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Detection and characterisation of microplastics and microfibres in fishmeal and soybean meal

Christopher Walkinshaw [a,b], Trevor J. Tolhurst [b], Penelope K. Lindeque [a], Richard Thompson [c], Matthew Cole [a,*]

[a] Plymouth Marine Laboratory, Prospect Place, Plymouth PL1 3DH, United Kingdom of Great Britain and Northern Ireland
[b] School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, Norfolk NR4 7TJ, United Kingdom of Great Britain and Northern Ireland
[c] Marine Institute, University of Plymouth, Level 3, Marine Building, Drake Circus, Plymouth PL4 8AA, United Kingdom of Great Britain and Northern Ireland

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ABSTRACT

Aquaculture is an increasingly important source of nutrition for global food security, which is reliant on animal- and plant-based feeds. Anthropogenic particles, including microplastics and semi-synthetic cellulosic fibres, are prolific marine pollutants that are readily consumed by marine organisms, including small pelagic fish commonly used in fishmeal. Conversely, there is no indication plants can accumulate anthropogenic microparticles. We explore whether aquaculture feed presents a route of contamination for farmed fish. Commercially-sourced aquaculture feedstocks, including fishmeals and soybean meal, were processed (KOH digestion and $ZnCl_2$ density separation) and anthropogenic particles characterised using microscopy and spectroscopic methods. Both fishmeal and soybean meals contained anthropogenic particles, with concentrations ranging 1070–2000 particles kg^{-1}. The prevalence of anthropogenic particles in plant-based feeds indicates that the majority of contamination occurs post-harvest. Based on our findings, farmed Atlantic salmon may be exposed to a minimum of 1788–3013 anthropogenic particles from aquaculture feed across their commercial lifespan.

1. Introduction

Fisheries and aquaculture provide over 15 % of the animal protein consumed by 4.5 billion people worldwide (Béné et al., 2015). With a rapidly expanding global population, aquaculture is becoming an increasingly important approach for supplying seafood to market, and intrinsic to marine food security; in 2019, aquaculture provided 52 % of fish production for human consumption with a value of 250 billion USD (FAO, 2020). Aquaculture can be used to grow a variety of species, including macroalgae, crustaceans and molluscs, however finfish dominates global production, contributing >54.3 million tonnes of food worth 139.7 billion USD (FAO, 2020). High value finfish species such as Atlantic Salmon and European seabass are typically maintained in open systems (e.g. sea pens), relying on aquaculture feed for sustenance and nutrition (Halwart et al., 2007). Aquaculture feed typically comprises protein-rich pellets, powders or cakes, prepared from animal (e.g. fishmeal) or plant (e.g. soybean meal) material. For fishmeal, feedstock derives from targeted capture of small marine fish such as Peruvian anchoveta (*Engraulis ringens*), Pacific sardine (*Sardinops sagax*), and Atlantic herring (*Clupea harengus*), by-catch, and by-products (i.e. offal, trimmings) from the processing of larger commercial fish species (Cashion et al., 2017). While herbivorous fish can consume a feed that is either partially or completely comprised of plant proteins and oils (Viola et al., 1988), carnivorous fish require the addition of animal-derived proteins and oils. In 2013, approximately 16.3 million tonnes of fish were reduced to fishmeal and fish oil (FAO, 2014), of which 60 % of total fishmeal and 80 % of total fish oil production were used in aquaculture (Boyd, 2013). In recent years, the use of fishmeal within aquaculture feeds has been diminishing, largely owing to economic and consumer pressure stemming from overfishing of lower trophic species for feeding commercial species (Naylor et al., 2009; Olsen and Hasan, 2012; Shannon and Waller, 2021); fishmeal is typically being replaced by plant-based meals, such as soybean, wheat and corn meal which is considered a cheaper and more sustainable option (Salin et al., 2018).

Microplastics, describing plastic particles and fibres 1 μm–5 mm in size, are a persistent, globally prevalent contaminant (Cole et al., 2011; Hale et al., 2020). These particles stem from industry (e.g. biobeads used in sewage treatment works, pre-production pellets), highways (e.g. tyre-

* Corresponding author.
E-mail address: mcol@pml.ac.uk (M. Cole).

