Title: *THE ABUNDANCE OF MICROPLASTICS IN CNIDARIA AND CTENOPHORA IN THE NORTH SEA*

Authors: Ria Devereux[1,3] Mark G. J. Hartl[1], Mike Bell[2] and Angela Capper[2,4]

1) Institute of Life & Earth Sciences, Centre for Marine Biodiversity & Biotechnology, Heriot-Watt University, Edinburgh EH14 4AS UK

2) International Centre for Island Technology (ICIT), Heriot-Watt University, Stromness, Orkney KW16 3AW UK

3) Sustainability Research Institute (SRI), University of East London, Docklands campus, 4-6 University Way, London E16 2RD UK

4) CQUniversity, Coastal Marine Ecosystems Research Centre (CMERC), Gladstone, QLD 4680 Australia

Abstract

Microplastic (MP) ingestion has been widely recorded in aquatic organisms, but few studies focus on cnidarians and ctenophores, which form a significant contribution to marine trophic interactions. Scyphozoans (*Cyanea capillata*, *C. lamarckii* and *Aurelia aurita*), hydrozoan (*Cosmetira pilosella*) and ctenophores (*Beroe cucumis* and *Pleurobrachia bachei*) collected opportunistically from Orkney, Shetland and the North Sea were thermally disintegrated, with a subsample of ingested plastics analysed using FTIR. A total of 1,986 MPs were counted (94% fibres), the majority (84.4%) in the four Cnidarian species. Highest MP concentrations were recorded in *B. cucumis* (0.956 ml^{-1}), whilst *C. pilosella* yielded the lowest (0.013 ml^{-1}). The main polymers in digestate were PET and PP, with 27% discounted as non-plastics. In feeding trials, *A. aurita* ingested a greater quantity of PET fibres (60-80%), compared to nylon (0%) and HDPE fibres (0%). This study demonstrates cnidarians and ctenophores, a largely overlooked group, are a potential route for MPs entry into food webs.

Highlights

- Ctenophore and cnidarian volume and microplastics were positively correlated.
- Microplastic fibre counts were significantly higher than microplastic particle counts per ml tissue
- Polyester terephthalate and polypropylene were the most commonly observed polymers.

Keywords: Microplastics, microfibres, microparticles, North Sea, jellyfish, Orkney, Shetland

1. Introduction

Concern for plastic contamination in marine ecosystems has been evident for some time (Mallory., 2008; Mascarenhas *et al*., 2004) and the ecological consequences of larger plastic debris is well publicised (Axelsson and van Sebille., 2017; Bucci *et al*., 2020; Tramoy *et al*., 2020; Woods *et al*., 2019). Our knowledge of the extent and impact of microplastic contamination lags somewhat behind, although it has greatly increased in the last decade (Bucci *et al*., 2020; Provencher *et al*., 2019). Microplastics (<5 mm) are produced by industrial processes and the breakdown of larger plastic debris (Cole *et al*., 2011). Plastic production has increased exponentially from 1950's levels of 1.5 million metric tons (MMT) to 359 MMT worldwide in 2018 (Plastics Europe., 2019). Their fate in the marine environment is determined by density, biofouling and further fragmentation to nanoplastics (<100 µm) that can disperse widely (Bond., 2018). Low-density microplastics can float or move through the water column, whilst high density or heavily biofouled microplastics may sink, potentially impacting sedentary or active benthos and demersal scavengers (Kooi *et al*., 2017). Microplastics may be incidentally ingested (consumption of prey already containing microplastics, filter-feeding or sifting sediment in benthic environments) (Brown *et al*.,2013) or mistakenly consumed as prey (e.g. zooplankton in the water column) (Cole *et al*.,2013; Hall *et al*.,2015). The extent of microplastic contamination across all levels of trophic food chains is becoming increasingly evident (Rouin *et al*., 2020; Pinheiro *et al*., 2020).

Cnidarians, including "jellyfish" (Scyphozoa and Hydrozoa), and ctenophores are exclusively found in aquatic environments and are predominantly predators. They form a significant contribution to marine trophic interactions in marine food webs, demonstrating a range of feeding mechanisms, e.g. filter feeding, suspension feeding, predation and absorption, and are consumed by many species, including jellyfish, bristle worms, crustaceans, fish and turtles (Alamaru *et al*., 2009; Ates., 2017; Bayha *et al*., 2012; Cardona *et al*., 2012; Hall *et al*., 2015). Cnidarians and ctenophores may ingest microplastics in the water column (Hall *et al*., 2015; lliff *et al*., 2020; Macali *et al*., 2018), with a few studies demonstrating uptake in the laboratory (Costa *et al*., 2020; Sucharitakul *et al*., 2020). However, the potential for cnidarian and ctenophore trophic transfer of microplastics has received little attention, considering how important they are to most marine food webs. As a burgeoning fishery in >15 countries, they are also increasingly consumed by humans (Brotz *et al*., 2016; Richardson *et al*., 2009).

This study aimed to identify microplastic ingestion in a range of cnidarians and ctenophores. This was achieved by: (1) quantifying microplastic ingestion in six species of cnidarians and ctenophores; (2) examining spatial variation in microplastic loading in pelagically-sourced (using nets and plankton tows) and beach-sourced (washed ashore) cnidarians and ctenophores; (3) confirmation of polymer-types of microplastics ingested using Fourier Transform Infra-Red (FTIR) spectroscopy; and (4) a laboratory feeding experiment to investigate microplastic ingestion in cnidarians in a controlled environment. The findings of this study will help determine the extent of microplastic ingestion and potential for trophic transfer of microplastics through marine food webs and may have important implications for human health associated with the consumption of jellyfish-related products.

2. Methods

2.1 Study area

Three collection locations were chosen for this study to encompass areas of dense vs. sparse human population. The North Sea is bordered by six European countries with >185 million people living in the catchment area and is subjected to riverine inputs from heavily industrialised areas with a significant potential for microplastic contamination (Leslie., 2004). Pelagic samples were opportunistically collected from 23 sites from the northeast of Scotland to the northeast coast of England (Fig 1, Supplementary Table 1) and by 'By the Ocean we Unite', a Dutch NGO that traverses the globe on sailing expeditions with the goal of documenting and reducing marine plastic litter[1]. The Orkney Islands are a remote Archipelago to the north of Scotland, made up of 70 islands with a sparse population of 22,000 (Harris, 2018). The largest island, known as the "mainland", has two major settlements: Kirkwall (8,000 people) and Stromness (2,000 people) (Orkney Government., 2020). Samples of individual organisms were collected from 14 sites around Orkney mainland: 7 sites in Scapa Flow; 4 sites in west Orkney; 3 sites in north Orkney and 5 sites in the vicinity of the island of Rousey, adjacent to Orkney mainland (Fig.1). Scapa Flow, the body of water that runs along the southern edge of Orkney mainland, acts as a funnel producing complex tidal streams at the entrance with strong currents and eddies which form in sheltered areas. These strong currents have the potential to disperse and deposit microplastics vertically and horizontally. Shetland (4 sites) is an archipelago that is 80 km northeast of Orkney with a population of 22,990 (Fig. 1). Its capital, Lerwick (7,500 people), is the main port, and its harbour supports the offshore oil and fishing industries.

Throughout this paper, sample "site" is defined as the geographical location of sampling in one of the three collection areas (North Sea, Orkney, Shetland), whereas "mode of collection" refers to the method of collection at a given site, i.e. cnidarians sourced from the water column by boat using plankton tows (pelagically-sourced), or use of hand-held nets from land-based infrastructure such as jetties (shoreline-sourced), or picked up by hand from the beach (beach-sourced).

[1] https://www.bytheoceanweunite.org [date of last access: 30 July 2021]

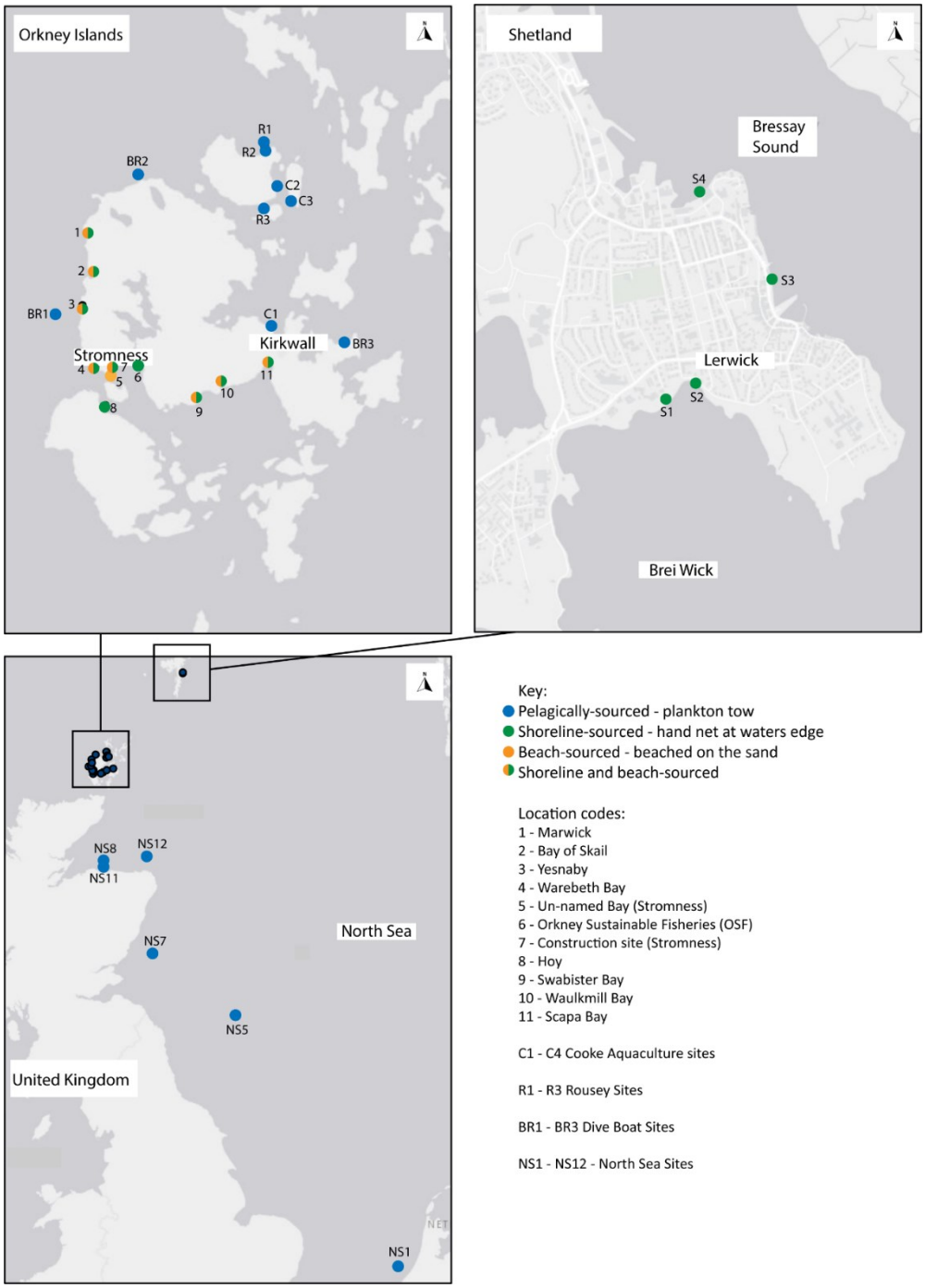

Fig.1 Boat-based and land-based collection sites of cnidaria and ctenophores in the Orkney archipelago, Shetland and the North Sea off the eastern coast of the UK

2.2.1 Shoreline-sourced samples

All specimens were collected between June and July 2017. Where practical, either plankton tows or a hand net was used to collect the specimens from the shoreline (Supplementary Table 1).

