Evaluating exposure of northern fur seals, *Callorhinus ursinus*, to microplastic pollution through fecal analysis

Mary J. Donohue[a,b]*, Julie Masura[c], Thomas Gelatt[d], Rolf Ream[d], Jason D. Baker[e], Kayleigh Faulhaber[c], and Darren T. Lerner[a,b]

[a] University of Hawai‘i Sea Grant College Program, School of Ocean and Earth Science and Technology, University of Hawai‘i at Mānoa, 2525 Correa Road, Hawai‘i Institute of Geophysics 238, Honolulu, Hawai‘i, 96822, USA

[b] University of Hawai‘i Water Resources Research Center, University of Hawai‘i at Mānoa, 2540 Dole St., Holmes Hall 283, Honolulu, HI 9682, Honolulu, Hawai‘i, 96822, USA

[c] Center for Urban Waters, University of Washington, Tacoma, 1900 Commerce St., Tacoma, WA 98402, USA

[d] Marine Mammal Laboratory, Alaska Fisheries Science Center/NOAA, 7600 Sand Point Way N.E. F/AKC3, Seattle, WA 98115-6349, USA

[e] Protected Species Division, Pacific Islands Fisheries Science Center, NOAA IRC, 1845 Wasp Boulevard, Building 176, Honolulu, HI 96818, USA

*Corresponding author

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Abstract

Environmental microplastics are widely documented in marine life and bioaccumulation may present risks to marine predators. Investigations of microplastics in marine mammals are increasing, though none have examined animals routinely consumed by humans. Here, we investigate microplastic exposure in the northern fur seal (*Callorhinus ursinus*), a species consumed by humans, using fecal material. We examined 44 feces (scat) at sites encompassing the seals' eastern Pacific range. Multiple contamination control measures were implemented, including field and laboratory controls. Fragments were the most common microplastic recovered, in 55% (24/44) of scat and no controls (range 1 to 86 fragments/scat, mean 16.6, sd 19.1). Microplastic fibers were recovered from 41% of scats (18/44), though some controls contained fibers confounding fiber results. Fecal analysis documented northern fur seal exposure to microplastics throughout their eastern Pacific range.

Keywords: northern fur seal, microplastics, marine debris, bioaccumulation, Pacific Ocean, trophic transfer.

Introduction

Synthetic plastic particles of generally less than 5mm in size, broadly regarded as microplastics (Andrady, 2011; Arthur et al., 2009; Gregory and Andrady, 2003), are widely characterized as environmental contaminants (Andrady, 2011; Barnes et al., 2009;

Moore, 2008; Rilling, 2012; Rochman et al., 2013). Pollution of aquatic ecosystems by microplastics has received considerable attention (Anderson et al., 2016; Brown et al., 2010; Eriksen et al., 2013; Jambek et al., 2015; Law and Thompson, 2014; Thompson, 2015) as have the absolute and relative threats presented by microplastic pollution (Koelmans et al., 2017; Lithner et al., 2011; Rochman et al., 2016; Wilcox et al., 2015). Microplastics are reported from the marine environment worldwide (Andrady, 2011; Arthur et al., 2009; Browne et al. 2011; Cole et al., 2010; Eriksen et al., 2013; GESAMP, 2015; Ivar do Sul and Costa, 2014; Law et al., 2010; Lusher et al., 2014) and in marine animals (Boerger et al., 2010; Foekema et al., 2013; Lusher et al., 2015).

Multiple attributes of microplastic pollution are of concern. Ingestion can be problematic simply due to the internal mechanical impacts of these durable particles (Wright et al., 2013) as has been shown in invertebrate (Cole et al., 2015; Sussarellu et al., 2016) and vertebrate studies (Pedà et al., 2016). Some chemicals used to manufacture plastic polymers pose environmental and health hazards (Lithner et al., 2011) and can leach from ingested plastics into animal tissues (Engler, 2012; Jarasova et al., 2009; Koelmans et al., 2014; Teuten et al., 2007). Accretion of pathogenic bacteria on “sticky” microbial biofilms that develop on environmental microplastics is an emerging concern (Galloway et al., 2017; Kirstein et al., 2016; Rummel et al., 2017). Further, some persistent organic pollutants present in the environment preferentially adsorb and become concentrated on plastic in the marine environment (Mato et al., 2001; Rios et al., 2007; Rochman et al., 2012; Teuten et al., 2007). Hydrophobic, microplastic-associated toxins may have lethal and non-lethal developmental effects on organisms exposed to them including via trophic

transfer of contaminated particles (Batel et al., 2016; Engler, 2012). For example, polychlorinated biphenyls impact physiology and survival in Atlantic salmon (Iwanowicz, et al., 2005; Lerner et al., 2007) and pinnipeds, the latter via carcinogenic action (Ylitalo et al., 2005).

