Oil fouling in three subsistence-harvested ringed (Phoca hispida) and spotted seals (Phoca largha) from the Bering Strait region, Alaska: Polycyclic aromatic hydrocarbon bile and tissue levels and pathological findings

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\textbf{ABSTRACT}

Oil spills of unknown origin were detected in three oil-fouled, ice-associated seals from the Alaska Bering Strait region collected by Alaska Native subsistence hunters during fall 2012. Bile analyses of two oiled seals indicated exposure to fluorescent polycyclic aromatic hydrocarbon (PAH) metabolites but levels of some metabolites were similar to or lower than biliary levels in harvested unoiled ice seals. Oiled seals had elevated tissue PAH concentrations compared to tissue levels of PAHs determined in unoiled ice seals. However, regardless of oiling status, tissue PAH levels were relatively low (< 50 ng/g, wet weight) likely due to rapid PAH metabolism and elimination demonstrated previously by vertebrates. Hepatic, pulmonary, and cardiac lesions were observed in oiled seals in conjunction with measurable PAHs in their tissue and bile. This is the first study to report tissue and bile PAH concentrations and pathologic findings of oiled ice seals from the U.S. Arctic.

1. Introduction

Although direct links between exposure to pollutants and effects on marine mammals have been difficult to establish, environmental contaminants have been associated with a number of deleterious effects (reviewed in O’Hara and O’Shea, 2001). Two classes of contaminants that occur frequently in marine ecosystems, persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs), have been linked to biological effects in marine mammals. For example, immuno-suppressive effects, reproductive dysfunction, and increased incidences of disease have been observed in marine mammals that have high blubber concentrations of POPs, such as polychlorinated biphenyls (PCBs) and chlorinated pesticides (Ross et al., 1995; Beckmen et al., 2003; Murphy et al., 2015; Randhawa et al., 2015). PAHs measured in environmental samples can be derived from a number of sources including crude oils and other petroleum products (petrogenic PAHs), forest fires, combustion of fossil fuel and wood products (pyrogenic PAHs), as well as formed biogenically (Collier et al., 2014). Lower molecular weight PAHs (containing 2 to 3 fused aromatic rings) and their alkylated homologues, PAHs containing at least one or more alkyl group (e.g., methyl, ethyl), are frequently measured in petroleum products whereas pyrogenic sources of these contaminants contain nonalkylated, higher molecular weight PAHs (containing 4 to 7 fused aromatic rings) (Collier et al., 2014). In contrast to POPs, PAHs are metabolized efficiently by vertebrates and are found usually at low concentrations in the tissues of these animals (Varanasi et al., 1989; Meador et al., 1995). However, previous studies have demonstrated both biochemical and biological effects associated with exposure to petroleum-related PAHs in marine mammals (Frost et al., 1994; O’Hara and O’Shea, 2001; Peterson et al., 2003). Because crude oil and...
petroleum products are used around the world, PAHs may pose risks to
populations of marine mammals and other protected species that occur
in marine waters that receive unintentional PAH releases via petroleum
spills or stormwater runoff, as well as atmospheric or current transport
from other PAH-polluted regions. Recent monitoring and modeling ef-
forts in the Arctic indicate that PAHs may be an emerging class of
contaminants in this region of the world (De Laender et al., 2011).

Industrial maritime activities in the Arctic (e.g., offshore oil and gas
development, commercial shipping, fishing, tourism) pose a significant
trans-boundary threat to marine mammals through accidental petro-
leum discharge (Geraci and St. Aubin, 1980). Apart from the wildlife
health and conservation concerns (Geraci and St. Aubin, 1988), oil-
spilled marine present a serious food security and potential food safety
risk for Alaskan coastal communities. Ice-assocated seal species, ringed
(Phoca hispida), bearded (Erignathus barbatus), spotted (Phoca largha),
and ribbon seals (Histriophoca fasciata) are important nutritional, cul-
tural, and economic resources for coastal communities throughout
northern and western Alaska – and are collectively known as “ice seals”.

During the fall of 2012, oil spills of unknown origin were detected in the
Bering Strait region of Alaska by the Alaska Native subsistence hunters including three oil-fouled female ice seals. Concurrently, sev-
eral oiled seabirds, common murre (Uria aalge), crested auklet (Aethia
cristatella), and black-legged kittiwake (Rissa tridactyla), were found
near Saint Lawrence Island, Alaska (2012 ADEC Wildlife Tracker, un-
published results). Limited data exist on the toxicologic pathology of oil
foiling on ice-assocated seal species from experimental exposure stu-
dies on ringed seals (Smith and Geraci, 1975; Engelhardt et al., 1977;
Engelhardt, 1982; Helm et al., 2015). Our objective was to provide the
first chemical analyses of petroleum-related PAHs in conjunction with
gross pathology and histologic findings of three oil-fouled spotted and
ringed seals in Alaska. These PAH findings were compared to those of
unouled harvested ringed and spotted seals collected in the region from
2012 to 2014 as part of a baseline contaminant Arctic marine mammal
study.

