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Assessing size-based exposure to microplastic particles and ingestion pathways in zooplankton and herring in a coastal pelagic ecosystem of British Columbia, Canada

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ABSTRACT: Microplastic particles (hereafter 'microplastics') are a widespread class of pollutants in marine environments that can become embedded in food webs. Due to their diverse composition and size, microplastics can enter food webs both directly through consumption and indirectly via trophic transfer. In this study, we investigated potential ingestion pathways of microplastics in an important pelagic food web in coastal British Columbia, Canada. Between March and September 2019, we completed repeat surveys of water, zooplankton, and larval Pacific herring *Clupea pallasii* at 11 locations in Baynes Sound, Strait of Georgia. Five zooplankton taxa were isolated from each zooplankton sample for specific analysis. Juvenile herring were sampled once in September. Samples were cold-digested with KOH or H_2O_2 and suspected microplastics isolated. Suspected microplastics were confirmed using μ-Raman spectroscopy and were subsequently identified from the collected samples. The average microplastic concentration in surface waters was 0.59 microplastic particles l^{-1}, and no clear spatial pattern was evident. Average microplastic particle loads were 0.0007 ind.$^{-1}$ in zooplankton, 0.0017 ind.$^{-1}$ in larval herring, and 0.089 ind.$^{-1}$ in juvenile herring. There was a clear difference in the biological:microplastic particle ratio across size fractions (125–250, 250–500, 500–1000, 1000–2000, 2000–4000 μm) in the water column. In size classes <1000 μm, biological particles outnumbered microplastic particles by up to 4 orders of magnitude, whereas for size classes >1000 μm, the ratio decreased to nearly 1. Zooplanktivorous consumers like juvenile herring are more likely to consume microplastics than prey since the ratio of microplastic particles >1000 μm to potential food, and therefore encounter rate, is higher.

KEY WORDS: Microplastic · Microplastic ingestion · Zooplankton · Forage fish · Herring · Food web

1. INTRODUCTION

Over the past decade, microplastic particles (hereafter 'microplastics') have emerged as one of the most pervasive and persistent global pollutants. Research has demonstrated that microplastics are ingested by zooplankton, benthic invertebrates, fish, seabirds, and mammals across marine environments (e.g. Cole et al. 2013, Lusher et al. 2013, Van Cauwenberghe & Janssen 2014, Desforges et al. 2015,

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Lusher et al. 2015, 2018, Steer et al. 2017, Nelms et al. 2019, Wieczorek et al. 2019, Moore et al. 2020). Microplastics are typically classified as plastics ranging from 100 nm to 5 mm in size (Duis & Coors 2016), the same size range as the planktonic portion of marine food webs. The size spectrum of microplastics overlaps with microphytoplankton (20–100 µm; e.g. diatoms), microzooplankton (20–200 µm; e.g. dinoflagellates), mesozooplankton (200 µm–20 mm; e.g. copepods), and early life stages of macrozooplankton (200 µm–20 mm; e.g. krill, larval fish). This opens up the possibility of plastic dietary transfer to higher trophic levels via both direct (including incidental) consumption of microplastics and indirect intake via predation on zooplankton that have consumed plastics (Cole et al. 2013, Setälä et al. 2014, Alava 2020). The size (and shape) of microplastic particles may be expected to affect the probability of ingestion in 2 ways. Firstly, the size of the microplastic determines whether it falls within the range of biological particles (i.e. food or prey item) likely to be ingested by a consumer. Secondly, the size of the microplastic will determine its relative encounter rate with respect to natural particles (i.e. plankton and detritus). In the Gulf of Mexico, Di Mauro et al. (2017) reported that the upper 50% size range of microplastics sampled (335 µm–5 mm) overlapped with all zooplankton species collected, and in these larger size classes, microplastic densities were similar in concentration to most zooplankton taxa. Globally, studies have reported a relatively high abundance of zooplankton-sized microplastics and mention the potential for zooplanktivores to mistake these microparticles for prey (e.g. Collignon et al. 2012, Frias et al. 2014, Kang et al. 2015, Panti et al. 2015, Gove et al. 2019, Ryan et al. 2019). However, most studies that report microplastic size distributions tend to ignore the abundance and particle size of microplastics relative to natural particles — and hence the biological relevance of microplastic concentrations — particularly for organisms feeding on smaller particles (i.e. those smaller than mesozooplankton). This has implications throughout the food web for microplastic exposure, uptake, and bioaccumulation potential, in which zooplankton exposed to plastic particles are the fundamental link for the initial trophic transfer of microplastics in marine environments (Alava 2020).

The direct and indirect impacts on biota from microplastic exposure may vary depending on the source, type, and time of exposure and mode of toxic action (e.g. acute, chronic, and sublethal toxicity). Microplastics are a diverse and unique class of chemicals (Rochman et al. 2019) and are modified by the chemical additives with which they are manufactured as well as organic pollutants (i.e. persistent organic pollutants, phthalates, hydrocarbons) or trace metals that adsorb to them from the environment (Rochman 2015). Organisms that consume microplastics are simultaneously exposed to the physical and physiological stress of attempting to capture and digest the microparticles as well as the exposure to their associated suite of chemical contaminants (Campanale et al. 2020, Zimmermann et al. 2020). Laboratory experiments have demonstrated that high concentrations of microplastics can readily impact feeding rates of copepods (Cole et al. 2013) and decrease reproductive rates in shellfish (Sussarellu et al. 2016), indicating there are potential negative consequences for marine food webs. In some marine taxa, particularly fish, first feeding is considered to play an important role in larval survival (May 1974, Hay 1981). In areas where concentrations of microplastics are high, there is the potential for an animal's first prey item to be plastic rather than nutritious prey. The prevalence of plastic ingestion in plastic-contaminated areas, therefore, has implications for fish health and survival (Jovanović 2017, Azevedo-Santos et al. 2019).

In British Columbia (BC), Canada, microplastic ingestion is of particular concern for Pacific herring *Clupea pallasii*, a forage fish with cultural, economic, and ecological importance that is a critical conduit of zooplankton biomass to top predators, including many commercially important fish species, seabirds, and marine mammals. The largest of BC's 7 herring stock management units spawns and rears in the Strait of Georgia. Herring eggs are deposited on substrate such as eelgrass, kelp, and shallow macrophytes (Humphreys & Hourston 1978) and hatch approximately 2 wk later (Outram 1955, Alderdice & Velsen 1971, Alderdice & Hourston 1985). Larvae tend to be concentrated nearshore, though they are also dispersed to open-water habitats (Hay & McCarter 1997). Juveniles remain nearshore until the fall and may overwinter in the Strait of Georgia before moving out onto the continental shelf in their second summer (Hay et al. 2003). Within the Strait of Georgia, the primary spawning area is in and around Baynes Sound, a channel on the western side of the central strait (Bendell 2019).

