Marine Pollution Bulletin 136 (2018) 448–453

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Pre-oil spill baseline profiling for contaminants in Southern Resident killer whale fecal samples indicates possible exposure to vessel exhaust

Jessica I. Lundin[a,*], Gina M. Ylitalo[b], Deborah A. Giles[c], Elizabeth A. Seely[a], Bernadita F. Anulacion[b], Daryle T. Boyd[b], Jennifer A. Hempelmann[e], Kim M. Parsons[d,e], Rebecca K. Booth[a], Samuel K. Wasser[a]

[a] Center for Conservation Biology, Department of Biology, University of Washington, Seattle, WA 98195, United States of America
[b] Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd E, Seattle, WA 98112, United States of America
[c] University of Washington Friday Harbor Labs, Friday Harbor, WA 98250, United States of America
[d] Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 7600 Sand Point Way N.E., Building 4, Seattle, WA 98115, United States of America
[e] Conservation Biology Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd E, Seattle, WA 98112, United States of America

ARTICLE INFO

Keywords:
Killer whale
Salish Sea
Polycyclic aromatic hydrocarbons
Oil spill
Vessel exhaust

ABSTRACT

The Southern Resident killer whale population (*Orcinus orca*) was listed as endangered in 2005 and shows little sign of recovery. Exposure to contaminants and risk of an oil spill are identified threats. Previous studies on contaminants have largely focused on legacy pollutants. Here we measure polycyclic aromatic hydrocarbons (PAHs) in whale fecal (scat) samples. PAHs are a diverse group of hazardous compounds (e.g., carcinogenic, mutagenic), and are a component of crude and refined oil as well as motor exhaust. The central finding from this study indicates low concentrations of the measured PAHs (< 10 ppb, wet weight), as expected; however, PAHs were as high as 104 ppb prior to implementation of guidelines mandating increased distance between vessels and whales. While causality is unclear, the potential PAH exposure from vessels warrants continued monitoring. Historical precedent similarly emphasizes the importance of having pre-oil spill exposure data available as baseline to guide remediation goals.

1. Introduction

Marine traffic of oil tankers and other vessels to ports in Washington State and British Columbia, as well as oil transportation by rail and pipeline, increases the vulnerability of inland marine waters to a catastrophic event such as a spill or grounding. Current shipping lanes transect areas designated as critical habitat for wildlife listed as endangered and threatened under the U.S. Endangered Species Act, including the Southern Resident killer whales (SRKWs) (Ecology, 2015). This area of inland waters is partially separated from the open Pacific Ocean, with few places for the oil to disperse. Historic events (e.g., "*Exxon Valdez*" oil spill in Alaska and "*Deepwater Horizon*" oil spill in the Gulf of Mexico) have demonstrated that oil spills in the marine environment can have population-level consequences for aquatic species. Specifically, exposure to oil in marine mammals has been associated with adrenal dysfunction and increased lung disease (Schwacke et al., 2013; Venn-Watson et al., 2015), cardiac, pulmonary, adrenal and gastric lesions (Stimmelmayr et al., 2018), reduced reproductive success (Kellar et al., 2017), immune system impairment (De Guise et al., 2017), and population decline (Matkin et al., 2008). A common component of oil is polycyclic aromatic hydrocarbons (PAHs), a diverse group of compounds known to be both carcinogenic and mutagenic that rank in the top 10 hazardous substances by the United States Agency for Toxic Substances and Disease Registry (ATSDR, 2018). Marine mammal exposure to PAHs and other compounds following a spill can initially occur through inhalation as volatile components of the oil slick evaporate, followed by contact and ingestion in the water column, and persistence in the marine environment once in sediment (Rosenberger et al., 2017).

Combustion of fuel from boat motors is another source of PAHs, and an additional risk for the Southern Resident killer whales because of shipping and recreational boat traffic, ferries, fishing vessels, etc.

* Corresponding author at: Department of Biology, Center for Conservation Biology, Johnson Hall 231, Box 351800, Seattle, WA 98195, United States of America.
E-mail address: jessica.lundin.uwalumni@gmail.com (J.I. Lundin).

https://doi.org/10.1016/j.marpolbul.2018.09.015
Received 11 May 2018; Received in revised form 7 September 2018; Accepted 9 September 2018

commonly found in the inland waters. Marine mammals are inherently vulnerable to poor air quality due to their extended time spent at the water's surface, and their deep breaths. A previous estimate demonstrated safe pollutant (PAH) levels of vessel exhaust exposure in Southern Resident killer whales are exceeded under certain conditions (Lachmuth et al., 2011). Concerns related to vessel traffic as a risk factor for Southern Resident killer whales is not a new concept (NMFS, 2008); however much of the focus to date has been on foraging behavior (Lusseau et al., 2009) and the effects of vessel noise (Holt et al., 2009).

