Organic nutrients and contaminants in seals and fish

ORIGINAL ARTICLE

ORGANIC NUTRIENTS AND CONTAMINANTS IN SUBSISTENCE SPECIES OF ALASKA: CONCENTRATIONS AND RELATIONSHIP TO FOOD PREPARATION METHOD

Sara K. Moses 1, Alex V. Whiting 2, Derek C.G. Muir 3, Xiaowa Wang 3, Todd M. O’Hara 1

1 Department of Biology and Wildlife and Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, USA
2 Native Village of Kotzebue, Kotzebue, USA
3 Aquatic Ecosystem Protection Research Division, Environment Canada, Burlington, Canada

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ABSTRACT

Objectives. To determine nutrient and contaminant concentrations, document concentration changes related to common preparation methods and provide a basic risk-benefit analysis for select subsistence foods consumed by residents of Kotzebue, Alaska.

Study design. Eleven organic nutrients and 156 persistent organic pollutants (POPs) were measured in foods derived from spotted seals and sheefish.

Methods. Nutrients in foodstuffs were compared to Daily Recommended Intake criteria. POPs were compared to Tolerable Daily Intake Limits (TDIL).

Results. Cooking, as well as absence/presence of skin during sheefish processing, altered nutrient and contaminant concentrations in seals and fish. Sheefish muscle and seal blubber were particularly rich in omega-3 fatty acids and seal liver in vitamin A. Seal liver exceeded the recommended upper limit for vitamin A. POP contribution to TDIL was <25% in all tissues except blubber, in which 4 POPs were present at >25% TDIL. No POPs exceeded TDIL in a serving of any tissue studied. The most prominent concerns identified were levels of vitamin A in spotted seal liver and certain POPs in blubber, warranting consideration when determining how much and how often these foods should be consumed.

Conclusions. Preparation methods altering tissues from their raw state significantly affect nutrient and contaminant concentrations, thus direct evaluation of actual food items is highly recommended to determine risk-benefits ratios of traditional diets. Traditional foods provide essential nutrients with very limited risk from contaminants. We encourage the consumption of traditional foods and urge public health agencies to develop applicable models to assess overall food safety and quality.

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INTRODUCTION

Fish and wildlife are important resources to the residents of north-west Alaska (AK). In Kotzebue, AK, fish and marine mammals comprise the majority (70%) of subsistence harvested foods with sheefish representing 45% of the total fish harvest and ice seals (spotted, ringed, bearded) accounting for 98% of the marine mammal harvest (1).

Obesity, cardiovascular disease and diabetes were rarely reported among AK Natives historically. Today, chronic disease is emerging as a major concern in this population as obesity rates increase (2,3). A shift from traditional subsistence-based diets to Western store-bought foods has decreased the nutrient intake and in combination with a decrease in physical activity has increased the risk of obesity, diabetes and cardiovascular disease. Biomedical professionals have documented that negative impacts have resulted, or will likely result, from a decrease in subsistence food use (2,4–10).

Although health benefits from the consumption of traditional foods exist, there is concern about the presence of environmental contaminants in those foods. Contaminants enter the arctic food chain from both local (11,12) and global sources (13). Anxiety about contaminants may be steering residents away from traditionally healthy subsistence diets to store-bought, processed foods that as a whole are less nutritious and are also known to contain contaminants (14–16). Decreased nutritional quality as a result of such changes in diet may actually be more harmful than consuming the contaminants in subsistence foods themselves (17), given the levels of contaminants present and the quantities consumed.

Previous studies have documented the presence of contaminants in wildlife tissues, but many are incomplete in that they do not account for the nutritional value of the tissues or how food processing may affect the chemical composition. Investigators that have previously examined contaminants and nutrients in subsistence-use species (18–23) did not primarily conduct their studies from the perspective of the consumer. Studies on actual marine food items prepared as they are consumed by AK Natives are limited (14,24). Without these data, intake quantities of contaminants and nutrients cannot be adequately estimated for subsistence communities consuming these foods.

Our study adds to existing wildlife studies in Alaska by examining the nutrient and contaminant content of tissues from 2 species commonly consumed by subsistence users in Kotzebue, AK. The selected species can also be used as models for related species and tissues that are consumed. We expand the previous studies by evaluating additional tissues (foods) and including the effects of food processing on these tissues. Both animal health and human intake perspectives are used by intensively examining animals taken and consumed by subsistence hunters. Spotted seal (Phoca largha) and sheefish (Stenodus leucicthys) were selected with input from local project participants, because they represent 2 major food groups (fish and marine mammals) and because their top trophic positions allow them to be good indicators of contaminants present in the marine food web.

The unique focus of this study is the measuring of contaminants and nutrients in an integrated fashion, utilizing both the raw product and the actual food items consumed. This research is necessary in order to provide
balanced information regarding contaminants and nutrients and to provide the information needed to develop integrated, quantitative models that public health officials can use to develop effective recommendations and interventions based on actual food items consumed. We emphasize that animal health and human health are intimately linked in this scenario.

The specific aims of the current study were: (1) to determine the concentrations of select nutrients and contaminants in spotted seal and sheefish tissues commonly utilized as subsistence foods, (2) to determine the effects of traditional food processing methods on the concentrations of these nutrients and contaminants, and (3) to relate the levels of nutrients and contaminants in these subsistence foods to established nutrient and contaminant intake criteria. The study presented here focuses on organic nutrients and contaminants. Inorganic nutrients and contaminants in these species were reported by Moses et al. (25). Together, these studies provide a comprehensive evaluation of the nutritional benefits and subsequent toxicological risks associated with the consumption of 2 important subsistence species in north-west AK.

MATERIAL AND METHODS

Sample collection, morphometrics and aging
Sample collection and permission (MMHSRP permit #932-1489-05), storage, morphometric measurements and age estimation have been described in detail and previously reported (25). Samples were collected in October 2004 and March 2005 at Kotzebue, AK. Blubber, muscle, liver and kidney samples from spotted seals (Phoca largha; n=5) and muscle from sheefish (Stenodus leucichthys; n=8) were collected from legally subsistence harvested animals for chemical analyses.

Food processing
A portion of each tissue was “food processed” to mimic traditional cooking methods as described in Moses et al. (25). Processing methods for seal tissues included rendering of blubber; boiling and drying of muscle; frying of liver; and boiling of kidney. Sheefish muscle was baked, dried and smoked both with and without skin on the filets.

