



## Original Research Article

## Survey of vitamin D and 25-hydroxyvitamin D in traditional native Alaskan meats, fish, and oils

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## ABSTRACT

Greater consumption of traditional foods has been associated with improved vitamin D status in Arctic and sub-Arctic populations, including Alaskan Native Americans. However, lack of vitamin D food composition data impairs epidemiological studies on health outcomes, and development of specific dietary recommendations. Vitamin D, including 25(OH)D<sub>3</sub> was quantified in samples of native fish, fish eggs, meats (caribou, goose, whale, seal) and traditionally prepared whale and seal oil collected from Alaskan tribes. Vitamin D<sub>3</sub>, 25(OH)D<sub>3</sub>, and vitamin D<sub>2</sub> were assayed in alkaline-saponified samples by UPLC-MS, after derivatization with 4-phenyl-1,2,4-triazole-3,5-dione, with in-house control materials and/or NIST SRM<sup>®</sup> 1546a Meat Homogenate included in each analytical batch. All but the land animals and bearded seal meat contained  $\geq 2 \mu\text{g}$  vitamin D<sub>3</sub>/100 g, with  $> 10 \mu\text{g}/100 \text{ g}$  in steelhead trout; dried sheefish, whitefish, smelt; smoked/dried salmon; fermented sheefish eggs; whale and seal oils. Large between-sample differences in bearded seal oil suggested possible effects of season and/or maturity on vitamin D content. 25(OH)D<sub>3</sub> was  $> 0.3 \mu\text{g}/100 \text{ g}$  in many foods, notably smoked salmon, beluga whale skin/fat and oil and spotted seal (but not other seal) oil, with the highest levels in dried beluga whale meat, skin/fat, and oil (up to 1.2). Vitamin D<sub>2</sub> was  $< 0.2 \mu\text{g}/100 \text{ g}$  in all foods.

## 1. Introduction

Vitamin D is an essential nutrient for bone health and has been increasingly recognized as a factor in immune system modulation, musculoskeletal health, cancer, and other aspects of health and disease (Pludowski et al., 2013). Vitamin D<sub>3</sub> is readily formed in the epidermis with sun (ultraviolet light) exposure, from its precursor, 7-dehydrocholesterol (Wacker and Holick, 2015). However, many individuals and populations have suboptimal vitamin D levels, as measured by serum 25-hydroxyvitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] concentration (Holick, 2005; Ross et al., 2011), due to insufficient sun exposure or dietary intake. Populations at higher latitudes are particularly susceptible to deficiency (Barake et al., 2010; Chen et al., 2007; Webb et al., 1988), and in these groups, dietary sources of vitamin D become more significant. Studies have found higher serum 25(OH)D<sub>3</sub> in individuals than what would be predicted by ultraviolet light (UV) exposure and dietary intake determined by food intake surveys and food composition data, and part of the explanation might be underestimation of dietary intake due to a lack of reliable and complete data for vitamin D in foods, particularly for 25(OH)D<sub>3</sub> (Roseland et al., 2016; Taylor et al., 2016). Since 25(OH)D<sub>3</sub> has a higher biological activity than D<sub>3</sub> and is not routinely

measured in foods or included in food composition databases, it may be an overlooked component of vitamin D intake (Cashman et al., 2012; Norman, 2008; Ovesen et al., 2003).

Suboptimal vitamin D status has been documented in a variety of Arctic and sub-Arctic populations, including Alaskan and Canadian Northwest Native Americans (Frost and Hill, 2008; Lebrun et al., 1993; Sharma et al., 2011; Singleton et al., 2015; Weiler et al., 2007). There has been a trend toward westernization of the diet of Native populations (Ballew et al., 2006; Johnson et al., 2009; Murphy et al., 1995; Nobmann et al., 2005), although greater consumption of traditional foods in North American Arctic and sub-Arctic populations has been associated with better vitamin D status (Andersen et al., 2013; Bersamin et al., 2007; Luick et al., 2014; Mansuri et al., 2016; Mohatt et al., 2007; Ryman et al., 2015; Sheehy et al., 2014). “Traditional” foods are defined as those “composed of items from the local, natural environment that are culturally acceptable” (Kuhnlein and Receveur, 1996). However, a lack of food composition data for traditional Native foods impairs both epidemiological studies on vitamin D status and health and the development of specific dietary recommendations for increasing vitamin D intake in these populations (Johnson et al., 2009).

Research on the role of vitamin D intake and health requires reliable

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food composition data, including sampling that is relevant to the population being studied (Ahuja et al., 2013). Food sampling for the USDA National Food and Nutrient Analysis Program (NFNAP) (Haytowitz et al., 2008; Haytowitz and Pehrsson, 2018) has provided analytical food composition data for the USDA National Nutrient Database for Standard Reference (SR) (USDA, 2016), including foods for the What We Eat In America (WWEA) National Health and Nutrition Examination Survey (NHANES) (U.S. Department of Agriculture, Food Surveys Research Group, 2017). Sampling is based on statistical models that account for market share and sources of variability in the food supply, to obtain representative population-wide average food composition data (Perry et al., 2002). However, these models cannot be translated to Native populations, since traditionally harvested and prepared foods must be obtained from remote locations, directly from the tribes who may not have excess to spare. Thus, efforts to develop robust vitamin D data for traditional foods need to be focused on those that are significant sources of the nutrient. There is a lack of information on vitamin D in these foods to serve as a basis to narrow the number of foods for further investigation.

In general, foods naturally rich in vitamin D include fish and fish oils, and some meats and organ tissues. However, the level can vary widely among specific foods in these groups, and the content can vary based on location of sampling (Roseland et al., 2018). While some fish are high in vitamin D (e.g., salmon, trout), many others contain negligible vitamin D (Mattila et al., 1995; Roseland et al., 2018; Schmid and Walther, 2013). Thus, it is reasonable to expect that some Native Alaskan fish and meats might be high in vitamin D, including 25(OH)D<sub>3</sub>. Kuhlein et al. (2006) reported on the vitamin D<sub>3</sub> content of several traditional native North American meats and fish sampled from Canadian tribes, but did not include 25(OH)D<sub>3</sub>. Foods which are potentially good sources of 25(OH)D<sub>3</sub> include some fish and organ meats (Roseland et al., 2018), pork fat (Clausen et al., 2003), and eggs from vitamin D supplemented hens (Exler et al., 2010). Furthermore, the vitamin D content of a particular species of fish or mammal can be influenced by diet or environment (Liu et al., 2013; Mattila et al., 1997; Rao and Raghuramulu, 1996; Roseland et al., 2018). Processing prior to consumption also can influence vitamin D; variation has been observed based on removal of the skin from meats and fish is removed (Pierens and Fraser, 2015), sun drying (Barnkob et al., 2016), and differences in cooking methods (Jakobsen and Knuthsen, 2014; Mattila et al., 1999; Roseland et al., 2018). Specific environmental conditions and methods of preserving (e.g. smoking, drying), storing, and cooking foods likely vary among different indigenous populations, which have localized food supplies and often unique techniques for handling and preparation and that deserve consideration when evaluating dietary intake in specific populations.

An effort to obtain food composition data specifically for Alaskan and American Indian Native (AIAN) traditional foods was initiated by the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) Nutrient Data Laboratory (NDL) (Beltsville, MD), with funding from the National Institutes of Health Office of Research on Minority Health (ORMH) and the Indian Health Service (IHS) (Rockville, MD). The main focus was to obtain food composition data for key nutrients, for inclusion in the American Indian/Alaskan Native database in SR, to support efforts on health studies of Native Americans (Amy and Pehrsson, 2003; Johnson et al., 2009; Pehrsson et al., 2005a, b; Perry et al., 2002). Meanwhile, efforts were being made in general to add data on vitamin D to SR (Holden et al., 2008), including studies on analytical methods and quality control (Byrdwell et al., 2008; Byrdwell, 2009; Phillips et al., 2008), vitamin D content of fish and shellfish (Byrdwell, 2009; Byrdwell et al., 2013), milk (Patterson et al., 2010), eggs (Exler et al., 2013), and mushrooms (Phillips et al., 2011). A key part of this work was validating methods for vitamin D and 25(OH)D. Until recently, methods for vitamin D were generally not standardized and often gave disparate results between laboratories using similar methods (Byrdwell et al., 2008; Phillips et al.,

2008). More recently, methodology for measurement of vitamin D and 25(OH)D by LC–MS has been validated (Byrdwell, 2009; Huang et al., 2009). The USDA Nutrient Data Laboratory (Beltsville, MD) has demonstrated that it is possible to obtain consistent results for vitamin D<sub>3</sub> and 25(OH)D<sub>3</sub> among laboratories (Roseland et al., 2016). Matrix-specific control materials and certified reference materials are, however, critical to include to account for the many factors that impact accuracy and precision of vitamin D quantification in different types of foods (Roseland et al., 2018).