2. Materials and methods

2.1. Sample collection for pathology and chemical analyses

Two spotted seals (N52-2012 and 2012-166) and one ringed seal
(N55-2012) with visible signs of potential oil fouling were harvested for
subistence purposes and submitted for post mortem examination to the
North Slope Borough, Department of Wildlife Management at Utqiagvik
(Barrow), Alaska (71.2906° N, 156.7886° W) and to the Alaska
Department of Fish and Game at Fairbanks, Alaska (64.8378° N,
147.7164° W). Complete necropsies and selective tissue sampling for
histopathology following a standard pinniped post mortem protocol
(Dierauf, 1994) and for polycyclic aromatic hydrocarbon (PAHs) ana-
sysis (NMFS Marine Mammal Oil Spill Sampling Guidelines 2007)
were conducted for each seal. Age class of each seal was categorized based on
Alaskan species-specific body size morphometrics (Quakenbush et al.,
2009, 2011) and claw band counts (McLaren, 1958). External oil
identification was based on the Marine Mammal Oil Spill Response
Guidelines (Johnson and Ziccardi, 2006). Neither brain nor blood
samples were collected due to harvest method and extended time from
death to transport to laboratory. Samples of major organs and tissues
were fixed in 10% neutral-buffered formalin, routinely processed, em-
bedded in paraffin by conventional methods, sectioned at 4–6 μm, and
stained with hematoxylin and eosin (H&E) (Histology Consultation
Services, Everson, Washington, 98247). Bile from N55-2012 and N52-
2012 were collected in 4-ml amber glass vials (SUN-Sri 15 × 45 mm
Screw Thread Vials). Additional tissue samples (i.e., skin, lung, liver,
blubber, kidney, muscle, trachea content, stomach content, and feces)
were collected in pre-cleaned 4-oz. glass jars (250 mL I-Chem clear glass
certified jar) and stored at −20 °C until analyzed for PAHs. For com-
parison, bile was collected from unoiled, subsistence-harvested Alaskan
ringed (n = 10) and spotted seals (n = 3) collected during 2012–2014
of mixed age and sex composition (adults (n = 6), subadults (n = 3),
pup (n = 3), unknown (n = 1); Sex: male (n = 5), female (n = 7), un-
known (n = 1)) during routine post mortem examination (2012–2014).
Additionally, blubber (n = 3), liver (n = 3), lung (n = 4), muscle
(n = 4) and stomach content (n = 2) samples from subsistence-har-
vested, unoiled Alaskan ringed seals sampled in 2012–2014 near the
Utqiagvik, Alaska region were collected using the same sampling and
storage protocols as described above.

2.2. Analysis of fluorescent PAH metabolites in bile

Chemical analyses were conducted at the National Marine Fisheries
Services's Northwest Fisheries Science Center in Seattle, Washington.
Seal bile was analyzed for fluorescent PAH metabolites using high-
performance liquid chromatography/fluorescence detection (HPLC-F)
method (Krahm et al., 1984; da Silva et al., 2006). Bile was injected
directly onto a Waters high-performance liquid chromatography/fluorescence
system equipped with a C18 reverse-phase column (Phe-
omenex Synergi Hydro). The PAH metabolites were eluted with a
linear gradient from 100% water (containing a trace amount of acetic
acid) to 100% methanol at a flow of 1.0 mL/min. Chromatograms
were recorded at the following wavelength pairs: 1) 292/335 nm where
many 2 benzene ring aromatic compounds (e.g., naphthalenes) fluo-
resce, 2) 260/380 nm where several 3 ring compounds (e.g., phenan-
threnes) fluoresce and 3) 380/430 nm where 4–5 ring compounds (e.g.,
fluoranthenes, benzo[a]pyrene) fluoresce. Peaks eluting after nine
minutes were integrated and the peak areas were summed. Con-
centrations of fluorescent PAHs in the bile samples of the seals were
determined using naphthalene (NPH), phenanthrene (PHN) or benzo[a]
pyrene (BaP) as external standards and converting the fluorescence
response of bile to phenanthrene (ng PHN equivalents/g bile), naph-
thalene (ng PHN equivalents/g bile), and BaP equivalents (ng BaP
equivalents/g bile) equivalents. Protein analysis as described in da Silva
et al. (2006) was completed for all bile samples. Biliary PAH metabolite
concentrations were normalized to bile protein content using a simple
ratio, which has been shown to be an indicator of feeding status in a
marine flatfish species (Collier and Varanasi, 1991).

As part of the analytical laboratory quality assurance (QA) plan
(Sloan et al., 2006), a method blank and a fish bile control sample (bile of
Atlantic salmon exposed to 25 μg/mL of Monterey crude oil for 48 h)
were analyzed with the sample set. The results of all QA samples met
established laboratory criteria.

2.3. PAH analyses of external, tissue, and gastroenteric samples

Seal external, gastroenteric and tissue samples were extracted and
analyzed for PAHs, including alkylated homologues, using the gas
chromatography/mass spectrometry (GC/MS) method of Sloan et al.
(2014). This method involves: (1) extraction of tissues using di-
chloromethane in an accelerated solvent extraction procedure, (2)
clean-up of the dichloromethane extract on a single stacked silica gel/
alumina column, (3) separation of PAHs from lipid or other biogenic
material by high-performance size exclusion liquid chromatography,
and (4) analysis on a low resolution quadrupole GC/MS system
equipped with a 60-m DB-5 GC capillary column. The instrument was
calibrated using sets of up to ten multi-level calibration standards of
known concentrations. Sum “low molecular weight PAHs” (sum
LMWAHs) included summing the concentrations of naphthalene, C1-
through C4-naphthalenes, acenaphthylene, acenaphthene, fluorene, C1-
through C3-fluorenes, anthracene, phenanthrene, C1– through C4-
phenanthenes/anthracenes, dibenzothiophene, and C1– through C4-
dibenzothiophenes. Sum “high molecular weight PAHs” (sum
HMWAHs) include adding the levels of fluoranthenes, pyrene, C1-
through C4-fluoranthenes/pyrenes, benzo[a]anthracene, chrysene/tri-
phenylene, C1– through C4-chrysenes/benzo[a]anthracenes, benzo[b]
fluoranthene, benzo[j]fluoranthene/benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-cd]pyrene, dibenz[a,h + a,c]anthracene, and benzo[ghi]perylene. Sum PAH concentrations were calculated by summing the levels of sum LMWAHs and sum HMWAHs. A subsample of extract was removed prior to the cleanup steps for gravimetric percent lipid determination. In some cases, low levels of certain PAHs (e.g., naphthalene, fluorene, phenanthrene, perylene) and/or their alkylated homologues were measured in a field sample and the method blank from the same sample set. If the concentration of an individual PAH was < 3 times lower than the level measured in method blank analyzed in the same sample set, the concentration of the compound in the field samples was not included in the associated sum LMWAHs, sum HWMAHs and sum PAH calculations.

