ABSTRACT

Objectives. To determine inorganic nutrient and contaminant concentrations in subsistence foods consumed by Alaska Natives, concentration changes related to common preparation methods and provide a basic risk-benefit analysis for these foods.

Study design. Eleven essential and six non-essential elements were measured in foods derived from spotted seals and sheefish.

Methods. Essential nutrients in foodstuffs were compared to Daily Recommended Intake (DRI) criteria. Non-essential elements were compared to Tolerable Daily Intake Limits (TDIL). These comparisons serve as a risk-benefit analysis, not as consumption advice.

Results. Cooking altered nutrient and contaminant concentrations. Spotted seal muscle and kidney are rich in Fe and Se; liver in Cu, Fe, Mo and Se; and sheefish muscle in Se. TDIL was exceeded in a 100 g serving of seal for THg in raw and fried liver and boiled kidney; MeHg in dried muscle and raw and fried liver; Cd in raw and boiled kidney; and As in raw and rendered blubber. Arsenic exceeded TDIL in sheefish muscle. However, toxicity potential is likely reduced by the element form (i.e., organic As, inorganic Hg) and the presence of protective nutrients such as Se.

Conclusions. Preparation methods alter wildlife tissues from their raw state, significantly affecting element concentrations. Direct evaluation of actual food items is warranted to determine risk-benefit ratios of traditional diets. Traditional foods provide many essential nutrients with a very limited risk from contaminants. We encourage continued consumption of traditional foods, and urge public health agencies to develop applicable models for providing consumption advice, incorporating food processing considerations.

Keywords: spotted seal, sheefish, essential elements, heavy metals, subsistence
INTRODUCTION

Marine mammals and fish are well-known resources for subsistence users of Northwest Alaska (AK). Residents of these communities depend on these and other wildlife species for nutritional, economic, cultural and spiritual reasons. However, several issues threaten food security in this region, including contaminants, climate change, access to animals, industrial development and integration of Western culture into traditional life-styles (1,2).

Until recently, obesity, cardiovascular disease (CVD) and diabetes were rarely reported among AK Natives. Today, chronic disease is emerging as a major concern in this population. Over the past decade, the prevalence of obesity has increased among AK Natives (3,4). Diet and physical activity, both linked to subsistence activities, are key factors in the prevention or development of obesity and other chronic diseases. Potential consequences of a shift from traditional subsistence-based diets to Western store-bought foods include decreased nutritive value and increased risk of obesity, diabetes and CVD. Biomedical professionals have documented that negative impacts have, or will likely, result (5–9), but these relationships are largely unknown.

Changes to traditional diets in AK result from both local and global factors. Local choices are significantly altered by the availability of store-bought foods, which can often be less nutritious than subsistence alternatives. At the same time, global sources are “contaminating” the arctic food chain with various chemicals (e.g., chlorinated pesticides, heavy metals, radioisotopes). Although known health benefits are associated with the consumption of traditional foods, there is concern about the presence of environmental contaminants.

Northwest AK receives contaminants from both local and global sources. These contaminants have been detected in fish, wildlife and local store-bought foods (10–12). Although the impact on local health has not been fully determined, fear of contaminants may be steering residents away from traditionally healthy subsistence diets to store-bought, processed foods (13–15). It has been suggested that changes in diet as a result of such fears may be more harmful than the contaminants themselves (16). Further study must be completed before these relationships can be soundly determined.

Although reports document the presence of contaminants in wildlife tissues, they are incomplete in many ways. Few account for the nutritive value of the tissues in which the contaminants are measured or how food processing affects chemical composition. Most wildlife contaminants studies have focused on tissues that are convenient to sample or biomagnify contaminants and are not conducted from the perspective of a consumer (i.e., do not included tissues specifically utilized as food). Nutrients have rarely been addressed, or they have been addressed only in raw tissues for basic nutrients. Although some did examine nutrients and contaminants in subsistence use (17–22), these studies dealt primarily with the North Slope of AK and did not focus on the consumer. Studies on actual marine food items, as they are consumed by AK Natives, are limited (12,23). Without these data, intake of contaminants and nutrients cannot be adequately estimated for subsistence communities consuming these foods.

Here, we examine nutrients and contaminants in two species commonly consumed
by subsistence users in Kotzebue, AK. We expand previous studies by evaluating additional tissues (foods) and including the effects of food processing. Both animal health and human intake perspectives are employed by intensively examining animals taken and consumed by subsistence hunters. We emphasize that animal health and human health are intimately linked in this scenario. The unique focus of this study is measuring contaminants and nutrients in an integrated fashion, utilizing both the raw product and the actual food items consumed. We evaluate changes in food composition as a result of various preparation methods. Such research is necessary to provide the balanced information regarding nutrients and contaminants that is needed to develop integrated, quantitative models that public health officials can use for effective interventions based on actual food items consumed. The information presented here is intended to serve as a basic risk-benefit analysis. We do not intend to provide consumption advice, as that is the responsibility of public health agencies.

Spotted seal (*Phoca largha*) and sheefish (*Stenodus leucichtys*) were selected based on availability of subsistence animals with input from local project participants, the community and hunters regarding the most frequently consumed species. Fish and marine mammals comprise the majority of subsistence harvested foods in Kotzebue. The Native Village of Kotzebue 2002–2004 Harvest Survey (24) reports that fish represent 27% of the subsistence harvest by weight, with sheefish making up 45% of the total fish harvest. Marine mammals account for another 26% of the total harvest by weight, with ice seals (spotted, ringed, bearded) accounting for 98% of this harvest.

**MATERIAL AND METHODS**

**Sample collection**

Samples were collected in October 2004 and March 2005 at Kotzebue, AK (66.90°N, 162.59°W) under Marine Mammal Health and Stranding Response Program (MMHSRP) permit #932-1489-05. Blubber, muscle, liver and kidney samples from spotted seals (*Phoca largha*; n=5) and muscle from sheefish (*Stenodus leucichtys*; n=8) were collected from legally subsistence harvested animals for chemical analyses using Whirl-Pak or Scienceware polyethylene bags. Blubber and liver subsamples were provided to the Alaska Marine Mammal Tissue Archival Project (AMMTAP) according to the methods of Becker et al. (5). Skin samples (1 cm²) were provided to the Alaska Department of Fish and Game Arctic Marine Mammal Program for genetic analyses, including confirmation of species identification.

All animals were assessed for gross general health prior to sampling to allow for data interpretation in the context of animal condition. Collection of nutrient and contaminant samples was performed as previously described (9). Samples were immediately frozen at -20°C, shipped to the University of Alaska Fairbanks (UAF) and stored at -80°C until analysis.

**Morphometrics and age estimation**

Spotted seal and sheefish morphometrics appear in Table I. Seal length was measured as the straight line distance from the tip of the nose to both the base and the tip of the tail. Girth was measured at the axillary and umbilical positions. Blubber thickness was measured as the distance from below the epidermis to the blubber-muscle interface at the axillary

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and umbilical positions via an incision along the ventral midline from the thoracic inlet to anus. Sheefish length was measured as the straight line distance from tip of mandible to fork of tail. Sex and body mass were determined for both species.

Seal age was estimated by counting annual growth layers in the cementum of teeth as described by Dehn et al. (17). Sheefish were aged by counting otolith annual growth increments as described in Brown et al. (26). Each slide was read in triplicate by each of three independent readers.

**Food processing**

A portion of each tissue was “food processed” to mimic traditional cooking methods.

**Spotted Seal**

Spotted seal blubber (ventral midline) was rendered to produce oil. 125 g of full thickness blubber from each individual was wrapped in cheese cloth and suspended within a 1,000 mL glass beaker at room temperature. Oil was allowed to drip from the blubber until no further appreciable oil was produced (approximately 30 days). Rendered oil was transferred to 40 mL clear borosilicate trace clean I-Chem vials (Chase Scientific Glass) and stored at -20°C. Muscle and kidney were boiled by placing approximately 125 g into 600 mL of ultrapure water in a 1000 mL glass beaker, and boiling for 20 minutes on a VWR model 355 hotplate. Muscle was also dried by placing 125 g of muscle strips (1x3x15 cm) in a Precision model

**Table 1.** Animal identification (ID), AMMTAP* ID, harvest date, sex, age, mass, length, girth and blubber thickness of spotted seals and sheefish sampled in Kotzebue, Alaska (2004–2005).

