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journal homepage: www.elsevier.com/locate/envpolTwo forage fishes as potential conduits for the vertical transfer of microfibres in Northeastern Pacific Ocean food webs[☆]J. Mark Hipfner^{a, *}, Moira Galbraith^b, Strahan Tucker^c, Katharine R. Studholme^d, Alice D. Domalik^e, Scott F. Pearson^f, Thomas P. Good^g, Peter S. Ross^h, Peter Hodumⁱ^a Wildlife Research Division, Environment and Climate Change Canada, RR#1 5421 Robertson Road, Delta, BC, V4K 3N2, Canada^b Institute of Ocean Sciences, Fisheries and Oceans Canada 9860 West Saanich Road, Sidney, BC, V8L 4B2, Canada^c Pacific Biological Station, Fisheries and Oceans Canada 3190 Hammond Bay Road, Nanaimo, BC, V9T 6N7, Canada^d Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, NS, B3H 4R2, Canada^e Centre for Wildlife Ecology, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 1S6, Canada^f Washington Department of Fish and Wildlife, 1111 Washington Street SE, Olympia, WA, 98501, USA^g Northwest Fisheries Science Center, National Marine Fisheries Service 2725 Montlake Boulevard East, Seattle, WA, 98112, USA^h Coastal Ocean Research Institute, Ocean Wise Conservation Association, P.O. Box 3232, Vancouver, BC, V6B 3X8, Canadaⁱ Department of Biology, University of Puget Sound Tacoma, WA, 98416, USA

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ABSTRACT

We assessed the potential role played by two vital Northeastern Pacific Ocean forage fishes, the Pacific sand lance (*Ammodytes personatus*) and Pacific herring (*Clupea pallasii*), as conduits for the vertical transfer of microfibres in food webs. We quantified the number of microfibres found in the stomachs of 734 sand lance and 205 herring that had been captured by an abundant seabird, the rhinoceros auklet (*Cerorhinca monocerata*). Sampling took place on six widely-dispersed breeding colonies in British Columbia, Canada, and Washington State, USA, over one to eight years. The North Pacific Ocean is a global hotspot for pollution, yet few sand lance (1.5%) or herring (2.0%) had ingested microfibres. In addition, there was no systematic relationship between the prevalence of microplastics in the fish stomachs vs. in waters around three of our study colonies (measured in an earlier study). Sampling at a single site (Protection Island, WA) in a single year (2016) yielded most (sand lance) or all (herring) of the microfibres recovered over the 30 colony-years of sampling involved in this study, yet no microfibres had been recovered there, in either species, in the previous year. We thus found no evidence that sand lance and herring currently act as major food-web conduits for microfibres along British Columbia's outer coast, nor that the local at-sea density of plastic necessarily determines how much plastic enters marine food webs via zooplanktivores. Extensive urban development around the Salish Sea probably explains the elevated microfibre loads in fishes collected on Protection Island, but we cannot account for the between-year variation. Nonetheless, the existence of such marked interannual variation indicates the importance of measuring year-to-year variation in microfibre pollution both at sea and in marine biota.

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1. Introduction

There is growing awareness that the vast quantities of debris polluting the world's oceans pose a serious threat to a wide range of marine organisms (Law, 2017). The debris gets into the ocean from both marine and terrestrial sources, and in a plethora of forms,

colours, shapes and sizes. Once there, physical abrasion and UV irradiation can cause much of the debris to degrade into smaller and smaller fragments (Auta et al., 2017). Microfibres of a variety of types, both natural and manufactured (the latter including microplastics), enter marine food webs when small pieces are ingested by planktivores, detritivores, suspension-feeders and filter-feeders (Goldstein and Goodwin, 2013; Setälä et al., 2014; Hall et al., 2015; Remy et al., 2015; Gusmão et al., 2016). These organisms can, in turn, transfer the microfibres on to their predators (Eriksson and Burton, 2003; Farrell and Nelson, 2013; Tosetto et al., 2017). Once ingested, the microfibres can have both physical (Wright et al.,

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2013) and toxicological (Cole et al., 2011a,b) effects on marine predators, with deleterious effects most evident at organismal and sub-organismal levels (Rochman et al., 2016).

Forage fishes often act as the key trophic links between zooplankton and the broad suite of piscivorous taxa that inhabit the oceans (Smith et al., 2011). Zooplanktivorous fishes can both incidentally take up small particles of debris ingested by or attached to their zooplankton prey (Cole et al., 2011a, b; Desforges et al., 2015), and actively consume larger particles that resemble natural food items (Lusher et al., 2013; Ory et al., 2017). Consequently, forage fishes could act as primary conduits through which microfibrils, and any associated contaminants, are transferred vertically into piscivores in marine food webs.