A method blank and a National Institute of Standards and Technology (NIST) blue mussel Standard Reference Material (SRM 1974b) were analyzed with each sample set as part of a performance-based quality assurance program (Sloan et al., 2006). Concentrations of individual analytes measured in SRM 1974b were in agreement with the certified or reference values published by NIST. Other quality control samples met established laboratory criteria.

3. Results

A subadult female spotted seal (N52-2012) was harvested on 5 September 2012 near Shishmaref, Alaska (66.2556° N, 166.0722° W), Alaska, and a second subadult female spotted seal (2012-166) was harvested near Gambell on St. Lawrence Island, Alaska (63.7761° N, 171.7008° W) on 16 October 2012 (Fig. 1). The carcass of N52-2012 was kept under a tarp outside and allowed to freeze naturally and was then shipped from Gambell, Alaska to the North Slope Borough's Department of Wildlife Management necropsy facility in Utqiagvik, Alaska. The carcass of 2012-166 was shipped unfrozen from Gambell, Alaska to the Alaska Department of Fish and Game's necropsy facility in Fairbanks, Alaska. A third animal, a subadult female ringed seal (N55-2012) was harvested near Gambell on 11 November 2012 (Fig. 1). The carcass of N55-2012 was shipped unfrozen from Gambell, Alaska to Utqiagvik, Alaska. No necropsies were performed on these animals. Table 1 provides a summary of the information obtained during the necropsies.

![Map of Alaska showing locations of three coastal villages](image)

**Fig. 1.** Locations of three coastal villages in Alaska where subsistence-harvested oil-fouled and unoiled ice seals were collected during 2012–2014.
2012, was harvested on 12 November 2012 near Gambell, Alaska (Fig. 1) and shipped unfrozen to the North Slope Borough's Department of Wildlife Management necropsy facility in Utqiagvik, Alaska. Ambient daily temperature during the respective months in Shishmaref and Gambell is at or below freezing (0 °C). Summarized information on the daily temperature during the respective months in Shishmaref and Gambell is at or below freezing (0 °C).

### Summary of key histopathological findings

#### Table 2

<table>
<thead>
<tr>
<th>Species and field identification number</th>
<th>Spotted seal</th>
<th>Spotted seal</th>
<th>Ringed seal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smell</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Oil visible</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Oil color</td>
<td>Brown</td>
<td>Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>Percent oiled</td>
<td>51–75%</td>
<td>51–75%</td>
<td>51–75%</td>
</tr>
<tr>
<td>Area oiled</td>
<td>Body (ventral/ lateral) with staining on fore flippers</td>
<td>Body (ventral) with staining on fore flippers</td>
<td>Body (dorsal) with staining on hind flippers</td>
</tr>
<tr>
<td>Depth of oiling</td>
<td>Deep</td>
<td>Deep</td>
<td>Deep</td>
</tr>
</tbody>
</table>

### 3.1. Chemical contaminant results

#### 3.1.1. Biliary fluorescent PAH metabolites

Detectable levels of fluorescent PAH metabolites were measured in the bile samples of two visibly oiled ice seals. Biliary NPH and PHN equivalent concentrations measured in the oiled spotted seal N52-2012 and ringed seal N55-2012 were approximately an order of magnitude higher than those determined in oiled ringed seal N55-2012 (Fig. 3). Protein-corrected concentrations of NPH and PHN equivalents determined in seal N52-2012 were at least three times higher than those measured in seal N55-2012 (Fig. 4) whereas the biliary protein concentration (99 mg/mL) of seal N52-2012 was approximately two times higher than the value of seal N55-2012 (48 mg/mL) (data not shown). In contrast to the NPH and PHN equivalents, BaP equivalents determined in the bile of these two oiled seals were similar (Figs. 3 and 4), with values ranging from 1000 to 1200 ng/g bile, wet weight. However, the protein-corrected BaP equivalent concentration was 2.5 times higher in the spotted seal (N52-2012) compared to the ringed seal (N55-2012) value.

We compared the levels of biliary fluorescent PAH metabolites (protein-corrected and non-protein corrected) measured in two visibly oiled seals to those determined in unoiled ice seals collected from the U.S. Arctic during 2012–2014 (Figs. 3 and 4). The NPH and PHN equivalent concentrations (protein and non-protein corrected) of the oiled spotted seal were approximately three times higher than the mean values of the unoiled spotted seals. In contrast, the levels of these fluorescent PAH metabolites (protein and non-protein corrected) measured in the bile of the ringed oil- inged ringed seal were somewhat lower than those measured in unoiled ringed seals. For both oiled spotted and ringed seals, the protein and non-protein corrected BaP equivalent concentrations were higher or comparable to those determined in the unoiled seals. The biliary protein values of the unoiled ringed seals ranged from 32 to 75 mg/mL whereas the values of the unoiled spotted seals ranged from 33 to 120 mg/mL.

#### 3.1.2. PAH concentrations in external, tissue, and gastroenteric samples

Concentrations of sum LMWAHs, sum HMWAHs and sum PAHs, as well as percent lipid, measured in tissues of oiled ice seals and unoiled ringed seals are reported in Table 4. A wide range of PAH concentrations was measured in the external, gastroenteric and tissue samples, with sum PAH values ranging from 0.2 (ringed seal N55-2012 muscle) to 280 ng/g, wet weight (spotted seal N55-2012 skin). In general, lower concentrations of sum LMWAHs, sum HMWAHs, and sum PAHs (mean values ranging from < lower limit of quantitation (LOQ) to 6.4 ± 7.8 ng/g, wet weight) were determined in tissue and stomach content samples obtained from our unoiled ringed seals compared to the oiled ice seals.

Elevated levels of sum PAHs were measured in skin and blubber samples collected from visibly oiled sites on individual ice seals compared to the same tissue type collected from a non-visibly oiled location of the same seal (Table 4). For example, the sum PAH concentration (25 ng/g, wet weight) measured in blubber taken from a visibly oiled portion of the ringed seal N55-2012 was approximately two times higher than the concentration (12 ng/g, wet weight) of blubber taken from an unoiled location of the same animal. A visibly oiled skin sample from N55-2012 had elevated sum PAHs (44 ng/g, wet weight) compared to a non-visibly oiled skin sample (30 ng/g, wet weight) from this same seal.