The objective of this work was to measure vitamin D<sub>3</sub> and 25(OH)D<sub>3</sub> in samples of traditional meats and fish obtained from Native Alaskan tribes, to screen for foods that could be a rich dietary source of vitamin D, as a basis for focusing more detailed food composition studies as well as to add to the dearth of published data for traditional foods, using validated analytical methodology and including traceable quality control materials.

## 2. Materials and methods

### 2.1. Sample collection

The foods sampled included raw, preserved (dried, frozen, fermented, canned, smoked) and prepared (boiled or baked) fish, land and marine mammals, and marine mammal oils. Samples were collected from Alaskan Native tribes, according to the sampling framework described previously (Perry et al., 2002), from the locations shown in Fig. 1. The foods sampled were selected based on published research, informal surveys and interviews with tribal council, elders, and members (e.g., at clinical setting), collaboration (e.g., food frequency questionnaires from other government or academic studies and focus groups. Handling of Alaskan native endangered species samples was authorized under permit 782–1694 from the National Marine Mammal Laboratory of the Alaska Fisheries Science Center of the National Oceanic and Atmospheric Agency (Seattle, WA). All food collection was approved by the councils of each tribe, and where presentations and supportive documents were required they were submitted to the tribe.

Samples were obtained within and among villages and tribes, from festivals or markets, homes, senior centers (food parties) and schools, as summarized in Table 1. Multiple samples of each food were obtained when possible, although the number of samples was necessarily limited by the availability of extra food for this study. Prepared foods (smoked, boiled, dried, etc.) had been processed by traditional methods, but details of these procedures were not always available. The food samples were, as much as possible, weighed on site (as the amount collected was often considered a common portion), frozen, and then packed on dry ice or blue ice packs and shipped via overnight express to the Food Analysis Laboratory Control Center (FALCC) in the Biochemistry Department at Virginia Tech (Blacksburg, VA). Upon receipt at the FALCC, samples were inspected to confirm integrity and then stored frozen at  $< -15 \pm 5^\circ\text{C}$  until prepared for analysis.

### 2.2. Sample preparation

Samples were thawed in the refrigerator ( $4 \pm 2^\circ\text{C}$ ) prior to homogenization (meats and fish overnight, fish eggs 4–6 h, and oils until completely liquefied). Canned fish samples were homogenized using a Robot Coupe® industrial stainless steel food processor (RSI 6 V Blixer, Robot Coupe USA, Jackson, MS). Other fish, meat, and fish egg samples were homogenized in the same manner, except with the addition of liquid nitrogen to aid the homogenization process. Oils were thawed at  $4 \pm 2^\circ\text{C}$  until liquefied, then stirred in a stainless steel bowl with a stainless steel spoon to homogenize. After homogenization, subsamples were dispensed in 15–30 g aliquots among 30-mL or 60-mL glass jars with Teflon™-lined lids (Qorpak®, Bridgeville, PA; #GLC-07098 and GLC-08640, respectively). The jars were sealed under nitrogen, surrounded with aluminum foil, and stored at  $< -55^\circ\text{C}$  until



Samples of a Pork/Egg control material (“Pork/Egg CC, a mixture of bratwurst sausage and cooked egg yolks), and/or NIST SRM® 1546a Meat Homogenate [National Institute of Standards and Technology (NIST), Gaithersburg, MD] that were part of the inter-laboratory study ([Roseland et al., 2016](#)), and/or an in-house Salmon control material (“Salmon D CC”, canned salmon) previously reported ([Phillips et al., 2008](#)) were analyzed with each batch of samples. Some of the Native

**Table 1**  
Description of samples. (See Supplementary Material for photographs).

Sample Code <sup>a</sup>	Description	Additional information	Sampling Location <sup>b</sup>	Date <sup>b,c</sup>	Sample Amount <sup>b,d</sup>
<b>Fish:</b>					
F1	Halibut, wild - raw		Palmer, AK	1/31/2003	5 fish
F2	Halibut, with skin - raw		Aleutian Islands, AK	n.i.	1 fish
F3	Halibut, with skin - raw		Aleutian Islands, AK	n.i.	1 fish
F4	Halibut, with skin - raw		Aleutian Islands, AK	n.i.	1 fish
F5	Halibut, with skin - cooked		Aleutian Islands, AK	n.i.	1 fish
F6	Salmon, Chum - dried	Skin was removed prior to analysis.	Buckland River, AK	March 2004	n.i.
F7	Salmon, Chum - dried	Multiple fillets from adult male and female fish. At time of harvest, fish were processed for drying, then dried about one week. Samples of this type are typically eaten dry with seal oil or black bear oil.	Shugnak Village, Kobuk River, AK	September 2003	n.i.
F8	Salmon, Red - smoked, canned	Fillets, with skin, were brined, then smoked 6 days. Extra salt was added and the fish was canned. Skin was removed prior to analysis.	Chignik Lagoon, AK	June 2003	2 cans
F9	Salmon, Red - smoked, canned	Fillets, with skin, were brined, smoked 12 days, then canned.	Chignik Lagoon, AK	Harvested in 2003 (no month given), obtained 8/28/2003	1 can
F10	Sheefish - baked	Midsection of fish	n.i.	Received 3/3/2005	1
F11	Sheefish - raw	Young female fish. Cleaned at lake, frozen the day after harvesting. Typically skin is removed prior to eating.	Kobuk Lake (40 miles from Buckland, AK)	May 2003	n.i.
F12	Smelt		Buckland River, AK	March 2004	n.i.
F13	Trout, Steelhead, inner flesh - dried		n.i.	November 2003	n.i.
F14	Trout, Steelhead - boiled, canned		n.i.	11/2003	n.i.
F15	Whitefish, Broad, filet -boiled	Fillet (~ 0.9 kg) from side of female fish. Cooking: Frozen filet was thawed at room temperature in a sink full of water. Blood was washed out and filet was cut into 2 pieces, then boiled, with skin and bones included, for 50 min in water with no added ingredients. Meat was removed from bones and skin, then flaked. Flakes would typically be used to make agutuk (ice cream) by mixing with berries, sugar, and fat.	Kobuk River, AK (Shugnak Village)	Harvested 9/21/2003, frozen at -20 °C, cooked in September 2004	1
F16	Whitefish - baked		n.i.	n.i.	n.i.
F17	Whitefish - raw, dried	Fillet from side of adult female fish. Dried for one week after caught, then frozen. Typically eaten dried, often dipped in seal oil.	Kobuk River, Chungnak Village, AK	9/21/2003	1
<b>Fish Eggs:</b>					
FE1	Sheefish eggs - fermented	Fermented 7 days prior to freezing. Typically eaten frozen or cooked.	Shugnak Village (Kobuk River), AK	10/8/2003	1 unit (~ 150 g)

(continued on next page)

Table 1 (continued)