The Pacific sand lance (*Ammodytes personatus*) and the Pacific herring (*Clupea pallasii*) are two abundant, widely-distributed forage fishes that play vital roles in food webs of the North-eastern Pacific Ocean. Diets in both species consist of zooplankton, particularly calanoid copepods (Foy and Norcross, 1999; Hipfner and Galbraith, 2013). Sand lance and herring are themselves consumed by a wide array of predators including many commercially valuable fishes (Brodeur, 1991; Coutre et al., 2015), and many species of marine mammals (Friedlaender et al., 2009; Tollit et al., 2015) and seabirds (Gjerdrum et al., 2003; Gladics et al., 2015). Compared to other marine taxa, the frequency at which microfibrils are ingested, and the consequences of their ingestion, are especially well documented for seabirds, a taxonomically and ecologically diverse group that is widely distributed throughout the world's oceans (Wilcox et al., 2015; Provencher et al., 2017).

Here, we quantify spatial and temporal variation in the amount and types of microfibrils ingested by sand lance and herring in waters off the coasts of British Columbia, Canada and Washington State, USA. Fishes were collected directly from a widely-distributed and abundant North Pacific seabird, the rhinoceros auklet (*Cerorhinca monocerata*), on six island breeding colonies in July or August in up to eight years, their stomachs were excised, and the contents quantified. The rhinoceros auklet is an ideal forage-fish predator for the purposes of this study for several reasons. First, they are known to ingest microfibrils; microplastic was found in the stomachs of four of 68 rhinoceros auklets recovered from various sources in the Northeastern Pacific over recent decades (Day, 1980; Robards et al., 1995, 1997; Blight and Burger, 1997; Avery-Gomm et al., 2013). Second, these birds are central-place foragers while breeding, so they sample prey within a restricted range around their colonies. Based on 63 day-long foraging trips taken by provisioning auklets equipped with GPS tags on islands in British Columbia, maximum linear travel distances away from colonies averaged 59.7 km (3.8 SE), and ranged from 5.8 to 119.4 km (A. Domalik, unpubl. data). Third, these birds dive to catch bill-loads of up to 30 whole fishes at dusk, mainly within the top 10 m of the water column (Kato et al., 2003), which they then deliver intact to their single nestlings (Davoren and Burger, 1999). It is a simple matter to collect the captured fishes when the birds return to the colony *en masse* (Bertram et al., 2002). The stomachs of fishes obtained in this manner contain zooplankton prey ingested within a short period of time prior to collection from within the auklets' foraging range (Hipfner and Galbraith, 2013). Information on the retention time of fibres in the stomachs of sand lance and herring is lacking, but laboratory experiments with goldfish (*Carassius auratus*), a zooplanktivorous fish of similar size, show that microfibrils only rarely accumulate in the gut contents over successive meals (Grigorakis et al., 2017).

Our primary objective in undertaking this research was to assess the potential role that these two forage fishes play as conduits for the vertical transfer of microfibrils to piscivores in Northeastern Pacific Ocean food webs. In addition, our multi-colony sampling

protocol enabled us to test the hypothesis that the local at-sea density of microplastic predicts its prevalence in marine zooplanktivores (Wilcox et al., 2015; Schuyler et al., 2016; Güven et al., 2017). Our test of that hypothesis rested on the results of Desforges et al. (2014), who measured the density of microplastic debris in sub-surface waters at 4.5 m depth across the southern portion of our study area in August and September of 2012.

The North Pacific Ocean is a global hotspot for small debris (van Sebille et al., 2015), but the local at-sea density of debris can vary due to small-scale oceanographic and anthropogenic factors. Desforges et al. (2014) found that microplastic density was 2.5–3 times higher around Pine Island, British Columbia (~8000 m⁻³) than around Triangle Island, BC (~2600 pieces m⁻³) or Protection Island, Washington (~3200 m⁻³). Therefore, we specifically predicted that we would find more microplastic in forage fish stomachs collected from auklets on Pine Island than on Protection or Triangle islands. Those authors attributed the high at-sea density of plastic debris in southern Queen Charlotte Sound, where Pine Island is located, to the convergence of pan-Pacific currents with outflow from Johnstone and then Queen Charlotte Straits, creating a zone of accumulation, combined with the actions of a clockwise gyre that tends to retain seawater, and any entrained plastic, for extended periods of time. They attributed the lower plastic density in the Salish Sea around Protection Island, despite the close proximity of large, land-based sources of plastic, to the short residency time of surface waters due to strong outflow through Johnstone and Queen Charlotte straits to the north, and Juan de Fuca Strait to the west. Low plastic density near Triangle Island, located 45 km offshore, was attributed to the tendency for plastic density to decline with distance from the mainland coast, as it does in other marine systems (Rudduck et al., 2017). At-sea plastic density has not been measured across the northern part of our study region, but there are no obvious oceanographic or anthropogenic forces that would be expected to produce high density around S'Gang Gwaay, Moore Island or Lucy Island, all along BC's outer coast.