The hand net was used to scoop specimens out of the water in Orkney (Stromness un-named bay, Stromness construction site, Orkney Sustainable Fisheries (OSF), Warebeth Bay, Swanbister, Marwick, Hoy and Shetland (Fig. 1). All samples were transferred to International Centre for Island Technology (ICIT), Heriot-Watt (HWU) Orkney campus for storage (-20^{o}C) and processing.

2.2.2 Pelagically-sourced samples

Specimens collected using a boat (Birsay, Birsay-Rousay and Cooke Aquaculture (Fig.1) were collected with plankton tows (212 µm mesh size) which were carried out for 2 minutes and repeated three times at each site.

Any relevant organisms collected with tows or opportunistically in hand-held nets were transferred to containers (50 ml for hydroids and ctenophores; 5 L buckets for larger cnidarians). One species, *P. bachei*, was opportunistically sourced from salmon farm enclosures at a Cooke Aquaculture site in Rousey (Orkney, Fig. 1), following an extensive bloom in the area. Samples were transferred and stored as above.

North Sea samples were opportunistically collected by 'By the Ocean We Unite', using a 0.5 mm trawl net with a transparent mesh, yielding cnidarians (Cyanea *lamarckii*, *Aurelia aurita,* moon jellyfish) and ctenophores (*Pleurobrachia bachei,* sea gooseberry). Tows lasted 30 minutes, the cod-end was removed, and contents passed through a 1 mm wire drum sieve. The retained material was transferred to HDPE containers. These were transferred to the Scottish government marine laboratory in Aberdeen (MarLab, Fisheries Research Services, Aberdeen Marine Laboratory), where they were frozen (-20^{o}C), then transported to Heriot-Watt University Edinburgh campus.

2.2.3. Beach-sourced samples

Beached cnidarians were opportunistically collected between Oct 2016 and May 2017 at two sites on mainland Orkney, Scapa Bay (2 *C. capillata)* and Waulkmill Bay (4 *C. capillata*) (Fig. 1). Samples were placed in individual Ziploc bags (pre-rinsed with distilled water) and returned to the ICIT, HWU and frozen at -20^{o}C, then transported to Edinburgh HWU campus and stored at -20°C until further processing. Further sample collections were made between June and August 2017, samples (22 cnidarians and ctenophores) were collected at 8 additional beach locations on Orkney: Orkney Sustainable Fisheries (OSF), Marwick, Scapa Bay, Waulkmill Bay, Construction site, Stromness unknown Bay, Hoy, Swanbister. Seven of these beaches were located within Scapa Flow (Fig. 1). Samples were frozen and stored as above.

2.2.4 Water samples

Water samples were collected between June and July 2017 at seven sites in Scapa Flow (Stromness caravan site, Stromness construction site, Stromness Orkney Sustainable Fisheries (OSF), Scapa Bay, Waulkmill Bay, Swanbister Bay, Warebeth Bay); two sites on the west side of Orkney (Yesnaby and Bay of Skail); one site in Hoy (Hoy-Orkney ferry terminal); and three sites in Lerwick, Shetland (Fig. 1). Water samples were obtained from sites where cnidarian and ctenophore samples were collected (except North Sea sites) using pre-rinsed 50 ml centrifuge tubes (3 per site). The centrifuge tubes were submerged into the water by hand to collect the surface layer of water. These were stored at HWU at 4^{o}C until processed.

2.3 Digestion and filtering

At the time this research was carried out (2017), no methods had been published on microplastic extraction from cnidarians or ctenophores. Cnidarian and ctenophore samples were washed thoroughly using distilled water to remove any potential microplastics adhered to the outside of the organism. Tentacles were removed to make sure only ingested microplastic in the gastrovascular cavity were counted and not plastic caught in the oral arms or tentacles (Classens *et al*., 2013; Iliff *et al*., 2020). The remaining tissue was transferred to glass beakers, heated to 40^{o}C and agitated (plate mixer Model US152D with magnetic stirrers) until liquefied (time taken was dependent on specimen size, but ranged from 3 minutes to 2 hours). This method was used for all specimens except *A. aurita* (moon jellyfish*),* which liquified when defrosted so did not require further processing. Once liquefied, sample volume was recorded, 30 ml distilled water was added to cool the liquefied suspension to prevent disintegration of the filter paper (Whatman filters pore size 2 µm), then the sample was filtered using a vacuum pump. Forensic style laboratory procedures (Blumenröder *et al*., 2017) were utilised throughout all laboratory sample processing to avoid cross-contamination: surfaces cleaned daily, glassware washed in a dishwasher and rinsed with distilled water prior to use; cotton lab coats were worn, digestion carried out in a fume hood, and samples covered at all times when not being processed. Filter papers dampened with distilled water were placed in Petri dishes on the work surface and exposed to the air during processing to account for any atmospheric fall out.

2.4 Laboratory feeding trials

To determine ingestion of microplastics (MPs) of varying densities by cnidarians in a controlled environment, juvenile *A. aurita* (n=25) 5-6 cm in diameter were acquired from the London Aquarium. Five *A. aurita* from London aquarium were tested for background MPs. No microplastics were observed in digestates suggesting no background levels of MP contamination were present. The 20 individuals used for the feeding trial were separated into 300 ml beakers (with 250-260 ml of water so the water neared the top of the beaker once one individual jellyfish and an air stone was placed inside). The 20 individual *A. aurita* were placed into groups of five which yielded three treatment groups and one control group each consisting of five individuals. Each treatment group was exposed to one type of microplastic: (1) moulded plastic particle suspensions - polyester terephthalate (MPS-PET) (5

MPS-PET particles per beaker); (2) recycled homopolymer PET (5 PET particles per beaker); and (3) nylon fibres (5 plastic fibres per beaker). The microplastics were analysed with FTIR to check polymer composition prior to use in the feeding trial.

A control group was fed ~2 ml live red plankton (n=10) or enough until their stomachs became red indicating that they were sated, whilst treatment individuals were fed 5 pieces of plastic each day for the course of the trial. Seawater was aerated (by air stone) and maintained at 17^{o}C. Treatment and control individuals were not removed from their beakers at any point during the experiment and no water changes took place. The test was terminated after 5 days and *A. aurita* were digested and filtered to determine microplastic ingestion.

2.5 Cross-contamination controls

To determine potential cross-contamination, specimen holding containers, plastic bags and glass test tubes were rinsed three times with distilled water, which was then filtered and observed under the microscope. Plankton nets (mesh and rope) were also rinsed with distilled water to check for cross-contamination after use and analysed under the microscope (Supplementary Table 2). Dampened filter papers from laboratory worktops exposed to the atmosphere when samples were exposed were analysed daily and whilst no plastic particles were recovered, fibres were only observed between 15th -19th June and 21st -30th July 2017, when multiple users were present in the lab (Supplementary Table 2).

2.6 Classifying microplastics

All MPs observed were classified in categories: fibre (referred to as microfibres (MPF) from here on in); and flake, pellet, fragment and 'other' (referred to as microplastic particles (MPP) from here on in). Pellets refer to cylindrical/spherical particles, whereas fibres are the same thickness throughout; although the length may vary and sometimes fraying can be seen. Fragments have a hard, sometimes straight or relatively straight sharp edge, and flakes refer to very thin particles. These were further distinguished by colour (Fig. 2). When both the number of microfibres and particles are quantified together this is referred to as microplastics total (MPT). 'The Guide for Microplastic Identification' by the Marine and Environmental Research Institute (2020) (Centre for Environmental Studies., 2020) was used to determine the type of microplastic. Samples were corrected for cross-contamination in the laboratory by comparing with daily counts on dampened filter papers held open to record atmospheric deposition. When *x* fibres of a specific colour were observed on dampened filter papers, the same number (*x*) were subtracted from sample counts (Supplementary Table 2).

The polymer type of a representative cross-section of plastic items (n=36) were identified using Fourier-Transformed Infrared Spectroscopy (FTIR). Attenuated Total Reflection (ATR) (Bruker Alpha fitted with a platinum ATR module and the software Opus 8.2) was used for pieces of microplastic that were too large or not transparent enough for the micro FTIR.