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particles) and household effluent (e.g. microfibres released during laundry cycles, scrubbing agents) (Andrady, 2011; Napper and Thompson, 2016), or form through the degradation of macroplastic litter (Napper et al., 2022). In the natural environment, microplastics degrade slowly and can persist for decades (Andrady, 2011). They are found in almost every environment worldwide, including freshwater, marine, benthic and terrestrial environments, and throughout the atmosphere resulting in their transport and deposition into remote ecosystems (Bergmann et al., 2019; Peeken et al., 2018; Rochman, 2018). While microplastics are among the most commonly studied marine pollutants, there are other types of anthropogenic microparticles that may also pose a risk to the marine environment; these include cellulosic microfibres comprised of cotton and semi-synthetic polymers manufactured from regenerated cellulose (e.g. rayon). Herein, we use the umbrella term 'anthropogenic particles' to refer to microplastics, semi-synthetic polymers and cotton particles. Cotton and semi-synthetic polymers are commonly used in textiles, such as clothing and agricultural fleece, and can enter the marine environment through household effluent, agricultural runoff and aeolian deposition (Napper and Thompson, 2016). Determining the environmental prevalence of these microfibres has been challenging, owing to the difficulties in differentiating between anthropogenic and natural cellulosic materials and issues with contamination, for example fibres shedding from operators' lab coats or contamination from clothing or atmospheric fallout during sample collection. Nevertheless, numerous studies point to the presence of these fibres in considerable quantities alongside plastic microfibres (Halstead et al., 2018; Nunes et al., 2021; Remy et al., 2015; Savoca et al., 2021; Talvitie et al., 2017).

Owing to their ubiquity in the marine environment, anthropogenic particles are inevitably taken up into living organisms, through ingestion or inhalation (Galloway et al., 2017). The presence of anthropogenic particles within commercially exploited aquatic species is well evidenced (Choy and Drazen, 2013; Foekema et al., 2013; Rummel et al., 2016). Chronic exposure to microplastics can have negative effects on commercially important marine organisms, with evidence of reduced growth and reproductive outputs (Cormier et al., 2021); such effects could reduce the productivity and profitability of commercial aquaculture facilities (Walkinshaw et al., 2020). Recent studies have identified the presence of both plastic and semi-synthetic microfibres in farmed Sea bream and Common carp (Savoca et al., 2021), and current evidence suggests that farmed fish typically contain more microplastic than wild-caught fish (Wootton et al., 2021). Yet, despite the importance of farmed seafood for human health and food security, the prevalence and effects of anthropogenic particles on farmed fish remain poorly elucidated. Farmed aquaculture species can be subject to anthropogenic particle exposure via their natural environment (e.g. through seawater and atmospheric deposition), release from equipment, infrastructure and clothing, and their food. Several studies have identified microplastics within fishmeals (Gündoğdu et al., 2021; Hanachi et al., 2019; Karbalaei et al., 2020; Thiele et al., 2021; Wang et al., 2022; Yao et al., 2021), however cellulosic microfibres were not considered in the majority of these studies. Contamination of aquaculture feed can occur where anthropogenic particles are present in source material (Hanachi et al., 2019). For example, fishmeal is typically manufactured using planktivorous fish (commonly termed forage fish) which have been widely identified to contain high body burdens of anthropogenic particles (Collard et al., 2017; Lusher et al., 2013; Tanaka and Takada, 2016; Walkinshaw et al., 2020; Welden et al., 2018). While studies have identified plastic particles ≤45 μm can adsorb onto aquatic plants (Dovidat et al., 2020; Mateos-Cárdenas et al., 2019), there is currently no evidence that anthropogenic particles can permeate into plant material; therefore, anthropogenic particles in plant-based feeds (e.g. soybean meal) are unlikely to derive from source material. However, anthropogenic particles can also contaminate feeds during processing, transport and packaging; for example, anthropogenic particles may be released through mechanical abrasion of equipment, shedding of fibres from clothing and airborne deposition (Dris et al., 2017; Roblin et al., 2020). In comparing anthropogenic particle concentrations in both animal- and plant-based feedstocks, the origin of these contaminants can be elucidated.