Previous studies on the health risks of exposure to toxic contaminants on the killer whale population have focused on legacy persistent organic pollutants (Krahn et al., 2007; Lundin et al., 2016; Ross et al., 2000), with few studies that have evaluated exposure to other environmental contaminants (Lachmuth et al., 2011; Rayne et al., 2004; Rosenberger et al., 2017). The objective of this study was to measure PAH concentrations in fecal (scat) samples from this endangered killer whale population. Floating killer whale fecal samples were collected and analyzed using gas chromatography–mass spectrometry. Results from this study indicate low current levels of PAH exposure in the whales. Prior to the change in guidelines that increased the protective distance of boats from this population, the PAH exposure may have been higher.

2. Methods

2.1. Sample collection

A total of 263 SRKW fecal samples were collected May through October from 2010 to 2013; of these, 70 samples were > 2 g, which was the minimum mass for the chemical analysis used in this study. All samples were collected within 30 linear miles of Mosquito Bay, San Juan Island, Washington, USA (Fig. 1). Samples were located by detection dogs trained to locate SRKW fecal floating on the water surface (Ayres et al., 2012; Lundin et al., 2016; Wasser et al., 2004). Samples were scooped off the surface of the water using a 1 L polypropylene beaker, collected in a 50 mL polypropylene tube, immediately centrifuged using a small field centrifuge, and all sea-water was decanted. The remaining pellet was kept on ice, frozen the day of collection, and then transferred to a −20 °C freezer until processed in the lab. Collections occurred from mid-May through mid-October, the time period when the SRKWs appear with regularity in the waters around the San Juan Islands and Puget Sound of Washington state, collectively referred to as the Salish Sea (Fig. 1).

2.2. Life history data

Age, sex, family lineage, and reproductive status of whales genotyped in this study (described below) were determined using annual population census data collected through photo-identification since 1976 by the Center for Whale Research (CWR, 2018). Age-sex class was defined as juveniles (either sex, < 10 years), adult males (≥10 years), reproductive-age females (≥10 to < 40 years) (CWR, 2018; Robeck et al., 2004), and post-reproductive females (40+ years).

2.3. Laboratory methods

Samples were thawed, homogenized, and sub-sampled for high throughput genotyping to determine individual identification (Ford et al., 2011) and for toxicant analysis. Individual identification was linked with the population census data to determine pod and age-sex class (CWR, 2018). Seventy samples were analyzed for PAHs and, as reported previously, persistent organic pollutants (POPs) (Lundin et al., 2016). These samples were extracted, purified, and analyzed for PAH concentrations using the procedures of Sloan et al. (2014). In brief, samples were extracted with dichloromethane using an accelerated