Bioaccumulation of microplastic particles and associated toxins within marine food webs is a potential threat (Engler, 2012; Eriksson and Burton, 2003; Wright et al., 2013). Microplastic trophic transfer has been observed experimentally in zooplankton (Setälä et al., 2014) and from mussels to crabs (Farrell and Nelson, 2013). Trophic transfer has also been reported in a predatory fish (Ferreira et al., 2018) and microplastics have been documented in fish (Rochman et al., 2015) and bivalves (Rochman et al., 2015; van Cauwenberghe and Janssen, 2014) for sale for human consumption. A recent study by Nelms et al. (2018) is the first to experimentally demonstrate trophic transfer of microplastic particles from fish to a captive marine mammal.

Understanding of the fate and impacts of microplastics ingested by animals directly or indirectly through prey is increasing. Laboratory experiments show ingestion of polystyrene particles negatively affects oysters through a suite of ecophysiological effects (Sussarellu et al., 2016) and microplastic uptake in the mussel, *Mytilus edulis*, results in particle circulatory and tissue deposition (Brown et al., 2008; Farrell and Nelson, 2013; von Moos et al., 2012). Detrimental effects of ingestion are also known in marine zooplankton (Cole et al., 2013; Cole et al., 2015; Lee et al., 2013), lugworms (Besseling et al., 2013; Wright et al., 2013) and corals (Hall et al., 2015). European sea bass,

Dicentrarchus labrax, fed microplastics showed abnormal intestinal histology (Pedà et al., 2016) and Japanese medaka fish, *Oryzias latipes*, exposed to virgin polyethylene particles and polyethylene with chemicals absorbed from the marine environment expressed hepatic stress (Rochman et al., 2013). Such reported effects in omnivores and predators (Pedà et al. 2016; Rochman et al. 2013; 2015) highlight concerns regarding bioaccumulation of microplastics and associated toxins in upper trophic-level organisms.

A handful of studies have examined microplastic pollution in wild marine mammals, primarily through necropsy of stranded individuals. All 21 odontocete digestive tracts examined from animals that stranded or died in fisheries by-catch in Ireland contained microplastic (Lusher et al. 2017). The gastrointestinal tract of a stranded True's beaked whale, *Mesoplodon mirus*, calf also yielded microplastics (Lusher et al., 2015). Fossi et al. (2012) examined the blubber of five stranded fin whales, *Balaenoptera physalus*, and documented relevant phthalate concentrations in four of the five whales, indicating pre-mortem exposure to plastic; phthalate concentrations in fin whale tissues were subsequently proposed as a proxy for pelagic microplastic pollution (Fossi et al., 2014).

Fewer studies have investigated microplastic pollution in living, wild, free-ranging marine mammals. Reports of small plastic present in scat (feces) were mentioned in diet studies of Hooker's sea lions (McMahon et al.,1999) and Eriksson and Burton (2003) recovered plastic from the scat of southern hemisphere fur seals, *Arctocephalus* spp. Notably, Eriksson and Burton (2003) postulated the bioaccumulation of plastics in these seals via a near-island, oceanic foodweb with the proximate prey species fish of the

family Myctophidae. These authors were among the first to recognize the parallel between the bioaccumulation of plastic in marine mammal foodwebs to that of pesticides, portending the future nexus of these two types of anthropogenic pollution.

Understanding the extent of biologically meaningful impacts of microplastic pollution on the health or fitness of individuals or populations remains nascent and to date primarily focused on the hypotheses of microplastics as a vector for the transport of contaminants or rafting species (Teuten et al., 2007; Teuten et al., 2009; Wright et al., 2013). Investigations of microplastic pollution are challenged by the potential for sample contamination both in the field and laboratory, as microplastics (particularly fibers) are widespread (Lusher et al., 2017; Nelms et al., 2018; Nuelle et al., 2014; Woodall et al., 2015). Rigorous, transparent, field-based and experimental investigations that contribute to understanding the exposure of wildlife to microplastic pollution are warranted particularly for species of conservation and cultural concern.

Here, we examine fecal material to investigate exposure of wild northern fur seals to microplastic pollution. Bioaccumulation of microplastic in this species is of interest as these seals are harvested for subsistence by indigenous communities. Our research objective is to investigate if microplastics are present in northern fur seal scat and describe any microplastics recovered.

Materials and Methods

Field Methods

Northern fur seal scat was collected for microplastic analyses from 16 July to 14 October 2015 at three colonies throughout the species' United States (U.S.) geographic range. Locations included St. Paul Island, AK (57.18° N, 170.27° W), Bogoslof Island, AK (53.93° N, 168.03° W), and San Miguel Island CA (34.03° N, 120.44° W) (Figure 1). A total of 44 northern fur seal scats were collected by hand using medical grade nitrile gloves and placed into sterile, polyethylene "Whirl-Pak" 207ml sample collection bags. Due to the high site fidelity of individual northern fur seals on shore (Baker et al., 1995) scat collection across sites was employed to avoid the possibility of collecting multiple scats from the same animal; as such, scats are assumed to be from individual fur seals. Scat was visually inspected *in situ* and samples selected to minimize inclusion of sediment. For example, samples deposited on rock were favored over those found on sand or soil. Care was taken to avoid plastic fiber contamination of samples during collection in the field by preventing contact of collection bags and gloves with plastic items, such as synthetic fleece clothing.

