ E 22° 35’	0.9	22.0
13	8042	2	F	2007 Nov.	N 65° 40’ E 22° 59’	1.2	45.0
14	8633	1	M	2008 May	N 65° 38’ E 23°07’	1.1	36.4
15	5330	13	F	2009 May	N 65° 45’ E 22° 43’	1.3	54.0
16	5377	14	M	2010 May	N 65° 45’ E 22° 43’	1.5	59.2
17	5053	0-1	F	2008 Oct.	N 65° 45’ E 22° 43’	0.9	28.5
18	5424	6	F	2008 May	N 65° 45’ E 22° 43’	1.3	42.3
19	5299	12	M	2009 May	N 65° 45’ E 22° 43’	1.3	46.0
20	5026	1	F	2008 June	N 65° 45’ E 22° 43’	1.1	26.9
21	5003	1	F	2008 May	N 65° 27’ E 22° 25’	1.0	26.2
22	5305	20	F	2008 June	N 65° 45’ E 22° 43’	1.2	37.8
23	5312	12	F	2008 May	N 65° 45’ E 22° 43’	1.2	44.8
24	44	7	M	2008 Nov.	N 65° 45’ E 22° 43’	1.4	61.1
25	8853	3	F	2008 June	N 65° 39’ E 23° 05’	1.1	28.2
26	8043	0-1	F	2007 Nov.	N 65° 47’ E 22° 46’	0.9	33.7
27	5327	4	M	2008 May	N 65° 20’ E 21° 54’	1.4	66.5
28	8995	25	M	2009 Oct.	N 65° 44’ E 22° 34’	0.0	0.0
29	5297	0-1	M	2008 May	N 65° 27’ E 22° 25’	1.0	30.9
30	5355	3	M	2009 May	N 65° 45’ E 22° 43’	1.3	47.5
31	8590	2	F	2009 Oct.	N 65° 45’ E 22° 43’	0.0	0.0
32	8911	1	M	2008 May	N 65° 40’ E 22° 42’	1.1	26.6
33	5325	1	F	2008 June	N 65° 20’ E 21° 54’	1.1	36.1
34	3749	12	F	2008 May	N 65° 27’ E 22° 25’	1.3	43.9
35	5326	0-1	M	2009 May	N 65° 27’ E 22° 25’	0.9	18.7
36	7994	4	F	2010 May	N 65° 24’ E 22° 29’	1.1	39.3
37	5469	1	F	2008 June	N 65° 49’ E 22° 47’	1.0	30. 7
38	5376	2	F	2008 May	N 65° 44’ E 22° 34’	1.2	41.7
39	7795	4	F	2009 May	N 65° 44’ E 22° 34’	1.2	33.5
40	5328	7	M	2008 June	N 65° 25’ E 22° 21’	1.3	50.5
41	5347	7	F	2008 May	N 65° 27’ E 22° 25’	1.3	51.2
42	5333	1	F	2008 June	N 65° 46’ E 22° 42’	1.0	27.7
43	9025	7	M	2008 Oct.	N 65° 46’ E 22° 42’	1.1	49.7
44	45	4	M	2008 Nov.	N 65° 43’ E 22° 36’	1.3	66.8
45	8858	5	M	2008 Oct.	N 65° 43’ E 22° 36’	1.3	66.3

3.2 Prey samples

Prey items were chosen based on their occurrence in previous dietary studies of grey seals and Baltic ringed seals (Tormosv & Rezvov 1978, Helle 1983, Sinisalo *et al.* 2006, 2008). Prey items were collected and classified into three major groups by Mänttäri (2011): ‘crustaceans’, ‘pelagic fish’ and ‘predator fish’. The group crustaceans included isotope values of the isopod (*Saduria entomon* L.) and the group represented species lower in the food chain. Three-spined stickleback (*Gasteroteus aculeatus* L.) can be included into these group crustaceans due to its similar nitrogen isotope value (9.8‰) (Sinisalo *et al.* 2006). The group pelagic fish included herring (*Clupea harengus membras* (L.), vendace (*Coregonus albula* (L.)), whitefish (*Coregonus lavaretus* (L.), ruffe (*Gymnocephalus cernuus* (L.), smelt (*Osmerus eperlanus* (L.)), perch (*Perca fluviatilis* L.), roach (*Rutilus rutilus* (L.), bleak (*Alburnus alburnus* (L.) and lamprey (*Lampetra fluviatilis* (L.). The predator group contained salmon (*Salmo salar* L.) and fourhorn sculpin (*Myoxocephalus quadricornis* L.) For these three prey groups the isotopic ratios for carbon and nitrogen of muscle tissue were calculated based on averages and standard deviations of individual prey items (Fig. 1). The carbon and nitrogen stable isotope values were for group salmon δ^{13}C 21.1 ‰ ± s.d. 1. 4 and δ^{15}N 12.7 ‰ ± s.d. 0.7, for group pelagic fish δ^{13}C 24.0 ‰ ± s.d. 1.4 and δ^{15}N 10.7 ‰ ± s.d. 1.9 and for group crustacean δ^{13}C 21.7 ‰ ± s.d. 1.6 δ^{15}N 8.1 ‰ ± s.d. 0.4 (Mänttäri 2011).

3.3 Stable isotope analysis of muscle and liver samples

Frozen samples were processed for stable isotope analysis with sterilized equipment to avoid contamination of samples. Liver and muscle samples were cut on glass (2 cm x 1cm) to smaller pieces so that any contaminated edges were removed; only the centre of the tissue was used for the SI analysis. The small sample pieces were stored in glass vials, closed with parafilm and kept in a freezer (-20 ^{o}C) before drying. Liver and muscle samples were freeze dried in a Christ ALPHA 1-4 LD Plus freeze drier. Before adding the samples to the drier the parafilm was pierced with a sharp spike. The samples were dried 48 h in -31 ^{o}C with a pressure of 0.34 bars. Samples were ground to a homogenous powder and every glass vial was closed with a plastic cap. Samples and standards were weighed (0.6 mg) into tin capsules using a microbalance. Two parallel samples were weighed to minimize experimental error. Laboratory standard (dried and homogenized pike muscle, *Esox lucius* L.) was used between the samples in the analysis so that every run contained 36 samples and 11 laboratory standards.

Stable isotope values of nitrogen (^{15}N/^{14}N) and carbon (^{13}C/^{12}C) were analyzed at the University of Jyväskylä using a Carlo Erba Flash EA1112 elemental analyzer connected to a Thermo Finnigan DELTA^{PLUS} Advantage continuous flow isotope ratio mass spectrometer (Thermo Electron Corp, Waltham, USA). To ensure linearity of isotopic results any drifting noticed during the run was corrected after runs. Muscle and liver tissues are lipid-rich and δ^{13}C values of lipids are lower than for fatless tissues and will affect isotopic results (Thompson *et al.* 2000). Carbon (δ^{13}C) values were therefore lipid-corrected based on sample carbon-nitrogen ratios (C/N) (Kiljunen *et al.* 2006).

3.4 Fatty acid analysis of blubber samples

The method of extraction of total fatty acid by Taipale *et al.* (2013) was used. The middle layer of blubber samples (n=45) were cut into smaller pieces samples were preserved in centrifuge tubes and stored in -20 ^{o}C. Dorsal blubber thickness was measured. The samples were kept frozen to avoid melting. The samples were placed into a cenfrifuge tube containing 2 ml of chloroform, flushed with nitrogen, sealed and stored in -20 ^{o}C.

Extraction was carried out by adding 1 ml of methanol, 1 ml of 2:1 (chloroform: methanol) and 1 ml of NaCl (0.9 %). The tubes were recapped and sonicated for 10 minutes. The samples were vortexed for 1 minute and then centrifuged for 5 minutes at 2500 rpm in 4 ^{o}C. The bottom organic layer was removed into a new centrifuge tube with a glass pasteur pipette. The pipette was washed twice inside and outside with chloroform. The old tube was recapped, vortexed for 1 minute and centrifuged for 5 minutes at 2500 rpm in 4 ^{o}C. The bottom organic layer was again removed to the same centrifuge tube and washed with chloroform. The procedure was repeated if the organic bottom layer was not fully removed. The samples were concentrated under a stream of nitrogen for 3 h after which the tubes were recapped and stored in -20 ^{o}C.

For methylation lipids were dissolved with 1 ml of toluene, and 2 ml of methylation reagent (methanol-sulphuric) and the tubes were shaken to mix the solvents. The samples were flushed under nitrogen for 10 minutes, and the tubes were then recapped and vortexed. The samples were methylated for 90 minutes at 80 ^{o}C in a water bath. After samples were cooled, 2 ml of 2 % $KHCO_3$, 5 ml of (1:1, hexane: diethyl ether) was added. The tubes were recapped, shaken and vortexed and the caps were gently removed. The samples were centrifuged at 1500 rpm for 2 minutes. The upper organic layer was removed to a new centrifuge tube. 5 ml of hexane was added into the centrifuge tube and again the samples were shaken, vortexed and centrifuged at 1500 rpm for 2 minutes. The upper organic layer was transferred into the new centrifuge tube; again if organic and inorganic layers were not fully separated the procedure was repeated. The solvent was evaporated under nitrogen. The samples were transferred into GC vials and 900 µl of hexane was added and stored in -20 ^{o}C.

The samples were too fatty for analysis, as samples were not originally weighed, too much fat was extracted. Therefore 28 samples were diluted 240-fold and 17 samples 360-fold. The samples were analyzed with a gas chromatograph (Shimadzu Ultra) equipped with mass detector (GC-MS). Helium gas was used as a carried gas with an average velocity of 34 cm s^{-1}. Calibration curves were used to determine fatty acid concentrations, the curves were based on standard solution of a FAME standard mixture.

3.5 Data analysis

3.5.1 Stable isotope analysis

Mixing models can provide quantitative dietary data and specific data from proportions of different food items in consumer diets (Phillips & Gregg 2003). The mixing model analysis was determined using the software package SIAR 4.0 e.g SIAR V 4 (Stable isotope analysis in R) by Parnell & Jackson (2013). SIAR calculates the most likely quantitative diet proportions based on Bayesian models, which tests all the distribution probabilities forming the final proportions (%) of the different prey groups in the seal diets. The model includes assumptions and limitations, so outputs should be treated with caution (Parnell *et al.* 2010). In this study liver and muscle tissues and their δ^{13}C and δ^{15}N isotopic values were used in mixing models to determine proportions (%) of different food items. Seals were divided into juveniles from 0 to 4 years old (n=30), and adults > 4 years old (n=15). Seasonal differences were examined between spring and autumn such that seals shot in May–June (n=36) and individuals shot in October–November (n= 9) were included into group autumn. In May–June there were 25 juveniles and 11 adults, in October–November 5 juveniles and 4 adults were studied. Fractionation factors used in this study included standard deviations from the study of McCutchan *et al.* (2003). Fractionation factors for muscle tissue were for carbon 1.3 ‰ ± s.d. 1.23 and for nitrogen 2.4 ‰ ± s.d. 1.07, and for liver tissue were for carbon 0.6 ‰ ± s.d. 1.23 and for nitrogen 3.1 ‰ ± s.d. 1.07 (Hobson *et al.* 1996, McCutchan *et al.* 2003).

3.5.2 Fatty acid analysis

The total of 45 Baltic ringed seals their middle blubber layer were analyzed. Identification of calibration curve and fatty acids was based on retention time, mass spectrum and authentic standard mixes. Identification of calibration curve was estimated with a GCMS Analysis editor. Calibration curve was determined using GCMS Postrum analysis program. Identification of fatty acids was based on spectrum database guidelines by Christie (2013). The database identification was based on the McLafferty rearrangent ion, molecular weight ion, omega ion and delta ion in a mass spectrum (Christie 2013). Omega ion represents the first double bond from the terminal group (Taipale *et al.* 2013). Analyzed fatty acids were in a *cis*- configuration.

Averages and standard deviations for individual fatty acid composition of seals were calculated in excel version 2010. Multivariate principal component analysis (PCA) was used to determine differences in multivariate fatty acids content between seals (Taipale *et al.* 2013). In addition, differences between seals were determined in excel version 2010 to detect divergence between individuals. PCA was carried out using IBM SPSS Statistics 22. PCA was used to transform original variables (individual fatty acids) into a smaller number of orthogonal variables, which were uncorrelated (Karlsson 2003). To compare the fatty acid composition of the seals to their diet, eight fatty acid categories were calculated: saturated fatty acids, monounsaturated fatty acids, sum of $C_{18}\omega3$ PUFAs, EPA plus DHA, sum of $C_{18}\omega6$ PUFAs, arachidonic acid, and sum of branched fatty acids and ratio of ω3 to ω6 fatty acids (Brett *et al.* 2006). The three largest principal components (PCs) were rotated using the normalized varimax strategy in SPSS, and new component coefficients were calculated. Positive Pearson correlation between three major components was determined to visualize results.