Sample Code <sup>a</sup>	Description	Additional information	Sampling Location <sup>b</sup>	Date <sup>b,c</sup>	Sample Amount <sup>b,d</sup>
FE2	Whitefish, Broad, eggs - raw	Typically consumed raw or frozen.	Kobuk River (down river from Shungnak River), AK	10/15/2003	n.i.
<b>Land Animals:</b>					
LA1	Caribou, rump, meat & fat - boiled	Stored at -20 °C between harvest and cooking. Cooking: Thawed at room temperature for a few hours, then boiled 2 h in water with no added ingredients. Cooled and frozen. This type of meat is normally cooked as a soup or stew with other ingredients.	Shungnak Village, AK	Harvested 10/14/2003; frozen; cooked in September 2004	n.i.
LA2	Caribou, rump & hind - half dried	Adult caribou. Rump and hind were half dried, then frozen. This type of dried meat is cooked or eaten as is.	Chungnak village, Upper Kobuk River, AK	10/6/2003	n.i.
LA3	Caribou, hind quarter flesh - boiled	From adult female. Cleaned outside, in the tundra, then frozen in home chest freezer as soon as hunter reached home. Cooking: Thawed at room temperature a few hours, then boiled 2 h in water with no other ingredients. This type of meat would normally be cooked in salted water along with macaroni, rice, potatoes, tomatoes, etc. to make soup or stew, or fried in shortening and eaten with gravy and rice.	Point Hope, AK (close to Cape Thompson)	Collected 8/1/2003; frozen, cooked in September 2004	1 piece (~0.5 kg)
LA4	Caribou, ground - raw		Kotzebue, AK	8/11/2003	n.i.
LA5	Caribou, hind quarter - raw	Male, approximately 2–3 years old. Meat was cut up and frozen on day of harvest and stored in home chest freezer. Prior to eating, meat of this type would be thawed then roasted or boiled.	Kumuk, AK (35 miles SE of Point Hope)	7/30/2003	n.i.
LA6	Caribou, meat/muscle - dried	Female of unknown age. Upon harvesting, animal was gutted and drained in the field, then meat was cut and hung on racks in house to dry. Typically, no further preparation would be done prior to consumption.	Buckland, AK	Harvested 8/15/2003	n.i.
LA7	Caribou, middle back - raw	Female animal. Cut up in river area where hunted down; placed in Ziploc bags, stored in home chest freezer (approximately 1 year before collected for analysis). This meat is typically eaten frozen and/or with seal oil.	Noatak, AK	Harvested August 2002 and frozen; collected 7/29/2003	n.i.
LA8	Caribou, shoulder blade/arm - dried	Preparation: Salt was put on meat before cutting it up and hanging to dry for 2 weeks. This type of meat is typically cut up and put into seal oil and can be used to make salad (with vegetables, carrots, celery, seal oil).	4 miles south of Shungnak Village, AK	Harvested summer 2003	n.i.
LA9	Goose, Canadian, flesh, boned - boiled	Adult male goose. Frozen with feathers on for ~1 year prior to cooking (see dates at right). Cooking: Feathers and guts, wings, and legs were removed. Boiled for 1 h in water with no added ingredients, then cooled before removing meat from bones. Typically, this type of bird is cooked with salt and rice, macaroni, onions, and potatoes to make soup.	Stebbins, AK	Harvested 9/27/2003; frozen at ~ -20 °C, cooked 9/22/2004	1 goose (~300 g meat)
LA10	Goose, Snow, flesh, boned - boiled	Breast, thigh, and leg meat. Bird was frozen intact. Cooking: Feathers, skin, and guts were discarded. Meat/bones were cut into pieces and boiled for 1 h in water with no added ingredients. Typically, this type of bird is boiled with rice and onions (and sometimes seal oil). Seal oil may be used as a dipping sauce.	Stebbins, AK	Harvested 5/15/2003; frozen at ~ -20 °C, cooked in September 2004	1 goose (0.5 kg meat)
<b>Marine Mammals:</b>					
MM1	Seal, Bearded, meat - dried	Back and leg meat were removed, cut into long strips, dried for 5 days, then frozen. This type of meat would typically be eaten plain or dipped into seal oil.	Buckland, AK (Buckland River)	Harvested June 2003	n.i.

(continued on next page)

Table 1 (continued)

Sample Code <sup>a</sup>	Description	Additional information	Sampling Location <sup>b</sup>	Date <sup>b,c</sup>	Sample Amount <sup>b,d</sup>
MM2	Seal, Bearded, meat - dried, in oil	"Black meat" bearded seal; unknown age/sex. Meat was dried outside [ $\sim 54^{\circ}\text{F}$ ( $\sim 12^{\circ}\text{C}$ )] for a couple of weeks. Oil was rendered from the blubber without added heat. Meat was cut into small pieces. Typically salt would be added prior to eating.	Kotzebue, AK	Harvested June 2003; collected 8/11/2003	n.i.
MM3	Whale, Beluga, meat - dried		n.i.	9/30/2003	n.i.
MM4	Whale, White Beluga (Muktuk), skin & fat - boiled	After harvest, rinsed to remove blood, then cut into pieces and frozen within an hour. Cooking: Thawed overnight in refrigerator, cut into pieces $\sim 3 \times 3$ (7.6 x 7.6 cm). Some fat was trimmed. Boiled for 1 h until fork-tender, then frozen. Typically would be eaten with salt.	Emmonak, AK	Harvested 8/2004; frozen; cooked in September 2004	1
MM5	Whale, White Beluga (Muktuk), skin & fat - boiled	Cooking: Thawed overnight in refrigerator, then cut into pieces $\sim 3 \times 3$ ( $\sim 7.6 \times 7.6$ cm). Some fat was trimmed off. Boiled for 1 h and 15 min until fork-tender, then frozen. Typically eaten with salt.	Stebbins, AK	Harvested 9/2003; frozen; cooked in September 2004	1
<b>Marine Mammal Oils:</b>					
MO1	Oil, Bearded Seal	Male adult seal. Seal was cut up, and blubber was cut into strips and put in plastic pails in a cool area to render for a week. After rendering, the oil was poured into jars and frozen. This oil is typically used for dipping foods or mixing into soups.	Stebbins, AK (near Stuart Island coast)	Harvested 5/18/2003	1
MO2	Oil, Bearded Seal, young (Muktuk)		Near Emmonak, AK	August 2004	1 unit ( $\sim 210$ g)
MO3	Oil, Bearded Seal, young (Muktuk)		Near Emmonak, AK	August 2004	1 unit ( $\sim 230$ g)
MO4	Oil, Ringed Seal		n.i.	n.i.	1
MO5	Oil, Spotted Seal	Male seal about 4 years old. Cleaned on the beach, cut and processed locally according to tradition. Seal fat was rendered for 4 weeks until it became liquid oil. Stored in a large glass jar and frozen. This oil is typically stored frozen.	Noatok, AK	Harvested 6/16/2003; collected 7/30/2003	1
MO6	Oil, Beluga Whale	Unknown age and sex. Blubber was removed from whale, cut into pieces, and rendered about 7–10 days, then frozen.	Kotz, AK	Harvested June 2003; collected 8/11/2003	1

<sup>a</sup> Correspond to Sample Codes in Table 3.<sup>b</sup> n.i. = information not available.<sup>c</sup> Month/day/year.<sup>d</sup> Total units (e.g. individual fish) in if a sample contained more than one individual.

Alaskan samples were analyzed in replicate, in independent analytical runs as a measure of day-to-day precision. Precision was estimated by percent relative standard deviations (RSD) for the samples assayed in replicate and for the control materials assayed over multiple runs. Acceptability of the RSD in each case was evaluated using the Horwitz ratio (HorRat), the assayed RSD/expected RSD, where the expected RSD was calculated as the assayed mean( $\mu\text{g}/100\text{ g}$ )/(100/1,000,000)<sup>a</sup>  $\sim 0.1505$  (Horwitz and Albert, 2006).

Accuracy was validated by results for three commercially available relevant food matrix reference materials assayed in a previous inter-laboratory study on quantitation of vitamin D and 25(OH)D (Roseland et al., 2016): NIST SRM<sup>®</sup>1546a Meat Homogenate, NIST SRM<sup>®</sup>1577c Freeze-Dried Bovine Liver, and NIST SRM<sup>®</sup>1845a Whole Egg Powder, analyzed in select batches during the period of this study. The accuracy of 25(OH)D<sub>3</sub> measurements was established in a previous report on eggs, which included recovery tests and the Pork/Egg CC (Exler et al., 2013), and also relative to consensus values for NIST SRM<sup>®</sup>1546a Meat Homogenate, SRM<sup>®</sup>1845a Whole Egg Powder, and SRM<sup>®</sup>1577c Freeze-Dried Bovine Liver, obtained in the previous interlaboratory study (Roseland et al., 2016).

## 2.6. Data analysis

Means, standard deviations (SD), and RSDs were calculated using Microsoft<sup>®</sup> Excel<sup>®</sup> 2016 (16.0.4266.1001) (Microsoft Corporation; Redmond, WA, USA). Analysis of Variance, Tukey's pairwise comparison of means, and Pearson correlation coefficients ( $\rho$ ) (Ott and Longnecker, 2016) were performed using XLSTAT (version 19.4.45826; Addinsoft, New York), with  $\alpha = 0.05$ . However, statistical means comparisons were not possible in most cases, due to the necessarily limited number of samples (Section 2.1).

## 3. Results and discussion

### 3.1. Quality control data

The assayed vitamin D<sub>3</sub> concentration in all certified reference materials was within the certified mean  $\pm$  uncertainty (Table 2). There are no food matrix reference materials with certified value for 25(OH)D<sub>3</sub> (although, more recently NIST has reported a reference value for 25(OH)D<sub>3</sub> in SRM<sup>®</sup>1549a Whole Milk Powder). Therefore, we relied on data for the Pork/Egg CC, SRM<sup>®</sup>1546a Meat Homogenate, SRM<sup>®</sup>1845a Whole Egg Powder, and SRM<sup>®</sup>1577c Freeze-Dried Bovine Liver previously characterized (Roseland et al., 2016), also validated by previously reported recovery studies for the Pork/Egg CC (Exler et al., 2013), to validate the accuracy of results during the period of this study. The assayed 25(OH)D<sub>3</sub> was within the previously established tolerance limits for all of these materials (Table 2).