A minimum of two substrate samples were collected, where available, from each study site to serve as controls. Not all areas within each study site could be sampled. For example, in areas with exclusively rock cobble or large boulder substrate, sample collection was not possible. As such, substrate sample collection was necessarily opportunistic and focused on study site areas characterized by sandy substrate (beaches). Substrate samples were collected to explore the potential contamination of scat samples with any microplastics present in substrate, as well as serve as controls for scat sample

collection techniques. Substrate was collected using sterile polyethylene "Whirl-Pak" sample collection bags ranging in size from 207ml to 384ml and gloves as described above for scat samples. Substrate samples were collected from the mid-intertidal to high-tide line with sampling conducted as near the high-tide line as possible. Approximately 200g of substrate was collected from the surface to 5cm depth.

All samples were labeled with date of collection, sample type (scat or substrate), and location. Samples were collected under the authority of the U.S. National Marine Fisheries Service (NMFS), Marine Mammal Marine Protection Act research permit #14327-01 issued to the Marine Mammal Laboratory.

Laboratory Methods

Scat samples were frozen and stored in the sample collection bags until thawed in the unopened bags at room temperature overnight prior to processing. All material in each fecal sample was processed. Thawed fecal material was homogenized in the sample bags by adding 1 to 3 drops of Dawn® Ultra Original Dishwashing Liquid soap and approximately 75ml of water followed by manual agitation of re-sealed sample bags. The resulting fecal slurry was rinsed with water through two stacked, cylindrical stainless-steel mesh sieves each of 20cm diameter, 5cm depth and with mesh sizes of 500µm and 250µm, respectively. Sieving of the fecal slurry was facilitated by the use of a clean metal spatula and sieve walls were carefully rinsed. Material remaining in the 500µm sieve was visually inspected using a LUXO brand, Taskmaster IM12D model Fluorescent Magnifier at 2.2 times magnification to identify conspicuous microplastics. Microplastics

identified at this stage were extracted from the 500µm sieve with antimagnetic stainless steel ultra-fine tip forceps and archived in new 20ml glass scintillation vials.

To investigate the presence of smaller, cryptic microplastics, the remaining material in both sieves, inclusive of sieve walls, was carefully rinsed with water into conical unbleached paper filters fitted over glass jars. Filter paper containing the sieved sample material was folded tightly effectively creating a sealed filter paper "envelope." The envelope with sample inside was placed in a drying room overnight to desiccate the sieved sample matrix. Initially, the dried sample matrix was decanted from the filter paper into new 20ml glass scintillation vials used to store samples until further processing, but concern arose regarding the ability to ensure full sample transfer. To reduce the possibility of sample loss during transfer the entire folded filter paper envelope with processed sample intact was subsequently placed into the glass scintillation vials for storage prior to further processing.

Further processing of sieved samples to identify microplastics was adapted from methods detailed in Masura et al. (2015) and summarized below. All material in vials was emptied using distilled water and the mass of dried, labeled vials and caps measured. Samples were dried to determine dry mass, exposed to 30% hydrogen peroxide (H_2O_2) at 75°C to digest most organic material, and placed in a saline solution to increase the solution density. Samples remained in a density separator (glass conical funnel covered with aluminum foil sheeting) overnight to allow lower density material to rise and higher density material to sink. Higher density material at the bottom of the funnel was drained

off and the remaining lower density, suspended material including putative microplastics sieved through a custom-made sieve with Nytex mesh (20cm diameter, 5cm depth) with pore size of 330µm and dried while covered with aluminum foil sheeting overnight. Sample remaining was visually inspected using an Olympus C0111 dissecting microscope at 40X magnification.

All recovered microplastics were visually classified to one of four particle types: fragments, fibers, film, and foam. Each particle, regardless of type, was subsequently assigned to one of five size ranges: <1mm, 1 to 2mm, 2 to 5mm, 5 to 10mm, and >10mm using the longest axis. Recovered particle color was also determined visually and recorded. Microplastics recovered after full processing were archived in their original sample vials.

Substrate samples were also processed using the methods detailed in Masura et al. (2015) as summarized below. Two hundred grams of substrate, or the entire sample if less than 200g, was used and mass recorded. The wet sediment was then dried and dry mass determined. The dry sample material was exposed to an equal volume of potassium metaphosphate to disaggregate the sediment, facilitating the presentation of individual sediment particles, rather than clumps. This sample matrix was washed and the material was dried again after being rinsed in a cylindrical stainless-steel sieve with pore size of 330µm. The sample was then mixed in 150ml of lithium metatungstate to allow for separation of lower density material from higher density material. All liquid was then poured off and the solution containing lower density material rinsed again in a 330µm

sieve. The material remaining on the sieve was transferred to a beaker and dried. The sample material remaining was subject to the wet peroxide oxidation, visual inspection, and recovery of microplastics as described above for scat samples.