Blubber content and condition index were calculated for 43 seals. Percentage of blubber content was calculated with the equation of Ryg *et al.* (1990): $B= 4.44 + 5.693 (L/M)^{0.5*}D$, where L is a standard length in meters, M is a body mass in kilograms and D is a dorsal blubber thickness in meters. Condition index was calculated according to Kraft *et al.* (2006): $CI= BIM/ M^{0.75}$, blubber content was multiplied by M/100 to get seal's BIM (Kraft *et al.* 2006). Condition index is used to determine energetic status and degree of fatness of seals (Kraft *et al.* 2006).

4. RESULTS

4.1 Stable isotope results

4.1.1 Long-term diet

Isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of muscle tissue of seals differed between individuals (Table 4). The $\delta^{13}C$ value varied from -22.13 ‰ to -19.82 ‰ and $\delta^{15}N$ ranged from 13.89 ‰ to 12.23 ‰ indicating some variation in diet of seals (Table 4).

Table 4. Isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of muscle tissue of studied seals (n=45). $\delta^{13}C$ (‰) original value, $\delta^{13}C'$ (‰) lipid corrected value, $\delta^{13}C$ fract. (‰) fractionation corrected value and $\delta^{15}N$ (‰) original value, $\delta^{15}N$ fract. (‰) fractionation corrected value, C/N. Carbon ($\delta^{13}C$) values were lipid-corrected based on sample carbon-nitrogen ratios. The $\delta^{13}C$ and $\delta^{15}N$ values of ringed seal muscle tissues are corrected for dietary isotopic fractionation. Baltic ringed seals (n=45) were shot between 2007 and 2009 from the Swedish side of the Bothnian Bay.

Seal No.	Seal	δ13C (‰)	δ13C' (‰)	δ13C fract. (‰)	δ15N (‰)	δ15N fract.(‰)	C/N
1	5298	-20.789	-19.928	-21.228	13.237	10.837	3.411
2	5336	-22.140	-21.417	-22.717	12.331	9.931	3.338
3	5334	-22.157	-21.355	-22.655	12.264	9.864	3.380
4	5300	-22.061	-21.283	-22.583	12.458	10.058	3.367
5	5046	-21.764	-20.950	-22.250	12.909	10.509	3.386
6	5017	-22.777	-21.873	-23.173	13.266	10.866	3.434
7	5009	-22.093	-21.206	-22.506	12.642	10.242	3.425
8	5052	-22.823	-22.021	-23.321	12.644	10.244	3.380
9	5004	-22.051	-21.154	-22.454	12.208	9.808	3.431
10	5023	-21.601	-20.689	-21.989	13.406	11.006	3.439
11	5323	-21.825	-21.092	-22.392	12.539	10.139	3.343
12	8900	-22.601	-21.653	-22.953	12.754	10.354	3.458
13	8042	-22.094	-21.385	-22.685	12.481	10.081	3.331
14	8633	-21.190	-20.384	-21.684	13.133	10.733	3.382
15	5330	-22.311	-21.677	-22.977	12.443	10.043	3.293
16	5377	-22.264	-21.563	-22.863	13.191	10.791	3.327
17	5053	-22.948	-21.905	-23.205	12.506	10.106	3.512
18	5424	-22.061	-21.477	-22.777	12.294	9.894	3.268
19	5299	-21.381	-20.758	-22.058	12.876	10.476	3.287
20	5026	-22.009	-21.222	-22.522	12.392	9.992	3.371
21	5003	-22.765	-21.992	-23.292	12.404	10.004	3.364
22	5305	-23.004	-22.125	-23.425	12.757	10.357	3.421
23	5312	-21.642	-21.009	-22.309	12.422	10.022	3.292
24	44	-21.831	-21.199	-22.499	12.934	10.534	3.292
25	8853	-22.259	-21.541	-22.841	13.106	10.706	3.335
26	8043	-20.780	-19.817	-21.117	13.247	10.847	3.467
27	5327	-21.983	-21.284	-22.584	12.704	10.304	3.326
28	8995	-21.785	-21.059	-22.359	12.382	9.982	3.340
29	5297	-21.663	-20.715	-22.015	12.447	10.047	3.458
30	5355	-22.187	-21.311	-22.611	12.639	10.239	3.419
31	8590	-22.393	-21.676	-22.976	12.635	10.235	3.335
32	8911	-21.993	-21.224	-22.524	12.297	9.897	3.362
33	5325	-21.977	-21.106	-22.406	12.433	10.033	3.417
34	3749	-22.270	-21.496	-22.796	12.615	10.215	3.365
35	5326	-22.430	-21.546	-22.846	13.885	11.485	3.423
36	7994	-22.693	-21.891	-23.191	13.006	10.606	3.379
37	5469	-21.736	-20.878	-22.178	12.235	9.835	3.410
38	5376	-21.759	-20.951	-22.251	12.758	10.358	3.383
39	7795	-22.397	-21.804	-23.104	12.680	10.280	3.272
40	5328	-21.324	-20.627	-21.927	12.433	10.033	3.325
41	5347	-22.034	-21.251	-22.551	12.682	10.282	3.369
42	5333	-22.318	-21.367	-22.667	13.225	10.825	3.461
43	9025	-21.048	-20.260	-21.560	12.543	10.143	3.372
44	45	-21.416	-20.535	-21.835	12.804	10.404	3.422
45	8858	-22.353	-21.576	-22.876	12.937	10.537	3.366

The majority of the ringed seals consumed similar proportions of the three prey groups in their long-term diets, but there were some differences between individual seals (Appendix 1). In long-term diet, the average proportional distribution of crustacean prey group was 32 % and both pelagic and predator prey groups 34 % (Table 5).

Table 5. Mean proportions and standard deviations of three prey groups from different trophic levels in long- term diet. Baltic ringed seals (n=45) were shot between 2007 and 2009 from the Swedish side of the Bothnian Bay.

	percentage (%)	s.d.
Crustacean	32.17	5.42
Pelagic fish	33.95	3.55
Predator fish	33.88	5.19

All Baltic ringed seals (n=45) ate the three prey groups (Fig. 1). Seal 1 ($\delta^{13}C$ -21.23, $\delta^{15}N$ 10.84), seal 26 ($\delta^{13}C$ -21.12, $\delta^{15}N$ 10.85), and seal 35 ($\delta^{13}C$ -22.85, $\delta^{15}N$ 11.49) probably ate more predator fish group according their higher $\delta^{15}N$- values. Seal 22 ($\delta^{13}C$ -23.43, $\delta^{15}N$ 10.36) had higher $\delta^{13}C$ suggesting more pelagic fish group in its diet (Fig. 1). According to mixing model results seals 1, 26 and 35 had used predator fish group, 43 %, 48 % and 40 %, respectively (Appendix 1). Seal 22 had used pelagic fish group 40 % and predator fish group 35 % (Appendix 1). In spring adult and juvenile seals ate similar diets, crustacean groups 32 %, pelagic fish 34 % and predator fish 33 % (Fig. 2). In autumn adult seals concentrated more on predator fish (36 %) than juveniles (34 %) (Fig. 2). Long-term diet of male seals consisted on slightly more predator fish compared to females (35 % and 33 %, respectively) (Table 6). Males and females contained equal proportions of species in the lower food chain (32 %), but females concentrated more on pelagic fish compared to males (35 % and 33 % respectively) (Table 6).

Table 6. Mean proportions (%) and standard deviations of three prey groups in females (n=25) and males (n=20) long-term diet. The Baltic ringed seals (n=45) were shot between 2007 and 2009 from the Swedish side of the Bothnian Bay.

	Crustacean	s.d.	Pelagic fish	s.d.	Predator fish	s.d.
Females (n= 25)	32.11	4.95	34.57	3.61	33.31	5.31
Males (n=20)	32.23	6.09	33.18	3.41	34.59	5.08

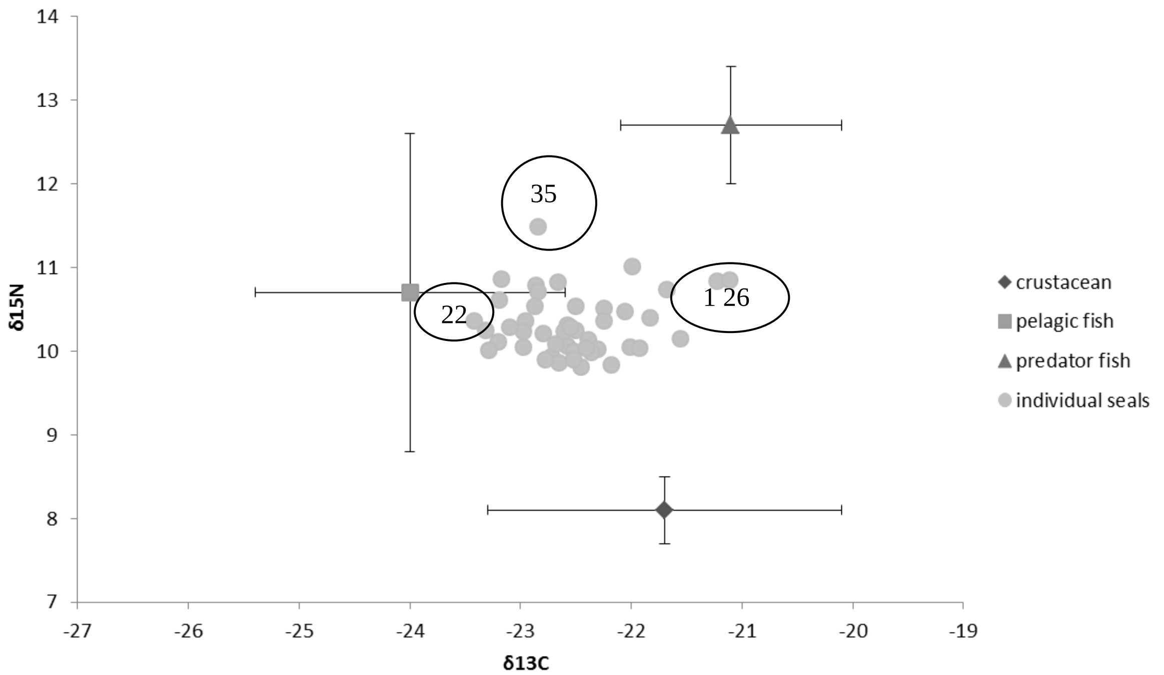

Figure 1. Dual isotope plot (see Phillips & Gregg 2003) of δ^{13}C and δ^{15}N values (‰) for individual ringed seals (●) and mean δ^{13}C and δ^{15}N values for three prey groups (Mänttäri 2011).The δ^{13}C and δ^{15}N values of ringed seal muscle tissues are corrected for dietary isotopic fractionation. Four circles are seal 1 (δ^{13}C -21.23, δ^{15}N 10.84), seal 22 (δ^{13}C -23.43, δ^{15}N 10.36) seal 26 (δ^{13}C -21.12, δ^{15}N 10.85) and seal 35 (δ^{13}C -22.85, δ^{15}N 11.49). Baltic ringed seals (n=45) were shot between 2007 and 2009 from the Swedish side of the Bothnian Bay.

