Author(s): Auttila, Miina; Sinisalo, Tuula; Valtonen, Mia; Niemi, Marja; Viljanen, Markku; Kurkilahti, Mika; Kunnasranta, Mervi

Title: Diet composition and seasonal feeding patterns of a freshwater ringed seal (Pusa hispida saimensis)

Year: 2015

Version:

Please cite the original version:

Auttila, M., Sinisalo, T., Valtonen, M., Niemi, M., Viljanen, M., Kurkilahti, M., & Kunnasranta, M. (2015). Diet composition and seasonal feeding patterns of a freshwater ringed seal (Pusa hispida saimensis). Marine Mammal Science, 31(1), 45-65. https://doi.org/10.1111/mms.12133

Marine Mammal Science

MARINE MAMMAL SCIENCE, **(*): ***–*** (*** 2014)

DOI: 10.1111/mms.12133

Diet composition and seasonal feeding patterns of a freshwater ringed seal (*Pusa hispida saimensis*)

Miina Auttila,[1] Department of Biology, University of Eastern Finland, PO Box 111, FI-80101 Joensuu, Finland; **Tuula Sinisalo**, Department of Biological and Environmental Sciences, University of Jyväskylä, PO Box 35, FI-40014 Jyväskylä, Finland; **Mia Valtonen**, **Marja Niemi** and **Markku Viljanen**, Department of Biology, University of Eastern Finland, PO Box 111, FI-80101 Joensuu, Finland; **Mika Kurkilahti**, Finnish Game and Fisheries Research Institute, Itäinen Pitkäkatu 3, FI-20520 Turku, Finland; **Mervi Kunnasranta**, Department of Biology, University of Eastern Finland, PO Box 111, FI-80101 Joensuu, Finland.

Abstract

The Saimaa ringed seal (*Pusa hispida saimensis*) is one of the few freshwater seal populations worldwide. The major conservation issue of this critically endangered population is bycatch mortality. We used digestive tract content and stable isotopes (δ^{13}C and δ^{15}N) to estimate the diet and seasonal feeding patterns for gaining better understanding of the seals feeding habits and potential conservation implications. The diet was similar across age groups. Altogether 15 fish species were identified and the most important were smelt (*Osmerus eperlanus*), ruff (*Gymnocephalus cernuus*), perch (*Perca fluviatilis*), vendace (*Coregonus albula*) and cyprinids. The high δ^{15}N values suggested that the seals lose weight during winter and spring. Additionally the drop in δ^{15}N values indicated that pups start to recover from postweaning stress and gain weight around the age of 6 mo. The isotope values differed regionally, which emphasizes that samples from consumers and prey should be collected from the same regions to improve interpretation of the stable isotopic results. Overall, diet composition suggests minimal to nonexistent competition with commercial or recreational fishing. However, observed weight loss of pups during summer may be related to higher risk of bycatch and this should be taken into account when planning temporal fishing closures.

Key words: *Phoca*, stable isotopes, digestive tract analysis, seal-fishery interaction, nutritional status, conservation, Saimaa ringed seal.

A ringed seal (*Pusa hispida saimensis*) population has lived in geographic isolation for at least 9,500 yr in the freshwater lake, Lake Saimaa, in Finland (Hyvärinen and Nieminen 1990). This subspecies differs morphologically, behaviorally, and genetically from other ringed seal subspecies (Hyvärinen and Nieminen 1990, Kunnasranta 2001, Palo *et al.* 2003, Valtonen *et al.* 2012). Currently, the population size is around 300 individuals (Metsähallitus 2012) and it is categorized as critically endangered (Kovacs *et al.* 2012). Major threats are connected to various anthropogenic

[1]Corresponding author (e-mail: miina.auttila@uef.fi).

influences, the most acute threat being bycatch mortality, especially entanglement in gill nets of recreational fishermen (Sipilä *et al.* 1990; Ranta *et al.* 1996; Sipilä 2003; Kovacs *et al.* 2012; Niemi *et al.* 2012, 2013). Despite various fishing restrictions (see Niemi *et al.* 2012, 2013), bycatch is still the most commonly observed cause of death for Saimaa ringed seals, especially for juveniles (Sipilä *et al.* 1990; Sipilä 2003; Niemi *et al.* 2012, 2013).

In previous centuries, local people have considered the seals to be competitors with fisheries (Auvinen *et al.* 2005) and nowadays skeptical attitudes have been expressed towards the extension of fishing restrictions related to seal conservation in Lake Saimaa (Salmi *et al.* 2000, Salmi and Muje 2001, Tonder and Jurvelius 2004, Tonder and Salmi 2004). Although the ringed seal diet in the marine environment has been studied comprehensively (*e.g.,* McLaren 1958; Lowry *et al.* 1978, 1980; Gjertz and Lydersen 1986; Weslawski *et al.* 1994; Wathne *et al.* 2000; Holst *et al.* 2001; Labansen *et al.* 2007, 2011), only a few studies concerning the diet and foraging behavior of freshwater ringed seals have been conducted so far (Kunnasranta *et al.* 1999, Sinisalo 2007).

The most common and widely used method of studying diet composition in pinnipeds is identification of hard remains from feces or digestive tracts (*e.g.,* Tollit *et al.* 2007, 2010; Bowen and Iverson 2013). It is the only method that gives data on the number of individuals of given types of prey eaten, while allowing the volumes of consumed food types to be estimated (Hyslop 1980, Tollit *et al.* 2010). However, this method also has biases, such as underestimation of prey species with delicate otoliths or other hard parts (Bowen and Iverson 2013). Additionally the high assimilation efficiency of seals means that the digestive tract contents only represent the diet over a few hours prior to sampling (Parsons 1977, Helm 1984, Lawson *et al.* 1997). Analyses of stable isotope values from different tissues with different turnover rates have become standard procedures to study the diet of marine mammals in recent decades (Kurle and Worthy 2002, Newsome *et al.* 2010). Furthermore, naturally occurring stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) in consumers and in their food sources have revealed assimilated diet and provided information on the trophic level and nutritional status of consumers (Hobson *et al.* 1997; Polischuk *et al.* 2001; Phillips and Gregg 2001, 2003; Sinisalo *et al.* 2008). The δ^{15}N values of tissues are not only affected by diet but also by the nutritional status of the consumers, and thus, δ^{15}N values can be used to indicate changes in the animals' body condition (Hobson *et al.* 1993). This means that when seals use their own blubber as an energy source or when pups are suckling, their tissues become ^{15}N enriched (Gannes *et al.* 1997, 1998; Fuller *et al.* 2004, 2005; Newsome *et al.* 2010).

This study examined the diet of the Saimaa ringed seal using two complementary methods; digestive tract content and stable isotope analyses. The main aim was to gain a more accurate picture of dietary patterns including prey composition, seasonal variation, and potential age-related differences in the feeding habits of this freshwater ringed seal. Greater knowledge of the feeding ecology of the seals is currently crucial both for their appropriate conservation and for mitigating conflict with fisheries.

Materials and Methods

Study Area

This study covers the main distribution area of the seals, dividing the lake into three regions: northern, central, and southern Saimaa (Fig. 1). Lake Saimaa