2. Materials and methods

2.1. Study sites

Our study took place on six rhinoceros auklet breeding colonies, five of them in British Columbia, Canada: Lucy Island (54°17' N 130°37' W) and Moore Island (52°57' N 129°34' W) along BC's North Coast; Pine Island (50°35' N 127°26' W) along BC's Central Coast; S'Gang Gwaay (52°05' N 131°13' W) off the southwestern tip of the Haida Gwaii archipelago; and Triangle Island (51°52' N 129°05' W), the outermost island in the Scott Islands archipelago. Sampling also occurred at one colony in Washington State, U.S.A.: Protection Island (48°07' N 122°55' W), in the protected inner waters of the Salish Sea (Fig. 1).

2.2. Field methods

Forage fishes were collected from rhinoceros auklets on 5–7 day visits to breeding colonies in early July to early August of 2009–2016. Auklets returning to the colony to deliver bill-loads of prey to their nestlings were induced to drop their bill-loads using bright lights, or were captured on the ground either by hand or with long-handled nets. The bill-loads were collected and placed in Whirl-Pak bags. For each bill-load, individual prey items were identified and enumerated to species, and whole specimens of sand lance (2009–2016) and herring (2014–2016) were selected for stomach sampling. The stomach contents of the individual fishes present in the same bill-load would not be independent if, as is likely, the fishes were feeding together when captured. Therefore,

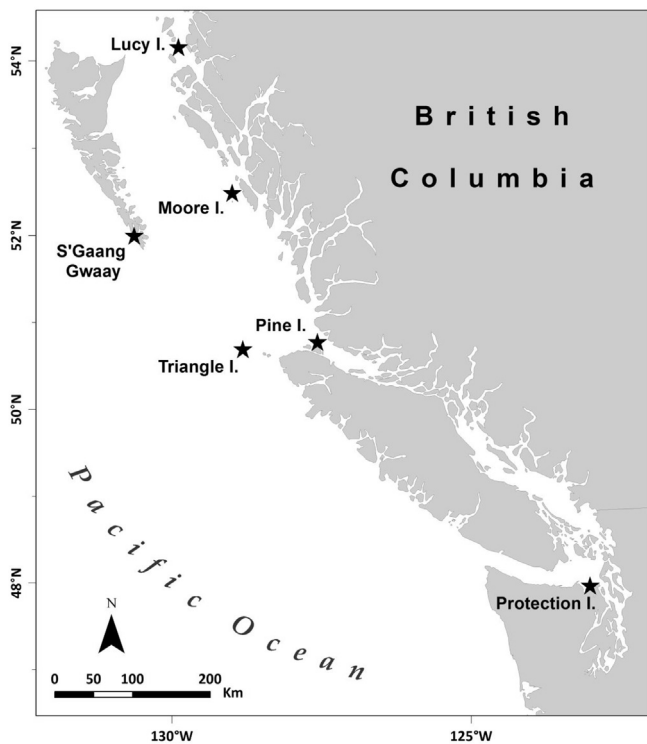


Fig. 1. Map of the British Columbia and northern Washington State coastlines, showing the locations of the six rhinoceros auklet colonies on which we collected predated Pacific sand lance and Pacific herring. Sampling for at-sea plastic density (Desforges et al., 2014) occurred in the southern half of the region, in the vicinities of Protection, Pine and Triangle islands.

with few exceptions, mostly on Protection Island, only the single largest specimen of sand lance and/or herring in a bill load was selected for stomach content analysis. The sand lance and herring were measured (fork length, ± 1 mm with a ruler) and weighed (± 0.1 g on an electronic balance) within 2 h of collection, and the gastro-intestinal tract of each fish was excised with scalpel and forceps and placed in a 30 ml screw-top bottle filled with 15% buffered formalin. The years in which sampling occurred at each of the six colonies, and the number of fish stomachs examined in each colony-year, are listed in Table 1.

2.3. Laboratory methods

Analytical methods used in the laboratory followed those in Desforges et al. (2014, 2015) with the exception that no acid digestion was performed. As in these studies, all lab work was done by M. Galbraith in the Fisheries and Oceans Canada lab at the Institute of Ocean Sciences in Sidney, British Columbia. The air entering the laboratory wing at IOS is filtered as it enters the air circulation plant for the building. The air passes through a second set of filters as it leaves the plant, and through a third set at the duct work entry to each lab. All work was performed in clothing made of 100% cotton, almost always when no other people were in the lab. A fresh, moist filter paper was placed in a petri dish in the working area and checked after each stomach was processed to see if any microfibrils had fallen out of the air. To date, only some fine dust particles have been collected on the filter paper.