**Table 2**  
Results for certified reference materials and control materials assayed with samples.

Material	Assayed				Expected		
	<i>n</i>	Mean	%RSD <sup>a</sup>	HorRat <sup>b</sup>	Low	High	Range (mean $\pm$ uncertainty) <sup>c</sup>
<b>Vitamin D<sub>3</sub></b>							
NIST SRM <sup>®</sup> 1546a Meat Homogenate	5	0.23	13.0	0.7	0.19	0.27	0.199–0.267
NIST SRM <sup>®</sup> 1577c Freeze-Dried Beef Liver	3	< 0.05			< 0.05	< 0.05	0.018–0.068
NIST SRM <sup>®</sup> 1845a Whole Egg Powder	3	4.42	1.8	0.1	4.33	4.48	4.04–4.94
<b>25(OH)D<sub>3</sub></b>							
NIST SRM <sup>®</sup> 1546a Meat Homogenate	6	0.08	19.7	0.8	0.06	0.10	0.07–0.11
NIST SRM <sup>®</sup> 1577c Freeze-Dried Beef Liver	3	1.42	5.3	0.4	1.34	1.49	1.30–1.72
NIST SRM <sup>®</sup> 1845a Whole Egg Powder	3	1.29	4.1	0.3	1.23	1.33	1.05–1.45

<sup>a</sup> Relative standard deviation.

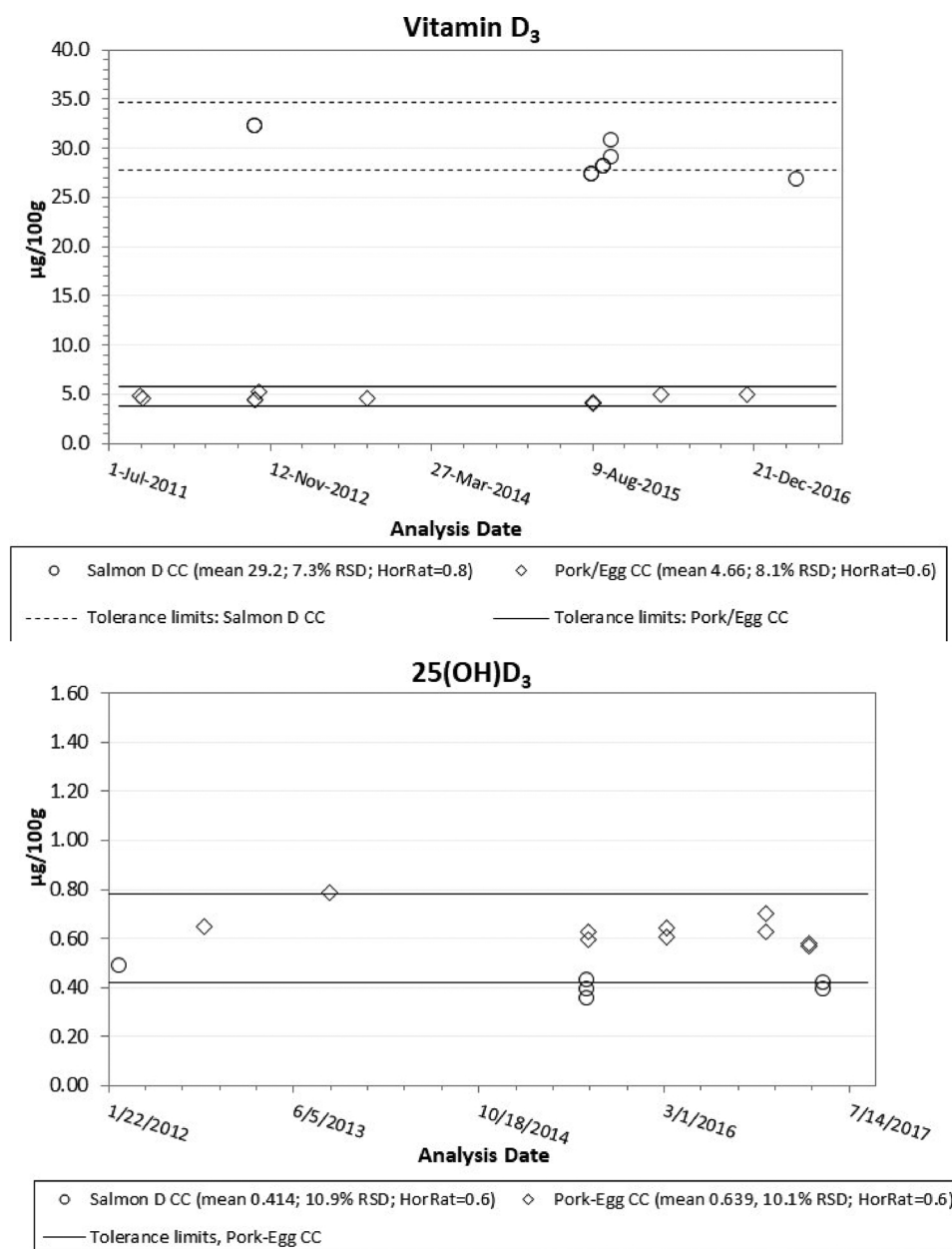
<sup>b</sup> Assayed RSD/Expected RSD, with Expected RSD = [(Mean/100/1,000,000)<sup>a</sup>  $\sim 0.1505$ ] (Horwitz and Albert, 2006).

<sup>c</sup> Roseland et al. (2016).

Because the Native food samples were analyzed over multiple assay batches, it was important to define and demonstrate the inter-assay precision, to establish uncertainty in mean values and enable comparison of vitamin D content among independent samples of a particular food or among different foods. Fig. 2 illustrates the precision of vitamin D<sub>3</sub> and 25(OH)D<sub>3</sub> analysis for the Salmon and Pork/Egg control materials; both had excellent precision over multiple analytical runs, with HorRat < |1.0|. The HorRat is a measure of the analytical precision demonstrated, relative to what is expected based on the concentration of the nutrient in the matrix, and should be between |1.0| and |2.0| (Horwitz and Albert, 2006). The HorRats for replicate analyses of samples are given in italics after the RSDs, in Table 3, and ranged from 0.05 to 1.5 for vitamin D<sub>3</sub> (mean, 0.6; median, 0.7), and from 0.06 to 1.4 for 25(OH)D<sub>3</sub> (mean, 0.6; median, 0.5). These data demonstrate excellent precision of analyses across the range of sample matrices and analyte concentrations, and that data for different foods assayed in different batches can be compared with confidence that differences are not due to run-to-run analytical variability.

### 3.2. Vitamin D content of foods

Vitamin D<sub>2</sub> was < 0.1  $\mu\text{g}/100\text{ g}$  in all cases. Although some vitamin D<sub>2</sub> could be present in marine mammals and fish, due to diet (Roseland et al., 2018), it was not detected in samples analyzed in this study. Vitamin D<sub>3</sub> was  $\geq 2\text{ }\mu\text{g}/100\text{ g}$  [ $\geq 13\%$  of the DRI of 15  $\mu\text{g}$  (600 IU) set by IOM (2010)] in all of the foods except the land animals, bearded seal meat, and mature (but not young) bearded seal oil. More than 10  $\mu\text{g}$  (400 IU) vitamin D<sub>3</sub> per 100 g was present in steelhead trout; dried sheefish, whitefish, and smelt; fermented sheefish eggs; salmon (including smoked salmon); and beluga whale and seal oils [except, interestingly, only in young bearded seal (20  $\mu\text{g}/100\text{ g}$ ) but not mature bearded seal oil (0.29  $\mu\text{g}/100\text{ g}$ )]. Many of the foods were significant sources of 25(OH)D<sub>3</sub>. The biological activity of 25(OH)D<sub>3</sub> has been hypothesized to be potentially 1.7–5 times that of D<sub>3</sub> (Bischoff-Ferrari et al., 2012; Jakobsen, 2007; Ovesen et al., 2003; Taylor et al., 2016), at least in part due to greater absorption relative to vitamin D<sub>3</sub> (Borel et al., 2015). Using a potency of 5x (to account for foods which cumulatively might contribute significant vitamin D activity), a 25(OH)D<sub>3</sub> level  $\geq 0.3\text{ }\mu\text{g}$  would represent at least 10% of the DRI for vitamin D. Foods containing  $\geq 0.3\text{ }\mu\text{g}$  25(OH)D<sub>3</sub> per 100 g were dried and smoked salmon; beluga whale dried meat, boiled skin and fat, and oil; and spotted seal oil. The highest levels of 25(OH)D<sub>3</sub> (0.7–1.2  $\mu\text{g}/100\text{ g}$ ) were in beluga whale skin and fat, dried meat, and oil, and in smoked red salmon. All of the land animals had vitamin D<sub>3</sub> contents < 0.1 and 25(OH)D<sub>3</sub> contents < 0.2  $\mu\text{g}/100\text{ g}$ . There was no correlation between vitamin D<sub>3</sub> and 25(OH)D<sub>3</sub> concentrations within any of the groups of all samples, fish and marine mammal products only, or marine mammal products only ( $\rho = 0.177$ ,  $\rho = 0.127$ , and  $\rho = -0.278$ , respectively),



**Fig. 2.** Quality control data for vitamin D<sub>3</sub> and 25-hydroxyvitamin D [25(OH)D<sub>3</sub>] in two matrices, a composite of bratwurst sausage and egg yolks (Pork/Egg CC) and canned sockeye salmon (Salmon D CC). SD = standard deviation; RSD = relative standard deviation. HorRat = ratio of assayed to expected RSD, with expected RSD = assayed mean/ $((100/1,000,000)^{-0.1505})$ , according to Horwitz and Albert (2006).

but the relationship was generally positive among samples of fish ( $\rho = 0.759$ ).