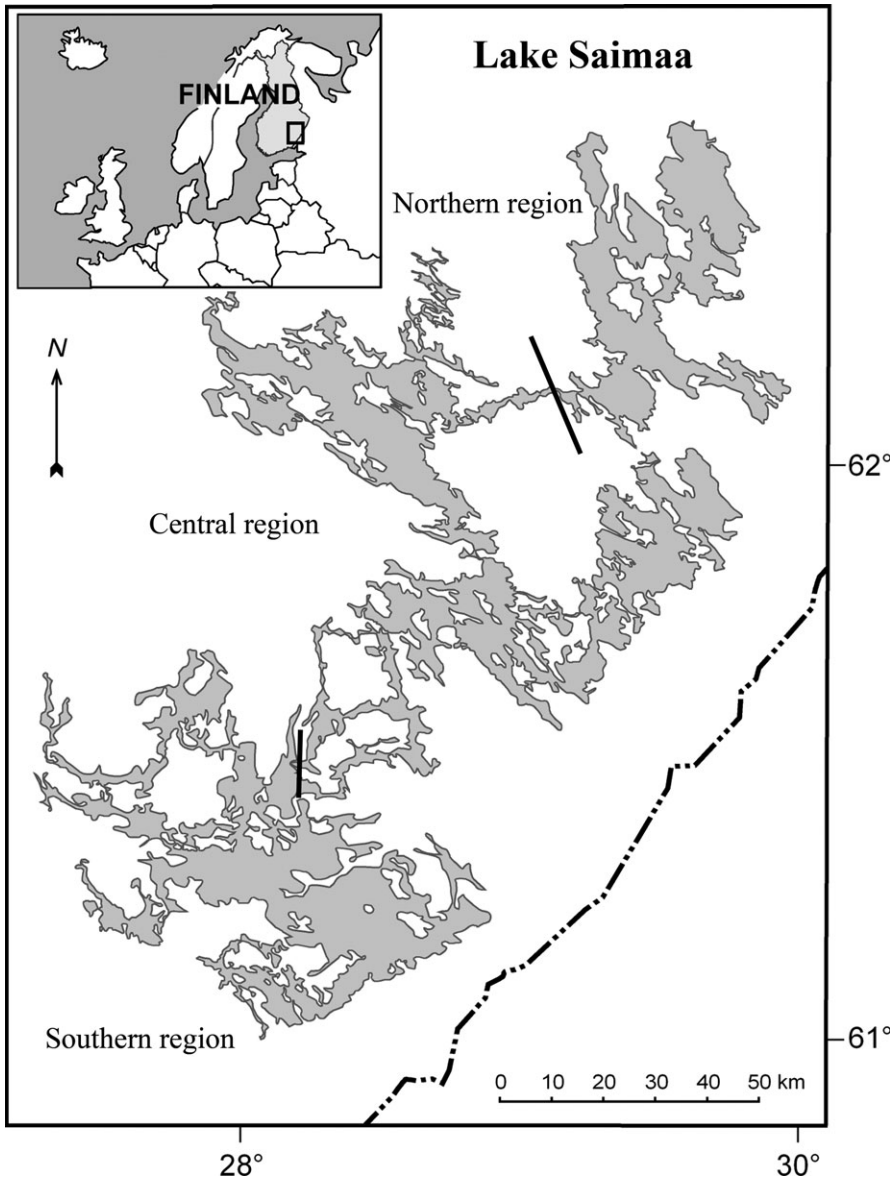

Figure 1. Lake Saimaa study area is divided into northern, central, and southern regions.

(61°05′–62°36′N, 27°15′–30°00′E) is about 180 km in length and 140 km in width, with approximately 14,000 islands. The mean depth of the lake is 12 m (maximum 85 m) and the total surface area is 4,400 km^2. The lake is inhabited by about 30 fish species (Auvinen *et al.* 1999) and four macrocrustacean species (Hynynen *et al.* 1999), which could be potential prey species for the seals. The most commercially valuable species are vendace (*Coregonus albula*), pikeperch

(*Sander lucioperca*), whitefish (*Coregonus lavaretus*), perch (*Perca fluviatilis*), and pike (*Esox lucius*) (Finnish Game and Fisheries Research Institute 2010).

Seal and Prey Samples

Given the critically endangered status of the Saimaa ringed seal population, the availability of seal samples for this study was necessarily limited to bycaught and stranded animals. Samples for digestive tract content and stable isotope (δ^{13}C and δ^{15}N) analyses were obtained from a tissue bank maintained by the University of Eastern Finland and the Natural Heritage Services of Metsähallitus. The seal carcasses (n = 54) were collected during the years 2002–2010, apart from one subadult seal found in 1985. The seals were examined, and causes of death were determined by the Finnish Food Safety Authority Evira. The samples were stored at −20°C until analysis. The age of the seals was determined by counting the cementum layers in the lower canine teeth (Stewart *et al.* 1996): individuals without a tooth sample (n = 5), were assessed *via* body mass (≤30 kg = pup, n = 1; 30–42 kg = subadult, n = 3; and >42 kg = adult, n = 1). Subsequently the seals were divided into two age groups: pups (<1 yr, n = 31), and older seals (≥1 yr, n = 23). The majority of pups (87%) were 6 mo old or younger, half of the older seals were subadults (1–4 yr) and the other half were adults (>4 yr). Tissue samples from five stillborn pups were also used in stable isotope analyses. We obtained samples of the potential prey species of the seals (Kunnasranta *et al.* 1999) collected by local fishermen from Lake Saimaa during the years 2010 and 2011. The carbon and nitrogen stable isotope values of these species were analyzed and used to form a reference database of prey species.

Digestive Tract Content Analysis

The analysis of the seals' digestive tract contents was based on identifying the undigested food items and hard parts. All food items were identified to the lowest possible taxonomic level using literature (Härkönen 1986, Koli 1998) and our reference collection of fish, otoliths, and pharyngeal arches. The stomach and intestines were cut open and the contents were rinsed into a dish using running cold tap water. Undigested prey items were picked up and the remaining material was rinsed with tap water and mixed in the dish. The mixture was left undisturbed so that all the particulate matter settled to the bottom of the dish and the fluid became clear. This rinsing procedure was repeated 5–20 times, until there were no floating particles in the water and hard parts remained at the bottom of the dish. Otoliths and other hard parts were recovered and stored dry until identification. Undigested prey individuals were counted and the fish were measured (length from snout to tail). Digested fish were identified by sagittal otoliths or pharyngeal arches (in cyprinids). Dextral and sinistral otoliths were separated and the number of otoliths in the most frequently occurring side was used to determine the number of individual fish in each prey category. The estimated number of individual cyprinids was based on the number of pharyngeal arches. The number of consumed invertebrates was determined by adding the number of whole individuals and the number of unique structures or paired structures divided by two.

Diet indices based on digestive tract contents (Hyslop 1980) were calculated according to: (1) the frequency of occurrence (FO%), *i.e.*, the percentage of digestive tracts containing a given prey category; and (2) the relative frequency (N%), where

the number of individuals in each prey category is recorded for all the digestive tracts and the total is expressed as a percentage (%) of the individuals in all prey categories. Prey-specific abundance (P%) was calculated as:

$$P_i = (\sum S_i / \sum S_{ti}) \times 100$$

where P_i is the prey-specific abundance of prey i, S_i the number of individuals in the digestive tract contents of prey i, and S_{ti} is the total digestive tract content in only those seals with prey i in their digestive tract.

Stable Isotope Analysis

δ^{13}C and δ^{15}N values were analyzed from the muscle of 11 fish species from the three different regions of the lake (Table S1). Additionally, the biggest crustacean species (*Gammaracanthus lacustris*) existing in the lake was included in the potential prey species and the stable isotope values were calculated based on the whole amphipod. The five key fish species, identified as important prey from the digestive tract contents, were divided into three groups based on their isotope values and predominant habitats in Lake Saimaa: benthic fish (ruff *Gymnocephalus cernuus*), pelagic fish (vendace and smelt *Osmerus eperlanus*), and littoral fish (roach *Rutilus rutilus* and bleak *Alburnus alburnus*). Two different seal tissues were chosen for stable isotope analysis due to their different turnover rates; the liver reflects assimilation of the diet over a period of a few weeks and muscle represents a period of 2–3 mo prior to sampling (Tieszen *et al.* 1983, Dalerum and Angerbjörn 2005). δ^{13}C and δ^{15}N values were determined from muscle (n = 53) and liver (n = 51) from the seals whose digestive tract contents were also analyzed. In addition, δ^{15}N values were determined from muscle and liver from five stillborn pups. We also examined the seasonal changes in δ^{15}N values of seals by dividing the age groups further into temporal groups (sample sizes given in Fig. 2). The older seals were divided into four seasonal groups: (1) December–February: the lake is ice covered and the first pups are born in February; (2) March–May: all pups have been born by mid-March, ice break up and weaning occurs, and molting begins in May; (3) June–August: molting ends in mid-June; and (4) September–November: fall (Helle *et al.* 1984; Sipilä and Hyvärinen 1998; Kunnasranta *et al.* 1999, 2002; Niemi *et al.* 2013). The fall was excluded from further analysis because of its small sample size (n = 1). The pups were divided into groups according to their age classes: February–March (stillborns), May (3 mo), June (4 mo), July (5 mo), August (6 mo), and September–January (≥7 mo).