The instruments and dishes to be used were cleaned and inspected before each stomach was processed. To start, the formalin was decanted off the stomach over a fine mesh sieve (0.063 mm) and captured for neutralization treatment and disposal. The stomach was washed with tap water to remove any traces of formalin, rinsed with Milli-Q (double filtered) water, placed in a petri dish, and the surface carefully inspected for foreign matter. The stomach was then cut open, and its contents washed out with double-filtered water over a 0.063 mm mesh. Using a Wild M420 dissecting microscope (www.leica-microsystems.com) with 20 \times oculars and 6.3–32 zoom capability, prey items found in the stomach were counted, the life stage of each determined, and each item identified to the lowest taxonomic level possible. Any foreign items present in the stomachs, whether natural or artificial, were separated out and enumerated.

All foreign items recovered from herring in 2016 (see below) were set aside for further analysis. These items were measured (length, width, ± 1 mm), and assigned to a colour category (Provencher et al., 2017). Currently, the sources and biological effects of microplastics are a major focus of the global research effort on pollution in marine ecosystems (Law, 2017). Therefore, from among the foreign items found in a single stomach, all of those suspected to be microplastic particles, based on visible features, plus 1–4 non-plastic items, were analyzed for material composition. This was done with a Cary 670 Fourier Transform Infrared Spectrometer (FTIR) equipped with a Cary 620 microscope (Agilent Technologies, Mulgrave, Australia) using a micro-ATR accessory equipped with a Germanium crystal. Each suspected microplastic particle was manually affixed to a glass microscope slide which had been coated with a thin layer of 2% dextrose (Sigma-Aldrich, St

Table 1

Prevalence of plastic pieces found in stomachs of Pacific sand lance and Pacific herring collected from chick-provisioning Rhinoceros auklets on colonies in British Columbia and Washington State. Data are represented as: number of stomachs examined (number with ≥ 1 plastic piece, total number of plastic pieces).

Species	Year	Location						
		Lucy I.(BC)	Moore I.(BC)	S'Gaang Gwaay (BC)	Pine I.(BC)	Triangle I.(BC)	Protection I.(WA)	
Sand lance	2009	38 (0)	–	7 (0)	50 (0)	23 (0)	–	
	2010	35 (0)	–	11 (0)	40 (0)	15 (0)	–	
	2011	27 (0)	–	14 (0)	28 (0)	3 (0)	–	
	2012	29 (0)	–	–	28 (1,1)	9 (0)	–	
	2013	30 (0)	–	–	24 (0)	20 (2,5)	–	
	2014	30 (0)	–	30 (0)	25 (0)	3 (0)	–	
	2015	29 (0)	7 (0)	19 (0)	29 (0)	–	41 (0)	
	2016	30 (0)	–	–	30 (0)	–	30 (8,49)	
	ALL YEARS	248 (0)	7 (0)	81 (0)	254 (1,1)	73 (2,5)	71 (8,49)	
Herring	2014	30 (0)	–	21 (0)	22 (0)	0 (0)	–	
	2015	30 (0)	0 (0)	3 (0)	21 (0)	–	19 (0)	
	2016	31 (0)	–	–	9 (0)	–	19 (4,51)	
	ALL YEARS	91 (0)	0 (0)	24 (0)	52 (0)	0 (0)	38 (4,51)	

Louis, USA) as an adhesive agent. Background and sample scans were collected with 16 co-added scans at a resolution of 8 cm^{-1} in the range of 3800 to 900 cm^{-1} . Spectra were matched against a commercial polymer database with 4520 selected ATR-FTIR spectra of polymers, plastics, polymer additives, plasticizers and packing materials (S.T. Japan-USA, LLC) and matches were subsequently confirmed using the FT-IR specific KnowItAll ID expert software (BioRad).

2.4. Statistical methods

To investigate links between the body condition of individual fishes and their propensity to ingest microfibres, we followed Miller et al. (2013) and Tucker et al. (2016) in calculating residuals from the regression of $\ln(\text{mass})$ against $\ln(\text{fork length})$ for individual fishes that had vs. had not ingested microfibres. The two sets of residuals were compared using t-tests. Permutation ANOVA was applied to test for an association between microfibre length and colour. The permutation approach is impervious to imbalances in sample sizes between factor levels. All analyses were conducted in the R statistical environment [R[®] version 3.2.5]. Because of the unusual features of the dataset – most notably, highly unequal sampling in relation to species, colonies, and years; as well as an extreme preponderance of zeroes, and extreme aggregation in the non-zero values - we did not statistically model the frequency of ingestion or number of microfibres recovered from fish stomachs.