Nutrients
Nutrient analyses were conducted by Maxxam Analytics (Mississauga, Ontario, Canada). All samples of the same species/tissue/processing method were pooled (spotted seals, n=5; sheefish, n=8), homogenized and a single nutrient concentration obtained in order to meet the large sample size requirement for these analytes. Similar sample masses from each individual were included in the pooled sample, thus analytic results represent a proximate average concentration. Nutrients investigated include total fat, saturated fat, cis-monounsaturated fatty acids (MUFA), cis-polyunsaturated FA (PUFA), trans-FA, omega-3 PUFA, omega-6 PUFA, cholesterol, beta carotene and vitamins A and C.

FA profiles were generated according to the Association of Analytical Communities (AOAC) method 996.06. Cholesterol was analysed according to AOAC method 976.26; vitamin A and beta carotene according to AOAC methods 922.04 and 922.06; and vitamin C according to AOAC methods 967.22 and 984.26 (26).
Persistent organic pollutants (POPs)

Persistent organic pollutants (POPs), including organochlorine pesticides, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), were determined in spotted seal and sheefish tissues according to previously described quantitative methods (27–29). Briefly, samples were extracted with dichloromethane (DCM), followed by drying and concentration of extracts. Extracts were analysed using single column capillary GC. Analyses were carried out at the National Laboratory for Environmental Testing (NLET) at the National Water Research Institute (NWRI, Burlington, Ontario, Canada).

Quantification of POPs was performed using internally (NLET) prepared spiking standards containing known concentrations of POPs. Standard reference materials (SRM 1588a: organics in cod liver oil) from the National Institutes of Standards and Technology (NIST, Gaithersburg, MD, USA) were used to confirm the accuracy and reproducibility of the analytical methods. A calibration check standard was run every 6 samples, followed by a spike or SRM for further QA/QC. Method blanks were run in order to blank correct analysed contaminant concentrations. The minimum detection limit (MDL) for POPs in tissues varied depending upon analyte and sample size. Individual MDLs are reported in Supplementary Tables II and III (http://ijch.fi/issues/684/684.html).

POPs determined in spotted seal tissues were 1,3,5-, 1,2,4- and 1,2,3-trichlorobenzene (TCB); hexachlorobutadiene; 1,2,4,5- and 1,2,3,4-tetrachlorobenzene (TTCB); pentachlorobenzene (PECB); hexachlorobenzene (HCB); 3,4,5,6-tetrachloroveratrole; pentachloroanisole; α-, β- and γ-hexachlorocyclohexane (HCH); heptachlor; octachlorostyrene; heptachlor epoxide; oxychlordane; α- and γ-chlordane; cis- and trans-nonachlor; dieldrin; o,p- and p,p-dichlorodiphenyldichloroethylene (DDE); o,p- and p,p-dichlorodiphenyl dichloroethane (DDD); o,p- and p,p-dichlorodiphenyltrichloroethane (DDT); methoxychlor; mirex; PCB congeners (IUPAC designations in order of elution: 1, 3, 4/10, 7/9, 6, 8/5, 19, 12/13, 18, 15/17, 24/27, 16/32, 54/29, 26, 25, 31/28, 50, 33/20, 53, 51, 22, 45, 46, 52, 43, 49, 47/48, 44, 59, 42, 71/41/64, 40, 100, 63, 74, 70/76/98, 66, 95, 91, 55, 56/60, 92, 84, 101, 99, 119, 83, 97, 81/87, 85, 136, 110, 82, 151, 135/144, 147, 107, 149, 118, 133, 114, 134/131, 146, 153, 132, 105, 141, 179, 137, 176, 130, 163/138, 158, 129, 178, 175, 182/187, 183, 128, 167, 185, 174, 177, 202/171, 156, 173, 157/200, 172, 197, 180, 193, 191, 199, 170/190, 198, 201, 203/196, 189, 208/195, 207, 194, 205, 206, 209 (“/” indicates co-eluters); and PBDE congeners (in order of elution: 17, 28/33, 49, 47, 66, 100, 99, 85, 154, 153, 138, 183, 190). POPs determined in sheefish were the same as above, except that 1,3-, 1,2- and 1,4-dichlorobenzene (DCB) were additionally analysed but PBDE congeners were not analysed in fish.

Some concentrations are reported as sums of contaminant groups. These include ΣPCB (sum of all PCB congeners detected), ΣPCB_{10} (sum of PCB congeners 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180), ΣDDT (sum of o,p- and p,p-substituted DDT, DDE and DDD), ΣCHL (sum of oxychlordane; methoxychlor; heptachlor; heptachlor epoxide; α- and γ-chlordane; and cis- and trans-nonachlor), ΣHCH (sum of α-, β- and γ-HCH isomers), ΣCBZ (sum of 1,2-, 1,3-, and 1,4-DCB; 1,3,5-, 1,2,4- and 1,2,3-trichlorobenzene [TCB]; 1,2,4,5- and 1,2,3,4-tetrachlorobenzene [TTCB]; PECB and HCB), and ΣPBDE (sum of all PBDE congeners detected).
Calculations and statistics

Concentrations and summary statistics
POP concentration summary statistics are reported for each tissue type/processing method combination for which the analyte was above the MDL in >50% of samples of that group. When >50% but <100% of samples were above the MDL, a value of ½ MDL was used for all samples that were below the detection limit (BDL) to calculate summary statistics.

Concentration changes due to food processing
Nutrient and POP concentrations (ww) were determined in tissues prior to and after food processing, as described above. Concentration changes due to food processing were calculated as:

\[
\% \text{ Change} = \left( \frac{C_p - C_r}{C_r} \right) \times 100
\]

where \( C_r \) is the concentration of the raw tissue and \( C_p \) is the concentration of the processed tissue. Thus, \( (C_p - C_r) \) represents the absolute concentration change due to food processing, where positive values indicate an increase and negative values indicate a decrease. Percent change was not calculated if either the raw or processed samples were BDL. To account for changes in water or lipid content during processing, absolute and percent change were calculated on a ww, dry weight (dw) and lipid weight (lw) basis. Significance was determined using a paired t-test (p<0.05). In addition, t-tests were repeated with the inclusion of a Bonferroni correction for multiple comparisons to account for potential Type I error arising from the use of multiple t-tests within a family (i.e., tissue/processing combination).