In order to avoid biased interpretation of the carbon isotope results (DeNiro and Epstein 1978, Focken and Becker 1998), a general lipid correction model (Kiljunen *et al.* 2006) was used to correct the δ^{13}C values of all samples. All isotope values were analyzed from two replicates of each sample. The deviations between the replicates were found to be lower than 0.2‰ for both δ^{13}C and δ^{15}N. The analysis was conducted using a Flash EA112 elemental analyzer (Carlo Erba) connected to a Finnigan Delta Plus Advantage continuous flow isotope ratio mass spectrometer (CFIRMS) (http://www.thermoscientific.com). The stable isotope values were expressed using the delta notation (expressed in units per mil, ‰): $\delta X = (R_{sample}/R_{standard} - 1) \times 1{,}000$, where $X = {}^{13}C$ or ${}^{15}N$, $R_{sample} = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$, and $R_{standard}$ = Vienna Pee Dee belemnite (VPDB) for δ^{13}C and atmospheric N_2 (air) for δ^{15}N (see Bond and Hobson 2012). The isotopic reference materials were IAEA

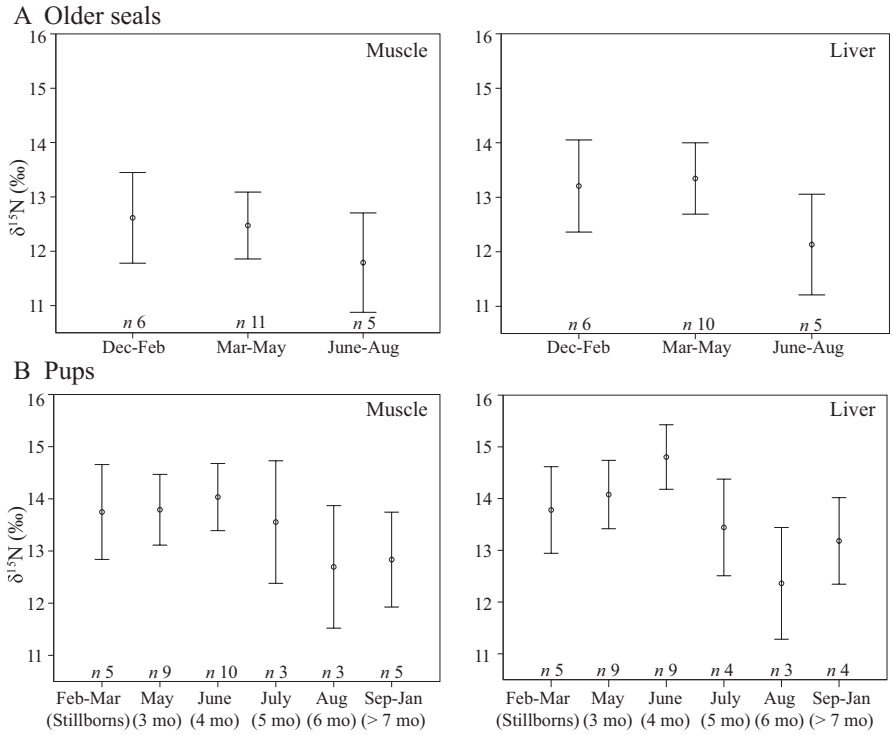

Figure 2. The changes in estimated (ANOVA) mean δ^{15}N values (whiskers show 95% confidence intervals) of the muscle and liver of (A) the older (≥1 yr) and (B) the pup Saimaa ringed seals found dead at different times of year. The older seals' δ^{15}N values are higher during the ice covered season (December–May) and the pups' values decrease towards the end of summer.

standards (CH6 sucrose, CH7 polyethylene, N1 and N2 ammonium sulfate) and the laboratory standard was made from freeze dried pike muscle. The δ^{13}C and δ^{15}N values for laboratory standard were −30.1‰ and 16.1‰, respectively and the standard deviation (SD) was <0.096 in all runs. Five laboratory standards were included at the beginning of the analysis and two after every 10 samples.

Statistical Methods

Differences between seal age groups in fish length and the numbers of fish species and individuals in the digestive tracts were analyzed using the Mann-Whitney *U* test. The frequency of occurrence of the most important prey categories were compared between the seal age groups using Fisher's exact test. The differences in δ^{13}C and δ^{15}N values of the prey habitat groups between the regions were analyzed by a Kruskal-Wallis nonparametric analysis of variance, because for these data the assumptions of ANOVA were not met. Differences in δ^{13}C and δ^{15}N values for the seals' muscle and liver were tested using analysis of variance between age groups, seasons (older seals), pup age classes, and regions within age groups. For these data assumptions of normal distribution and homogeneity of variances were tested from residuals after analysis of variance (Levene's test for variances and the Shapiro-Wilks test for normal

distribution) and the assumptions of ANOVA were met. Because the seals' δ^{13}C and δ^{15}N values were correlated (Table S2), a repeated ANOVA model with an unstructured covariance structure and the seal as the subject level was used. Kenward–Roger's approximation was used for determining the correct standard error estimates and degrees of freedom (SAS 2011). *Post hoc* comparisons between the pup age classes and between the older seals during different seasons were adjusted by a Tukey's test. Preplanned pairwise comparisons (contrasts, *t*-test) between regions within age groups were performed without adjustment. The statistical analyses were performed with SPSS PASW 18 (SPSS Inc., Chicago, IL) and SAS 9.3 software (SAS Institute Inc., Cary, NC).

Results

Digestive Tract

The majority (69%) of the studied seals had died during the open water season (May–November). Bycatch (*i.e.,* entanglement mainly in gill nets) was the main cause of death both in pups (87%) and in seals older than one year (65%). Ninety-four percent of the studied digestive tracts had some dietary contents and empty digestive tracts (n = 3) were excluded from further content analysis. Altogether, 15 fish and 4 invertebrate species were found (Table 1). The mean number of fish species in the digestive tracts was around four and the median number of fish individuals was 137 (Table 2). There were no statistically significant age related differences in the number of fish species or individuals eaten (Mann-Whitney U test: $U = 256.5$, $P = 0.255$; $U = 285.5$, $P = 0.579$, respectively). Most fish were small, averaging 8.6 cm ± 3.4 (± SD) in length, but size did vary according to the age group of the seals ($U = 7015.500$, $P = 0.002$): the pups fed on slightly smaller fish (Table 2). In addition, 33% of the digestive tracts contained sand or small stones and 28% contained plant material.

Detected prey items (n = 9,089) were grouped into 13 different categories (Table 1). The five most frequently occurring (FO%) prey (ruff, smelt, perch, vendace, and cyprinids) were the same in both age groups, but their actual frequencies varied. The FO% of all other prey categories was 20% or less. About 70% of all digestive tracts contained the remains of unidentified fish species, but their relative frequencies (N%) were less than 5.5% (Table 1). All the studied seals had fed on fish; invertebrates were found in 20% of the digestive tracts of the pups and in 10% of those of the older seals. According to the relative frequencies, the proportion of invertebrates was less than 1%. The five prey categories with the highest N% were the same as the most frequently occurring prey categories. In both age groups the relative frequencies of those prey categories that were not mentioned above were less than 1%.

A statistically significant difference between the age groups was found in the case of vendace (Table 1). Despite the fact that the older seals had fed more frequently on vendace than the pups, the relative frequency of vendace in the older seals' diet was only 8.4%, whereas in the pups' diet it was 14.7%. A graphical analysis (Costello 1990, modified by Amundsen *et al.* 1996, Fig. 3) shows that both age groups have generalist feeding strategies. Using the diagram, the five fish categories were further combined into three groups according to their importance in the seal diet: main (FO % > 75 and P% > 20), common (FO% 35–70 and P% 5–35) and minor (FO% < 50 and P% < 3) prey species. An age related difference in prey importance was only

Table 1. Diet composition of the Saimaa ringed seal based on digestive tracts: pups (n = 30) and ≥1-yr-old seals (n = 21). The statistical test (Fisher's exact test, 2-sided) of age related differences in frequencies of occurrence of the five most preferred prey categories. Statistically significant values ($P \leq 0.05$) are in bold.