In this study, we investigate the potential exposure of commercially exploited finfish species to anthropogenic particles via aquaculture feed. We apply optimised methods for isolating and characterising >25 μm anthropogenic particles in ten commercially-available aquaculture feeds, including a variety of fish meals and a soybean meal. We hypothesise that there are a wide range of anthropogenic particles present in aquaculture feed, including both microplastics and semi-synthetic cellulosic fibres. The analysis of both fishmeal and soybean meal will allow us to explore the hypothesis that anthropogenic particle contamination of aquaculture feed is predominantly driven by the level of contamination in the source material. Finally, we test the hypothesis that the use of aquaculture feed in fish farming increases risk of anthropogenic particulate exposure in farmed finish as compared to wild stock by calculating the additional anthropogenic particle load that farmed salmon will incur from the consumption of aquaculture feed.

2. Methods

2.1. Contamination control and blanks

All sample processing took place within a laminar flow hood in the ultraclean microplastics laboratory in Plymouth Marine Laboratory (Plymouth, UK). The laboratory minimizes microplastic contamination through use of a HEPA filtered positive pressure airflow system (which removes 99.95 % of airborne particles with a diameter of 0.3 μm), controlled personnel entry, tack mats to remove footwear contamination and cotton labcoats to suppress release of polymeric clothing fibres. Wherever possible, glass apparatus and consumables were used to avoid plastic contamination. All flasks were sealed with aluminium foil and parafilm whenever taken out of the laminar flow hood and when in the orbital shaker incubator. Procedural blanks (n = 3) were performed and analysed in the same way as test samples to identify and eliminate background contamination. Positive controls (n = 3), spiked with a known quantity of 250 μm nylon fibres and 30 μm polystyrene beads, were taken through the process to determine methodological efficacy.

2.2. Digestion and density separation

Ten commercially-available aquaculture feeds were chosen for investigation, comprising nine fishmeals of marine origin and one soybean meal, bulk masses 1–5 kg (Table 1). Fishmeal is a complex organic matrix, comprising dehydrated flesh, bone and abiotic material. Therefore, it was necessary to employ an optimised two-step process, including chemical digestion and density separation, to effectively

Table 1
Aquaculture feed details including country of origin and main species within the feed. Samples are referred to throughout the text by the name designated in brackets.

Sample	Country of origin	Main species
LT-94 (LT94a)	Norway	Atlantic herring (*Clupea harengus*)
LT-94 (LT94b)	Norway	Atlantic herring (*Clupea harengus*)
Provimi 66 (Pv66a)	UK	White fish and salmon trimmings
Provimi 66 (Pv66b)	UK	White fish and salmon trimmings
Pre-digested fish protein (CP70)	UK	Pre-digested white fish and fish trimmings
White fish (WF)	Scotland	White fish
Sardine and anchovy (SA)	South America	Sardine and anchovy
Squid (Sq)	Unknown	Dried whole squid
Krill (Kr)	Antarctic krill	Antarctic krill (*Euphausia superba*)
Soybean meal (soy)	Unknown	Defatted heat treated soya (*Glycine max*)