There was a wide range in vitamin D content among different samples for some foods, for which multiple samples were possible to collect [halibut ( $n = 4$ ), dried and smoked salmon ( $n = 4$ ), beluga whale skin and fat ( $n = 2$ ), and young bearded seal oil ( $n = 2$ )]. There was a striking difference in vitamin D<sub>3</sub> content among samples of beluga whale skin and fat (2.97 and 10.0  $\mu\text{g}/100\text{g}$ ), although 25(OH)D<sub>3</sub> content (1.2  $\mu\text{g}/100\text{g}$ ) did not vary. Both D<sub>3</sub> and 25(OH)D<sub>3</sub> varied among samples of dried and smoked salmon (32.6–53.6 and 0.081–0.836  $\mu\text{g}/100\text{g}$ , respectively) and were not related to moisture content (39.8–67.8  $\mu\text{g}$  D<sub>3</sub> and 0.112–1.06  $\mu\text{g}$  25(OH)D<sub>3</sub> per 100 g dry weight, respectively).

There are some publications that discuss possible reasons for variability in vitamin D between species and within different samples of food species (Kenny et al., 2004; Mattila et al., 1997; Pierens and

Fraser, 2015; Rao and Raghuramulu, 1996; Roseland et al., 2018), including variation in diet. Reports on vitamin D metabolism and content of marine mammals are more limited (Keiver et al., 1988; Kenny et al., 2004; Routti et al., 2010; Wilske and Arnbom, 1996). Seals belong to the suborder Pinnipedia, comprising flipper-footed marine animals [seals (Phocidae family), sea lions (Otariid family), and walruses (Odobenidae family)]; whales belong to the order Cetacea, that also includes dolphins and porpoises (Marine Mammal Center, 2016). Pinnipeds spend some time on land, and thus (in contrast to whales and fish), have the potential for cutaneous synthesis of vitamin D in addition to dietary sources. Wilske and Arnbom (1996) studied elephant seals and found two annual peaks in serum 25(OH)D<sub>3</sub>, occurring after periods of intensive exposure to UV radiation. Because the hunting season in far northern latitudes would be in the late spring-early fall, vitamin D stores in seals could be expected to build up over the summer and be higher in animals caught in late summer or fall. Thus, the low vitamin D

**Table 3**Vitamin D<sub>3</sub> and 25-hydroxyvitamin D<sub>3</sub> [25(OH) D<sub>3</sub>] in traditional native meats, fish, and oils (SD, standard deviation; RSD, relative standard deviation).

Food	Sample Code <sup>a</sup>	Moisture (g/ 100 g)	Total Fat (g/ 100 g)	Vitamin D <sub>3</sub> (µg/100 g)					25(OH)-Vitamin D <sub>3</sub> (µg/100 g)				
				n <sup>b</sup>	mean	SD	%RSD	(HorRat <sup>c</sup> )	n <sup>b</sup>	mean	SD	%RSD	(HorRat <sup>c</sup> )
<b>Fish</b>													
Halibut, wild - raw (Palmer, AK)	F1	77.3	0.74	2	3.66	0.48	13.1	(1.0)	1	0.128			
Halibut, with skin -raw (Aleutian Islands)	F2	76.3	1.46	1	6.54				1	< 0.100			
	F3	71.0	3.58	2	5.82				1	0.039			
	F4	69.2	5.92	1	4.85				1	< 0.100			
Halibut, with skin - cooked (Aleutian Islands)	F5	73.3	1.14	1	5.47				na				
Salmon, Chum - dried	F6	18.1	18.2	1	32.6				1	0.292			
	F7	27.4	10.6	2	43.8	3.5	8.0	(0.9)	1	0.081			
Salmon, Red - smoked	F8	26.9	11.0	4	40.3	2.2	5.6	(0.6)	1	0.778			
	F9	21.0	11.8	2	53.6	2.6	4.9	(0.6)	2	0.836	0.190	22.8	(1.4)
Sheefish, midsection - baked	F10	66.2	7.68	2	12.7	0.4	3.3	(0.3)	2	0.068	0.015	22.3	(0.9)
Sheefish, raw	F11	74.6	2.84	2	13.8	0.1	0.5	(0.05)	2	0.032			
Smelt - dried	F12	16.9	17.9	3	25.4	2.8	11.0	(1.1)	2	< 0.100			
Trout, Steelhead - boiled, canned	F14	70.6	8.26	1	11.1				1	0.155			
Trout, Steelhead - dried	F13	6.49	8.06	2	19.2	0.3	1.5	(0.1)	1	0.088			
Whitefish, filet - boiled	F15	75.7	2.38	1	2.11				1	< 0.100			
Whitefish - baked	F16	68.4	10.8	2	8.80	0.75	8.5	(0.7)	1	0.053			
Whitefish, raw - dried	F17	20.6	13.4	2	12.3	1.0	8.0	(0.7)	1	0.110			
<b>Fish Eggs</b>													
Whitefish, Broad - raw	FE1	68.9	0.76	2	3.62	0.45	12.5	(0.9)	1	< 0.100			
Sheefish - fermented	FE2	67.7	5.86	2	17.7	1.7	9.6	(0.9)	2	< 0.100			
<b>Land Animals</b>													
Caribou, raw: ground	LA4	73.5	1.44	2	< 0.1				2	< 0.100			
hind quarter	LA5	72.6	3.37	2	< 0.1				2	< 0.100			
middle back	LA7	74.4	5.19	1	< 0.1				2	< 0.100			
Caribou- boiled: flesh	LA3	58.4	6.26	2	< 0.1				2	< 0.100			
rump, meat & fat	LA1	26.2	58.5	2	< 0.05				2	< 0.100			
Caribou - dried: meat/muscle	LA6	22.3	8.52	2	< 0.1				2	0.024	0.009	38.1	(1.4)
shoulder blade/arm	LA8	33.8	3.67	3	< 0.1				2	< 0.100			
rump & hind - half dried	LA2	38.04	5.46	2	< 0.1				2	< 0.100			
Goose, Canadian, boned flesh - boiled	LA9	59.9	6.29	2	< 0.1				2	0.028	0.001	4.8	(0.2)
Goose, Snow, boned flesh - boiled	LA10	57.4	8.63	2	< 0.1				2	0.037	0.005	14.0	(0.5)
<b>Marine Mammals</b>													
Beluga Whale, meat - dried	MM3	21.5	8.37	2	2.01	0.10	4.9	(0.3)	1	0.986			
Seal, Bearded, meat - dried	MM1	34.9	2.31	2	< 0.1				2	0.102	0.002	2.1	(0.1)
Seal, Bearded, meat - dried, in oil	MM2	35.5	25.1	2	< 0.1				2	< 0.055			
Whale, Beluga, skin & fat - boiled	MM4	54.4	27.5	3	10.0	0.7	7.4	(0.7)	2	1.24	0.14	11.5	(0.7)
	MM5	52.21	31.40	3	2.87	0.58	20.1	(1.5)	2	1.15	0.02	2.1	(0.1)
<b>Marine Mammal Oils &amp; Fats</b>													
Whale oil, Beluga, oil	MO6	0.15	97.5	4	7.11	0.34	4.8	(0.4)	4	0.739	0.067	9.1	(0.5)
Seal oil, Bearded	MO1	< 0.1	100	3	0.37				3	< 0.100			
Seal oil, Bearded, young	MO2	< 0.1	99.4	1	19.5				1	< 0.100			
Seal oil, Bearded, young	MO3	< 0.1	99.0	1	20.2				1	< 0.100			
Seal oil, Ringed	MO4	< 0.1	98.0	2	29.0	0.3	1.0	(0.1)	2	< 0.100			
Seal oil, Spotted	MO5	0.25	99.3	1	16.1				1	0.628			

<sup>a</sup> See Table 1 for descriptive information for the corresponding sample codes, where available.<sup>b</sup> Number of analytical replicates.<sup>c</sup> Ratio of assayed RSD to expected RSD, with expected RSD = assayed mean/((100/1000000)<sup>-0.1505</sup>), according to Horwitz and Albert (2006).

content of oil from the mature bearded seal but high content in the oil from young bearded seal (Table 3) might be due to the time of harvest (May for the mature seal, August for the young seals; see Table 1), rather than age of the animal. Kenny et al. (2004) found significant differences in vitamin D<sub>3</sub> content of blubber among cetaceans and pinnipeds that was correlated with diet, with those primarily consuming invertebrates (e.g., bowhead whale, Pacific walrus) having lower vitamin D<sub>3</sub> levels than those consuming primarily fish (e.g., beluga whale, ringed seal). However, in the present study, the vitamin D content of beluga whale oil, while high (mean, 7.11 µg/100 g), was substantially lower than that of ringed seal oil (29.0 µg/100 g). One limitation of the present study (necessary due to the factors stated in Section 2.1) and others is that there are few samples per type of animal and lack of details on age, sex, time of harvest, and diet, so there are not

enough data to draw conclusions about the relationship between vitamin D content and time of harvest, maturity of animal or other variables. These areas are worthy of further study.