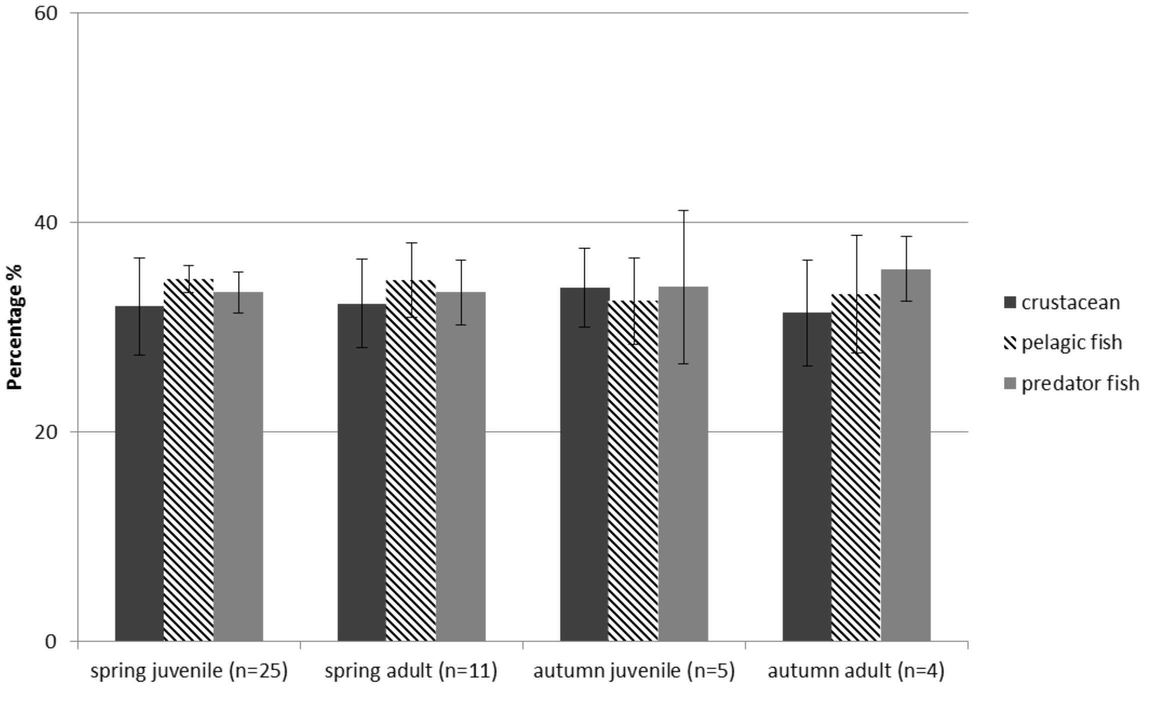

Figure 2. Mean proportions (%) of long- term diet of adult (>4 years) and juvenile (0-4 years) seals hunted in spring and in autumn. Percentages of prey items in diet are calculated from seal muscle tissues using R mixing model. Standard deviations of mean proportions are added to bars.

4.1.2 Short-term diet

Isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of liver tissue of seals differed between individuals (Table 7). The $\delta^{13}C$ value varied from -22.62 ‰ to -20.42 ‰ and $\delta^{15}N$ ranged from 11.09 ‰ to 8.52 ‰ indicating some variation in diet of seals (Table 7).

Table 7. Isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of liver tissue of studied seals (n=45). $\delta^{13}C$ (‰) original value, $\delta^{13}C'$ (‰) lipid corrected value, $\delta^{13}C$ fract. (‰) fractionation corrected value and $\delta^{15}N$ (‰) original value, $\delta^{15}N$ fract. (‰) fractionation corrected value, C/N. Carbon ($\delta^{13}C$) values were lipid-corrected based on sample carbon-nitrogen ratios. The $\delta^{13}C$ and $\delta^{15}N$ values of ringed seal liver tissues are corrected for dietary isotopic fractionation. Baltic ringed seals (n=45) were shot between 2007 and 2009 from the Swedish side of the Bothnian Bay.

Seal No.	Seal	δ13C (‰)	δ13C' (‰)	δ13C fract. (‰)	δ15N(‰)	δ15N fract.(‰)	C/N
1	5298	-21.820	-20.120	-20.720	13.490	10.390	3.935
2	5336	-23.787	-21.506	-22.106	13.185	10.085	4.405
3	5334	-23.256	-21.193	-21.793	13.375	10.275	4.216
4	5300	-23.534	-21.326	-21.926	11.624	8.524	4.340
5	5046	-22.609	-20.795	-21.395	13.401	10.301	4.019
6	5017	-24.423	-21.818	-22.418	13.718	10.618	4.722
7	5009	-23.387	-21.397	-21.997	13.418	10.318	4.155
8	5052	-23.812	-21.971	-22.571	13.251	10.151	4.039
9	5004	-23.699	-21.520	-22.120	13.150	10.050	4.314
10	5023	-22.843	-20.923	-21.523	13.599	10.499	4.101
11	5323	-23.152	-21.065	-21.665	13.908	10.808	4.235
12	8900	-23.254	-21.535	-22.135	13.457	10.357	3.948
13	8042	-23.299	-21.281	-21.881	13.334	10.234	4.179
14	8633	-23.295	-20.730	-21.330	13.778	10.678	4.680
15	5330	-23.438	-21.390	-21.990	13.333	10.233	4.203
16	5377	-23.588	-21.544	-22.144	13.883	10.783	4.200
17	5053	-23.747	-21.365	-21.965	12.603	9.503	4.499
18	5424	-23.914	-21.595	-22.195	13.081	9.981	4.440
19	5299	-23.131	-20.877	-21.477	14.051	10.951	4.381
20	5026	-23.941	-21.495	-22.095	13.412	10.312	4.560
21	5003	-23.936	-21.880	-22.480	13.298	10.198	4.210
22	5305	-24.131	-22.024	-22.624	13.484	10.384	4.253
23	5312	-22.990	-21.012	-21.612	13.500	10.400	4.146
24	44	-23.800	-21.943	-22.543	13.616	10.516	4.052
25	8853	-23.439	-21.723	-22.323	13.915	10.815	3.946
26	8043	-21.675	-19.820	-20.420	13.680	10.580	4.050
27	5327	-23.140	-21.156	-21.756	13.481	10.381	4.150
28	8995	-22.480	-20.589	-21.189	13.284	10.184	4.078
29	5297	-23.178	-20.791	-21.391	13.435	10.335	4.503
30	5355	-23.275	-21.336	-21.936	13.484	10.384	4.116
31	8590	-23.224	-21.246	-21.846	13.448	10.348	4.147
32	8911	-23.098	-21.063	-21.663	13.464	10.364	4.192
33	5325	-23.341	-21.196	-21.796	13.091	9.991	4.285
34	3749	-23.011	-21.076	-21.676	13.688	10.588	4.112
35	5326	-23.673	-21.353	-21.953	14.192	11.092	4.441
36	7994	-23.839	-21.517	-22.117	13.623	10.523	4.443
37	5469	-23.181	-21.035	-21.635	13.052	9.952	4.286
38	5376	-23.266	-21.181	-21.781	13.238	10.138	4.234
39	7795	-23.888	-21.662	-22.262	13.346	10.246	4.356
40	5328	-22.702	-20.476	-21.076	13.275	10.175	4.355
41	5347	-23.236	-21.152	-21.752	12.816	9.716	4.233
42	5333	-23.499	-21.378	-21.978	13.416	10.316	4.264
43	9025	-22.685	-20.693	-21.293	12.714	9.614	4.158
44	45	-22.435	-20.477	-21.077	13.353	10.253	4.130
45	8858	-23.388	-21.261	-21.861	13.184	10.084	4.269

The majority of the ringed seals consumed similar proportions of the three prey groups in their recent diets, but there were some differences between individual seals (Appendix 2). Recently 45 seals had consumed on average group predator 38 %, group pelagic fish 34 % and group crustaceans 28 % (Table 8).

Table 8. Mean proportions and standard deviations of three prey groups from different trophic levels in short- term diet. Baltic ringed seals (n=45) were shot between 2007 and 2009 from the Swedish side of the Bothnian Bay.

	percentage (%)	s.d.
Crustacean	27.82	4.47
Pelagic fish	34.43	3.21
Predator fish	37.75	4.41

The short-term diet varied between individual seals (Fig. 3). Seal 1 (δ^{13}C -20.72, δ^{15}N 10.38) seal 26 (δ^{13}C -20.42, δ^{15}N 10.58) and 35 (δ^{13}C -21.95, δ^{15}N 11.09) probably ate more predator fish group according their higher δ^{15}N values (Fig. 3). Seal 4 (δ^{13}C -21.93, δ^{15}N 8.52) had lower δ^{15}N suggesting that diet consisted more crustacean group (Fig. 3). According to mixing model results seals 1, 26 and 35 had used predator fish group 44 %, 48 % and 43 %, respectively (Appendix 2). Seal 4 had used crustacean group 45 % and group pelagic fish 35 % (Appendix 2).

In spring adult seals concentrated on species from the higher food web (38 %) and pelagic fish group (35 %) (Fig. 4). Consumption of lower trophic level prey increased towards autumn for both juveniles and adults (Fig. 4). The increase was greater for adult seals from 24 % to 30 % than for juveniles (28 % and 29 %). Group predator fish consumption by juveniles increased towards autumn from 37 % to 39 % and decreased for adults (from 38 % to 36.5%) (Fig. 4). Overall, juveniles used pelagic fish more in spring than in autumn (from 35 % to 32 %) (Fig. 4). Males used little more group salmon compared to females (39 %, 37 %, respectively) (Table 9).

Table 9. Mean proportions (%) and standard deviations of three prey groups in females (n=25) and males (n=20) short-term diet. The Baltic ringed seals (n=45) were shot between 2007 and 2009 from the Swedish side of the Bothnian Bay.

	Crustacean	s.d.	Pelagic fish	s.d.	Predator fish	s.d.
Females (n= 25)	28.53	4.73	34.76	3.28	36.71	5.03
Males (n=20)	26.93	4.06	34.01	3.16	39.05	3.16

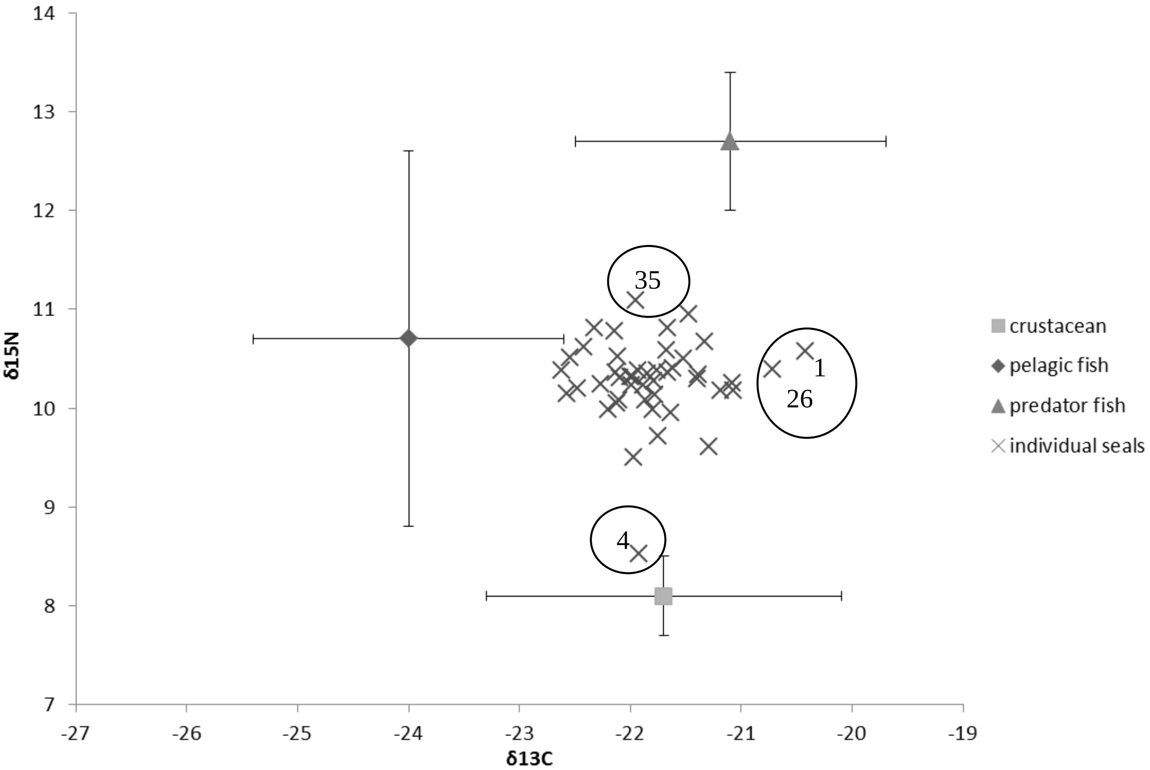

Figure 3. Dual isotope plot (see Phillips & Gregg 2003) of δ^{13}C and δ^{15}N values (‰) for individual ringed seals (x) and mean δ^{13}C and δ^{15}N values for three prey groups (Mänttäri 2011).The δ^{13}C and δ^{15}N values of ringed seal liver tissues are corrected for dietary isotope fractionation. Three circles are seal 1 (δ^{13}C -20.42, δ^{15}N 10.58), seal 4 (δ^{13}C -21.93, δ^{15}N 8.52), seal 26 (δ^{13}C -20.72, δ^{15}N 10.38) and seal 35 (δ^{13}C -21.95, δ^{15}N 11.09). Baltic ringed seals (n=45) were shot between 2007 and 2009 from the Swedish side of the Bothnian Bay.