A previous study found that Baynes Sound had high concentrations of microplastics, particularly microfibres (mean ± SD: 3210 ± 628 microplastic particles m^{-3}; detected particles >65 µm) in subsurface waters (Desforges et al. 2014). The degree of microplastic pollution consumption by low trophic level biota near Baynes Sound has been assessed in 2

species of zooplankton (*Neocalanus cristatus* and *Euphausia pacifica*), with ingestion rates of 1 microplastic particle per 34 copepods and 1 microplastic particle per 17 euphausiids (Desforges et al. 2015). More recent research has focussed on the prevalence and role of microplastics in sediments, shellfish, and nearshore waters of Baynes Sound (Cluzard et al. 2015, Kazmiruk et al. 2018, Collicutt et al. 2019, Covernton et al. 2019a,b). However, detailed analysis of pelagic microplastic distributions, and analysis of the uptake of these microplastics by the wider food web, has yet to be performed. To date, no analysis has examined microplastic uptake by juvenile Pacific herring, despite microplastics being acknowledged as one of the emerging contaminants of concern in BC's marine environment (Desforges et al. 2014, 2015, Gies et al. 2018, Kazmiruk et al. 2018, Alava 2019, 2020). Herring have 2 feeding modes: indiscriminate filter feeding of small prey (<400 µm) and particle feeding on larger prey (Blaxter 1985). For both feeding modes, higher microplastic:prey ratios might be expected to increase the probability of consumption, although a previous study of blueback herring *Alosa aestivalis* indicated that they discriminated against microplastics (Ryan et al. 2019).

The aim of this study was to provide a baseline of microplastic concentrations and composition in a region that supports critical habitat for an important planktivorous fish species and determine the uptake of microplastics by the pelagic food web. Specifically, we aimed to (1) determine the spatial distribution of microplastics in a dynamic coastal system, (2) determine the occurrence, composition, and size of microplastics ingested by 5 species of zooplankton and 2 life history stages of Pacific herring (larval and juvenile), and (3) evaluate the contribution of microplastics to plankton size classes to provide insight into consumer encounter rates.

2. MATERIALS AND METHODS

2.1. Study area

This study was conducted in the waters in and around Baynes Sound, BC, Canada (Fig. 1). Baynes Sound is an approximately 20 km long body of water located between Vancouver Island and Denman Island in the Strait of Georgia. Oceanographic conditions in Baynes Sound are largely controlled by tides and freshwater (Morris et al. 1979). The main freshwater source is the Courtney River, which contributes to the ultimate net outflow of surface water from Baynes Sound, moving from north to south. Deep water is also presumed to flow from north to south, with exchange occurring through the southern entrance due to topographic restrictions to the north. The residence time of bottom water is predicted to be approximately 2 mo (Morris et al. 1979).

2.2. Field collection of samples

A set of 11 stations where we collected CTD measurements, microplastics, zooplankton, and larval herring were routinely and systematically sampled from March–September 2019 (Fig. 1, Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m683p139_supp.pdf). Sampling frequency was weekly from 15 March until 18 April, spanning the period of herring spawn (peak spawn on March

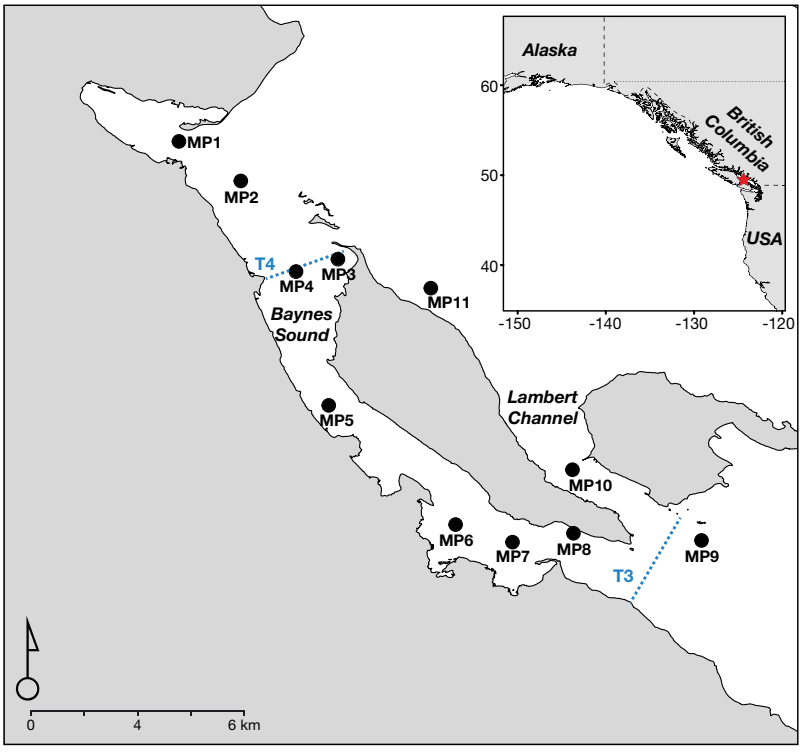

Fig. 1. Baynes Sound, showing the location of sampling stations (MP1–MP11). Dashed lines: transects (T3, T4) for sampling juvenile herring near Baynes Sound; inset: northwest coast of North America, with Baynes Sound indicated by the red star

14–15 in 2019) and early larval development, and monthly from May until July, with an additional sampling event in September.

Full water column CTD data were collected at each station using an RBR XR-420 or RBR XR-620. Chlorophyll *a* (chl *a*) data were collected using fluorescence data from the downcast of the CTD. Water samples were collected with a Niskin bottle. Surface water was collected by submerging the Niskin horizontally below the surface and manually closing the bottle. Sub-surface water samples were collected by attaching the Niskin bottle to a nylon rope, deploying the bottle vertically to the designated depth (5, 10, or 35 m), and closing the bottle using a messenger. Water samples were transferred from the closed Niskin bottle to fill a 4 l glass carboy via a metal tube, with tinfoil covering the glass carboy to minimize background contamination. The carboy was sealed immediately after water collection and remained sealed until inside the laboratory clean room the following day. Though the Niskin bottles were composed of PVC, we did not detect any PVC particles in our samples. Only one nylon fibre was found in all water samples analysed, indicating that the rope used for water collection had a near-negligible contribution to contamination of samples.

Zooplankton vertical net tows were conducted using a 3 m length ring net with a mouth diameter of 0.5 m, a mesh size of 250 µm, and equipped with a General Oceanics flowmeter positioned in the mouth of the net to calculate volume filtered. Nets were deployed to 5 m above bottom depth (~35 m) and were subsequently retrieved vertically at 1 m s^{-1}. After each tow, the net was rinsed down from the outside using a seawater hose, and the contents were transferred to a glass jar and preserved in a 5–10% buffered formaldehyde–seawater solution. No water was allowed to enter the net during wash-down.

Larval herring were collected with a 3 m length ring net with a mouth diameter of 0.5 m and a mesh size of 350 µm. The net was deployed in nearshore waters to a depth of <5 m and was hauled in obliquely at ~0.5 m s^{-1}. Once surfaced, the contents in the cod end were quickly transferred to a glass jar and preserved in a 5–10% buffered formaldehyde–seawater solution. The net was towed at a slower rate than zooplankton tows and was not rinsed down to minimize disturbance to the larval herring, which have been shown to void their gut contents when stressed (Hay 1981).

Juvenile herring were obtained from Fisheries and Oceans Canada's annual purse seine survey conducted in the Strait of Georgia (Schweigert et al. 2009, Thompson et al. 2020a,b). The survey comprises 10 fixed-core transects sampled in mid- to late September each year, 2 of which (Transects 3 and 4) are located on the southern and northern sides of Baynes Sound, respectively (Fig. 1). A total of 10 fish from each of these 2 transects were obtained from samples taken in 2016, 2017, and 2018 (when available) for stomach content analysis to quantify microplastic ingestion by juvenile herring. Samples were stored frozen until analysis.

