

Examination of Blubber Fatty Acids in Pregnant and Lactating Alaskan Harbor Seals (*Phoca vitulina*)

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Abstract

Pregnancy and lactation are energetically expensive for female mammals and greatly influence the evolution of species-specific reproductive strategies. Phocid (“true” seals) lactation is generally short in duration and relies heavily on stored energy, whereas otariid (sea lions and fur seals) lactation is generally much longer, and energy is supplemented by foraging. While a phocid, the smaller body size of the harbor seal (*Phoca vitulina*) is assumed to preclude the maintenance of lactation solely from stored energy. For this reason, their lactation strategy is believed intermediate to that of otariids and phocids. The purpose of this study was to characterize blubber fatty acids (FAs) of pregnant and lactating harbor seals and determine if lactating blubber FA profiles more closely resemble phocids or otariids. Blubber FA differences between female reproductive states (lactating, $n = 18$; pregnant, $n = 7$; non-lactating–non-pregnant, $n = 17$), mother–pup pairs ($n = 6$), and families (otariid, $n = 3$; phocid, $n = 3$) were evaluated using permutation analysis of variance (PERMANOVA). Compared to lactating females, pregnant harbor seals had elevated polyunsaturated FAs (PUFAs) and decreased monounsaturated FAs (MUFAs) in their blubber, suggesting pregnant harbor seals may prioritize PUFA storage in the blubber. Additionally, when compared to their pregnant counterparts, lactating harbor seals had lower PUFA, as well as saturated FAs (SFAs) and MUFA $\leq 16C$, suggesting lactating harbor seals may utilize blubber FAs similar to other phocids. Lastly, while blubber SFA and MUFA concentrations may be conserved among pinniped families, PUFA concentrations among lactating phocids and otariids appear to be similar, suggesting lactating species may selectively mobilize PUFA from the blubber in a similar way despite family or lactation strategy. Understanding

how family and body size influence the lactation strategy of a species provides greater insight into the physiological and behavioral limitations a species may have to both internal and external forces during such a critical time in its life history.

Key Words: harbor seal, *Phoca vitulina*, phocid, blubber, fatty acid, lactation, pregnancy

Introduction

Pregnancy and lactation are energetically expensive for female mammals. Pregnant females, for example, have increased energy costs associated with embryo development and the development of tissues associated with pregnancy such as mammary glands, the gravid uterus, and the placenta (Laws, 1959; Hewer & Backhouse, 1968). Lactation is an additional cost of reproduction, normally exceeding all other reproductive costs combined (Gittleman & Thompson, 1988). It can buffer the transition to offspring independence by providing energy stores to offspring or extending the duration of the developmental period (Crocker & McDonald, 2016).

In pinnipeds, two general lactation strategies have evolved to cope with the energetic trade-off of providing adequate nutrition to offspring during pupping and lactation (Bonner, 1984; Oftedal et al., 1987). Otariids (sea lions, fur seals) lactate during an initial perinatal fast of 5 to 9 days, then begin alternating regular foraging trips with suckling periods of 1 to 2 days, with lactation periods lasting 4 months to several years (Crocker & McDonald, 2016). By contrast, phocids (“true” seals), and specifically larger phocids, store adequate energy reserves as blubber prior to parturition to forego foraging trips during a brief, intense lactation period ranging from 4 days in the hooded seal (*Cystophora cristata*) to up to 7 weeks in the Weddell seal

(*Leptonychotes weddellii*) (Crocker & McDonald, 2016). For strategies typically employed by otariids, the milk delivered to the pup is initially synthesized from substrates derived from maternal tissues and subsequently from nutrients acquired while foraging during lactation (Iverson, 1993; Crocker & McDonald, 2016), whereas the phocid strategy relies on substrates for milk synthesis being derived from maternal tissues throughout lactation (Bowen et al., 1992; Iverson, 1993).

The harbor seal (*Phoca vitulina*) is a relatively smaller phocid species (130 to 170 kg; Reeves et al., 2002) that inhabits coastal marine environments throughout the Northern Hemisphere (Boness et al., 1994). In contrast to their larger relatives, the smaller size of the harbor seal is assumed to preclude the maintenance of lactation solely from stored energy (Bowen et al., 1992). For this reason, their lactation strategy is reportedly intermediate to that of otariids and other phocids, with an intermittent foraging cycle that resembles otariids but a lactation length, rate of mass gain in pups, and milk fat content that closely resembles larger phocids (Boness et al., 1994).

Throughout pinniped lactation, a lipid-based milk containing large amounts of fatty acids (FAs), transferred from the mother to the pup, is produced either directly from dietary sources, from the mobilization of FA stored in the adipose tissue, or from biosynthesis (Oftedal, 1984; Jensen, 1989; Iverson et al., 1995). In response to the high physiological demands of lactation, specific FAs may be mobilized or sequestered to accommodate the physiological requirements of both mother and pup (Samuel & Worthy, 2004) often resulting in changes of the mother's blubber FA composition (Iverson et al., 1995; Grahl-Nielsen et al., 2000). In addition, because FAs can be differentially mobilized according to their molecular structure (Connor et al., 1996; Herzberg & Farrell, 2003; Raclot, 2003), loss of FAs from adipose tissue is not merely a function of the relative abundance of individual FAs (Wheatley et al., 2008). Given that female blubber is the primary source of FAs for milk synthesis in phocids, its composition, mobilization, and metabolism can affect the composition of milk and, therefore, the developing neonate (Jump, 2002; Crocker & McDonald, 2016).

While blubber FAs have been investigated in several lactating phocids and otariids (Iverson et al., 1995; Wheatley et al., 2007; Arriola et al., 2013; Fowler et al., 2014; Meynier et al., 2014), only milk FAs have been reported for the harbor seal (Smith et al., 1997). The purpose of this study was to qualitatively investigate blubber FAs of pregnant and lactating harbor seals to determine how blubber stores may be impacted by reproductive state. Blubber FA profiles were compared among

lactating, pregnant, and non-lactating–non-pregnant (NLNP) seals to examine differences in pre- and post-parturition blubber stores. In addition, FA profiles of six mother–pup pairs were compared to previously published milk FA values (Smith et al., 1997) to speculate on possible FA mobilization of blubber stores from mothers for milk synthesis. Lastly, blubber FAs of lactating harbor seals were compared to published blubber FA values of lactating phocid and otariid species to determine if the intermediate lactation strategy of the harbor seal is reflected in its blubber FA profile. Understanding how the harbor seal stores and utilizes blubber FAs during pregnancy and lactation may discern how their body size impacts their metabolism and behavior during a crucial life history event.

Methods

Sample Collection

Seventy-eight ($N = 78$) harbor seals were captured, sampled, and released between April 2000 and October 2010 (Table 1; MMPA NMFS Permit #s 1000, 358-1585, and 358-1787; and ADFG ACUC 07-16) from ten locations on or near rookeries and haulouts from Prince William Sound and Southeast Alaska (Figure 1). Mother–pup pairs ($n = 6$) were sampled together during the 2009 lactation season (June through August) at Tracy and Endicott Arms in Southeast Alaska prior to weaning.

Harbor seals were captured using multifilament seine nets (Jeffries et al., 1993) and monofilament gillnets (Blundell et al., 2011) for terrestrial and glacial fjord captures, respectively. Animals were manually restrained and subsequently sedated with 0.25 mg/kg of diazepam administered intravenously using a 2.5- or 3.5-inch 18-g spinal needle (Blundell et al., 2014). Harbor seals were weighed to the nearest 0.1 kg using a digital hanging scale. Sex was determined visually, and basic morphometrics (e.g., curvilinear length, axillary girth; Blundell & Pendleton, 2008) were measured to the nearest centimeters. A small incision was made in the skin, and full-depth blubber cores (surface to muscle interface with no epidermal tissue) were collected from the right hip using a 6-mm biopsy punch after prepping the area with betadine and 70% ETOH. All blubber core samples were immediately placed into 100% chloroform and stored at -20°C while in the field (< 14 d), then transferred into a -80°C ultra-cold freezer until processing.

Harbor seal age was estimated using a validated model that incorporates sex, curvilinear length, and mass (Blundell & Pendleton, 2008). All adult female harbor seals were estimated to be older than 3.75 years of age based off the age estimation model (Blundell & Pendleton, 2008) and age of sexual maturity (Lydersen & Kovacs, 2005), and

Table 1. Number of female harbor seal (*Phoca vitulina*) blubber samples collected between 2001 and 2010 ($N = 38$) by reproductive state (lactating, pregnant, and non-lactating–non-pregnant [NLNP]) for each year and season sampled within Prince William Sound (PWS) and Southeast Alaska (SEA). Spring = March 1 to May 31; Summer = June 1 to August 31.