| Animal ID | Species | AMMTAP ID | Harvest date | Sex | Age [median (range)] | Mass (kg) | Length (cm)* | Girth (cm)^ | Blubber thickness (cm)^ |
|-----------|---------|-----------|--------------|-----|---------------------|-----------|--------------|-------------|------------------------|
| KOTZ-01-04 | Spotted Seal | 692-SPSL-015 | 25-Oct-2004 | Male | 6 (3–8) | 95.2 | 122/129 | 92/90 | 5.5 / 5.2 |
| KOTZ-02-04 | Spotted Seal | 692-SPSL-016 | 25-Oct-2004 | Female | 5 (4–6) | 87.1 | 119/125 | 93/80 | 3.5 / 3.6 |
| KOTZ-03-04 | Spotted Seal | 692-SPSL-017 | 25-Oct-2004 | Male | 5 (4–7) | 105.2 | 131/137 | 98/89 | 5.2 / 6.0 |
| KOTZ-04-04 | Spotted Seal | 692-SPSL-018 | 25-Oct-2004 | Male | 6 (5–8) | 57.0 | NA^ | NA^ | 4.0 / 4.0 |
| KOTZ-05-04 | Spotted Seal | 692-SPSL-019 | 25-Oct-2004 | Male | 3 (2–4) | 60.3 | 106/115 | 70/68 | 4.4 / 4.8 |
| KOTZ-01-05 | Sheefish | NA | 22-Mar-2005 | Male | 14 (14–19) | 5.2 | 83.0 | NA | NA |
| KOTZ-02-05 | Sheefish | NA | 22-Mar-2005 | Male | 15 (15–18) | 5.0 | 79.9 | NA | NA |
| KOTZ-03-05 | Sheefish | NA | 22-Mar-2005 | Female | 20 (19–21) | 5.2 | 81.0 | NA | NA |
| KOTZ-04-05 | Sheefish | NA | 22-Mar-2005 | Male | 22 (22–25) | 5.5 | 83.1 | NA | NA |
| KOTZ-05-05 | Sheefish | NA | 22-Mar-2005 | Female | 20 (19–23) | 6.5 | 87.7 | NA | NA |
| KOTZ-06-05 | Sheefish | NA | 22-Mar-2005 | Female | 22 (20–25) | 6.7 | 90.1 | NA | NA |
| KOTZ-07-05 | Sheefish | NA | 22-Mar-2005 | Female | 23 (23–23) | 4.8 | 86.6 | NA | NA |
| KOTZ-08-05 | Sheefish | NA | 22-Mar-2005 | Female | 17 (17–18) | 5.6 | 79.8 | NA | NA |

*Alaska Marine Mammal Tissue Archival Project (AMMTAP).

*Spotted seal length is the straight line distance from tip of nose to base of tail/tip of the tail. Sheefish length was measured from tip of mandible to fork of tail.

*Girth is reported here as axillary/umbilical (cm).

*Blubber thickness is reported here as axillary/umbilical (cm), measured along the ventral midline.

*No length or girth measurements available because body was distorted during transport and storage (could not be appropriately positioned for measurement).
45EG gravity convection oven for 12 hours at 65°C. Liver was fried by placing 14 g of butter into a stainless steel frying pan and heating it on a VWR model 355 hot plate on the highest setting. When the butter was melted, 125 g of liver was placed in the pan for ten minutes, flipping it every two minutes with a stainless steel spoon. Processed muscle, liver and kidney were subsampled and stored at -80°C until analysis.

Sheefish
Sheefish muscle was baked, dried and smoked both with and without skin on the filets. Baking was carried out at 425°F (218°C) for 20 minutes in a conventional oven (Kenmore Model 911.9349180). Muscle was dried as described above for seals. Finally, muscle was smoked with 50/50 mesquite/hickory wood chips in an electric smoker (Brinkman Model 810-7080-K) for two hours. Processed sheefish muscle was subsampled and stored at -80°C until analysis.

Elements analyses
Raw and food processed tissues were analysed for essential elements [calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), potassium (K), selenium (Se), sodium (Na), zinc (Zn)] and non-essential elements [arsenic (As), cadmium (Cd), lead (Pb), total mercury (THg), methyl mercury (MeHg), silver (Ag)]. Elements in tissues are reported on a ppm (µg/g) or ppb (ng/g) ww basis.

Elements were analysed at Texas A&M University (TAMU) and/or the UAF according to U.S. Environmental Protection Agency (EPA) procedures (27) as previously described (18) with minor modifications. Briefly, 0.8 g (ww) of homogenized, subsampled tissue was digested by a microwave procedure using nitric acid (HNO₃), hydrogen peroxide (H₂O₂) and hydrochloric acid (HCl). In preparation for Se analysis, an aliquot of each digest was heated (95°C, 1 hour) with excess HCl to reduce all Se(VI) to Se(IV).

Ag, As, Cd, Cr, Cu, Mn, Mo and Pb in seals and Ag, As, Cd, Cr, Cu, Fe, Mn, Mo, Pb, Se and Zn in sheefish were analysed at TAMU using a Perkin Elmer Sciex Elan Model 6100 DRC-II inductively coupled plasma mass spectrometer (ICP-MS). Ca, Fe, K, Mg, Na and Zn in seal samples were analysed at TAMU using a Spectro Ciros Vision ICP optical emissions spectrometer (ICP-OES). Ca, K, Mg, and Na in sheefish were analysed at the UAF on a Perkin Elmer A Analyst 800 atomic absorption spectrometer (AAS) using flame ionization. Finally, Se in seals was determined at TAMU by hydride generation with a PSA Millennium System atomic fluorescence (AF) detector.

An aliquot of each digest was diluted 1:4 with 7% HCl for THg analysis. THg in seal tissues was measured at TAMU with a CETAC Quick Trace Mercury Analyzer. THg in sheefish was analysed at the UAF using a purge-and-burn technique with cold vapour atomic fluorescence spectrometric (CVAFS) detection on an Amalgamation Control Module equipped with a Model III detector (Brooks Rand) as previously described (10,11,17,18).

MeHg analyses of seals and sheefish were carried out at the UAF using a purge-and-burn technique with CVAFS detection, as established previously by Bloom (28). In short, approximately 0.25 g dry weight (dw) of tissue was digested in 25% KOH in methanol. Aqueous phase ethylation was activated using 1% sodium tetraethylborate (NaB(C₂H₅)₄) to
produce methylethyl mercury. Methylethyl mercury was purged from solution with N₂ gas and collected on Tenax® traps (Brooks Rand). MeHg was thermally desorbed from the traps, analogs separated using isothermal (100°C) gas chromatography, and detected via CVAFS on a Model III detector.

The proportion of THg present as MeHg (%MeHg) in each tissue was determined according to the equation:

\[ \% \text{MeHg} = \frac{\text{MeHg (ng/g)}}{\text{THg (ng/g)}} \times 100 \%
\]

**Detection limits**

The minimum detection limit (MDL) for each element was determined in terms of tissue ww concentrations. In spotted seal tissues, the MDL (ng/g) were 500 (Ca, Mg, K, Na), 125 (Fe), 50 (Cu, Zn), 25 (Mo), 12.5 (Cr, As), 10 (Mn, Se), 8 (THg), 7 (MeHg), 5 (Cd), 2.5 (Ag) and 1.25 (Pb). In sheefish tissues, the MDL (ng/g) were 200,000 (Na), 4,000 (Ca), 3,000 (Mg), 750 (K), 200 (Fe), 80 (Cu, Mn, Zn), 40 (Mo), 20 (Cr, Se, As, Pb), 8 (Cd), 7 (MeHg) and 4 (THg, Ag).

**QA/QC**

Element analyses were held to strict QA/QC standards to assure the accuracy and precision of the results. For every twelve samples, at least five QA/QC samples were run, including a method blank, method blank spike, sample spike, sample duplicate and standard reference materials (SRM). SRM were DOLT-2 dogfish liver tissue (Institute for Environmental Chemistry, National Research Council), 1577b Bovine Liver and 1946 Lake Superior Fish Tissue (National Institute of Standards and Technology). Method blanks were held <10% of MDL for the element analysed. Method blank spikes, sample spikes and SRM were kept within 80–120% recovery. Sample duplicate validation criterion was percent difference <20%.

**Calculations and statistics**

**Concentration changes due to food processing**

Element concentrations were determined in tissues before and after food processing, as described above. Concentration changes due to food processing were calculated as:

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

where \( C_r \) is the concentration (ppm) of the raw tissue, and \( C_p \) is the concentration (ppm) 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. To account for changes in water content during food processing, absolute and percent change were calculated on both a ww and dw basis. Significance was determined using a paired t-test (p<0.05). Water content was determined by freeze drying a 1 g sample to a constant mass on a Labconco FreeZone 4.5 L Benchtop Freeze Dryer.