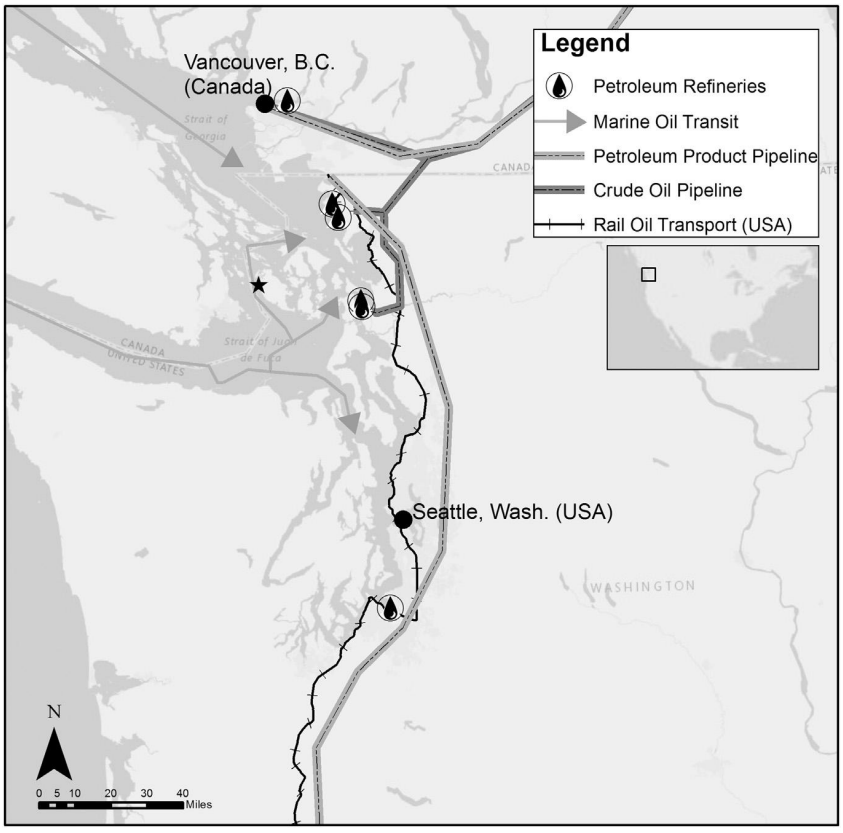

Fig. 1. Map of Southern Resident killer whale habitat within the Salish Sea, showing locations of petroleum refineries together with areas of pipeline, marine, and rail transport of oil. The star (★) marks Mosquito Bay, San Juan Island, Washington, USA; all fecal samples were collected within 30 linear miles of this point. Service layer credits: Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community; Data source for map layers: https://www.eia.gov/mapslayer_info-m.php, http://www.wsdot.wa.gov/mapsdata/geodatacatalog/.

Table 1
Descriptive data of fecal samples; 70 samples from 34 unique whales, with number of samples by collection year, collection month, pod, and age-sex class.

Year		2010	2011	2012	2013
Total samples measured		19	20	13	18
Number of unique whales		14	15	10	11
Month	June	3	2	3	7
	July	3	5	2	2
	August	7	7	4	4
	September	6	6	4	4
	October	0	0	0	1
Pod	J	6	6	7	7
	K	6	3	3	2
	L	5	6	1	4
	Unknown[a]	2	5	2	5
Age-sex class	Juvenile	1	2	1	1
	Adult female	7	6	6	4
	Adult male	2	5	3	6
	Post-reproductive female	7	2	1	2
	Unknown[a]	2	5	2	5

[a] Identity unknown; individual identification from genotype analysis not available for all whales.

solvent extractor. The extracts were then purified using a gravity flow cleanup column containing alumina/silica to remove highly polar compounds, followed by a second cleanup step to remove lipids and other biogenic compounds using size exclusion high-performance liquid chromatography. The sample extracts were then analyzed using gas chromatography–mass spectrometry (GC/MS). In total, 25 PAHs were analyzed (naphthalene, NPH; 1-methylnaphthalene, MN1; 2-methylnaphthalene, MN2; 2,6-dimethylnaphthalene, DMN; 2,3,5-trimethyl naphthalene, TMN; acenaphthylene, ACY; acenaphthene, ACE; fluorene, FLU; dibenzothiophene, DBT; phenanthrene, PHN; anthracene, ANT; 1-methylphenanthrene, MP1; fluoranthene, FLA; pyrene, PYR; retene (methyl isopropyl phenanthrene); benz[*a*]anthracene, BAA; chrysene + triphenylene (co-elution), CHR; benzo[*b*]fluoranthene, BBF; benzo[*j*]fluoranthene + benzo[*k*]fluoranthene (co-elution), BKF; benzo[*e*]pyrene, BEP; benzo[*a*]pyrene, BAP; perylene, PER; indeno[1,2,3-*cd*]pyrene, IDP; dibenz[*a,h*]anthracene + dibenz[*a,c*]anthracene (co-elution), DBA; and benzo[*ghi*]perylene, BZP. All PAH concentrations are reported as ng/g, wet weight.

2.4. Statistical methods

Genetic identification was known for 56 of the 70 samples. Analysis of variance was performed for year (2010, 2011, 2012, and 2013), pod (J, K, L, unknown), Julian day of sample collection (continuous), and age-sex class (juvenile, adult male, adult female, post-reproductive female, and unknown), with whale identity included as a random effect to account for repeat sampling from an individual whale. This analysis was performed for summation of all PAHs measured (sum PAHs), as well as the summation of low molecular weight (LMWAHs; NPH, MN1, MN2, DMN, TMN, ACY, ACE, FLU, DBT, PHN, ANT, MP1, retene) and high molecular weight (HMWAHs; FLA, PYR, BAA, CHR, BBF, BKF, BAP, BEP, PER, IDP, DBA, BZP) compounds. All levels below the lower limit of quantification were reported as zero. When levels in field samples were less than three times those measured in the analysis-set method blank, they were reported as zero. All analyses were conducted in R (version 3.2.2, R Development Core Team, 2015). The level of significance used for all statistical tests was $p < 0.05$.