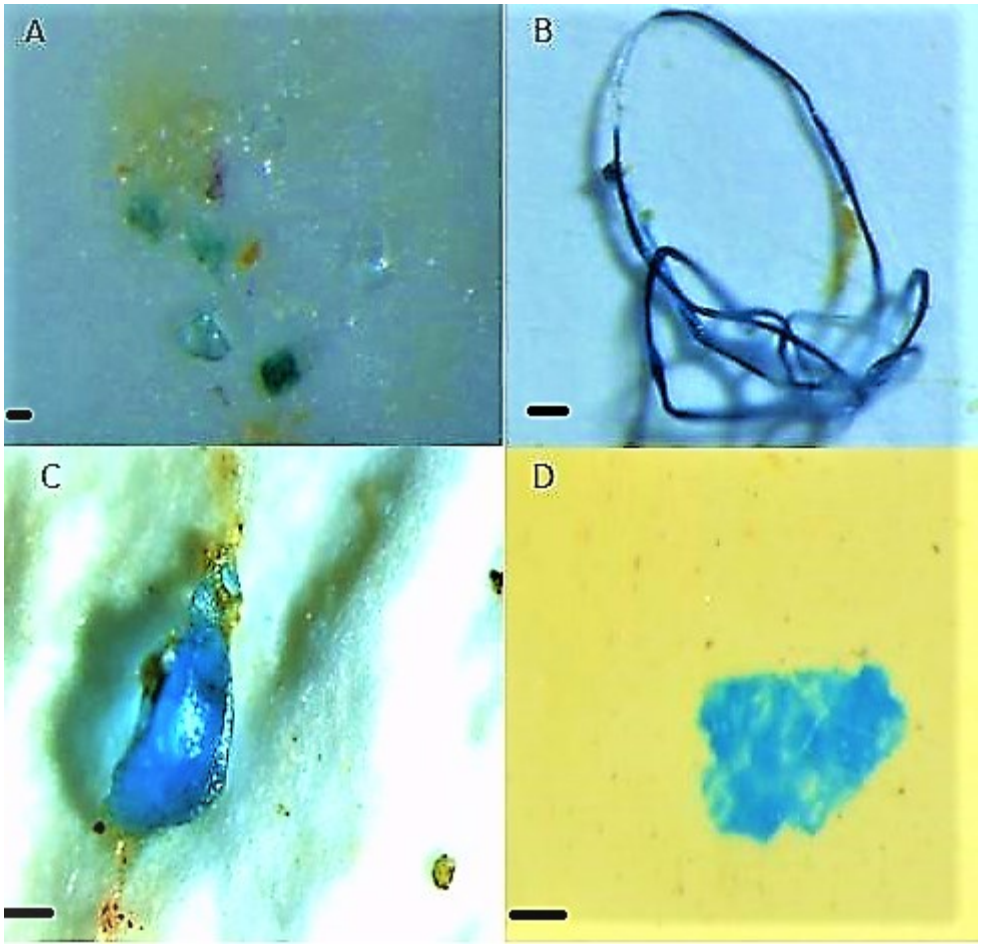

Fig.2 Microplastics classified into: (A) pellets; (B) fibres; (C) fragments; and (D) flakes. Scale bar for (A) and (D) is 50 µm, scale bar for (B) and (C) is 100 µm

2.7 Data analysis

Statistical analysis was undertaken using the R statistical package (R Core Team, 2021). Data were standardised to MPs per ml of digestate (ml-1) for tissue samples, and MPs ml-1 of water for water samples (based on 50 ml of water collected per replicate and summed over replicates). Whenever MP quantities are stated as either MPF, MPP or MPT they are the mean of the individuals unless otherwise specified. Prior to analysis, standardised MP values for both tissue and water samples were fourth-root transformed for normality of residuals and homogeneity of variances. Analysis of variance (ANOVA) was used to compare standardised microplastic concentrations between sites and mode of capture within species. ANOVA models were simplified by removal of non-significant ($P > 0.05$) interactions between site and mode of capture. Post-hoc Tukey HSD tests (e.g. Lepˇs and ˇSmilauer, 2020, pp. 113-114) were used to test for differences between individual sites. In the case of significant differences in MPs between sites, concentration of MPs in water was considered as a simplification of the site effect in the ANOVA model. All non-significant terms were removed from the base (site) model and omitted also from the simplified (water) model. Model comparisons showing a significant change in residual mean square were interpreted

as water MPs not accounting for between-site differences in tissue MPs. Model simplification was only possible where water samples were available for more than two sites at which a species had been collected. Comparison of standardised MPs between water samples was similarly carried out by ANOVA, considering differences between regions (Hoy, rest of Orkney and Shetland) and between shoreline and pelagic samples. Owing to the sampling design, these two factors were confounded, but each was considered on its own as a possible explanation of differences among samples.

3. Results

A total of 1,986 microplastics (MPs) were recorded (1,640 in 120 individual cnidarians and 346 in 353 individual ctenophores) (Table 1). From these 1,986 MPs, the majority (84.4%) were observed in the cnidarian species with a large number (1259) classified as fibres, of which 94% were black and blue in colour. Comparisons between cnidarian species demonstrated that microplastic fibres (MPF) were dominant in all individuals sampled (using a percentage of the total MP counted for each cnidarian species): *C. capillata* (*n* = 55, where n refers to the number of individuals sampled) 88% MPF; *C. lamarckii* (*n* = 34) 84% MPF; *A. aurita* (*n* = 12) 97% MPF; hydroid *Cosmetira pilosella* (*n* = 16) 98% MPF. Fibres were again predominant in ctenophores as a percentage of the total count: *Pleurobrachia bachei* (*n* = 46) 84% MPF and *Beroe cucumis* (*n* = 7) 92% MPF.

Table 1. Location of cnidarian and ctenophore samples and quantity of microplastics found within each group normalised to microplastics per ml of tissue

Phyla	**Class**	**Species**	**Location**	**Number collected**	**MPF[1] Average pieces per ml (± StdDev)**	**MPP[2] Average pieces per ml (± StdDev)**
Cnidaria	*Scyphozoa*	*Cyanea capillata*	Hoy	18	0.241 (0.19)	0.04 (0.05)
			Orkney	37	0.118 (0.11)	0.016 (0.04)
		Cyanea lamarckii	Hoy	12	0.142 (0.08)	0.04 (0.11)
			Orkney	16	0.005 (0.61)	0.04 (0.4)

			Aberdeen	6	0.23 (0.13)	0.044 (0.06)
		Aurelia aurita	Orkney	4	0.139 (0.16)	0.007 (0.01)
			Aberdeen	8	0.085 (0.07)	0 (0)
	Hydrozoa	*Cosmetira pilosella*	Orkney	12	0.013 (0.013)	0.001 (0.001)
			Hoy	1	0.005 (0)	0 (0)
			Shetland	3	0.018 (0.008)	0 (0)
Ctenophora	*Nuda*	*Beroe cucumis*	Orkney	7	0.854 (0.76)	0.102 (0.11)
	Tentaculata	*Pleurobrachia bachei*	Orkney	250-333*	0.442 (1.47)	0.038 (0.05)
			Aberdeen	12	0.244 (0.61)	0 (0)
			Hoy	1	0.031 (0)	0 (0)

[1] MPF-Total microfibres

[2]MPP-Total microplastic other than fibres found, i.e. particles, fragments

*Orkney *P. bachei* (sea gooseberries) collected in 50 ml vials began to disintegrate upon return to Heriot-Watt University therefore, an estimate is provided of numbers based on the smallest, mid-size and largest *P. bachei* in each vial.

3.1 Cnidaria

3.1.1 *Cyanea capillata*

MPFs were the predominant plastic observed (88% fibres) in *C. capillata*. The majority were black (59%) and blue (19%) in colouration. MP total counts (MPF + MPP = MPT) were significantly higher beach-sourced individuals (537 MPT in 16 individuals) compared to shoreline-sourced using nets (415 MPT in 32 individuals). When the data was normalised to MPs per ml of tissue digestate (ml-1 tissue digestate), the mean for beached individuals was 0.150 MPT ml-1 compared to shoreline collected individuals with a mean of 0.219 MPT ml-1 (Fig. 3A and B). The lowest mean MP concentration (0.024 MPT ml- 1 of tissue digestate) was observed in Orkney at the southern site of Waulkmill Bay from beach-sourced individuals (site comparisons outside of Orkney were not possible as this species was not found in North Sea or Shetland sites) (Fig. 3A). MPT differed significantly among sites (ANOVA, $F_{7,39} = 3.43$, $P = 0.006$), but not according to mode of capture (ANOVA, $F_{1,39} = 0.156$, $P = 0.694$). Post-hoc comparisons between sites showed no strong pattern, indicating that only the distinction between Hoy and Waulkmill Bay was significant (Tukey HSD, $P = 0.040$). Replacement of site effect by MPT in water results in significant loss of explanatory power (ANOVA, $F_{4,37} = 5.71$, $P = 0.001$), thus MPT concentration in water does not account for between site differences (Fig.3.F)

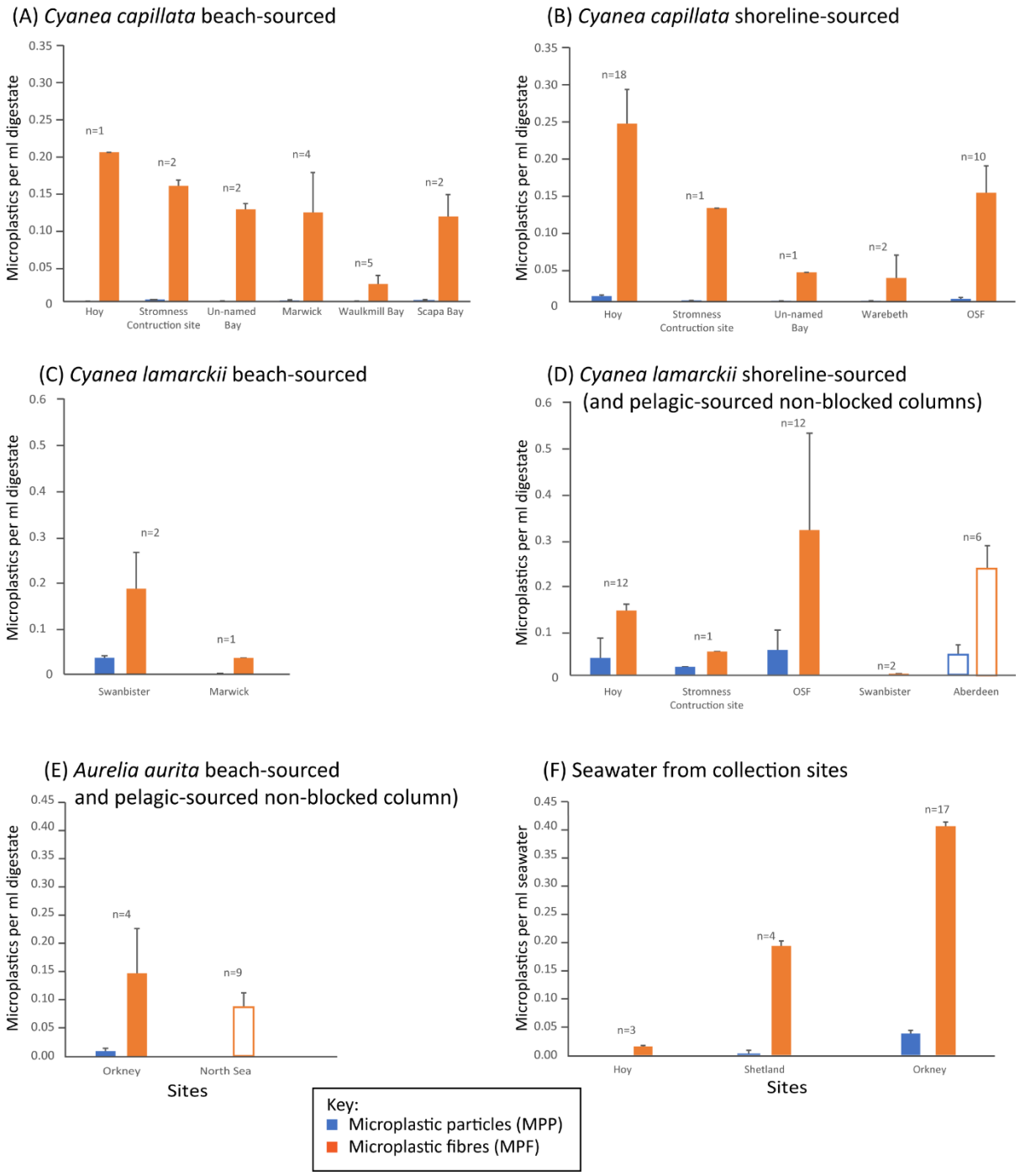

Fig. 3. Mean microplastic fibres (MPF) and particles (MPP) per individual per ml digestate in (A) *Cyanea capillata* beach-sourced from Orkney sites; (B) *Cyanea capillata* shoreline-sourced from Orkney sites; (C) *Cyanea lamarckii* beach-sourced from Orkney sites; (D) *Cyanea lamarckii* shoreline-sourced from Orkney sites and pelagic-sourced from North Sea sites; (E) *Aurelia aurita* beach-sourced from Orkney sites and pelagic-sourced from North Sea sites; and mean MPF and MPP in (F) water samples (50 ml) from Hoy, Orkney and Shetland. For (A-E) n = the number of individuals sampled. For (F) n = number of sites water was collected from.