Reference intake values for organic nutrients
Nutrient concentrations (ww) in raw and processed tissues were compared to reference intake values (Daily Reference Intake [DRI]/Upper Limit [UL], Acceptable Macronutrient Distribution Range [AMDR] or Daily Value [DV], as appropriate) (30). The contribution (%) of one serving (100 g) of each food product to the reference values was determined. The recommendation for trans-FA intake is “as low as possible while consuming a nutritionally adequate diet”; thus, % contributions to recommended intakes need not be calculated. Reference intake values for cis-MUFA and cis-PUFA were not available; therefore, these nutrients were not included in this analysis. The reference group used for calculations was adult men, ages 31–50 years. It should be noted that recommended intakes vary by cohort (i.e., age, sex, pregnancy/lactation status).

Tolerable Daily Intake Limits (TDIL) for persistent organic pollutants
POP concentrations (ww) in raw and processed tissues were compared to established tolerable daily intakes (TDI). Assuming a reference consumer body weight (BW) of 70 kg, tolerable daily intake limits (TDIL) of food products for each POP were calculated according to:

\[
\text{TDIL (g)} = \frac{\text{TDI (ng/kg/day)} \times \text{BW (kg)}}{C_t \text{ (ng/g, ww)}}
\]

where, \( C_t \) is the mean concentration of the contaminant in the food tissue. The TDIL represents the amount of a particular food a 70-kg consumer could eat safely on a daily basis throughout his/her entire lifespan without the risk of an adverse effect from a given contaminant.
The oral reference dose (RfD) established by the United States Environmental Protection Agency (31) was utilized as the TDI for hexachlorobutadiene, dieldrin, mirex, $\Sigma$CHL and $\Sigma$HCH. TDI guidelines established by Health Canada (32) were utilized for $\Sigma$PCB, $\Sigma$DDT and $\Sigma$CBZ. The TDI value for DDT was used to calculate the TDIL for $\Sigma$DDT, a group which contains both DDT and its DDE and DDD metabolites. The TDI used to calculate the TDIL for $\Sigma$CBZ was the mean of all contaminants included in this group. TDIL calculations for tetrachloroveratrole, octachlorostyrene, pentachloroanisole and $\Sigma$PBDE are not presented here because no official intake criteria exist for these compounds.

RESULTS

Organic nutrient and contaminant concentrations and changes due to food processing

Concentrations of nutrients and POPs in raw and food processed spotted seal and sheefish tissues are summarized in Supplementary tables I, II and III published online (http://ijch.fi/issues/684/SupplementaryMoses.pdf). In spotted seals, all fat and fatty acid classes were highest in raw and rendered blubber. Cholesterol was highest in organ meats (liver and kidney) and lowest in muscle and blubber products. Vitamin A was highest in liver followed by blubber, kidney and finally muscle. Vitamin C and beta carotene were very low or below MDL in all tissues. Generally, spotted seal and sheefish muscle had similar organic nutrient concentrations.

Changes in concentrations as a result of food processing on a ww, dw and lw basis are shown in Tables I–IV. Seven statistically significant changes were noted, ranging from $-95.6\%$ (vitamin A in boiled seal kidney) to $+583\%$ (octachlorostyrene in sheefish muscle dried without skin) on a ww basis, from $-97.2\%$ (vitamin A in boiled seal kidney) to $+468\%$ (tetrachloroveratrole in rendered blubber) on a dw basis, and from $-96.7\%$ (vitamin A in boiled seal kidney) to $+413\%$ (tetrachloroveratrole in rendered blubber) on a lw basis. When a Bonferroni correction was applied to account for multiple pair-wise comparisons, only the change in $\Sigma$PBDE in seals remained statistically significant, and only on a dw basis for muscle when boiled ($-38.1\%$) or dried ($-69.4\%$) and kidney when boiled ($-56.2\%$).

Contributions to Daily Intake Reference Values and Tolerable Daily Intake Limits (TDIL)

Nutrient concentrations (ww) in a meal-sized portion (100g) of each tissue were compared to recommended daily intake criteria for those nutrients for which guidelines were available (Table V). Nutrients present at $>100\%$ of the minimum daily intake (MDI) reference values in spotted seal tissues were total fat and omega-3 PUFA in raw and rendered blubber, vitamin A in raw and fried liver and raw blubber. No nutrients were present at $>100\%$ of MDI value in sheefish muscle processed by any method investigated.

In 8 cases, seal tissues exceeded nutrient ULs for a single serving: total fat in rendered blubber (104%), omega-3 PUFA in raw and rendered blubber (511% and 393%, respectively), vitamin A in raw and fried liver (913% and 1050%), and cholesterol in fried liver (162%) and raw and boiled kidney (149% and 240%). No raw or processed sheefish muscle exceeded the UL for any nutrient in a single serving.
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Table I. Absolute and percent change (% Δ) in organic nutrients on a wet weight basis as a result of food processing tissues of spotted seal (pooled, n=5) and sheefish (pooled, n=8) harvested in Kotzebue, Alaska (2004–2005)^a.

|                | Total Fat | Saturated Fat | cis-MUFA^c | cis-PUFA^c | trans-FA^c | Omega-3 PUFA^c | Omega-6 PUFA^c | Cholesterol | Vitamin A | Vitamin C |
|----------------|-----------|---------------|------------|------------|------------|----------------|----------------|-------------|-----------|-----------|
| Spotted Seal   |           |               |            |            |            |                |                |             |           |           |
| Blubber Δ with Rendering | +5.7      | +2.0          | +6.7       | +3.3       | +0.20      | -3.2           | -0.23          | -2.0        | -653      | NA^d      |
| Muscle Δ with Boiling   | -1.64     | -0.419        | -1.12      | +0.012     | -0.037     | +0.022         | -0.009         | +26.0       | NA^d      | NA^d      |
| Muscle Δ with Drying    | +0.99     | +0.245        | +0.45      | +0.333     | +0.019     | +0.164         | +0.068         | +125        | NA^d      | NA^d      |
| Muscle Δ with Drying    | +28.4%    | +26.8%        | +20.9%     | +132%      | +22.9%     | +143%          | +110%          | +208%       |           |           |
| Liver Δ with Frying     | +0.91     | +0.478        | +0.28      | +0.10      | +0.018     | +0.026         | +0.072         | +196        | +4100     | -388      |
| Liver Δ with Frying     | +26.4%    | +48.7%        | +23.5%     | +9.43%     | +25.4%     | +3.72%         | +19.7%         | +67.4%      | +15.0%    | -95.6%    |
| Kidney Δ with Boiling   | +1.15     | +0.404        | +0.63      | +0.048     | +0.017     | -0.036         | +0.084         | +273        | +0.2      | -0.3      |
| Kidney Δ with Boiling   | +36.4%    | +47.8%        | +38.7%     | +9.76%     | +29.3%     | -16.1%         | +31.3%         | +61.1%      | +28.6%    | -37.5%    |