**Daily recommended intakes (DRI) and upper limits (UL) for essential elements**

Essential element concentrations (ww) in raw and food processed tissues were compared to Daily Recommended Intakes (DRI) and Upper Limits (UL) (29). The contribution (%) of one serving (100 g) of each food product to element DRI/UL was determined. Serving size was chosen as an intermediate to the serving sizes for meats and fish established by the United
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States Department of Agriculture (57–85 g) and the Food and Drug Administration (140 g). K and Na were not included in this analysis since these macronutrients do not have DRIs. The reference group for DRI/UL 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 non-essential elements*

Non-essential element concentrations (ww) in raw and processed tissues were compared to the provisional tolerable daily intake (PTDI) for As, Cd, Pb, THg and MeHg and to the reference dose (RfD) established by the U.S. EPA for Ag (0–4).

Assuming a reference consumer body weight (BW) of 70 kg, tolerable daily intake limits (TDIL) of food products for each non-essential element were calculated according to:

\[
TDIL (g) = \frac{PTDI \text{ or } RfD (\mu g/kg/day) \times BW (kg)}{C_t (\mu g/g, \text{ww})}
\]

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 safely eat daily throughout their entire lifespan without risk of adverse effect from a given contaminant.

**RESULTS**

**Essential and non-essential element concentrations and changes due to food processing**

Concentrations of essential (Ca, Cr, Cu, Fe, Mg, Mn, Mo, K, Se, Na, Zn) and non-essential (As, Cd, Pb, THg, MeHg, Ag) elements in raw and food processed spotted seal and sheefish tissues are summarized in Tables II and III. Changes in element concentrations as a result of food processing on a ww and dw basis are shown in Tables IV and V. Statistically significant changes ranged from -96.4% (K with rendering of seal blubber) to +217% (As with drying of seal muscle) on a ww basis and from -96.4% (K with rendering of seal blubber) to -13.9% (Mg with frying of seal liver) on a dw basis.

**Contribution to daily reference intakes (DRI) and upper limits (UL)**

The contribution of a 100 g serving of each food product to the DRI for essential elements is shown in Table VI. Elements present at >100% of DRI in spotted seal tissues included Cu and Mo in raw and fried liver, and Fe and Se in all raw and processed muscle, liver and kidney. In sheefish, Se exceeded 100% in muscle dried with skin.

In two cases tissues exceeded the UL for an element for a single serving. Dried seal muscle contributes 122% of the UL for Fe. Raw kidney provides 132% of the UL for Se. No element exceeded the UL in a serving of sheefish prepared by any method investigated.

**Contribution to tolerable daily intake limit (TDIL)**

Tolerable daily intake levels (TDIL) for non-essential elements are shown in Table VII. In spotted seal tissues, elements present above the TDIL in a 100 g serving were THg in raw and fried liver, MeHg in dried muscle and raw and fried liver, Cd in raw and boiled kidney, and As in raw and rendered blubber. In sheefish muscle, As was above the TDIL in all raw and processed samples.
Table IIa. Mean (±SD), median and range of essential elements (ppm\textsuperscript{a} or ppb\textsuperscript{b} ww) in various raw and processed tissues of spotted seals (n=5) harvested in Kotzebue, Alaska (2004)\textsuperscript{c}.

| Element | Blubber raw | Blubber rendered | Muscle raw | Muscle boiled | Muscle dried | Liver raw | Liver fried | Kidney raw | Kidney boiled |
|---------|-------------|------------------|------------|---------------|--------------|-----------|-------------|------------|---------------|
| Ca\textsuperscript{a} | 7.34 (±0.60) | 1.09 (±0.16) | 32.0 (±4.8) | 35.1 (±8.2) | 85.0 (±15.8) | 36.8 (±4.2) | 67.6 (±25.2) | 65.1 (±2.4) | 73.3 (±22.9) |
| Cu\textsuperscript{b} | 0.12 (±0.01) | 1.30 (±0.20) | 1.48 (±0.22) | 3.13 (±0.75) | 16.4 (±8.6) | 17.4 (±7.2) | 4.09 (±0.42) | 3.52 (±1.83) |
| Fe\textsuperscript{a} | 4.83 (±2.80) | 204 (±53) | 271 (±74) | 549 (±146) | 392 (±151) | 43.1 (±149) | 136 (±30) | 182 (±39) |
| Mg\textsuperscript{b} | 8.10 (±0.91) | 1.13 (±0.39) | 233 (±12) | 214 (±25) | 602 (±115) | 200 (±9) | 225 (±15) | 154 (±10) | 141 (±47) |
| Mn\textsuperscript{b} | 38.4 (±2.3) | 16.7 (±2.1) | 169 (±48) | 173 (±31) | 381 (±12) | 5306 (±469) | 6408 (±407) | 1066 (±92) | 1027 (±359) |
| Mo\textsuperscript{b} | 138 (±25) | 4.4 (±10.0) | 649 (±191) | 700 (±202) | 1655 (±606) | 2992 (±1010) | 3806 (±607) | 5274 (±652) | 3776 (±1407) |
| K\textsuperscript{a} | 140 (±35) | 4.66 (±1.62) | 3234 (±253) | 2120 (±377) | 8922 (±1628) | 3084 (±246) | 3172 (±325) | 2568 (±211) | 1344 (±401) |
| Se\textsuperscript{b} | 1.4 (±10.0) | 0.26 (±19) | 700 (±202) | 1655 (±606) | 2992 (±1010) | 3806 (±607) | 5274 (±652) | 3776 (±1407) |
| Na\textsuperscript{a} | 150 (±13) | 48.1 (±5.2) | 569 (±129) | 357 (±78) | 1592 (±326) | 883 (±69) | 1044 (±186) | 1058 (±133) | 1036 (±337) |
| Zn\textsuperscript{b} | 3.30 (±0.68) | 1.82 (0.54) | 19.6 (±3.6) | 27.6 (±5.8) | 54.4 (±10.0) | 42.1 (±4.8) | 54.3 (±3.1) | 26.2 (±1.4) | 35.8 (±11.5) |

\textsuperscript{a}Ca, Cu, Fe, Mg, K, Na and Zn are reported in ppm ww.
\textsuperscript{b}Mn, Mo and Se are reported in ppb ww.
\textsuperscript{c}BDL = Below Detection Limit. All samples were BDL for Cr.
### Table IIb. Mean (±SD), median and range of essential elements (ppm<sup>a</sup> or ppb<sup>b</sup> ww) in raw and processed muscle tissue of sheefish (n=8) harvested in Kotzebue, Alaska (2005)<sup>c</sup>.

| Element | Raw | Baked no skin | Baked with skin | Dried no Skin | Dried with Skin | Smoked no Skin | Smoked with Skin |
|---------|-----|---------------|----------------|---------------|----------------|---------------|-----------------|
| Ca<sup>a</sup> | 32.6 (±4.0) | 15.4 (±5.8) | 12.3 (±4.5) | 43.6 (±64.6) | 100 (±39) | 13.0 (±4.8) | 21.2 (±24.3) |
| Cu<sup>b</sup> | 21.2 | 15.2 | 11.4 | 20.3 | 103 | 12.0 | 13.2 |
| Mn<sup>b</sup> | 5.9-129 | 6.8-23.4 | 7.9-22.1 | 16.3-203 | 20.2-303 | 7.8-23.1 | 8.7-81.1 |
| Mg<sup>a</sup> | 323 (±96) | 480 (±199) | 394 (±130) | 675 (±296) | 836 (±904) | 482 (±141) | 430 (±165) |
| Mn<sup>b</sup> | 304 | 430 | 352 | 614 | 468 | 460 | 415 |
| Mn<sup>b</sup> | 246-544 | 245-731 | 262-623 | 385-1151 | 365-3007 | 309-716 | 239-770 |
| Fe<sup>a</sup> | 3.75 (±1.13) | 3.39 (±3.75) | 6.51 (±3.13) | 4.35 (±2.15) | 3.30 (±2.19) | 3.39 (±1.40) |
| Mn<sup>b</sup> | 2.85 (±1.11) | 2.45 | 1.92-5.20 | 1.44-5.67 | 2.22-13.5 | 2.18-11.7 | 3.11-9.58 |
| Mn<sup>b</sup> | 414 (±58) | 431 (±74) | 389 (±51) | 704 (±162) | 815 (±151) | 511 (±136) | 374 (±33) |
| Mn<sup>b</sup> | 385 | 435 | 386 | 751 | 843 | 529 | 373 |
| Mn<sup>b</sup> | 368-538 | 279-509 | 318-462 | 431-875 | 601-1067 | 353-736 | 337-432 |
| Mn<sup>b</sup> | 188 (±56) | 219 (±43) | 208 (±40) | 307 (±82) | 386 (±227) | 252 (±66) | 253 (±20) |
| Mn<sup>b</sup> | 145 | 232 | 211 | 298 | 270 | 237 | 253 |
| Mn<sup>b</sup> | 123-300 | 144-264 | 160-278 | 179-416 | 213-883 | 186-378 | 211-274 |
| Mn<sup>b</sup> | 5486 (±379) | 5760 (±550) | 5394 (±587) | 9474 (±1343) | 10968 (±1860) | 6884 (±1139) | 6499 (±1407) |
| Mn<sup>b</sup> | 5536 | 5760 | 5394 | 9474 | 10968 | 6884 | 6499 |
| Mn<sup>b</sup> | 4881-5957 | 5167-6676 | 4802-6394 | 7495-11301 | 8708-14595 | 5510-9163 | 5100-9182 |
| Mn<sup>b</sup> | 293 (±30) | 320 (±36) | 301 (±32) | 513 (±134) | 584 (±50) | 385 (±47) | 368 (±42) |
| Mn<sup>b</sup> | 291 | 317 | 295 | 474 | 594 | 372 | 362 |
| Mn<sup>b</sup> | 265-344 | 277-379 | 251-354 | 347-730 | 505-655 | 333-476 | 310-452 |
| Mn<sup>b</sup> | 565 (±118) | 658 (±180) | 623 (±168) | 1063 (±328) | 1026 (±319) | 640 (±158) | 610 (±143) |
| Mn<sup>b</sup> | 605 | 607 | 565 | 989 | 981 | 603 | 609 |
| Mn<sup>b</sup> | 414-704 | 449-920 | 439-856 | 765-1825 | 642-1717 | 493-980 | 360-825 |
| Mn<sup>b</sup> | 3.81 (±0.34) | 4.83 (±0.79) | 4.40 (±0.55) | 7.87 (±1.61) | 8.07 (±1.35) | 5.07 (±0.54) | 5.27 (±0.94) |
| Mn<sup>b</sup> | 3.81 | 4.63 | 4.20 | 8.34 | 7.62 | 5.29 | 4.93 |
| Mn<sup>b</sup> | 3.47-4.47 | 4.01-6.15 | 3.78-5.42 | 4.95-9.52 | 6.73-10.4 | 4.24-5.64 | 4.31-7.26 |