MPP (ANOVA, F7,39 = 2.52, *P* = 0.031) and MPF (ANOVA, F7,39 = 3.16, *P* = 0.0096) differed significantly among sites but not according to mode of capture in either MPP (ANOVA, F1,39 = 0.305, *P* = 0.584) or MPF (ANOVA, F1,39 = 0.202, *P* = 0.656). No clear pattern between site differences was seen in post-hoc tests for either MPF or MPP. Replacement of site effect by MPP in water results in significant loss of explanatory power (ANOVA, F4,37 = 2.81, *P* = 0.039), thus MPP concentration in water does not account for between site differences. The same was also observed for MPF in water (ANOVA, F4,37 = 4.60, *P* = 0.004) (Fig. 3F). There appeared to be no correlation between volume of digested tissue and the MP concentration in the digestate, the opposite of what was expected: the larger the tissue mass, the higher the MPs count (R2 = 0.002, Fig. 4A).

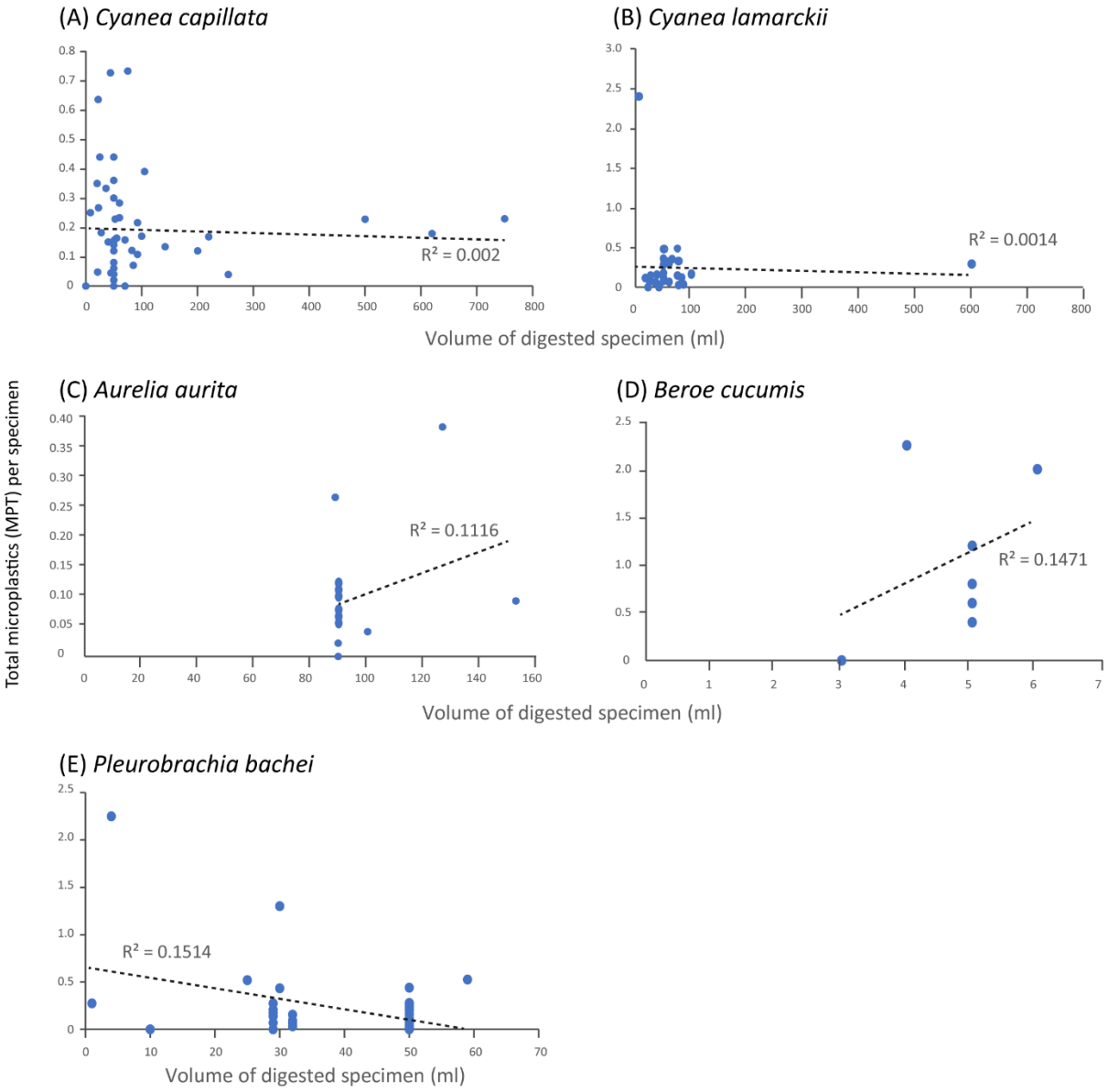

Fig. 4. Total quantity of microplastics (MPT) in digested tissue samples of: (A) *Cyanea capillata*; (B) *Cyanea lamarckii*; (C) *Aurelia aurita*; *(*D) *Beroe Cucumis* and; (*E) Pleurobrachia bachei as* a function of total homogenate volume (ml).

3.1.2 *Cyanea lamarckii*

In 34 individual *Cyanea lamarckii,* a total of 501 MPs were counted (MPT). When split into MPF vs. MPP, this species had the second highest MPF count (84% fibres). The majority of MPFs were black (49.1%) and blue (23.4%) in colouration.

Sustainable Fisheries on the south coast of Orkney, in Stromness (Fig. 3D). Larger (100-600 ml) beach-sourced *C. lamarckii* had greater MPT ml-1 tissue digestate (3 individuals with 0.52 MPT ml-1 tissue digestate) than smaller pelagically sourced *C. lamarckii* (75-100 ml, 7 individuals, 0.28 MPT ml-1 tissue digestate) and shoreline-sourced *C. lamarckii* (5-65 ml, 28 individuals, 0.22 MPT ml-1 tissue digestate) (Fig. 3C and D). MPT ml-1 tissue digestate was significantly different between modes of capture (ANOVA, $F_{1,28} = 10.4$, $P = 0.003$) and significantly different among sites (ANOVA, $F_{4,28} = 4.038$, $P = 0.010$). Post-hoc comparisons did not identify a significant pattern for either factor. Replacement of site effect by MPT ml-1 tissue digestate in water results in significant loss of explanatory power (ANOVA, $F_{3,37} = 4.42$, $P = 0.014$), thus MPT concentration in water does not account for between site differences (Fig. 3F).

A significant difference was observed in MPF ml-1 tissue digestate and between modes of capture (ANOVA, $F_{1,28} = 6.92$, $P = 0.014$, Fig. 3C and D) with pelagically-sourced yielding 0.23 MPF ml-1 tissue digestate whilst shoreline-sourced and beach sourced were 0.2 and 0.014 MPF ml- 1 tissue digestate respectively (Table 2). However, it was not significantly different among sites (ANOVA, $F_{4,28} = 2.41$, $P = 0.073$), and post-hoc comparisons did not identify a significant pattern. MPP ml-1 tissue digestate on the other hand was not significantly different according to mode of capture (ANOVA, $F_{1,28} = 2.63$, $P = 0.116$) or site (ANOVA, $F_{4,28} = 0.927$, $P = 0.462$) or (Fig. 3C and D, Table 2).

Similar to *C. capillata*, MPT ml-1 tissue digestate showed no correlation ($R^2 = 0.0014$).

Table 2. A comparison of microplastics observed in cnidarian and ctenophore species per ml of tissue digestate based on mode of capture: in pelagically-caught (by boat using nets); opportunistically beach-sourced (washed up on the beach); shoreline-sourced (caught using nets from man-made structures on land).

Species	Source	Sampled	Average MPF[1] (± StdDev)	Average MPP[2] (± StdDev)
Cyanea capillata	Beach-sourced	16 Scapa Bay = 2, Waulkmill Bay = 5, Marwick = 4, Stromness Construction site = 1, Stromness Unknown Bay = 1, Hoy = 1,	0.127 (0.11)	0.02 (0.002)
	Shoreline-sourced	32 Stromness Unknown Bay = 1, Stromness Construction Site = 1, Orkney Sustainable Fisheries = 10, Warebeth Bay = 2, Hoy = 18	0.175 (0.18)	0.005 (0.06)
Cyanea lamarckii	Beach-sourced	3 Swanbister Bay = 2, Marwick = 1	0.014 (0.12)	0.024 (0.02)
	Pelagically-sourced	6 North Sea = 6	0.23 (0.13)	0.044 (0.06)
	Shoreline-sourced	25 Stromness Construction Site = 1, Orkney Sustainable Fisheries = 9, Swanbister Bay = 2, Marwick = 1, Hoy = 12	0.2 (0.36)	0.041 (0.08)
Aurelia aurita	Beach-sourced	4 Marwick = 2, Orkney Sustainable Fisheries = 2	0.139 (0.16)	0.007 (0.01)
	Pelagically-sourced	8 North Sea = 8	0.09 (0.07)	0 (0)
Cosmetira pilosella	Beach-sourced	7 Scapa Bay = 7	0.006 (0.005)	0.001 (0.002)
	Shoreline-sourced	9 Shetland = 3, Hoy = 1, Orkney Sustainable Fisheries = 5	0.021 (0.01)	0 (0)
Pleurobrachia bachei	Pelagically-sourced	41 Aberdeen = 12, Birsay = 16, Birsay-Rousay = 13	0.147 (1.5)	0.034 (0.05)
	Hand netted in fish farm cage (Pelagically-sourced)	250-300 Cooke Aquaculture 250-300	0.241 (0.11)	0.035 (0.04)
	Shoreline-sourced	5 Hoy = 1, Orkney Sustainable Fisheries = 4	0.075 (0.05)	0 (0)
Beroe cucumis	Shoreline-sourced	7 Orkney Sustainable Fisheries = 7	0.854 (0.76)	0.102 (0.11)