		Lactating ($n = 18$)		Pregnant ($n = 7$)		NLNP ($n = 13$)	
		PWS	SEA	PWS	SEA	PWS	SEA
2001	Summer	1	--	--	--	--	--
2003	Spring	--	--	1	--	2	--
	Summer	4	--	--	--	2	--
2004	Spring	--	--	1	5	--	1
2005	Summer	5	--	--	--	6	--
2009	Summer	--	5	--	--	--	1
2010	Summer	--	3	--	--	--	1

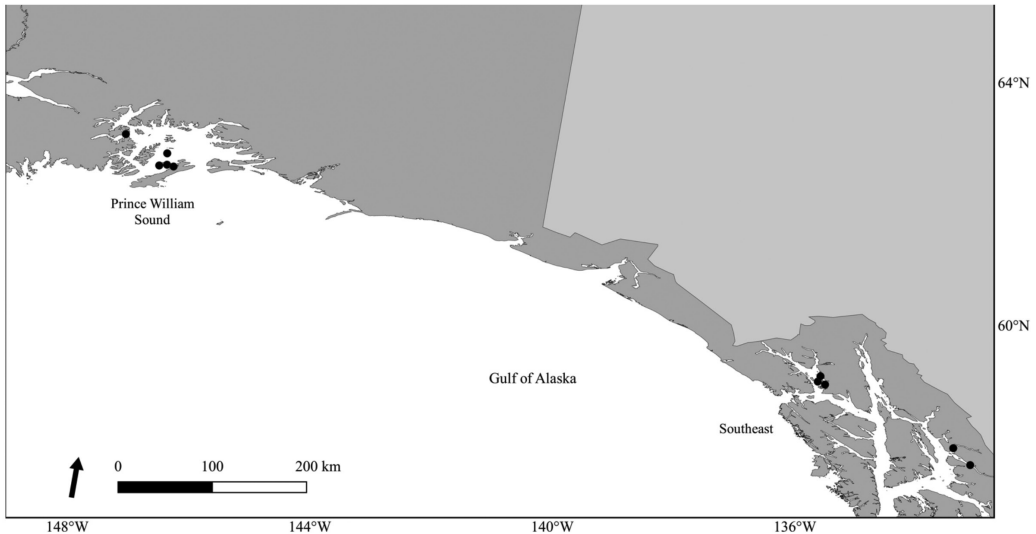


Figure 1. Alaskan harbor seal (*Phoca vitulina*) sampling locations

they were classified as lactating ($n = 18$), pregnant ($n = 7$), or NLNP ($n = 13$). Females were assigned as lactating if they were observed with a suckling pup or if milk could be manually expressed from the teat. Harbor seal females identified as pregnant were confirmed through abdominal distension, nipple and vulva enlargement, and ultrasound, when available. Trans-abdominal ultrasound images were collected with an Aloka SSD 500 portable ultrasound unit (IMAGO Medical, Montreal, Quebec, Canada) equipped with an Aloka UST-660-7.5 transducer (www.aloka.com) and were performed according to Adams et al.

(2007). Females identified as NLNP were confirmed to be neither lactating nor showing any signs of pregnancy through visual examination and/or ultrasound.

Laboratory Procedures

Harbor seal FA quantification analysis protocols followed Beck et al. (2007) and Neises et al. (2021). Briefly, lipids were extracted from full-depth blubber cores applying a modified Folch method using chloroform and methanol (Folch et al., 1957; Iverson et al., 2001). Extracted lipids were derivatized to FA methyl esters (FAME) as described in

Iverson et al. (1997). FAME were analyzed at the Applied Science, Engineering and Technology laboratory at the University of Alaska Anchorage as described in Dodds et al. (2004). Specific FAs were identified using known standard mixtures (Sigma, Supelco, Matrexa, and/or Nu-check Prep), silver nitrate chromatography, and gas chromatography/mass spectrometry (Beck et al., 2007). Individual FAs are reported as percent weight of the total FAs analyzed and are designated using shorthand nomenclature of carbon chain length: number of bonds and location (n-x) of the double bond nearest the terminal methyl group. For example, a FA with a carbon chain length of 16, one double bond, and the location of this double bond seven carbons back from the terminal methyl group would be designated as 16:1n-7 (Budge et al., 2006).

Statistical Analysis

Prior to analysis, harbor seal blubber FA signatures with concentrations less than 0.005 were removed and the remaining signatures were normalized (Budge et al., 2006). For both the female reproductive state and mother-pup pair analysis, a subset of 13 FAs was selected from a total set of 62 FAs (Table 2) based on those considered to be the most abundant (> 1% total of the sum of all samples; Noren et al., 2013), and these accounted for $89.43 \pm 2.04\%$ and $90.01 \pm 2.55\%$ of total FAs by weight, respectively.

For the family analysis, FA values of blubber samples for saturated FA (SFA; no double bonds), monounsaturated FA (MUFA; one double bond), and polyunsaturated FA (PUFA; more than one double bond) were recorded from published papers (Table 3). The species that made up the phocid family in analysis ($n = 3$) included the hooded seal, gray seal (*Halichoerus grypus*), and northern elephant seal (*Mirounga angustirostris*), while the species that made up the otariid family ($n = 3$) included the Steller sea lion (*Eumetopias jubatus*), New Zealand sea lion (*Phocartos hookeri*), and Cape fur seal (*Arctocephalus pusillus pusillus*). If inner and outer blubber FA and/or early and late lactation values were provided, values were averaged (Arnould et al., 2005; Arriola et al., 2013; Fowler et al., 2014). For New Zealand sea lions, FA values used in analysis were the total FA values (see Table 2 in Meynier et al., 2014). For the Cape fur seals, rump values were used for females sampled on land (see Table 2 in Arnould et al., 2005) to be consistent with the reported sampling protocols of the other species. To be consistent with the sampling regions used in this study, Steller sea lion FA class averages were calculated from individual FAs sampled from Lowrie Island (Southeast Alaska) and Fish Island (Prince William Sound) (as reported in Appendix A of Adams, 2000). FA values given in SD were converted to SE for bar graphs.

Blubber FA differences between female reproductive state (lactating, pregnant, NLNP), mother-pup pairs, and families (otariid, phocid) were evaluated using permutation analysis of variance (PERMANOVA) with a Bray-Curtis dissimilarity distance matrix with 999 permutations. Prior to conducting PERMANOVA, a resemblance-based permutation test (betadispr) was conducted to test the null hypothesis that average within-group dispersion is equivalent among groups (Anderson, 2006) as differences in multivariate dispersion, measured as the average distance to the group centroid, can affect PERMANOVA results (Anderson & Walsh, 2013). To determine which blubber FAs were driving differences, pairwiseAdonis with a Bonferroni correction was conducted on any significant PERMANOVA results.

All alpha levels were set to $p = 0.05$. All statistical analysis was conducted in R, Version 3.4.2 (R Core Team, 2017). PERMANOVA and betadispr were conducted using the adonis and betadispr functions in the 'Vegan' package, Version 2.5-7 (Oksanen et al., 2020). PairwiseAdonis was conducted using the 'pairwiseAdonis' package, Version 0.4 (Martinez Arbizu, 2020).

Results

In the blubber FA profiles for lactating females ($n = 18$), MUFAs accounted for $66.09 \pm 1.44\%$ of total blubber FAs, while PUFAs and SFAs accounted for $17.49 \pm 1.23\%$ and $16.42 \pm 0.73\%$, respectively (Table 2). Pregnant females ($n = 7$) had MUFA, PUFA, and SFA blubber concentrations of $52.70 \pm 1.74\%$, $29.92 \pm 2.19\%$, and $17.37 \pm 0.88\%$ total blubber FAs, respectively (Table 2). In NLNP females ($n = 13$), MUFAs accounted for $61.42 \pm 2.29\%$ of total blubber FAs, while PUFAs and SFAs accounted for $22.64 \pm 2.39\%$ and $15.94 \pm 0.35\%$, respectively (Table 2).