3. Results

3.1. Sand lance and herring in rhinoceros auklet bill-loads

Across all 30 colony-years of sampling (Table 1), we collected a total of 7301 individual prey items (fish, several squid) in 1582 rhinoceros auklet bill-loads. Pacific sand lance (61.9% of the total) and Pacific herring (19.9%) were the two most common prey species.

3.2. Prevalence of microfibres in sand lance and herring

The stomachs of 734 sand lance and 205 herring were examined (Table 1). All foreign items recovered in the fishes' stomachs were in the form of small fibres (i.e., we found no fragments, beads, etc.). The overall occurrence of microfibres was low in both forage-fish species: for sand lance, 11 stomachs (1.5% of those sampled) contained a total of 55 microfibres, with a range of 1–9 fibres per stomach. For herring, four stomachs (2.0% of those sampled) contained a total of 51 microfibres, with a range of 5–27 fibres per stomach.

At Pine Island, microfibres of any type were found in just one sand lance stomach in eight years of sampling (0.4% of those examined there) and in zero herring in three years of sampling (Table 1). At Protection Island, microfibres were found in eight sand lance (11.3%) and in four herring (10.5%) over two years of sampling. At Triangle Island, microfibres were found in two sand lance stomachs (2.7%) in six years of sampling; herring did not occur in rhinoceros auklet nestling diets there. No sand lance or herring stomachs contained microfibres in any year at Lucy Island (8 years), Moore Island (1 year), or S'Gang Gwaay (5 years; Table 1).

Of particular interest was the interannual variation in the occurrence of microfibres in fishes collected on Protection Island. None were found in any sand lance or herring stomachs in 2015, but microfibres were present in 27% of sand lance and 21% of herring in 2016 (Table 1). In fact, sampling on Protection Island in 2016 yielded both the vast majority of fishes that had ingested microfibres over the entirety of this study (73% of sand lance, 100% of herring),

and the vast majority of microfibres recovered (89% from sand lance, 100% from herring).

3.3. Characteristics of recovered microfibres

Among the 51 microfibres recovered in 4 herring stomachs on Protection Island in 2016, the distribution of colours was as follows: 25% clear/white; 24% pink/red; 20% black; 16% blue/purple; 10% orange/brown; 4% green; and 2% yellow. In terms of size, the 49 measurable fibres (two of the clear/white fibres were too severely tangled to measure) ranged from 0.75 mm to 142.4 mm in length; 82% were less than 5 mm in length, while 18% were longer than 5 mm. Based on a one-way ANOVA with permutation, the length and colour of the 49 fibres were unrelated ($F_{4,41} = 1.23$, $P = 0.25$; Fig. 2).

Twenty-five of the 51 fibres were suspected to be plastic based on visible features. A total of 34 fibres (all 25 suspected plastic items plus 9 others) was analysed by FTIR; useable spectra were obtained for 29 of the 34. Of these, 12 were plastic (5 polyester, 3 acrylic, 3 nylon, 1 polypropylene), with 9 found in one stomach and the remaining 3 in separate stomachs; 8 were cotton and 1 wool; 4 were rayon; 2 were composed of regenerated cellulose and 1 of modified cellulose; and 1 was a piece of hair or fur. Of the 12 plastic fibres, 5 were black, 5 red/pink, and 2 purple/blue; 8 were microplastics (1–5 mm) and 4 were mesoplastics (5–20 mm).

3.4. Size and condition of sand lance and herring that had ingested microfibres

All of the sand lance and herring that had microfibres in their stomachs were on the small end of the size range of sampled fish (Fig. 3). Whereas 'clean' sand lance ranged from 80 to 183 mm in fork length and 1.0–28.3 g in mass, the 11 sand lance that had ingested microfibres ranged only from 90 to 131 mm and 2.7–8.5 g. Likewise, 'clean' herring ranged from 57 to 171 mm in fork length and 1.6–50.2 g in mass, while the four herring that had ingested microfibres were 73–80 mm and weighed 3.0–3.5 g. Based on residuals of $\ln(\text{mass})$ against $\ln(\text{fork length})$, the 11 sand lance that had microfibres in their stomachs were in slightly negative body condition ($-0.020\text{ g mm}^{-1} \pm 0.024\text{ SE}$) compared to all 'clean' sand lance (Fig. 3). Likewise, the four herring that had ingested microfibres were in slightly negative condition ($-0.064\text{ g mm}^{-1} \pm 0.095\text{ SE}$) relative to all 'clean' herring. However, as mentioned, most