Sheefish Muscle

|                | Total Fat | Saturated Fat | cis-MUFA^c | cis-PUFA^c | trans-FA^c | Omega-3 PUFA^c | Omega-6 PUFA^c | Cholesterol | Vitamin A | Vitamin C |
|----------------|-----------|---------------|------------|------------|------------|----------------|----------------|-------------|-----------|-----------|
| Δ with Baking without Skin | -0.61     | -21.0%        | -0.363     | -0.095     | -0.021     | -0.083         | -0.012         | -2          | -1        | NA^d      |
| Δ with Baking with Skin   | +5.17     | +0.996        | +3.22      | +0.721     | +0.007     | +0.628         | +0.089         | +7          | +5        | NA^d      |
| Δ with Drying without Skin | +2.07     | +0.656        | +1.48      | +0.711     | +0.114     | +0.668         | +0.055         | +45         | +8        | NA^d      |
| Δ with Drying with Skin   | +71.1%    | +112%         | +109%      | +92.5%     | +156%      | +93.8%         | +96.5%         | +84.9%      | +114%     |           |
| Δ with Smoking without Skin | +0.49     | +0.103        | +0.16      | +0.201     | -0.013     | +0.193         | +0.015         | +6          | +10       | NA^d      |
| Δ with Smoking with Skin  | +16.8%    | +17.6%        | +11.8%     | +26.1%     | -17.8%     | +27.1%         | +25.3%         | +11.3%      | +143%     |           |
| Δ with Smoking with Skin  | +2.94     | +0.506        | +1.69      | +0.641     | -0.012     | +0.578         | +0.062         | +4          | +11       | NA^d      |

^a Fat (total and saturated) and fatty acid classes are reported in g/100g wet weight (ww), cholesterol in mg/100g (ww) and vitamin A and vitamin C in µg/100g (ww).
^b Bold text indicates changes of >50% and bold underline changes >100% as a result of food processing.
^c MUFA = monounsaturated fatty acid. FA = fatty acid. PUFA = polyunsaturated fatty acid.
^d Absolute and percent change not available (NA) because either raw, food processed or both tissues were below the minimum detection limit (BDL). Beta carotene was NA in all tissues.
Table II. Absolute* and percent change (%Δ) in organic nutrients on a dry weight basis as a result of food processing tissues of spotted seal (pooled, n=5) and sheefish (pooled, n=8) harvested in Kotzebue, Alaska (2004–2005)*.

|                | Total Fat | Saturated Fat | cis- MUFA | cis- PUFA | trans- FA | Omega-3 PUFA | Omega-6 PUFA | Cholesterol | Vitamin A | Vitamin C |
|----------------|-----------|---------------|-----------|-----------|-----------|---------------|---------------|--------------|-----------|-----------|
| **Spotted Seal** |           |               |           |           |           |               |               |              |           |           |
| Blubber Δ with Rendering* | +5.7      | +2.0          | +6.7      | +3.3      | +0.20     | -3.2          | -0.23         | -2.0         | -653      | NA*       |
| Muscle Δ with Boiling | -6.67     | -1.72         | -4.41     | -0.080    | -0.15     | -0.014        | -0.064        | +32.2        | NA*       | NA*       |
| Muscle Δ with Drying | -5.33     | -1.68         | -4.11     | -0.10     | -0.16     | -0.058        | -0.053        | +19.5        | NA*       | NA*       |
| Liver Δ with Frying | -0.39     | +0.42         | -0.22     | -0.55     | -0.010    | -0.46         | -0.10         | +25.4        | -10641    | -1702     |
| Kidney Δ with Boiling | -11.7     | -0.19         | -0.78     | -0.63     | -0.043    | -0.45         | -0.18         | +64.5        | -0.043    | -2.07     |
| **Sheefish Muscle** |           |               |           |           |           |               |               |              |           |           |
| Δ with Baking without Skin | -3.96     | -0.75         | -2.11     | -0.83     | -0.12     | -0.75         | -0.078        | -42.2        | -1.42     | NA*       |
| Δ with Baking with Skin | +13.6     | +2.60         | +8.85     | +1.59     | -0.015    | +1.35         | +0.23         | -23.3        | +1.95     | -5.12%    |
| Δ with Drying without Skin | -0.46     | -0.033        | -0.17     | -0.32     | +0.055    | -0.28         | -0.020        | -29.7        | -0.15     | NA*       |
| Δ with Drying with Skin | +726      | +1.52         | +4.61     | +0.89     | -0.071    | +0.75         | +0.14         | -10.8        | +11.7     | +63.0%    |
| Δ with Smoking without Skin | -1.80     | -0.35         | -1.04     | -0.25     | -0.10     | -0.23         | -0.020        | +41.2        | +20.9     | NA*       |
| Δ with Smoking with Skin | +5.87     | +0.93         | +3.68     | +1.15     | -0.11     | +1.02         | +0.13         | +40.3        | +25.8     | NA*       |

*Fat (total and saturated) and fatty acid classes are reported in g/100g wet weight (dw), cholesterol in mg/100g (dw) and vitamin A and vitamin C in µg/100g (dw).

**Bold text indicates changes of >50% as a result of food processing.

*MUFA = monounsaturated fatty acid. FA = fatty acid. PUFA = polyunsaturated fatty acid.

*Changes in blubber are based on wet weight values which are assumed to be essentially equivalent to dry weight values (i.e., 0% water content).