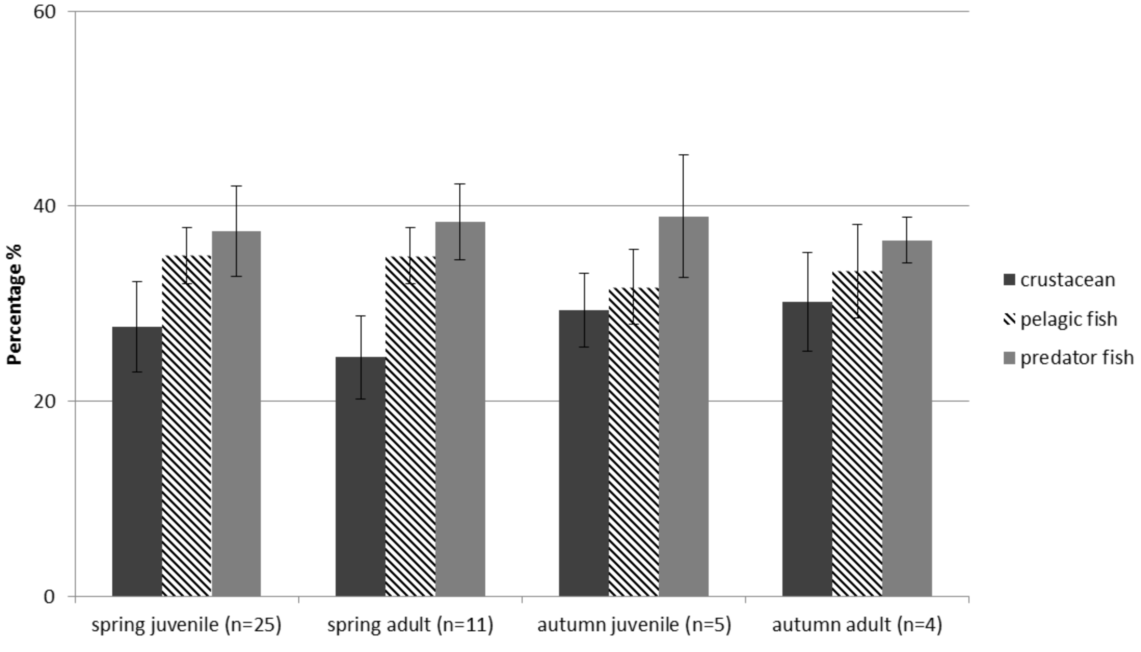

Figure 4. Mean proportions (%) of short- term diet of adult (>4 years) and juvenile (0-4 years) seals hunted in spring and in autumn. Percentages of prey items in diet are calculated from seal liver tissues using R mixing model. Standard deviations of mean proportions are added to bars.

4.2 Fatty acid analysis

4.2.1 Fatty acid profile

A total of 42 fatty acids were identified to examine long-term diet differences between 45 seals. The seven most common fatty acids were: 16:0, 16:1ω9, 16:1ω7c, 18:1ω9c, 20:5ω3, 22:5ω3 and 22:6ω3 in seals blubber tissues (Fig. 5). Studied fish species (n=13) (Table 11) and their 16:0, 16:1ω9, 16:1ω7c, 18:1ω9c, 20:5ω3, 22:5ω3 and 22:6ω3 proportions are also illustrated in Figure 5. Particularly, 16:1ω9 isomer was found abundant in seals but not so extensively from fish (Fig. 5). Abundance of fatty acids for 45 seals was analyzed with principal component analysis (PCA). The distribution of 42 fatty acids between two components is illustrated in Figure 6. C_{18} MUFAs and C_{20} PUFAs correlated positively with PC1, while the saturated fatty acids, palmitic acid (16:0) and MUFAs correlated positively with PC 2 (Fig. 6). The distribution of fatty acids is compared to the distribution of fatty acids in seals in PC1/PC2 and PC1/PC3 (Fig. 7).

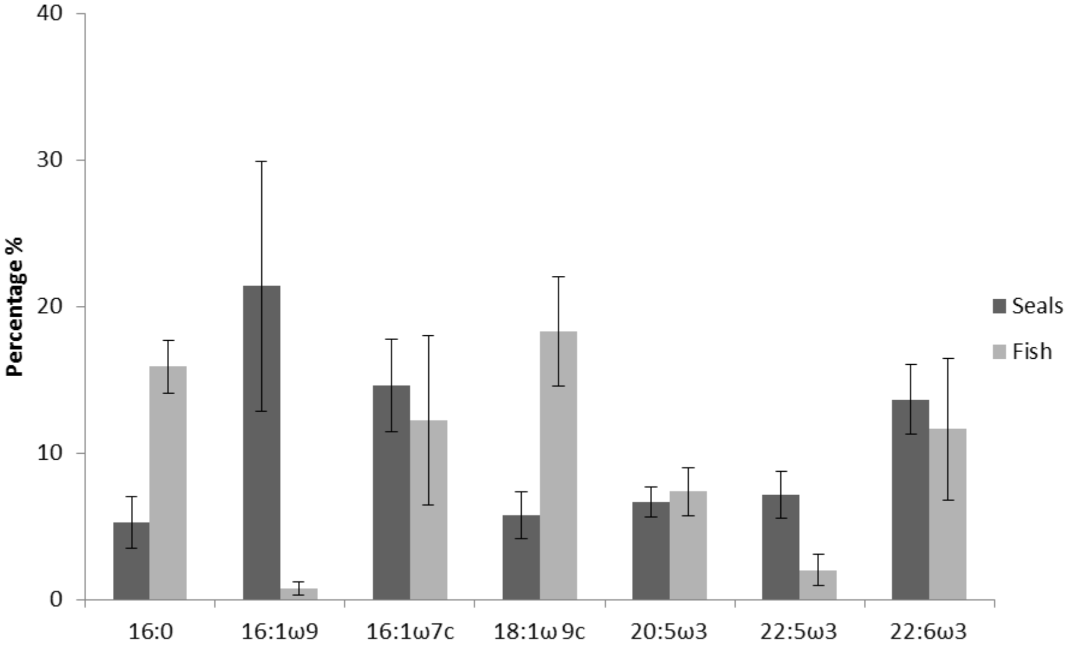

Figure 5. Seven most common fatty acids found from studied Baltic ringed seals and the same fatty acids from fish species (n=13) (Lundström 2012c). The mean and standard deviation for fatty acid proportion is calculated from individual seals and from studied fish species. Baltic ringed seals (n=45) were shot between 2007 and 2009 from the Swedish side of the Bothnian Bay.

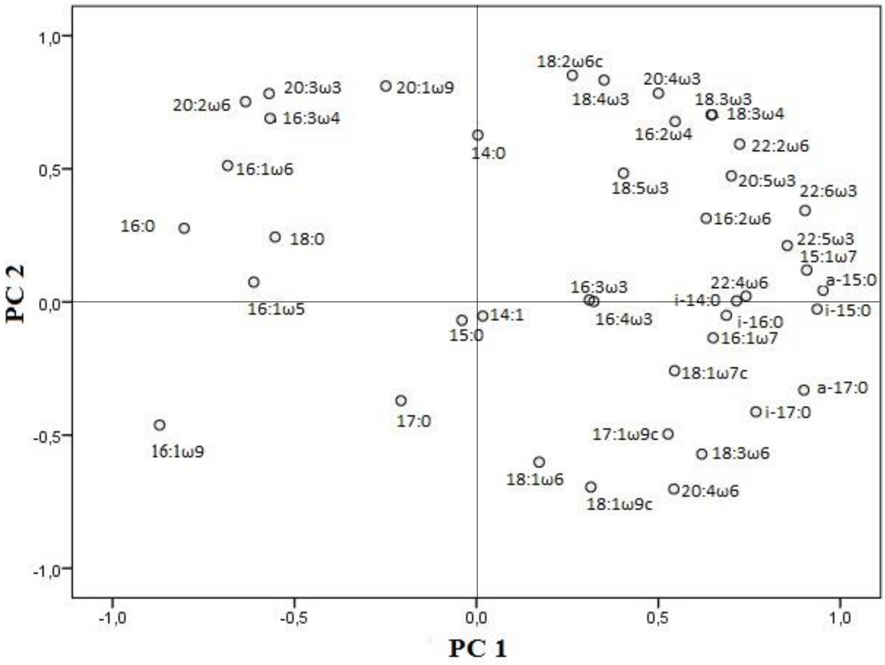

Figure 6. A principal component analysis (PCA) of fatty acid composition.

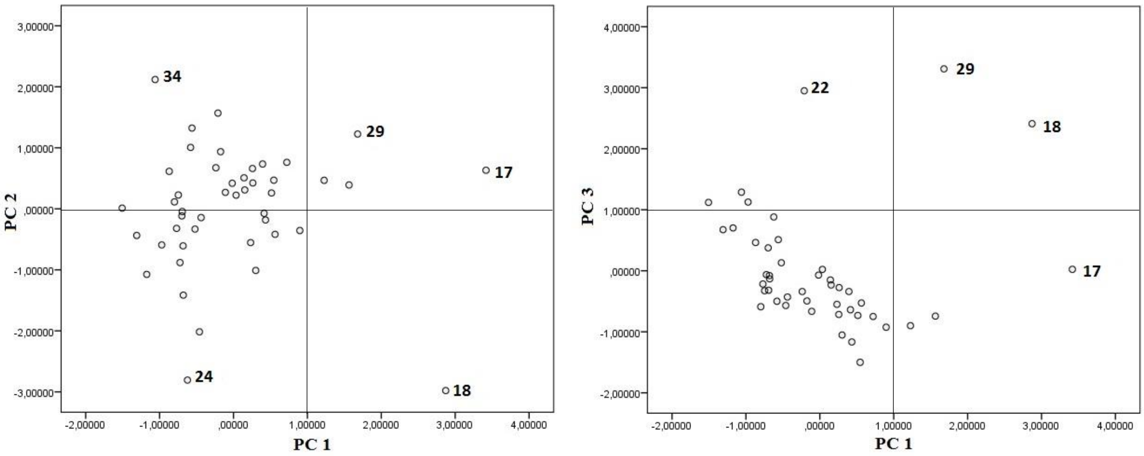

Figure 7. A principal component analysis (PCA) of fatty acid composition and diet of 45 seals; divergent individual seals are named. PC 1 explained 38 %, PC 2 25 % and PC 3 16 % of the variance.

Correlation coefficients between three principal components for eight fatty acid variables were calculated (Table 10). The first three principal components of the rotated PCA explained 38 %, 25 % and 16 % of the variance, when cumulative was 78 %. The first PC was most strongly associated with ω-3 fatty acids, in particular 18:3ω3 (Table 10, Appendix 4). Some individuals differed from the other seals (Fig. 7). Individuals 17 and 18 had positive correlation with PC 1 (Fig. 7). Seal 17 had a lot of DHA, EPA and 20:5ω3 fatty acids in its blubber, while seal 18 had 18:3ω3 and 18:2ω6 (Appendix 3). The second PC was most correlated with branched fatty acids, SAFAs (Table 10). Seal 34 correlated positively with PC 2 (Fig. 7). Blubber of seal 34 contained more 20:4ω6 isomer than that of the other seals (Appendix 3). The PC 3 was mostly correlated with normal saturated fatty acids and SAFAs (Table 10). Palmitic acid (16:0) was found high in numbers (*r* =0.94) from PC 3 (Appendix 4). Seal 22 correlated positively with PC 3, while blubber of seal 22 consisted 18:1ω7 isomer (Appendix 3, Fig. 7).

To determine which fatty acids have the most potential as dietary trophic markers for Baltic ringed seals, correlation coefficients of three principal components were compared to fatty acid composition of 13 possible prey items (Lundström 2012c) (Table 11). PC 1 had slight correlation with species higher in the food web, such as pike, salmon and sea trout (Table 11). PC 2 strongly correlated with burbot, while PC 3 did not correlate with any of the studied 13 prey items (Lundström 2012c) (Table 11). PC 1 was strongly associated with 18:3ω3 isomer (*r*=0.78) which was abundant in both vendace and sea trout (Appendix 4, Lundström 2012c). In addition, vendace was the only fish species which was caught from the Bothnian Bay and had the highest abundance of 18:3ω3 compared to other analyzed fish species (Lundström 2012c). PC 2 was highly correlated with 20:4ω6 isomer which was found to be abundant in benthic fish (Lundström 2012c). PC 3 did not correlate with studied prey items (Table 11).