Harbor seal blubber FA profiles differed by reproductive state (lactating, pregnant, NLNP) when comparing FA classes (PERMANOVA, $p < 0.01$; Table 4 & Figure 2A) and individual FAs (PERMANOVA, $p = 0.01$; Table 4 & Figure 2B). The degree of β dispersion did not differ among reproductive state when compared between FA classes (betadispr, $p = 0.43$; Table 4) or individual FAs (betadispr, $p = 0.46$; Table 4), providing confidence that results were not due to differences in dispersion among groups. Within FA classes, lactating females had significantly more MUFA and lower PUFA concentrations compared to pregnant females (pairwiseAdonis, MUFA, $p < 0.01$; PUFA, $p = 0.01$; Table 5 & Figure 2A). Lactating and NLNP females had significantly more MUFA 18:1n-11 compared to pregnant females (pairwiseAdonis, lactating, $p = 0.04$;

Table 2. Fatty acid (FA) composition of blubber tissue from lactating, pregnant, and non-lactating–non-pregnant (NLNP) females, as well as mother–pup pairs. Values are mean \pm SE percent of total FA by weight for all FAs that averaged $\geq 0.2\%$ in any group. Boldface indicates 13 FAs containing an average of $> 1\%$ total FA that were used in the female reproductive state analysis. (*) indicates 13 FAs containing an average of $> 1\%$ total FA that were used in the mother–pup analysis.

	Female reproductive state			Mother–pup pairs	
	Lactating (n = 18)	Pregnant (n = 7)	NLNP (n = 13)	Mother (n = 6)	Pup (n = 6)
<i>Saturated</i>					
14:0*	4.67 \pm 0.36	4.86 \pm 0.56	4.77 \pm 0.32	3.76 \pm 0.50	4.82 \pm 0.26
15:0	0.19 \pm 0.01	0.24 \pm 0.01	0.21 \pm 0.01	0.20 \pm 0.02	0.19 \pm 0.02
16:0*	8.87 \pm 0.45	10.18 \pm 0.41	8.95 \pm 0.25	8.40 \pm 0.76	10.99 \pm 0.17
7methyl 16:0	0.11 \pm 0.03	0.16 \pm 0.03	0.11 \pm 0.04	0.17 \pm 0.02	0.08 \pm 0.01
17:0	0.72 \pm 0.19	0.16 \pm 0.04	0.34 \pm 0.07	0.72 \pm 0.16	0.09 \pm 0.01
18:0*	1.31 \pm 0.09	1.25 \pm 0.06	1.12 \pm 0.04	1.07 \pm 0.12	0.88 \pm 0.02
20:0	0.28 \pm 0.04	0.08 \pm 0.01	0.13 \pm 0.03	0.32 \pm 0.10	0.12 \pm 0.05
<i>Monounsaturated</i>					
14:1n-5*	0.89 \pm 0.06	0.94 \pm 0.08	0.98 \pm 0.07	1.01 \pm 0.10	2.66 \pm 0.23
16:1n-11	0.28 \pm 0.03	0.35 \pm 0.04	0.33 \pm 0.05	0.38 \pm 0.06	0.29 \pm 0.04
16:1n-9	0.39 \pm 0.03	0.37 \pm 0.02	0.37 \pm 0.02	0.45 \pm 0.03	0.65 \pm 0.02
16:1n-7*	11.81 \pm 0.72	13.92 \pm 0.71	12.91 \pm 0.69	13.12 \pm 0.60	26.22 \pm 1.12
16:1n-5	0.18 \pm 0.01	0.09 \pm 0.04	0.19 \pm 0.02	0.18 \pm 0.02	0.22 \pm 0.01
17:1	0.28 \pm 0.07	0.34 \pm 0.07	0.21 \pm 0.07	0.57 \pm 0.06	0.48 \pm 0.03
18:1n-13	0.25 \pm 0.05	0.23 \pm 0.04	0.24 \pm 0.04	0.15 \pm 0.10	0.08 \pm 0.05
18:1n-11*	3.05 \pm 0.37	1.42 \pm 0.49	2.80 \pm 0.35	2.82 \pm 0.51	1.41 \pm 0.30
18:1n-9*	31.75 \pm 1.88	23.42 \pm 1.49	28.27 \pm 2.23	33.21 \pm 2.73	28.00 \pm 2.49
18:1n-7*	5.14 \pm 0.31	4.46 \pm 0.37	4.63 \pm 0.26	4.84 \pm 0.48	4.82 \pm 0.45
18:1n-5	0.24 \pm 0.06	0.37 \pm 0.06	0.30 \pm 0.07		
18:1n-3	0.15 \pm 0.04	0.09 \pm 0.09	0.15 \pm 0.06	0.31 \pm 0.09	0.20 \pm 0.06
20:1n-11*	5.86 \pm 1.00	2.86 \pm 0.81	4.87 \pm 0.77	3.36 \pm 0.48	1.20 \pm 0.27
20:1n-9*	2.56 \pm 0.18	1.83 \pm 0.20	2.31 \pm 0.28	2.66 \pm 0.24	0.97 \pm 0.14
20:1n-7	0.41 \pm 0.12	0.36 \pm 0.06	0.29 \pm 0.02	0.25 \pm 0.02	0.11 \pm 0.01
22:1n-11	2.05 \pm 0.42	1.02 \pm 0.31	1.81 \pm 0.42	0.85 \pm 0.16	0.12 \pm 0.03
22:1n-9	0.41 \pm 0.08	0.21 \pm 0.04	0.30 \pm 0.05	0.26 \pm 0.01	0.05 \pm 0.02
<i>Polyunsaturated</i>					
16:2n-4	0.45 \pm 0.04	0.22 \pm 0.02	0.38 \pm 0.03	0.41 \pm 0.07	0.36 \pm 0.05
16:3n-6	0.03 \pm 0.03	0.43 \pm 0.12	0.04 \pm 0.04	--	--
16:3n-4	0.03 \pm 0.02	0.28 \pm 0.06	0.05 \pm 0.03	--	--
16:4n-1	0.25 \pm 0.05	0.51 \pm 0.10	0.29 \pm 0.05	0.23 \pm 0.07	0.19 \pm 0.04
18:2n-6	0.90 \pm 0.05	0.94 \pm 0.09	1.06 \pm 0.07	1.01 \pm 0.16	0.69 \pm 0.07
18:2n-4	0.07 \pm 0.02	0.23 \pm 0.05	0.10 \pm 0.02	0.06 \pm 0.03	0.12 \pm 0.02
18:3n-4	0.16 \pm 0.02	0.22 \pm 0.02	0.15 \pm 0.02	0.16 \pm 0.03	0.17 \pm 0.03
18:3n-3	0.38 \pm 0.03	0.52 \pm 0.05	0.53 \pm 0.05	0.49 \pm 0.13	0.24 \pm 0.05
18:4n-3	0.72 \pm 0.05	1.01 \pm 0.09	1.01 \pm 0.11	0.76 \pm 0.17	0.46 \pm 0.06
18:4n-1	0.09 \pm 0.03	0.30 \pm 0.07	0.09 \pm 0.03	0.13 \pm 0.04	0.15 \pm 0.06
20:2n-6	0.14 \pm 0.01	0.20 \pm 0.02	0.18 \pm 0.01	0.17 \pm 0.04	0.17 \pm 0.04
20:4n-6	0.41 \pm 0.07	0.61 \pm 0.11	0.48 \pm 0.04	0.45 \pm 0.03	0.61 \pm 0.06
20:4n-3	0.36 \pm 0.05	0.62 \pm 0.09	0.51 \pm 0.07	0.54 \pm 0.14	0.28 \pm 0.05
20:5n-3*	3.78 \pm 0.40	8.71 \pm 1.40	4.72 \pm 0.67	4.09 \pm 0.37	3.07 \pm 0.43
21:5n-3	0.22 \pm 0.05	0.46 \pm 0.10	0.17 \pm 0.06	0.37 \pm 0.02	0.27 \pm 0.04
22:4n-6	0.09 \pm 0.03	0.13 \pm 0.03	0.12 \pm 0.01	0.04 \pm 0.02	0.04 \pm 0.02
22:5n-3*	3.23 \pm 0.31	5.39 \pm 0.60	3.98 \pm 0.50	4.03 \pm 0.64	2.32 \pm 0.30
22:6n-3*	5.85 \pm 0.52	8.57 \pm 0.94	8.39 \pm 1.09	7.18 \pm 1.28	5.05 \pm 0.61
% total FA					
Saturated	16.42 \pm 0.73	17.37 \pm 0.88	15.94 \pm 0.35	14.87 \pm 1.30	17.53 \pm 0.19
Monounsaturated	66.09 \pm 1.44	52.70 \pm 1.74	61.42 \pm 2.29	65.47 \pm 2.54	68.36 \pm 1.49
Polyunsaturated	17.49 \pm 1.23	29.92 \pm 2.19	22.64 \pm 2.39	19.66 \pm 2.74	14.12 \pm 1.50

Table 3. Summary of data from previous studies that investigated blubber fatty acid (FA) profiles of lactating females. SFA = saturated FA, MUFA = monounsaturated FA, and PUFA = polyunsaturated FA. Average female weights cited from Reeves et al. (2002).