### 3.3. Nutritional significance

In some Native Arctic populations, large quantities of wild caught foods, including salmon and other fish, contribute significantly to the diet (Ballew et al., 2006). Numerous researchers have reported improved vitamin D status and health outcomes in Native Alaskan and other Arctic populations consuming a diet rich in traditional foods (Andersen et al., 2013; Bersamin et al., 2007; Johnson et al., 2009; Kuhnlein et al., 2006; Luick et al., 2014; Mansuri et al., 2016; Mohatt et al., 2007; Murphy et al., 1995; Ryman et al., 2015; Sharma et al.,

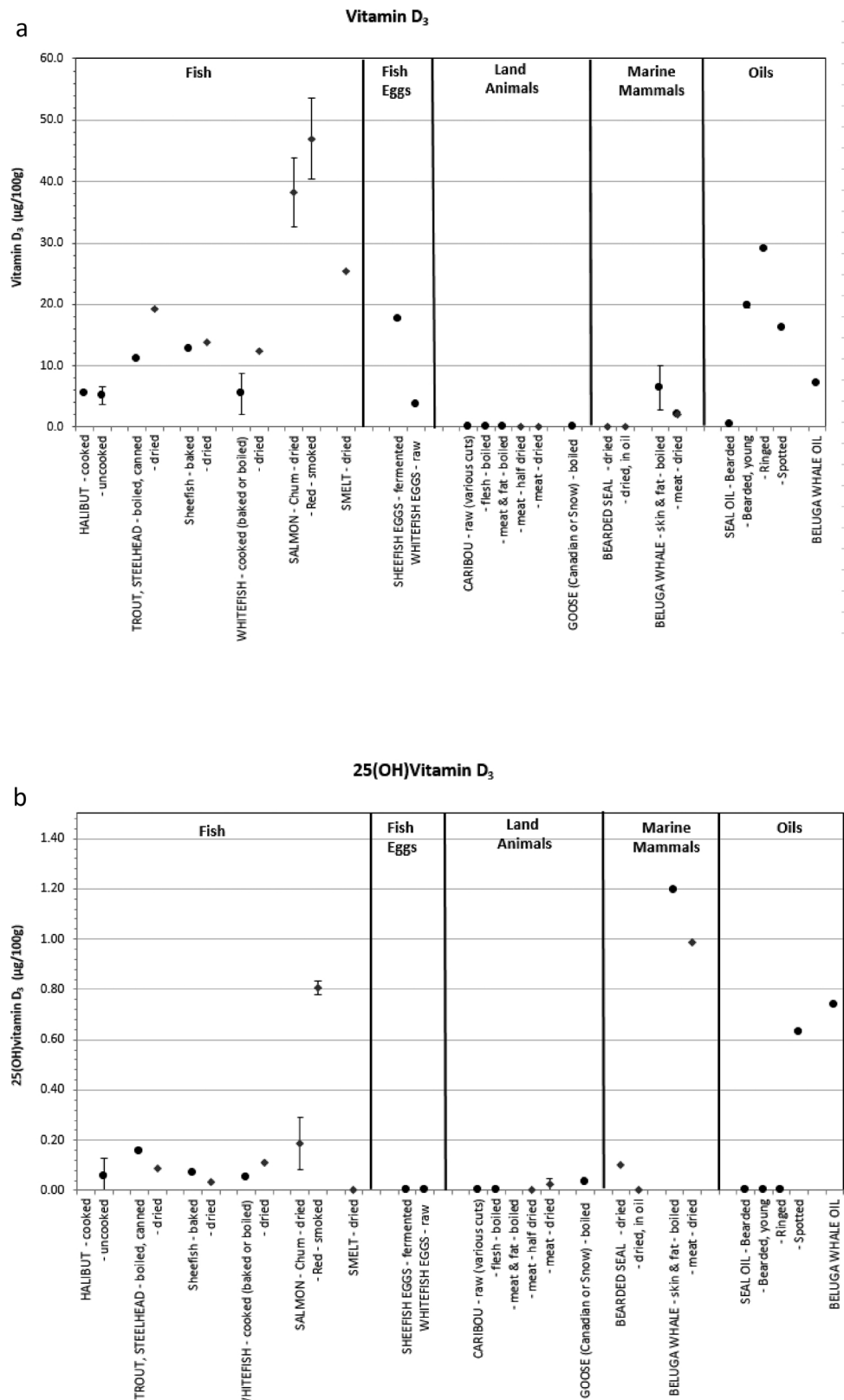


Fig. 3. Overview of vitamin D<sub>3</sub> (A) and 25-hydroxyvitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] (B) in traditional native foods. Error bars show the range in cases where more than one sample of a food was analyzed. ♦ indicates dried foods. The Daily Recommended Intake is 15 µg (600 IU) (Institute of Medicine, 2011).

2011; Sheehy et al., 2014; Singleton et al., 2015). However, the conclusions about specific nutrient and health outcomes rest on the quality and completeness of food composition data, which are lacking (Taylor et al., 2016). Sharma et al. (2011) reviewed the association between

lower risk of vitamin D deficiency and consumption of traditional foods in Arctic populations and suggested increasing the intake of fatty fish and marine mammals. However, in the present study, the vitamin D<sub>3</sub> and fat contents among fish and marine mammal samples were not

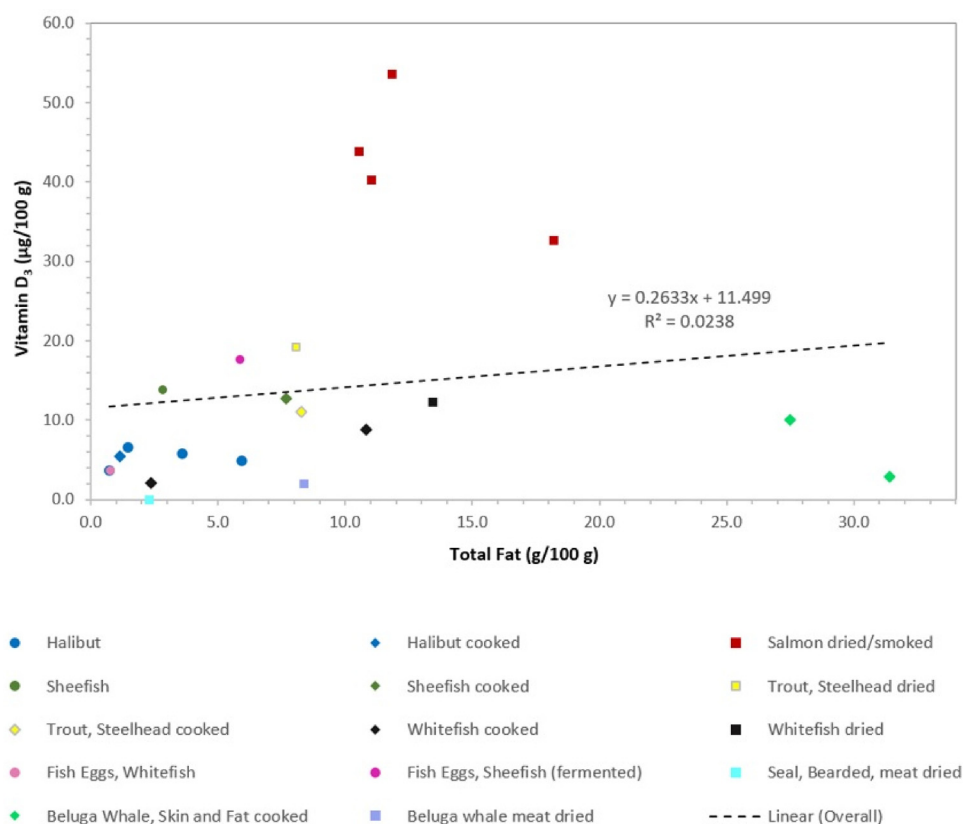


Fig. 4. Relationship between vitamin D<sub>3</sub> and total fat contents in Native Alaskan fish and marine mammal foods (excluding oils).

highly correlated ( $\rho = 0.154$ ; see Fig. 4), confounding the contention that “fatty fish” are the best sources of vitamin D, but consistent with a similar observation by Lu et al. (2007).