isolate anthropogenic particles from this substrate. Aquaculture feed was manually mixed within its container and 10 g subsamples weighed with a mass balance and placed into a clean conical flask with 200 mL of 10 % KOH. Flasks were sealed with aluminium foil and parafilm to prevent airborne contamination, and placed into an orbital shaker incubator (Sanyo Orbisafe orbital incubator) and digested for 48 h at 50 °C, 125 rpm. Treilles et al. (2020) show that both plastic and cotton are resistant to KOH degradation at this concentration. After digestion, undigested material was vacuum filtered sequentially onto 100 μm, 63 μm and 25 μm nylon filter mesh discs, rinsing filtration equipment with ultrapure water to ensure no loss of material; filter discs were dried overnight in a dehydrator set to 60 °C. Multiple pore sizes were utilised to dilute out remaining materials aiding in anthropogenic particle identification and to prevent the masking of smaller anthropogenic particles by larger materials. Samples were subsequently density separated using a sediment-microplastic isolation (SMI) unit (Coppock et al., 2017) filled with $ZnCl_2$ solution (solution density 1.5 g/cm^3); the solution was mixed and left to separate out for 30 min, and then the lower-density particulates in the supernatant were filtered back on to corresponding mesh discs to retain any anthropogenic particles <1.5 g/cm^3. The mesh disc was then placed into a Petri dish and dried for 12 h in a dehydrator at 60 °C. Between repeats, the SMI unit was cleaned with ultrapure water and the $ZnCl_2$ solution was recycled by filtering through a 0.2 μm GF/F glass fibre filter. $ZnCl_2$ solution density was checked, and if this was below 1.5 g/cm^3 a new solution was manufactured. The two-step protocol removed on average of 97.5 % of the sample material by mass, making identification of anthropogenic particles using microscopy viable, but precluding the use of scanning technologies (e.g. Raman, FT-IR imaging) as such methods require pristine microplastics absent of other detrital matter.

2.3. Anthropogenic particle identification

Anthropogenic particles were identified by performing a multi-stage identification process involving microscopic screening based on visual characteristics, supplemented with polymeric verification using Fourier-transform infrared (FTIR) spectroscopy. This approach aligns with the methodologies used for analysis of environmental samples elsewhere (Jones-Williams et al., 2020). Each mesh disc was systematically checked for potential anthropogenic particles manually using an Olympus SZX16 microscope and CellSens software (Olympus, version 2.1); mesh discs were placed onto a glass slide with a 3 mm^2 grid and each square analysed to identify particles of interest. Particles of interest were identified through morphology, colour, and texture that may allude to being anthropogenic in origin. For each of these particles, colour, shape (fibre, fragment or film), and length of longest dimension (μm) was recorded. A subset of 400 particles (48.5 % total identified particles) were randomly selected for polymeric analysis; these particles were placed on to 0.02 μm anodiscs for FTIR analysis. To reduce analytical effort, where particles showed a high degree of morphological similarity, only a randomly selected subset of these particles were analysed (33 % of selected particles); spectroscopic data was used to estimate the polymer composition of the other particles on the mesh disc.

Potential anthropogenic particles were verified using a Perkin Elmer Spotlight 400 imaging system comprised of a PerkinElmer Frontier FT-IR spectrometer (MCT detector, KBr window) and PerkinElmer Spotlight 400 microscope, with SpectrumIR software (PerkinElmer, 2017, version 10.6.0.893). The spectrometer was used in transmittance sampling mode, with 20 scans (range = 1250–4000 cm^{-1}) at a resolution of 4 cm^{-1}. Resultant spectra were compared with bespoke and publicly-available reference libraries, including the spectral library created by Primpke et al. (2018), who utilised a near identical spectral range in the creation of this reference library (1250–3600 cm^{-1}). Particles with spectral matches > 70 % were used as confirmation of particle composition. However, as organic soiling on the surface of plastic particles can reduce spectral match accuracy, particles with spectral matches < 70 % were also included in the results where physical characteristics (morphology, colour, structure) matched similar particles within the sample that were successfully characterised as being of anthropogenic origin. Following the polymer identification steps, polymers were assigned to one of three categories based on their origin: Petroleum-based plastic, semi-synthetic or cotton. Semi-synthetic polymers were defined as cellulose-based polymers manufactured synthetically from regenerated cellulose, such as rayon, cellophane, and cellulose acetate. There is added complexity in distinguishing anthropogenic cellulosic particles from natural cellulose in the samples. In order to distinguish anthropogenic cellulosic particles such as rayon, an extra step was added following FTIR analysis. If the resultant spectra identified the particle as cellulose, rayon, cellophane, or cellulose acetate, the particle was again screened visually and was only included if the colour and morphology was indicative of being of anthropogenic origin, i.e. a non-natural uniform colour and uniform shape with no organic structures visible.