References	Species	Sample location	Number of individuals	Family	Average female weight (kg)	SFA	MUFA	PUFA
Adams, 2000	<i>Eumetopias jubatus</i> (Steller sea lion)	Alaska	49	Otariid	350	15.68 ± 0.28 (SE)	60.64 ± 0.36 (SE)	23.69 ± 0.14 (SE)
Meynier et al., 2014	<i>Phocarcotus hookeri</i> (New Zealand sea lion)	New Zealand	26	Otariid	160	22.90 ± 3.10 (SD)	58.80 ± 2.90 (SD)	18.30 ± 2.20 (SD)
Arnould et al., 2005	<i>Arctocephalus pusillus pusillus</i> (Cape fur seal)	South Africa	2	Otariid	120	24.00 ± 0.95 (SE)	40.45 ± 1.80 (SE)	35.55 ± 0.85 (SE)
This study	<i>Phoca vitulina</i> (harbor seal)	Alaska	20	Phocid	130	16.42 ± 0.73 (SE)	66.09 ± 1.44 (SE)	17.49 ± 1.23 (SE)
Iverson et al., 1995	<i>Cystophora cristata</i> (hooded seal)	Canada	9	Phocid	300	16.32 ± 0.92 (SE)	64.14 ± 1.08 (SE)	19.50 ± 0.51 (SE)
Arriola et al., 2013	<i>Halichoerus grypus</i> (gray seal)	United Kingdom	57	Phocid	200	7.55 ± 2.30 (SD)	22.61 ± 5.99 (SD)	13.89 ± 4.11 (SD)
Fowler et al., 2014	<i>Mirounga angustirostris</i> (northern elephant seal)	California	41	Phocid	600	18.18 ± 1.06 (SD)	69.91 ± 2.50 (SD)	11.50 ± 1.58 (SD)

Table 4. PERMANOVA and degree of in-group β dispersion results for statistical analysis of blubber fatty acid (FA) profiles for female reproductive state analysis (lactating, $n = 18$; pregnant, $n = 7$; NLNP, $n = 13$), mother–pup pairs analysis (mom, $n = 6$; pup, $n = 6$), and family comparison analysis (phocid, $n = 3$; otariid, $n = 3$; see Table 3). Degrees of freedom for all analysis was 1. p values based on 999 permutations for PERMANOVA and 99 permutations for betadispr. Significant differences in bold ($p < 0.05$). SS = sum of squares. FA class comparison includes saturated FA (SFA), monounsaturated FA (MUFA), and polyunsaturated FA (PUFA). Individual FA analysis includes the 13 FA subsets identified in Table 2.

		PERMANOVA				betadispr	
		SS	F model	R ²	p value	F value	p value
Female reproductive state	FA class comparison	0.08	7.24	0.29	0.003	0.86	0.43
	Individual FAs	0.12	3.19	0.15	0.01	0.78	0.46
Mother–pup pairs	FA class comparison	0.01	3.18	0.24	0.10	3.18	0.18
	Individual FAs	0.11	11.87	0.54	0.005	1.08	0.26
Family comparison	FA class comparison	0.02	0.39	0.13	0.93	1.24	0.47

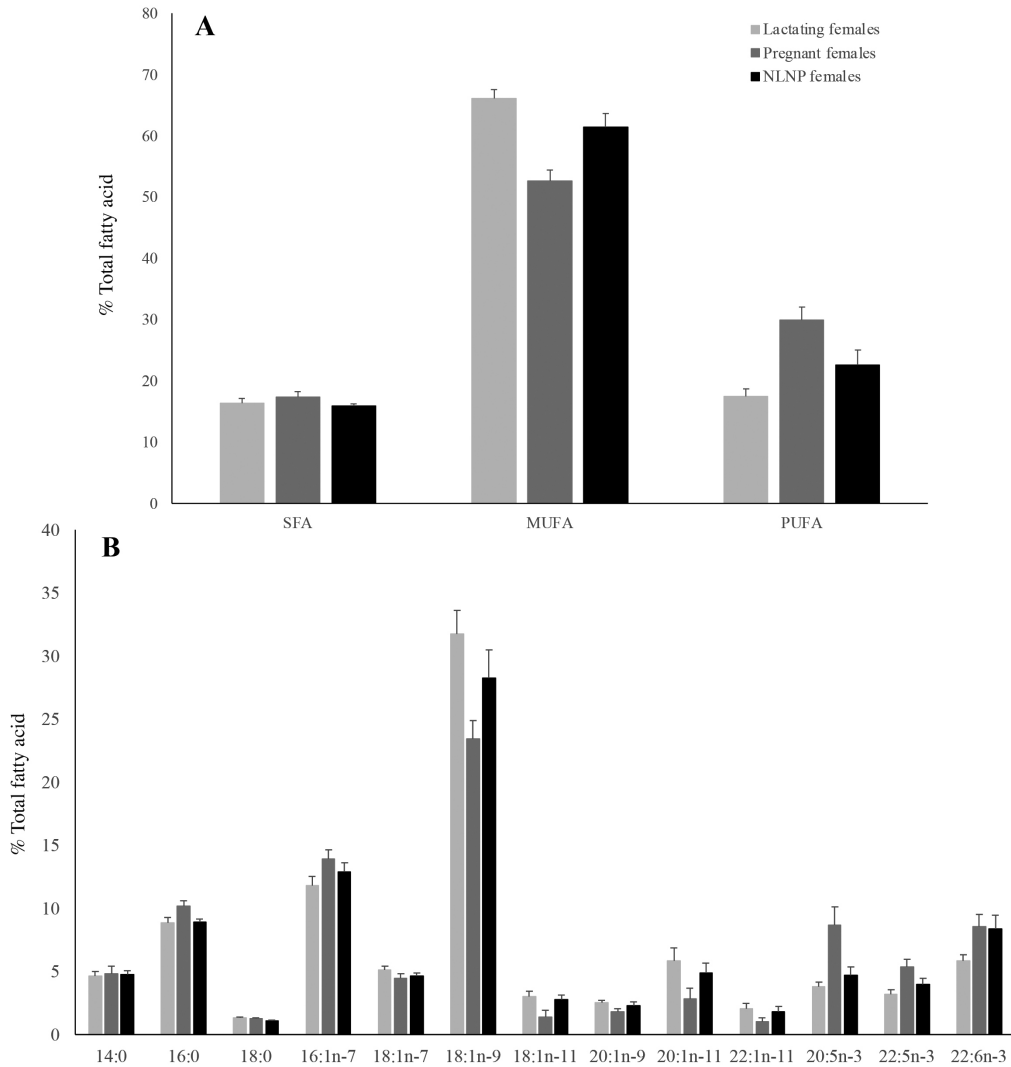


Figure 2. Fatty acid (FA) composition (mean \pm SE) of Alaskan harbor seal blubber from lactating (light grey, $n = 18$), pregnant (dark grey, $n = 7$), and non-lactating–non-pregnant females (NLNP; black, $n = 40$). Results of PERMANOVA showed FA differences between female reproductive state when examining FA classes (A, $p < 0.01$) and individual FAs (B, $p = 0.01$).

NLNP, $p = 0.04$; Table 5 & Figure 2A). Two of the three PUFAs examined were significantly lower in lactating females compared to pregnant females (pairwiseAdonis, 20:5n-3, $p = 0.01$; 22:5n-3, $p < 0.02$), while only 20:5n-3 was significantly lower in NLNP females compared to pregnant females (pairwiseAdonis, $p = 0.03$; Table 5 & Figure 2B).

When examining the overall class of FAs in mother blubber within the mother–pup pairs ($n = 6$), MUFAs accounted for $65.47 \pm 2.54\%$ of the total blubber FAs, while PUFAs and SFAs

accounted for $19.66 \pm 2.74\%$ and $14.87 \pm 1.30\%$, respectively (Table 2). For pups, MUFAs, PUFAs, and SFAs accounted for $68.36 \pm 1.49\%$, $14.12 \pm 1.50\%$, and $17.53 \pm 0.19\%$ of total blubber FAs, respectively (Table 2). Overall, FA classes did not differ significantly between mothers and pups (PERMANOVA, $p = 0.10$; Table 4 & Figure 3A); however, differences were detected when comparing individual FAs (PERMANOVA, $p < 0.01$; Table 4 & Figure 3B). The degree of β dispersion did not differ among mother–pup pairs when

Table 5. Pairwise Adonis results for significantly different blubber fatty acid (FA) profiles for lactating ($n = 18$), pregnant ($n = 7$), and NLNP ($n = 13$) females between FA groups (SFA vs MUFA vs PUFA, PERMANOVA, $F = 7.24$, $p < 0.01$; Table 4) and the FA subset (13 individual FAs identified, $F = 3.32$, $p < 0.01$; Table 4). Significant differences in bold ($p < 0.05$). * p values for pairwise comparisons are adjusted with Bonferroni correction. SFA = saturated FA, MUFA = monounsaturated FA, PUFA = polyunsaturated FA, and SS = sum of squares.