These methods are applicable for the determination of many common plastics including polyethylene (0.91-0.97 g/mL), polypropylene (0.94 g/mL), polyvinyl chloride (1.4 g/mL), and polystyrene (1.05 g/mL). Microplastic debris was thus operationally defined as any solid material in the appropriate size range (0.3 mm to 5 mm) that is resistant to wet peroxide oxidation, exhibits flotation in a 5 M NaCl (d=1.15 g/mL) or ~5.4 M lithium metatungstate (d=1.62 g/mL) solution, and subsequently passes positive visual inspection under a microscope at 40X power. Visual inspection was inclusive of putative microplastic particles remaining intact after physical manipulation and scoring using forceps.

Contamination control measures were implemented throughout project activities including focused attention on laboratory hygiene. During all laboratory work, cotton laboratory coats were worn over street clothing and nitrile gloves used. All materials used were carefully washed, dried with low-lint wipes, and inspected visually for any plastic material that could contaminate samples. As described above, metal and glass equipment and supplies were used wherever possible. A plastic brush was included as an identified laboratory control sample and brush tines and a scraping of the handle collected for analysis. In addition to the substrate sample controls, two additional types of laboratory controls were designed to specifically reveal any contamination of both scat and substrate

samples during processing. First, blank samples (procedural blanks) were processed contemporaneously and identically to actual samples with each batch of samples to evaluate and serve as controls for potential contamination during processing by nitrile gloves, sample collection bags, filter paper, dishwashing soap, faucet plumbing and rinse water, or other procedural sources. Second, white (bleached) filtration papers placed inside a standard glass petri dish were exposed to the air in the laboratory immediately adjacent to sample processing locations for the duration of sample processing. These filters were visually inspected daily using a dissecting microscope at 40X magnification for airborne microplastic deposition. When not being manipulated, a watch glass covered samples at all times during processing to further minimize potential contamination.

A Perkin-Elmer Spectrum 100 Fourier-transform infrared (FTIR) spectrometer was used to identify the polymer composition of two representative microplastics fragments from scat samples and the plastic laboratory brush control. Scans were compared to cataloged scans to determine the polymer-type.

Statistical analysis

Logistic regression analysis was used to evaluate potential differences in the prevalence (presence/absence) of microplastic in scat and substrate (by study site) and controls (blank and air). We also tested whether scat samples decanted from filter paper during processing differed from those processed with filter paper intact. The number of microplastic items recovered from each positive sample, defined as a sample containing

at least one microplastic particle, were also analyzed for differences among study site, particle type, and processing (with and without filter paper) using linear models. Normality was improved by log-transforming the number of microplastic particles per positive sample prior to fitting linear models.

Results

Microplastic fragments and fibers were recovered from northern fur seal scat at all study sites; no foams or films were recovered from scat. At total of 398 microplastic fragments and 186 fibers were documented in the 44 scats examined (Table 1 and 2). Contamination controls revealed no scat contamination with non-endogenous microplastic fragments, as evidenced by the absence of any microplastic fragments in substrate samples or laboratory controls. However, some fibers were recovered from controls as presented in Table 2.

Microplastic Fragments

Microplastic fragments were the most common type of particle recovered, present in 55% of scats (24/44) across all sites and accounting for 85% of all particles recovered (Table 1). Fifteen scat samples (10 from St. Paul Island and 5 from San Miguel Island) decanted into scintillation vials during initial processing had lower ($p = 0.01$) presence of fragments (4/15 samples or 27%) than those processed within their filter paper envelopes (20/29 samples or 69%). However, controlling for this difference in processing method,

there was no difference in the probability of presence of microplastic fragments in scat by location ($p = 0.28$).

The number of microplastic fragments per positive scat ranged from 1 to 86 and was highly variable at all locations (Table 1). The distribution of the number of fragments per positive scat was right-skewed and normality was improved by log-transforming the data. As noted above, four of the 15 scat samples (three from St. Paul Island and one from San Miguel Island) decanted into scintillation vials during initial processing contained microplastic fragments. The number of fragments found in those four samples was significantly lower than among samples processed in their filter paper envelopes ($p = 0.03$). Among the 20 samples positive for fragments and processed with their filter paper envelopes, those from St. Paul Island had significantly higher numbers of fragments per scat than those from Bogoslof Island or San Miguel Island ($p = 0.005$).

Of the 398 microplastic fragments recovered from scat, all but one were identified and recovered after wet peroxide oxidation and density separation treatments. All fragments recovered after these treatments ($N = 397$) were white in color. A single fragment, blue in color and 7mm long by 2mm wide, was recovered from a St. Paul Island scat after initial sieving at 500µm. All recovered fragments possessed irregular edges.

Most microplastic fragments recovered from scat (82%) were <1mm in size though all size classes up to 10mm were recovered (Table 1). The next most numerous fragment

size class was 2 to 5mm, representing 10% of all recovered fragments. Just two fragments were recovered from 5 to 10mm in size; no fragments were recovered >10mm in size.

No contamination of scat samples with non-endogenous microplastic fragments was found; no microplastic fragments were recovered from any laboratory controls (procedural blanks or filters exposed to air) or substrate samples at any site (Table 1).

The polymer composition of two fragments from two scats collected on St. Paul Island including the blue plastic fragment described above and one representative white fragment, was low density polyethylene. Funding limitations prevented comprehensive FTIR analysis of additional recovered fragments. The identified laboratory control sample (plastic brush) was composed of different polymers than recovered fragments tested; brush tines were polyvinyl chloride and brush handle scrapings were found to be polypropylene.