3. Results

The PAHs were measured in 70 fecal samples. Genetic identity was determined for 56 samples, representing 34 unique whales (Table 1). Nineteen samples were collected in 2010, 20 in 2011, 13 in 2012, and 18 in 2013. Samples represented all three SRKW pods; 37% of samples were from members of J pod (26/70), 20% from K pod, and 23% from L pod (Table 1). Seven percent of samples represented juvenile whales (5/70), while 33% were from reproductive-age females, 17% from post-reproductive females, and 23% from adult males.

The concentrations of sum PAHs in all fecal samples were below 10 ppb wet weight (Fig. 2; Table 2) except in four outliers collected in 2010 (discussed below). The sum PAHs for these outliers ranged from 11 to 104 ppb wet weight. In 2010, at least 25% of the samples had concentrations of MN2, DMN, ACE, FLU, and PHN that were above the lower level of quantitation, and more than three times those measured in the analysis-set method blank (Supplemental Table 1). In 2011, this list of compounds was MN2, ACY, ACE, FLU, PHN, ANT, MP1, FLA, PYR, CHR, and, in 2012 and 2013, this list only included NPH, MN1, and MN2. All other measured PAHs for each year were above the lower limit of detection or more than three times those measured in the analysis-set method blank in < 25% of the samples.

There was no significant effect of year, pod, or age-sex class for sum PAHs, sum LMWAHs, or sum HMWAHs. Similarly, there was no significant effect for Julian day of sample collection for sum PAHs, sum LMWAHs, or sum HMWAHs. However, a review of the data for each year revealed four 2010 samples with distinctively high signals compared to the rest of the samples (Fig. 2). Sample 72 (collected on June 13 from adult male J26) demonstrated a unique low-molecular weight signal. Samples 1, 18, and 19 (respectively collected June 13 from adult male J26, July 7 from adult female K16, and July 7 from post-reproductive female L7) demonstrated a distinct high-molecular weight signal.

4. Discussion

The Southern Resident killer whales are currently experiencing negligible exposure to the PAHs measured; with a few exceptions, all samples were below 10 ppb wet weight for sum PAHs. These concentrations reflect a profile of recent exposure because bioaccumulation of PAHs occurs less readily than for POPs due to their chemical properties (Nfon et al., 2008) and the ability of vertebrates to rapidly metabolize PAHs to more polar compounds that can be readily eliminated (Varanasi et al., 1993; Meador et al., 1995; Ylitalo et al., 2017). This PAH baseline data, in conjunction with other health and population metrics on the Southern Resident killer whale population (CWR, 2018; Fearnbach et al., 2011; Wasser et al., 2017), will be invaluable to guide remediation goals and damage assessment evaluations in the event of an oil spill. In particular, the collection and measurement of PAHs in SRKW fecal samples following an oil spill, relative to the baseline measures reported here, would provide vital information on recent exposure indicating the extent of the spill and the effectiveness of the

Table 2
Quartiles demonstrating levels of PAHs measured in fecal samples (ng/g, wet weight); in total, 25 PAHs were analyzed in 70 samples.

	Min	25%	50%	75%	Max
Sum PAHs	0.00	1.06	1.79	3.73	103.59
Sum LMWAHs	0.00	0.88	1.68	3.27	45.33
Sum HMWAHs	0.00	0.00	0.00	0.27	84.60

NOTE: sum PAHs: summation of all 25 PAHs measured; sum LMWAHs: NPH, MN1, MN2, DMN, TMN, ACY, ACE, FLU, DBT, PHN, ANT, MP1, retene; sum HMWAHs: FLA, PYR, BAA, CHR, BBF, BKF, BAP, BEP, PER, IDP, DBA, BZP.