	Pairwise comparison	SS	F	R ²	p^*
SFA	Lactating vs NLNP	0.001	0.17	0.01	1.00
	Lactating vs pregnant	0.01	1.30	0.05	0.78
	NLNP vs pregnant	0.01	5.25	0.23	0.08
14:0	Lactating vs NLNP	0.01	0.28	0.01	1.00
	Lactating vs pregnant	0.004	0.15	0.01	1.00
	NLNP vs pregnant	0.000	0.01	0.001	1.00
16:0	Lactating vs NLNP	0.003	0.31	0.01	1.00
	Lactating vs pregnant	0.03	2.69	0.10	0.32
	NLNP vs pregnant	0.02	6.98	0.28	0.08
18:0	Lactating vs NLNP	0.03	2.23	0.07	0.34
	Lactating vs pregnant	0.003	0.18	0.01	1.00
	NLNP vs pregnant	0.01	4.11	0.19	0.18
MUFA	Lactating vs NLNP	0.01	2.93	0.09	0.29
	Lactating vs pregnant	0.05	27.96	0.55	0.003
	NLNP vs pregnant	0.02	5.45	0.23	0.08
16:1n-7	Lactating vs NLNP	0.02	1.25	0.04	0.83
	Lactating vs pregnant	0.04	2.65	0.10	0.32
	NLNP vs pregnant	0.08	1.04	0.05	0.96
18:1n-7	Lactating vs NLNP	0.02	1.09	0.04	0.89
	Lactating vs pregnant	0.02	1.34	0.05	0.77
	NLNP vs pregnant	0.002	0.17	0.01	1.00
18:1n-9	Lactating vs NLNP	0.03	1.37	0.05	0.73
	Lactating vs pregnant	0.09	6.88	0.23	0.05
	NLNP vs pregnant	0.02	1.72	0.09	0.61
18:1n-11	Lactating vs NLNP	0.01	0.10	0.004	1.00
	Lactating vs pregnant	0.64	7.56	0.25	0.02
	NLNP vs pregnant	0.53	6.51	0.27	0.04
20:1n-9	Lactating vs NLNP	0.03	1.06	0.04	0.90
	Lactating vs pregnant	0.11	5.09	0.18	0.10
	NLNP vs pregnant	0.04	1.01	0.05	1.00
20:1n-11	Lactating vs NLNP	0.02	0.24	0.01	1.00
	Lactating vs pregnant	0.31	2.66	0.10	0.23
	NLNP vs pregnant	0.24	2.37	0.12	0.41
22:1n-11	Lactating vs NLNP	0.05	0.34	0.01	1.00
	Lactating vs pregnant	0.18	1.21	0.05	0.96
	NLNP vs pregnant	0.25	1.57	0.08	0.56
PUFA	Lactating vs NLNP	0.10	2.27	0.07	0.44
	Lactating vs pregnant	0.40	12.50	0.35	0.01
	NLNP vs pregnant	0.13	3.47	0.16	0.21
20:5n-3	Lactating vs NLNP	0.05	0.84	0.03	1.00
	Lactating vs pregnant	0.60	12.01	0.34	0.01
	NLNP vs pregnant	0.33	6.37	0.26	0.03
22:5n-3	Lactating vs NLNP	0.05	1.29	0.04	0.73
	Lactating vs pregnant	0.30	8.69	0.27	0.02
	NLNP vs pregnant	0.11	2.74	0.13	0.32
22:6n-3	Lactating vs NLNP	0.15	3.83	0.12	0.14
	Lactating vs pregnant	0.17	5.79	0.20	0.05
	NLNP vs pregnant	0.01	0.35	0.02	1.00

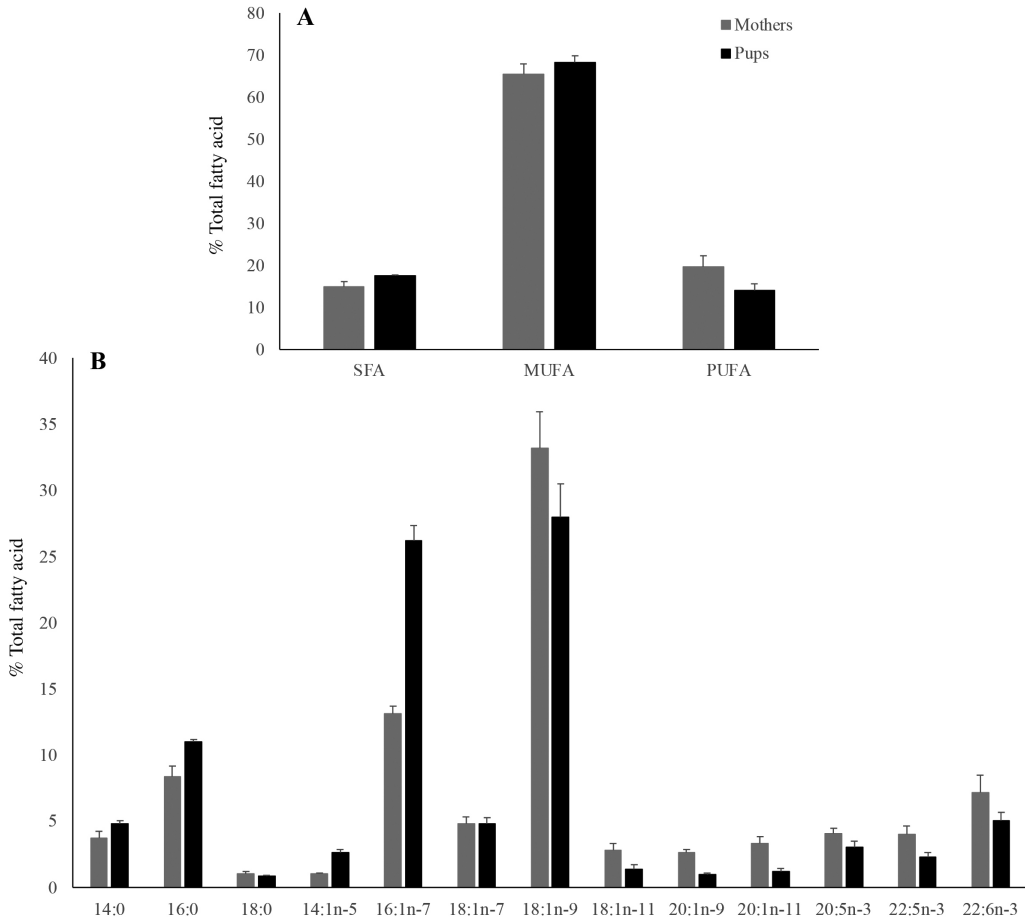


Figure 3. Fatty acid (FA) composition (mean \pm SE) of Alaskan harbor seal blubber from mother–pup pairs ($n = 6$). Results of PERMANOVA showed no FA differences between mothers and pups when examining FA classes (A, $p = 0.10$), but differences were found between individual FAs (B, $p = 0.005$). SFA = saturated FA, MUFA = monounsaturated FA, and PUFA = polyunsaturated FA.

compared between FA classes (betadispr, $p = 0.18$; Table 4) or individual FAs (betadispr, $p = 0.26$; Table 4). SFA 16:0 ($p = 0.01$) and MUFAs 14:1n-5 ($p < 0.01$) and 16:1n-7 ($p < 0.01$) were significantly elevated in pups, while MUFAs 20:1n-9 ($p < 0.01$) and 20:1n-11 ($p = 0.004$) and PUFA 22:5n-3 ($p = 0.04$) were significantly elevated in mothers (pairwiseAdonis; Table 6 & Figure 3B).

Finally, the blubber concentration of SFA, MUFA, and PUFA of lactating harbor seals in this study were not statistically different from the blubber of lactating otariids (Steller sea lion, New Zealand sea lion, Cape fur seal) or larger phocids (hooded seal, gray seal, northern elephant seal) (PERMANOVA, $p = 0.93$; Table 4 & Figure 4A).