<sup>a</sup>Ca, Fe, Mg, K, Na and Zn are reported in ppm ww.  
<sup>b</sup>Cu, Mn and Se are reported in ppb ww.  
<sup>c</sup>BDL = Below Detection Limit. All samples were BDL for Cr and Mo.
Table III. Mean (±SD), median and range of non-essential elements (ppb ww) in various raw and processed tissues of spotted seals (n=5) and sheefish (n=8) harvested in Kotzebue, Alaska (2004–2005).

|                | THg   | MeHg  | %MeHg | Cd    | As    | Ag    | Pb    |
|----------------|-------|-------|--------|-------|-------|-------|-------|
| **Spotted Seal** |       |       |        |       |       |       |       |
| Blubber        | BDL<sup>a</sup> | BDL<sup>a</sup> | -----  | BDL<sup>a</sup> | 2704 (±254) | 41.5 (±13.4) |
| Raw            |       |       |        |       |       |       |       |
| Spotted Seal   | 2580 (±348) | 33.1 (±9.3) |       |       |       |       |       |
| Blubber        | BDL<sup>a</sup> | BDL<sup>a</sup> | -----  | BDL<sup>a</sup> | 2570 | BDL<sup>a</sup> |
| Rendered       | 2110-3070 | 25.3-49.3 |       |       |       |       |       |
| **Spotted Seal** |       |       |        |       |       |       |       |
| Blubber        | BDL<sup>a</sup> | BDL<sup>a</sup> | -----  | BDL<sup>a</sup> | 182 (±46) | 149 (±17) | 86.4 (±23.9) | 197 (±70) | 5.73 (±0.95) |
| Raw            | 2410-3060 | 26.1-60.2 |       |       |       |       |       |
| Spotted Seal   | 2580 (±348) | 33.1 (±9.3) |       |       |       |       |       |
| Blubber        | BDL<sup>a</sup> | BDL<sup>a</sup> | -----  | BDL<sup>a</sup> | 2630 | BDL<sup>a</sup> |
| Rendered       | 2110-3070 | 25.3-49.3 |       |       |       |       |       |
| **Sheefish**   |       |       |        |       |       |       |       |
| Muscle         | 196 | 146 | 72.0 | BDL<sup>a</sup> | 172 | BDL<sup>a</sup> |
| Raw            | 133-242 | 128-172 | 64.9-119 | 141-319 |       |       |
| Spotted Seal   | 261 (±74) | 179 (±42) | 71.2 (±21.0) | 200 (±21) | 200 (±24) | 4.34 (±0.89) |
| Muscle         | 294 | 182 | 61.4 | BDL<sup>a</sup> | 209 | BDL<sup>a</sup> |
| Raw            | 161-343 | 112-226 | 57.8-108 | 175-219 |       |       |
| Spotted Seal   | 406 (±111) | 415 (±125) | 103 (±17) | 583 (±249) | 61.8 (±14) |       |
| Muscle         | 413 | 430 | 110 | BDL<sup>a</sup> | 532 | BDL<sup>a</sup> |
| Raw            | 248-559 | 289-596 | 73.0-116 | 370-991 |       |       |
| Spotted Seal   | 1991 (±1170) | 314 (±35) | 22.9 (±15.2) | 478 (±155) | 415 (±154) | 43.0 (±1.1) | 10.5 (±4.4) |
| Liver          | 2500 | 326 | 13.6 | 374 | 362 | 44.1 |       |
| Raw            | 615-3140 | 253-340 | 10.4-41.1 | 349-671 | 304-686 | 20.9-69.3 | 9.4-68.9 |
| Spotted Seal   | 2510 (±1524) | 415 (±60) | 26.4 (±21.3) | 456 (±111) | 557 (±159) | 61.8 (±32.1) | 16.1 (±9.8) |
| Liver          | 3440 | 442 | 12.9 | 440 | 493 | 60.6 |       |
| Boiled         | 835-3730 | 336-482 | 9.91-57.8 | 351-626 | 432-828 | 24.9-112 | 16.5 (±4.4) |
| **Sheefish**   |       |       |        |       |       |       |       |
| Muscle         | 444 | 83.9 | 13.2 | 3488 (±538) | 281 (±58) | 16.5 (±4.4) |       |
| Kidney         | 437 | 84.4 | 19.0 | 3740 | 296 | BDL<sup>a</sup> |       |
| Raw            | 336-661 | 63.8-99.8 | 13.6-29.4 | 2830-4040 | 182-333 | 11.1-21.3 |       |
| Spotted Seal   | 576 (±200) | 113 (±26) | 20.4 (±4.4) | 2417 (±1273) | 541 (±559) | 7.79 (±1.45) |       |
| Kidney         | 526 | 100 | 21.1 | 2270 | 279 | BDL<sup>a</sup> |       |
| Boiled         | 399-908 | 90.9-151 | 15.2-25.1 | 614-4090 | 245-1540 | 7.47 |       |
| **Sheefish**   |       |       |        |       |       |       |       |
| Muscle         | 87.7 (±34.5) | 69.0 (±23.9) | 80.0 (±13.9) | 6236 (±3030) |       |       |
| Raw            | 76.7 | 64.5 | 76.2 | BDL<sup>a</sup> | 6466 | BDL<sup>a</sup> |       |
| Sheefish       | 62.5-169 | 45.5-117 | 64.1-99.8 | 2059-10547 |       |       |
| Muscle         | 94.8 | 87.7 | 85.5 | BDL<sup>a</sup> | 6147 | BDL<sup>a</sup> |       |
| Baked (No Skin)| 58.2-105 | 66.3-160 | 76.2-148 | 1979-8309 |       |       |
| Sheefish       | 102 (±40) | 91.9 (±35.4) | 90.0 (±7.3) | 5810 (±2949) |       |       |
| Muscle         | 96.9 | 81.7 | 89.6 | BDL<sup>a</sup> | 6086 | BDL<sup>a</sup> |       |
| Baked (With Skin)| 62.5-191 | 56.4-169 | 79.7-98.5 | 1695-10455 |       |       |
| Sheefish       | 158 (±63) | 161 (±66) | 104 (±17) | 10175 (±4863) |       |       |
| Muscle         | 133 | 159 | 108 | BDL<sup>a</sup> | 10978 | BDL<sup>a</sup> |       |
| Dried (No Skin)| 97.8-268 | 102-306 | 75.7-125 | 3326-16697 |       |       |
| Sheefish       | 161 (±66) | 156 (±46) | 99.8 (±15.2) | 10945 (±5720) |       |       |
| Muscle         | 140 | 153 | 97.5 | BDL<sup>a</sup> | 11543 | BDL<sup>a</sup> |       |
| Dried (With Skin)| 106-311 | 94.5-256 | 82.3-123 | 2816-18962 |       |       |
| Sheefish       | 108 (±39) | 85.6 (±28.5) | 80.6 (±14.6) | 6772 (±3658) |       |       |
| Muscle         | 101 | 83.5 | 76.5 | BDL<sup>a</sup> | 7101 | BDL<sup>a</sup> |       |
| Smoked (No Skin)| 68.4-195 | 49.1-148 | 65.9-109 | 2120-11827 |       |       |
| Sheefish       | 105 (±43) | 84.6 (±27.5) | 82.4 (±7.8) | 5735 (±2646) |       |       |
| Muscle         | 95.8 | 80.0 | 84.8 | BDL<sup>a</sup> | 5819 | BDL<sup>a</sup> |       |
| Smoked (With Skin)| 70.0-198 | 57.4-135 | 68.4-92.2 | 2486-10272 |       |       |