2.3. Laboratory analysis

2.3.1. Filtration of water samples

Water samples collected in the field were transported to a laboratory clean room and filtered within 36 h of sample collection. For each water sample, a measured amount (ranging from 3.5–3.9 l) was filtered onto a 10 µm pore, 47 mm diameter Sterlitech polycarbonate filter using glass funnels and a vacuum pump and stored in a clean PetriSlide dish. Samples were stored in a −20°C freezer in clean PetriSlides until chemical digestions.

2.3.2. Selection of zooplankton species and larval herring

Five zooplankton taxonomic groups were selectively picked from the vertical zooplankton tows to investigate microplastic consumption. The zooplankton taxa were *Calanus* spp., *Eucalanus bungii*, euphausiid furcilia (mainly *Euphausia pacifica*), *Metridia pacifica*, and *Neocalanus plumchrus*. These zooplankton taxa were selected since they are common and ecologically important in the Strait of Georgia (Harrison et al. 1983) and comprise a portion of typical Pacific herring diets (Foy & Norcross 1999). Zooplankton samples were transferred out of the formalin solution, and each species was picked out with stainless steel forceps and placed in Milli-Q water. Each individual was inspected for external microplastics before being added to groups of 12–100 individuals, depending on the abundance of taxa in the water (Table S2). Grouped individuals were transferred to a glass beaker and covered with tinfoil.

Individual larval herring from the oblique nearshore tows and from the vertical zooplankton tows

were collected in 3 subsamples when possible (ranging from 13–50 ind. subsample^{-1}; Table S2) from each net tow. Individuals were collected and processed as described above.

2.3.3. Dissection of juvenile herring stomachs

Juvenile herring (approximately 6 mo old) were dissected and entire stomachs were preserved in 5–10% formalin until gut processing. Stomachs were removed from the formalin solution and the exterior of each stomach was rinsed thoroughly with Milli-Q water. Stomachs were transferred to a glass Petri dish under a microscope and gut contents were processed. Each stomach was assigned a percent fullness index (0–25, 25–50, 50–75, or 75–100%) and a measure of digestive state (1, 2, or 3), and the main taxa contributing to the diet were recorded. The main taxa were primarily small–medium calanoid and cyclopoid copepods (1–4 mm), barnacle nauplii and cyprids, juvenile and adult euphausiids, cladocerans, and amphipods. Gut contents were transferred to a 50 ml glass beaker and covered with tinfoil.

2.3.4. Digestion of water samples

To break down the biological matter in water samples, chemical digestions were used. A 10% potassium hydroxide (KOH) solution was added to each sample (>3× sample volume, approximately 20–30 ml), and samples were left for 2–3 wk at room temperature (~20°C) to digest (Foekema et al. 2013). Samples were subsequently filtered onto a 10 µm, 47 mm Sterlitech polycarbonate filter using a vacuum pump with glass funnels.

Due to the prevalence of biological material remaining on filters after the KOH digestion, an additional digestion using 30% H_2O_2 was done using the same methods as described above.

2.3.5. Digestion of zooplankton species, herring stomachs

To break down the biological matter in zooplankton and herring samples, a 10% KOH solution was added to each sample (>3× sample volume, approximately 20–30 ml) and samples were left for 2–3 wk at room temperature ~20°C) to digest (Foekema et al. 2013). Samples were then filtered onto a 10 µm, 47 mm Sterlitech polycarbonate filter using a vacuum pump with glass funnels.

2.4. Quality assurance/quality control: procedural blanks

All activity and lab procedures that exposed samples to the environment occurred in a designated clean room with a laminar flow hood. Clothing in the clean room was restricted to bright yellow Tyvex suits or clothing composed of 100% natural fibres to avoid synthetic fibre contamination of samples. Three lab procedural blanks were conducted with each batch of water samples from the field. For each procedural blank, 100 ml of Milli-Q water was filtered onto a 10 µm, 47 mm polycarbonate filter and stored in a clean PetriSlide dish and were subsequently processed in the same manner as samples. Final microplastic concentrations were blank-corrected (described below). Three lab procedural blanks were also conducted with each batch of animal samples, where approximately 25 ml of 10% KOH was added to 50 ml beakers covered in tinfoil and processed alongside organism samples.

2.5. Visual identification of potential microplastics

Post-digestion samples were dried and stored at room temperature. Each PetriSlide containing a sample or a procedural blank was inspected (top cover removed) under a dissecting light microscope (Zeiss Stemi 508) encased in a clear garbage bag within a clean room with a laminar flow hood. Only suspected microplastics within the range of 100–5000 µm were included in this study. Each potential microplastic was inspected visually and prodded with metal forceps to ensure it was a suspected microplastic (i.e. not glass, sediment). Each suspected microplastic was characterized by type (i.e. fibre, fragment, film, granule, foam) and colour (i.e. blue, red, green, brown, black, purple, clear), and measured along its largest dimension using the Zeiss Zen software. Once each filter was fully processed, the top cover was put back and all samples were stored for future spectrometry analyses to confirm suspected microplastics were described as actual microplastics. All suspected microplastics remained on the filter to reduce contamination and/or loss of particles before polymer identification.

2.6. Spectroscopic identification

A subsample of the suspected microplastics in water samples (all samples from Stn MP5, 161 suspected microplastics; approximately 22% of all suspected microplastics from all stations) and all zooplankton, larval herring, and juvenile herring (48, 51, and 56 suspected microplastics, respectively) were identified using μ-Raman spectroscopy. A correction value was applied to water samples collected from stations where suspected microplastics were not confirmed (see Section 3.3). The particles were moved from the membrane filters using metal forceps onto a clean aluminum slide. A 785 nm laser was focused onto the sample using a BX-51 Olympus microscope fitted with a Reichert Plan Achro 40×/0.17 NA Infinity Objective. Raman-scattered light entered a spectrometer (Princeton Instruments Acton SP2300) with 600 grooves mm^{-1} grating with 4.5 cm^{-1} resolution.

Spectra were collected in the range 400–3000 cm^{-1}. In addition to the 18 reference spectra collected in-house (Text S1), our reference library included the publicly available SLoPP and SLoPP-E Raman database (Munno et al. 2020), which contains Raman spectra from plastic and other materials commonly found in the environment. In total, our reference library contained 278 unique spectra from 28 different materials. Each sample spectrum was compared to reference spectra and assigned a 'match' that was accepted when certain thresholds were met. For a detailed description of the process identifying each spectrum, see Text S1 and Figs. S1–S4.

2.7. Comparison of biological and microplastic particle densities across size classes

Samples from Stn MP2 (one sample from each sampling date) were later size-fractionated using a sieve column into 4 logarithmically equal size classes: 250–500, 500–1000, 1000–2000, and 2000–4000 μm (Table S3). To obtain total wet weight, each fraction was weighed to the nearest 0.01 g using a Mettler Toledo (XS205) microbalance and then divided by the volume filtered. For each size class (i), minimum (WW_{min_i}) and maximum (WW_{max_i}) wet weight were calculated assuming near-neutral density and ellipsoid shape, where the minimum and maximum length corresponds to the equivalent spherical diameter (ESD) (Suthers et al. 2004, Suthers et al. 2006) as follows:

$$\text{Biomass (WW)} = \frac{4\pi}{3} \times \left(\frac{\text{ESD}}{2}\right)^2 \quad (1)$$

To obtain a representative weight for an individual within each size class, we calculated the geometric mean wet weight (WW_{GM_i}) as follows:

$$WW_{GM_i} = \sqrt{WW_{min_i} \times WW_{max_i}} \quad (2)$$

Total abundance of individuals in each size class was then determined by dividing the total wet weight of size class i (mg) by the geometric mean wet weight (WW_{GM_i}; mg $ind.^{-1}$).