[1] MPF-Total microfibers per ml

[2]MPP-Total microplastic other than fibres found, i.e. particles, fragments per ml

3.1.3. Aurelia aurita

In *A. aurita*, MP counts were dominated by MPF (97% fibres). North Sea *A. aurita* yielded 43% blue and 35% black fibres, while Orkney samples yielded 49% black and 20% blue. No MPPs were observed in *A. aurita* from the same North Sea samples. There were no significant differences between MPT ml-1 between North Sea and Orkney sites (ANOVA, $F_{1,11} = 0.632$, $P = 0.443$) (Fig. 3E). The highest count of MPF ml-1 tissue digestate was observed at Orkney Sustainable Fisheries (OSF) (0.23 ml-1 tissue digestate) (Table 2). However, the mean across 4 sites in Orkney was 0.14 MFP ml-1 tissue digestate (Table 1). Whilst North Sea samples of *A. aurita* had a lower mean MPF ml-1 tissue digestate than Orkney (0.085 ml-1 tissue digestate) (Fig. 3E, Table 1). Orkney beach-sourced individuals yielded 0.139 MPF ml-1 tissue digestate compared to pelagically-sourced specimens with MPF of 0.09 ml-1 tissue digestate (Table 2). However, there were no significant differences in MPF ml-1 tissue digestate (ANOVA, $F_{1,11} = 0.505$, $P = 0.492$) (Fig. 3E). As MPPs were only observed in Orkney and not in the North Sea, *A. aurita* statistical testing was not relevant.

It was not possible to test for differences by both site and mode of capture as specimens were either beach-sourced (Orkney) at sites or pelagically-sourced (North Sea) but not both. Also due to a lack of water samples taken from North Sea sites there were no effects of water MPs considered because only two water samples were collected both from Orkney. There was no correlation between specimen volume and MPT ml-1 ($R2 = 0.1116$; Fig. 4C).

3.1.4. Cosmetira pilosella

MPF were the dominant microplastic form counted in *C. pilosella* (93% fibres). At Hoy 100% of MPF were blue, whereas Orkney samples consisted of 54% blue and 34% black MPF, and MPFs in Shetland samples were 55% blue and 36% black. As only one mode of capture was utilised for *C. pilosella*, no comparisons could be made between mode of capture and site. No significant difference was observed between site and MPT ml-1 tissue digestate (ANOVA, $F_{3,13} = 2.92$, $P = 0.074$), MPP ml-1 tissue digestate (ANOVA, $F_{3,13} = 0.328$, $P = 0.805$) or MPF ml-1 tissue digestate (ANOVA, $F_{3,13} = 3.19$, $P = 0.059$). Shoreline-sourced *C. pilosella* had a mean of 0.021 MPF ml-1 tissue digestate compared to mean of 0.006 MPF ml-1 tissue digestate in beach-sourced specimens (Table 2). No correlations could be made between specimen volume and MPT ml-1 as all specimens were estimated to be of equal size.

3.1.5 *Beroe cucumis*

Whilst seven *B. cucumis* specimens were opportunistically sourced from one site at OSF Stromness (shoreline-sourced) only one specimen was sourced from Hoy.

B. cucumis digestate contained mainly MPF (97% fibres), 42% of which were black and 33% blue. Out of the eight specimens, only one contained no MPs. Compared with all the other

shoreline-sourced species, *B. cucumis* had the highest MPT concentration of 0.956 ml-1 tissue digestate (Table 2). Due to all specimens being shoreline-sourced, only interactions between sites could be investigated for this species. There was no significant difference between sites of MPT ml-1 tissue digestate (ANOVA, F1,6 = 0.0276, *P* = 0.873), MPP ml-1 tissue digestate (ANOVA, F1,6 = 0.982, *P* = 0.360) or MPF ml-1 tissue digestate (ANOVA, F1,6 where *B. cucumis* were sampled, both in Orkney, no effects of water MPs were considered. There was also no correlation between specimen volume and MPT ml-1 (R2 = 0.1471) (Fig. 4D).

3.1.6. Pleurobrachia bachei

In samples from the North Sea (Aberdeen) and Hoy, *Pleurobrachia bachei* yielded only MPF in tissue digestate. Taken as a percentage of the total MPs for this species, 42% were black and 33% blue, and 100% blue, respectively. In Orkney, fibres were again dominant accounting for 81% of the total count, of which 36% were blue and 32% black.

P. bachei were both pelagically-sourced (41 individuals) and shoreline-sourced (255-305 individuals), as a result mode of capture was not relevant. Of the latter, approx. 300 individuals were sourced from black-netted cages during a bloom in a salmon fish farm. Black fibres (potentially from nets) were not higher in individuals from this site than any other colours nor were black fibre counts in tissue higher at this site than any other sites sampled.

No MPP were observed in shoreline-sourced *P. bachei* at OSF and Hoy, whereas a total of 51 MPP were observed in pelagically-sourced individuals (at sites:C1-C4 Cooke Aquaculture Sites, R1-R3 Rousay Sites and BR1-BR3 Dive Boat Sites, Fig. 1). This resulted in significant differences of MPP ml-1 tissue digestate among sites (ANOVA, F5,36 = 7.70, *P* < 0.0001). Post-hoc test separates Rousay samples from OSF and North Sea samples, with Cooke, Birsay and Hoy overlapping these two groups. Replacement of site effect by MPP in water results in non-significant loss of explanatory power (ANOVA, F3,24 = 2.43, *P* = 0.090), thus providing (weak) evidence that MPP concentration in water does account for between site differences (Fig. 3F).

Pelagically-sourced individuals had a higher MPF (0.147 ml-1 tissue digestate) than shoreline-sourced individuals (0.075 ml-1 tissue digestate, Table 2). However, there was no significant difference between sites and MPF ml-1 tissue digestate (ANOVA, F5,36 = 0.370, *P* = 0.866) or MPT ml-1 tissue digestate (ANOVA, F5,36 = 0.657, *P* = 0.658).

There was no correlation between specimen volume and MPT ml-1 (R2 = 0.1514).

3.2 Water samples

In total 1,495 pieces of plastic were counted in water samples from Hoy, Orkney and Shetland. MPF represented 93% of all plastic found. In water samples from Orkney, the most common were black (50%) and blue (23%), Shetland were blue (64%) and black (22%), whilst Hoy were blue (52%) and red (22%). Other MPF colours found at Hoy, Marwick, Rousay and sites 1-6 in the North Sea included white, green, purple, pink, yellow and orange.

All water samples, regardless of mode of capture (shoreline or pelagically-sourced) were collected in the same manner (i.e. 50 ml centrifuge tubes). Even though overall, water samples from Shetland had the highest mean MPT at 0.583 ml-1, whereas Orkney samples had 0.408 MPT ml-1 and Hoy the least at 0.06 MPT ml-1, there were no significant difference among sites (ANOVA, $F_{2,21} = 2.53$, $P = 0.104$) or between shoreline and pelagic samples (ANOVA, $F_{1,22} = 0.633$, $P = 0.435$).

No significant difference was observed in MPF ml-1 counts in water samples between shoreline and pelagic samples (ANOVA, $F_{1,22} = 0.349$, $P = 0.561$) or when comparing Shetland, Hoy and other Orkney Sites (ANOVA, $F_{2,21} = 2.16$, $P = 0.140$).

Water samples collected from the shorelines had fewer MPF ml-1 and, with the exception of four sites in Orkney (Cooke Aquaculture site 1 (C1), Cooke Aquaculture site 3 (C3), Dive Boat Birsay (B2), Dive Boat Birsay-Rousay (BR2)), had more MPP than pelagically sourced samples.

When comparing MPP ml-1 at sites in Shetland, Hoy and Orkney a significant difference was observed among sites (ANOVA, $F_{2,21} = 5.36$, $P = 0.013$). This was largely accounted for by the difference between shoreline and pelagic samples (ANOVA, $F_{1,22} = 20.5$, $P = 0.0002$). A post-hoc test indicated Hoy as significantly different from rest of Orkney, with Shetland intermediate between these two.

3.3 Feeding trial results

Of the three types of plastic fibres (polyester terephthalate (MPS-PET); nylon and recycled homopolymer) offered to *Aurelia aurita* (five individuals per treatment type) in feeding trials, only MPS-PET was recovered from *A. aurita* tissues (3-4 pieces per individual equating to 0.04 MPT ml^{-1}), demonstrating a 60-80% ingestion rate. In the ten individuals from the control group one black high-density polyethylene (HDPE) fibre was recovered (likely from cross-contamination).

3.4 FTIR

Fourier Transform Infra-Red (FTIR) spectroscopy was used to identify plastics recovered from water, cnidarian and ctenophore samples. PET was the most common polymer observed. Over 50% of these were found in *C. capillata* collected from the OSF site in Stromness as well as in one water sample from the same site. The remaining 50% were found in sites in Shetland and Waulkmill Bay. However, FTIR analysis also highlighted the issue related to plastic identification by observation alone which is subject to human error as some of the 36 particles initially identified as plastic were later verified as non-plastics (27%) (Table 3).

Table 3 Fourier Transform Infra-Red (FTIR) spectroscopy results for a representative sample of microplastics in cnidarians, ctenophores and water samples.