Discussion

Pregnancy and lactation are energetically expensive for female mammals and greatly influence the evolution of species-specific reproductive strategies. Based on our results, pregnant females had elevated PUFA and decreased MUFA compared to lactating females, suggesting pregnant harbor seals may prioritize PUFA storage in the blubber during pregnancy in preparation for lactation. Additionally, when compared to their pregnant counterparts, lactating harbor seals had decreased PUFA, as well as SFA and MUFA $\leq 16C$, and elevated amounts of SFA and MUFA $\geq 18C$, implying lactating harbor seals may utilize blubber FAs similar to other phocids (Wheatley et al., 2007,

Table 6. Pairwise Adonis results for significantly different blubber fatty acid (FA) profiles for mom ($n = 6$) and pup ($n = 6$) pairs between FA groups (SFA vs MUFA vs PUFA, PERMANOVA, $F = 3.18$, $p = 0.1$; Table 3) and the FA subset (13 individual FAs identified, $F = 11.82$, $p = 0.005$; Table 3). Significant differences in bold ($p < 0.05$). * p values for pairwise comparisons are adjusted with Bonferroni correction. SFA = saturated FA, MUFA = monounsaturated FA, PUFA = polyunsaturated FA, and SS = sum of squares.

	SS	F	R ²	p^*
SFA	0.02	5.21	0.34	0.05
14:0	0.16	5.09	0.34	0.05
16:0	0.06	11.06	0.53	0.01
18:0	0.02	1.87	0.16	0.197
MUFA	0.002	1.14	0.10	0.29
14:1n-5	0.57	47.53	0.83	0.001
16:1n-7	0.33	117.17	0.92	0.003
18:1n-7	0.00001	0.0007	0.00007	0.99
18:1n-9	0.02	1.68	0.14	0.21
18:1n-11	0.23	3.14	0.24	0.08
20:1n-9	0.63	41.11	0.80	0.002
20:1n-11	0.53	7.88	0.44	0.004
PUFA	0.06	2.93	0.23	0.11
20:5n-3	0.06	2.85	0.22	0.10
22:5n-3	0.16	5.08	0.34	0.04
22:6n-3	0.06	1.94	0.16	0.20

2008; Fowler et al., 2014). Lastly, while blubber SFA and MUFA concentrations may be conserved among pinniped families, PUFA concentrations among lactating phocids and otariids appear to be similar, suggesting lactating species may selectively mobilize PUFA from the blubber in a similar way, despite family or lactation strategy.

Recent studies report pregnant phocids respond to the increase in metabolic demand of pregnancy through changes in foraging behavior and energy requirements (Hückstädt et al., 2018; Shero et al., 2018). For example, when examining foraging behavior, pregnant northern elephant seals will initially increase dive durations to increase prey encounter rates, presumably to gain energy needed to increase the blood volume and mass of the mother to increase oxygen stores (Hückstädt et al., 2018). Similarly, pregnant Weddell seals increase time spent foraging to meet the additional energetic cost of the growing fetus (Shero et al., 2018). Metabolically, previous work suggests that pregnancy in pinnipeds is associated with hypometabolism (Renouf & Gales, 1994; Sparling et al., 2006). When compared to their non-pregnant counterparts, pregnant harbor, Weddell, and northern elephant seals make physiological adjustments to conserve energy while foraging by decreasing their diving metabolic rate (Maresh et al., 2015; Shero et al., 2018). Lowering diving

metabolic rate would not only allow pregnant females to lengthen the aerobic dive limit, enhancing foraging capacities, but it would also decrease maternal maintenance costs and spare energy for fetal growth and energy storage as lean and lipid tissue (Prentice et al., 1989; Shero et al., 2015).

Compared to lactating and NLNP females, pregnant harbor seals in this study had elevated PUFA and less MUFA in their blubber FA profiles. All blubber samples acquired for the pregnant females in this study were collected in mid-April, and while the exact time of gestation is unknown, the majority of harbor seals in Alaska give birth in June and July (Hoover-Miller, 1994), suggesting these females are likely within 2 to 3 months of giving birth. The elevated level of PUFA in pregnant females and the increase in MUFA levels when comparing pregnant and lactating females (53% pregnant to 66% lactating) suggest that the pregnant harbor seals in this study are likely in an anabolic state at the time they were sampled. While blubber FAs in pregnant seals is unstudied, the first two trimesters of pregnancy in humans (0 to 6 mo gestation) begins with an anabolic phase marked by an increase in lipid synthesis and fat storage (Grimes & Wild, 2018), particularly long-chain PUFA $\geq 20C$ (Al et al., 2000; Otto et al., 2001). While this cannot be directly confirmed in these harbor seals without sampling blubber FAs

throughout pregnancy, the elevated level of blubber PUFA compared to lactating females, particularly those $\geq 20C$, suggests a similar physiological response could be occurring in harbor seals. In addition, the elevated amount of PUFA suggests harbor seals may prioritize PUFA storage in the blubber during gestation. Additional studies that sample blubber FAs of pregnant seals throughout gestation would provide a more complete view of harbor seal

lipid stores during gestation and whether or not priority is given to PUFA storage in the blubber.

When compared to pregnant females, the blubber FA profile of lactating harbor seals had a higher concentration of MUFAs ($\geq 18C$) and relatively less PUFAs. When comparing the FA profiles of mothers and pups, mothers were found to have significantly more MUFAs 20:1n-9 and 20:1n-11, and significantly less SFA 16:0 and

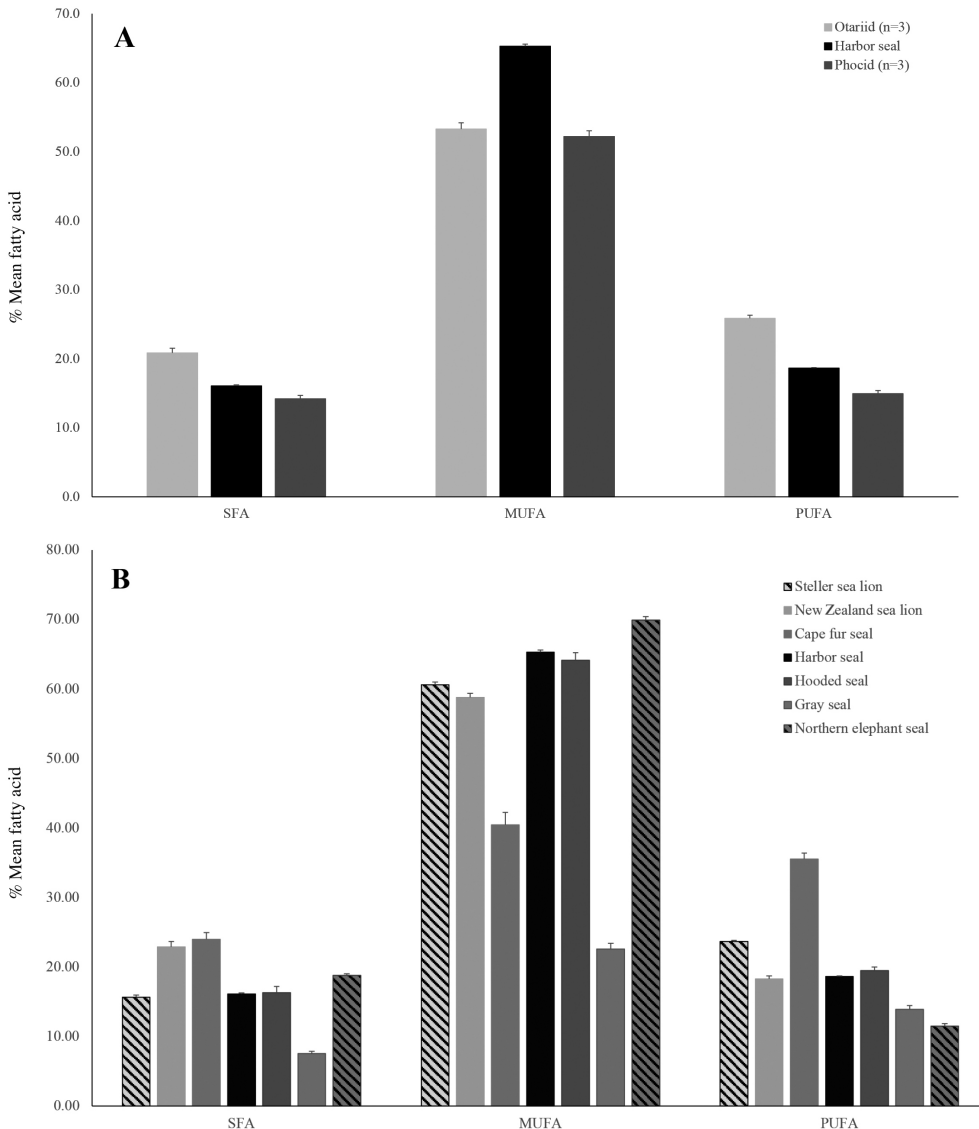


Figure 4. Blubber fatty acid (FA) composition (mean \pm SE) of (A) lactating harbor seals alongside other lactating otariids ($n = 3$) and phocids ($n = 3$) and (B) individual species ($n = 1$ for each). Results of family comparison PERMANOVA showed no blubber FA differences between lactating harbor seals and other lactating otariids or phocids (A, $p = 0.93$). SFA = saturated FA, MUFA = monounsaturated FA, and PUFA = polyunsaturated FA.