#### Table 3

Summary of key histopathological findings in oil-fouled, subsistence-harvested subadult female ice seals (n = 3), in the Bering Strait, Alaska, 2012. The severity of the lesions was recorded as mild, moderate, or severe, with severity scores of 1, 2, or 3 respectively.

<table>
<thead>
<tr>
<th>System</th>
<th>Necropsy and pathologic findings</th>
<th>Spotted seal</th>
<th>Spotted seal</th>
<th>Ringed seal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatitis</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>Oiled and non-oiled</td>
</tr>
<tr>
<td>Mucocutaneous erosion</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bacterial and fungal colonization</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Congestion</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Tracheal edema</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Interstitial emphysema</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Interstitial pneumonia</td>
<td>1 chronic active</td>
<td>1 chronic presumed</td>
<td>1 acute</td>
<td></td>
</tr>
<tr>
<td>Pulmonary nematodiasis</td>
<td>2</td>
<td>Presumed</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Myocarditis</td>
<td>1 chronic</td>
<td>0</td>
<td>1 acute</td>
<td></td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>NA</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Gastric ulcers/erosions</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Enteritis/colicitis</td>
<td>NA</td>
<td>1 (acanthocephalans)</td>
<td>1 nematode migration</td>
<td></td>
</tr>
<tr>
<td>Lymphophagocytosis</td>
<td>0</td>
<td>0</td>
<td>1 spleen</td>
<td></td>
</tr>
<tr>
<td>Lymphoid hyperplasia – mesenteric lymph node</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Splenic extramedullary hematopoiesis</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Vascular change, adrenal gland</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Adrenocortical hyperplasia</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Sum LMWAHs and sum PAHs in tissues of the oiled spotted seals were elevated compared to the concentrations measured in the same tissues of oiled and unoiled ringed seals (Table 4). Regardless of oiling status, the LMWAHs were the major contributors to sum PAHs, contributing 70% to 100% to the sum PAHs for all samples except liver (30% of spotted seal 2012-166) and the stomach content samples of spotted seal N52-2012 and ringed seal N55-2012 (37% and 45%, respectively). Sum HMWAHs contributed between 0 and 70% to the sum PAH values. Sum LMWAHs contributed 73% and 92% to sum PAHs in unoiled ringed seals that had detectable levels of PAHs (stomach content and muscle) (Table 4).

The composition patterns of PAHs (percent contribution of parent and alkylated PAHs to summed PAHs) of the visibly oiled seal samples were compared to the patterns of two petroleum samples — a sample of 20% weathered Alaska North Slope crude oil (ANSCO) and a bunker oil — to help identify PAH sources to which the ice seals were potentially exposed. The composition patterns on the skin of spotted seal N52-2012 resembled those of the oil/fuel samples except for decreased percentages of naphthalene and alkylated naphthalenes and increased percentages of alkylated phenanthrenes/anthracenes and alkylated dibenzothiophenes (Fig. 5). In contrast, the percentages of naphthalene and alkylated naphthalenes in ringed seal N55-2012 skin and hair samples were similar to the oil samples though percentages of high molecular PAHs differed from the oil sources and skin of spotted seal N52-2012 (Fig. 5). Examination of the internal tissues (e.g., blubber, kidney, liver) and gastroenteric samples (i.e., stomach contents, feces) of the oiled ice seals were less similar to the composition patterns of the weathered ANSCO or bunker oils (liver samples shown in Fig. 6). The tissue and stomach content PAH composition patterns of unoiled ringed...
seals did not resemble those of petroleum samples or the tissues/gastroenteric samples of the visibly oiled ringed seal (data not shown). For example, concentrations of all parent and alkylated PAHs determined in the bladder, liver, and lung samples of the unoiled ringed seals from Utqiaġvik, Alaska were < LOQ.

### 3.1.3. Gross pathology and histopathologic findings

For spotted seal N52-2012 (case 1), molt had been completed, and no skin or mucocutaneous lesions were observed. On internal examination, the lungs were congested and the liver was soft and pale. The stomach contained a scant amount of green oily mucoid material. Small –0.5 cm mucocutaneous erosions were present on the left lip margin. On internal examination, the lungs were congested, the airways thickened and filled with clear mucus, with 4 adult nematodes were present. Parasites were not further identified. Multiple, well-demarcated (0.2–0.5 cm) yellow nodules were embedded in the lung parenchyma adjacent to the airways. The liver was swollen, firm and very pale with well-demarcated, mottled red areas present on multiple lobes. The stomach was filled with a scant amount of green oily mucoid material. Small (~2 mm) gastric erosions were present. The spleen on cross section had prominent white pulp and the mesenteric lymph node was enlarged (reactive). Based on histopathology, this seal had a variety of chronic changes in the haired skin and mucous membranes typical of the disease process seen in the ongoing, federally designated Northern Alaska Pinniped Unusual Mortality Event (UME) of unknown etiology. The changes observed in the skin were the same between oilied and non-oiled regions. The lungs had changes consistent with chronic pulmonary nematodiasis. Similar to spotted seal N52-2012, this oiled ringed seal had mild vacuolar hepatopathy characterized by multiple small, clear, well-delineated cytoplasmic vacuoles distributed randomly. Of note, a moderate, multifocal random to portal small, clear, well-delineated cytoplasmic vacuoles distributed randomly. Of note, a moderate, multifocal random to portal small, clear, well-delineated cytoplasmic vacuoles distributed randomly. Of note, a moderate, multifocal random to portal