To estimate the concentration of microplastic particles, we used the density of confirmed microplastic particles (i.e. number of confirmed microplastic particles per liter of water) at Stn MP5 across all 10 sampling dates and 4 depths (n = 40). First, microplastic samples from each sampling date and depth strata at Stn MP5 were grouped into equal logarithmic size classes (i.e. 125–250, 250–500, 500–1000, 1000–2000, 2000–4000 μm). To calculate microplastic density for each logarithmic size class, the total microplastic count was divided by the total volume filtered. To compare across size classes for the entire water column, we took the average density of microplastics in each size class across depth strata. The ratio of estimated zooplankton to microplastic densities in each size class was then calculated for each sampling event.

Because we were able to quantify microplastics down to 100 μm, we estimated the abundance of zooplankton within the 125–250 μm size fraction using normalized biomass size spectra (NBSS). Organisms were grouped into equal logarithmic size bins and the total biomass within each size bin was divided by the width of the size bin. NBSS were plotted as the least-squares linear regression between log normalized biomass and log body size (Kerr & Dickie 2001). The linear regression equation of each NBSS was then used to infer total abundance within the 125–250 μm size fraction for comparison with microplastics.

2.8. Statistical analysis

To assess differences in microplastic particle concentrations at different depths in central Baynes Sound as well as differences in microplastic particle concentrations and various environmental variables across stations and time in and around Baynes Sound, we applied separate 1-way ANOVA tests. A Shapiro-Wilk test indicated that the surface microplastic data across all stations were not normally distributed even with a logarithmic transformation (p =

0.003), but a Bartlett test indicated there was a common variance ($p = 0.55$). Since ANOVA is relatively robust to deviations from normality, we proceeded with the test (Schmider et al. 2010). Microplastic data from discrete depths collected in central Baynes Sound were normally distributed and showed a common variance using a Shapiro-Wilks test and Bartlett test, respectively. A Tukey's HSD post hoc test was applied to determine differences detected by the 1-way ANOVA tests. All statistical analyses were conducted using R v.3.5.2 (R Core Team 2018); results were considered significant at $p \leq 0.05$.

3. RESULTS

3.1. Environmental conditions in Baynes Sound

The 1-way ANOVAs indicated there were no statistically significant spatial patterns ($p > 0.05$) in mean temperature ($p = 0.63$), salinity ($p = 1$), or chl *a* ($p = 0.72$) at 2 m between any of the 11 stations sampled when averaged over 10 sampling events from March–September 2019 (Fig. 2). Seasonally, sea surface temperatures ranged from 7.35–18.45°C, surface salinities ranged from 25.18–29.96, and surface chl *a* ranged from 0.15–15.97 µg l^{-1} across the sampling period. Average surface (2 m) water temperature was highest from May–September, and salinities were lowest in June. Chl *a* at 2 m was generally highest in March–April and September and lowest in May–June.

3.2. Suspected microplastic particle characteristics

The vast majority of suspected microplastics in water, zooplankton, larval herring, and juvenile herring were fibrous particles (94.3, 88.4, 90.0, and 90.0%, respectively; Table S4). The remaining suspected microplastics were either fragments, films, or foams. Most suspected microplastics in water, larval, and juvenile herring (64.9, 70.0, and 57.8%, respectively) were clear in colour, while in zooplankton most suspected microplastics were black (44.2%) followed by the observation of blue, red, brown, green, and purple particles, as reported in Table S5. Overall, most suspected microplastic particles were <1 mm, and secondary consumers (particularly juvenile herring) had higher proportions of larger suspected microplastics compared to smaller suspected microplastics than were observed in the water (Table S6).

3.3. Procedural blanks

Procedural blanks run in the lab concurrently with samples contained particles (mean ± SE) on the order of 0.300 ± 0.535 suspected microplastics (water samples) and 1.111 ± 1.023 suspected microplastics (organism blanks) per sample. Since 3 out of 8 (i.e. 37.5%) of the suspected microplastics in lab blanks for water samples were identified as plastic, each water sample was corrected for potential contamination by subtracting the mean suspected microplastics value in water samples × 3/8 from each sample (i.e. 0.113; to a minimum value of 0). Since all suspected microplastics from organisms and their respective lab blanks were analysed using µ-Raman spectroscopy, confirmed plastics in lab blanks were compared with confirmed plastics in samples, and final plastic concentrations in animals excluded counts where lab blanks contained the same confirmed polymer as the corresponding animal sample.

3.4. Confirmation of suspected microplastic particles using Raman analysis

The µ-Raman analysis confirmed that 82 out of the 316 (26.0%) suspected microplastic particles analysed were plastic (Fig. 3, Table S7). However, when broken down by group, water had a higher proportion of plastic to total suspected microplastic (including non-plastic and unknown) particles (70 out of 161 particles, 43.5%). Zooplankton, larval herring, and juvenile herring had comparatively lower ratios of plastic to total suspected microplastic particles (6.3, 5.9, and 10.7%, respectively). Remaining particles were confirmed as non-plastic material or classified as 'unknown'. Due to the strong Raman signal and high signal-to-noise ratio of confirmed plastic particles, it is unlikely that samples categorized as 'unknown' were plastic (Fig. S2).

Confirmed plastic particles were overwhelmingly polyethylene terephthalate (PETE)/polyester (82.9%), but also included some particles composed of polyamide, polyethylene, polypropylene, cellulose acetate, nylon, polystyrene, and polylactic acid (Table 1). Particles categorized as 'non plastic' were mostly cellulose and cotton, with rare occurrences of cashmere and rayon.

Only 3 of the 48 suspected microplastics (6.25%) from zooplankton were confirmed as plastic particles (one PETE/polyester fibre each in *Metridia pacifica*, *Eucalanus bungii*, and *Calanus pacificus*).