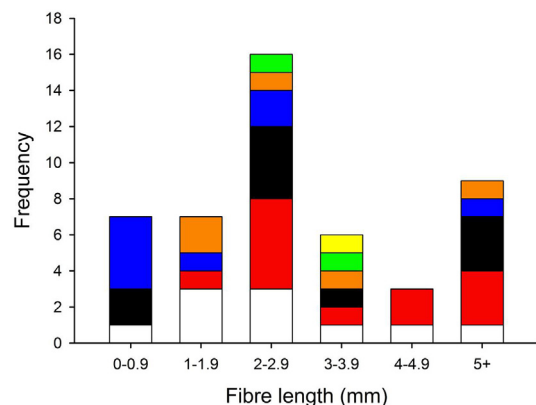


Fig. 2. Colour in relation to size for the 51 microfibres recovered from Pacific herring stomachs at Protection Island in 2016. The white bars represent clear plastic. There was no statistically significant association between fibre colour and fibre length.

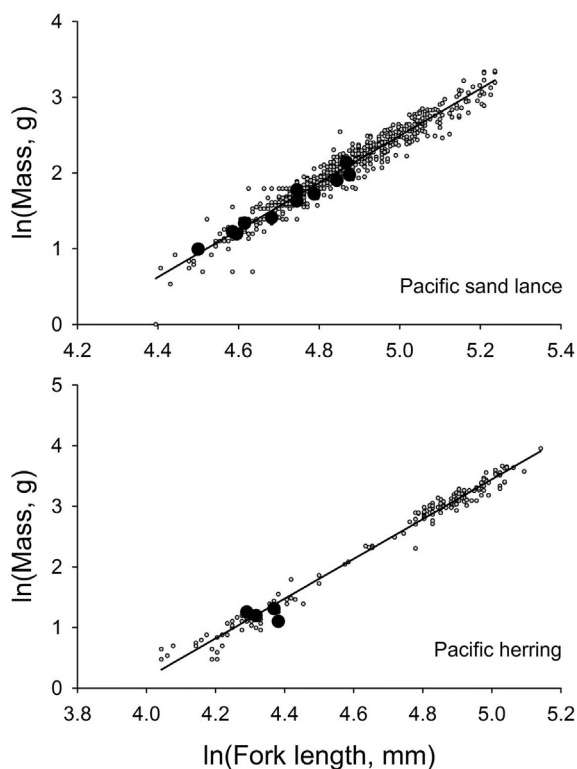


Fig. 3. Mass against fork length for Pacific sand lance and Pacific herring collected on Rhinoceros auklet colonies in British Columbia and Washington State in 2009–2016, with linear regression. Individuals that had not ingested plastic are represented by small grey dots, individuals that had ingested plastic are represented by large black dots. Comparison of residuals between these groups suggests that body condition did not vary between individuals that had ingested plastic and those that had not.

(sand lance) or all (herring) fibre-contaminated fishes were collected on Protection Island in 2016. At that site in that year, mean body condition in the eight sand lance that had ingested microfibres ($-0.015 \text{ g mm}^{-1} \pm 0.029 \text{ SE}$) did not differ significantly from that of 22 fishes that had not ($+0.047 \text{ g mm}^{-1} \pm 0.031 \text{ SE}$; $t_{28} = 1.13$, $P = 0.27$). Likewise, the four herring that had ingested microfibres ($-0.077 \text{ g mm}^{-1} \pm 0.095 \text{ SE}$) did not differ significantly in mean condition from the 15 that had not ($-0.109 \text{ g mm}^{-1} \pm 0.330 \text{ SE}$; $t_{17} = 0.41$, $P = 0.69$).

4. Discussion

4.1. Prevalence of microfibres in sand lance and herring

Reflecting their importance in food webs of the Northeastern Pacific Ocean, Pacific sand lance and Pacific herring combined to form over 80% of prey items delivered to rhinoceros auklet nestlings on six breeding colonies in British Columbia and Washington State. Despite the extensive amounts of micro-debris polluting the North Pacific Ocean (van Sebille et al., 2015), just 1.5% of sand lance and 2.0% of herring that we examined had ingested microfibres. In addition, we found no systematic relationship between the local prevalence of plastic at-sea around Pine, Triangle and Protection islands in 2012 vs. in the stomachs of the fishes collected at those three sites. Most notably, just a single sand lance collected at Pine Island in eight years, and no herring collected there in three years, had microfibres of any type in their stomachs, despite the fact that this island is situated in southern Queen Charlotte Sound, where microplastic debris accumulates and is retained during summer

(Desforges et al., 2014). In fact, microfibres were equally rare in fish stomachs at all of our study sites in British Columbia: just two sand lance in six years had ingested microfibres at offshore Triangle Island (where, in 2012, the local at-sea density of microplastic was one-third that at Pine Island), and in no years had any sand lance or herring collected at Lucy Island, Moore Island, or S'Gang Gwaay ingested microfibres.