### 4. Discussion

This is the first documentation of tissue and bile PAH concentrations and pathologic findings of visibly oiled ringed and spotted seals from the U.S. Arctic. Analyses of bile collected from two visibly oiled seals indicated exposure to PAHs as determined by measuring fluorescent PAH metabolites—compounds that have been previously measured in visibly oiled marine mammals after the Exxon Valdez spill (Varanasi et al., 1993; Frost et al., 1994) and sea turtles following the 2010 Deepwater Horizon spill (Ylitalo et al., 2017). The oiled spotted seal N52-2012, with a ventral oiling pattern, generally had higher levels of these interstitial pulmonary emphysema, tracheal edema (Fig. 7A), pulmonary congestion, and edema. In addition, typical chronic interstitial and bronchopneumonia consistent with the known pulmonary nematodiasis (infection with nematodes) was also noted. There was moderate lymphoid hyperplasia of the mesenteric lymph node, splenic extramedullary hematopoiesis (hematopoiesis occurring in organs outside of the bone marrow) and hemosiderosis (excessive accumulation of iron deposits) and very mild vacuolar change in the adrenal cortex concentrated in the zona fasciculate with fewer vacuoles in the zona glomerulosa (Fig. 7B). In the heart of the spotted seal, there was a regionally extensive area of fibrosis adjacent to and around a small arteriole, myofibers drop out, as well as atrophy (Fig. 7C).

For spotted seal 2012-166 (case 2), molt had been completed, and no skin or mucocutaneous lesions were observed. On internal examination, the lungs were congested; the liver was soft and pale; the stomach was empty, and the mesenteric lymph node was enlarged (reactive). Acanthocephalans (thorny-headed worms) were present in the colon. On histopathology of this animal, there were no significant findings in the hair skin or mucosa. There was a mild chronic lymphoplasmacytic interstitial pneumonia and mucoid bronchopneumonia characteristic of pulmonary nematodiasis. Rare, multifocal random vacuolar change in the liver characterized by multiple, clear, sharply delineated cytoplasmic vacuoles distributed randomly, most consistent with fatty change. Moderate lymphoid hyperplasia of the mesenteric lymph node and splenic hemosiderosis was also observed.

Patchy hair loss (alopecia) was present on the fore flippers, insertion of the hind flippers, tail, and ventral surface for ringed seal N55-2012 (case 3). Multiple small (~0.1 cm) nodules were present on the interdigital webbing of both hind flippers. Multiple small (~0.5 cm) mucocutaneous erosions were present on the left lip margin. On internal examination, the lungs were congested, the airways thickened and filled with clear mucus, with 4 adult nematodes were present. Parasites were not further identified. Multiple, well-demarcated (0.2–0.5 cm) yellow nodules were embedded in the lung parenchyma adjacent to the airways. The liver was swollen, firm and very pale with well-demarcated, mottled red areas present on multiple lobes. The stomach was filled with a scant amount of green oily mucoid material. Small (~2 mm) gastric erosions were present. The spleen on cross section had prominent white pulp and the mesenteric lymph node was enlarged (reactive). Based on histopathology, this seal had a variety of chronic changes in the haired skin and mucous membranes typical of the disease process seen in the ongoing, federally designated Northern Alaska Pinniped Unusual Mortality Event (UME) of unknown etiology. The changes observed in the skin were the same between oilied and non-oiled regions. The lungs had changes consistent with chronic pulmonary nematodiasis. Similar to spotted seal N52-2012, this oiled ringed seal had mild vacuolar hepatopathy characterized by multiple small, clear, well-delineated cytoplasmic vacuoles (Fig. 7D) distributed multifocally random. Of note, a moderate, multifocal random to portal hepatitis was observed, also typical of symptoms associated with the UME. There was an enterotyphlitis associated with nematode migration, which could account for the reactive mesenteric lymph node and splenic extramedullary hematopoiesis. Lastly, mild adenocortical hyperplasia and a mild acute myocarditis were detected.
flourescent metabolites compared to the concentrations in the oiled ringed seal (dorsal oiling pattern) or the unoiled ice seals (see Figs. 3 and 4) analyzed for comparison. In addition, concentrations of biliary PAH metabolites reported in the oiled spotted seal N52-2012 are comparable to or considerably higher than those reported in visibly oiled harbor seals (Phoca vitulina) from Prince William Sound (PWS), Alaska that stranded in 1989/1990 after the Exxon Valdez oil spill (Varanasi et al., 1993; Frost et al., 1994). For example, the NPH fluorescent metabolite findings may be confounded by feeding status. A previous laboratory exposure study on English sole (Parophrys vetulus) demonstrated that levels of PAH metabolites, as well as protein and biliverdin, concentrate in the bile of non-feeding fish (Collier and Varanasi, 1991). The relatively high biliary protein values together with visible confirmation of empty stomachs of some of the oiled and unoiled ice seals indicated that these animals had not fed recently. Thus, the PAH metabolites may have concentrated in bile of the ice seals over an extended time period and may not necessarily represent recent exposure (< 48 h) to these contaminants.

### 4.1. Concentrations of PAHs in external, tissue, and gastroenteric samples of ice seals

As expected, low levels of PAHs (< 50 ng/g, wet weight) were measured in tissues of oiled seals agree with previous studies that vertebrates, such as fish, do not accumulate high levels of PAHs in their tissues. This is due to the rapid metabolism of PAHs to more polar forms that are transferred to bile and are then eliminated rapidly from the body (Krahn et al., 1984; Varanasi et al., 1989; Beyer et al., 2010). Furthermore, tissues of oilied ice seals (e.g., blubber, skin) with higher percent lipid values (> 10% lipid) had higher concentrations of sum PAHs compared to tissues (e.g., muscle, kidney, liver) with lower lipid content (<10% lipid). Additionally, high lipid-containing blubber samples of visibly oiled PWS harbor seals had higher sum PAHs (ranging from 5.0 to 800 ng/g, wet weight) compared to those in the corresponding lower lipid-containing muscle and liver samples (ranging from < LOQ to 17 ng/g, wet weight) (Varanasi et al., 1993; Frost et al., 1994). Previous PAH exposure studies in fish (Bruner et al., 1994; Meador et al., 1995; Hellou and Leonard, 2004) and field studies conducted on northern Gulf of Mexico sea turtles (Ylitalo et al., 2017) have shown that higher PAH concentrations are associated with tissues with higher percent lipid content. In tissues of unoiled ice seals, the PAH concentrations were either < LOQ or, when detected, were lower (< 20 ng/g, wet weight) than the levels measured in the corresponding