Table 1. Number and size of microplastic fragments recovered from northern fur seal scat and control samples (procedural blanks, filters exposed to air in the laboratory near sample processing, and substrate samples). Mean fragments per positive sample calculations include only those scats containing at least one microplastic fragment.

Location	Sample Type	Sample size	Samples with fragments	Mean number of fragments/positive sample (sd)	Number of fragments/size class (mm)					Total
					<1	1-2	2-5	5-10	>10	
St. Paul Is., AK	Scat	18	10	28.0 (sd 26.4)	237	24	17	2	0	280
Bogoslof Is., AK	Scat	17	10	9.3 (sd 7.4)	66	7	20	0	0	93
San Miguel Is., CA	Scat	9	4	6.3 (sd 3.0)	22	1	2	0	0	25
Subtotal	**Scat**	**44**	**24**	**16.6 (sd 19.1)**	**325**	**32**	**39**	**2**	**0**	**398**
Substrate control (all sites pooled)	Substrate	6	0	0 (0)	0	0	0	0	0	0
Laboratory control	Blank	19	0	0 (0)	0	0	0	0	0	0
Laboratory control	Filter-air	19	0	0 (0)	0	0	0	0	0	0

Microplastic fibers

Seventy-one microplastic fibers were recovered from northern fur seal scat at all study sites (Table 2). Fibers were present in 41% of all scat samples (18/44). The proportion of scats positive for microplastic fibers did not differ depending upon whether samples were decanted into scintillation vials or processed with their filter paper envelopes ($p = 0.46$). There was also no difference in the proportion of scats positive for microplastic fibers among locations ($p = 0.37$). The number of fibers per positive scat ranged from 1 to 18.

Fibers recovered from scat samples included all size classes though smaller fibers were more common (Table 2). Over 70% of fibers recovered were in the smallest two size classes, together accounting for fibers <2mm in size. An additional 28% of fibers

recovered from scat were between 2 and 10mm in size. The color of fibers recovered from scat samples included black, white, purple, blue, red, yellow, and clear.

A total of 45 microplastic fibers were also recovered from substrate samples for both Alaska locations; no fibers were found in San Miguel Island substrate (Table 2). The number of fibers per positive substrate sample ranged from 11 to 18. The color of fibers recovered from substrate included black, white, blue, and red.

Unlike microplastic fragments, fibers were found in laboratory controls, including procedural blanks and filters exposed to air (Table 2). Forty-seven percent (9/19) of procedural blanks contained one or more fibers. In positive blanks, from 1 to 6 fibers were recovered per sample; on average 2.4, *sd* 2.1 fibers per positive blank. In total, 22 fibers were recovered from procedural blanks. The majority of fibers recovered from procedural blanks were <1mm in size (13/22) (Table 2). The color of fibers recovered from procedural blanks included black, white, purple, blue, and green.

Microplastic fibers were also recovered from filters exposed to ambient laboratory air near locations where samples were being processed. Seventy-four percent (14/19) of air filters were positive for fibers. Of positive filters, from 1 to 10 fibers were recovered per filter, on average 3.5, *sd* 2.6 fibers per positive filter. Of the 49 fibers recovered from air filters, 41 or 84% were <1mm in size, with the remaining 8 fibers 1 to 2mm in size (Table 2). The color of fibers recovered from filters included black, white, purple, blue, red, and clear.

As there were multiple control samples in which microplastic fibers were found, we tested whether there were any differences in prevalence (presence/absence) among the study locations and the three control classes (procedural blank, filter exposed to air, and substrate). Among all sample types, air filter controls were found to have a significantly higher prevalence of microplastic fibers than scats, substrate, or procedural blanks ($p = 0.04$). We also tested the log-transformed number of fibers per positive sample, i.e. limited to samples with at least one fiber, for all sample types. Substrate had significantly higher numbers of fiber than other sample types ($p = 0.002$).

Table 2. Number and size of microplastic fibers recovered from northern fur seal scat and control samples (procedural blanks, filters exposed to air in the laboratory near sample processing, and substrate). The mean number of fibers per sample calculation includes only those samples positive for fibers.

Location	**Sample type**	**Sample size**	**Samples with fibers**	**Mean number of fibers/positive sample (sd)**	**Number of fibers/size class (mm)**					**Total**
					<1	**1-2**	**2-5**	**5-10**	**>10**	
St. Paul Is., AK	Scat	18	9	3.1 (sd 3.2)	20	6	2	0	0	28
Bogoslof Is., AK	Scat	17	7	5.1 (sd 3.9)	16	14	5	1	0	36
San Miguel Is., CA	Scat	9	2	3.5 (sd 0.7)	3	1	3	0	0	7
Subtotal	**Scat**	**44**	**18**	**3.78 (sd 3.4)**	**39**	**21**	**10**	**1**	**0**	**71**
St. Paul Is., AK	Substrate	2	2	11.5 (sd 0.7)	5	9	5	3	1	23
Bogoslof Is., AK	Substrate	2	2	11.0 (sd 4.2)	4	7	8	3	0	22
San Miguel Is., CA	Substrate	2	0	0 (sd 0.0)	0	0	0	0	0	0
Laboratory control	Blank	19	9	2.4 (sd 2.1)	13	3	0	6	0	22
Laboratory control	Filter-air	19	14	3.5 (sd 2.6)	41	8	0	0	0	49
Total					**102**	**48**	**23**	**13**	**1**	**187**

Microplastic foam and film

No microplastic foam was recovered from scat or laboratory controls. Two foam particles were recovered from one substrate sample from San Miguel Island. These two foam particles were 1 to 2mm, and 2 to 5mm, in size respectively. No microplastic film was recovered from any sample type or control sample.