	Frequency of occurrence					Relative frequency				Prey specific abundance			
	Pups		≥1 yr		Fisher's test	Pups		≥1 yr		Pups		≥1 yr	
Prey category	n	FO%	n	FO%	P	n	N%	n	N%	n	P%	n	P%
Smelt *Osmerus eperlanus*	25	83	16	76	0.722	1,972	36.5	1,241	33.6	4,518	43.6	2,840	43.7
Ruff *Gymnocephalus cernuus*	26	87	16	76	0.460	1,899	35.2	651	17.6	4,433	42.8	2,966	21.9
Perch *Perca fluviatilis*	19	63	17	81	0.221	253	4.7	792	21.5	3,464	7.3	2,572	30.8
Vendace *Coregonus albula*	11	37	14	67	**0.048**	791	14.7	309	8.4	2,474	32.0	2,735	11.3
Cyprinids *Cyprinidae*[a]	11	37	10	48	0.565	181	3.4	436	11.8	1,416	12.8	2,075	21.0
Other fish[b]	12	40	10	48	–	58	1.1	39	1.6	2,942	2.0	2,539	1.5
Percids *Percidae*[c]	6	20	4	19	–	18	0.3	21	0.6	–	–	–	–
Cottids *Cottidae*[d]	4	13	2	10	–	29	0.5	10	0.3	–	–	–	–
Burbot *Lota lota*	1	3	5	24	–	1	0.0	6	0.2	–	–	–	–
Pikeperch *Sander lucioperca*	2	7	1	5	–	9	0.2	1	0.0	–	–	–	–
Nine-spined stickleback *Pungitius pungitius*	–	–	1	5	–	–	–	1	0.0	–	–	–	–
Salmonids *Salmonidae*[e]	1	3	–	–	–	1	0.0	–	–	–	–	–	–
Unidentified fish	20	67	15	71	–	204	3.8	198	5.4	–	–	–	–
Invertebrates[f]	6	20	2	10	–	41	0.8	24	0.7	–	–	–	–

[a]*Rutilus rutilus*, *Alburnus alburnus*, *Scardinius erythrophthalmus* and *Blicca bjoerkna*.
[b]Created for a graphical analysis (Fig. 3); includes all prey categories below except unidentified fish and invertebrates.
[c]*Perca fluviatilis*, *Sander lucioperca* and *Cymnochephalus cernuus*, if not possible to identify into species.
[d]*Cottus gobio*, *C. poecilopus* and *Myoxocephalus quadricornis*.
[e]*Salmo trutta* or *S. salar*.
[f]*Gammaracanthus lacustris*, *Pallaseopsis quadrispinosa*, *Mysis relicta* and *Pisidium sp.*

Table 2. Characteristics of fish found in digestive tracts of the Saimaa ringed seal: pups ($n = 30$) and ≥1-yr-old seals ($n = 21$).

	Fish length (cm)				Fish species			Fish individuals		
Age group	*n*	Median	Range	Mean ± SD	Median	Range	Mean ± SD	Median	Range	Mean ± SD
Pups	106	7.6	3.0–17.0	7.8 ± 3.2	4.0	1–7	3.6 ± 1.6	153.5	1–628	180.0 ± 142.0
≥1 yr	171	9.0	2.5–17.0	9.1 ± 3.4	4.0	1–7	4.1 ± 1.4	129.0	1–680	175.7 ± 175.0
All	277	8.4	2.5–17.0	8.6 ± 3.4	4.0	1–7	3.8 ± 1.5	137.0	1–680	178.2 ± 154.8

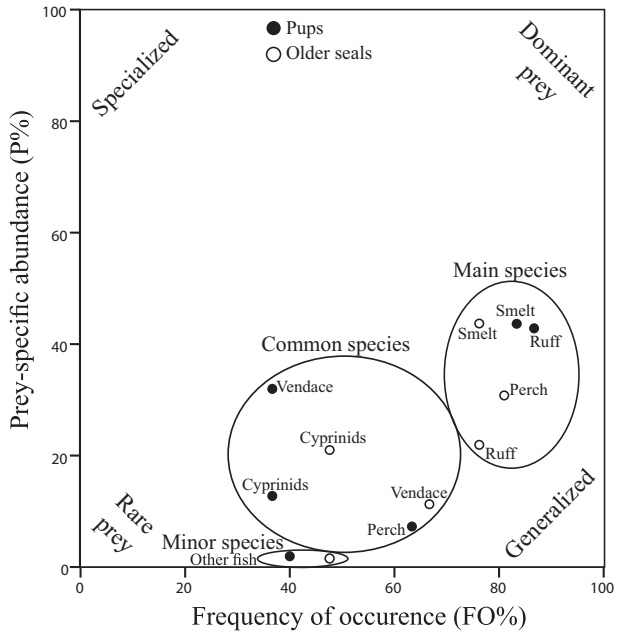

Figure 3. A graphical analysis (Costello 1990, modified by Amundsen *et al.* 1996) of feeding strategy shows that the Saimaa ringed seal is a generalist. Prey are divided into three groups (main, common, and minor species) based on their importance in the seals' diet.

found for perch; in older seals it was categorized as a main prey species, whereas in pups it was a common species.

Stable Isotopes

The mean δ^{15}N values of pups' muscle and liver were higher than those of the older seals (1.2‰ and 0.8‰ higher, respectively, *t*-test: $t = 4.334_{(54)}$, $P < 0.001$ and, $t = 3.029_{(54.1)}$, $P = 0.004$, respectively; Table 3). Also the mean δ^{13}C value of muscle of pups was slightly higher (0.4‰) than that of the older seals (*t*-test: $t = -2.024_{(53.8)}$, $P = 0.048$), but there was no statistically significant difference in the carbon values of liver (Table S3). The δ^{13}C values of seal muscle and liver differed significantly among the regions (overall ANOVA: $F = 263.6_{(23, 104.7)}$, $P < 0.001$; Table 3). There was a statistically significant difference in the δ^{13}C values of older seals between the northern and southern regions and in both age groups between the central and southern regions (preplanned comparisons after ANOVA; Fig. 4, Table S4). However, there were no statistically significant differences in the δ^{15}N values within age groups among the regions.

The older seals' δ^{15}N values in the muscle and liver did not differ statistically significantly by season (Table S5). However, the mean δ^{15}N values of liver were 1.1‰ higher in winter and 1.2‰ higher in spring than in summer (Fig. 2). In pups, a significant difference was observed among age classes in the δ^{15}N liver values, but not in the δ^{15}N muscle values (Table S6). In addition, their nitrogen values decreased towards the end of the summer (Fig. 2). The mean δ^{15}N values of the pups' liver

Table 3. $\delta^{15}N$ and $\delta^{13}C$ values (‰) in the muscle and liver of ringed seals from different regions of Lake Saimaa.

				$\delta^{15}N$			$\delta^{13}C$		
Tissue	Age group	Region	*n*	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Muscle	Pups	All	30	13.6 ± 1.0	11.4	15.8	−25.4 ± 0.8	−27.0	−24.0
		Northern	4	12.9 ± 0.6	12.2	13.5	−25.1 ± 0.7	−25.6	−24.2
		Central	21	13.8 ± 1.1	11.4	15.8	−25.6 ± 0.8	−27.0	−24.0
		Southern	5	13.3 ± 0.6	12.9	14.3	−24.9 ± 0.7	−25.9	−24.3
	≥1 yr	All	23	12.4 ± 1.0	10.2	13.7	−25.0 ± 0.8	−26.4	−23.5
		Northern	3	13.3 ± 0.3	13.0	13.7	−26.0 ± 0.3	−26.4	−25.7
		Central	13	12.3 ± 1.1	10.2	13.7	−25.2 ± 0.6	−26.4	−24.4
		Southern	7	12.2 ± 0.9	10.8	13.7	−24.1 ± 0.4	−24.8	−23.5
Liver	Pups	All	29	13.9 ± 1.2	11.1	16.6	−25.4 ± 0.9	−26.9	−23.6
		Northern	4	13.1 ± 0.7	12.3	13.7	−25.1 ± 0.7	−25.8	−24.3
		Central	21	14.0 ± 1.3	11.1	16.6	−25.6 ± 0.9	−26.9	−23.6
		Southern	4	13.9 ± 0.8	13.3	15.0	−24.8 ± 0.7	−25.7	−24.1
	≥1 yr	All	22	13.1 ± 1.1	11.2	14.9	−25.0 ± 0.8	−26.3	−23.9
		Northern	3	14.1 ± 0.7	13.5	14.9	−25.8 ± 0.2	−26.1	−25.7
		Central	12	12.9 ± 1.1	11.3	14.6	−25.3 ± 0.6	−26.3	−24.0
		Southern	7	12.8 ± 0.9	11.2	13.7	−24.2 ± 0.4	−25.0	−23.9

