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

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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 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) 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 $\delta^{15}\text{N}$ values suggested that the seals lose weight during winter and spring. Additionally the drop in $\delta^{15}\text{N}$ 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

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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 (Kunnastranta *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 ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) 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 $\delta^{15}\text{N}$ values of tissues are not only affected by diet but also by the nutritional status of the consumers, and thus, $\delta^{15}\text{N}$ 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

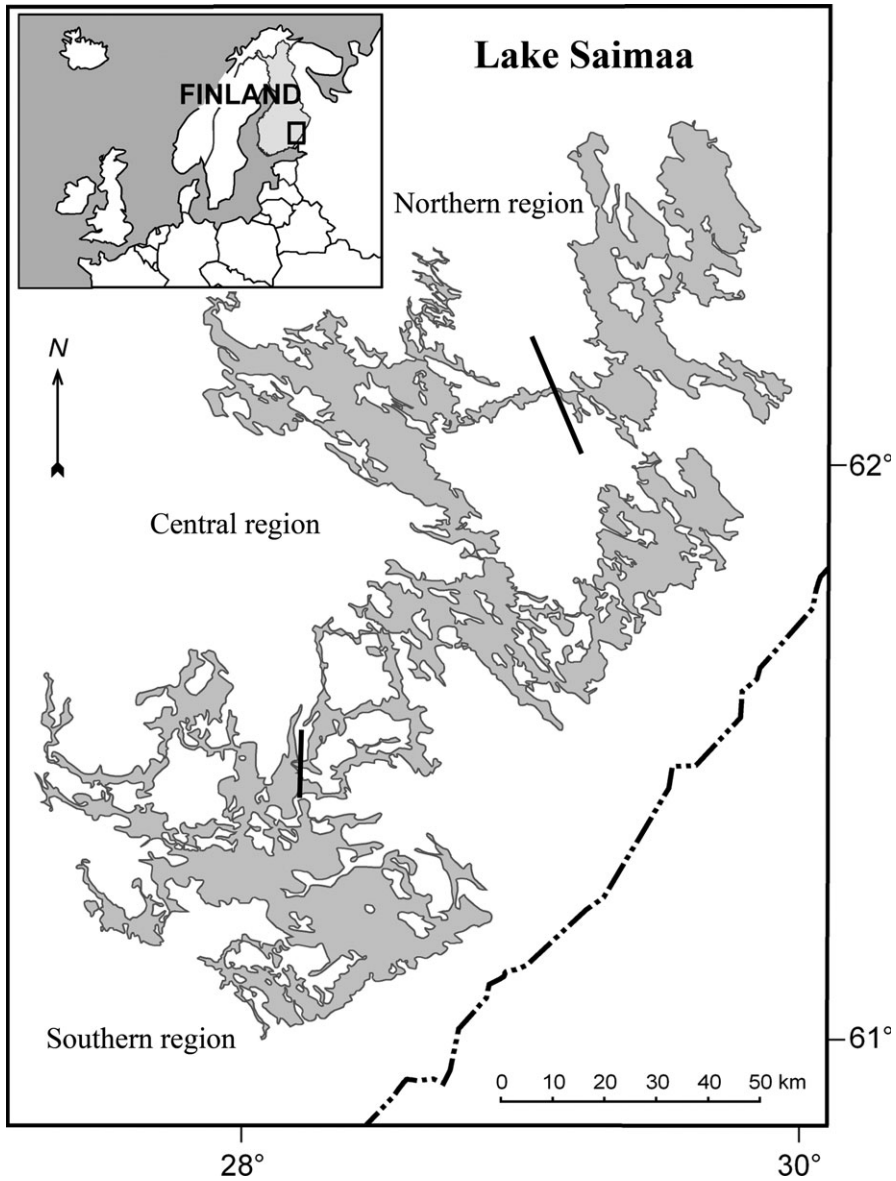


Figure 1. Lake Saimaa study area is divided into northern, central, and southern regions.

($61^{\circ}05'–62^{\circ}36'N$, $27^{\circ}15'–30^{\circ}00'E$) 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 km². 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 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) 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:

$$P_i = \left(\frac{\sum S_i}{\sum S_{ii}} \right) \times 100$$

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_{ii} is the total digestive tract content in only those seals with prey i in their digestive tract.