Discussion

This study is the first to investigate and document the presence of microplastic pollution in northern fur seals, a North Pacific Ocean pinniped of conservation and cultural importance. The Eastern Pacific Stock of the northern fur seal is depleted due to a persistent decline in abundance owing to unknown causes (Muto et al., 2016; Towell et al., 2006). Northern fur seals are also a subsistence food source and cultural resource for Native Aleut communities. The presence of microplastic pollution in seals at all study sites and in over one-half of all scats examined corroborate the occurrence of this type of pollution in upper trophic-level marine predators.

The finding that the proportion of scats containing microplastic fragments, and number of fragments found per positive scat, was significantly lower for the minority of samples processed by decanting into scintillation vials versus those processed in their filter paper envelopes suggest some microplastic fragments were likely lost during the decanting process, perhaps remaining attached to the filter paper. An alternative explanation, that the filter paper itself was contaminated with microplastic fragments, is refuted by the fact

that no fragments were found in any of the procedural blanks, which included filter paper. In consideration of these findings, the reported overall prevalence of microplastic fragments in scat (55%) is likely to be negatively biased. Actual prevalence of fragments in scat may be more closely approximated by the 69% of scats processed in their filter paper envelopes (20/29) that contained fragments. Due to suspected fragment loss, the number of fragments per positive scat at St. Paul Island and San Miguel Island is likely underestimated.

The prevalence of microplastic pollution we report is difficult to place in context as few studies of this anthropogenic pollution in wild marine mammals have been conducted. Eriksson and Burton (2003) recovered a total of 164 small plastic particles from 145 scats of Southern Ocean fur seals, *Arctocephalus spp.,* in the early to mid-1990s though only reported data for scat samples positive for plastic, precluding comparison of frequency of occurrence. A later study of *Arctocephalus spp.* (Ryan et al., 2016) at different locations using the methods of Eriksson and Burton (2003) found no plastic. Microplastics were recovered *post mortem* from the digestive tracts of 12% of 107 harbor seals, *Phoca vitulina*, in the Netherlands, but none recovered from scat (Bravo Rebolledo et al., 2013). However, the harbor seals examined had all died as a result of a phocine distemper virus mass mortality (Härkönen et al., 2006) and interpretation of these results in relation to healthy animals uncertain. In a study of captive grey seals, *Halichoerus grypus*, fed wild-caught Atlantic mackerel, *Scomber scombrus*, Nelms et al. (2018) showed that nearly one-half (48%) of grey seal scats contained microplastics, somewhat lower than the 55% to 69% prevalence of microplastic fragments we report for wild, free-ranging northern fur

seals. The contamination protocols employed by Nelms et al. (2018) and in this study increase confidence in these estimates.

Our predominance of fragments is consistent with that reported for pinnipeds (Eriksson and Burton, 2003; Nelms et al., 2018). The number of fragments per positive scat we report is greater than the 1 to 4 reported for wild Southern Ocean fur seals, possibly as a result of Eriksson and Burton (2003) focusing on plastic ≥ 500µm. Our recovery of fragments sized 330µm to 500µm allowed for a greater size range, and likely number, of fragments recovered. This is also reflected in our smallest size class representing over 80% all fragments recovered. Our second most common fragment size class at 2 to 5mm is the most common fragment size recovered from Southern Ocean fur seals (Eriksson and Burton, 2003). The predominance of smaller microplastic fragments is biologically meaningful as the bioavailability of microplastics increases with decreasing particle size allowing uptake by organisms in lower trophic guilds (Galloway et al., 2017; Wright et al., 2013). The majority of fragments recovered here in the smallest size category indicate high bioavailability at multiple trophic tiers which may facilitate trophic transfer of this pollution.

With the exception of the single blue microplastic fragment recovered during the initial 500µm sieving, the white color of all fragments recovered after full processing is unexplained. Virgin experimental polyethylene microplastic particles have been shown to be resistant to color bleaching during oxidation of organic material with 30% H_2O_2 at 55°C for 7 days (Avia et al., 2015), though we cannot eliminate the possibility that our

higher H_2O_2 processing temperature of 75°C resulted in particle color bleaching. Particles recovered here have also been ingested and passed through the gastrointestinal tracts of, at minimum, one mammalian predator (northern fur seal). Gastrointestinal residence time of microplastics transported in food webs and associated exposure to animal generated digestive acids might also affect color, though this is unknown. Environmental conditions, including photo-degradation, that result in macroplastic fragmentation to microplastic may also fade polymer coloration (Andrady, 2015). The above notwithstanding, white was also the most common color of plastic recovered from Southern Ocean fur seal scat (Eriksson and Burton, 2003) as well as North Pacific pelagic predatory fish (Choy and Drazen, 2013). It is possible fish are disproportionately ingesting white-colored microplastics, perhaps due to enhanced visibility/contrast of this color particle, and this is subsequently reflected in predators' scat.