<sup>a</sup>BDL = Below Detection Limit.
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Table IVa. Absolute (ppm\textsuperscript{a} or ppb\textsuperscript{b}) and percent change (%Δ) [mean (±1 SD)]\textsuperscript{c} in essential and non-essential elements on a wet weight basis as a result of food processing for various tissues of spotted seals (n=5) harvested in Kotzebue, Alaska (2004)\textsuperscript{d}.

| Element | Blubber Δ with rendering | Muscle Δ with boiling | Muscle Δ with drying | Liver Δ with frying | Kidney Δ with boiling |
|---------|--------------------------|----------------------|---------------------|---------------------|----------------------|
| Ca      | -6.25 (±0.69)            | +3.18 (±6.73)        | +53.0 (±16.5)       | +30.8 (±23.1)       | +8.20 (±21.4)        |
| Cu      | -85.0 (±3.1)%            | +9.9 (±22.7)%        | +170 (±59)%         | +81.8 (±55.7)%      | +12.0 (±33.4)%       |
| Fe      | -2.88 (±0.55)            | +67.0 (±33.1)        | +26.9 (±6.7)        | +20.8 (±57.6)%      | -14.2 (±41.4)%       |
| Mg      | -89.7 (±5.0)%            | +33.5 (±14.1)%       | +170.8 (±36.4)      | +43.4 (±119.8)%     | +36.7 (±31.8)%       |
| Mn      | -6.97 (±1.10)            | -19.2 (±31.6)        | +369 (±112)         | +24.8 (±20.2)       | +12.3 (±40.2)        |
| Mo      | -58.6 (±2.7)             | +4.80 (±25.5)        | +212 (±106)         | +1102 (±464)        | -38.8 (±299)         |
| K       | -135 (±36)               | -1114 (±523)         | +5688 (±1785)       | +88.0 (±464)        | -1224 (±420)         |
| Se      | -6.94 (±2.0)%            | -33.9 (±14.1)%       | +179 (±64)%         | +3.5 (±15.0)%       | -47.5 (±16.4)%       |
| Na      | -103 (±11)               | -212 (±133)          | +1007 (±487)        | +814 (±387)         | -1498 (±1849)        |
| K       | -68.0 (±3.2)%            | -35.1 (±17.0)%       | +185 (±52)%         | +18.8 (±22.3)%      | -43.6 (±19.8)%       |
| Zn      | -1.48 (±0.99)            | -9.80 (±5.65)        | +34.8 (±12.0)       | +12.2 (±7.3)        | +9.6 (±12.1)         |
| THg     | -42.2 (±23.2)%           | -44.3 (±40.4)%       | +188 (±95)%         | +30.8 (±20.1)%      | +37.7 (±45.6)%       |
| MeHg    | -124 (±26)               | +51.6 (±135)         | +1007 (±487)        | +814 (±387)         | -1498 (±1849)        |
| Cd      | -103 (±11)               | -212 (±133)          | +1007 (±487)        | +814 (±387)         | -1498 (±1849)        |
| As      | -68.0 (±3.2)%            | -35.1 (±17.0)%       | +185 (±52)%         | +18.8 (±22.3)%      | -43.6 (±19.8)%       |
| Ag      | -124 (±26)               | +51.6 (±135)         | +1007 (±487)        | +814 (±387)         | -1498 (±1849)        |
| Pb      | -8.40 (±38.3)%           | -36.1 (±22.2)%       | +46.0 (±53.7)%      | +118.0 (±191)%      | -40.1 (±26.3)%       |

\textsuperscript{a}Ca, Cu, Fe, Mg, K, Na and Zn are reported in ppm ww.
\textsuperscript{b}Mn, Mo, Se, THg, MeHg, As, Ag, Cd and Pb are reported in ppb ww.
\textsuperscript{c}Bold text represents statistically significant changes (p < 0.05).
\textsuperscript{d}NA = Not available because both raw and processed samples were below the analytical detection limit. All samples NA for Cr.
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| Element | Δ with baking (no skin) | Δ with baking (with skin) | Δ with drying (no skin) | Δ with drying (with skin) | Δ with smoking (no skin) | Δ with smoking (with skin) |
|---------|------------------------|--------------------------|------------------------|--------------------------|------------------------|--------------------------|
| Ca      | -17.1 (±37.4)          | -20.3 (±36.2)            | 11.0 (±27.1)           | 67.4 (±101)              | -19.6 (±40.5)           | -11.4 (±51.4)             |
| Cu      | +157 (±229)            | +70.5 (±185)             | +352 (±297)            | +513 (±906)              | +164 (±267)             | +62.7 (±72.6)             |
| Fe      | +899 (±1778)           | +1543 (±4352)            | +3658 (±1387)          | +1502 (±2818)            | +457 (±2091)            | +542 (±1782)              |
| Mg      | +16.7 (±75.3)          | -25.2 (±74.5)            | +290 (±154)            | +401 (±151)              | +97.5 (±134)            | -39.9 (±40.5)             |
| Mn      | +31.1 (±47.8)          | +20.0 (±35.7)            | +119 (±246)            | +198 (±219)              | +63.6 (±72.2)           | +65.3 (±52.0)             |
| K       | +274 (±751)            | -92.8 (±538)             | +3988 (±1325)          | +5482 (±1770)            | +1397 (±1181)           | +1012 (±1450)             |
| Se      | +263 (±35.2)           | +7.50 (±27.5)            | +220 (±118)            | +291 (±66)               | +91.3 (±39.7)           | +75.0 (±31.0)             |
| Na      | +93.3 (±124)           | +58.9 (±130)             | +499 (±296)            | +461 (±284)              | +75.0 (±138)            | +45.6 (±145)              |
| Zn      | +1.02 (±0.87)          | +0.59 (±0.67)            | +4.06 (±1.47)          | +4.26 (±1.56)            | +1.25 (±0.74)           | +1.46 (±0.89)             |
| THg     | +17.9 (±19.7)          | +16.9 (±11.9)            | +80.4 (±37.9)          | +83.9 (±19.2)            | +24.3 (±14.0)           | +19.2 (±15.1)             |
| MeHg    | +24.8 (±12.5)          | +22.9 (±17.1)            | +91.8 (±47.7)          | +87.0 (±33.9)            | +16.6 (±14.0)           | +15.6 (±15.2)             |
| As      | +0.97 (±1.17)          | +0.43 (±0.77)            | +3.94 (±1.97)          | +4.71 (±3.27)            | +0.54 (±1.57)           | -0.50 (±0.78)             |

*Ca, Fe, Mg, K, Na, Zn and As are reported in ppm ww.*

*Cu, Mn, Se, THg and MeHg are reported in ppb ww.*

**Bold** text represents statistically significant changes (p < 0.05).

NA = Not available because both raw and processed samples were below the analytical detection limit. All samples were NA for Cr, Mo, Cd, Ag and Pb.
Table Va. Absolute (ppm\(^a\) or ppb\(^b\)) and percent change (%Δ) [mean (±SD)]\(^c\) in essential and non-essential elements on a dry weight basis as a result of food processing for various tissues of spotted seals (n=5) harvested in Kotzebue, Alaska (2004)\(^e\).