*Absolute and percent change not available (NA) because either raw, food processed or both tissues were below the minimum detection limit (BDL). Beta carotene was NA in all tissues.
## Table III. Absolute\(^a\) and percent change (\(\%\Delta\)) in organic nutrients on a lipidd adjusted weight basis as a result of food processing tissues of spotted seal (pooled, \(n=5\)) and sheefish (pooled, \(n=8\)) harvested in Kotzebue, Alaska (2004–2005)\(^bc\).

|                  | Saturated Fat | cis-MUFA\(^c\) | cis-PUFA\(^c\) | trans-FA\(^c\) | Omega-3 PUFA\(^c\) | Omega-6 PUFA\(^c\) | Cholesterol | Vitamin A | Vitamin C |
|------------------|---------------|----------------|----------------|---------------|--------------------|--------------------|--------------|-----------|-----------|
| **Spotted Seal** |               |                |                |               |                    |                    |              |           |           |
| Blubber \(\Delta\) with Rendering\(^d\) | +0.013 | +0.045 | -0.055 | +0.0001 | -0.052 | -0.005 | -0.069 | -9.00 | NA\(^f\) |
|                 | +7.51% | +8.21% | -26.4% | +0.16% | -28.6% | -17.5% | -10.9% | -65.7% |          |
| Muscle \(\Delta\) with Boiling | +0.007 | -0.058 | +0.052 | +0.001 | +0.041 | +0.011 | +29.5 | NA\(^f\) | NA\(^f\) |
|                 | +2.52% | -9.39% | +102% | +4.82% | +125% | +61.7% | +171% |          |          |
| Muscle \(\Delta\) with Drying | -0.003 | -0.036 | +0.041 | -0.001 | +0.029 | +0.011 | +24.1 | NA\(^f\) | NA\(^f\) |
|                 | -1.30% | -5.85% | +80.3% | -4.33% | +88.9% | +63.2% | +140% |          |          |
| Liver \(\Delta\) with Frying | +0.050 | -0.008 | -0.041 | -0.002 | -0.036 | -0.006 | +27.3 | -717 | +0.004 |
|                 | +17.6% | -2.25% | -13.4% | -0.81% | -17.9% | -5.31% | -32.3% | -9.03% | +1.74% |
| Kidney \(\Delta\) with Boiling | +0.022 | +0.009 | -0.030 | -0.001 | -0.027 | -0.003 | +25.6 | -124 | -0.14 |
|                 | +8.33% | +1.66% | -19.5% | -5.19% | -38.5% | -3.70% | +18.1% | -96.7% | -54.2% |
| **Sheefish Muscle** |               |                |                |               |                    |                    |              |           |           |
| \(\Delta\) with Baking without Skin | +0.005 | -0.034 | +0.029 | -0.003 | +0.029 | +0.0002 | +3.96 | +1.07 | NA\(^f\) |
|                 | +2.69% | -7.25% | +10.9% | -9.88% | +11.0% | -0.11% | +21.7% | +44.6% |          |
| \(\Delta\) with Baking with Skin | -0.005 | +0.10 | -0.080 | +0.014 | -0.079 | -0.002 | +10.8 | -0.92 | NA\(^f\) |
|                 | -2.56% | +21.3% | -30.2% | +56.6% | -32.2% | -7.75% | -59.2% | -38.3% |          |
| \(\Delta\) with Drying without Skin | +0.005 | +0.004 | -0.018 | +0.006 | -0.015 | -0.001 | -1.93 | +0.006 | NA\(^f\) |
|                 | +2.64% | +0.94% | -6.97% | +23.8% | -6.31% | -5.02% | -10.6% | +3.58% |          |
| \(\Delta\) with Drying with Skin | +0.004 | +0.065 | -0.055 | +0.014 | -0.055 | +0.0001 | -7.62 | -0.31 | NA\(^f\) |
|                 | +1.75% | +13.8% | -20.6% | -53.8% | -22.4% | -0.26% | -41.8% | -12.8% |          |
| \(\Delta\) with Smoking without Skin | +0.001 | -0.020 | +0.023 | -0.006 | +0.022 | +0.002 | -0.86 | +2.59 | NA\(^f\) |
|                 | +0.68% | -4.34% | +8.74% | -22.6% | +8.79% | +8.11% | -4.72% | +108% |          |
| \(\Delta\) with Smoking with Skin | -0.014 | +0.054 | -0.023 | -0.015 | -0.024 | +0.001 | -8.47 | +0.67 | NA\(^f\) |
|                 | -7.16% | +11.6% | -8.79% | -58.4% | -9.88% | +3.85% | -46.5% | +27.9% |          |

\(^a\)Saturated fat and fatty acid classes are reported in g/g lipid weight (lw), cholesterol in mg/g (lw) and vitamin A and vitamin C in \(\mu\)g/g (lw).

\(^b\)Bold text indicates changes of >50% as a result of food processing.

\(^c\)MUFA = monounsaturated fatty acid. FA = fatty acid. PUFA = polyunsaturated fatty acid.

\(^d\)Absolute and percent change not available (NA) because either raw, food processed or both tissues were below the minimum detection limit (BDL). Beta carotene was NA in all tissues.
Table IV. Absolute (ng/g) and percent change (%Δ) [mean (±1 SD)] in organic contaminants on a wet weight (ww), dry weight (dw) and lipid adjusted weight (lw) basis as a result of food processing various tissues of spotted seals (n=5) and sheefish (n=8) harvested in Kotzebue, Alaska (2004)\textsuperscript{a}.