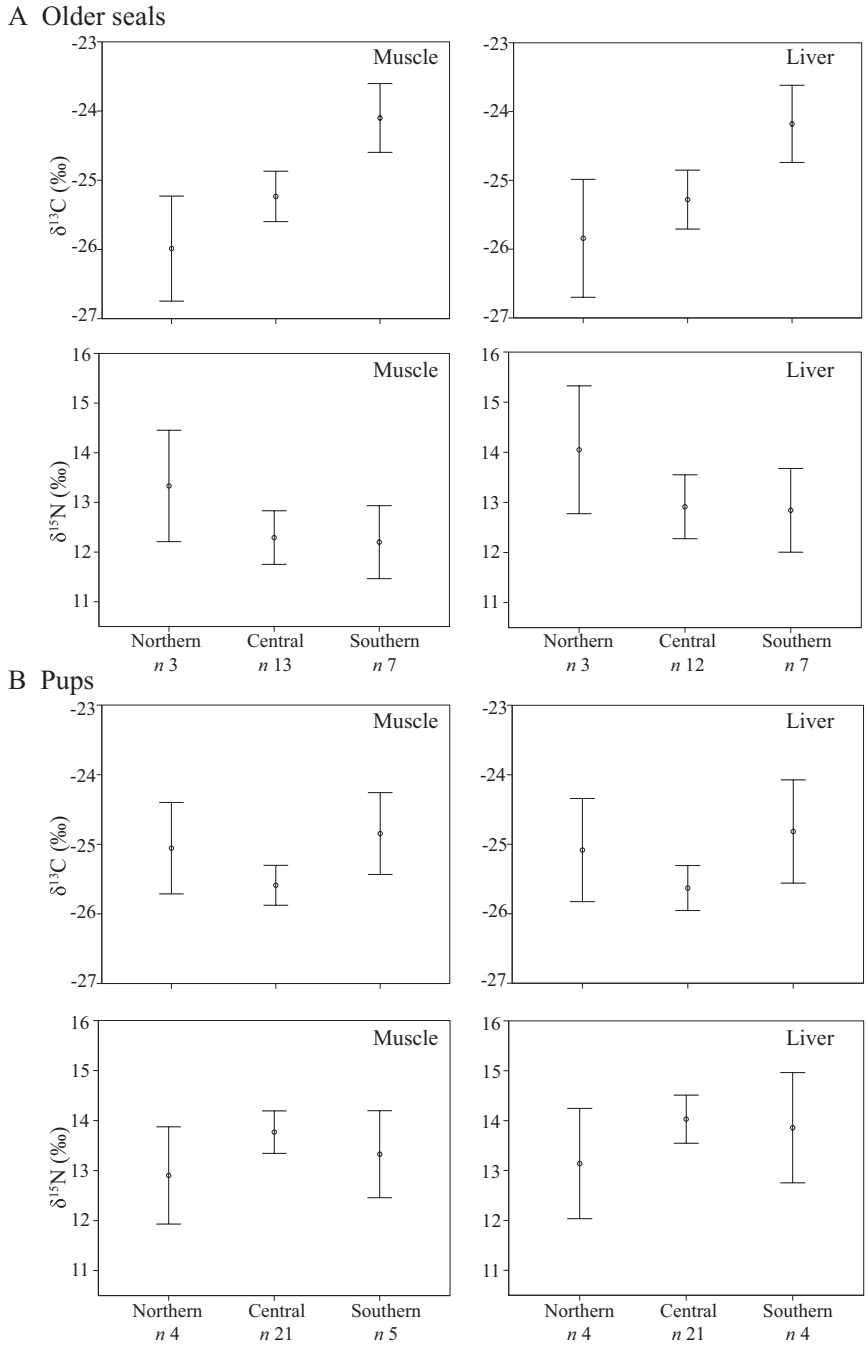

Figure 4. The regional differences are seen in estimated (ANOVA) mean δ^{15}N and δ^{13}C values (whiskers show 95% confidence intervals) of the muscle and liver of (A) the older (≥1 yr) and (B) pup Saimaa ringed seals in the three regions of the lake.

were statistically significantly higher in June than in August and September–January (Table S6).

The reference database of the δ^{13}C and δ^{15}N values of potential prey species was created to show the regional and interspecies variation in stable isotope values (Table S1). Differences were apparent among the three regions of the lake in both carbon and nitrogen isotopic values in the three prey habitat groups (Fig. 5). A significant difference among regions was detected in the δ^{13}C values of the benthic (Kruskal-Wallis test: χ^2 = 8.521, df = 2, P = 0.014) and littoral fish groups (χ^2 = 10.395, df = 2, P = 0.006) as well as in the δ^{15}N values of the benthic (χ^2 = 16.186, df = 2, P < 0.001) and pelagic fish groups (χ^2 = 18.287, df = 2, P < 0.001). However, the carbon values of the pelagic fish groups (χ^2 = 1.004, df = 2, P = 0.605) and the nitrogen values of the littoral fish groups (χ^2 = 0.579, df = 2, P = 0.749) did not differ by region.

Discussion

Diet Composition

Our results show that the Saimaa ringed seal is a generalist feeder that feeds exclusively on fish; a minimum of 15 different species were consumed. Although ringed seals utilize a wide variety of prey, the diet is typically dominated by a few key species (*e.g.,* Lowry *et al.* 1980; Weslawski *et al.* 1994; Wathne *et al.* 2000; Labansen *et al.* 2007, 2011). This study confirms the earlier findings of Kunnasranta *et al*. (1999) that Saimaa ringed seals clearly rely on the five most abundant small schooling fish species: smelt, ruff, perch, vendace, and cyprinids. Moreover, diet was similar across age groups, but pups fed on somewhat smaller fish than older seals. This deviates from the findings of some studies of marine ringed seals in which all age classes fed on similar sized fish (Lowry *et al.* 1980, Holst *et al.* 2001).

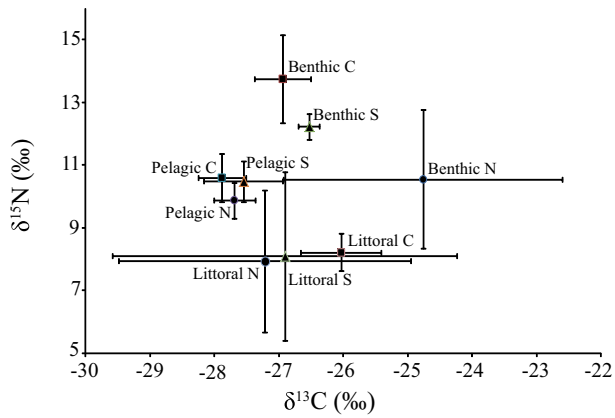

Figure 5. The biplot of the δ^{13}C and δ^{15}N values (mean ± SD) for the prey habitat groups (benthic: ruff; pelagic: vendace and smelt; littoral: roach and bleak) in the three regions (N = northern, C = central and S = southern) of Lake Saimaa. The figure shows regional differences especially in the benthic and littoral prey groups of the Saimaa ringed seal.

On the other hand, the feeding habits of ringed seals can also vary among age classes in marine environment where crustaceans compose an important proportion of their diet (McLaren 1958, Lowry *et al.* 1980, Wathne *et al.* 2000, Holst *et al.* 2001, Labansen *et al.* 2011): crustaceans comprise a large proportion of the pup diet, whereas fish are more important to adults (Lowry *et al.* 1980, Holst *et al.* 2001). Crustaceans are almost absent from the diet of the Saimaa ringed seal (Kunnasranta *et al.* 1999, this study). The number of invertebrates found in the digestive tracts was very low and they were small sized (<2 cm). Although consumption of invertebrates can be underestimated (Lawson *et al.* 1995), they most probably ended up in the seals' digestive tracts by accident or as secondary prey (released from the stomachs of consumed fish). It is possible that in Lake Saimaa crustaceans do not exist in sufficient density to make them a viable prey, or that the seals prefer fish over crustaceans, as Wathne *et al.* (2000) observed in the Barents Sea. The minimal consumption of crustaceans by Saimaa ringed seals may explain the lack of conspicuous age-related differences in diet composition of this freshwater seal.