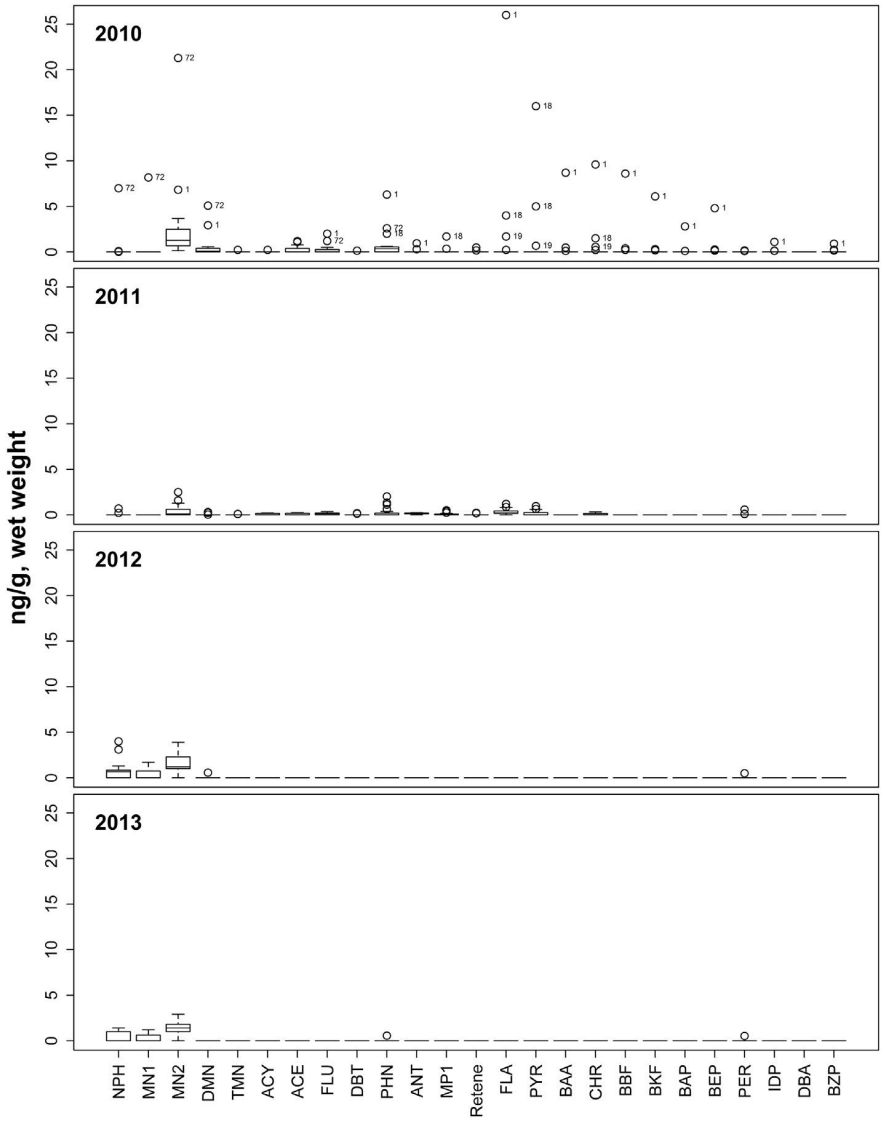

Fig. 2. Boxplots of 25 PAHs (ng/g, wet weight) measured in SRKW fecal samples, by year. Notations for 2010 denote sample number of 4 outliers, samples 1, 18, 19, and 72.
NOTE: naphthalene, NPH; 1-methylnaphthalene, MN1; 2-methylnaphthalene, MN2; 2,6-dimethylnaphthalene, DMN; 2,3,5-trimethyl naphthalene, TMN; acenaphthylene, ACY; acenaphthalene, ACE; fluorene, FLU; dibenzothiophene, DBT; phenanthrene, PHN; anthracene, ANT; 1-methylphenanthrene, MP1; retene (methyl isopropyl phenanthrene); fluoranthene, FLA; pyrene, PYR; benz[*a*]anthracene, BAA; chrysene + triphenylene (co-elution), CHR; benzo[*b*]fluoranthene, BBF; benzo[*j*]fluoranthene + benzo[*k*]fluoranthene (co-elution), BKF; benzo[*a*]pyrene, BAP; benzo[*e*]pyrene, BEP; perylene, PER; indeno[1,2,3-*cd*]pyrene, IDP; dibenz[*a,h*]anthracene + dibenz[*a,c*]anthracene (co-elution), DBA; and benzo[*ghi*]perylene, BZP.

clean-up efforts. Further validation of these methods, along with the inclusion of alkylated and hydroxylated PAH compounds, would increase confidence in the source and environmental pathway of the PAH measurements. The historical precedent of oil spill impacts on marine ecosystems has emphasized the importance and utility of having pre-spill census and exposure data on different species, particularly wild cetaceans. Pre-spill data was not available for the *Exxon Valdez* spill, making it challenging to evaluate individual- and population-level impacts on resident killer whale population recovery (Matkin et al., 2008).