Location	Sample	FTIR result
Scapa Bay	*Cyanea capillata*	Polyethylene
Hoy	*Cyanea capillata*	Polypropylene
	Aurelia aurita	Polyethylene
OSF Stromness	Water sample	Alkyd resin
		Not plastic
		Not plastic
OSF	*Cyanea capillata*	Alkyd resin
		Polyester terephthalate
		Polystyrene vinylidene chloride
		Polyetherimide
		Polyester terephthalate
		Polyethylene propylene
		Polyacrylonitrile
		Polyvinyl chloride
		Polycarbonate
		Polycarbonate
		Alkyd resin
		Alkyd resin
	Water sample	Polyester terephthalate
	Aurelia aurita	Polyester terephthalate
Waulkmill bay	*Cyanea Capillata*	Polyester, tere-iso-phthalate
Sampling site 3	*Cosmetira pilosella*	Not plastic
		Not plastic
		Not plastic
Sampling site 5	*Cosmetira pilosella*	Cascamite 14 powdered resin
		Cascamite 14 powdered resin
		Not plastic
		Not plastic
		Not plastic
Shetland 1	*Cosmetira pilosella*	Polyethylene terephthalate
	Water sample	Not plastic
Shetland 4	*Cosmetira pilosella*	Not plastic
	Water sample	Polyethylene terephthalate
Warebeth	*Cyanea capillata*	Polypropylene
		Polypropylene
		Polypropylene

4. Discussion

One might expect that the larger the species of cnidarian or ctenophore, the greater the number of microplastics (MPs) likely to be consumed. However, MP abundance between species is more

10

comparable when normalising to MPs per ml tissue digestate (Rochman et al., 2015; Naidoo et al., 2020; Zhang et al., 2019). Once normalised per unit tissue digestate, relatively more MPs would be expected to be ingested when smaller species occur in substantial blooms, such as *B. cucumis* or *P. bachei* (Jaikumar et al., 2019; Vesela and Vijverberg, 2006). It has been suggested that larger species with an increased size of bell and a greater number of tentacles will increase the likelihood of coming into contact with MPs so we might expect there to be a positive correlation between MPs ingested and volume of species (Costello and Colin, 1994; Hansson and Kultima, 1995; Hansson, 1997; Titelman et al., 2007). However, that was not the case in this study. Whilst *Cyanea* yielded the highest counts of MPs, when normalised per ml of digestate counts were lower than smaller species. The source of the cnidarians and ctenophores (i.e. mode of capture) also influenced microplastic counts. Individuals were opportunistically sampled: pelagically-sourced (by boat); beach-sourced (picked up by hand on the beach); or shoreline sourced (caught from the shore in a net). Out of all the groupssampled only two species (*C. capillata* and *C. lamarckii*) were caught using multiple modes of capture. Whilst microplastic totals (MPT) were not significantly different based on mode of capture in *C. capillata,* MPT and microplastic fibres (MPF) in *C. lamarckii* were. Mean MPT and MPF were higher per ml in pelagically-sourced individuals compared to beach-sourced or shore-line sourced for *C. lamarckii* particularly MPF.

described and the intertidal zone sediments are generally considered to contain higher loadings than any other area of the shore (Blumenr¨oder et al., 2017; Hidalgo-Ruz et al., 2012). In cases where beach-sourced individuals had higher MP counts, cross-contamination

with MPs from the sediment may have occurred (Blumenr¨oder et al., 2017; Merga et al., 2020). Although specimens were rinsed before processing, absolute removal of exterior MPs could not be guaranteed. However, beach-sourced specimens of *C. capillata* had less MPF and microplastic particles (MPP) than shore-line sourced *C. capillata*. Another beached species, *C. pillosella*, also had lower mean MPF per ml-1 than shore-line sourced individuals. However, beach-sourced *A. aurita* had a higher mean MPF ml-1 digestate concentration than pelagically-sourced. This demonstrates that there is variation in ingested microplastics depending on mode of capture and species and there could be potential cross-contamination from sediments depending on location. The representation of beached individuals within this study provides essential knowledge that can be used for tracing MPs further within food webs as the stranded specimens can be consumed by seabirds, crabs and other scavengers (Gershwin, 2016)

The smallest species, *B. cucumis*, contained the highest MPF ml^{-1} digestate. This may be related to feeding strategies as *B. cucumis* consume prey whole without the use of tentacles, unlike *C. capillata, C. lamarckii* and *P. brachei* (Haddock.,2007; Tamm.,1983). However, it does not explain why *C. pilosella* would have the lowest relative MPF and MPP, as it also consumes prey whole.

Aurelia aurita had the second lowest mean MP ml^{-1} digestate with levels similar to *C. lamarcki*, *C. capillata* and *P. bachei*. There were concerns that individuals sampled from the Cooke Aquaculture salmon fish farm may have accumulated MPF from the black fish nets, however there was no significant difference between fibres found at this site and those observed in tissue or water samples. The MP counts from this site could be an over-estimate, because the organisms partially disintegrated during sample collection, which prevented the rinsing of any externally attached MPs.

All species of cnidarians and ctenophores utilised in this study inhabit the upper water column and as a result will be exposed to plastics that are buoyant. Even particles that are negatively buoyant which eventually sink still spend time at the surface due to surface tension and upwelling. As a result, pelagic species that inhabit the upper water column may be exposed to higher levels of plastic (Choy *et al*., 2019). Whilst some cnidarians can have tentacles up to 30 m in length, the majority of smaller cnidarians are still likely to only come into contact with low density MPs in the upper water column as they float or move about in the current. Conversely, they are less exposed to denser MPs that sink or are fouled (Botterell *et al*., 2019; Kaiser *et al*., 2017). To date, no experimental research has been carried out on the 'sticking' capability of different types of plastic to nematocysts, which catch particles, and tentacles which then transport food to the mouth.

4.1 Microplastics (MPs) in water and tissue samples

In the present study, cnidarians and ctenophores had similar MP loading to the pelagic waters they were collected from suggesting no bioconcentration of MPs. Whilst lab studies reported here demonstrated active uptake through ingestion, bioaccumulation does not appear to occur in the environment at this trophic level. Further studies are needed to determine how long MPs remain in the individuals and whether they are excreted from the

individuals over time. Alcazo *et al*., (2019) found *A. aurita* may recognise MPs and expel them during feeding experiments before it reached the gastric cavity. Therefore, some species may reject particular types of MPs (based on shape, size or taste/smell) but ingest others (Botterell *et al*., 2020; Lehtiniemi *et al*., 2018).

Black and blue fibres were the most abundant colours across all species and in all water samples, which is consistent with previous studies across the world that record fibres as the most common type of microplastic (Brown *et al*., 2011; Iliff *et al*., 2020; Lots *et al*., 2017; Thompson *et al*., 2004a). Most studies have indicated that washing machines are the most likely source of fibres being released in wastewater (Fendall and Sewell., 2009; Napper and Thompson., 2016; Bruce *et al*., 2017; Kelly *et al.,* 2019), others have suggested the use of ropes and fishing equipment as major sources (Andrady., 2011). Both are feasible within the area studied due to fishing and marine industry near sampling sites, as well as wastewater outfall pipes from urbanised areas of Orkney and Shetland. Future studies could investigate fibre output from local outfall pipes in proximity to some of the study sites to confirm this. MPPs were found in significantly higher numbers per ml of water around Orkney than in Hoy or Shetland, with Hoy being significantly different from the other Orkney sites. MPPs are typically heavier than fibres and so water samples can have significantly fewer particles per volume, as MPPs settle out to the sediment sooner (Doyle *et al*., 2011). Differences in MPP concentrations in water samples may be related to the MPP density and the unique current regimes in Orkney and the associated sediment mobilisation closer to shore. The higher abundance of MPs from sites around Orkney compared to Shetland is unlikely to be related to population size, which is similar in both archipelagos (ONS UK., 2020). However, Lerwick (Shetland) has a bigger port and is more exposed than most sites sampled in Orkney. Whilst a large number of the Orkney sites are located in Scapa Flow, which is more "sheltered" than Lerwick, they are also exposed to strong water currents that pass through the area which may bring MPs from other locations and trap them within the area.

4.2 Feeding trials

In spiked feeding trials using *A. aurita*, moulded plastic polyethylene terephthalate (MPS-PET) fibres were recovered from tissue samples, unlike nylon and recycled homopolymer PET fibres which were not. This may be due to the size of the container used to accommodate *A. aurita* (i.e. small species in a large beaker) which resulted in a decreased chance of opportunistic feeding or coming into contact with the MPs. It may also be that the individuals were stressed or that as this species actively feeds and "chemically senses" prey, immotile MPs did not entice them (Botterell *et al*., 2019; Macali *et al*., 2018; Sullivan *et al*., 1997). This is supported by Alcazo (*et al*., 2019), who also found that, in the absence of prey, A. *aurita* were not motivated to ingest MPs. The same study also found that plastic microspheres could be expelled by the manubrium, suggesting that the organism may be able to recognise MPs as inedible (Alcazo *et al*., 2019).

In the present study, fibres were most commonly observed in MP counts, especially in cnidarians. As fibres were recovered more frequently than particles from cnidarians and ctenophores (84% in *P. bachei* to 97% in *C. pilosella*), fibres may be preferentially consumed compared to particles, fragments or flakes. However, it could also be that fibres are more

frequently observed in the environment in general (Brown *et al*., 2011; Iliff *et al*., 2020; Lots *et al*., 2017; Thompson *et al*., 2004a), and that the higher aspect ratio means they are more likely to catch on tentacles or get incidentally caught in mucus rather than being specifically sought after.

4.3 FTIR

The major polymer found in this survey was polyester terephthalate (PET) (15%). This type of plastic is widely used for packaging as well as in clothing (Piccardo *et al*., 2020). Polypropylene was the second most common polymer (10%) used in textiles and packaging (Henry *et al.,* 2019). *Cosmetira pilosella* obtained from site 5 (off the coast of Aberdeen) contained two microplastic particles characterised as Cascamite 14 powdered resin. This is most commonly used as a resin glue in boat building, external joinery and cabinet making (Polyvine., 2009). Although sampling site 5 is not near a boat yard, it is in an area surrounded by marine transport routes, suggesting that resin may have flaked off passing boats. There may therefore be a link between location of sites and types of polymers found. This was generally seen for sampling sites further away from land, whereas samples from sites closer to land contained polymers consistent with packaging and textile industries.

4.4 Digestion method

The method of digestion used during this study had, to the best of the authors' knowledge, not been used previously at the time the research was carried out. Comparable studies involving the digestion of cnidarians or ctenophores used acid digestion, heat and mechanical means (stirring). There are reports that nitric acid detrimentally affects nylon, PET and biopolymers (Iliff *et al*., 2020; Plastics international., 2021). Potassium hydroxide has also been used with suggestions it can destroy cellulose acetate, polyethylene sheets and some biodegradable plastic (Alcazo *et al*., 2019; Kühn *et al*., 2017). Strong oxidising agents used at high temperatures for varying periods of time to digest tissue and biological material can also potentially damage polymers (Hurley *et al*., 2018; Munno *et al*., 2017). Other methods proposed in this study to reduce this potential loss/damage of polymers such as heat, freezing/defrosting, and enzyme digestion (Classens *et al*., 2013). Enzyme digestion for digestion was ruled out due to budgetary constraints. As a result, the authors looked for an alternative method. Preliminary tests found no acids, bases or enzymes were required to digest cnidarians and ctenophores and that moderate heat and stirring, and in some cases freezing and thawing, were sufficient.