### Table 4

Concentrations (ng/g, wet weight) of sum low molecular weight polycyclic aromatic hydrocarbons (sum LWMAHs), sum high molecular weight polycyclic aromatic hydrocarbons (sum HMWAHs), sum polycyclic aromatic hydrocarbons (sum PAHs) and percent lipid measured in tissues of subsistence-harvested oiled and unoiled ice seals collected in Alaska.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Species</th>
<th>Field identification number</th>
<th>Collection site</th>
<th>Collection date</th>
<th>Percent lipid</th>
<th>ng/g, wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blubber</td>
<td>Spotted seal</td>
<td>N52-2012</td>
<td>Shishmaref</td>
<td>9/5/2012</td>
<td>89</td>
<td>35, 0.6, 36</td>
</tr>
<tr>
<td></td>
<td>Spotted seal</td>
<td>2012-166</td>
<td>Gambell</td>
<td>10/16/2012</td>
<td>85</td>
<td>48, 0.4, 48</td>
</tr>
<tr>
<td></td>
<td>Ringed seal</td>
<td>NS5-2012</td>
<td>Gambell</td>
<td>11/12/2012</td>
<td>94</td>
<td>12, &lt; LOQ, 12</td>
</tr>
<tr>
<td></td>
<td>Ringed seal</td>
<td>NS5-2012</td>
<td>Gambell</td>
<td>11/12/2012</td>
<td>92</td>
<td>25, &lt; LOQ, 25</td>
</tr>
<tr>
<td></td>
<td>Unoiled ringed</td>
<td>(n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Feces</td>
<td>N52-2012</td>
<td>Shishmaref</td>
<td>9/5/2012</td>
<td>1.0</td>
<td>&lt; LOQ, &lt; LOQ, &lt; LOQ</td>
</tr>
<tr>
<td></td>
<td>Hair</td>
<td>NS5-2012</td>
<td>Gambell</td>
<td>11/12/2012</td>
<td>18</td>
<td>32, 8.2, 40</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>NS5-2012</td>
<td>Gambell</td>
<td>9/5/2012</td>
<td>1.6</td>
<td>3.7, &lt; LOQ, 3.7</td>
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<tr>
<td></td>
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<td>2012-166</td>
<td>Gambell</td>
<td>10/16/2012</td>
<td>1.0</td>
<td>0.7, &lt; LOQ, 0.7</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>NS52-2012</td>
<td>Shishmaref</td>
<td>9/5/2012</td>
<td>3.2</td>
<td>15, 2.6, 18</td>
</tr>
<tr>
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<td>Gambell</td>
<td>11/12/2012</td>
<td>4.1</td>
<td>2.2, 5.1, 7.3</td>
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<tr>
<td></td>
<td>Unoiled ringed</td>
<td>(n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>NS5-2012</td>
<td>Shishmaref</td>
<td>9/5/2012</td>
<td>0.8</td>
<td>5.8, &lt; LOQ, 5.8</td>
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<td>Muscle</td>
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<td>Shishmaref</td>
<td>9/5/2012</td>
<td>0.4</td>
<td>6.7, 1.2, 7.9</td>
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<tr>
<td></td>
<td>Ringed seal</td>
<td>NS5-2012</td>
<td>Gambell</td>
<td>10/16/2012</td>
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<td>2.2, 0.2, 2.4</td>
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<td></td>
<td>Skin</td>
<td>NS5-2012</td>
<td>Shishmaref</td>
<td>9/5/2012</td>
<td>1.3</td>
<td>5.9 ± 6.8, 5.3 ± 1.1, 6.4 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>Ringed seal</td>
<td>NS5-2012</td>
<td>Gambell</td>
<td>11/12/2012</td>
<td>1.3</td>
<td>0.2, &lt; LOQ, 0.2</td>
</tr>
<tr>
<td></td>
<td>Stomach contents</td>
<td>NS5-2012</td>
<td>Shishmaref</td>
<td>9/5/2012</td>
<td>NA</td>
<td>3.9, 4.8, 8.7</td>
</tr>
<tr>
<td></td>
<td>Trachea contents</td>
<td>NS5-2012</td>
<td>Shishmaref</td>
<td>9/5/2012</td>
<td>NA</td>
<td>3.3, &lt; LOQ, 3.3</td>
</tr>
</tbody>
</table>

1 Sum of low molecular weight PAHs (LMWAHs) containing 2–3 ring compounds.
2 Sum of high molecular weight PAHs (HMWAHs) containing 4–5 ring compounds.
3 Sum of LMWAHs and HMWAHs.
4 Sample collected from a non-visibly oiled area of seal carcass.
5 Sample collected from a visibly oiled area of seal carcass.
tissues of the oiled ice seals.

Tissues of the oiled spotted seals had elevated concentrations of sum LMWAHs and sum PAHs compared to the levels determined in the same tissues of the oiled and unoiled ringed seals (see Table 2). However, the unoiled ringed seals had elevated biliary NPH and PHN equivalent concentrations, as well as muscle sum PAHs levels, compared to the values for the oiled ringed seal. These differences in PAH exposure may be related to differences in metabolism, the timing and type of PAH source exposure, as well as variations in foraging habitats and prey consumed. For example, adult ringed seals are associated with sea ice throughout the year (Kelly et al., 2010) whereas spotted seals are associated with sea ice from late fall through spring during breeding and nursing season and move toward ice-free coasts during the summer months to feed on schooling fish species (Boveng et al., 2009).

The skin PAH composition patterns of the oiled spotted seal N52-2012 and oiled ringed seal N55-2012 resembled the patterns of the weathered ANSCO (see Fig. 5). Seal N52-2012 was likely exposed to a more weathered petroleum source due to the decreased proportions of naphthalene and alkylated naphthalenes and increased proportions of alkylated dibenzothiophenes compared to the weathered oil samples. In contrast, the percentages of naphthalenes and alkylated naphthalenes in seal N55-2012 were similar to the weathered petroleum samples but, the lack of alkylated chrysenes and certain high molecular weight PAHs (e.g., benzo[a]pyrene, IDP = indeno[1,2,3-cd]pyrene, DBA = dibenz[a,h]anthracene + dibenz[a,c]anthracene, BZP = benzo[g,h,i]perylene).