Stable Isotope Analysis

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were determined from muscle ($n = 53$) and liver ($n = 51$) from the seals whose digestive tract contents were also analyzed. In addition, $\delta^{15}\text{N}$ values were determined from muscle and liver from five stillborn pups. We also examined the seasonal changes in $\delta^{15}\text{N}$ 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; Kunasranta *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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. 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, ‰): $\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000$, where $X = {}^{13}\text{C}$ or ${}^{15}\text{N}$, $R_{\text{sample}} = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$, and $R_{\text{standard}} =$ Vienna Pee Dee belemnite (VPDB) for $\delta^{13}\text{C}$ and atmospheric N_2 (air) for $\delta^{15}\text{N}$ (see Bond and Hobson 2012). The isotopic reference materials were IAEA

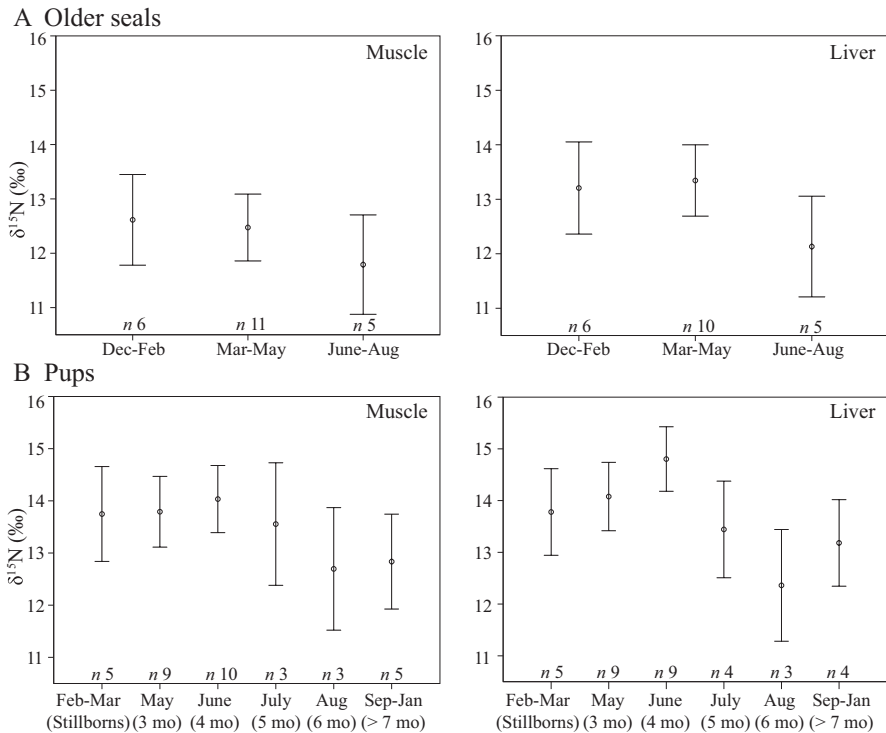


Figure 2. The changes in estimated (ANOVA) mean $\delta^{15}\text{N}$ values (whiskers show 95% confidence intervals) of the muscle and liver of (A) the older (≥ 1 yr) and (B) the pup Saimaa ringed seals found dead at different times of year. The older seals' $\delta^{15}\text{N}$ values are higher during 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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' $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $8.6 \text{ cm} \pm 3.4$ (\pm 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.

Prey category	Frequency of occurrence				Fisher's test	Relative frequency				Prey specific abundance			
	≥ 1 yr		≥ 1 yr			Pups		≥ 1 yr		Pups		≥ 1 yr	
	n	FO%	n	FO%		n	N%	n	N%	n	P%	n	P%
Smelt <i>Osmerus eperlanus</i>	25	83	16	76	0.722	1,972	36.5	1,241	33.6	4,518	43.6	2,840	43.7
Ruff <i>Gymnocephalus cernuus</i>	26	87	16	76	0.460	1,899	35.2	651	17.6	4,433	42.8	2,966	21.9
Perch <i>Perca fluviatilis</i>	19	63	17	81	0.221	253	4.7	792	21.5	3,464	7.3	2,572	30.8
Vendace <i>Coregonus albula</i>	11	37	14	67	0.048	791	14.7	309	8.4	2,474	32.0	2,735	11.3
Cyprinids <i>Cyprinidae</i> ^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 <i>Percidae</i> ^c	6	20	4	19	-	18	0.3	21	0.6	-	-	-	-
Cottids <i>Cottidae</i> ^d	4	13	2	10	-	29	0.5	10	0.3	-	-	-	-
Burbot <i>Lota lota</i>	1	3	5	24	-	1	0.0	6	0.2	-	-	-	-
Pikeperch <i>Sander lucioperca</i>	2	7	1	5	-	9	0.2	1	0.0	-	-	-	-
Nine-spined stickleback	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pungitius pungitius</i>	-	-	1	5	-	-	-	1	0.0	-	-	-	-
Salmonids <i>Salmonidae</i> ^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*, *Scardinus erythrophthalmus* and *Blicca bjoerkna*.