| Wet Weight | Tissue/Processing | Contaminant | Δ with Processing | Dry Weight | Tissue/Processing | Contaminant | Δ with Processing | Lipid Adjusted Weight | Tissue/Processing | Contaminant | Δ with Processing |
|------------|-------------------|-------------|-------------------|------------|-------------------|-------------|-------------------|-----------------------|-------------------|-------------|-------------------|
| Spotted Seal | Blubber/Rendered | Tetrachloroveratrole | +1.12 (±0.81) | Blubber/Rendered | Tetrachloroveratrole | +1.12 (±0.81) | Blubber/Rendered | Tetrachloroveratrole | +1.08 (±0.79) |
| | | | +468 (±305)\% | | | | | | +413 (±283)\% |
| | Blubber/Rendered | \(\Sigma\)CBZ | +2.75 (±2.11) | Blubber/Rendered | \(\Sigma\)CBZ | +2.75 (±2.11) | Blubber/Rendered | \(\Sigma\)CBZ | -4.43 (±0.58) |
| | | | +22.9 (±18.2)\% | | | | | | -44.0 (±20.0)\% |
| | Muscle/Dried | \(\Sigma\)PBDE | -0.07 (±0.05) | Muscle/Boiled | \(\Sigma\)PBDE | -0.54 (±0.21) | Muscle/Dried | \(\Sigma\)PBDE | -66.4 (±38.1) |
| | | | -16.6 (±11.8)\% | | | | | | -48.3 (±16.0)\% |
| | Liver/Fried | Dieldrin | +0.22 (±0.17) | Muscle/Dried | PCB | -17.0 (±11.5) | Muscle/Dried | \(\Sigma\)PBDE | -16.2 (±11.5) |
| | | | +15.4 (±10.6)\% | | | | | | -7.2 (±12.0)\% |
| Sheefish Muscle | Muscle/Dried | \(\Sigma\)PBDE | -0.97 (±0.16) | Liver/Fried | \(\Sigma\)DDT | -0.97 (±0.16) | Liver/Fried | \(\Sigma\)DDT | -13.3 (±6.2) |
| | | | -69.4 (±8.8)\% | | | | | | -15.9 (±11.3)\% |
| Dried (No Skin) | Dieldrin | +0.16 (±0.19) | Liver/Fried | \(\Sigma\)HCH | -1.36 (±1.06) | Liver/Fried | \(\Sigma\)HCH | -7.05 (±4.01) |
| | | | +154 (±261)\% | | | | | | -25.7 (±18.6)\% |
| Dried (No Skin) | Octachlorostyrene | +1.11 (±0.13) | Kidney/Boiled | \(\Sigma\)PBDE | -1.30 (±0.39) | Kidney/Boiled | Mirex | -0.71 (±0.34) |
| | | | +583 (±126)\% | | | | | | -54.5 (±24.8)\% |
| Dried (No Skin) | \(\Sigma\)CHL | +2.07 (±2.28) | Sheefish | \(\Sigma\)PBDE | -1.30 (±0.39) | None | Sheefish | -15.3 (±8.5) |
| | | | +270 (±503)\% | | | | | | -7.4 (±9.1) |
| Dried (No Skin) | \(\Sigma\)HCH | +0.40 (±0.29) | None | Sheefish | | | | | |
| | | | +213 (±354)\% | | | | | | |
| Dried (No Skin) | \(\Sigma\)CBZ | +1.71 (±1.77) | None | Sheefish | | | | | |
| | | | +173 (±289)\% | | | | | | |
| Smoked (With Skin) | Octachlorostyrene | +0.07 (±0.06) | None | Sheefish | | | | | |
| | | | +278 (±354)\% | | | | | | |
| Smoked (With Skin) | \(\Sigma\)HCH | +0.30 (±0.33) | None | Sheefish | | | | | |
| | | | +175 (±339)\% | | | | | | |

\textsuperscript{a}Only statistically significant changes (p < 0.05) are shown. For all changes displayed, none remain significant if a Bonferroni corrections for multiple comparisons is included.
Table V. Mean percent (%) contribution (of minimum reference value/maximum reference value) of 1 serving (100g ww) of spotted seal (pooled, n=5) and sheefish (pooled, n=8) tissue to the Daily Reference Intake (DRI)/Upper Limit (UL), Acceptable Macronutrient Distribution Range (AMDR) or Daily Value (DV) for select organic nutrients

| DRI/UL, AMDR, or DV | Total Fat 44-78 g/day | Saturated Fat ND/20 g/day | Omega-3 PUFA 1.6-2.7 g/day | Omega-6 PUFA 11-22 g/day | Cholesterol ND/300 mg/day | Vitamin A 900-3000 µg/day | Vitamin C 90-2000 mg/day |
|---------------------|-----------------------|---------------------------|---------------------------|---------------------------|-------------------------|--------------------------|--------------------------|
| **Spotted Seal**    |                       |                           |                           |                           |                         |                          |                          |
| Raw Blubber         | 172/96.8              | ND/64.0                   | 863/511                   | 18.6/9.32                 | ND/16.0                 | 114/34.3                 | BDLd                     |
| Rendered Blubber    | 185/104               | ND/74.0                   | 663/393                   | 16.5/8.27                 | ND/15.3                 | 41.9/12.6                | BDLc                     |
| Raw Muscle          | 7.91/4.46             | ND/4.58                   | 7.19/4.26                 | 0.56/0.28                 | ND/20.0                 | 0.22/0.07                | BDLc                     |
| Boiled Muscle       | 4.18/2.36             | ND/2.48                   | 8.56/5.07                 | 0.48/0.24                 | ND/28.7                 | BDLd                     |
| Dried Muscle        | 10.2/5.73             | ND/5.80                   | 17.4/10.3                 | 1.18/0.59                 | ND/61.7                 | BDLd                     |
| Fried Liver         | 7.84/4.42             | ND/4.91                   | 43.6/25.9                 | 3.33/1.66                 | ND/97.0                 | 3044/913                 | <0.01/0.01               |
| Raw Liver           | 9.91/5.59             | ND/7.30                   | 45.3/26.8                 | 3.98/1.99                 | ND/162                  | 3500/1050                | 0.01/0.01                |
| Raw Kidney          | 7.18/4.05             | ND/4.23                   | 14.0/8.30                 | 2.44/1.22                 | ND/149                  | 45.1/13.5                | 0.01/0.01                |
| Boiled Kidney       | 9.80/5.53             | ND/6.25                   | 11.8/6.96                 | 3.20/1.60                 | ND/240                  | 2.00/0.60                | <0.01/0.01               |
| **Sheefish Muscle** |                       |                           |                           |                           |                         |                          |                          |
| Raw                 | 6.61/3.73             | ND/2.92                   | 44.5/26.4                 | 0.52/0.26                 | ND/17.7                 | 0.78/0.23                | BDLc                     |
| Baked without Skin  | 5.23/2.95             | ND/2.37                   | 39.3/23.3                 | 0.41/0.20                 | ND/17.0                 | 0.89/0.27                | BDLc                     |
| Baked with Skin     | 18.4/10.4             | ND/7.90                   | 83.8/49.6                 | 1.33/0.66                 | ND/20.0                 | 1.33/0.40                | BDLc                     |
| Dried without Skin  | 13.7/7.72             | ND/6.20                   | 86.3/51.1                 | 1.02/0.51                 | ND/32.7                 | 1.67/0.50                | BDLc                     |
| Dried with Skin     | 20.6/11.6             | ND/9.25                   | 108/63.7                  | 0.80/0.40                 | ND/32.0                 | 2.11/0.63                | <0.01/0.01               |
| Smoked without Skin | 7.73/4.36             | ND/3.44                   | 56.6/33.5                 | 0.65/0.33                 | ND/19.7                 | 1.89/0.57                | BDLc                     |
| Smoked with Skin    | 13.3/7.50             | ND/5.45                   | 80.6/47.8                 | 1.08/0.54                 | ND/19.0                 | 2.00/0.60                | BDLc                     |