2.4. Analysis and statistics

Concentrations of anthropogenic particles within fishmeal samples were calculated as mean number of particles per 10 g sample, with the total of all three meshes (100, 63 and 25 μm) comprising each replicate. Data was then used to calculate the mean number of particles kg^{-1}. Exposure of farmed Atlantic salmon to anthropogenic particles (P_E) was calculated by taking the approximate weight of a salmon upon harvest and multiplying this by the feed conversion ratio (FCR, a measure of the weight of feed needed for 1 kg biomass gain in the farmed organism) to calculate the total feed consumed (F_C). This value is then multiplied by the approximate percentage inclusion of fishmeal or soybean meal in salmon feed ($\%I_{FM}$ and $\%I_{SBM}$ for fishmeal and soybean meal, respectively) to calculate the mass of each feed included in the meal. The resulting value is also multiplied by the mean number of anthropogenic particles kg^{-1} identified in each feed (AP_{FM} and AP_{SBM} for fishmeal and soybean meal, respectively) to calculate the estimated number of anthropogenic particles ingested by Atlantic salmon through fishmeal and soybean meal. This calculation is shown below:

$$F_C = Salmon\ mass\ (\mathrm{kg}) \times FCR \tag{1}$$

$$P_E = (F_C \times \%I_{FM} \times AP_{FM}) + (F_C \times \%I_{SBM} \times AP_{SBM}) \tag{2}$$

Data is presented as mean with standard errors of the mean, unless otherwise stated. Statistical analyses were performed using R (version 4.1.0). Data were tested for normality using Shapiro-Wilk tests, and normally-distributed data were tested by ANOVA with Tukey's post-hoc testing. Where data violated assumptions of normality, non-parametric Kruskal-Wallis tests with Dunn's post-hoc pairwise testing were performed to investigate whether individual experimental groupings differ significantly. The significance level for both tests was set at $\alpha = 0.05$.

3. Results

3.1. Anthropogenic particle identification

Analysis of process blank samples showed a mean of five particles per sample, all of which were fibres, of which 4.67 fibres filter^{-1} were semi-synthetic and 0.33 fibres filter^{-1} were identified as polyester. Mean blank results were removed from each replicate. Positive controls found mean recovery rates of 100 % and 94 % for nylon fibres and polystyrene beads respectively. Owing to the high recovery rates, no corrective factor was applied to the results.

Across all aquaculture feed subsamples, 865 suspected anthropogenic particles were identified via microscopy, with 64 % of selected particles identified as being anthropogenic in origin using FT-IR. For all particles assessed: the most prevalent morphology was fibres (82.5 %), followed by fragments (16.8 %) and films (0.8 %); the most common colour of anthropogenic particle was blue (70 %), followed by red (11.8

%) and black (6.5 %); and the longest dimension of particles and fibres ranged from 24 to 11,400 μm, with a mean throughout all samples of 1218 μm (median 732 μm).

Accounting for contamination in procedural blanks, the mean number of anthropogenic particles, including semi-synthetic, cotton and petroleum-based polymers, ranged from 10.7 to 20 particles per 10 g (Table 2), equating to 1070–2000 particles kg^{-1}.

3.2. Particle characteristics

Fibres were predominant in all samples regardless of feed origin. When comparing fibre prevalence between samples, only sample LT94a and LT94b were significantly different (Kruskal-Wallis/Dunn test, $P < 0.05$). Only one anthropogenic particle fragment (comprised of polyamide) was identified across all soybean meal samples, compared to an average concentration of 1.0–5.3 fragments per 10 g fishmeal sample; statistical analysis revealed significantly (Kruskal-Wallis/Dunn test, $P < 0.05$) more fragments in Pv66a, Pv66b, LT94b, and Krill meal when compared with the soybean meal. When comparing LT94a/LT94b and Pv66a/Pv66b a difference in the number of anthropogenic particles can be observed, with LT94a containing considerably more fibres and less fragments than LT94b. Pv66a has a similar number of fragments but more fibres on average than Pv66b.