Additionally, within fish and marine mammals, certain species appear to be far better sources of vitamin D than others (Figs. 3 and 4). Of the fish, halibut and whitefish were relatively lower in vitamin D ( $< 10 \mu\text{g D}_3$  and/or  $< 0.2 \mu\text{g } 25(\text{OH})\text{D}_3$  per 100 g) than the other species [salmon, steelhead trout, sheefish, and dried fish (salmon, whitefish, sheefish, smelt)] that contained as much as  $53.6 \mu\text{g D}_3$  and nearly  $1 \mu\text{g } 25(\text{OH})\text{D}_3$  per 100 g, equivalent to, per 85 g serving, more than 300% of the  $15 \mu\text{g}$  DRI specified by the Institute of Medicine (2011). Among different marine mammals and also within species, there were large inter-sample differences in some cases. For example, two samples of beluga whale skin and fat had  $10.0$  and  $2.87 \mu\text{g D}_3$  and  $1.24$  and  $1.15 \mu\text{g } 25(\text{OH})\text{D}_3$  per 100 g, respectively. The sample of dried beluga whale meat was rich in vitamin D ( $8.37$  and  $0.986 \mu\text{g D}_3$  and  $25(\text{OH})\text{D}_3$  per 100 g, respectively) compared to dried bearded seal meat, which had negligible vitamin D [ $\leq 0.1 \mu\text{g}/100 \text{ g}$  of both  $\text{D}_3$  and  $25(\text{OH})\text{D}_3$ ]. In Fig. 5, vitamin D levels in the Alaskan Native foods and values in conventional alternatives [data from SR (USDA, 2016)] are compared.

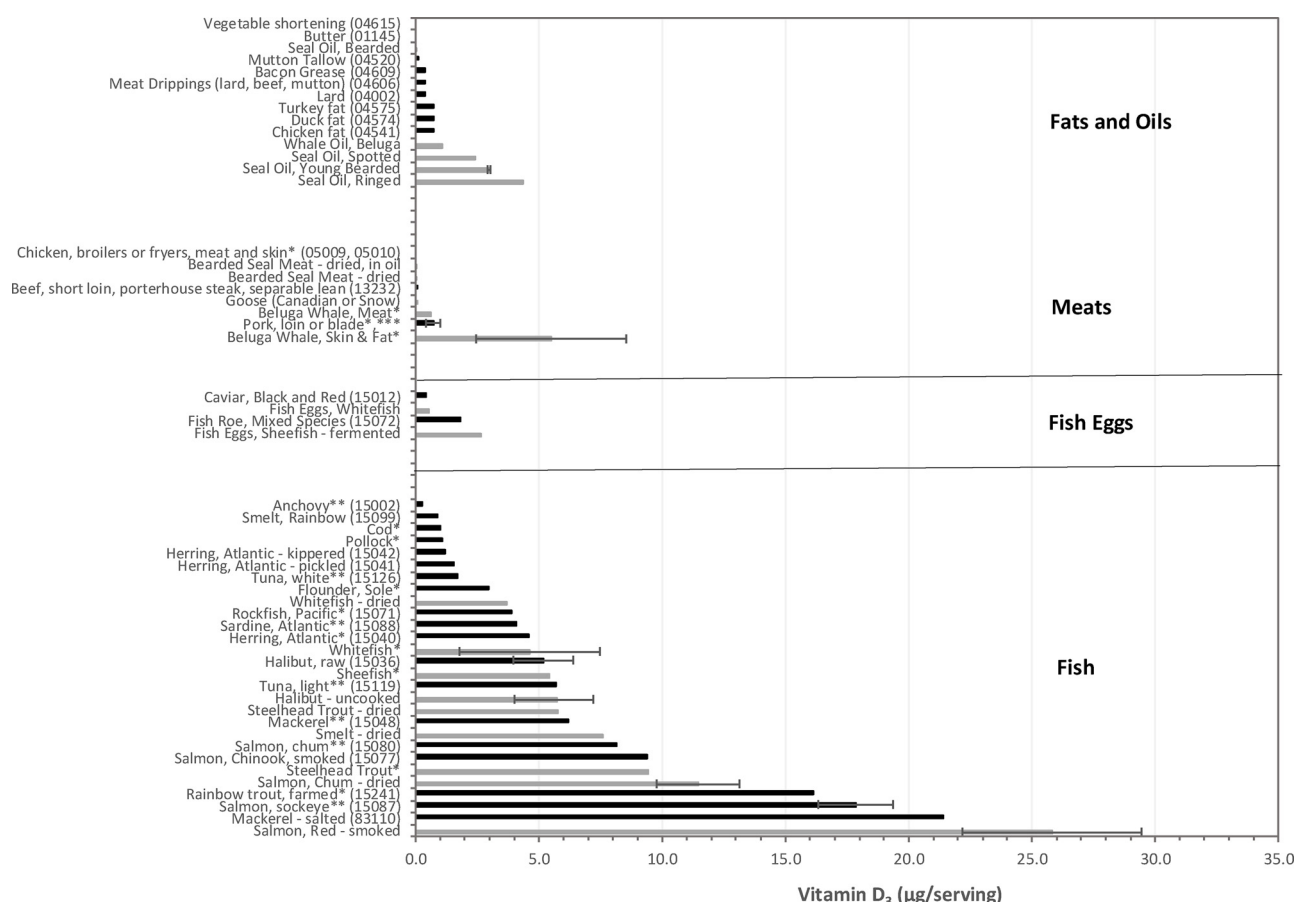
Probably one of the most significant sources of vitamin D that would be unique to the Native diet is seal oil. In many traditional Native Alaskan households (e.g. Inuit and Yup'ik), it is used as a dipping sauce for bread and meats/fish, added to soups or stews, and in some traditional medicinal preparations (Magdanz and Wolfe, 1988 and Table 1). Among all samples analyzed, the ringed seal oil was one of the highest in vitamin D, with  $29.0 \mu\text{g D}_3/100 \text{ g}$ , equivalent to  $4.35 \mu\text{g}$  per one 15 g ( $\sim 1$  tablespoon) serving (29% of the DRI). The content was substantially higher than beluga whale oil, although the latter was also a good source of vitamin D [ $7.11 \mu\text{g D}_3$  and  $0.739 \mu\text{g } 25(\text{OH})\text{D}_3$  100 g]. However, there was one sample of bearded seal oil with  $< 0.5 \mu\text{g D}_3/100 \text{ g}$ . Given the limited number of samples, it is possible that differences are not necessarily inter-species necessarily, but due to variation

among individuals within a species, as discussed in Section 3.2.

The results of this survey study suggest the importance of collecting detailed information on intake of wild caught foods, including season, maturity, and preparation, and correlating those intakes with the corresponding food composition data reflecting factors that influence vitamin D content in individual animals, in epidemiological studies seeking to correlate nutrient intake with health outcomes in Native populations. For example, the average vitamin D<sub>3</sub> content among all seal oil samples was  $17.0 \mu\text{g}/100 \text{ g}$ , but the range was  $0.37$ – $29$  ( $< 1$  to  $4.35$  per 15 g serving), and  $25(\text{OH})\text{D}_3$  was  $< 0.1 \mu\text{g}/100 \text{ g}$  in all samples but one, which contained a meaningful amount ( $0.628 \mu\text{g}/100 \text{ g}$ ) (section 3.3). Considering its varied and widespread use and that it would be consumed in variable amounts and up to more than tens of grams per day, just monitoring intake of “seal oil” and an average value for vitamin D in seal oils could over- or underestimate vitamin D intake by more than 50% of the DRI if 30 g were consumed, based on the range found in samples in this study.

One point worth noting is the risk of pesticide residues (e.g., organohalogenes) and heavy metals (e.g., mercury) that can accumulate in sea mammals, and in the case of organohalogenes, concentrate in the fat (for example, see Brown et al., 2016; Gmelch et al., 2017; Laird et al., 2013; Pedro et al., 2017; Reiner et al., 2016). Data on potential toxic components in the marine mammal oils and fats consumed in particular populations would be beneficial to determine prior to recommending massive increases in consumption. Additionally, there have been cases of botulism from improper storage and handling of seal oil and dried meats (Bendinger, 2014; Centers for Disease Control and Prevention and Alaska Department of Health and Social Services, 2016; Dankmeyer, 2016) so attention should be paid to education in handling for food safety.