*Bold text highlights a contribution of >100% of the minimum or maximum reference value of a given nutrient by a 100g meal of the specified tissue.

bRecommendation for trans-fatty acids is “as low as possible while consuming a nutritionally adequate diet.” No criteria for cis-monounsaturated or cis-polysaturated fatty acids exist. Thus % contributions to recommended intakes could not be calculated for these nutrients.

cDRI/UL criteria used for vitamin A and vitamin C. AMDR criteria used for total fat, omega-3 and omega-6 polyunsaturated fatty acids. DV criteria used for saturated fat and cholesterol (MND = Minimum required intake has not been determined).

dBDL = No contribution to DRI calculated because element was below detection limit in tissue. Beta carotene was BDL in all tissues.

eND = Not determined because no minimum intake criteria exists.
Tolerable daily intake limits (TDIL) for POPs are shown in Table VI. No POP exceeded TDIL in a 100 g serving of any of the seal or sheefish tissues studied. Only in blubber did any POP exceed 25% of the respective TDIL.

**DISCUSSION**

Results of the current study indicate that foods derived from spotted seal and sheefish are nutritious, containing particularly substantial amounts of omega-3 PUFA and vitamin A. Contaminants in these food items were present at relatively low levels, posing a very minimal threat to the consumer. The levels of nutrients and contaminants in the wildlife tissues were significantly affected by tissue type and food preparation method.

**Organic nutrient and contaminant concentrations**

Increasing POP concentrations were observed with increasing fat content in tissues, as would be expected for the lipophilic contaminants. Thus, blubber, both raw and rendered, consistently had the highest concentration of POPs. In general, the next highest concentrations were found for liver, followed by kidney and with the lowest concentrations found in muscle, with...
some exceptions. POP concentrations have been reported for numerous arctic marine mammal and fish species (21–23,33–37), although rarely in food processed tissues. The concentrations determined in spotted seals and sheefish were well within reported ranges for similar species and tended to be relatively low by comparison.

Changes due to food processing
In several cases, significant changes in either nutrient or contaminant concentrations of tissues resulted from food processing. These changes were determined not only on a wet weight basis, but also on a dry weight and lipid weight basis to account for concentration changes resulting from changes in basic composition such as water or lipid content. This is a critical consideration for determining risks and benefits to human consumers as the contribution to recommended daily intake criteria or TDIL is subsequently affected and potential mechanisms for compositional changes can be proposed as well.

Some analytes, while showing a significant change with processing on a ww basis, did not change significantly on a dw basis, indicating a likely concentration change due to variations in water content, as dehydration results in increased concentration. For example, cis-PUFA, omega-3 FA, omega-6 FA and cholesterol increased in seal muscle on a ww basis when dried, but showed no significant change on a dw basis in the same tissue, suggesting that the increase was a result of decreased water content. Similarly, some analytes showed significant changes with processing on a ww basis, but not on a lw basis. For example, sheefish muscle (without skin) increased significantly in dieldrin, octachlorostyrene, ΣCHL, ΣHCH and ΣCBZ when dried on a ww basis, but did not change significantly on a lw basis, indicating the ww changes observed resulted from variations in total lipid content during processing. Finally, other analytes showed significant changes on a ww, dw and lw basis, thus simple water loss or change in % lipid cannot explain the results. For example, vitamin A significantly decreased in spotted seal tissues on a ww, dw and lw basis when boiled, suggesting that the change was independent of variations in water or lipid content of the tissue and that vitamin A is actually lost during processing and may explain why vitamin A toxicosis is not a common health problem in this population or for others who consume internal organs from certain marine mammals.

It is worth noting that the presence or absence of skin on the sheefish fillets during processing affected the nutrient and contaminant concentrations in the final muscle product. With the exception of cholesterol in dried and smoked muscle and trans-FA in dried muscle, all nutrients were higher in fillets processed with skin left on versus those prepared without skin. With the exception of hexachlorobutadiene and pentachloroanisole in baked muscle, all POPs were higher in fillets baked or smoked with skin on the fillets versus their counterparts processed without skin. In contrast, muscle dried with skin left on had higher POP concentrations in all cases except ΣHCH. Thus, the presence of skin during food processing may be an important consideration when providing consumption advice for sheefish.

Concentration changes on a ww basis, along with meal size, are the most important factors when determining human intake of nutrients and contaminants, as they represent the tissue as it is actually consumed. Thus, preparation
method must be considered when assessing the nutritional benefits provided by traditional foods. By basing calculations of contributions to recommended daily intake, upper intake limits (nutrients) and TDIL (contaminants) only on concentrations in raw tissues, contributions may be grossly under- or overestimated for the actual food items consumed. These very basic findings support an “end-of-the fork” approach to assessing nutrient and contaminant risks and benefits.

**Contributions to Daily Intake**

**Reference Values**

As expected, traditionally prepared foods provide a number of nutrients at >100% of recommended daily intake per 100 g serving. In addition, these foods provide many nutrients in moderate amounts (i.e., 10–100% per serving) while others, such as vitamin C and beta carotene, are not present in any tissue at ≥10% of the recommended intake value. These results support the assertion that traditional foods represent an important, nutritious part of a balanced diet.

Certain nutrient ratios, such as omega-6 to omega-3 PUFA, are also important considerations. An optimal omega-6 to omega-3 ratio has not yet been established, but it is known that shifts from traditional diets based on fish and marine mammals to Western diets rich in saturated fats from dairy, meats and vegetable oils greatly increases omega-6 PUFA to omega-3 PUFA ratio (38). The omega-6 to omega-3 PUFA ratios found in Western diets can be as high as 10–25:1 and may increase inflammatory processes, promoting chronic diseases (39). In contrast, traditional diets based on fish and game meats provide an omega-6 to omega-3 ratio closer to 1:1 or less (38,40). In this study, the ratio in spotted seal tissues ranged from 0.15:1 (raw blubber) to 1.96:1 (boiled kidney) and from 0.07:1 to 0.15:1 in sheefish muscle. The high intake of omega-3 PUFA relative to omega-6 PUFA likely provides protection to consumers against chronic diseases such as diabetes and metabolic syndrome (41).