Table 10. Correlation coefficients between the three principal components and the eight fatty acid categories used to generate them; classification of categories is illustrated in Table 1.

	PC 1	PC 2	PC 3
SAFAs	0.23	4.94	4.97
MUFAs	1.14	0.40	0.17
C18 ω3 PUFAs	2.45	0.23	0.57
EPA plus DHA	1.43	0.17	-0.43
C18 ω6 PUFAs	1.49	0.78	0.40
Arachidonic acid	-0.24	0.61	-0.02
Branched Fas	0.34	4.78	4.07
ω3/ω6 FA ratio	1.87	-9.54	0.37

Table 11. Correlations between possible prey items (Lundström 2012c) and the three principal components analyzed from seals (Table 10).

	PC 1	PC 2	PC 3
Fourhorn sculpin	-0.02	-0.19	-0.33
Burbot	-0.02	0.61	-0.43
Eelpout	0.06	-0.14	-0.29
Herring	0.02	-0.24	-0.24
Ide	0.01	-0.11	-0.23
Perch	0.03	-0.14	-0.33
Pike	0.13	-0.13	-0.22
Roach	-0.09	-0.12	-0.26
Salmon	0.11	-0.13	-0.33
Sea trout	0.10	-0.15	-0.27
Smelt	0.02	-0.21	-0.30
Whitefish	0.03	-0.12	-0.23
Vendace	0.01	-0.17	-0.03

4.2.2 Blubber content and condition index

Condition index was calculated for 43 seals; only the nine seals with blubber thicker than 3 cm are illustrated in Table 12. Eight of these seals were shot in autumn when blubber should be the thickest, as Baltic ringed seals double their body weight for winter. Degree of fatness was high for the nine seals (>1) (Table 12). The lowest condition index was found from juvenile seals hunted in May, when blubber thickness was less than 1 cm.

Table 12. Age, sex (F=female, M=male), month when shot, length, body mass, dorsal blubber thickness, blubber content and condition index of 9 Baltic ringed seals.

Seal. No	Age (yr)	Sex	Month	Length (cm)	Body Mass (kg)	Blubber thickness (cm)	Blubber content (%)	Condition index
13	2	F	Nov07	117	45	4	41.2	1.07
17	0-1	F	Oct08	92	29	4	49.4	1.14
24	7	M	Nov08	137	61	4	34.2	0.96
26	0-1	F	Nov07	95	34	5	47.3	1.14
28	25	M	Oct09			6		
33	1	F	June08	110	36	3	34.3	0.84
43	7	M	Oct08	109	50	6	55.0	1.46
44	4	M	Nov08	125	67	5	43.4	1.24
45	5	M	Oct08	125	66	6	47.4	1.35

5. DISCUSSION

5.1 Diet of Baltic ringed seals

The aim of the thesis was to study the feeding and foraging behaviour of Baltic ringed seals. Stable isotope analysis determined proportional diet, which was evenly distributed between three prey groups in long-term diet. The diet of seals consisted of a mixture of prey groups. No clear specialization amongst 45 seals for one prey group was evident from the isotope results, although there were slight differences between individual seals. Fatty acid analysis identified more species-specific components in the seal diets. Some individuals might have concentrated more on species higher in the food web, while some individuals concentrated on pelagic or benthic fish species. In addition, due to intra-specific differences in fatty acid composition within the Baltic Sea, possible prey items analyzed in previous studies were evaluated (Käkelä *et al.* 1993, Kirsch *et al.* 1998, Lundström 2012c). In addition, fish species analysed by Lundström (2012c) were mainly from the Bothnian Sea, and analysed fish did not strongly correlate positively with components 1 and 3 analysed from seals (Table 11). However, biomarker fatty acids of common prey items for seals in the Bothnian Bay (Table 2) supported findings from fatty acid composition and isotopes values of seals. The fatty acid composition of the studied seals was dominated by saturated and polyunsaturated fatty acids, and included high amounts of ω- 3 PUFAs such as DHA (22:6ω3), EPA (20:5ω3) and DPA (22:5ω3). According to biochemical methods the majority of seals may have used pelagic fish such as herring due to the abundance of the 18:1ω9 isomer. Some seals might have used species higher in the food such as fourhorn sculpin, eelpout, burbot, sea trout or even salmon. However, this conclusion needs further research to clarify and confirm it. It is unlikely that ‘filter feeding’ Baltic ringed seals ate salmonids. The most commonly found fish species from stomach contents of studied seals were vendace, herring and three-spined stickleback.

5.2 Diet variation between individual seals

Diet variation between individuals was determined based on stable isotope, fatty acid and stomach content analysis. Long-term diet of Baltic ringed seals was determined from stable isotope values of muscle tissues and fatty acid composition of blubber tissue. Isotope values of muscle tissue will reflect diet within months (Phillips & Elridge 2006, Sinisalo *et al.* 2008). Furthermore, fatty acid composition of blubber will also reflected diet over weeks to months; therefore the methods were compared to each other to get the most reliable long-term dietary data of Baltic ringed seals (Thiemann *et al.* 2007, Tucker *et al.* 2008). Data from stomach content analysis was used to support findings from the biochemical methods of liver tissue.

In fatty acid analysis individuals 17 and 18 were positively correlating with PC 1. According to fatty acid composition of seals, they ate prey from the higher food web (Table 11). Stable isotope results revealed that consumption of group pelagic fish was highest for both individuals. Seal 17 had consumed group pelagic fish 37 % and predator fish group 27 %. High abundances of 20:1ω9, 18:2ω6, 18:3ω3 and 18:4ω3 isomers were found from PC 1 (Appendix 4) and these isomers are related to pelagic prey (Käkelä *et al.* 2005). Based on the fatty acid, stable isotope and stomach analyses seal 17 consumed mostly vendace. Vendace is abundant in the Bothnian Bay and have relatively high proportions of ALA (18:3ω3) (Budge *et al.* 2003, Käkelä *et al.* 2005, Lundström 2012c). Vendace and PC 1 did not correlate strongly in fatty acid analysis (Table 11), but the stomach content of seal 17 included a large amount of vendace. This individual was a juvenile female seal, with high energetic status according to the condition index (Table 12) (Kraft *et al.* 2006). In addition, ARA (20:4ω6) was abundant in vendace, burbot and in eelpout, ARA is essential to the growth and development of young individuals such as seal 17 (Käkelä *et al.* 1993). Unsaturated fatty acids were common in the fatty acid composition of seal 17, and salmon slightly correlated with PC 1, it is still unlikely that a young individual such as seal 17 consumed salmon. Also other fish species such as Baltic cod (*Gadus morhua* L.) has been found to be rich in DHA (22:6ω3) (Käkelä *et al*. 1993, Kirsch *et al.* 1998), the Baltic cod is not abundant in the Bothnian Bay. Furthermore, there was no salmon found in stomachs of seals. However, the majority of seals did not have a positive correlation with PC 1 and according to long-term diet results the majority of seals did not use vendace as major prey, due to the low proportion of the 18:3ω3 isomer in their blubber. The 18:3ω3 isomer is also found from other fish species such as sea trout in the Bothnian Bay. Nevertheless, the stomach contents of twenty-seven seals revealed that they had consumed vendace as their recent diet, while stable isotopes revealed that the pelagic fish was one of the main prey groups. Pelagic fish such as vendace might be an ideal prey item for juvenile seals in early summer due to hatching and migration of vendace fry to the coast of the Bothnian Bay (Muus & Dahlstrom 2005); the majority of seals (n=36) were shot in May and June and according to short-term diet consumption pelagic fish group was higher in spring compared to autumn.

According to stomach content analysis three-spined stickleback and herring were the recent diet for seal 18. Seal 18 had used group pelagic fish 36 %, predator fish 34 % and group crustaceans 30 %. According to prey items (Lundström 2012c) it appears that seal 18 did not use herring in its long-term diet according to the weak correlation between PC 1 and herring (Table 11). Even though, 18:1ω9 is abundant in the Baltic herring and in analysed fish species, it was not abundant in PC 1 (Käkelä *et al.* 1993). However, 18:1ω9 and 18:3ω3 were abundant fatty acids in seal 18 (Appendix 3), it is still likely that seal 18 consumed herring. Three-spined stickleback was not analyzed by Lundström (2012c), while based on isotopic values of three-spined stickleback it is classified into species lower in the food chain (Sinisalo *et al.* 2006) for which consumption by seal 18 did not differ from the other seals.

Seals 22 and 34 were both adult female seals and shot in spring 2008. Individual 34 was positively correlated with PC 2 and perhaps ate predator fish, while isomer 20:4ω6 was rich in burbot (Lundström 2012c). Burbot was the only fish species which had moderate correlation with the components analysed from seals (Table 11). It is still unlikely that seal 34 ate burbot as it has not been found in previous studies (Lundström 2012c). The results from Table 11 have to be evaluated with a care as prey items were not caught from the Bothnian Bay. According to isotope results seal 34 consumed both crustaceans and pelagic fish groups 35 %, and predator fish 30 %. Recent diet of seal 34 consisted of three-spined stickleback, herring and vendace according to the stomach content analyses. In addition, no burbot was found from the stomach content of any seals. PC 2 of seals was correlated positively with 17:0, 18:1ω7 and in particular 20:4ω6 which are both associated with benthic prey (Käkelä *et al.* 2005). Likely seal 34 used other benthic fish species such as eelpout or fourhorn sculpin.

Even though, PC 3 did not correlate positively with analyzed prey items, it still correlated with seal 22. Isotope results revealed that consumption of group pelagic fish was 40 % and suggested that the diet of seal 22 consisted of pelagic prey. Fatty acid results for seal 22 were contradictory and therefore diet was impossible to infer. Furthermore, the stomach content of seal 22 contained a large amount of herring. Herring is a common prey item for older seals for example for 20 years old female seal 22 (Härkönen *et al.* 2008). PC 3 of seals correlated with isomers 17:0 and 18:1ω7 which are related to benthic systems, so seal 22 might also have eaten some benthic species which were not studied (Käkelä *et al.* 2005).

Overall, the fatty acid results suggested that the studied seals did not eat crustaceans due to low abundance of 20:1ω9 isomer (Käkelä *et al.* 1993). The isomers 20:1ω9 and 22:1ω11 usually originate from marine crustaceans (Käkelä *et al.* 1993). Stomach content analysis revealed that seals 28 and 31 had slight evidence of crustaceans in their stomachs. Crustaceans might be a by-catch during foraging for fish species from the bottom. Stable isotopes reflected that seal 28 had consumed group crustaceans 30 % and seal 31 35 %. One species from the lower of the food web was three-spined stickleback and because it was abundant in the stomachs of 34 studied seals this could be one of the main prey items. In late summer and autumn Baltic ringed seals forage intensively (Härkönen *et al.* 2008). It is likely that seals then consumed fatty fish species such as three-spined stickleback.

Condition index was determined for nine seals with blubber thicker than three centimetres. Of these nine seals five were adult males shot in autumn, which reflects that male seals did not have stress at that time of year as they do not have to fight to defend their territory, which usually takes place in spring during the mating period (Helle 1983). On the other hand, adult females in spring have low energetic status due to lactation and the breeding season (Kraft *et al.* 2006). Individuals 13, 17, 26 and 33 were immature female seals with high condition index. Condition index reflected that female seals do not have stress, for example from foraging, lactation or parturition, and that individuals were in healthy condition (Helle 1983, Kraft *et al.* 2006). In addition, the majority of the studied seals were juveniles (n=30) and shot in spring when they are skinniest; therefore the condition index for the majority of young seals was below 1, and blubber thickness less than three centimetres (Helle 1983).

5.3 Long-term diet

Proportional diet from stable isotope analyses revealed that the diet of Baltic ringed seals was evenly distributed between the three prey groups. Short- and long-term diets did not differ greatly. In addition, the three prey groups were consumed similarly through spring and autumn. Previous studies have reported great seasonal variation in seal diets; it was not the result in this study (Helle 1983). Diet consisted more of species higher in the food web than found in previous studies (Tormosov & Rezvov 1987, Käkelä *et al.* 1993, 1995, Karlsson 2003, Sinisalo *et al.* 2008, Mänttäri 2011 & Lundström 2012a, 2012b).

Nitrogen isotope values of seals reflected that prey items were caught from the same level of the food web, but greater variation between carbon isotope values indicated that seals ate several different fish species from a mixture of prey groups (Vander Zanden *et al.* 1997, Fry 2006, Cipro *et al.* 2012). In spring adult and juvenile seals had similar diets. While towards autumn adults apparently concentrated more on species near the top of the food web. There was a small difference in feeding behaviour between male and female seals; males concentrated slightly more on predator fish, while females ate more pelagic fish. The results between sexes in this study are consistent with previous studies in which differences in diet between females and males have been negligible (Mänttäri 2011).