We know of no previous studies of microfibre ingestion by any species of sand lance, but three studies of Atlantic herring (*Clupea harengus*) reported similarly low (0.0–1.7%) prevalence of microplastic ingestion in the North and Baltic seas (Foekema et al., 2013; Rummel et al., 2016; Hermesen et al., 2017). Direct comparisons of our results to those from the North Atlantic Ocean are complicated – first, because we obtained Pacific herring from seabirds, which are selective in their choice of prey (Tucker et al., 2016), rather than in trawls, which are not selective, reflecting our focus on the food-web transfer of microfibres; and second, because we generally analysed just a single (largest) fish per bill-load rather than all fishes caught, in order to maintain statistical independence. As we have shown, large herring were less likely than small herring to ingest microfibres. Nonetheless, the consistently low rates of ingestion of microfibres by herring is noteworthy because the North Sea and North Pacific Ocean both are marine regions where the at-sea density of debris is high, and where similarly large proportions (62% and 54%) of northern fulmars (*Fulmaris glacialis*), widespread and abundant seabirds, had ingested plastic (van Franeker and Law, 2015).

The lone exception to the consistently low occurrence of microfibres in fish stomachs in our study was in collections made on Protection Island, WA. There, we found no fibres in any sand lance or herring stomachs examined in 2015, whereas fibres were present in 27% of sand lance and 21% of herring in 2016. Thus the sampling at Protection Island in 2016 yielded both the vast majority of fishes found to have ingested microfibres over the entirety of this study, and the vast majority of all microfibres recovered. Protection Island lies in the Salish Sea, which is unique among our marine study zones in having extensive urban development along its shores. Fishes living near urban areas can have elevated microfibre loads (Tanaka and Takada, 2016), because landfills, recycling and industrial facilities, and municipal wastewater are significant sources of microfibre pollution (Auta et al., 2017). Primary and secondary treatment remove most microfibres from municipal wastewater (Murphy et al., 2016), but seven municipalities on the south end of Vancouver Island, including the City of Victoria, located 42 km northwest of Protection Island, merely screen and then discharge untreated sewage offshore through outfall pipes. While effects of the untreated sewage on the local ecosystem appear to be relatively benign (Chapman, 2006), there has been, to our knowledge, no specific assessment of the quantity and environmental impacts of the microfibres that are being released. Many of the types of microfibres recovered in herring stomachs, such as acrylic, polyester, polypropylene, rayon, and cellulose-based, are commonly associated with wastewater (Browne et al., 2011; Cesa et al., 2016), and it is unlikely to be mere coincidence that these microfibre types were recovered in large numbers only at our Salish Sea study site. These seven municipalities plan to apply tertiary treatment to their wastewater beginning in 2020 (<https://www.crd.bc.ca/project/wastewater-treatment-project>).

Nonetheless, even if anthropogenic sources such as untreated sewage can account for the increased microfibre loads found in forage fishes taken by rhinoceros auklets on Protection Island in 2016, they do not explain the between-year variation that we observed. Excluding the possibility that the variation was due to random bias (there were only two years of data), potential explanations include year-to-year differences in behavioural traits such

as the diets of the forage fishes, or the auklets' relative harvest of fishes from areas closer to or farther from point sources of fibres, such as sewage outfall. Pazos et al. (2017) found more microplastics in the stomachs of fishes taken closer to sewage discharge. Year-to-year differences in the strength or direction of physical forcing due to ocean circulation patterns (Howell et al., 2012), winds (Browne et al., 2010), storminess, and freshwater runoff (Moore et al., 2002) could also be involved. We intend to continue this program, and may have an opportunity in the future to examine the behavioural and environmental factors that underlie variation in the ingestion of microfibrils by zooplanktivorous fishes, and to compare microfibril loads in sand lance and herring in runs of years before vs. after tertiary wastewater treatment is applied on southern Vancouver Island. In addition to having higher loads of microfibrils, at least in some years, the forage fish prey of rhinoceros auklets in the Salish Sea have higher contaminant loads than prey taken along Washington State's outer coast, and may act as vectors for the transfer of contaminants to piscivorous fishes, birds and mammals (Good et al., 2014).