^bCreated for a graphical analysis (Fig. 3); includes all prey categories below except unidentified fish and invertebrates.

^c*Perca fluviatilis*, *Sander lucioperca* and *Gymnocephalus 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 ($n = 21$).

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

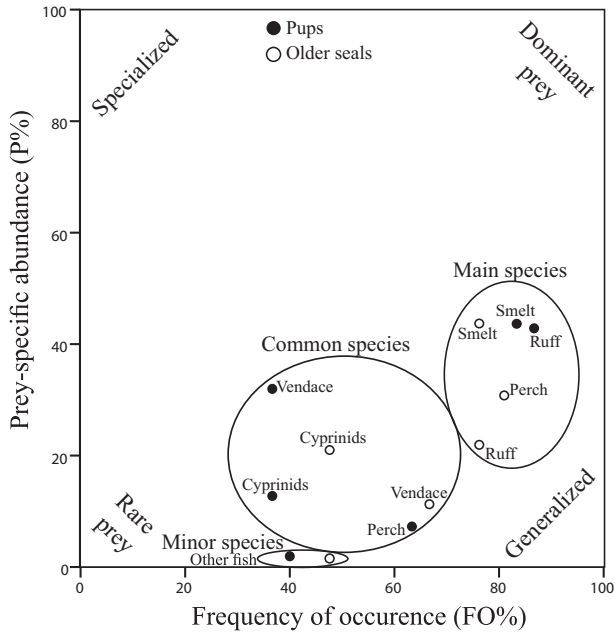


Figure 3. A graphical analysis (Costello 1990, modified by Amundsen *et al.* 1996) of feeding strategy shows that the Saimaa ringed seal is a generalist. Prey are divided into three groups (main, common, and minor species) based on their importance in the seals' diet.

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

Stable Isotopes

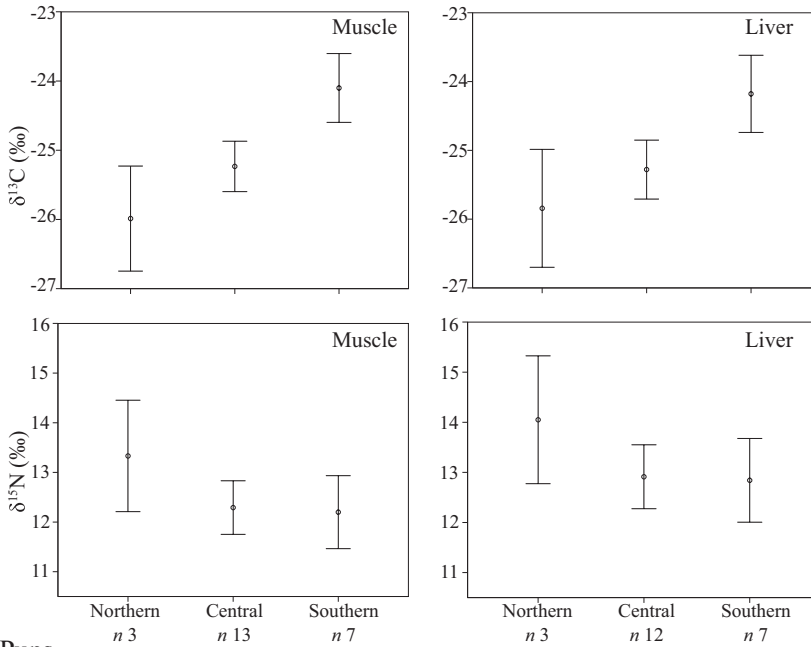
The mean $\delta^{15}\text{N}$ values of pups' muscle and liver were higher than those of the older seals (1.2‰ and 0.8‰ higher, respectively, t -test: $t = 4.334_{(54)}$, $P < 0.001$ and, $t = 3.029_{(54.1)}$, $P = 0.004$, respectively; Table 3). Also the mean $\delta^{13}\text{C}$ value of muscle of 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 $\delta^{13}\text{C}$ values of seal muscle and liver differed significantly among the regions (overall ANOVA: $F = 263.6_{(23, 194.7)}$, $P < 0.001$; Table 3). There was a statistically significant difference in the $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ values within age groups among the regions.