Fig. 5. Comparisons of percent contribution of individual and alkylated polycyclic aromatic hydrocarbons (PAHs) to summed PAHs determined in a weathered Alaska North Slope crude oil (ANSCO) (black) and a bunker oil sample (striped) (A) to those determined for skin samples of a spotted seal (Phoca largha) N52-2012 (case 1) harvested near Shishmaref, Alaska during September 2012 (B) and a ringed seal (Phoca hispida) N55-2012 (case 3) (C) harvested near Gambell, Alaska during November 2012. Percent contributions of C2- through C4-fluoranthenes/pyrenes are not shown as these compounds were not analyzed in the weathered ANSCO. Abbreviations: NPH = naphthalene, C1–C4NPH = naphthalenes containing one to four alkyl-substituted (e.g., methyl, ethyl, propyl, butyl) groups, ACY = acenaphthylene, ACE = acenaphthene, FLU = fluorene, C1–C3FLU = fluorenes containing one to three alkyl-substituted groups, PHN = phenanthrene, ANT = anthracene, C1–C4PHN/ANT = phenanthrenes/anthracenes containing one to four alkyl-substituted groups, DBT = dibenzothiophene, C1–C3DBT = dibenzothiophenes containing one to four alkyl-substituted groups, FLA = fluoranthene, PYR = pyrene, C1FLA/PYR = fluoranthenes/pyrenes containing one alkyl-substituted group, BAA = benz[a]anthracene, CHR = chrysene, C1–C4CHR = chrysenes containing one to four alkyl-substituted groups, BBF = benzo[b]fluoranthene, BKF/BJF = benzo[k]fluoranthene + benzo[b]fluoranthene, BAP = benzo[a]pyrene, DBA = dibenz[a,h]anthracene + dibenz[a,c]anthracene, BZP = benzo[g,h,i]perylene.
Fig. 6. Comparisons of percent contribution of individual and alkylated polycyclic aromatic hydrocarbons (PAHs) to summed PAHs determined in a weathered Alaska North Slope crude oil (ANSCO) (black) and a bunker oil sample (striped) (A) to those determined in liver samples of spotted seal (*Phoca largha*) N52-2012 (case 1) harvested near Shishmaref, Alaska during September 2012 (B), spotted seal 2012-166 (case 2) (D) harvested near Gambell, Alaska during October 2012 and Case 3 (ringed seal (*Phoca hispida*) N55-2012) (C) harvested near Gambell, Alaska during November 2012. Percent contributions of C2- through C4-fluoranthenes/pyrenes are not shown as these compounds were not analyzed in the weathered ANSCO. Abbreviations: NPH = naphthalene, C1–C4NPH = naphthalenes containing one to four alkyl-substituted (e.g., methyl, ethyl, propyl, butyl) groups, ACY = acenaphthylene, ACE = acenaphthene, FLU = fluorene, C1–C3FLU = fluorenes containing one to three alkyl-substituted groups, PHN = phenanthrene, ANT = anthracene, C1–C4PHN/ANT = phenanthrenes/anthracenes containing one to four alkyl-substituted groups, DBT = dibenzothiophene, C1–C3DBT = dibenzothiophenes containing one to three alkyl-substituted groups, FLA = fluoranthene, PYR = pyrene, C1FLA/PYR = fluoranthenes/pyrenes containing one alkyl-substituted group, BAA = benz[a]anthracene, CHR–CHR = chrysenes, C1-C4CHR = chrysenes containing one to four alkyl-substituted groups, BAF = benzo[b]fluoranthene, BKF/BJF = benzo[j]fluoranthene + benzo[k]fluoranthene, BAP = benzo[a]pyrene, IDP = indeno[1,2,3-cd]pyrene, DBA = dibenzo[a,h]anthracene + dibenzo[a,i]anthracene, BZP = benzo[ghi]perylene.
In the current study, the lung lesions (tracheal edema; interstitial emphysema) observed only in spotted seal N52-2012 are suggestive of effects of oil exposure rather than more chronic pathological conditions such as bacterial pneumonias observed in bottlenose dolphins that stranded in a heavily oiled region (e.g., Barataria Bay, Louisiana) of the northern Gulf of Mexico after the Deepwater Horizon oil spill (Schwacke et al., 2014; Venn-Watson et al., 2015). The acute lesions of marked interstitial pulmonary emphysema and tracheal edema noted in spotted seal N52-2012 were likely an acute manifestation of oil exposure rather than more chronic pathological conditions such as bacterial pneumonias observed in bottlenose dolphins that stranded in a heavily oiled region (e.g., Barataria Bay, Louisiana) of the northern Gulf of Mexico during and after the 2010 Deepwater Horizon oil spill (Venn-Watson et al., 2015). Other noted lung characteristics (i.e. fur density and thickness) are thought to function as a barrier to oil from making epidermal contact (St. Aubin, 1990). In addition, we did not observe kidney and ocular lesions as previously reported for oil exposed marine mammals including ringed seals (Engelhardt, 1982; Smith and Geraci, 1975) and sea otters (Lipscomb et al., 1993). Oil exposure in marine mammals can occur via various routes including physical contact, inhalation, aspiration, or ingestion (Geraci and St. Aubin, 1988; Schwacke et al., 2014; Rosenberger et al., 2017). In the current study, the lung lesions (tracheal edema; interstitial emphysema) observed only in spotted seal N52-2012 are suggestive of possible inhalation related damage due to the oil exposure. Additionally, this seal had elevated sum PAH levels in the lung, liver, muscle, kidney, and skin compared to the other seals (oiled or unoiled). The external oiling pattern on spotted seal N52-2012 suggests that the oiling occurred on land; however, this does not exclude the possibility that this seal may have also been exposed previously to petroleum-contaminated water and inhaled volatile oil components at the air/water interface. Although oil was not observed in the nares or mouth of spotted seal N52-2012, physical oiling of these orifices may also have occurred and contributed to increased exposure to oil via increased respiratory efforts. Lung lesions, attributed to the exposure to volatile fractions of petroleum products, have been well described in experimental and field studies on petroleum exposure in cattle (Coppock et al., 1996), sea otters (Lipscomb et al., 1993), and adrenal and lung lesions most recently in common bottlenose dolphins (Tursiops truncatus) from a heavily oiled site in the Gulf of Mexico after the Deepwater Horizon oil spill (Schwacke et al., 2014; Venn-Watson et al., 2015).
lesions (pulmonary congestion and hemorrhage; pulmonary nematodiases) found in visibly oiled ice seals in the current study are commonly found in lungs of healthy, subsistence-harvested ice seals (NSB DWM, unpublished results 2008–2016).