The vulnerabilities of this killer whale population to an oil spill is forecast to increase. Proposed expansion of oil pipelines (e.g., Kinder Morgan Trans Mountain Pipeline System carrying oil from Alberta) and the movement of oil by rail, which started bringing oil to the state of Washington from North Dakota in 2012 (with ~10% from Alberta and Saskatchewan), will increase the amount of oil transported within this region. Estimates across the last few years amount to > 2 billion gallons of oil transported quarterly in the state of Washington (Ecology, 2017, 2018); half of the oil transported by marine vessel, with a quarter by a rail and a quarter by pipeline transport systems (Ecology, 2015, 2017, 2018). However, the shift from historically predominant tank ship transportation of oil (90%) does not necessarily decrease the risks to aquatic ecosystems, as these pipelines terminate at marine ports, and the railways run along major waterways such as the Columbia River and Puget Sound (Fig. 1). Mitigation of potential oil spill risks through prevention and waterway management will be critical moving forward.

An unexpected finding from this study was four samples, all collected in 2010, that had levels of PAHs above 10 ppb (ranging from 11 to 104 ng/g, wet weight). Sample 72 was laden with low molecular-weight PAHs, a signature of crude or refined oil (versus combustion) source. This single 2010 sample may be due to leaked gas or oil on the surface of the water contaminating the sample. The remaining three 2010 outliers contained high molecular-weight PAHs, which is a signature of PAHs produced through combustion. The Be Whale Wise guidelines (www.bewhalewise.org), federal regulations under the Marine Mammal Protection Act and Endangered Species Act, increased the legal distance that boats must maintain from whales from 100 to 200 yards (yds) after 2010. The extent these three whales were exposed to boat exhaust during 2010 when vessels were allowed to be in closer proximity to the whales is unknown. However, empirical evidence has shown combustion engine emissions at 31% of source at 100 m (109 yd) distance, and at background levels by 200 m (219 yd) distance (Lachmuth et al., 2011). Following the implementation of the mandated 200 yd. distance between marine vessels and whales in 2011, our next three years of fecal collection showed no samples with PAHs above

10 ppb. This suggests that whales may have experienced a decrease in exposure to combustion engine emissions following the updated vessel regulations. Marine vessels around the whales continue to increase in size and number. Even with regulations protecting the distance between the vessels and the whales, the potential PAH exposure from vessels warrants PAH sampling in future years.

It is possible that the high molecular weight PAH levels in the three 2010 sample were deposited atmospherically on the fecal sample floating on the surface of the water. Alternatively, the increased PAHs in 2010 may simply have resulted from contamination during field sampling. We do not expect the latter to be the case as caution was taken to maintain a clean sampling station both onboard the research vessel, as well as in the laboratory. However, PAH compounds are pervasive and field contamination cannot be ruled out. PAH contamination could also have been from our own research vessel; although, that being the case, we would also have expected signals of elevated PAHs in later year samples since our sampling methodologies did not change across the 4-year study.

Killer whales are highly vulnerable to adverse risk from oil spills due to their small population size, slow rate of reproduction, and dietary specialization (NMFS, 2008; Rosenberger et al., 2017). Baseline PAH exposure data from this study will provide a reference point for remediation goals in the potential event of an oil spill in the inland waters of Washington state (USA) and British Columbia (Canada). However, more preventative action through preparation and management is recommended to diminish the risk of an oil spill in the Salish Sea, and particularly within the designated critical habitat for the Southern Resident killer whales (Fig. 1).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2018.09.015.

Acknowledgements

This work was supported by the Washington Sea Grant, University of Washington [pursuant to National Oceanic and Atmospheric Administration (NOAA), award no. NA10OAR417005], Seattle, Washington, USA. All samples were analyzed at the NOAA National Marine Fisheries Service Northwest Fisheries Science Center (NWFSC) in the Environmental and Fisheries Sciences Division. This publication was developed under STAR Fellowship Assistance Agreement no. 91735201 awarded by the U.S. Environmental Protection Agency (EPA). It has not been formally reviewed by the EPA. The views expressed in this publication are solely those of the authors, and the EPA does not endorse any products or commercial services mentioned in this publication. The authors thank Jennie Bolton for careful review of this manuscript. The authors also thank field and lab assistants and volunteers, and the killer whale community for continued support helping to make our project a success. Fecal samples from Southern Resident killer whales were collected in United States waters under National Marine Fisheries Service permits 10045. Fecal samples were collected in Canadian waters under Marine Mammal License numbers 2010-09 and 2012-08, as well as Species at Risk Act permits numbered 109 and 155.

References

ATSDR, 2018. Agency for Toxic Substances and Disease Registry. Priority list of hazardous substances. http://www.atsdr.cdc.gov/SPL/index.html, Accessed date: 7 March 2018.