The older seals' $\delta^{15}\text{N}$ values in the muscle and liver did not differ statistically significantly by season (Table S5). However, the mean $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ liver values, but not in the $\delta^{15}\text{N}$ muscle values (Table S6). In addition, their nitrogen values decreased towards the end of the summer (Fig. 2). The mean $\delta^{15}\text{N}$ values of the pups' liver

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

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

A Older seals



B Pups

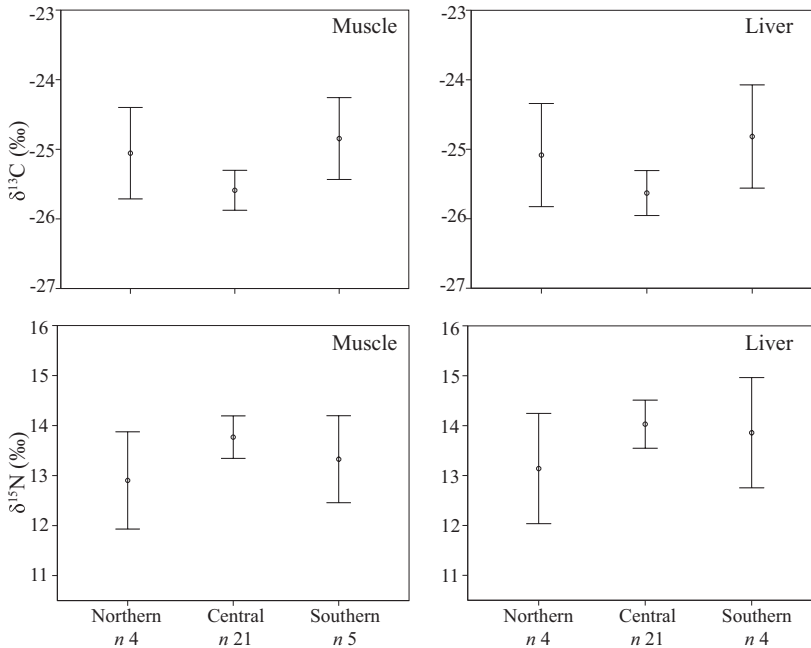


Figure 4. The regional differences are seen in estimated (ANOVA) mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (whiskers show 95% confidence intervals) of the muscle and liver of (A) the older (≥ 1 yr) and (B) pup Saimaa ringed seals in the three regions of the lake.

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

The reference database of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ values of the benthic (Kruskal-Wallis test: $\chi^2 = 8.521$, $df = 2$, $P = 0.014$) and littoral fish groups ($\chi^2 = 10.395$, $df = 2$, $P = 0.006$) as well as in the $\delta^{15}\text{N}$ values of the benthic ($\chi^2 = 16.186$, $df = 2$, $P < 0.001$) and pelagic fish groups ($\chi^2 = 18.287$, $df = 2$, $P < 0.001$). However, the carbon values of the pelagic fish groups ($\chi^2 = 1.004$, $df = 2$, $P = 0.605$) and the nitrogen values of the littoral fish groups ($\chi^2 = 0.579$, $df = 2$, $P = 0.749$) 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).

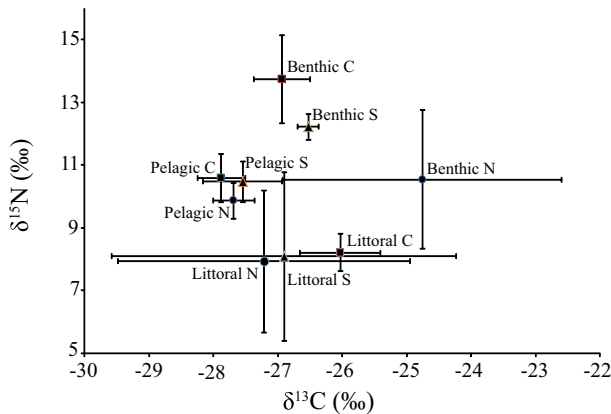


Figure 5. The biplot of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD) for the prey habitat groups (benthic: ruff; pelagic: vendace and smelt; littoral: roach and bleak) in the three regions (N = northern, C = central and S = southern) of Lake Saimaa. The figure shows regional 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values (Sears *et al.* 2009), but they still had substantially higher values than the older seals. This emphasizes the observed difference in $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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' $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the three prey groups were apparent among the different regions of the lake. There were also significant regional variations in the seals' $\delta^{13}\text{C}$ 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 rich² 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 alpinus*) 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. b. botnica*) diet did not contain salmonids, even when gray seals (*Halichoerus grypus*) fed on salmon in the same area.

²Personal 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.

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SUPPORTING INFORMATION

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Table S1. Reference database of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $d^{15}\text{N}$ and $d^{13}\text{C}$ 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 $d^{15}\text{N}$ 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 $d^{15}\text{N}$ values of Saimaa ringed seal pups between the time periods after ANOVA. The overall repeated ANOVA result was highly significant ($F = 3.8_{(11, 47.3)}$, $P < 0.001$).