| Element | Spotted Seal muscle Δ with Rendering\(^d\) | Spotted Seal muscle Δ with Boiling | Spotted Seal liver Δ with Frying | Spotted Seal kidney Δ with Boiling |
|---------|------------------------------------------|----------------------------------|---------------------------------|----------------------------------|
| Ca      | -6.25 (-0.69)                            | -12.1 (±21.1)%                  | +45.9 (±52.6)                  | -66.2 (±79.9)                   |
|         | -85.0 (-3.1)%                            | -5.4 (±11.9)%                   | +38.6 (±40.9)%                 | -23.6 (±27.9)%                  |
| Cu      | NA\(^e\)                                | -0.72 (±0.74)                   | -9.90 (±24.4)                  | -8.12 (±5.67)                   |
| Fe      | -2.88 (-0.55)%                           | +39.1 (±114)                    | -204 (±394)                    | -58.9 (±171)                    |
| Mn      | -89.7 (±5.0)%                            | +8.0 (±17.6)%                   | +11.9 (±99.6)%                 | -7.10 (±26.4)%                  |
| Mg      | -6.97 (-1.1)%                            | -211 (±288)                    | -89.9 (±65.6)                  | -245 (±145)                     |
| Mo      | -85.8 (-5.1)%                            | -26.1 (±8.9)%                   | -13.9 (±10.2)                  | -37.8 (±23.1)%                  |
| Na      | -56.4 (-5.5)%                            | -14.8 (±14.1)%                  | -7.36 (±8.70)                  | -34.2 (±26.1)%                  |
| K       | -135 (±36)                               | -5149 (±852)                    | -2090 (±1245)                  | -7485 (±1090)                   |
| Se      | -96.4 (-2.0)%                            | -47.3 (±8.5)%                   | -21.0 (±11.8)%                 | -66.5 (±9.9)%                   |
| Se      | -124 (-26)                               | -314 (±404)                    | -194 (±1284)                   | -12559 (±6779)                  |
| Na      | -89.5 (-6.5)%                            | -11.4 (±16.6)%                  | -1.06 (±11.2)%                 | -51.6 (±21.8)%                  |
| Mn      | -68.0 (-3.2)%                            | -48.3 (±11.4)%                  | -9.16 (±18.5)%                 | -62.6 (±13.9)%                  |
| Na      | -1.48 (-20.9)%                           | +7.31 (±20.3)                   | -2.11 (±20.5)                  | -13.9 (±44.5)                   |
| Zn      | -42.2 (-23.2)%                           | +16.7 (±35.8)%                  | +10.0 (±63.6)%                 | -10.3 (±36.2)%                  |
| Thg     | NA\(^e\)                                | +85.2 (±209)                    | -155 (±361)                    | -628 (±966)                     |
| MeHg    | NA\(^e\)                                | +20.8 (±46.5)%                  | -5.10 (±11.7)%                 | -21.6 (±35.8)%                  |
| Cd      | NA\(^e\)                                | -52.5 (±107)                    | +9.95 (±111)                   | -47.8 (±108)                    |
| Cd      | NA\(^e\)                                | -4.51 (±21.1)%                  | +1.00 (±11.0)%                 | -10.5 (±27.3)%                  |
| Cd      | NA\(^e\)                                | NA\(^e\)                       | NA\(^e\)                      | NA\(^e\)                       |
| Pb      | -8.40 (-1.83)                            | -7.75 (±6.23)                   | -9.85 (±11.4)                  | -54.5 (±25.7)                   |
| Pb      | -12.3 (-38.3)%                           | -36.1 (±22.2)%                  | -46.0 (±53.7)%                 | -68.0 (±14.7)%                  |

\(^a\)Ca, Cu, Fe, Mg, K, Na, Zn and Cd are reported in ppm dw.
\(^b\)Mn, Mo, Se, THg, MeHg, As, Ag and Pb are reported in ppb dw.
\(^c\)Bold text represents statistically significant changes (p < 0.05).
\(^d\)Changes in blubber are based on ww values which are assumed to be essentially equivalent to dw values (i.e., 0% water content).
\(^e\)NA = Not available because either raw or processed samples were below the analytical detection limit.
All samples were NA for Cr.
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Table Vb. Absolute (ppm\(^a\) or ppb\(^b\)) and percent change (%Δ) [mean (±SD)] in essential and non-essential on a dry weight basis as a result of food processing for muscle tissue of sheefish (n=8) harvested in Kotzebue, Alaska (2005)\(^c\).

| Element | Δ with baking no skin | Δ with baking with skin | Δ with drying no skin | Δ with drying with skin | Δ with smoking no skin | Δ with smoking with skin |
|---------|-----------------------|------------------------|----------------------|------------------------|-----------------------|------------------------|
| Ca      | -82.0 (±165)          | -94.6 (±159)           | -51.2 (±60.1)%       | +76.5 (±220)           | -96.2 (±175)          | -70.4 (±205)           |
|         | -22.7 (±72.6)%        | -50.4 (±27.4)%         | -31.0 (±46.1)%       | +168 (±311)%           | -48.4 (±39.9)%        | +10.6 (±159.9)%        |
| Cu      | +251 (±732)           | -62.2 (±703)           | -83.7 (±578)         | +276 (±1373)           | +121 (±776)           | -3.60 (±715)           |
|         | +30.1 (±68.0)%        | +4.88 (±51.2)%         | -1.24 (±39.2)%       | +27.0 (±101)%          | +21.9 (±61.1)%        | +5.26 (±46.7)%         |
| Fe      | +829 (±6051)          | +2462 (±14822)         | +190 (±6582)         | -2414 (±6900)          | -1601 (±7044)         | -1133 (±6536)          |
|         | +21.3 (±66.7)%        | +54.9 (±179)%          | +11.3 (±58.4)%       | -8.29 (±60.0)%         | -12.1 (±47.8)%        | -2.20 (±47.8)%         |
| Mg      | -208 (±344)           | -420 (±238)            | -353 (±274)          | +79.0 (±366)           | -150 (±423)           | -518 (±125)            |
|         | -11.8 (±20.7)%        | -23.4 (±13.5)%         | -21.4 (±16.4)%       | +4.51 (±23.0)%         | -6.83 (±26.8)%        | -31.9 (±8.3)%          |
| Mn      | -31.8 (±154)          | -92.4 (±114)           | -178 (±178)          | +56.7 (±400)           | -11.3 (±217)          | +4.85 (±230)           |
|         | -0.17 (±18.5)%        | -10.8 (±12.8)%         | -22.4 (±18.2)%       | +8.66 (±57.7)%         | +1.21 (±26.5)%        | +7.14 (±27.8)%         |
| K       | -2695 (±3377)         | -4755 (±2272)          | -4276 (±3363)        | +1363 (±4521)          | -1641 (±3733)         | -2196 (±5363)          |
|         | -11.9 (±15.0)%        | -22.5 (±11.2)%         | -19.5 (±14.8)%       | +5.40 (±21.1)%         | -7.72 (±17.6)%        | -10.6 (±24.6)%         |
| Se      | -109 (±203)           | -227 (±129)            | -231 (±168)          | +59.7 (±271)           | -45.2 (±186)          | -76.3 (±129)           |
|         | -8.3 (±16.5)%         | -19.0 (±9.2)%          | -19.8 (±15.2)%       | +6.6 (±25.5)%          | -3.3 (±16.6)%         | -5.9 (±10.4)%          |
| Na      | -100 (±445)           | -269 (±599)            | -269 (±703)          | -108 (±693)            | -391 (±487)           | -450 (±378)            |
|         | -2.95 (±17.58)%       | -11.7 (±23.0)%         | -9.56 (±30.7)%       | -1.01 (±31.38)         | -15.2 (±21.9)%        | -18.5 (±16.9)%         |
| Zn      | +0.71 (±3.19)         | -1.35 (±3.06)          | -0.78 (±3.21)        | +1.73 (±4.50)          | -0.41 (±3.12)         | +0.59 (±3.46)          |
|         | +6.27 (±22.9)%        | -8.01 (±18.5)%         | -4.10 (±22.2)%       | +13.3 (±32.8)%         | -1.35 (±21.7)%        | +4.07 (±21.3)%         |
| Thg     | -0.14 (±63.6)         | -23.2 (±50.2)          | -5.61 (±103)         | -0.26 (±56.5)          | -28.6 (±44.3)         | -29.9 (±40.6)          |
|         | -0.13 (±21.7)%        | -7.7 (±15.7)%          | -15.5 (±26.3)%       | -3.3 (±17.6)%          | -9.2 (±13.9)%         | -11.2 (±12.8)%         |
| MeHg    | +39.2 (±53.9)         | +18.6 (±71.1)          | +20.2 (±73.2)        | +59.7 (±86.7)          | -23.1 (±49.2)         | -17.9 (±58.6)          |
|         | +15.9 (±18.4)%        | +6.29 (±23.3)%         | +9.18 (±29.4)%       | +24.2 (±33.6)%         | -7.8 (±16.4)%         | -6.1 (±21.2)%          |
| As      | -6.28 (±6.51)         | -6.14 (±3.48)          | -5.33 (±3.23)        | -1.36 (±5.13)          | -4.81 (±4.44)         | -7.07 (±4.61)          |
|         | -23.0 (±17.0)%        | -27.1 (±12.8)%         | -23.8 (±10.8)%       | -9.44 (±18.9)%         | -22.1 (±16.9)%        | -28.9 (±11.0)%         |

\(^a\)Ca, Fe, Mg, K, Na, Zn and As are reported in ppm dw.

\(^b\)Cu, Mn, Se, THg and MeHg are reported in ppb dw.