Previous studies (Tormosov & Rezvov 1978, Sinisalo *et al.* 2008, Mänttäri 2011) have showed that Baltic ringed seals have concentrated mostly on pelagic prey. The stable isotope values in this study revealed that Baltic ringed seals were generalist feeders and ate a mixture of prey items from several levels of the food web. There are several possible fish species consumed from higher in the food web and it is likely that seals used several fish species on the top of the food web. In addition, predator fish such as sea trout, eelpout and burbot have similarly high nitrogen isotopic values (Cott *et al.* 2011). Pelagic fish consumption was also concentrated on several fish species, while consumption of group crustaceans was more concentrated on three-spined stickleback. Based on the isotope results it is not possible to evaluate species-specific prey composition (Iverson *et al.* 2004, Tucker *et al.* 2008, Bowen & Iverson 2013).

The distribution of fatty acids also revealed that the diet of studied seals (n=45) consisted of a mixture of prey items from several trophic levels. However, compared to previous studies the diet of the studied seals (n=45) did not contain extensive marine isopods (Käkelä *et al.* 1993); instead the diet was concentrated more on pelagic and benthic fish species. There was evidence that some seals had used species from higher trophic levels. Palmitic acids (C16 acids) were found in high numbers from seals. Usually C16 is found to be abundant in fresh- and brackish water environments where diatoms and green algae dominate the food web (Käkelä *et al.* 1993). On the other hand, individuals were mainly juvenile (n=30) seals, which will have more C16 acids and less C22 acid particularly in the outer blubber (Käkelä *et al.* 1993). 16:1ω7 was an abundant fatty acid in studied seals; it is a general product of biosynthesis of seal blubber (Budge *et al.* 2006). Fatty acid composition of seals is not only related to diet; other factors such as environmental conditions and their seasonal variation will affect fatty acid composition. In particular C16 is related to water temperature, which fluctuates strongly (4 °C -20 °C) with season in the Baltic Sea (Hietala no date). Overall, C16 and C18 MUFAs and 22:5ω6 and 18:2ω6 PUFAs are abundant in fish and seals in the Baltic Sea (Karlsson 2003). A great overlap in fatty acid composition among species has been noticed in the Baltic Sea perhaps due to the simple food web with a limited number of species (Lundström 2012c).

5.4 Short-term diet

Short-term diet was determined from liver tissue, which will reflect diet within the last weeks before the hunt (Phillips & Elridge 2006, Sinisalo *et al.* 2008). Short-term diet of Baltic ringed seals (n=45) revealed that all seals consumed the three prey groups used in the research. However, there was a difference between proportional consumption of some individuals. For example, seal 35 had used group crustaceans only 19 %, while the diet of seal 4 consisted of 45 % group crustaceans. Similar variation was found in consumption of group predator fish, as seal 4 had used it 20 % and seal 26 48 %. The variation in nitrogen isotope values reflected that seals ate prey items from several levels of the food web (Vander Zanden *et al*. 1997, Fry 2006, Cipro *et al.* 2012).

δ^{13}C- and δ^{15}N values of individual Baltic ringed seals differed more than in previous studies (Sinisalo *et al.* 2008, Mänttäri 2011). Overall, in this study the short-term diet of 45 seals were concentrated more on species higher in the food chain than was found in previous isotope studies (Sinisalo *et al.* 2008, Mänttäri, 2011). Seal 4 had concentrated more on group crustaceans, while its stomach contents included a large amount of herring and also three-spined stickleback which likely is its recent diet. On the other hand, seal 26 had used a lot of group predator fish, but its recent diet included a lot of vendace. However, some individuals such as 27 and 28 had consumed fourhorn sculpin as their recent diet. Fourhorn sculpin is included into group predator fish. Predator fish group consumption of seals 27 (39 %) and 28 (40 %) was slightly higher than the average consumption of seals (38 %) in short-term diet.

Fourhorn sculpin is a common fish species in the brackish water system, and it is abundant throughout the year (Kuparinen *et al.* 1996). It is likely that individual seals ate fourhorn sculpin during their foraging for small fishes from the bottom, as fourhorn sculpin can be found from the bottom of sea floor, as its diet consists mainly of crustaceans. In addition, the Bothnian Bay is relatively shallow, with average depth 40 metres, and maximum daily dive depths for Baltic ringed seal males has been reported to be 40 metres and for females 25 metres (Härkönen *et al.* 2008). Juvenile seals have the ability to forage for benthic prey in shallow coastal waters in the Bothnian Bay. Juvenile seals usually have restricted ability to capture prey, and their diet can be less specialized than that of adults. In addition, in shallow environments benthic production is more intense and dispersed, which will provide different exploitation opportunities for seals. Diet of juvenile and adult seals did only differ few percent between each other, which again can reflect the simple food web with a limited number of prey species.

Group predator fish consumption was highest compared to other prey groups in spring and in autumn for both seal age groups, while in autumn juveniles used more group predator fish than adult seals in their short-term diets. It is unlikely that the short-term diet of individual 26 included salmonids as one year old seals have limited ability to dive and capture prey, while their teeth are not ideal for eating predator fish species (Helle 1983, Strandberg *et al.* 2008, Lundström 2012b). Since the predator fish group included fourhorn sculpin, it appears more likely that these juvenile seals had been feeding on this species. However, the possible proportion of farmed smolt in the diet of Baltic ringed seals needs more investigation. In particular, in early summer the amount of salmon and sea trout increases temporarily due to migration of species, while farmed smolts are released in early summer in the Bothnian Bay.

Overall, Baltic herring is an important prey item for Baltic ringed seals (Härkönen *et al.* 2008). Consumption of group pelagic fish stayed steady through the study. Herring was also one of the most abundant fish species found from stomach content of seals. Some seals such as individual 22 had consumed 40 % of group pelagic fish. Herring is abundant through the year in the Bothnian Bay, and it is an ideal prey for seals particularly in late summer when seals are accumulating fat stores (Helle 1983, Härkönen *et al.* 2008, Rosen & Tollit 2012). Herring contains approximately 44 % of calories as fat (Budge *et al.* 2006). Baltic ringed seals are also active divers during the day, when herring is near the sea floor due to diurnal vertical migration (Härkönen *et al.* 2008). Three-spined stickleback was also one of the most abundant species found from the stomach contents of seals. In particular, seals 26 and 34 had consumed a lot of three-spined sticklebacks as their recent diet. In previous studies three-spined stickleback has also been found to be an important prey item for Baltic ringed seals in late summer due to its high fat content (Tormosov & Rezvov 1978). Furthermore, the three-spined stickleback is abundant through the year, except from March to June when it migrates to rivers to spawn (Muus & Dahlstrom 2005). In early summer when the majority of seals (n=36) were shot, the food web in the Bothnian Bay contained fewer species at the lower trophic level, and more species at the higher trophic level such as migratory salmonids.

5.5 Management of species in the future

The studied Baltic ringed seals (n=45) consumed pelagic and benthic prey which are not classified as valuable fisheries species (Laanikari 2013). In addition, as shown previously, ringed seals caught from fishing nets are trapped during their foraging (Lunneryd 2005). However, in the future if the herring population in the Baltic Sea decreases fishermen and Baltic ringed seals will be competing more for the same prey, as herring is quantitatively (kg) the most valuable prey in the Baltic Sea (Raitaniemi & Manninen 2013). More ethical ways to manage the protected species other than hunting permits would then be needed. For example, for grey seals a pontoon trap model is being developed to catch seals alive and then removed them from fish traps (Anonymous 2013). The pontoon trap model is useful for removing the individuals for fishing; at the moment Baltic ringed seals are removed from traps to avoid by-catch losses. Due to the increasing population of Baltic ringed seals further research is needed to determine how many ringed seals are actually visiting fish traps and whether they really are consuming prey. On the other hand, more species-specific diet information for Baltic ringed seals should be obtained to improve understanding of seal feeding and foraging behaviour in the Bothnian Bay. The key is to improve co-operation between Finnish and Swedish researchers to determine more completely diet of Baltic ringed seals.

5.6 Limitations of methods

Both biochemical methods used in the study relied on some assumptions made for fractionation factors for each consumer and their diets (Hobson *et al.*1996, Lesage *et al.* 2002, Kurle & Worthy 2002, Thiemann *et al.* 2007 & Rosen & Tollit 2012). Stable isotope results were mainly averages for the population, but differences in isotope values between individuals were also studied to investigate the diet of individuals. Prey items analyzed by Mänttäri (2011) had significant variation between individuals within a single group, which increased standard deviation between analyzed prey items within a group. The mixing model works best when stable isotope values of prey items are in a distance and fall close to predator values in the mixing model (Phillips & Gregg 2003). As the studied seals values fell close to values of prey items, the mixing model was applicable and the results can be considered reliable.

Prey items used in the study were not caught from the Swedish side of the Bothnian Bay, where the studied seals were shot. Prey items used in the stable isotope analysis were caught from the Finnish side of the Bothnian Bay, while for fatty acid analysis only vendace was from the Bothnian Bay and other fish species were from the Bothnian Sea. In addition, fish species were caught 2004 and 2005, three to five years earlier than seals. Intra-specific differences in fatty acid composition between the Bothnian Bay and the Bothnian Sea occur. In addition, there was only little variation in fatty acid composition of studied fish species (n=13) (Lundström 2012c). There are regional differences in salinity and temperatures which will affect zooplankton communities and then the fatty acid composition of fish species. Furthermore, fatty acid signatures of ringed seal prey should be stable across years, in particular when different species in the diet are evaluated (Thiemann *et al.* 2007).

The study involved 30 juveniles and 15 adult seals, which is a rather small sample and could misrepresent the average diet within the population. Ideally more seals of all ages and of both sexes would have been needed, but available samples of this protected seal species are restricted. For further research a more extensive number of possible prey items should be included as ringed seals consume at least 12 different fish species. 36 seals had blubber layer thickness less than 3 cm, which can affect fatty acid composition of long-chain PUFAs in the middle blubber layer (Strandberg *et al.* 2008). In future studies blubber samples should be weighed before extraction to allow quantitative fatty acid composition of seals to be determined, and to look at abundance of specific prey items. Abundance of prey groups was determined with stable isotope analysis, but species-specific abundance could not be determined as quantitative fatty acid signature analysis (QFASA) was not applied (Iverson *et al.* 2004, Rosen & Tollit 2012).

5.7 Conclusions

The aim of the study was to determine the feeding and foraging behaviour of individual Baltic ringed seals (n=45). According to stable isotope analysis the diets of the seals consisted of a mixture of prey groups, but there appeared to be some differences between individual seals. Furthermore, Baltic ringed seals consumed similarly three prey groups and diet did not show marked seasonal variation. Seals ate many different prey species from several levels of the food web. There was slightly more variation in short-term diet where consumption of the predator prey group by 45 seals was 38 % compared to long-term diet value of 34 %. In spring juvenile seals ate more species from the top of the food web in their long-term diet, perhaps the lactation is still effecting to the nitrogen values of juvenile seals. Male seals consumed more species from the top of the food web through the seasons analyzed. Fatty acid composition of seals was dominated by saturated, polyunsaturated fatty acids and ω-3 PUFAs. Fatty acid results showed that seals concentrated on pelagic and benthic fish species on the Swedish side of the Bothnian Bay, according to the profile of 18:1ω7, 18:1ω9, 22:6ω3 and C16 PUFAs and MUFAs. There was no evidence of consumption of isopods. According to the biochemical methods, seals diet may consist of three-spined stickleback, fourhorn sculpin and Baltic herring. Feeding behaviour between individual seals differed somewhat between different pelagic and benthic fish species. It is likely that seals 18 and 22 had used more Baltic herring. On the other hand, individual 17 differed from the rest of the population and this individual had perhaps specialized more on vendace. On the other hand it seems that seal 34 also ate some predator fish.

By combining the biochemical methods, the most reliable and species-specific dietary information was obtained. In general the two methods supported each other when the biomarker fatty acids for diet of seals were used. However, according to the stable isotope and fatty acid results it is impossible to say species-specific diet of studied seals.

The results showed that using conventional analysis such as stomach content analyses it will reflect only the recent diet, and that long-term consumption can be totally different (Bowen & Iverson 2013). However, stomach content analysis is still useful to support findings from biochemical methods. The methods used in this study reflect the simple food web in the Bothnian Bay inter alia overlapping in species fatty acid composition.