The lack of differences among study sites in the proportion of scats positive for microplastic fragments was surprising given the diversity of sites and underscores the pervasive nature of this pollution. The three study sites, spanning over 4,500km and 23 degrees latitude, vary in climate and relationship to human population centers. St. Paul Island is a subarctic Bering Sea island over 500 km from mainland Alaska with approximately 500 residents, primarily Native Alaskans (U.S. Census, 2010). Bogoslof Island is an uninhabited, undeveloped 0.62 km^2 subarctic island 379 km southeast of St. Paul Island and presently experiencing morphologic changes due to active vulcanism (USGS, 2018). In contrast, San Miguel Island is about 100km offshore of the heavily populated and developed California coastline. The consistency of microplastic fragments

in scat from these three locations, coupled with the absence of fragments in any substrate samples, suggests seals are ingesting microplastics at sea.

Summer foraging locations and associated prey assemblages of fur seals at the three study sites are geographically distinct (Kuhn et al., 2014; Zeppelin and Orr, 2010) implying the seals are exposed to microplastic pollution via diverse prey taxa. Distribution of seals from all the study locations do overlap to some degree during the winter through spring migration. Thus, while microplastics recovered from scat in this study seem likely to reflect foraging during July through October, the possibility exists that the microplastics derived from a common migratory region and were retained in the seals' digestive systems for some months.

Fur seals in the Eastern Pacific Ocean consume over 27 species of prey including cephalopods, polychaete worms, and fish (Zeppelin and Orr, 2010; Zeppelin and Ream, 2006). Among the numerous forage fish are those of the family Myctophidae, previously implicated in the trophic transfer of microplastics in Southern Ocean fur seals (Eriksson and Burton, 2003). The higher number of microplastic fragments in St. Paul Island seal scat may reflect ingestion of prey species with greater microplastic burden. Our data do not allow discernment of the origin of microplastics recovered to direct ingestion, indirect ingestion via prey, or incidental ingestion via contaminated seawater while foraging. However, the bioavailability of the smaller-sized fragments recovered, coupled with varied fur seal foraging locations and diet, are consistent with widespread trophic transfer of microplastic pollution to a top ocean predator. Further, the absence of any microplastic

fragments in any substrate samples or laboratory controls strongly support that recovered microplastic fragments were endogenous to scat and are not attributable to field or laboratory contamination.

Though microplastic fibers were recovered from a somewhat lesser proportion of scats than fragments, unlike fragments, fibers were also recovered from laboratory controls including procedural blanks confounding interpretation due to probable contamination. The finding that air filter controls were more likely to be positive for fibers than other sample types indicates airborne contamination by ambient fibers almost certainly occurred during laboratory processing. All fiber colors found in scat, except one (yellow), were also recovered from controls further suggesting contamination. It is possible fibers recovered from substrate and scat samples were endogenous. However, the four colors of fibers found in substrate, white, black, blue, and red were also found both in scat and controls, preventing definitive determination of endogenous fiber in substrate. The higher number of fibers per positive sample for Alaska substrate may also reflect higher substrate sample masses as compared to scat.

The difficulty in conducting microplastic studies due to potential contamination is increasingly acknowledged (Lusher et al., 2017; Nelms et al., 2018; Nuelle et al., 2014; Woodall et al., 2015). Laboratory processing of samples for microplastics within a positive pressure laminar flow hood as employed by Nelms et al. (2018) is optimal, though we show this may not be as critical for fragment recovery versus fiber. Reasonable laboratory contamination control measures as employed here appear effective

for microplastic fragment analysis. The absence of any microplastic fragments in any substrate samples or laboratory controls strongly support that microplastic fragments reported here were endogenous to scat and are not attributable to field or laboratory contamination. The difficulty in microplastic fiber analysis due to contamination has led some studies to omit fibers from consideration (Dekiff et al., 2014; Goldstein and Goodwin, 2013; van Cauwenberghe et al., 2013) and a forensic science approach to fiber analysis recommended (Woodall et al., 2015). Contamination may also occur during sample collection in the field, even when contamination measures are employed and collection dedicated to microplastic study (Lusher et al., 2017, Hidalgo-Ruz et al., 2012). As such, use of archived environmental samples collected opportunistically or for other investigations and subsequently analyzed for microplastics require conservative interpretation, particularly with regard to fibers. Contamination control measures and assessment, as well as the use of extensive sample controls, is warranted in microplastic research. Reporting of detailed methodology inclusive of contamination control measures and outcomes, as we report here, is important to advance research on microplastic pollution.