4.2. Characteristics of ingested microfibrils

Previous studies indicate that most of the micro-pollution in seawater tends to be in the form of fibres, and that it is fibres that are most commonly ingested by zooplanktivorous fishes (Lusher et al., 2013; Nadal et al., 2016; Güven et al., 2017; Jabeen et al., 2017; Pazos et al., 2017; Murphy et al., 2016; Vendel et al., 2017). Fibres constituted 75% of the small plastic debris recovered in marine waters off the coast of British Columbia (Desforges et al., 2014), and 100% of the debris that we recovered in sand lance and herring stomachs. Like Lusher et al. (2013), we found that microplastics constituted a minority of the fibres ingested by fishes. Further, only about one-half of the items that we suspected were plastic based on visual examination actually were plastic, an issue noted previously by Remy et al. (2015).

Zooplanktivorous fishes appear to ingest debris that resembles their natural prey in both size and colour (Ory et al., 2017). The size of the fibres that we recovered in sand lance and herring – 82% up to 5 mm in length, 18% longer than 5 mm – agrees with observations in many other zooplanktivorous fish communities (Lusher et al., 2013; Nadal et al., 2016; Rummel et al., 2016; Güven et al., 2017; Jabeen et al., 2017; Ory et al., 2017; Vendel et al., 2017). In terms of colour, we found microfibrils belonging to seven of eight colour categories (Provencher et al., 2017) in herring stomachs. Half of all of the ingested fibres were either clear/white or red/pink, while among plastic fibres, pink/red and black made up 83% of those ingested; most of the plastic fibres recovered internally or externally from zooplankton off the coast of British Columbia were black (Desforges et al., 2015). Colours ingested most frequently in other studies of zooplanktivorous fishes include black (Lusher et al., 2013; Murphy et al., 2016), clear (Jabeen et al., 2017), white (Boerger et al., 2010), and blue (Güven et al., 2017; Ory et al., 2017).

4.3. Size and condition of sand lance and herring that had ingested plastic

In contrast to most previous studies of fishes (Vendel et al., 2017; Güven et al., 2017; Mizraji et al., 2017), including a study of Atlantic herring (Foekema et al., 2013), we found that the propensity to ingest microfibrils varied with body size, being limited to smaller individuals in both sand lance and herring. Conversely, Boerger et al. (2010) found that ingestion increased with size in fishes of the North Pacific Gyre. In sand lance, smaller individuals select smaller prey (Hipfner and Galbraith, 2013), suggesting that as they grow, sand lance may adjust their prey field to target larger

prey items and thereby avoid directly ingesting small fibres. Based on length-at-age for sand lance (Robards et al., 1999) and herring (Batten et al., 2016) from other studies, both young-of-year and 2nd year sand lance had ingested microfibrils, but only young-of-year herring had done so. But there was little indication of a link between poor body condition and microfibril ingestion in either species, which is consistent with previous observations on Atlantic herring (Foekema et al., 2013; Rummel et al., 2016). However, the ingestion of microplastics has been linked to poor condition in juveniles in other zooplanktivorous fishes (Mizraji et al., 2017). Rhinoceros auklet nestling diets were somewhat anomalous in several respects on Protection Island in 2016, the year in which we found more fibres in fish stomachs. Following several months of above-normal sea-surface temperatures, the average mass of fish per bill-load was the lightest recorded in 14 years of sampling. For the eight years where we have fish-specific mass and length information, sand lance collected in 2016, for example, were 30% shorter and 24% lighter than the average of the previous seven years (S.F. Pearson et al. unpubl. data). To what extent these anomalies were linked to the increased consumption of microfibrils by forage fishes in 2016 we cannot say.

5. Conclusion

We found that two vitally important Northeastern Pacific Ocean forage fishes, the Pacific sand lance and Pacific herring, rarely ingested microfibrils. In fact, ingestion was consistently rare in individuals of both species that we had collected at sampling sites scattered across coastal British Columbia, over which the at-sea density of microfibrils varies markedly due to oceanographic and anthropogenic factors (Desforges et al., 2014). We therefore suggest that these two forage fishes are not currently acting as major conduits for the vertical transfer of microfibrils to marine piscivores, such as rhinoceros auklets, along British Columbia's outer coast. However, many individuals of both forage fishes were found to have ingested fibres in one of two years of study in the protected inner waters of the Salish Sea, which has extensive urban development along its shores. Future research should aim to quantify spatial and temporal variation in the occurrence of microfibrils at sea (Rudduck et al., 2017), and in the frequency of their ingestion by marine biota. To date, spatial variation in ingestion has been investigated in a variety of marine taxa (Ryan et al., 2016; van Franeker et al., 2011), including semi-pelagic fishes (Nadal et al., 2016; Brate et al., 2016). In contrast, while there have been several investigations of trends in plastic ingestion by marine organisms over decadal scales (Mrosovsky et al., 2009; van Franeker and Law, 2015), year-to-year variation, which we have shown can be appreciable, has received little attention.

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