4.5 Cross-contamination

Contamination is always an issue for this type of research as MPs are ubiquitous. Whilst forensic measures were used, cross-contamination did still occur. Glass equipment was used wherever possible however, these could not be used in the field due to potential breakage and weight issues with transportation. In the laboratory, pre-rinsed beakers were always covered with aluminium foil to prevent contamination from aerial particles (Mai *et al*., 2018). Water samples were collected in plastic tubes (pre-rinsed with distilled water). Nevertheless, a very small quantity of plastic fibres were still identified in these samples and accounted for in the final analysis. Clear fibres were found in the organisms from the field,

but not in any contamination controls, indicating the clear fibres came from the marine environment and not from contamination. Where possible, auto-digestions were carried out in a fume hood to mitigate contamination from precipitating atmospheric MPs (Wang *et al*.,2017; Wesch et al.,2017). Wesch *et al* (2017) found a fume hood and clean bench was the best way to reduce aerial contamination by 50%. Other studies recommend only wearing clothes made from cotton and limiting access to the lab (Prata *et al*.,2021; Wang and Wang.,2018). This can be difficult in laboratories with multiple users.

4.6 Future studies

In future studies, water samples, cnidarians and ctenophores could be collected from a range of sites along the UK coastline. Additional sites in Shetland and other islands in the Orkney archipelago should be sampled especially with focus on heavily populated islands vs. islands that are uninhabited. Specimens could also be sampled at the start of cnidarian and ctenophore blooms and compared to specimens caught towards the end of the bloom to identify differences in MP abundance or types of plastic found in specimens over time. Additional laboratory assays could be carried out to establish if species learn to recognise types of microplastic and, as a result, avoid consuming it. Chronic exposure and long-term accumulation should be examined in cnidarians, ctenophores and hydroids. This may provide important information to determine if sub-lethal effects such as fecundity, locomotion and feeding are affected by plastic ingestion. The impacts of sorbed chemicals from plastic and bioaccumulation of toxic compounds could also be examined. Ability to digest plastics with associated chemicals is also important and has been investigated in other species such as *Arenicolamarina (*Thompson *et al*.,2009) and *Eurytemora affinis* (Powell and Berry,1990).

Conclusion

This study demonstrated the presence of microplastics in cnidarians and ctenophores in Orkney, Shetland and North Sea locations. This has potential impacts for global food chains as they act as potential vectors for the trophic transfer of microplastics in a range of marine organisms. Cnidarians and ctenophores are becoming more abundant in the marine environment due to climate change and the overfishing of predator species leading to mass jellyfish blooms. Not only do many species, such as turtles, consume jellyfish and rely on them as a source of food and energy, but so do humans. In fact, jellyfish are becoming a popular delicacy in some regions of China (Pitt and Lucas, 2014) with the means to exploit jellyfish blooms, and human consumption could provide a direct route for MP consumption with potential health implications.

In conclusion, this study demonstrates plastic ingestion in short-lived ephemeral species and highlights the current issue of microplastic contamination in pelagic-based species and the need for a more extensive study on neuston species.

Acknowledgements

This research was funded by the Heriot-Watt University MSc in Marine Science

References

Alamaru, A., Bronstein, O., Dishon, G. and Loya, Y., 2009. Opportunistic feeding by the fungiid coral *Fungia scruposa* on the moon jellyfish *Aurelia aurita*. *Coral Reefs*, 28(4), pp.865-865.

Alcazo, J., Gomez Cabrera, M. and Herrera Ulibarri, A., 2019. *Impact of Microplastics In Jellyfish*. [online] Accedacris.ulpgc.es. Available at: <https://accedacris.ulpgc.es/bitstream/10553/69718/2/0764272_00000_0000.pdf> [Accessed 30 November 2020].

Andrady, A., 2011. Microplastics in the marine environment. Marine Pollution Bulletin, 62(8), pp.1596-1605.

Ates, R., 2017. Benthic scavengers and predators of jellyfish, material for a review. *Plankton and Benthos Research*, 12(1), pp.71-77.

Axelsson, C. and van Sebille, E., 2017. Prevention through policy: Urban macroplastic leakages to the marine environment during extreme rainfall events. *Marine Pollution Bulletin*, 124(1), pp.211-227.

Bayha, K., Graham, W., Higgins III, J. and Fletcher, H., 2012. Predation potential of the jellyfish *Drymonema larsoni* Bayha & Dawson (Scyphozoa: Drymonematidae) on the moon jellyfish *Aurelia* sp. in the northern Gulf of Mexico. *Hydrobiologia*, 690(1), pp.189-197.

Baztan, J., Jorgensen, B., Pahl, S., Thompson, R. and Vanderlinden, J., 2016. Fate and impact of microplastics in marine ecosystems. Saint Louis: Elsevier Science, pp.46-47.

Blumenröder, J., Sechet, P., Kakkonen, J. and Hartl, M., 2017. Microplastic contamination of intertidal sediments of Scapa Flow, Orkney: A first assessment. Marine Pollution Bulletin, 124(1), pp.112-120.

Botterell, Z., Beaumont, N., Cole, M., Hopkins, F., Steinke, M., Thompson, R. and Lindeque, P., 2020. Bioavailability of microplastics to marine zooplankton: Effect of shape and infochemicals. *Environmental Science & Technology*, 54(19), pp.12024-12033.

Bond, T., 2018. The occurrence and degradation of aquatic plastic litter based on polymer physicochemical properties: A review. [online] Taylor & Francis. Available at: https://www.tandfonline.com/doi/abs/10.1080/10643389.2018.1483155?scroll=top&needAccess=true&journalCode=best20 [Accessed 24 Oct. 2019].

Browne, M., Crump, P., Niven, S., Teuten, E., Tonkin, A., Galloway, T. and Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: Sources and sinks. Environmental Science and Technology, 45(21), pp.9175-9179.

Browne, M., Niven, S., Galloway, T., Rowland, S. and Thompson, R., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. *Current Biology*, 23(23), pp.2388-2392.

Brotz, L., Schiariti, A., López-Martínez, J., Álvarez-Tello, J., Peggy Hsieh, Y., Jones, R., Quiñones, J., Dong, Z., Morandini, A., Preciado, M., Laaz, E. and Mianzan, H., 2016. Jellyfish fisheries in the Americas: origin, state of the art, and perspectives on new fishing grounds. *Reviews in Fish Biology and Fisheries*, 27(1), pp.1-29.

Bruce, N., Hartline, N., Karba, S., Ruff.B., Sonar, S., 2017. Microfiber pollution and the apparel industry, [Online]http://www.esm.ucsb.edu/research/2016Group_Projects/documents/PataPlastFinalReport.pdf [accessed 14 aug 2017]

By the ocean we unite, 2021. Ahoy! Come aboard and join us on our journey to cleaner oceans. [online] By the Ocean we Unite. Available at: <https://www.bytheoceanweunite.org/> [Accessed 10 March 2021].

Bucci, K., Tulio, M. and Rochman, C., 2020. What is known and unknown about the effects of plastic pollution: A meta-analysis and systematic review. *Ecological Applications*, 30(2).

Cardona, L., Álvarez de Quevedo, I., Borrell, A. and Aguilar, A., 2012. Massive consumption of gelatinous plankton by mediterranean apex predators. *PLoS ONE*, 7(3), p.e31329.

Carpenter, E.J., Smith, K.L., 1972. Plastics on the Sargasso sea surface. Science, 4027, pp. 1240-1241.

Center for Environmental Studies., 2020. *Guide To Microplastic Identification*. [online] Sfyl.ifas.ufl.edu. Available at: <https://sfyl.ifas.ufl.edu/media/sfylifasufledu/flagler/sea-grant/pdf-files/microplastics/MERI_Guide-to-Microplastic-Identification.pdf> [Accessed 25 March 2020].

Choy, C., Robison, B., Gagne, T., Erwin, B., Firl, E., Halden, R., Hamilton, J., Katija, K., Lisin, S., Rolsky, C. and S. Van Houtan, K., 2019. The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. *Scientific Reports*, 9(1).

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M. and Janssen, C., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. *Marine Pollution Bulletin*, 70(1-2), pp.227-233.

Cole, M., Lindeque, P., Halsband, C. and Galloway, T., 2011. Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin*, 62(12), pp.2588-2597.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J. and Galloway, T., 2013. Microplastic Ingestion by Zooplankton. *Environmental Science & Technology*, 47(12), pp.6646-6655.

Costa, E., Gambardella, C., Piazza, V., Vassalli, M., Sbrana, F., Lavorano, S., Garaventa, F. and Faimali, M., 2020. Microplastics ingestion in the ephyra stage of Aurelia sp. triggers acute and behavioral responses. *Ecotoxicology and Environmental Safety*, 189, p.109983.

Costello, J. H. and Colin, S. P., 1994. Morphology, fluid motion and predation by the scyphomedusa *Aurelia aurita*. Marine Biology ,121, 327–334.

Doyle, M.J., Watson, W., Bowlin, N.M., Sheavly, S.B., 2011. Plastic particles in coastal pelagic ecosystems of the northeast Pacific Ocean. Mar. Environ. Res. 71, 41–52.

Fendall, L. and Sewell, M., 2009. Contributing to marine pollution by washing your face: Microplastics in facial cleansers. Marine Pollution Bulletin, 58(8), pp.1225-1228.

Fowler, C.W., 1987. Marine debris and northern fur seals: A case study, Marine Pollution Bulletin, 18(6), pp. 326–335. Doi: 10.1016/s0025-326x (87)80020-6.

Gershwin, L., 2016. *Jellyfish: A Natural History*. University of Chicago Press. p. 140. ISBN 978-0-226-28767-6.

Gouin, T., 2020. Toward an Improved Understanding of the Ingestion and Trophic Transfer of Microplastic Particles: Critical Review and Implications for Future Research. *Environmental Toxicology and Chemistry*, 39(6), pp.1119-1137.

Graham, E.R. and Thompson, J.T., 2009. Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. Journal of Experimental Marine Biology and Ecology 368, 22-29.

Haddock, S., 2007. Comparative feeding behavior of planktonic ctenophores. *Integrative and Comparative Biology*, 47(6), pp.847-853.

Hall, N., Berry, K., Rintoul, L. and Hoogenboom, M., 2015. Microplastic ingestion by scleractinian corals. *Marine Biology*, 162(3), pp.725-732.

Hansson, L., 1997. Capture and digestion of the scyphozoan jellyfish *Aurelia aurita* by *Cyanea capillata* and prey response to predator contact. Journal of Plankton Research, 19(2), pp.195-208.

Hansson, L. and Kultima, K., 1995. Behavioural response of the scyphozoan jellyfish *Aurelia aurita* (L.) upon contact with the predatory jellyfish *Cyanea capillata* (L.). Marine and Freshwater Behaviour and Physiology, 26(2-4), pp.131-137.

Harris, S., 2018. Investigating household expenditure in island communities - Office for National Statistics. [online] Ons.gov.uk. Available at: <https://www.ons.gov.uk/economy/regionalaccounts/grossdisposablehouseholdincome/articles/investigatinghouseholdexpenditureinislandcommunities/2018-10-31> [Accessed 10 March 2021].