Ayres, K.L., Booth, R.K., Hempelmann, J.A., Koski, K.L., Emmons, C.K., Baird, R.W., Balcomb-Bartok, K., Hanson, M.B., Ford, M.J., Wasser, S.K., 2012. Distinguishing the impacts of inadequate prey and vessel traffic on an endangered killer whale (*Orcinus orca*) population. PLoS One 7, e36842.

CWR, 2018. Center for Whale Research. About killer whales. http://www.whaleresearch.com/#!about-orcas/c1qa8, Accessed date: 7 March 2018.

De Guise, S., Levin, M., Gebhard, E., Jasperse, L., Hart, L.B., Smith, C.R., Venn-Watson, S., Townsend, F., Wells, R.S., Balmer, B., 2017. Changes in immune functions in bottlenose dolphins in the northern Gulf of Mexico associated with the Deepwater Horizon oil spill. Endanger. Species Res. 33, 291–303.

Ecology, 2015. Department of Ecology. Washington State 2014 Maine and Rail Oil Transportation Study. Publication Number: 15-08-010.

Ecology, 2017. Department of Ecology. Crude Oil Movement by Rail and Pipeline Quarterly Report: October 1, 2016 to December 31, 2016. Revised February 2017. Publication no. 17-08-002.

Ecology, 2018. Department of Ecology. Crude Oil Movement by Rail and Pipeline Quarterly Report: April 1, 2018 through June 30, 2018. July 2018. Publication 18-08-011.

Fearnbach, H., Durban, J.W., Ellifrit, D.K., Balcomb III, K.C., 2011. Size and long-term growth trends of endangered fish-eating killer whales. Endanger. Species Res. 13, 173–180.

Ford, M.J., Hanson, M.B., Hempelmann, J.A., Ayres, K.L., Emmons, C.K., Schorr, G.S., Baird, R.W., Balcomb, K.C., Wasser, S.K., Parsons, K.M., Balcomb-Bartok, K., 2011. Inferred paternity and male reproductive success in a killer whale (*Orcinus orca*) population. J. Hered. 102, 537–553.

Holt, M.M., Noren, D.P., Veirs, V., Emmons, C.K., Veirs, S., 2009. Speaking up: killer whales (*Orcinus orca*) increase their call amplitude in response to vessel noise. J. Acoust. Soc. Am. 125, EL27–EL32.

Kellar, N.M., Speakman, T.R., Smith, C.R., Lane, S.M., Balmer, B.C., Trego, M.L., Catelani, K.N., Robbins, M.N., Allen, C.D., Wells, R.S., 2017. Low reproductive success rates of common bottlenose dolphins *Tursiops truncatus* in the northern Gulf of Mexico following the Deepwater Horizon disaster (2010–2015). Endanger. Species Res. 33, 143–158.

Krahn, M.M., Hanson, M.B., Baird, R.W., Boyer, R.H., Burrows, D.G., Emmons, C.K., Ford, J.K.B., Jones, L.L., Noren, D.P., Ross, P.S., Schorr, G.S., Collier, T.K., 2007. Persistent organic pollutants and stable isotopes in biopsy samples (2004/2006) from southern resident killer whales. Mar. Pollut. Bull. 54, 1903–1911.

Lachmuth, C.L., Barrett-Lennard, L.G., Steyn, D.Q., Milsom, W.K., 2011. Estimation of southern resident killer whale exposure to exhaust emissions from whale-watching vessels and potential adverse health effects and toxicity thresholds. Mar. Pollut. Bull. 62, 792–805.

Lundin, J.I., Ylitalo, G.M., Booth, R.K., Anulacion, B.F., Hempelmann, J., Parsons, K.M., Giles, D.A., Seely, E.A., Hanson, M.B., Emmons, C.K., Wasser, S.K., 2016. Modulation in persistent organic pollutant concentration and profile by prey availability and reproductive status in Southern Resident killer whale scat samples. Environ. Sci. Technol. 50, 6506–6516.

Lusseau, D., Bain, D.E., Williams, R., Smith, J.C., 2009. Vessel traffic disrupts the foraging behavior of southern resident killer whales *Orcinus orca*. Endanger. Species Res. 6, 211–221.

Matkin, C.O., Saulitis, E.L., Ellis, G.M., Olesiuk, P., Rice, S.D., 2008. Ongoing population-level impacts on killer whales *Orcinus orca* following the 'Exxon Valdez' oil spill in Prince William Sound, Alaska. Mar. Ecol. Prog. Ser. 356, 269–281.

Meador, J., Stein, J., Reichert, W., Varanasi, U., 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms, Reviews of environmental contamination and toxicology. Springer, pp. 79–165.