Nutritional Status and Trophic Level of the Seals

In this study, we detected seasonal changes in δ^{15}N values of the subadults and adults; higher values were observed in winter and spring than in summer. This may indicate that the seals have used their adipose tissue as an energy source and lost weight during winter and molting. The nutritional status of marine ringed seals is known to vary markedly on a seasonal basis (McLaren 1958, Ryg *et al.* 1990, Smith *et al.* 1991). Female ringed seals lose approximately 27% of their body weight during lactation in the late winter–early spring (Smith *et al.* 1991) and in both sexes blubber thickness gradually declines through early spring and during the molting season in late spring (McLaren 1958, Ryg *et al.* 1990). On the other hand, the δ^{15}N values of tissues are affected not only by the nutritional status of the animal, but also by diet (Hobson *et al.* 1993). However, due to the small lake habitat where the same prey species of the Saimaa ringed seal are available year round, we assume that seasonal changes in the older seals δ^{15}N values result from their nutritional status instead of feeding on different trophic levels.

The studied pups were enriched in ^{15}N (muscle by 1.2‰ and liver by 0.8‰) above the older seals, suggesting that the pups (majority ≤6 mo old) were thus either feeding at a higher trophic level (using milk) or using their tissues as their main energy source. It is notable that the pups were growing, which tends to cause lower δ^{15}N values (Sears *et al*. 2009), but they still had substantially higher values than the older seals. This emphasizes the observed difference in δ^{15}N values between the age groups. Also the group of older seals included growing individuals (subadults), which may have had a lowering effect on their values, but our sample size was too small to allow testing adults and subadults separately. Suckling harbor seal (*Phoca vitulina*) pups were also reported to have ^{15}N-enriched tissues in comparison to adults and subadults (Germain *et al.* 2012). Suckling northern fur seal (*Callorhinus ursinus*) and polar bear (*Ursus maritimus*) cubs also have higher δ^{15}N values in their tissues than adults (1.9‰ and 1.0‰, respectively; Hobson *et al.* 1997, Polischuk *et al.* 2001). In contrast, ringed seal pups (<1 year old) in Hudson Bay have lower δ^{15}N values in muscle and liver than the juveniles and adults, which was seen as an indication of lower trophic level feeding (Young *et al.* 2010). Yet this earlier study did not report on the feeding status of these pups (suckling or weaned). Arctic ringed seal pups achieve over 90% of their first-year growth in body length during the nursing period,

and weaned pups appear to lose weight at the end of summer and fall (Smith *et al.* 1991). In Lake Saimaa the pups are born from February to March (Helle *et al.* 1984) and weaning occurs in mid-May (Niemi *et al.* 2013). In our study the pups' δ^{15}N values were high from February to July and decreased in August, which suggests that the pups started to recover from postweaning nutritional stress and gained weight at around 6 mo of age (*cf.* Gannes *et al.* 1997, 1998; Fuller *et al.* 2004; Newsome *et al.* 2010).

Regional Differences in the Stable Isotope Values

Both the δ^{13}C and δ^{15}N values have shown strong spatial isotopic gradients in marine habitats (Hobson and Schell 1998, Lee *et al.* 2005), proving useful in the study of marine mammal feeding ecology (Sinisalo *et al.* 2006, Newsome *et al.* 2010). The differences in the δ^{13}C and δ^{15}N values of the three prey groups were apparent among the different regions of the lake. There were also significant regional variations in the seals' δ^{13}C values, indicating that the Saimaa ringed seal has a sedentary life style, which is supported by behavioral and genetic studies (Kunnasranta 2001, Koskela *et al.* 2002, Valtonen *et al.* 2012). The regional differences in seal tissues indicated that Saimaa ringed seals feed predominantly in the same regions all year round and that large scale seasonal feeding migrations within the lake do not occur.

Observed regional differences in the stable isotope values of both prey species and seals imply that an accurate sampling protocol is essential in order to compare stable isotope values reliably. Both consumer and potential prey samples should be collected from the same regions to avoid regional differences in the stable isotope values of prey hampering the interpretation of results for the dietary assessment of the predators. Furthermore, there can be interannual variation in stable isotope values (Carroll *et al.* 2013, Chambellant *et al.* 2013), but the small sample size of this critically endangered subspecies prevented us from studying this aspect.

Saimaa Ringed Seal and Fishery

The Saimaa ringed seal was considered to be a competitor of commercial and recreational fishery in previous centuries and bounties were paid on seals as late as the 1940s (Kokko *et al.* 1999, Sipilä 2003, Auvinen *et al.* 2005). The conventional assumption is that the Saimaa ringed seal's primary prey is vendace (Hyvärinen *et al.* 1998, Sipilä and Hyvärinen 1998, Auvinen *et al.* 2005), which is the most commercially valuable fish species in the inland waters of eastern Finland (Finnish Game and Fisheries Research Institute 2010). During an earlier diet study (Kunnasranta *et al.* 1999), the vendace population in Lake Saimaa was low and this was proposed as one reason for the low percentage of vendace in the seal diet. In the present study, however, the vendace population was moderately rich[2] but it still comprised a rather small proportion of the diet, indicating that the seals do not compete markedly with the vendace fishery. In addition, the seals do not seem to feed on the endangered salmon species (*Salmo salar* m. *Sebago*, *S. trutta* m. *lacustris*, and *Salvelinus alpines*) of Lake Saimaa. This is in line with the observations of a dietary study in the Baltic Sea (Suuronen and Lehtonen 2012), where ringed seals (*P. h. botnica*) diet did not contain salmonids, even when gray seals (*Halichoerus grypus*) fed on salmon in the same area.

[2]Personal communication from Pentti Valkeajärvi, Finnish Game and Fisheries Research Institute, Survontie 9, FI-40500 Jyväskylä, Finland, February 2012.

This study used similar method to our own, which indicates that despite the fragile nature of salmonid otoliths (Härkönen 1986) they are likely to be found in the seals' digestive tract contents, if they have been eaten.

Dietary studies of top predators may have implications in conflict mitigation and conservation. Our study indicates minimal to nonexistent resource competition between the seals and fishermen in Lake Saimaa. However, another significant aspect of seal-fishery interaction exists: exceptionally high bycatch mortality of juveniles in fishing gear (Sipilä 2003; Niemi *et al.* 2012, 2013), which was also the main cause of death in the sampled seals. The most critical period for pup survival is the dispersal period in early summer (Niemi *et al.* 2013) and the risk of entanglement in gill nets is high, especially for light pups (Sipilä and Hyvärinen 1998, Sipilä 2003). Therefore gill net fishing is banned from 15 April to 30 June (Governmental Decree 294/2011) in closure areas established in the breeding areas of the Saimaa ringed seal (see Niemi *et al.* 2012). However, our results demonstrate that weaned pups start to gain weight as late as in August, emphasizing the need for extending temporal coverage of the springtime gill net closures to improve the survival of weaned pups in late summer.

Acknowledgments

We thank the fishermen T. Laitinen, E. Hirvonen, K. Marttinen, P. Naukarinen and M. Törrönen for the fish samples. Special thanks are given to A-M. Nakari for her extremely hard work in identifying prey items, and to R. Airivuo, O. Pöyhönen and T. Zetterberg for their help with the digestive tract content analysis. We are also thankful to H. Hyvärinen, R. Jones, S. Oksanen and two anonymous reviewers for their valuable comments to improve this manuscript, to K. Sotejeff-Wilson and H. Shiels for checking the language of the manuscript, and to K. Kyyrönen for help with constructing the map. We appreciate the pleasant cooperation we have enjoyed with Metsähallitus, especially with T. Sipilä and T. Kokkonen, and with M. Isomursu from the Finnish Food Safety Authority Evira. This study was supported financially by WWF Finland, the Raija and Ossi Tuuliainen Foundation, the Finnish Ministry of Agriculture and Forestry and the Finnish Ministry of the Environment.

Literature Cited

Amundsen, P.-A., H.-M. Gabler and F. J. Staldvik. 1996. A new approach to graphical analysis of feeding strategy from stomach contents data–modification of the Costello (1990) method. Journal of Fish Biology 48:607–614.

Auvinen, H., J. Karjalainen, V.-M. Kaijomaa, *et al*. 1999. Fish and fishing. Pages 112–125 *in* E. Kuusisto, ed. Saimaa, a living lake. Tammi, Helsinki, Finland.

Auvinen, H., J. Jurvelius, J. Koskela and T. Sipilä. 2005. Comparative use of vendace by humans and Saimaa ringed seal in Lake Pihlajavesi, Finland. Biological Conservation 125:381–389.

Bond, A. L., and K. A. Hobson. 2012. Reporting stable-isotope ratios in ecology: Recommended terminology, guidelines and best practices. Waterbirds 35:324–331.

Bowen, W. D., and S. J. Iverson. 2013. Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. Marine Mammal Science 29:719–754.