This study of Baltic ringed seals has indicated some new aspects of dietary behaviour of Baltic ringed seals in the Bothnian Bay. Perhaps dietary and foraging behaviour in brackish water systems is not as straightforward as sometimes assumed. Therefore, diets of endangered seal species need further research to help address problems with fisheries and maintaining sustainable management of seal species. For further research fatty acid composition for several fish species on the Swedish side of Bothnian Bay should be analyzed to detect rare prey species in the diet of seals. Also sulphur, hydrogen and oxygen stable isotopes could give more information about the diet of Baltic ringed seals. Very long chain polyunsaturated fatty acids such as 24:4ω3 and 28:7ω3 could be also analyzed from seals and prey items.

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Appendix 1. The mean diet proportions of prey items from three different trophic levels**.** The mixing model analysis was determined from muscle tissue using the software package SIAR 4.0 e.g SIAR V 4 (Stable isotope analysis in R) by Parnell & Jackson (2013). The mean value (+/- low-high 95%).

seal No.	seal	Crustacean	Pelagic fish	Predator fish
1	A2008/05298	0.32(0.05-0.53)	0.26(0-0.51)	0.43(0.13-0.70)
2	A2008/05336	0.38(0.08-0.65)	0.35(0.02-0.63)	0.27(0.02-0.49)
3	A2008/05334	0.39(0.08-0.67)	0.34(0.02-0.62)	0.27(0.01-0.48)
4	A2008/05300	0.37(0.07-0.67)	0.34(0.01-0.62)	0.29(0.02-0.50)
5	A2008/05046	0.34(0.06-0.57)	0.31(0-0.58)	0.35(0.06-0.59)
6	A2008/05017	0.28(0.02-0.49)	0.38(0.03-0.69)	0.34(0.0-0.60)
7	A2008/05009	0.36(0.06-0.60)	0.33(0-0.59)	0.32(0.03-0.55)
8	A2008/05052	0.34(0.04-0.59)	0.38(0.03-0.69)	0.28(0.01-0.50)
9	A2008/05004	0.41(0.09-0.68)	0.33(0.0-0.60)	0.27(0.02-0.48)
10	A2008/05023	0.29(0.03-0.50)	0.31(0.0-0.57)	0.40(0.11-0.69)
11	A2008/05323	0.37(0.08-0.63)	0.32(0.0-0.59)	0.31(0.03-0.53)
12	A2008/08900	0.34(0.05-0.57)	0.36(0.02-0.66)	0.31(0.03-0.53)
13	C2007/08042	0.37(0.07-0.62)	0.34(0.01-0.63)	0.29(0.03-0.51)
14	A2008/08633	0.32(0.06-0.54)	0.29(0-0.54)	0.39(0.20-0.66)
15	A2008/05330	0.37(0.06-0.63)	0.36(0.02-0.65)	0.28(0.01-0.50)
16	A2008/05377	0.29(0.03-0.51)	0.36(0.02-0.65)	0.35(0.04-0.60)
17	A2008/05053	0.35(0.05-0.61)	0.37(0.03-0.68)	0.27(0.01-0.49)
18	A2008/05424	0.30(0.03-0.53)	0.36(0.02-0.66)	0.34(0.03-0.58)
19	A2008/05299	0.21(0-0.40)	0.34(0.02-0.62)	0.45(011-0.77)
20	A2008/05026	0.27(0.02-0.48)	0.36(0.02-0.65)	0.37(0.02-0.50)
21	A2008/05003	0.28(0.02-0.49)	0.38(0.03-0.69)	0.34(0.03-0.60)
22	A2008/05305	0.25(0.07-0.47)	0.40(0.05-0.72)	0.35(0.02-0.61)
23	A2008/05312	0.27(0.02-0.47)	0.33(0.01-0.60)	0.40(0.09.0.69)
24	A2008/00044	0.24(0.01-0.47)	0.40(0.05-0.71)	0.36(0.03-0.64)
25	A2008/08853	0.21(0.0-0.41)	0.40(0.05-0.40)	0.39(0.04-0.70)
26	C2007/08043	0.26(0.02-0.46)	0.26(0.0-0.52)	0.48(0.16-0.78)
27	A2008/05327	0.27(0.02-0.47)	0.34(0.01-0.62)	0.39(0.07-0.67)
28	C2009/08995	0.30(0.04-0.51)	0.31(0.01-0.57)	0.40(0.09-0.67)
29	A2008/05297	0.28(0.03-0.49)	0.32(0.01-0.58)	0.40(0.10-0.69)
30	A2008/05355	0.27(0.01-0.47)	0.35(0.02-0.63)	0.38(0.06-0.66)
31	C2009/08590	0.35(0.05-0.59)	0.36(0.02-0.65)	0.29(0.02-0.52)
32	A2008/08911	0.39(0.08-0.66)	0.33(0.01-0.61)	0.28(0.02-0.49)

33	A2008/05325	0.38(0.08-0.64)	0.32(0.01-0.59)	0.30(0.03-0.52)
34	A2008/03749	0.35(0.06-0.60)	0.35(0.02-0.63)	0.30(0.02-0.52)
35	A2008/05326	0.22(0.01-0.41)	0.38(0.02-0.68)	0.40(0.05-0.71)
36	A2008/07994	0.30(0.02-0.53)	0.38(0.03-0.69)	0.32(0.02-0.56)
37	A2008/05469	0.40(0.10-0.68)	0.32(0.01-0.59)	0.28(0.05-0.57)
38	A2008/05376	0.35(0.05-0.58)	0.31(0.02-0.66)	0.33(0.02-0.52)
39	A2008/07795	0.34(0.10-0.66)	0.37(0.0-0.55)	0.29(0.04-0.53)
40	A2008/05328	0.39(0.07-0.60)	0.30(0.01-0.61)	0.31(0.03-0.54)
41	A2008/05347	0.35(0.03-0.51)	0.33(0.02-0.64)	0.32(0.05-0.62)
42	A2008/05333	0.29(0.03-0.51)	0.35(0.02-0.64)	0.36(0.05-0.62)
43	A2008/9025	0.39(0.11-0.64)	0.27(0-0.52)	0.33(0.06-0.57)
44	A2008/00045	0.36(0.08-0.60)	0.29(0.0-0.55)	0.36(0.07-0.60)
45	A2008/08858	0.32(0.04-0.55)	0.35(0.02-0.64)	0.33(0.04-0.57)

Appendix 2. The mean diet proportions of prey items from three different trophic levels. The mixing model analysis was determined from liver tissue using the software package SIAR 4.0 e.g SIAR V 4 (Stable isotope analysis in R) by Parnell & Jackson (2013). The mean value (+/- low-high 95%).

Seal No.	seal	Crustacean	Pelagic fish	Predator fish
1	A2008/05298	0.29(0.04-0.49)	0.27(0-0-0.53)	0.44(0.13-0.73)
2	A2008/05336	0.29(0.03-0.51)	0.36(0.03-0.64)	0.35(0.05-0.61)
3	A2008/05334	0.28(0.03-0.49)	0.34(0.02-0.61)	0.38(0.06-0.65)
4	A2008/05300	0.45(0.11-0.79)	0.35(0.1-0.64)	0.20(0-0.39)
5	A2008/05046	0.29(0.03-0.49)	0.32(0.01-0.58)	0.40(0.09-0.68)
6	A2008/05017	0.23(0.01-0.43)	0.39(0.04-0.69)	0.37(0.04-0.67)
7	A2008/05009	0.27(0.02-0.48)	0.36(0.02-0.64)	0.37(0.05-0.65)
8	A2008/05052	0.28(0.01-0.50)	0.39(0.03-0.68)	0.33(0.03-0.59)
9	A2008/05004	0.30(0.03-0.52)	0.35(0.02-0.64)	0.35(0.05-0.65)
10	A2008/05023	0.26(0.02-0.47)	0.33(0.01-0.61)	0.41(0.10-0.71)
11	A2008/05323	0.22(0.0-0.42)	0.35(0.02-0.63)	0.43(0.10-0.74)
12	A2008/08900	0.27(0.02-0.47)	0.36(0.03-0.66)	0.37(0.05-0.65)
13	C2007/08042	0.28(0.02-0.49)	0.35(0.02-0.63)	0.37(0.06-0.64)
14	A2008/08633	0.24(0.01-0.44)	0.33(0.01-0.59)	0.43(0.11-0.74)
15	A2008/05330	0.28(0.02-0.49)	0.35(0.02-0.64)	0.37(0.06-0.64)
16	A2008/05377	0.22(0.0-0.41)	0.38(0.03-0.68)	0.40(0.06-0.71)
17	A2008/05053	0.36(0.06-0.61)	0.34(0.01-0.62)	0.31(0.03-0.52)
18	A2008/05424	0.30(0.02-0.52)	0.36(0.02-0.64)	0.34(0.04-0.59
19	A2008/05299	0.21(0.01-0.40)	0.34(0.01-0.61)	0.45(0.11-078)
20	A2008/05026	0.27(0.02-0.48)	0.36(0.03-0.64)	0.37(0.05-0.65)
21	A2008/05003	0.28(0.02-0.49)	0.38(0.04-0.68)	0.34(0.03-0.60)
22	A2008/05305	0.25(0.0-0.46)	0.40(0.06-0.72)	0.34(0.02-0.62)
23	A2008/05312	0.27(0.02-0.48)	0.33(0.01-0.61)	0.40(0.09-0.69)
24	A2008/00044	0.24(0.01-0.45)	0.40(0.04-0.70)	0.36(0.02-0.64)
25	A2008/08853	0.21(0.01-0.41)	0.39(0.05-0.71)	0.39(0.05-0.70)
26	C2007/08043	0.26(0.02-0.46)	0.26(0.0-0.52)	0.48(0.17-0.78)
27	A2008/05327	0.27(0.02-0.48)	0.34(0.01-0.62)	0.39(0.06-0.67)

28	C2009/08995	0.30(0.04-0.52)	0.30(0.0-0.56)	0.40(0.10-0.67)
29	A2008/05297	0.28(0.03-0.49)	0.31(0.01-0.58)	0.40(0.09-0.69)
30	A2008/05355	0.27(0.02-0.47)	0.35(0.02-0.64)	0.38(0.07-0.66)
31	C2009/08590	0.27(0.02-0.48)	0.35(0.02-0.63)	0.38(0.07-0.66)
32	A2008/08911	0.27(0.02-0.48)	0.34(0.01-0.60)	0.39(0.08-0.68)
33	A2008/05325	0.31(0.04-0.53)	0.33(0.01-0.61)	0.36(0.06-0.61)
34	A2008/03749	0.24(0.01-0.44)	0.34(0.02-0.62)	0.41(0.08-0.71)
35	A2008/05326	0.19(0.0-0.38)	0.38(0.03-0.67)	0.43(0.07-0.76)
36	A2008/07994	0.25(0.01-0.45)	0.37(0.03-0.67)	0.38(0.05-0.67)
37	A2008/05469	0.32(0.05-0.54)	0.32(0.01-0.60)	0.36(0.05-0.61)
38	A2008/05376	0.30(0.04-0.51)	0.34(0.01-0.61)	0.37(0.05-0.63)
39	A2008/07795	0.28(0.02-0.49)	0.37(0.03-0.66)	0.36(0.04-0.62)
40	A2008/05328	0.30(0.05-0.52)	0.29(0.01-0.55)	0.40(0.10-0.68)
41	A2008/05347	0.34(0.06-0.58)	0.32(0.01-0.59)	0.33(0.05-0.57)
42	A2008/05333	0.27(0.02-0.48)	0.35(0.02-0.64)	0.38(0.05-0.65)
43	A2008/9025	0.36(0.08-0.61)	0.29(0.0-0.56)	0.34(0.06-0.57)
44	A2008/00045	0.29(0.04-0.50)	0.30(0.0-0.55)	0.41(0.10-0.69)
45	A2008/08858	0.30(0.03-0.52)	0.34(0.01-0.62)	0.36(0.05-0.61)

Appendix 3. Sums of fatty acid composition of 45 seals and the eight fatty acid categories used to profit them; classification of categories is illustrated in Table 1.