Acute and chronic cardiac lesions were present in two of the three visibly oiled ice seals. In spotted seal NS2-2012 (case 1), there was a chronic change of regionally extensive fibrosis, myofibers drop out, and myofiber atrophy. These areas of myocardial fibrosis can indicate previous focus of necrosis such as could occur with a wide variety of causes including parasite migration, various nutritional deficiencies such as Vitamin E, thiamine or copper, stress as occurs with catecholamine-induced myocardial necrosis, cardiovascular dysfunction such as myocardial infarction, previous myocarditis or toxic focal damage to the myocardium due to a wide variety of toxins (Maxie and Robinson, 2007). Small foci are generally incidental and the cause cannot be determined. It was a very chronic change and not likely related to the current acute exposure. Ringed seal NS5-2012 (case 3) had a small focus of acute supplicative myocarditis which is most often due to a systemic infection, either bacterial, viral, or protozoal; however, toxicity exposure cannot be ruled out. The cause could not be determined.

Cardiac tissue effects have been previously demonstrated in oil exposed mammals and fish including increased inflammatory cells and myofibril scarring in rats (Han et al., 2010), decreased heart size in zebrafish exposed as embryos (Hicken et al., 2011) associated with decreased cardiac function (Incarnado et al., 2004, 2014), periarteriolar cardiac fibrosis in rats after exposure to naphthenic acids (Rogers et al., 2002), and myocarditis in cattle (Stoeber, 1962 cited in Coppock et al., 1995). In oiled PWS harbor seals, Spraker et al. (1994) reported a single case of acute mild myocardial degeneration of undetermined cause.

Mild to moderate liver lesions were observed in two of the three visibly oiled seals and were not directly related to their liver PAH concentrations. One change seen was rare clear vacuoles in hepatocytes randomly scattered about the liver consistent with fatty change though without special stains glycogen accumulation cannot be entirely ruled out. This is seen occasionally in hunter-killed ice seals (K. Burek-Huntington, personal communication). Gastric ulcers and hemorrhages were also commonly seen in oiled sea otters in Alaska after the Exxon Valdez oil spill, but were thought to be related to the stress of captivity (Lipscomb et al., 1993). In oiled PWS harbor seals, observed digestive lesions were most likely associated with parasites and or parasite migration (Spraker et al., 1994). No additional evidence of underlying disease or trauma were noted for these subsistence-harvested ice seals.

In summary, the three ice-associated seals were exposed to petroleum, based on visible oiling and the presence of various organ lesions, as well as chemical analyses findings documenting detectable levels of biliary PAH metabolites and tissue parent and alkylated PAHs. Although tissue PAH levels were generally low (< 50 ng/g, wet weight) in both oiled and unoiled seals likely due to rapid PAH metabolism and elimination reported previously in fish and other vertebrates (Krahn et al., 1984; Varanasi et al., 1989; Parkinson, 2001; Beyer et al., 2010; Collier et al., 2014; Saengtienchai et al., 2015), tissue PAH concentrations were elevated in oiled ice seals compared to the tissue levels of unoiled ringed seals. The observed cardiac, pulmonary, adrenal, and gastric lesions in oil-exposed seals from this study (see Table 3) are absent or uncommon in unoiled subsistence-harvested ice seals (NSB DWM Marine Mammal Health Baseline monitoring program, unpublished results). For example, among 91 unoiled ringed seals that were subsistence-harvested (2011–2016) and examined, only one (1/91) had interstitial myocarditis and one had myocardial infarction (1/91) (R. Stimmelmayr and D. Rotstein, unpublished results). No cases of tracheal edema and interstitial emphysema were found (0/91); adrenal cortical hyperplasia was present in one animal (1/91); gastritis was found in four animals (4/91). Finally, similarity exists between some of the noted pulmonary, cardiac, and adrenal lesions observed in this study to those previously observed in naturally or experimentally exposed oiled pinnipeds and sea otters (Exxon Valdez oil spill), cattle, and laboratory animals.

As previously discussed by St. Aubin (1990) “we should have little difficulty in predicting the consequences of oil exposure for most pinniped species” in light of diverse records of encounters between oil and pinnipeds. Nevertheless, when determining oil spill related injury and exposure of marine mammals, field case studies continue to be challenged by sample size and sample quality, lack of pre-spill baseline data and limited data from experimental exposure studies (see Schwacke et al., 2014). Our results are no exception and with these caveats in mind, we guardedly propose that the observed pathologic pulmonary, cardiac, adrenal and gastric findings for the oiled ice seals in the current study are related to the documented oil exposure based on the presented chemical and pathologic evidence. Future work on Arctic marine mammals should include collecting baseline diagnostic and petroleum exposure data and rigorous diagnostic work up inclusive of PAH bile and tissue concentration determinations in cases of suspected oiling.

Acknowledgements

We thank the coastal community members of Shishmaref and Gambell for acting on their conservation, public safety, and health concerns. Without sharing their discoveries as well as their efforts to provide their harvested seals for examination, none of this information would be available. The photographs of the spotted seal 2012-166 were
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