MUFAs 14:1n-5 and 16:1n-7, compared to their pups. The difference in MUFA and PUFA concentrations between pregnant and lactating females, as well as between mothers and their pups, suggests that lactating harbor seals may be mobilizing SFA and MUFA $\leq 16C$ while conserving SFA and MUFA $\geq 18C$.

Previous studies involving marine mammals suggest the external blubber layer retains a higher proportion of medium-chain ($\leq 18C$) MUFAs, while the inner layer is enriched in SFA and long-chain ($\geq 20C$) MUFAs (Crocker & McDonald, 2016). The higher rate of mobilization of PUFA, as well as SFA and MUFA $\leq 16C$, is consistent with previous studies, indicating FA mobilization increases with unsaturation and shorter-chain length (Raclot & Groscolas, 1993; Fowler et al., 2014; Crocker & McDonald, 2016), such that there is an increase in PUFA and SFA mobilization from the blubber, while long-chain MUFAs ($\geq 20C$) are retained (Crocker & McDonald, 2016). The conservation of MUFAs $\geq 20C$ in harbor seal blubber is thought to be highly advantageous, providing greater insulative thermoregulatory capacity (Sinensky, 1974). This becomes increasingly important during lactation as blubber stores are depleted and thermoregulatory capacity diminishes. Similar blubber FA profiles of lactating mothers have been documented in the Weddell seal where the blubber FAs with the highest rate of mobilization are SFAs 14:0 and 16:0, and MUFAs 14:1n-5, 16:1n-5, and 16:1n-7 (Wheatley et al., 2007, 2008). Similarly, northern elephant seals and ringed seals (*Phoca hispida*) also appear to conserve more longer-chain MUFAs ($> 18C$) (Strandberg et al., 2008) while mobilizing PUFAs, SFAs, and medium-chain MUFAs to a much greater degree (Fowler et al., 2014).

While milk samples from lactating females in this study were not collected, comparing the blubber FAs of lactating harbor seals sampled within this study to published harbor seal milk FA values allows a hypothetical assessment of possible blubber FA mobilization and utilization in lactating harbor seals and pups. It is important to note that the milk samples that will be discussed were collected from a different population of harbor seals in 1997. We chose to mention these data with the assumption that milk FAs sampled from the Sable Island harbor seal population would have a similar FA distribution as Alaskan harbor seal populations. It is also important to note that the blubber FA data from the lactating females in this study would most certainly have different FA distributions to the Sable Island population due to varying diets. As such, the discussion below is presented with caution with the hope that more recent milk FA data may be collected from Alaskan harbor seals in the future.

As such, examination of milk FAs from harbor seals sampled on Sable Island, Nova Scotia, reveals that milk has elevated levels of SFA and PUFA, particularly FAs 16:0, 20:5n-3, and 22:6n-3 (Table 7; Smith et al., 1997). This supports the premise that phocids highly mobilize SFA and PUFA during lactation (Crocker & McDonald, 2016). In addition, lactating harbor seals on Sable Island appear to prioritize SFAs 14:0 and 16:0 in milk, as well as 16 and 18C MUFAs and PUFAs early in lactation, while longer-chained MUFA ($\geq 20C$) and PUFA ($\geq C22$) are increased later in lactation (Smith et al., 1997). When comparing pup blubber FAs from this study to the milk FA values reported by Smith et al. (1997), pup blubber FAs display a similar trend to lactating females, with pup's blubber exhibiting less SFA and PUFA compared to milk (Table 7). This suggests that while lactating females may mobilize SFA and PUFA to pups via milk, these FAs, particularly PUFAs, may be used by pups for immediate energy and thermoregulatory needs rather than incorporated into blubber. Additionally, while lactating harbor seals are potentially shuttling SFA and PUFA from the blubber to their pups, they may be meeting their own energetic requirements by intermittently feeding during the lactation period. This is contrary to what has been proposed for the larger northern elephant seals. Fowler et al. (2014) suggest elephant seal mothers may preferentially use PUFA and SFA for their own metabolism, decreasing availability for milk deposition.

The possible difference in FA mobilization between the relatively smaller, shallower diving harbor seal and larger, deeper diving northern elephant seal may be tied, in part, to their lactation strategies. While northern elephant seals fast during their ~26-day lactation period (Costa et al., 1986; Crocker et al., 2001), harbor seals are known to intermittently feed throughout their 24-day lactation period (Schulz & Bowen, 2004), with < 1 -day foraging trips beginning 7 to 14 days after parturition (Boness et al., 1994; Thompson et al., 1994). It is assumed that the need for harbor seals to feed during lactation is tied to body size as larger harbor seal females with increased blubber stores appear to remain onshore longer after parturition before foraging when compared to their smaller bodied counterparts (Costa, 1991; Thompson et al., 1994). During the lactation period, harbor seal mothers are believed to use approximately 78% of their blubber stores within 80% of their lactation period, with a reported maternal cost of 24.2 MJ/kg^{0.75} (Boness et al., 1994). In comparison, elephant seals use 46% of their total blubber stores with a higher maternal cost of 32.6 MJ/kg^{0.75} (Costa et al., 1986), suggesting the smaller harbor seal uses a larger fraction

Table 7. Milk fatty acid (FA) values from Smith et al. (1997) referenced alongside mother and pup blubber FA values. Fatty acid values are mean percentage composition by mass. Milk FAs are reported for Day 0 (day of parturition) and Days 19 to 21 (end of lactation) from harbor seals sampled from Sable Island, Nova Scotia.

	Mother blubber (n = 6)	Milk		Pup blubber (n = 6)
		Day 0 (n = 15)	Days 19 to 21 (n = 9)	
SFA				
14:0	3.76	4.57	3.48	4.82
16:0	8.40	15.65	11.92	10.99
18:0	1.07	2.50	2.34	0.88
MUFA				
14:1n-5	1.01	0.32	0.55	2.66
16:1n-7	13.12	9.69	9.41	26.22
18:1n-11	2.82	3.37	3.89	1.41
18:1n-9	33.21	13.49	16.94	28.00
18:1n-7	4.84	4.11	3.78	4.82
20:1n-11	3.36	1.21	1.72	1.20
20:1n-9	2.66	3.90	6.34	0.97
PUFA				
20:5n-3	4.09	9.95	5.97	3.07
22:5n-3	4.03	4.52	4.83	2.32
22:6n-3	7.18	10.27	12.08	5.05

of its stored fat to maintain energy expenditures that are similar in magnitude to those reported in larger species (Bowen et al., 1992).

While elevated levels of SFAs 14:0 and 16:0 have been reported in phocid milk relative to mothers' blubber (Iverson et al., 1995; Smith et al., 1997; Grahl-Nielsen et al., 2000; Arriola Ortiz, 2010; Fowler et al., 2014), the relative values of 14:0, 16:0, 14:1n-5, and 16:1n-7 found in harbor seal milk are 4.6, 15.7, 0.6, and 10.5% lower than in pups' blubber, respectively (Smith et al., 1997). This may indicate the elevated levels observed in the blubber of these harbor seal pups, particularly 14:1n-5 and 16:1n-7 (Table 7), likely originated via biosynthesis. Elevated fetal adipose levels of 16:0 are common with most mammalian systems, including humans (King et al., 1971; Hirsch, 2010), and elevated levels of FAs with 14:0 and 16:0 have been documented in hooded seals prior to first suckling (Iverson et al., 1995). Blubber biopsies taken from ten harbor seal pups directly after birth and prior to first suckling contained 4.1 to 4.4% of 14:0, 10.0 to 13.1% of 16:0, 4.0 to 5.0% of 14:1n-5, and 43.1 to 48.5% of 16:1n-7 (Iverson et al., 1995). Like hooded seals, harbor seal pups are born with a substantial blubber layer (11% body fat, 1.4 cm, 36% of body mass; Bowen et al., 1992), likely an adaptation to protect pups from the thermoregulatory stress of being born near the water's edge and entering the

cold water within hours of being born (Lawson & Renouf, 1985). In this study, blubber concentrations of 14:1n-5 and 16:1n-7 are approximately half of those reported by Iverson et al. (1995), suggesting harbor seal pups may be metabolizing MUFAs for daily energetic needs while conserving 14:0 and 16:0 in the blubber for possible thermoregulatory purposes.