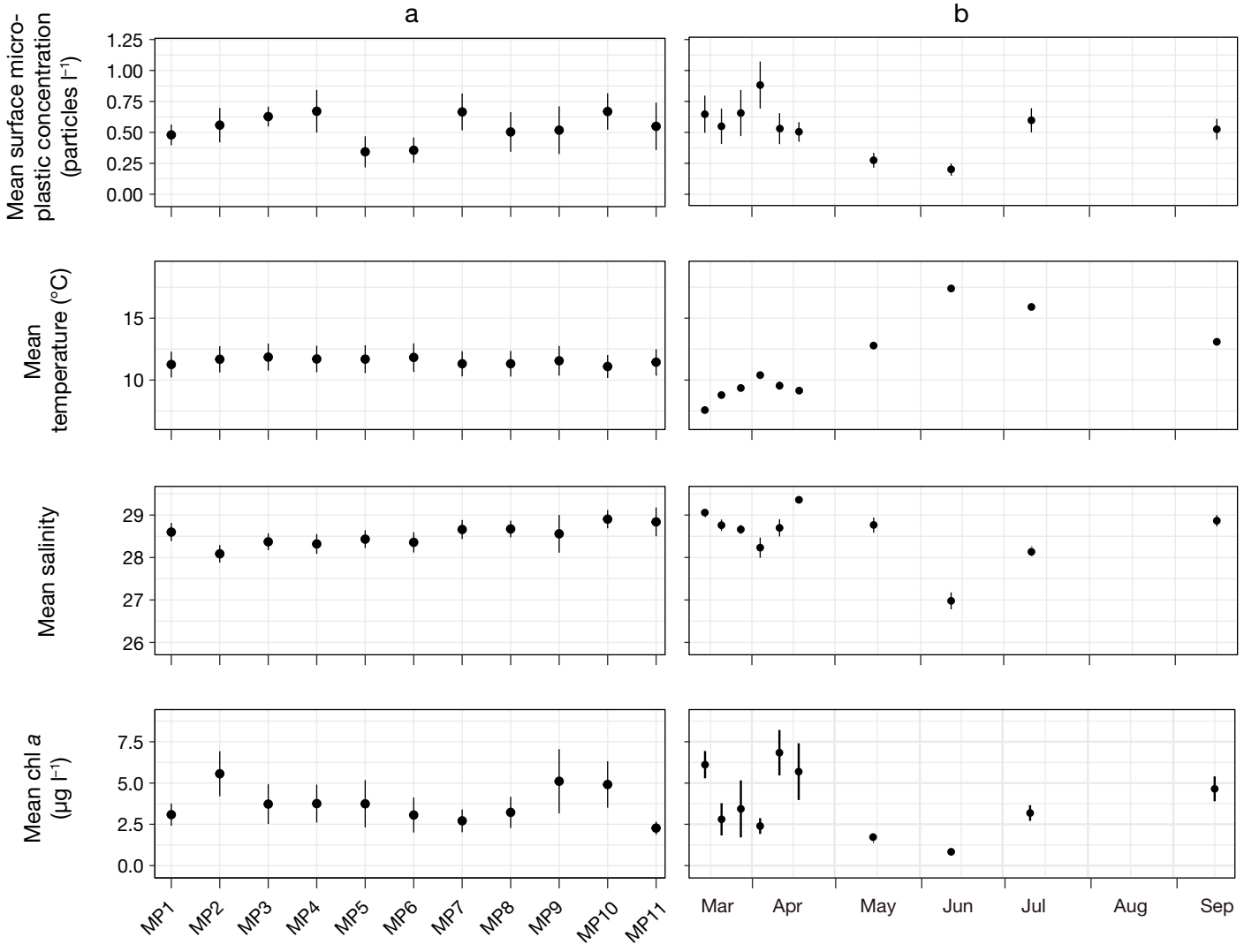

Fig. 2. Mean ± SE surface water confirmed microplastic particle concentrations, temperature, salinity, and chl *a* at 2 m, averaged across sampling dates for each station (left) and all stations for each sampling date (right). Data were collected from 11 stations sampled on 10 d from March–September 2019

Despite 77.5% of juvenile herring stomachs containing at least one suspected microplastic, only 12.8% of the herring stomachs had a confirmed plastic particle.

3.5. Confirmed microplastic particle concentration in water and marine biota

In surface waters, confirmed microplastic concentrations ranged from 0–2.12 microplastic particles l^{-1} and averaged (±SE) 0.59 ± 0.04 microplastic particles l^{-1} across all stations sampled. Surface concentrations of suspected microplastics did not statistically differ between stations when averaged over time (1-way ANOVA, $p = 0.78$). However, the lowest surface concentrations were in central Baynes Sound, i.e. Stns MP5 and MP6 (Fig. 2). When averaged by sampling date, surface microplastic concentrations were significantly lower in May and June compared to the April-04 sampling date (1-way ANOVA, $p < 0.05$; Tukey's HSD, $p = 0.04$ for May and $p = 0.01$ for June; Fig. 2).

The average water concentration of microplastics was 0.59 microplastic particles l^{-1} in central Baynes Sound (Stn MP5), where microplastics in water were sampled at 4 depths (0, 5, 10, and 35 m). There were no significant differences in microplastic density between depths when averaged over time (1-way ANOVA, $p = 1$; Fig. 4).

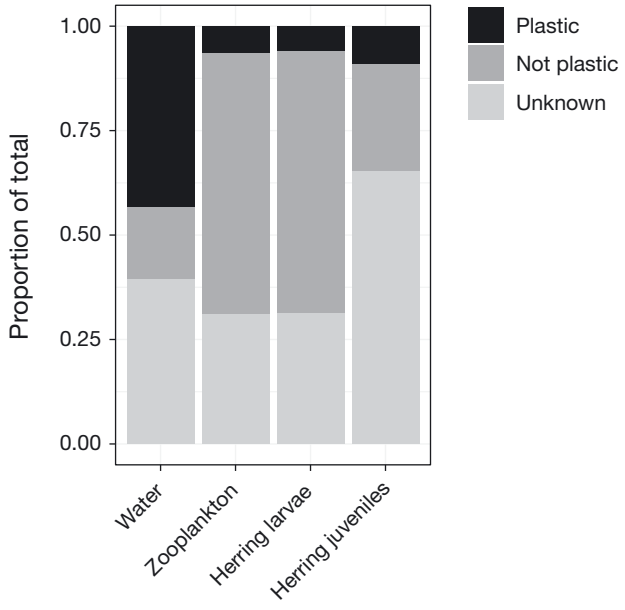

Fig. 3. Proportion of suspected microplastics identified as plastic, not plastic (i.e. cellulose, cotton), or unknown/unidentified using µ-Raman spectroscopy in samples from water, zooplankton, larval herring, and juvenile herring

Pooling all zooplankton taxa resulted in a mean of 3 microplastic particles per 4153 individuals, or 7 microplastic particles per 10 000 individuals (i.e. 0.0007 microplastic particles ind.$^{-1}$). Similarly, larval herring had a low prevalence of 3 microplastic particles per 1742 individuals (i.e. 0.0017 ind.$^{-1}$). Juvenile herring had a higher prevalence of 5 microplastic particles per 56 individuals (i.e. 0.089 ind.$^{-1}$).

Confirmed microplastics in the water column ranged from 0.18–3.88 mm in size, with more than 87% being <1 mm (Fig. 5). However, confirmed ingested microplastic particle size ranges were 0.56–0.73 mm for zooplankton, 0.39–1.17 mm for herring larvae, and 0.3–4.95 mm for juvenile herring.

3.6. Comparison of the prevalence of biological particles and microplastic particles across size classes

We were able to directly compare the ratio of zooplankton to microplastics in 4 size classes (250–500, 500–1000, 1000–2000, and 2000–4000 µm). For the 2 smallest comparable size classes (i.e. 250–500 and 500–1000 µm), the density of zooplankton exceeded that of microplastic particles by up to 4 orders of magnitude (Fig. 6). However, at larger size fractions (1000–2000 and 2000–4000 µm), the ratio decreased considerably, and at the largest size fraction (2000–4000 µm) investigated there were more microplastics m^{-3} than zooplankton. Our inferred estimates of zooplankton density in the 125–250 µm size fraction (i.e. those estimated using NBSS) were on average 4 orders of magnitude higher than the concentration of microplastics (ranging from 2–7 orders of magnitude difference; Fig. 6).