Nfon, E., Cousins, I.T., Broman, D., 2008. Biomagnification of organic pollutants in benthic and pelagic marine food chains from the Baltic Sea. Sci. Total Environ. 397, 190–204.

NMFS, 2008. National Marine Fisheries Service. Recovery Plan for Southern Resident Killer Whales (*Orcinus orca*). National Marine Fisheries Service, Northwest Region, Seattle, Washington.

R Development Core Team, 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rayne, S., Ikonomou, M.G., Ross, P.S., Ellis, G.M., Barrett-Lennard, L.G., 2004. PBDEs, PBBs, and PCNs in three communities of free-ranging killer whales (Orcinus orca) from the northeastern Pacific Ocean. Environ. Sci. Technol. 38, 4293–4299.

Robeck, T.R., Steinman, K.J., Gearhart, S., Reidarson, T.R., McBain, J.F., Monfort, S.L., 2004. Reproductive physiology and development of artificial insemination technology in killer whales (*Orcinus orca*). Biol. Reprod. 71, 650–660.

Rosenberger, A.L.J., MacDuffee, M., Rosenberger, A.G.J., Ross, P.S., 2017. Oil spills and marine mammals in British Columbia, Canada: development and application of a risk-based conceptual framework. Arch. Environ. Contam. Toxicol. 73, 131–153.

Ross, P.S., Ellis, G.M., Ikonomou, M.G., Barrett-Lennard, L.G., Addison, R.F., 2000. High PCB concentrations in free-ranging pacific killer whales, Orcinus orca: effects of age, sex and dietary preference. Mar. Pollut. Bull. 40, 504–515.

Schwacke, L.H., Smith, C.R., Townsend, F.I., Wells, R.S., Hart, L.B., Balmer, B.C., Collier, T.K., De Guise, S., Fry, M.M., Guillette Jr., L.J., 2013. Health of common bottlenose dolphins (*Tursiops truncatus*) in Barataria Bay, Louisiana, following the Deepwater Horizon oil spill. Environ. Sci. Technol. 48, 93–103.

Sloan, C.A., Anulacion, B.F., Baugh, K.A., Bolton, J.L., Boyd, D., Boyer, R.H., Burrows, D.G., Herman, D.P., Pearce, R.W., Ylitalo, G.M., 2014. Northwest Fisheries Science Center's Analyses of Tissue, Sediment, and Water Samples for Organic Contaminants by Gas Chromatography/mass Spectrometry and Analyses of Tissue for Lipid Classes by Thin Layer Chromatography/flame Ionization Detection. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-125.

Stimmelmayr, R., Ylitalo, G.M., Sheffield, G., Beckmen, K.B., Burek-Huntington, K.A., Metcalf, V., Rowles, T., 2018. 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. Mar. Pollut. Bull. 130, 311–323.

Varanasi, U., Casillas, E., Arkoosh, M.R., Hom, T., Misitano, D.A., Brown, D.W., Chan, S., Collier, T.K., McCain, B.B., Stein, J.E., 1993. Contaminant exposure and associated biological effects in juvenile chinook salmon (Oncorhynchus tshawytscha) from urban and nonurban estuaries of Puget Sound. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northwest Fisheries Science Center, Environmental Conservation Division.

Venn-Watson, S., Colegrove, K.M., Litz, J., Kinsel, M., Terio, K., Saliki, J., Fire, S., Carmichael, R., Chevis, C., Hatchett, W., 2015. Adrenal gland and lung lesions in Gulf of Mexico common bottlenose dolphins (Tursiops truncatus) found dead following the Deepwater Horizon oil spill. PLoS One 10, e0126538.

Wasser, S.K., Davenport, B., Ramage, E.R., Hunt, K.E., Parker, M., Clarke, C., Stenhouse,

G., 2004. Scat detection dogs in wildlife research and management: applications to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. Can. J. Zool. 82, 475–492.
Wasser, S.K., Lundin, J.I., Ayres, K., Seely, E., Giles, D., Balcomb, K.C., Hempelmann, J., Parsons, K.M., Booth, R.K., 2017. Population growth is limited by nutritional impacts on pregnancy success in endangered Southern Resident killer whales (*Orcinus orca*). PLoS One 12, e0179824.
Ylitalo, G.M., Collier, T.K., Anulacion, B.F., Juaire, K., Boyer, R.H., da Silva, D.A., Keene, J.L., Stacy, B.A., 2017. Determining oil and dispersant exposure in sea turtles from the northern Gulf of Mexico resulting from the Deepwater Horizon oil spill. Endanger. Species Res. 33, 9–24.