Conclusions

We demonstrate that northern fur seals are exposed to microplastic pollution throughout their U.S. range as evidenced by fragment excretion in scat. The non-invasive collection and examination of fecal material for evaluating exposure of northern fur seals to microplastic pollution was effective, though contamination control measures indicated an

unknown level of contamination with microplastic fibers. Due to unknown ingestion and egestion rates of microplastic by seals, exploration of alternative, humane sample matrices to detect exposure to environmental plastic in marine mammals, inclusive of phthalate tracers, is recommended. Equally important is the ongoing evaluation of the relative threat posed by microplastic bioaccumulation in top predators, including humans.

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Figure Caption

Figure 1. Location of study sites where northern fur seal fecal samples were collected in 2015 for microplastic analyses: (1) St. Paul Island,

AK (57.18° N, 170.27° W); (2) Bogoslof Island, AK (53.93° N, 168.03° W); and (3) San Miguel Island, CA (34.03° N, 120.44° W). Adapted from Zeppelin and Orr (2010).

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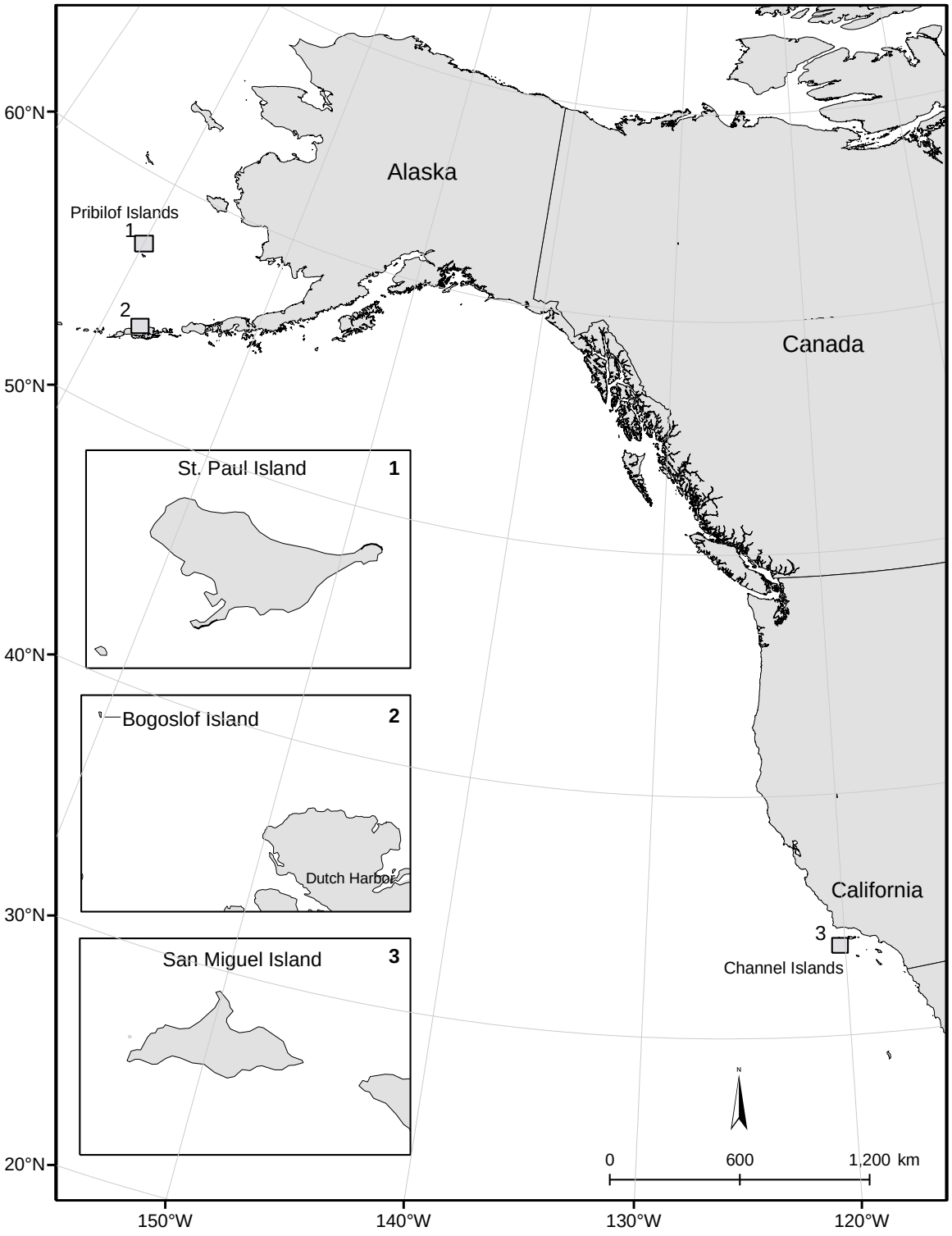
60°N
50°N
40°N
30°N
20°N
150°W
140°W
130°W
120°W
Alaska
Canada
California
Pribilof Islands
1
2
3
Channel Islands
St. Paul Island
1
Bogoslof Island
2
Dutch Harbor
San Miguel Island
3
N
0
600
1,200 km