4. DISCUSSION

4.1. Microplastic particle concentration varies seasonally but not spatially within the study area

Our estimates of microplastic density in the surface waters of Baynes Sound (0.59 ± 0.04 microplastic particles l^{-1}) were slightly lower than previous concentrations reported for the region (ranging from 0.63 ± 0.52 to 5.28 ± 4.17 microplastic particles l^{-1}; Desforges et al. 2014, Collicutt et al. 2019, Covernton et al. 2019b). However, only one of the studies (Covernton et al. 2019b) confirmed plastic identity using chemical analysis, and that study reported concentrations closer to ours (0.72 ± 0.57 microplastic particles l^{-1}). Notably, microplastics in these studies were collected using a combination of bottle samples and neuston nets, which can lead to very different microplastic density estimates (Covernton et al. 2019a). Even after accounting for procedural blank contamination, our suspected microplastic concentration in surface waters was 2.2 times that of confirmed microplastic concentration in water, emphasizing the need

Table 1. Composition of confirmed plastic particles identified using µ-Raman spectroscopy. A total of 81 particles were identified as plastic in this study collected from water, zooplankton, and larval and juvenile herring. PETE: polyethylene terephthalate; PLA: polylactic acid

Plastic polymer	n	Total plastics (%)	Water (n)	Zooplankton (n)	Larval herring (n)	Juvenile herring (n)
Acrylic	1	1.22	1	0	0	0
Cellulose acetate	3	3.66	2	0	0	1
Nylon	1	1.22	1	0	0	0
PETE/polyester	68	82.93	60	3	2	3
PLA	1	1.22	1	0	0	0
Polyamide	3	3.66	3	0	0	0
Polyethylene	2	2.44	1	0	0	1
Polypropylene	1	2.44	1	0	0	0
Polystyrene	1	1.22	0	0	1	0

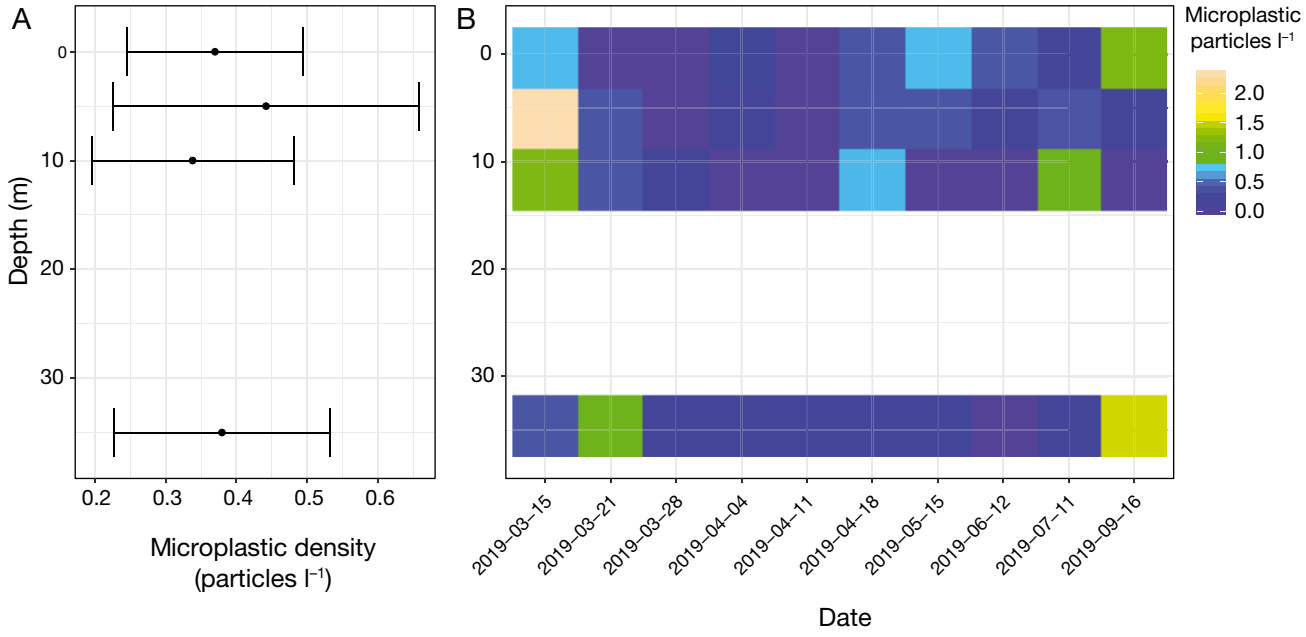

Fig. 4. Mean ± SE confirmed microplastic density at 0, 5, 10, and 35 m depth sampled in central Baynes Sound (Stn MP5) (A) averaged over the 10 sampling dates from March–September in 2019 and (B) on the 10 sampling dates

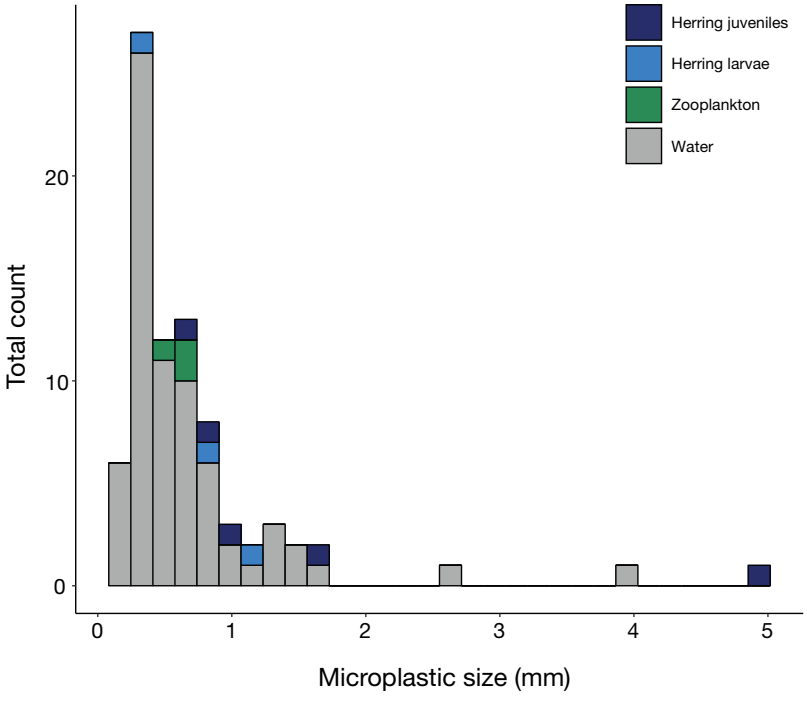

Fig. 5. Size frequency distribution of confirmed microplastics from Baynes Sound in water, zooplankton, larval herring, and juvenile herring. Histogram bin widths are smaller than size bins used for other analyses to show more detail in the differences in microplastic particle sizes consumed by animals

for chemical confirmation of suspected microplastics.