Carroll, S. S., L. Horstmann-Dehn and B. L. Norcross. 2013. Diet history of ice seals using stable isotope ratios in claw growth bands. Canadian Journal of Zoology 91: 191–202.

Chambellant, M., I. Stirling and S. H. Ferguson. 2013. Temporal variation in western Hudson Bay ringed seal *Phoca hispida* diet in relation to environment. Marine Ecology Progress Series 481:269–287.

Costello, M. J. 1990. Predator feeding strategy and prey importance: A new graphical analysis. Journal of Fish Biology 36:261–263.

Dalerum, F., and A. Angerbjörn. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–658.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506.

Finnish Game and Fisheries Research Institute. 2010. Commercial inland fishery 2008. Finnish Game and Fisheries Research Institute, Helsinki, Finland 25 pp.

Focken, U., and K. Becker. 1998. Metabolic fractionation of stable carbon isotopes: Implications of different proximate compositions for studies of aquatic food webs using δ^{13}C data. Oecologia 115:337–343.

Fuller, B. T., J. L. Fuller, N. E. Sage, D. A. Harris, T. C. O'Connell and R. E. M. Hedges. 2004. Nitrogen balance and δ^{15}N: Why you're not what you eat during pregnancy. Rapid Communications in Mass Spectrometry 18:2889–2896.

Fuller, B. T., J. L. Fuller, N. E. Sage, D. A. Harris, T. C. O'Connell and R. E. M. Hedges. 2005. Nitrogen balance and δ^{15}N: Why you're not what you eat during nutritional stress. Rapid Communications in Mass Spectrometry 19:2497–2506.

Gannes, L. Z., D. M. O'Brien and C. M. del Rio. 1997. Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. Ecology 78: 1271–1276.

Gannes, L. Z., C. Martinez del Rio and P. Koch. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. Comparative Biochemistry and Physiology 119A:725–737.

Germain, L. R., M. D. McCarthy, P. L. Koch and J. T. Harvey. 2012. Stable carbon and nitrogen isotopes in multiple tissues of wild and captive harbor seals (*Phoca vitulina*) off the Californian coast. Marine Mammal Science 28:542–560.

Gjertz, I., and C. Lydersen. 1986. The ringed seal (*Phoca hispida*) spring diet in northwestern Spitsbergen, Svalbard. Polar Reserch 4:53–56.

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

Helle, E., H. Hyvärinen and T. Sipilä. 1984. Breeding habitat and lair structure of the Saimaa ringed seal *Phoca hispida saimensis* Nordq. in Finland. Acta Zoologica Fennica 182: 125–127.

Helm, R. C. 1984. Rate of digestion in three species of pinnipeds. Canadian Journal of Zoology 62:1751–1756.

Hobson, K. A., and D. M. Schell. 1998. Stable carbon and nitrogen isotope patterns in baleen from eastern Arctic bowhead whales (*Balaena mysticetus*). Canadian Journal of Fisheries and Aquatic Sciences 55:2601–2607.

Hobson, K. A., R. T. Alisauskas and R. G. Clark. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: Implications for isotopic analyses of diet. Condor 95:388–394.

Hobson, K. A., J. L. Sease, R. L. Merrick and J. F. Piatt. 1997. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. Marine Mammal Science 13:114–132.

Holst, M., I. Stirling and K. A. Hobson. 2001. Diet of ringed seals (*Phoca hispida*) on the east and west sides of the North Water Polynya, northern Baffin Bay. Marine Mammal Science 17:888–908.

Hynynen, J., A. Palomäki, H. Veijola, *et al*. 1999. Planktonic and zoobenthic communities in an oligotrophic, boreal lake inhabited by an endemic and endangered seal population. Boreal Environment Research 4:145–161.

Hyslop, E. J. 1980. Stomach contents analysis–a review of methods and their applications. Journal of Fish Biology 17:411–429.

Hyvärinen, H., and M. Nieminen. 1990. Differentiation of the ringed seal in the Baltic Sea, Lake Ladoga and Lake Saimaa. Finnish Game Research 47:21–27.

Hyvärinen, H., T. Sipilä, M. Kunnasranta and J. T. Koskela. 1998. Mercury pollution and the Saimaa ringed seal (*Phoca hispida saimensis*). Marine Pollution Bulletin 36:76–81.

Kiljunen, M., J. Grey, T. Sinisalo, C. Harrod, H. Immonen and R. I. Jones. 2006. A revised model for lipid-normalizing ^{13}C values from aquatic organisms, with implications for isotope mixing models. Journal of Applied Ecology 43:1213–1222.

Kokko, H., E. Helle, J. Lindström, E. Ranta, T. Sipilä and F. Courchamp. 1999. Backcasting population sizes of ringed and grey seals in the Baltic and Lake Saimaa during the 20th century. Annales Zoologici Fennici 36:65–73.

Koli, L. 1998. Suomen kalat [Fish in Finland]. WSOY, Porvoo, Finland.

Koskela, J. T., M. Kunnasranta, E. Hämäläinen and H. Hyvärinen. 2002. Movements and use of haul-out sites of radio-tagged Saimaa ringed seal (*Phoca hispida saimensis* Nordq.) during the open-water season. Annales Zoologici Fennici 39:59–67.

Kovacs, K. M., A. Aguilar, D. Aurioles, *et al*. 2012. Global threats to pinnipeds. Marine Mammal Science 28:414–436.

Kunnasranta, M. 2001. Behavioral biology of two ringed seal (*Phoca hispida*) subspecies in the large European lakes Saimaa and Ladoga. Ph.D. thesis, University of Joensuu, Joensuu, Finland. 86 pp.

Kunnasranta, M., H. Hyvärinen, T. Sipilä and J. T. Koskela. 1999. The diet of the Saimaa ringed seal *Phoca hispida saimensis*. Acta Theriologica 44:443–450.

Kunnasranta, M., H. Hyvärinen, J. Häkkinen and J. T. Koskela. 2002. Dive types and circadian behaviour patterns of Saimaa ringed seals *Phoca hispida saimensis* during the open-water season. Acta Theriologica 47:63–72.

Kurle, C. M., and G. A. J. Worthy. 2002. Stable nitrogen and carbon isotope ratios in multiple tissues of the northern fur seal *Callorhinus ursinus*: Implications for dietary and micratory reconstructions. Marine Ecology Progress Series 236:289–300.

Labansen, A. L., C. Lydersen, T. Haug and K. M. Kovacs. 2007. Spring diet of ringed seals (*Pusa hispida*) from northwestern Spitsbergen, Norway. ICES Journal of Marine Science 64:1246–1256.

Labansen, A. L., C. Lydersen, N. Levermann, T. Haug and K. M. Kovacs. 2011. Diet of ringed seals (*Pusa hispida*) from Northeast Greenland. Polar Biology 34:227–234.

Lawson, J. W., G. B. Stenson and D. G. McKinnon. 1995. Diet of harp seals (*Phoca groenlandica*) in nearshore waters of the northwest Atlantic during 1990–1993. Canadian Journal of Zoology 73:1805–1818.

Lawson, J. W., J. A. Hare, E. Noseworthy and J. K. Friel. 1997. Assimilation efficiency of captive ringed seal (*Phoca hispida*) fed different diets. Polar Biology 18:107–111.

Lee, S. H., D. M. Schell, T. L. McDonald and W. J. Richardson. 2005. Regional and seasonal feeding by bowhead whales *Balaena mysticetus* as indicated by stable isotope ratios. Marine Ecology Progress Series 285:271–287.

Lowry, L. F., K. J. Frost and J. J. Burns. 1978. Food of ringed seal and bowhead whales near Point Barrow, Alaska. The Canadian Field-Naturalist 92:67–70.

Lowry, L. F., K. J. Frost and J. J. Burns. 1980. Variability in the diet of ringed seals, *Phoca hispida*, in Alaska. Canadian Journal of Fisheries and Aquatic Science 37: 2254–2261.

McLaren, I. A. 1958. The biology of the ringed seal (*Phoca hispida* Schreber) in the eastern Canadian Arctic. Bulletin of the Fisheries Research Board of Canada no. 118, Ottawa, Canada.

Metsähallitus, Natural Heritage Services. 2012. Hyljekanta 2012 [Saimaa ringed seal population 2012]. Available at http://www.metsa.fi/sivustot/metsa/fi/Luonnonsuojelu/Lajitjaluontotyypit/Uhanalaisetelaimet/Saimaannorppa/Hyljekanta2012/Sivut/default.aspx.