Henry, B., Laitala, K. and Klepp, I., 2019. Microfibres from apparel and home textiles: Prospects for including microplastics in environmental sustainability assessment. *Science of The Total Environment*, 652, pp.483-494.

Hidalgo-Ruz, V., Gutow, L., Thompson, R. and Thiel, M., 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environmental Science and Technology, 46(6), pp.3060-3075.

Hurley, R., Lusher, A., Olsen, M. and Nizzetto, L., 2018. Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices. *Environmental Science & Technology*, 52(13), pp.7409-7417.

Jaikumar, G., Brun, N., Vijver, M. and Bosker, T., 2019. Reproductive toxicity of primary and secondary microplastics to three cladocerans during chronic exposure. *Environmental Pollution*, 249, pp.638-646.

Kaiser, D., Kowalski, N. and Waniek, J., 2017. Effects of biofouling on the sinking behavior of microplastics. *Environmental Research Letters*, 12(12), p.124003.

Kelly, M., Lant, N., Kurr, M. and Burgess, J., 2019. Importance of water-volume on the release of microplastic fibers from laundry. Environmental Science and Technology, 53(20), pp.11735-11744.

Kooi, M., Nes, E., Scheffer, M. and Koelmans, A., 2017. Ups and Downs in the Ocean: Effects of biofouling on vertical transport of microplastics. *Environmental Science & Technology*, 51(14), pp.7963-7971.

Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E. and van Franeker, J., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. *Marine Pollution Bulletin*, 115(1-2), pp.86-90.

Lehtiniemi, M., Hartikainen, S., Näkki, P., Engström-Öst, J., Koistinen, A. and Setälä, O., 2018. Size matters more than shape: Ingestion of primary and secondary microplastics by small predators. *Food Webs*, 17, p.e00097.

Lepš, J. & Šmilauer, P. (2020). Biostatistics with R. An Introductory Guide for Field Biologists. Cambridge University Press, Cambridge. 365 pp.

Iliff, S., Wilczek, E., Harris, R., Bouldin, R. and Stoner, E., 2020. Evidence of microplastics from benthic jellyfish (*Cassiopea xamachana*) in Florida estuaries. *Marine Pollution Bulletin*, 159, p.111521.

Lots, F., Behrens, P., Vijver, M., Horton, A. and Bosker, T., 2017. A large-scale investigation of microplastic contamination: Abundance and characteristics of microplastics in European beach sediment. Marine Pollution Bulletin, 123(1-2), pp.219-226.

Lusher, A., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I. and Officer, R., 2015. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: The True's beaked whale *Mesoplodon mirus*. Environmental Pollution, 199, pp.185-191.

Macali, A., Semenov, A., Venuti, V., Crupi, V., D'Amico, F., Rossi, B., Corsi, I. and Bergami, E., 2018. Episodic records of jellyfish ingestion of plastic items reveal a novel pathway for trophic transference of marine litter. Scientific Reports, 8(1).

Maes, T., Jessop, R., Wellner, N., Haupt, K. and Mayes, A., 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Scientific Reports*, 7(1).

Mai, L., Bao, L., Shi, L., Wong, C. and Zeng, E., 2018. A review of methods for measuring microplastics in aquatic environments. *Environmental Science and Pollution Research*, 25(12), pp.11319-11332.

Merga, L., Redondo-Hasselerharm, P., Van den Brink, P. and Koelmans, A., 2020. Distribution of microplastic and small macroplastic particles across four fish species and sediment in an African lake. *Science of The Total Environment*, 741, p.140527.

Munno, K., Helm, P., Jackson, D., Rochman, C. and Sims, A., 2017. Impacts of temperature and selected chemical digestion methods on microplastic particles. *Environmental Toxicology and Chemistry*, 37(1), pp.91-98.

Naidoo, T., Sershen, Thompson, R. and Rajkaran, A., 2020. Quantification and characterisation of microplastics ingested by selected juvenile fish species associated with mangroves in KwaZulu-Natal, South Africa. *Environmental Pollution*, 257, p.113635.

Napper, I. and Thompson, R., 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. Marine Pollution Bulletin, 112(1-2), pp.39-45.

Orkney Government., 2020. History And Character Of Stromness. [online] Orkney.gov.uk. Available at: <https://www.orkney.gov.uk/Service-Directory/H/History-and-Character-of-Stromness.htm> [Accessed 25 March 2020].

Petresin.org., 2019. Fact Sheet - An Introduction to PET (polyethylene terephthalate) | PETRA: Information on the Use, Benefits & Safety of PET Plastic. [online] Available at: http://petresin.org/news_introtopet.asp [Accessed 24 Apr. 2019].

Piccardo, M., Provenza, F., Grazioli, E., Cavallo, A., Terlizzi, A. and Renzi, M., 2020. PET microplastics toxicity on marine key species is influenced by pH, particle size and food variations. *Science of The Total Environment*, 715, p.136947.

Pinheiro, L., Ivar do Sul, J. and Costa, M., 2020. Uptake and ingestion are the main pathways for microplastics to enter marine benthos: A review. *Food Webs*, 24, p.e00150.

Pitt, K. and Lucas, C., 2014. *Jellyfish Blooms*. Springer, p.Chapter 6: Living with Jellyfi sh: Management and Adaptation Strategies.

Plasticseurope., 2019. Plastics-The Facts 2019 An Analysis of European Plastics Production, Demand and Waste Data. [online] Available at: <https://www.plasticseurope.org/application/files/9715/7129/9584/FINAL_web_version_Plastics_the_facts2019_14102019.pdf> [Accessed 25 March 2020].

Plastics international, 2021. *Search Results | Plastics International | Plastics International*. [online] Plasticsintl.com. Available at: <https://www.plasticsintl.com/chemical-resistance-chart> [Accessed 4 May 2021].

Polyvine, 2009. *a TECHNICAL DATA SHEET: Cascamite*. [online] Fiddes.co.uk. Available at: <https://fiddes.co.uk/app/uploads/2016/11/Cascamite-MSDS-1.pdf> [Accessed 4 May 2021].

Powell, M. and Berry, A.,1990. Ingestion and regurgitation of living and inert materials by the estuarine copepod *Eurytemora affinis* (Poppe) and the influence of salinity. Estuarine, Coastal and Shelf Science, 31(6), pp.763-773.

Prata, J., Reis, V., da Costa, J., Mouneyrac, C., Duarte, A. and Rocha-Santos, T., 2021. Contamination issues as a challenge in quality control and quality assurance in microplastics analytics. *Journal of Hazardous Materials*, 403, p.123660.

PRIMER-E Ltd, 2015. *PRIMER-e V7: Plymouth Routines In Multivariate Ecological Research*. PRIMER-E Plymouth, URL https://www.primer-e.com/.

Provencher, J., Ammendolia, J., Rochman, C. and Mallory, M., 2019. Assessing plastic debris in aquatic food webs: what we know and don't know about uptake and trophic transfer. *Environmental Reviews*, 27(3), pp.304-317.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Richardson, A., Bakun, A., Hays, G. and Gibbons, M., 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. *Trends in Ecology & Evolution*, 24(6), pp.312-322.

Rochman, C., Tahir, A., Williams, S., Baxa, D., Lam, R., Miller, J., Teh, F., Werorilangi, S. and Teh, S., 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Scientific Reports*, 5(1).

Sfriso, A., Tomio, Y., Rosso, B., Gambaro, A., Sfriso, A., Corami, F., Rastelli, E., Corinaldesi, C., Mistri, M. and Munari, C., 2020. Microplastic accumulation in benthic invertebrates in Terra Nova Bay (Ross Sea, Antarctica). *Environment International*, 137, p.105587.

Statista., 2017. "Global Plastic Production. https://www.statista.com/statistics/282732/global-production-of- Sundt, Peter and Schulze, Per-Erik: "Sources of microplastic-pollution to the marine environment", "Mepex for the Norwegian Environment Agency", 2008plastics-since-1950/ Last accessed 14/03/2017

Sucharitakul, P., Pitt, K. and Welsh, D., 2020. Limited ingestion, rapid egestion and no detectable impacts of microbeads on the moon jellyfish, *Aurelia aurita*. *Marine Pollution Bulletin*, 156, p.111208.

Sullivan, B., Suchman, C. and Costello, J., 1997. Mechanics of prey selection by ephyrae of the scyphomedusa *Aurelia aurita*. *Marine Biology*, 130(2), pp.213-222.

Tamm, S., 1983. Motility and mechanosensitivity of macrocilia in the ctenophore Beroë. *Nature*, 305(5933), pp.430-433.
Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic?. Science, 304, pp. 838

Tramoy, R., Gasperi, J., Colasse, L. and Tassin, B., 2020. Transfer dynamic of macroplastics in estuaries — New insights from the Seine estuary: Part 1. Long term dynamic based on date-prints on stranded debris. *Marine Pollution Bulletin*, 152, p.110894.

Thompson, R., Swan, S., Moore, C. and vom Saal, F., 2009. Our plastic age. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), pp.1973-1976.

Titelman, J., Gandon, L., Goarant, A. and Nilsen, T., 2007. Intraguild predatory interactions between the jellyfish *Cyanea capillata* and *Aurelia aurita*. Marine Biology, 152(4), pp.745-756.

Vesela, S. and Vijverberg, J., 2006. Effect of Body Size on Toxicity of Zinc in Neonates of Four Differently Sized Daphnia Species. *Aquatic Ecology*, 41(1), pp.67-73.

Wang, W., Ndungu, A., Li, Z. and Wang, J., 2017. Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China. *Science of The Total Environment*, 575, pp.1369-1374.

Wang, W. and Wang, J., 2018. Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis. *TrAC Trends in Analytical Chemistry*, 108, pp.195-202.

Welden, N. and Lusher, A., 2017. Impacts of changing ocean circulation on the distribution of marine microplastic litter. Integrated Environmental Assessment and Management, 13(3), pp.483-487.

Wesch, C., Elert, A., Wörner, M., Braun, U., Klein, R. and Paulus, M., 2017. Assuring quality in microplastic monitoring: About the value of clean-air devices as essentials for verified data. Scientific Reports, 7(1).

Woods, J., Rødder, G. and Verones, F., 2019. An effect factor approach for quantifying the entanglement impact on marine species of macroplastic debris within life cycle impact assessment. *Ecological Indicators*, 99, pp.61-66.

Zhang, F., Wang, X., Xu, J., Zhu, L., Peng, G., Xu, P. and Li, D., 2019. Food-web transfer of microplastics between wild caught fish and crustaceans in East China Sea. *Marine Pollution Bulletin*, 146, pp.173-182.

Zebe, E. and Schiedek, D., 1996. The lugworm *Arenicola marina*: A model of physiological adaptation to life in intertidal sediments. Helgoländer Meeresuntersuchungen, 50(1), pp.37-68.