\(^c\)Bold text represents statistically significant changes (p < 0.05).

\(^d\)NA = Not available because both raw and processed samples were below the analytical detection limit. All samples were NA for Cr, Mo, Cd, Ag and Pb.
Table VI. Mean percent (%) contribution of one serving (100g ww) of spotted seal (n=5) and sheefish (n=8) tissue to the Daily Reference Intake (DRI) for select essential elements.

| Species   | Tissue          | Processing  | Ca 1000 mg/day | Cu 900 μg/day | Fe 8 mg/day | Mg 420 mg/day | Mn 1.8 mg/day | Mo 45 μg/day | Se 55 μg/day | Zn 11 mg/day |
|-----------|-----------------|-------------|----------------|---------------|-------------|---------------|---------------|--------------|--------------|--------------|
| Spotted   | Blubber         | Raw         | 0.07           | 1.28          | 6.04        | 0.19          | 0.21          | BDL          | 25.1         | 3.00         |
| Spotted   | Blubber         | Rendered    | 0.01           | BDL          | 0.03        | 0.09          | BDL          | 2.62         | 17.8         |
| Spotted   | Muscle          | Raw         | 0.32           | 14.5         | BDL         | 5.54          | 0.94          | BDL          | 118          |
| Spotted   | Muscle          | Boiled      | 0.35           | 16.4         | BDL         | 5.09          | 0.96          | BDL          | 127          |
| Spotted   | Muscle          | Dried       | 0.85           | 34.7         | BDL         | 14.3          | 2.12          | BDL          | 301          |
| Spotted   | Liver           | Raw         | 0.37           | 183          | BDL         | 4.77          | 29.5          | BDL          | 123          |
| Spotted   | Liver           | Fried       | 0.68           | 193          | BDL         | 5.36          | 35.6          | BDL          | 178          |
| Spotted   | Kidney          | Raw         | 0.65           | 45.4         | BDL         | 3.66          | 5.92          | BDL          | 26.2         |
| Spotted   | Kidney          | Boiled      | 0.73           | 39.1         | BDL         | 3.37          | 5.71          | BDL          | 41.0         |
| Sheefish  | Muscle          | Raw         | 0.33           | 3.59         | BDL         | 3.56          | 9.86          | 1.05         | BDL          | 53.3         |
| Sheefish  | Muscle          | Baked (no skin) | 0.15       | 5.33         | 4.68        | 10.3          | 1.22          | BDL          | 58.2         |
| Sheefish  | Muscle          | Baked (with skin) | 0.12      | 4.38         | 5.49        | 9.26          | 1.16          | BDL          | 54.7         |
| Sheefish  | Muscle          | Dried (no skin) | 0.44      | 7.49         | 8.13        | 16.8          | 1.70          | BDL          | 93.3         |
| Sheefish  | Muscle          | Dried (with skin) | 1.00      | 9.29         | 5.44        | 19.4          | 2.15          | BDL          | 106         |
| Sheefish  | Muscle          | Smoked (no skin) | 0.13      | 5.36         | 4.13        | 12.2          | 1.40          | BDL          | 70.0         |
| Sheefish  | Muscle          | Smoked (with skin) | 0.21      | 4.77         | 4.24        | 8.90          | 1.41          | BDL          | 66.9         |

Table VII. Mean percent (%) contribution of one meal (100g ww) of spotted seal (n=5) and sheefish (n=8) tissue to the toxicological reference dose for select non-essential elements.

| Species   | Tissue          | Processing  | Thg 0.7| MeHg 0.27| Cd 1.0| As 2.1| Ag 5.0| Pb 3.57|
|-----------|-----------------|-------------|--------|----------|------|-------|-------|-------|
| Spotted   | Blubber         | Raw         | BDL    | BDL      | BDL  | (184) | BDL   | 1.7   |
| Spotted   | Blubber         | Rendered    | BDL    | BDL      | BDL  | (176) | BDL   | 1.3   |
| Spotted   | Muscle          | Raw         | 36.6   | 78.8     | BDL  | (13.4) | BDL   | 0.2   |
| Spotted   | Muscle          | Boiled      | 52.5   | 94.7     | BDL  | (13.6) | BDL   | 0.2   |
| Spotted   | Muscle          | Dried       | 81.7   | 220      | BDL  | (39.7) | BDL   | 0.3   |
| Spotted   | Liver           | Raw         | 401    | 166      | 68.3 | (28.2) | 1.2   | 0.4   |
| Spotted   | Liver           | Fried       | 505    | 220      | 65.1 | (37.9) | 1.8   | 0.6   |
| Spotted   | Kidney          | Raw         | 89.3   | 44.4     | 499  | (19.1) | BDL   | 0.7   |
| Spotted   | Kidney          | Boiled      | 116    | 59.8     | 346  | (36.8) | BDL   | 0.3   |
| Sheefish  | Muscle          | Raw         | 17.7   | 36.5     | BDL  | (424) | BDL   | BDL   |
| Sheefish  | Muscle          | Baked (no skin) | 20.7   | 49.6     | BDL  | (378) | BDL   | BDL   |
| Sheefish  | Muscle          | Baked (with skin) | 20.5   | 48.6     | BDL  | (395) | BDL   | BDL   |
| Sheefish  | Muscle          | Dried (no skin) | 31.8   | 85.2     | BDL  | (694) | BDL   | BDL   |
| Sheefish  | Muscle          | Dried (with skin) | 32.4   | 82.5     | BDL  | (748) | BDL   | BDL   |
| Sheefish  | Muscle          | Smoked (no skin) | 21.7   | 45.3     | BDL  | (461) | BDL   | BDL   |
| Sheefish  | Muscle          | Smoked (with skin) | 21.1   | 44.8     | BDL  | (390) | BDL   | BDL   |

*Bold text highlights contributions of >100% of DRI of a given element by a 100g meal of the specified tissue.

**Bold text highlights contributions of >100% of PTDI/RfD of a given element by a 100g meal of the specified tissue.

**THg, MeHg, Cd, As, Pb: Provisional Tolerable Daily Intake (PTDI): Joint FAO/WHO Expert Committee on Food Additives (JECFA)

*Ag: Reference Dose (RfD): United States Environmental Protection Agency (US EPA).

(1) indicate that the PTDI for As refers to inorganic As, while total As was measured for this study and used for RfD contribution calculations. Because As is expected to be primarily organic in these tissues, contribution is likely overestimated here (see Discussion).

*BDL = No contribution to toxicological reference dose calculated because element was below detection limit in tissue.
Percent methylmercury (% MeHg)
%MeHg in raw and food processed tissues is shown in Table III. Muscle had the highest %MeHg, followed by liver and kidney. Both THg and MeHg were below the MDL in all blubber samples, thus %MeHg was not determined. %MeHg in muscle (71.2–104% for seals and sheefish) was significantly higher than both seal liver (22.9% raw and 26.4% fried) and kidney (19.9% raw and 20.4% boiled), but the %MeHg in liver and kidney were not significantly different. %MeHg did not change significantly in any spotted seal tissue when food was processed by any method. Three processing methods (baking with skin, drying without skin, and drying with skin) resulted in significant increases in %MeHg in sheefish muscle. No concurrent significant change in THg concentration was observed. There were no significant differences in %MeHg in sheefish muscle processed by the same method whether skin was present during processing or not.

DISCUSSION

Alaska is unique within the United States in that a significant proportion of its population depends on fish and wildlife as major food sources (35). Shifts from traditional subsistence diets to “Westernized,” store-bought diets have coincided with increases in adverse health issues among AK Natives, including obesity, cardiovascular disease and diabetes (3,4,6–9). Although contaminants are generally lower in arctic species than their counterparts from lower latitudes, they remain a concern due to the importance of these species as food sources. The relative benefits of nutritional contributions must be weighed against potential risks posed by the presence of contaminants. Store-bought alternatives to subsistence foods are often limited, may not provide the same level of nutrition and may contain appreciable levels of contaminants themselves (12). Numerous studies have concluded that the risks of consuming nutritionally inferior commercial foods outweigh the risks posed by the contaminant intake associated with a subsistence diet (5,16,20,36,37). However, the recent release of an AK-based fish consumption advisory (38) for Hg has blurred this issue.

Contaminant and nutrient studies in AK have focused primarily on establishing baseline concentration data for use in species monitoring over space and time. Thus, the tissues studied have not necessarily been those utilized most frequently by human consumers for food. Nor have they investigated how contaminants or nutrients may change when a tissue is processed for food. Therefore, true intake of nutrients and contaminants by subsistence users, critical components of a risk-benefit analysis of traditional foods, is unknown. This study focuses on known food tissues in important subsistence species of Northwest AK and documents that these foods contain numerous important nutrients with very limited risk from contaminants, and that element concentrations can be significantly altered through food processing.