Newsome, S. D., M. T. Clementz and P. L. Koch. 2010. Using stable isotope biochemistry to study marine mammal ecology. Marine Mammal Science 26:509–572.

Niemi, M., M. Auttila, M. Viljanen and M. Kunnasranta. 2012. Movement data and their application for assessing the current distribution and conservation needs of the endangered Saimaa ringed seal. Endangered Species Research 19:99–108.

Niemi, M., M. Auttila, M. Viljanen and M. Kunnasranta. 2013. Home range, survival and dispersal of endangered Saimaa ringed seal pups: Implications for conservation. Marine Mammal Science 29:1–13.

Palo, J. U., H. Hyvärinen, E. Helle, H. S. Mäkinen and R. Väinölä. 2003. Postglacial loss of microsatellite variation in the landlocked Lake Saimaa ringed seal. Conservation Genetics 4:117–128.

Parsons, J. L. 1977. Metabolic studies on ringed seals (*Phoca hispida*). M.Sc. thesis, University of Guelph, Ontario, Canada. 82 pp.

Phillips, D. L., and J. W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia 128:171–179.

Phillips, D. L., and J. W. Gregg. 2003. Source partitioning using stable isotopes: Coping with too many sources. Oecologia 136:261–269.

Polischuk, S. C., K. A. Hobson and M. A. Ramsay. 2001. Use of stable-carbon and -nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Canadian Journal of Zoology 79:499–511.

Ranta, E., J. Lindström and H. Kokko. 1996. Ecological risk analysis: The case of the Saimaa ringed seal. Ambio 25:363–365.

Ryg, M., T. G. Smith and N. A. Øritsland. 1990. Seasonal changes in body mass and body composition of ringed seals (*Phoca hispida*) on Svalbard. Canadian Journal of Zoology 68:470–475.

Salmi, P., and K. Muje. 2001. Local owner-based management of Finnish lake fisheries: Social dimensions and power relations. Fisheries Management Ecology 8:435–442.

Salmi, P., H. Auvinen, J. Jurvelius and M. Sipponen. 2000. Finnish lake fisheries and conservation of biodiversity: Coexistence or conflict? Fisheries Management and Ecology 7:127–138.

SAS. 2011. SAS, OnlineDoc, Version 9.3. SAS Institute Inc., Cary, NC.

Sears, J., S. A. Hatch and D. M. O'Brien. 2009. Disentangling effects of growth and nutritional status on seabird stable ratios. Oecologia 159:41–48.

Sinisalo, T. 2007. Diet and foraging of ringed seals in relation to helminth parasite assemblages. Ph.D. thesis, University of Jyväskylä, Jyväskylä, Finland. 38 pp.

Sinisalo, T., E. T. Valtonen, E. Helle and R. I. Jones. 2006. Combining stable isotope and intestinal parasite information to evaluate dietary differences between individual ringed seal (*Phoca hispida botnica*). Canadian Journal of Zoology 84:823–831.

Sinisalo, T., R. I. Jones, E. Helle and E. T. Valtonen. 2008. Changes in diets of individual Baltic ringed seals (*Phoca hispida botnica*) during their breeding season inferred from stable isotope analysis of multiple tissues. Marine Mammal Science 24:159–170.

Sipilä, T. 2003. Conservation biology of Saimaa ringed seal (*Phoca hispida saimensis*) with reference to other European seal populations. Ph.D. thesis, University of Helsinki, Helsinki, Finland. 116 pp.

Sipilä, T., and H. Hyvärinen. 1998. Status and biology of Saimaa (*Phoca hispida saimensis*) and Ladoga (*Phoca hispida ladogensis*) ringed seals. Pages 86–99 *in* M. P. Heide-Jørgensen and C. Lydersen, eds. Ringed seals in the North Atlantic. NAMMCO, Tromsø, Norway.

Sipilä, T., E. Helle and H. Hyvärinen. 1990. Distribution, population size and reproductivity of the Saimaa ringed seal (*Phoca hispida saimensis* Nordq.) in Finland, 1980–84. Finnish Game Research 47:3–10.

Smith, T. G., M. O. Hammil and G. Taugøl. 1991. A review of the developmental, behavioural and physiological adaptations of the ringed seal, *Phoca hispida*, to life in the arctic winter. Arctic 44:124–131.

Stewart, R. E. A., B. E. Stewart, I. Stirling and E. Street. 1996. Counts of growth layer groups in cementum and dentine in ringed seals (*Phoca hispida*). Marine Mammal Science 12:383–401.

Suuronen, P., and E. Lehtonen. 2012. The role of salmonids in the diet of grey and ringed seals in the Bothnian Bay, northern Baltic Sea. Fisheries Research 125–126:283–288.

Tieszen, L. L., T. W. Boutton, K. G. Tesdahl and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37.

Tollit, D. J., S. G. Heaslip, R. L. Barrick and A. W. Trites. 2007. Impact of diet-index selection and the digestion of prey hard remains on determining the diet of the Steller sea lion (*Eumetopias jubatus*). Canadian Journal of Zoology 85:1–15.

Tollit, D. J., G. J. Pierce, K. A. Hobson, W. D. Bowen and S. J. Iverson. 2010. Diet. Pages 165–190 *in* I. L. Boyd, W. D. Bowen and S. J. Iverson, eds. Marine mammal ecology and conservation: A handbook of techniques. Oxford University Press, Oxford, U.K.

Tonder, M., and J. Jurvelius. 2004. Attitudes toward fishery and conservation of the Saimaa ringed seal in Lake Pihlajavesi, Finland. Environmental Conservation 31:122–129.

Tonder, M., and P. Salmi. 2004. Institutional changes in fisheries governance: The case of the Saimaa ringed seal, *Phoca hispida saimensis*, conservation. Fisheries Management and Ecology 11:283–290.

Valtonen, M., J. U. Palo, M. Ruokonen, M. Kunnasranta and T. Nyman. 2012. Spatial and temporal variation in genetic diversity of an endangered freshwater seal. Conservation Genetics 13:1231–1245.

Wathne, J. A., T. Haug and C. Lydersen. 2000. Prey preference and niche overlap of ringed seals *Phoca hispida* and harp seals *P. groenlandica* in the Barents Sea. Marine Ecology Progress Series 194:233–239.

Weslawski, J. M., M. Ryg, T. G. Smith and N. A. Oritsland. 1994. Diet of ringed seals (*Phoca hispida*) in a fjord of West Svalbard. Arctic 47:109–114.

Young, B. G., L. L. Loseto and S. H. Ferguson. 2010. Diet differences among age classes of Arctic seals: Evidence from stable isotope and mercury biomarkers. Polar biology 33:153–162.

Received: 12 February 2013
Accepted: 7 March 2014

Supporting Information

The following supporting information is available for this article online at http://onlinelibrary.wiley.com/doi/10.1111/mms.12133/suppinfo.

Table S1. Reference database of the $\delta^{13}C$ and $\delta^{15}N$ values (‰) of the potential prey species of ringed seals from the three regions of Lake Saimaa.

Table S2. Pearson correlation estimates of observed stable isotope values of ringed seals in Lake Saimaa.

Table S3. Preplanned comparisons of the mean $\delta^{13}C$ and $\delta^{15}N$ values between pups and older (>1 yr) Saimaa ringed seals after analysis of variance. The overall repeated ANOVA result was highly significant ($F = 5{,}515.2_{(7,\ 68.2)}$ $P < 0.001$).

Table S4. Preplanned pairwise comparisons of the Saimaa ringed seal $d^{15}N$ and $d^{13}C$ values in different lake regions (N = northern, C = central, and S = southern) after ANOVA. The overall repeated ANOVA result was highly significant ($F = 2{,}363.6_{(23,104.7)}$, $P < 0.001$).

Table S5. Tukey's adjusted pairwise comparisons (all *P*-values were nonsignificant; $P > 0.05$) of $d^{15}N$ values of older (>1 yr) Saimaa ringed seals between seasons after

ANOVA. The overall repeated ANOVA result was highly significant ($F = 7.2_{(5,\ 25)}$, $P < 0.001$).

Table S6. Tukey's adjusted pairwise comparisons of d^{15}N values of Saimaa ringed seal pups between the time periods after ANOVA. The overall repeated ANOVA result was highly significant ($F = 3.8_{(11,\ 47.3)}$, $P < 0.001$).