In addition to the health consequences posed by a lack of proper nutrition, some nutrients can become detrimental at excessive doses. Therefore, upper limits (UL) have been developed in addition to the minimal requirements. In 8 cases, spotted seal tissues exceeded the UL for a nutrient for a single serving, including total fat in rendered blubber, omega-3 PUFA in raw and rendered blubber, vitamin A in raw and fried liver and cholesterol in fried liver, raw and boiled kidney.

It should be noted that the UL are daily limits. Because of the seasonal nature of subsistence foods, it would be extremely unlikely that any given food item would be eaten every day of the year. In addition, 100 g may be an overestimate of a typical serving size for items such as blubber, which is commonly consumed as a dipping oil, and an underestimate for other tissues such as muscle. The major concern in terms of nutrient ULs based on the current data would be an excessive vitamin A intake resulting from the consumption of spotted seal liver products. Serving size and intake frequency should be kept in mind when consuming these items. For some processing methods, vitamin A was greatly reduced providing a potential mechanism for protection against excessive vitamin A intake. Interactions of nutrients with each other as well as with contaminants are other important considerations but are not within the scope of this work.
Contribution to Tolerable Daily Intake Limits (TDIL)

Although Alaskan wildlife is generally less contaminated than wildlife at lower latitudes, several contaminants are still detectable in all tissues of these animals. Contaminant levels approached TDIL in some cases, but never exceeded TDIL.

In spotted seals, all POPs were at <10% of their respective TDIL per serving of any type of muscle, liver or kidney. Only raw and rendered blubber, the tissues with the highest lipid content, contained POPs approaching TDIL in a single serving. Raw and rendered blubber contained >25% of the TDIL per serving for dieldrin, ΣPCB, ΣCHL and ΣHCH. Sheefish did not exceed 3% TDIL for any POP. The rank order of apparent risks posed by contaminants in the subsistence foods studied here are dieldrin, followed by ΣPCB, ΣCHL and ΣHCH in blubber products.

When interpreting TDIL, one must remember that these values are very conservative, they include a large safety factor and they represent the amount that can be consumed every day over an entire lifetime without risk of adverse health effects. On the one hand, because of the seasonality of subsistence foods, it is extremely unlikely that any of the food items studied here would be eaten every day of the year for an entire lifetime. On the other hand, the risks mentioned here outline only those originating from the specified individual food items. Human consumers ingest many foods and thus must also consider the intake of contaminants from multiple sources. To assess overall exposure to a human consumer would require detailed diet survey data and proper biosampling of human tissues such as serum, whole blood or subcutaneous fat.

POPs in store-bought foods

To place these findings on spotted seal and sheefish into context, we compare concentrations to what may be found in alternative store-bought foods. It is well known that store-bought foods contain a wide range of nutrient levels. But, in addition, these foods are also known to contain organic contaminants at levels that may be similar to or exceed those in some subsistence foods. For example, Hites et al. (42) found that several organic contaminants, including ΣPCB, dioxins, toxaphene and dieldrin, were consistently higher in farmed than wild salmon. Farm raised salmon on the Canadian market were found to have ΣPCB concentrations as high as 45.1 ng/g ww and 29.1 ng/g ww in farm raised trout (43). These levels exceed ΣPCB found in all sheefish samples, with the exception of two samples of muscle dried without skin which had ΣPCB levels of 34 ng/g ww. O’Hara et al. (14) also quantified POPs in store-bought foods from Barrow, AK, and found concentrations to be comparable to many marine-based subsistence foods such as fish and marine mammals. The single exception is any blubber-based subsistence food. Because lipid content significantly affects POP concentrations, blubber consistently has the highest concentration of POPs of any subsistence or store-bought food item, as there is no blubber-like alternative available commercially in AK.

Conclusions

Cooking method and tissue type can have significant effects on the concentration of nutrients and contaminants, illustrating the importance of considering the actual food items consumed when assessing the risks and benefits of a traditional diet. In addition to tissue type and food processing method, it was found that the pres-
ence or absence of skin on sheefish filets during food processing had consistent effects on nutrient and contaminant concentrations, justifying this as another important consideration when providing fish consumption advice.

Spotted seal and sheefish tissues were abundant sources of several nutrients, particularly vitamin A in seal liver and omega-3 PUFA in seal muscle and liver and sheefish muscle. The consumption of these traditional foods does not appear to pose any significant threat of nutrients exceeding their established UL, except for the cases of vitamin A in spotted seal liver and omega-3 PUFA in blubber, which may be greatly reduced during cooking. Some preparation methods greatly reduce vitamin A content. Although certain POPs approached their respective TDIL in blubber-based food items, none exceeded TDIL. The seasonal nature of subsistence food use and the fact that blubber is frequently consumed in servings less than 100 g lead to the conclusion that the risk posed by contaminant intake resulting from these items is relatively low in most cases. Individuals should be cognizant of the levels of vitamin A in seal liver and omega-3 PUFA and certain POPs in spotted seal blubber and take the associated risks into consideration when making decisions about portion size and intake frequency of these foods for themselves and their families. But, we stress that outright avoidance is likely not a good choice because of the obvious nutritional benefits provided by these foods.

Overall, these results suggest that the traditional foods investigated provide an array of nutrients accompanied by a limited risk of contaminant exposure. Therefore, we encourage the continuation of traditional food consumption as a nutritious part of a balanced diet. The lack of certain nutrients, such as vitamin C and beta carotene, in all tissues studied underline the importance of eating a balanced and varied diet in order to meet the minimum daily intake criteria for all important nutrients. Because the current work was interpreted in terms of a 70-kg male human consumer, the data would need to be re-evaluated for other consumer cohorts, particularly children and women of childbearing age who may have different nutrient requirements or vulnerabilities to contaminants. The data presented here have been provided to and could be used by public health agencies in the future to support the development of cohort-specific consumption advice in a format that enhances communication for local residents. Finally, we encourage public health agencies to develop models or algorithms to assess overall food safety and quality for under-represented diets, such as the marine-based subsistence diet of many AK residents.

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Sara K. Moses
Institute of Arctic Biology
University of Alaska Fairbanks
P.O. Box 757000
Fairbanks
AK 99775-7000
USA
Email: skmoses@alaska.edu