Table 4 summarizes vitamin D data for Arctic fish and marine mammal products from the literature compared to values from the present study of Alaskan Native foods. Differences in vitamin D<sub>3</sub> were



**Fig. 5.** Comparison of vitamin D<sub>3</sub> per serving in Native Alaskan traditional fish and marine mammal foods (n) with values for conventional alternatives (n) from the USDA National Nutrient Database for Standard reference (SR) (USDA, 2016) and the Dietary Reference Intake (---) (Institute of Medicine, 2011). NDB numbers from SR are given in parentheses for the conventional foods. Error bars show the range for traditional foods for which more than one sample was analyzed, and the standard error for values from SR. \* cooked; \*\* canned; \*\*\*data are for cuts with similar fat content and are the mean and range for NDB numbers 10983, 10984, 10985, 10986, 10987, 10988, 10990, 10991, 10992, 10993. Serving sizes are the Reference Amounts Customarily Consumed Per Eating Occasion (U.S. Food and Drug Administration, 2017 for fish and fish eggs; U.S. Food and Drug Administration, 2012) for meats and animal oils and fats: uncooked meats and fish, 110 g; cooked meats and fish, 85 g; smoked salmon, 55 g; dried fish, 30 g; fish eggs, 15 g; oils and fats, 15 g. (No data for 25(OH)D<sub>3</sub> available in SR as of 2017).

notable among studies/samples for seal oils and blubber (1.6–160 µg/100 g for ringed seal and < 0.1–29.2 µg/100 g for bearded seal) and whale oil and blubber (< 1.6–74.4 µg/100 g). It is difficult to determine whether the variability is due to actual within-species variation due to environment or maturity, for example (as discussed for variation among samples in this study, section 3.2 and in Roseland et al., 2018) or analytical differences, due to lack of reference samples in common that would quantify the contribution of any analytical variability, as discussed in other publications (Phillips et al., 2006; Phillips and Rasor, 2016; Roseland et al., 2016). The existing literature do not include data on 25(OH)D<sub>3</sub>, and some of the foods can be significant contributors (> 0.6 µg/100 g) (see Fig. 3). Thus, 25(OH)D<sub>3</sub> should be included in food composition databases used to assess vitamin D intake in native populations.

The scarcity of data on vitamin D in Native foods makes it difficult to draw definitive conclusions about vitamin D intake and health in Native populations. Yet, hypotheses about diet and health, and epidemiological associations between food components and health outcomes, depend on complete and valid food composition data that are representative of the food supply of the population being studied. Without such data, it is not possible to reliably estimate nutrient intake or possible covariation of different components. For example, Adler et al. (1994) reported decreased prevalence of impaired glucose tolerance and diabetes associated with daily seal oil or salmon consumption by Alaska Natives, and attributed the effect to omega-3 fatty acids.

However, in the present study, both of these foods were among the highest in vitamin D<sub>3</sub> (32.6–53.6 and 16.1–29.9 µg/100 g (bearded, ring, and spotted seal), respectively) and 25(OH)D<sub>3</sub> [0.081–0.836 and 0.628 (spotted seal), respectively]. Whereas this does not prove cause and effect, possible associations between co-varying nutrients, particularly omega-3 fatty acids and vitamin D, may be overlooked when food composition data are lacking.

Incomplete data on vitamin D in foods also means potential misestimation of vitamin D intake, particularly underestimation of the contribution of 25(OH)D<sub>3</sub>. Currently, SR does not contain data for 25(OH)D<sub>3</sub>, although it is planned to be included in future releases (Taylor et al., 2016). Data for vitamin D<sub>3</sub> for only some of the samples were incorporated into SR beginning with release 19 (USDA, 2006) as part of the American Indian and Alaskan Native (AIAN) food composition database (Pehrsson et al., 2005a), due to the limited resources and quality of analytical methods available at the time. Many other food composition databases that have been used for dietary intake studies (e.g., Jakobsen and Saxholt, 2009; Lamberg-Allardt, 2006; Milešević et al., 2018; Neufingerl et al., 2016; Ovesen et al., 2003) also have not contained data for 25(OH)D<sub>3</sub>. Recently, improved analytical methodology, including for 25(OH)D<sub>3</sub>, has allowed USDA to analyze more foods for both vitamin D<sub>3</sub> and 25(OH)D<sub>3</sub> (Roseland et al., 2016). Currently, the USDA is transitioning to a larger, more comprehensive food composition database, that will eventually include 25(OH)D<sub>3</sub> values.

**Table 4**  
Summary and comparison of data from this study to data from the literature for Arctic marine mammals and fish.

Food	Vitamin D <sub>3</sub> µg/100 g						25(OH)D <sub>3</sub> µg/100 g			
	Kuhnlein et al. (2006)			Kenny et al. (2004)			This study			
	n	Mean	(Range)	n	Mean	(Range)	n	Mean	(Range)	Mean (Range)
<b>Marine Mammal Meats</b>										
<b>Seal</b>										
Bearded seal:										
Flesh, boiled	1	1.8								
Flesh, dried							1	< 0.1		0.102
Ringed seal:										
Flesh, raw	3	< 1.6								
<b>Beluga Whale</b>										
Flesh, dried							1	2.01		0.986
Skin, boiled	2	< 1.6								
Skin, raw	1	1.8								
<b>Fish</b>										
<b>Trout</b>										
Lake	3	19.7	(3.7–37.1)							
Steelhead, boiled							1	11.1		0.155
Steelhead, dried							1	19.2		0.088
<b>Whitefish</b>										
Flesh, raw	2	4.4	(3.5–5.0)	6	5.5	(4.2–7.3)				
Flesh, raw, dried							1	12.3		0.110
Flesh, baked or boiled							2	5.45	(2.11–8.80)	0.053 (< 0.1–0.053)
<b>Arctic char</b>										
Salmon	4	25.8	(9.3–62.0)	2	4.2	(4.0–4.3)				
Pink, raw				4	8.975	(6.1–13.4)				
Red, dried or smoked							2	47.0	(40.3–53.6)	0.807 (0.778–0.836)
Chum, dried							2	38.2	(32.6–43.8)	0.187 (0.081–0.292)
<b>Sheefish</b>										
Baked							2	6.4	(< 0.1 - 12.7)	0.068 (0.057–0.079)
Dried							1	13.8		0.032
<b>Smelt, dried</b>										
							1	25.4		< 0.1
<b>Halibut, raw</b>										
							4	5.22	(3.66–6.54)	0.057 (< 0.1–0.128)
<b>Sculpin</b>										
Raw	1	14.1		5	4.78	(2.1–8.1)				
Boiled	1	2								
<b>Cisco</b>										
Raw				5	4.7	(2.5–7.1)				
<b>Fish Eggs</b>										
Loche	2	15.2	(13.0–17.4)							
Cisco	2	6.5	(5.2–7.8)							
Whitefish (broad)							1	3.62		< 0.1
Sheefish, fermented							1	17.7		< 0.1
<b>Marine Mammal Oils and Fats:</b>										
<b>Seal</b>										
Bearded:										
Blubber, raw				6	15.7	(1.1–29.2)				
Oil (from mature seal)							1	< 0.1		< 0.1
Oil (from young seal)							2	19.9	(19.5–20.2)	< 0.1
Ringed:										
Blubber, raw	1	1.6		7	74.7	(13.8–160)				
Oil							1	29		< 0.1
Spotted										
Oil							1	16.1		0.628
<b>Walrus</b>										
Blubber, raw				7	0.54	(0.3–0.9)				
<b>Whale</b>										
Beluga:										
Blubber, boiled	1	13.3					2 <sup>a</sup>	6.46	(2.87–10.0)	1.20 (1.15–1.24)
Blubber, raw	2	< 1.6		6	42.6	(22.5–74.4)				
Oil	1	26.7					1	7.11		0.739
Bowhead:										
Blubber, raw				5	0.42	(0.3–0.6)				

<sup>a</sup> Skin and fat.

### 3.4. Limitations and future research

The number of samples analyzed was necessarily limited by the fact that the foods given for analysis were from the Native hunted food supply, and intensive sampling would impact tribal food security.

Nonetheless, the wide range of vitamin D content among the marine mammal products, likely reflecting influences of species, maturity, environment, and season and the seasonality of food harvesting and consumption in these populations, suggests the importance of population-specific sampling of the food supply that reflects these variables.

The preliminary data from this study has pointed towards more intensive sampling of marine mammals and oils as most critical in obtaining accurate estimates of vitamin D intake, along with addressing other challenges that have been described for nutritional epidemiology in Native populations (Amy and Pehrsson, 2003; Fialkowski et al., 2010; Pehrsson et al., 2005a, b; Ryman et al., 2015; Sheehy et al., 2013; Standlee-Strom, 2016).

## Declarations of interest

None.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jfca.2018.09.008>.

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