Essential and non-essential element concentrations
All elements, except As and Pb, were lower in seal blubber than in muscle, liver or kidney. Typically, either liver or kidney contained the highest concentration of a given element.
In most cases, element concentrations in raw seal tissues were similar to those previously reported for northern ice seals (11,17,39–45). Concentrations of Ca, Cr, K, Na and MeHg in blubber and Ca and K in kidney were not sufficiently represented in the literature to make an adequate comparison. In this study, As was approximately twofold higher in blubber and muscle of spotted seals than previously reported for ringed and harp seals (11,44). This result could be a marker for piscivory or locally elevated As levels from geologic sources and/ or activities such as mining. Mg was one-third to one-half that reported in the same studies. Very little data on elements in sheefish exists in published literature. Therefore, the data in this study fill an important gap and were not compared with existing information for this species and region.

**Changes due to food processing**

Significant changes in nutrient and contaminant concentrations of tissues resulted from food processing in several cases. Changes were determined not only on a ww basis but also on a dw basis to account for concentration changes resulting from changes in water content. This is a critical consideration for determining risks and benefits to human consumers, as the contribution to DRI or TDIL is subsequently affected and potential mechanisms for compositional changes can be proposed.

As an example, raw sheefish muscle provides 53.3% of the DRI for Se per serving. This value does not change significantly for baked fish, but if the fish consumed is smoked or dried, the contribution to DRI increases to 70.0/66.9% (cooked without/with skin) and 93.3/106%, respectively. Similarly, the contribution to TDIL for Cd in spotted seal kidney decreases significantly from 449% to 368% when kidney is boiled as compared to when it is raw. Therefore, the contribution to TDIL is overestimated if only raw kidney is considered.

Thus, preparation method must be considered when assessing nutrients and contaminants in traditional foods. By basing DRI and TDIL determinations only on concentrations in raw tissues, the contribution to DRI or TDIL may be grossly under- or overestimated for the actual food items consumed.

**Contributions to daily reference intakes (DRI) and upper limits (UL)**

As expected, traditionally prepared foods provide a number of nutrients at >100% of the DRI per 100 g serving. In addition, these foods provide many nutrients in moderate amounts (i.e., 10–100% per 100 g serving) while others, such as Ca and Cr, were not represented in any tissue at ≥10% of their respective DRI’s. These results support the assertion that traditional foods represent an important, nutritious part of a balanced diet.

In addition to the danger posed by a lack of nutrition, some essential elements can become harmful at excessive doses. Therefore, UL have been developed in addition to minimal requirements. Fe in dried seal muscle and Se in raw kidney exceeded the UL for these elements. It should be noted though that UL are daily limits. The seasonal nature of subsistence foods makes it 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 dried seal meat. Finally, raw kidney is not an abundant food item as compared to the mass of other tissues and is included in this study primarily for comparison to processed kidney. Based on
these considerations, there does not appear to be a significant threat of essential element toxicosis from consuming these traditional foods. Element interactions are another important consideration, but are beyond the scope of this work.

**Contribution to tolerable daily intake limits**

Although Alaskan wildlife is generally less contaminated than wildlife at lower latitudes, several contaminants are still detectable in all tissues of these animals. The non-essential elements investigated may have natural and/or anthropogenic sources. Contaminant levels approached or exceeded TDIL in some cases.

When interpreting TDIL, one must remember that these values are very conservative and represent the amount that can be consumed *every day over an entire lifetime* without risk of adverse health effects. Due to the seasonality of subsistence foods, it is extremely unlikely that any food item studied would be eaten every day of the year for an entire lifetime. On the other hand, these risks are only those originating from individual food items. Humans consume a range of foods and must also consider contaminant intake from multiple sources. Further, the above assessments do not take into account the chemical form of some elements, a critical factor for assessing potential toxicity.

It is not our intent that comparisons of element concentrations to DRI and TDIL be interpreted as consumption advice. This analysis was used to put concentration values into a useful context in terms of human consumption guidelines and to facilitate comparisons between tissues and between the current study and the available published literature. We recognize that it is the responsibility of public health agencies to consider the information presented here and to provide consumption advice accordingly. The data have been provided to public health agencies in Alaska, including the Alaska Division of Public Health, Department of Health and Social Services and the Alaska Native Tribal Health Consortium.

**Arsenic speciation considerations**

Only total As was measured in this study. The chemical form of the As present was not determined. Like Hg, As can exist in organic or inorganic forms, yet the PTDI does not take into consideration the relative amounts of each form present. Inorganic As is of greater concern in terms of toxic effects to a consumer. It is well known that As in marine mammals is primarily in the organic form (46). Studies have shown that >90% of the As present is organic with >70% being organic arsenobetaine (47–50). Similarly, fish muscle contains 75–100% organic As (51–53). Therefore, the fact that As was above TDIL in seal blubber and sheefish muscle should be interpreted carefully. It is likely that the levels present in this study do not pose a toxicological risk, but a complete investigation of the As speciation in these tissues is needed to make this conclusion with greater certainty.

**Percent methylmercury (％MeHg)**

When considering implications of Hg in foods, it is critical to take into account the chemical and physical forms represented. MeHg is the main form of concern, since it can be present in appreciable amounts, is relatively bioavailable and is a known neurotoxicant (54). This
is particularly true for the developing central nervous systems of fetuses and children (55), making them the cohort of greatest concern. Inorganic mercury is considered less toxic because it has lower bioavailability and may be bound to selenium in insoluble Hg-Se complexes. MeHg is present in fish and marine mammals, but whether it occurs at levels that may cause subtle neurotoxic effects in human consumers of these species has been widely debated. Long-term studies indicate that the benefits obtained from the nutrients (e.g., Se, ω-3 fatty acids) in these foods outweigh any danger posed by the presence of MeHg (7).

%MeHg must be considered together with the THg concentration. A tissue with low %MeHg can still contribute significant levels of MeHg if the THg concentration is substantial. For example, the %MeHg in spotted seal liver (22.9% and 26.4% for raw and fried, respectively) is much lower than that in muscle (86.4%, 71.2% and 103% for raw, boiled and dried). However, because the THg level in liver (1991 and 2510ng/g for raw and fried) is greater than in muscle (182, 261 and 406ng/g for raw, boiled and dried), a serving of liver contributes more MeHg to the diet than an equivalent serving of muscle.

### Mercury–Selenium Interactions

Another important consideration for determining potential toxicity of Hg in foods is the relative ratio of Hg to Se in the tissue. Studies suggest Se may be highly effective in reducing Hg toxicity, though human data are lacking (56). The exact mechanism of the protective role of Se against Hg toxicity is unknown. The leading hypothesis is that Se protects against Hg by forming insoluble complexes with Hg, rendering it non-bioavailable (57). Others suggest the mechanism may be related to the antioxidant properties of Se, for example as glutathione peroxidase (GSH-Px), which may protect against Hg toxicity and be involved in demethylation of MeHg (58). For human consumers, intake of tissues with Se in excess of Hg could potentially aid in reducing the bioavailability of Hg and/or mitigating the systemic toxic effects of Hg.

In all tissues studied, Se:THg molar ratio was significantly >1 (student t-test; p<0.05). In spotted seals, Se:Hg was lowest in liver (4.84–5.22), followed by muscle (7.05–10.7) and highest in kidney (17.8–31.5). Se:Hg in sheefish muscle was similar (8.35–10.3) to that found in seals. These results indicate that although these traditional foods contain Hg, they are also rich in Se, which may help to counteract any toxic effects of Hg.

### Conclusions

Cooking can have significant effects on the concentration of elements in a tissue, illustrating the importance of looking at the actual food items consumed when considering the risks and benefits of a traditional diet.

Spotted seal and sheefish tissues were abundant sources of several nutrients. The consumption of these traditional foods does not appear to pose a significant threat due to essential elements exceeding their established UL. Although certain non-essential elements exceeded their respective TDIL in certain food items, considerations of element interactions (Se:Hg), bioavailability (Hg-Se complexes) and chemical form (organic vs. inorganic Hg or As) as well as the seasonal nature of subsistence food use, lead to the conclusion that the risk posed by contaminant intake via these items is relatively low.
Overall, the results suggest that the traditional foods investigated provide an array of nutrients accompanied by a very limited risk of contaminant toxicosis. Therefore, we encourage the continuation of traditional food consumption as a nutritious part of a balanced diet. 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 capacity to tolerate contaminants without ill effect. The data presented here could be used by public health agencies in the future to support the development of cohort-specific consumption advice. It is our intent that the data presented here be treated as a risk-benefit analysis, not consumption advice, which should be provided only by appropriate public health agencies. Finally, we encourage public health agencies to develop models or algorithms to assess overall food safety and quality for underrepresented diets, such as the subsistence diet of many Alaska residents.

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