seal nro.	seal nro.	SAFAs	MUFAs	C18 ω3 PUFAs	EPA plus DHA	C18 ω6 PUFAs	ARA	Branched Fas	ω3/ω6 ratio
1	A2008/05298	11.66	52.48	3.47	15.58	1.41	1.00	10.98	8.63
2	A2008/05336	9.38	48.86	3.91	18.87	2.05	0.49	8.79	10.40
3	A2008/05334	8.23	48.62	4.27	17.81	2.42	1.44	7.62	7.26
4	A2008/05300	9.46	46.68	4.14	19.27	2.03	0.92	8.84	9.42
5	A2008/05046	10.58	49.24	4.06	17.70	1.65	0.91	9.84	9.33
6	A2008/05017	12.84	47.45	3.33	18.25	1.58	0.76	12.01	9.82
7	A2008/05009	9.59	44.14	4.31	21.03	2.00	0.68	8.89	10.53
8	A2008/05052	7.36	48.34	4.29	21.63	2.23	0.94	6.81	8.95
9	A2008/05004	10.22	49.77	3.53	19.65	1.42	0.56	9.65	12.36
10	A2008/05023	12.91	50.81	3.42	16.04	1.56	0.52	11.94	9.80
11	A2008/05323	8.78	42.10	4.13	21.00	2.42	0.92	8.11	9.16
12	A2008/08900	7.11	40.87	4.35	22.45	2.43	0.85	6.58	9.85
13	C2007/08042	6.82	39.10	4.97	22.82	2.31	0.92	6.24	10.61
14	A2008/08633	10.68	51.11	4.10	18.85	2.31	0.88	7.80	7.23
15	A2008/05330	10.25	42.64	4.99	23.35	2.18	0.83	7.29	9.40
16	A2008/05377	11.34	50.49	3.85	19.56	2.35	1.19	8.19	6.41
17	A2008/05053	12.10	25.94	7.34	30.31	2.84	0.65	8.98	10.19
18	A2008/05424	17.08	26.82	8.20	22.03	4.51	0.47	13.90	5.12
19	A2008/05299	12.17	45.39	4.26	20.53	2.08	1.05	9.14	7.94
20	A2008/05026	14.93	53.26	3.30	17.94	1.33	0.75	11.99	8.82
21	A2008/05003	12.93	50.02	4.25	18.59	2.10	0.99	9.44	7.22
22	A2008/05305	22.89	33.52	5.52	14.31	3.66	1.38	17.57	4.15
23	A2008/05312	12.28	49.75	4.73	18.39	1.96	0.83	9.48	8.51
24	A2008/00044	12.30	53.29	4.72	14.51	2.49	0.41	10.14	5.67

25	A2008/08853	11.34	45.64	4.44	21.84	2.19	0.75	8.29	8.77
26	C2007/08043	12.03	50.10	3.89	19.16	2.10	0.88	9.18	7.66
27	A2008/05327	9.48	47.33	4.15	22.70	2.04	0.99	6.94	8.69
28	C2009/08995	9.82	44.25	4.87	22.60	2.40	1.05	7.15	8.19
29	A2008/05297	22.84	17.89	5.89	27.07	2.46	0.99	17.94	8.19
30	A2008/05355	9.63	51.83	4.13	19.03	2.08	0.93	6.73	7.94
31	C2009/08590	7.83	42.43	5.33	23.65	2.27	0.61	5.25	9.85
32	A2008/08911	10.07	46.73	4.22	22.06	1.95	0.81	6.97	9.38
33	A2008/05325	8.93	44.71	5.02	21.01	2.40	0.78	5.89	9.00
34	A2008/03749	12.23	52.64	4.17	16.91	1.68	1.71	9.06	6.21
35	A2008/05326	8.40	49.17	5.25	20.29	2.24	1.11	5.66	7.91
36	A2008/07994	6.42	45.74	4.82	24.99	2.38	1.32	4.14	8.17
37	A2008/05469	8.70	44.94	4.96	23.42	2.39	0.66	5.96	9.46
38	A2008/05376	9.26	46.66	4.49	20.30	2.50	1.06	6.00	7.59
39	A2008/07795	10.01	48.70	4.72	20.79	2.03	1.01	6.76	8.51
40	A2008/05328	11.27	51.24	4.30	16.86	2.11	1.23	7.86	6.49
41	A2008/05347	9.01	49.87	4.30	18.81	2.29	0.89	5.94	7.89
42	A2008/05333	8.95	51.42	4.18	20.30	2.02	1.23	6.32	8.00
43	A2008/9025	9.08	47.18	5.06	21.56	2.45	0.96	6.24	7.86
44	A2008/00045	9.24	51.73	3.99	18.95	2.10	0.89	6.25	7.88
45	A2008/08858	8.72	46.66	4.82	22.01	2.39	0.82	6.11	8.49

Appendix 4. A principal component analysis (PCA) of fatty acid composition and diet of 45 seals. PC 1 explained 38 %, PC 2 25 % and PC 3 16 % of the variance.

FA	i-14:0	14:00	14:01	i-15:0	a-15:0	15:00	15:1w7	i-16:0	16:00	16:1w9	16:1w7c	16:1w6c	16:1w5c	16:2w6	i-17:0	a-17:0	16:2w4	17:00	16:3w4	16:3w3	17:1w9c
PC1	0.10	0.01	0.23	0.19	0.40	-0.08	0.41	0.12	-0.05	-0.80	0.20	0.47	0.11	0.39	-0.12	-0.04	0.39	-0.20	0.26	-0.16	0.01
R^2	0.01	0.00	0.05	0.04	0.16	0.01	0.17	0.02	0.00	0.64	0.04	0.22	0.01	0.15	0.01	0.00	0.15	0.04	0.07	0.03	0.00
PC2	0.62	-0.33	0.25	0.78	0.74	0.24	0.17	0.66	-0.02	-0.07	0.00	-0.39	-0.26	0.02	0.80	0.84	-0.09	0.44	-0.52	-0.11	0.38
R^2	0.39	0.11	0.06	0.61	0.55	0.06	0.03	0.44	0.00	0.00	0.00	0.15	0.07	0.00	0.64	0.71	0.01	0.19	0.27	0.01	0.14
PC3	0.17	0.49	0.31	0.26	-0.17	0.88	-0.41	0.40	0.97	-0.19	-0.19	0.57	0.09	0.11	0.19	0.12	-0.26	0.77	0.16	0.01	0.00
R^2	0.03	0.24	0.10	0.07	0.03	0.77	0.17	0.16	0.94	0.03	0.03	0.33	0.01	0.01	0.03	0.01	0.07	0.59	0.03	0.00	0.00

FA	16:4w3	18:00	18:1w9c	18:1w7c	18:1w6	18:2w6c	18:3w6	18:3w4	18:3w3	18:4w3	18:5w3	20:1w9	20:2w6	20:4w6	20:3w3	20:4w3	20:5w3	22:2w6	22:4w6	22:5w3	22:6w3
PC1	-0.19	-0.11	-0.03	0.18	0.17	0.67	-0.07	0.88	0.88	0.76	0.35	0.20	0.50	-0.24	0.58	0.80	0.67	0.89	0.15	0.74	0.76
R^2	0.03	0.01	0.00	0.03	0.03	0.45	0.00	0.78	0.78	0.57	0.12	0.04	0.25	0.06	0.33	0.64	0.45	0.79	0.02	0.55	0.58
PC2	-0.09	0.16	-0.20	0.23	0.44	-0.21	0.26	0.07	0.08	-0.18	-0.44	-0.15	-0.55	0.61	-0.55	-0.35	-0.08	-0.06	0.72	0.30	0.25
R^2	0.01	0.03	0.04	0.05	0.19	0.04	0.07	0.00	0.01	0.03	0.19	0.02	0.31	0.38	0.30	0.12	0.01	0.00	0.52	0.09	0.06
PC3	0.01	0.90	-0.82	0.20	0.03	0.26	-0.05	0.10	0.10	0.42	0.08	0.57	0.52	-0.02	0.43	0.11	-0.17	-0.15	0.18	-0.43	-0.27
R^2	0.00	0.81	0.67	0.04	0.00	0.07	0.00	0.01	0.01	0.17	0.01	0.32	0.27	0.00	0.18	0.01	0.03	0.02	0.03	0.18	0.07

Appendix 5. The original copy of stomach content analysis of Baltic ringed seals (n=45), analyzed by Annika Strömberg in Swedish Museum of Natural History, Department of Environmental Research & Monitoring.

Seal	Perch	4-horned sculpin	Small sandeel	Smelt	Sand goby	Whitefish	Vendace	Sprat	Isopod	3-spined stickleback	Herring	Eelpout
A2008/05297	0	0	0	0	0	0	0	0	0	0.161367194	0.8386328	0
A2008/05298	0	0	0	0	0	0	0	0	0	1	0	0
A2008/05299	0	0	0	0	0	0	0	0	0	1	0	0
A2008/05300	0	0	0	0	0	0	0.048065236	0	0	0.178026078	0.7739087	0
A2008/05305	0	0	0	0.037487541	0	0.014848406	0.295739145	0	0	0.02794243	0.6239825	0
A2008/05312	0	0	0	0.002342417	0	0	0.845434586	0	0	0.113833344	0.0383897	0
A2008/05325	0	0	0	0	0	0	0	0	0	1	0	0
A2008/05327	0	0.308638372	0	0.008013688	0	0	0.135832829	0	0	0.000252658	0.508672	0.0385904
A2008/05328	0	0	0	0	0	0	0	0	0	1	0	0
A2008/05330	0	0	0	0	0	0	0.038945982	0	0	0.107693109	0.8533609	0
A2008/05333	0	0	0	0	0	0	0	0	0	0.039601256	0.9603987	0
A2008/05334	0	0	0	0.039854997	0.000405512	0.153715076	0.338572563	0.0101066	0	0.012749792	0.4445954	0
A2008/05336	0	0	0	0	0	0	0.057414493	0	0	0.059595764	0.8829897	0
A2008/05347	0	0	0	0	0	0	0	0	0	0.15644939	0.8435506	0
A2008/05355	0	0	0	0.011617467	0	0	0	0	0	0.103808771	0.8845738	0
A2008/05376	0.0299961	0	0	0	0	0	0.529564398	0	0	0.044394284	0.3960452	0
A2008/05377	0	0	0	0.012870571	0	0	0.779975182	0	0	0.000272376	0.2068819	0
A2008/05424	0	0	0	0	0	0	0	0	0	0.239251242	0.7607488	0
A2009/05003	0	0	0	0.046097755	0	0	0	0	0	0	0.9539022	0
A2009/05004	0	0	0	0	0	0	0.130579993	0	0	0.103485472	0.7659345	0
A2009/05009	0	0	0	0.004776259	0	0	0	0	0	0.003207351	0.9920164	0
A2009/05017	0	0	0	0.038946609	0	0	0.361960927	0	0	0.126786187	0.4723063	0
A2009/05023	0	0	0	0	0	0	0	0	0	0.479265547	0.5207345	0
A2009/05026	0	0	0	0	0	0	0.490490235	0	0	0.509509765	0	0
A2009/05046	0	0	0	0	0	0	0	0	0	0.752098319	0.2479017	0
A2009/05052	0	0	0	0	0	0	0.895325141	0	0	0.104674859	0	0
A2009/05053	0	0	0	0	0	0	0.915547995	0	0	0	0.0202762	0.0641758
C2007/08042	0	0	0	0	0	0	0.845704828	0	0	0	0.1060141	0.0482811
C2007/08043	0	0	0	0	0	0	0.573590225	0	0	0.06656962	0.3598402	0
C2008/03749	0	0	0	0	0	0	0.06696272	0	0	0.826763438	0.1062738	0
C2008/05469	0	0	0	0	0	0	0.978974122	0	0	0	0.0210259	0
C2008/07795	0	0	0	0	0	0	0.958315055	0	0	0.009300249	0.0323847	0
C2008/07994	0	0	0	0	0.000544014	0	0	0	0	0	0.999456	0
C2008/08633	0	0	0	0	0	0	0.72601702	0	0	0	0.273983	0
C2008/08853	0	0	0	0.013219035	0	0	0.986780965	0	0	0	0	0
C2008/08858	0	0	0	0	0	0	0	0	0	0.001355066	0.0074414	0.9912036
C2008/08900	0	0	0	0	0	0	0.86369869	0	0	0.078885328	0.057416	0
C2008/08911	0	0	0	0	0	0	0.206713321	0	0	0.651810448	0.1414762	0
C2008/09025	0	0	0	0	0	0	0.997301054	0	0	0.002698946	0	0
C2009/00044	0	0	0.002863344	0.004428484	0	0	0.864795496	0	0	0.000572464	0.1273402	0
C2009/00045	0	0	0	0	0	0	0.902241572	0	0	0.001638369	0.0961201	0
C2009/08590	0	0.139500652	0	0	0.00239492	0	0.848781447	0	0.009323	0	0	0
C2009/08995	0	0.379725056	0	0	0	0	0	0	0.0192239	0	0.601051	0