Harbor seal pups are known to spend considerable time in the water relatively soon after parturition during the lactation period (Ofteidal et al., 1991). The elevated levels of PUFAs reported in harbor seal milk (Smith et al., 1997) and the lack of that representation in the pup's blubber relative to their mothers may indicate that pups are utilizing PUFAs in the skeletal muscle for oxygen conservation as well as for thermoregulation. This is corroborated by Boness et al. (1994) who determined that harbor seal pups deposit less fat during lactation compared to other species, with the harbor seal pups containing only 34% body fat at weaning compared to 50, 46, and 45% documented in northern elephant harp (*Pagophilus groenlandicus*), and hooded seals (Ortiz et al., 1978; Worthy & Lavigne, 1983; Ofteidal et al., 1993). In addition, only 50% of the mass lost by harbor seal mothers is stored by the pup compared to 77, 76, and 67% in the harp, hooded, and ringed seals (Boness et al., 1994), respectively, suggesting a high degree of FA metabolism

and low rate of energy deposition by harbor seal pups. Trumble et al. (2010) found that the skeletal muscle of Weddell seal pups switches from primarily SFAs and MUFAs to increased PUFA as the percentage of blubber increases, which was suggested to contribute to the development of oxidative capabilities for diving and to provide non-shivering thermoregulatory benefits (Noren et al., 2008; Trumble et al., 2010). The similarities with harbor and Weddell seal pup blubber FAs support interesting physiological or thermoregulatory patterns. Additional studies designed to sample blubber and milk FAs of harbor seals and their pups throughout lactation would provide clarity to how harbor seals, in particular, are mobilizing blubber FA during lactation.

Other phocid species known to intermittently feed during lactation include the ringed, bearded (*Erignathus barbatus*), and Weddell seals (Schulz & Bowen, 2004). When compared to otariids, these phocids spend a comparable amount of their lactation period foraging but have shorter lactation periods. For example, the harbor seal spends roughly 55% of its 25-day lactation period foraging, while the South American sea lion (*Otaria flavescens*) and Galapagos fur seal (*Arctocephalus galapagoensis*) spend 59 and 50% of their 548- and 540-day lactation periods at sea, respectively

(Schulz & Bowen, 2004). While this analysis was unable to determine differences between lactating harbor seal blubber FAs and those of other lactating phocids or otariids, the data trends suggest that SFA and MUFA may be conserved within families, with harbor seals showing a more similar MUFA distribution to other lactating phocids compared to otariids. PUFAs, however, do not appear to be conserved among families during lactation, with harbor seal PUFA concentrations intermediate to phocids and otariids.

Due to sample size constraints, no statistical analysis was able to be conducted to examine species blubber FA differences; however, when examining how PUFA concentrations differ by mass, the majority of PUFA concentrations appear to fall between 10 to 25% (Figure 5). Comparing blubber FA concentrations among phocid and otariid species suggests that lactating harbor seal PUFA concentrations are most similar to lactating New Zealand sea lions and hooded seals (within 2% total PUFA by weight). The similarity in PUFA concentrations between harbor seals, hooded seals, and New Zealand sea lions was interesting considering the differences in their lactation strategies. For example, hooded seals fast during their 4-day lactation period (Bowen et al., 1985), while New Zealand sea lions feed throughout their

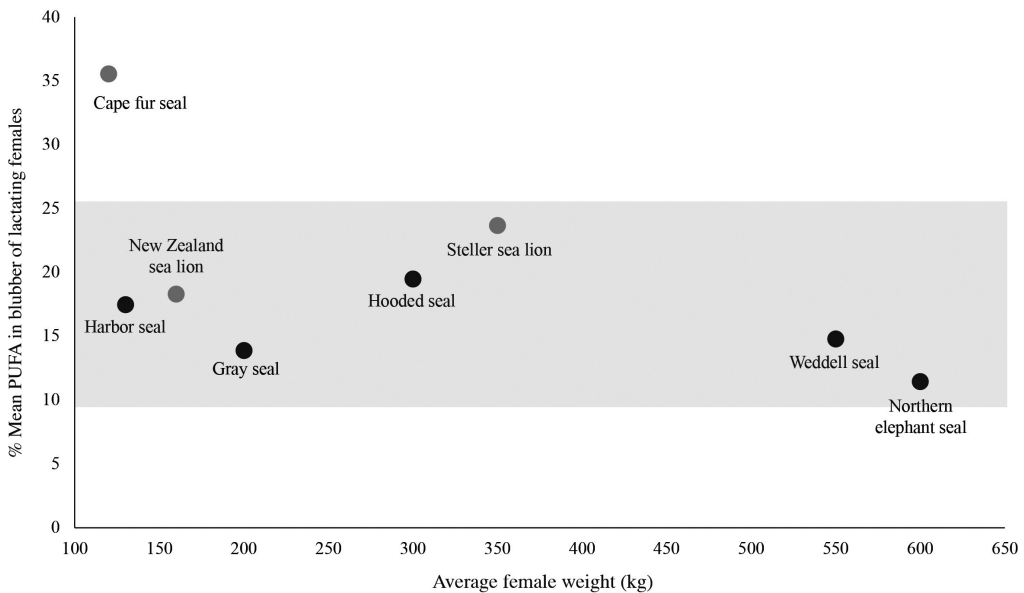


Figure 5. Mean % total polyunsaturated fatty acid (PUFA) concentration of lactating females by average female weight of various phocids (black circles) and otariids (grey circles). Shaded area represents range at which PUFA concentrations of lactating females appear to be similar despite family or lactation strategy. Average female weights (kg) recorded from Reeves et al. (2002).

10-month lactation period (Gales, 1995; Costa & Gales, 2000). When compared to the more similarly sized gray seal, which fasts during its 15-day lactation period (Reeves et al., 2002), and larger Weddell seal (550 kg; Reeves et al., 2002), which has a similar blubber PUFA concentration as the northern elephant seal (14.8% total blubber FA; Wheatley et al., 2007), despite intermittently feeding during their 50-day lactation period (Schulz & Bowen, 2004), harbor seal PUFA concentrations were within 4% total PUFA by weight (Figure 5). This could suggest that lactating phocids and otariids may selectively mobilize PUFA from the blubber in a similar way (Arriola et al., 2013) despite family or lactation strategy. The exception to this trend appears to be the Cape fur seal, with PUFA values reported to be 1.5 times greater than the Steller sea lion. While it is possible that over one-third of blubber FAs in lactating Cape fur seals are PUFAs, the authors believe these values may be inflated due to a small sample size ($n = 2$; Arnould et al., 2005). Increased sampling efforts of other lactating phocids and otariids would allow greater insight and further clarification on the use of PUFAs in lactating pinnipeds.

As one of the smallest members of the phocid family, the harbor seal has successfully adapted its lactation strategy to maximize energy expenditure at a rate similar to larger phocids. As the first study to document blubber FAs in pregnant harbor seals, we propose pregnant harbor seals may prioritize PUFA storage in the blubber. In addition, comparison of lactating harbor seals to their pregnant counterparts suggests lactating females may have a similar lactation strategy as other phocids, potentially mobilizing SFA $\leq 16C$, MUFA $\leq 16C$, and PUFA to their pups, and conserving SFA and MUFA $\geq 18C$ for their own thermoregulatory needs while utilizing intermittent feeding to maintain energy requirements during lactation. In addition, harbor seal pups spend a considerable amount of time swimming prior to weaning, and these findings suggest pups may be utilizing a large portion of the FAs available in milk, particularly PUFAs, for energetic needs rather than directly incorporating them into the blubber like other phocids. Lastly, while harbor seal blubber SFA and MUFA proportions appear similar to other lactating phocids, PUFA concentrations among species appear similar, suggesting lactating phocids and otariids may mobilize PUFA in a comparable way. While this study was only able to make theoretical assessments as to possible FA mobilization, additional studies designed to sample pregnant and lactating harbor seals throughout their respective states would provide a more definitive view of how harbor seals may or may not differ from other pinnipeds.

Understanding how body size influences the lactation strategy of a species provides greater insight into the physiological and behavioral limitations a species may have to both internal and external forces during such a critical time in its life history.

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