Baynes Sound is a region with several potential point and non-point sources for microplastics to enter the marine environment. It has the highest density of BC's shellfish farms, a plastic-intensive industry, and is also adjacent to several urban and rural areas, including Courtney/Comox, which can introduce microplastics to the ocean via sewage output and imperfect wastewater treatments as well as run-off (Browne et al. 2011, Chae & An 2018). Despite these different point sources, we detected no consistent horizontal patterns in the concentration of microplastics in the waters around Baynes Sound. Though there may have been very small-scale and short-lived 'hot spots' of microplastics, we did not have enough intra-seasonal replication to statistically test this possibility, and this is an area of microplastics research that should be investigated further. Additionally, no vertical structure in the concentration of microplastics was detected in central Baynes Sound. The lack of spatial pollution gradient in microplastics may be due to the strong tides and currents in the

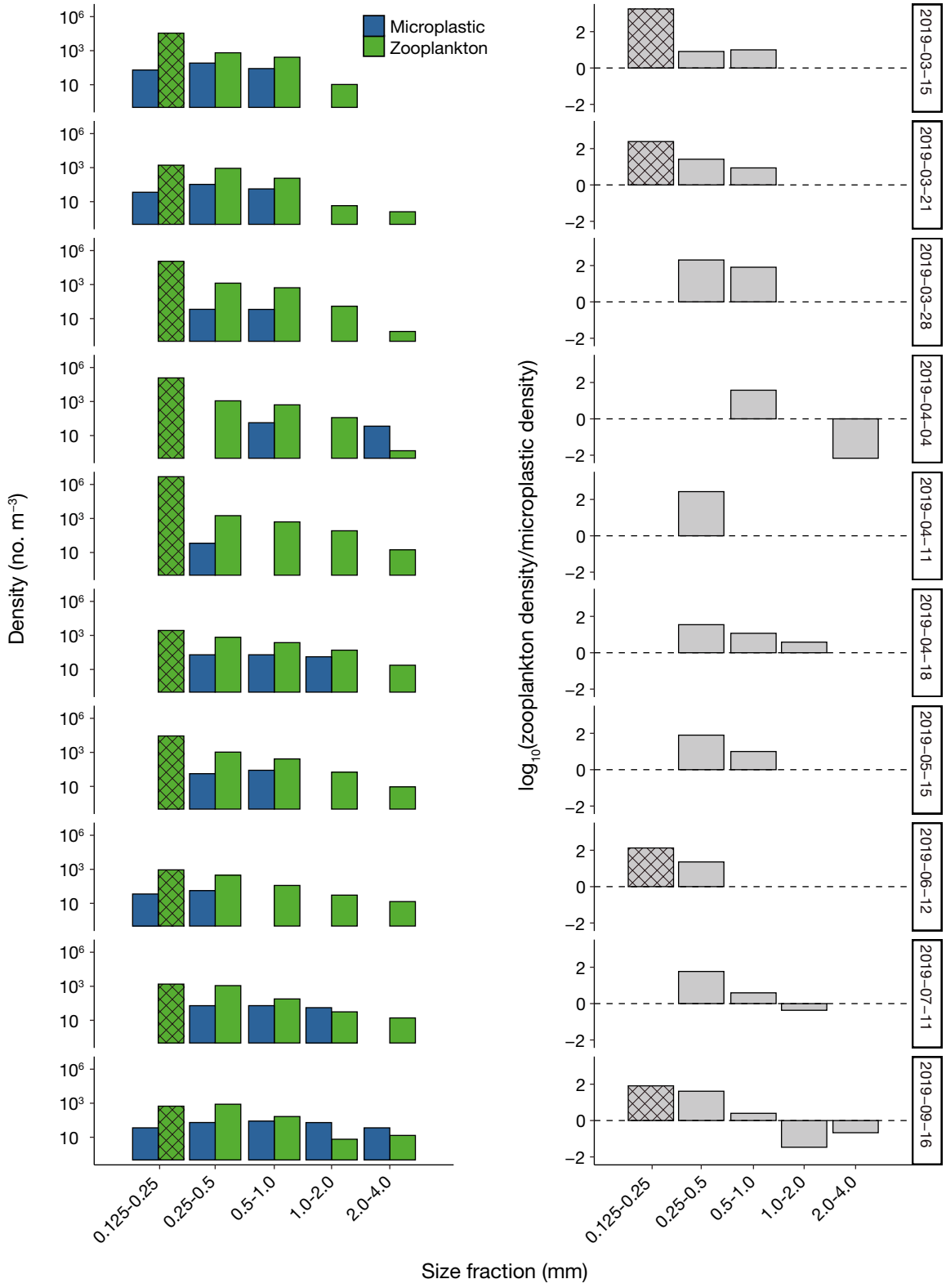

Fig. 6. Comparison of microplastic and zooplankton densities (left) and the log-transformed ratio of zooplankton to microplastic density (right) by size fraction for each sampling date in central Baynes Sound (Stn MP5). Inferred estimates of zooplankton density using normalized biomass size spectra for the smallest size fraction (i.e. 0.125–0.25 mm) are identified with cross-hatching

region that disperse and homogenize their distribution. Baynes Sound experiences a net outflow of surface water towards the south due to freshwater input from the north, and tidal exchange through the northern and southern entrances (Morris et al. 1979). Similarly, Covernton et al. (2019b) found that surface water microplastic concentrations did not vary with distance to aquaculture sites in different locations across BC, including several sites in Baynes Sound. In regions with strong tidal flushing and/or vertical mixing, point sources may not be discernible by concentration gradients due to the rapid dispersal and mixing of particles through the water column.

The seasonality of microplastics in marine environments remains largely unexplored. This study indicated the potential for seasonality in the microplastic concentrations in the surface waters of Baynes Sound, with the lowest values being recorded in early summer. Similar to other studies in nearshore waters, we observed changes in microplastic concentrations over the sampling period, which we attributed to local environmental factors (e.g. freshwater input via rivers or rainfall, storm activity) working at various timescales (Moore et al. 2002, Lima et al. 2015, Cheung et al. 2016). Long-term (multi-year) and high-frequency (e.g. biweekly) sampling is needed to fully investigate the potential mechanisms influencing changes in microplastic concentrations within dynamic coastal environments and how those mechanisms may vary with season.

It is possible that when the ratio of microplastic particles to biological particles increases, higher encounter rates lead to increased biological removal from the water column by particle grazers. Laboratory experiments have demonstrated that the abundance of phytoplankton can influence the uptake or ingestion of microplastics by zooplankton, including copepods (Cole et al. 2013). Recently, there has been evidence that the eco-corona or plastisphere harbouring a biodiverse epiplastic community (coating of biomaterial on nanomaterials and plastic particles in the environment) may, in fact, stimulate consumer uptake of microplastics (Zettler et al. 2013, Debeljak et al. 2017, Galloway et al. 2017, Ramsperger et al. 2020). Future studies should take seasonality into account, either by including several time points taken during more than one season when reporting microplastic densities in an area, or considering the seasonal context of sample collection (e.g. wet or dry season, as suggested in Cheung et al. 2016).

4.2. Uptake pathways of microplastics in marine food webs and considering microplastic:biological particle ratios in exposure assessment

Since microplastics overlap in size with the planktonic portion of marine food webs, there is the potential for organisms associated with the plankton to be exposed to microplastics via both direct (i.e. mistaking a microplastic as food) and indirect (i.e. consuming prey that has eaten a microplastic) consumption. The low prevalence of microplastic particles in zooplankton and larval herring (1 microplastic particle per 1384 and 581 individuals, respectively), but higher density of microplastics in juvenile herring (1 microplastic particle per 11 individuals) indicated that larger consumers may encounter microplastics more frequently. The wider range of confirmed microplastic sizes in juvenile herring indicated a stronger contribution of both indirect and direct ingestion pathways than in zooplankton and larval herring. While some zooplankton can selectively feed based on particle type, the feeding by juvenile herring can be passive or active depending on the prey field (Blaxter 1985). Thus, feeding by a juvenile herring may be less selective compared to feeding by zooplankton. Another factor potentially contributing to the higher prevalence of microplastics in larger organisms is size-related increases in gut passage and, hence, retention time (Bautista & Harris 1992). Retention time may also be affected by microplastic particle size and shape, although the nature of this effect appears highly variable (Rist et al. 2017, Kinjo et al. 2019, Ohkubo et al. 2020, Yu et al. 2021).

In addition to affecting ingestion pathways, the probability of microplastic ingestion is expected to vary by particle size and shape. The ratio of biological particles to microplastic particles varied by orders of magnitude across plankton size classes. During our study there were, on average, 4 orders of magnitude more biological particles than microplastic particles when considering the smallest size class we investigated (125–250 µm; i.e. the size range of microzooplankton and mesozooplankton). Estimating zooplankton biomass using NBSS may lead to some degree of error, as NBSS may not be perfectly linear (Atkinson et al. 2021), particularly when a limited size range of plankton are used. However, given the magnitude of difference between zooplankton and microplastic densities for the estimated size fraction, it is unlikely our overall finding would change. Our estimates of biological particles only included micro- and mesozooplankton, and if phytoplankton were included then the difference between biological and

plastic particles in smaller size classes not captured in this study could be even higher. In productive regions with high phytoplankton and microzooplankton biomass, the ratio of microplastics to biological particles is probably such that encounter rates for consumers of microplankton (most zooplankton taxa and fish larvae) would be extremely low. Importantly, when comparing larger particles (>500 µm), the ratio of microplastics to biological particles is much closer to 1; in Baynes Sound, 2–4 mm microplastic particles can actually be more abundant than zooplankton. The juvenile herring in this study were feeding on zooplankton ranging from 0.5–15 mm, but the vast majority (>90%) of prey taxa were between 1–4 mm. This supports the notion that zooplanktivorous consumers were more likely to encounter prey-sized microplastic particles, increasing the potential for exposure and direct ingestion.

Notably, the ratios of biological particles to microplastics reported here were average values for spring–summer in a productive coastal system and did not take into account the patchiness of zooplankton distribution or the seasonality of plankton communities that exists in the region (Mahara et al. 2019). Since phytoplankton, microzooplankton, zooplankton, and microplastic densities all vary on seasonal scales, the microplastic encounter rate will also change. In temperate coastal environments, we expect consumers to encounter relatively more microplastics during periods and areas of low biomass (e.g. winter and tidal mixing zones) and fewer microplastics during plankton blooms or in high-productivity areas. In periods or areas of low plankton biomass, the health impacts of microplastics on animals (e.g. increased mortality, decreased reproductive capacity, decrease growth, decreased feeding; Cole et al. 2013, Lee et al. 2013, summarized in Botterell et al. 2019, Fulfer & Menden-Deuer 2021) may be more prevalent. Importantly, even the use of average concentration values in this study demonstrated that animals feeding on zooplankton are likely more susceptible to ingesting microplastics than consumers feeding on phytoplankton or microzooplankton.

Previous estimates of suspected microplastic consumption in zooplankton and juvenile salmon have been published in the same region as this study and are higher than our estimates (Desforges et al. 2015, Collicutt et al. 2019). Similar to other studies (Lusher et al. 2013, Hipfner et al. 2018), most suspected microplastics in zooplankton and herring in Baynes Sound were not plastic (only 7.7% were confirmed as plastic), emphasizing the need for chemical confirmation of microparticles. Microplastics have been observed to be present in 0–22% of sampled herring in other studies, with reported non-zero microplastic concentrations ranging from 0.25–0.90 microplastic particles ind.$^{-1}$ (Foekema et al. 2013, Rummel et al. 2016, Hermsen et al. 2017, Hipfner et al. 2018, Ogonowski et al. 2019). Notably, Hipfner et al. (2018) concluded that smaller herring were more likely to consume microplastics than large herring in their study in BC; since we collected stomachs from juvenile herring, it may explain why nearly 10% of our stomachs contained microplastics. Relatively low concentrations of microplastics (i.e. mean ± SD: 1.2 ± 1.4 microplastic particles ind.$^{-1}$, with microfibers accounting for 95% of total plastic particles identified) were also found in juvenile Chinook salmon *Oncorhynchus tshawytscha* from southeastern Vancouver Island, indicating that this salmonid species is exposed to these micropollutants (Collicutt et al. 2019). Although we are beginning to understand the ubiquitous distribution of microplastics across marine environments and biota, the impacts of microplastics on ecosystems and individuals is scarcely known (Cole et al. 2011, Galloway et al. 2017). A recent study indicated that blueback herring *Alosa aestivalis* may select against microplastics in favour of zooplankton prey (Ryan et al. 2019), an important consideration when considering consumption. However, this study did not take into account size distributions of prey and microplastics. We recommend that future studies take into account the size of particles consumed by fish when estimating microplastic selectivity.

4.3. Polymer composition of microplastics in Baynes Sound

Globally, microfibres are the most common and dominant microplastic type (e.g. >90%) found in marine environments and biota (Barrows et al. 2018). Our results indicate that the microplastics identified in Baynes Sound water and biota were overwhelmingly fibrous PETE/polyester (i.e. 83% of confirmed microplastics). Fibres are the most common microplastic type reported in the Northeast Pacific water (coastal and offshore), zooplankton, bivalves, and fish (Desforges et al. 2014, 2015, Hipfner et al. 2018, Collicutt et al. 2019, Covernton et al. 2019a,b). The high prevalence of PETE fibres indicates that sewage outputs of textile emissions, aerial dust from cities, or coastal landfills may be important point sources of microplastics to Baynes Sound and other

coastal environments. For instance, a pilot study in a municipal wastewater treatment plant estimated that despite a 99% rate of microplastic retention after treatment, 0.3 ± 0.01 trillion microplastic particles were released into the receiving aquatic environment annually (Gies et al. 2018). Though the majority of our suspected microplastics were not confirmed as plastic, it is important to highlight that anthropogenic inputs are not limited to synthetic particles. We found many cellulose and cotton fibres in our samples, as have many other studies conducted globally (e.g. Suaria et al. 2020, Athey & Erdle 2021). We did not focus on non-synthetic anthropogenic inputs in this study, but the addition of cellulosic fibres to marine environments should be investigated more extensively in future work.

5. CONCLUSIONS

This study determined microplastic concentrations in the water, zooplankton, and larval and juvenile herring in an environmentally and economically important region of BC's coastal waters. The average water concentration of microplastics (0.59 microplastic particles l^{-1}) was one order of magnitude lower than the estimated safe concentration threshold of 6.65 plastic particles l^{-1}, below which adverse effects are unlikely to occur for marine invertebrates, as reported by Everaert et al. (2018). No significant spatial differences were found through the region or vertically in the water column, but there is an indication of seasonal variation in microplastics that should be explored further. Microplastic consumption and accumulation was low in zooplankton and larval herring but relatively higher in juvenile herring. We suggest that juvenile herring were more likely to ingest microplastics due to the high ratio of microplastics to prey encountered in the size range of particles consumed by herring. Conversely, zooplankton (and to a lesser extent larval herring), which feed on smaller prey size classes, encountered orders of magnitude higher concentrations of biological particles relative to microplastics. These findings show that the size distribution of plastics vs. prey is an important consideration when evaluating microplastic exposure.

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