Petroleum-based polymers were identified in all feeds tested, with the number of particles and the number of different polymer types identified varying between samples (Fig. 1). In total, 18 different petroleum-based polymers were identified. In order to simplify results, the most commonly identified microplastic pollutants were split out (polyamide, polyester, polyethylene, polypropylene and polystyrene), while the rest of the plastic polymers were identified as 'other'. This category included plastics such as polyvinyl chloride, polytetrafluoroethylene (PTFE), epoxy and alkyd urea resins, and copolymers (see Supplementary Tables 1 and 2 in Appendix for full details of all polymers identified in each sample). Polyester was the most common petroleum-based polymer identified in the samples, being present in all samples except for CP70 at a concentration of 66.7–633.3 particles kg^{-1}. The least diversity in polymer type identified within the sample is observed with the soybean meal sample, which only had particles from four of the polymer categories used here (polyamide, polyester, semi-synthetic and other); marine meals contained polymers from 5 to 9 categories.

Semi-synthetic polymers were found in all samples tested and ranged from 8 to 73 % of all particles identified (Figs. 1 & 2). Semi-synthetics were most predominant in LT94a, Krill and soybean meal, where they represented >50 % of the total number of anthropogenic particles identified. Cotton was found in all samples except for soybean meal, and was most prevalent in CP70, where it represented 53 % of the total particles identified. Petroleum-based polymers were the most prevalent in all other samples. Cotton contamination ranged from 0 to 700 particles kg^{-1}, with semi-synthetic contamination varying from 133 to 1467 particles kg^{-1} and contamination by petroleum-based plastics ranging from 267 to 1267 particles kg^{-1}.

Table 2
Anthropogenic particles identified in feed meals of different origin. Mean results (n = 3) with result range displayed in brackets. Refer to Table 1 for detail on aquaculture feeds.

Aquaculture feed meal	Mean particles per 10 g replicate	Mean fibres per 10 g replicate	Mean fragments per 10 g replicate	Mean films per 10 g replicate
LT94a	20.0 (8–35)	18.3 (8–33)	1.7 (0–3)	0.0
LT94b	10.7 (6–16)	5.3 (1–13)	5.3 (3–9)	0.0
Pv66a	14.3 (9–17)	11.0 (9–13)	3.3 (0–6)	0.0
Pv66b	11.0 (10–12)	7.3 (4–9)	3.3 (1–6)	0.3 (0–1)
CP70	13.0 (1–31)	11.0 (0–29)	2.0 (1–3)	0.0
WF	14.7 (7–26)	12.7 (5–23)	1.7 (1–3)	0.3 (0–1)
S&A	12.7 (7–16)	11.3 (7–14)	1.3 (0–2)	0.0
Sq	11.3 (7–15)	10.0 (5–14)	1.0 (0–2)	0.3 (0–1)
Kr	13.3 (9–19)	11.0 (7–16)	2.3 (2–3)	0.0
Soy	12.3 (8–17)	12.0 (8–17)	0.3 (0–1)	0.0

3.3. Size fractionation of particles

The total number of particles captured on each mesh did not correlate with mesh size and was not consistent between samples (Fig. 2). This was also the case for the number of petroleum-based plastics, semi-synthetics and cotton particles identified on each mesh. The number of fragments identified correlated with mesh size in five out of the ten sample types (LT94a, LT94b, Pv66a, Pv66b, CP70), with decreasing numbers of fragments identified with decreasing mesh size. The number of fibres showed no correlation to mesh size, and not enough films were identified for trends to emerge. The size of particles captured also did not correlate with mesh pore size (Fig. 3). The 25 μm mesh captured fibres with lengths up to 1700 μm, which had passed through both the 100 μm and 63 μm meshes. This may be because, while fibres are measured by their longest dimension (length), they are very small in diameter and have the capacity to pass through larger mesh sizes lengthways (Barrows et al., 2017; Covernton et al., 2019). The diameter of a subset of microfibres from sample LT94a were measured and ranged from approximately 10–30 μm, many of which would pass through a 25 μm mesh if oriented appropriately. Fragments >100 μm were also identified on the 25 μm meshes in this study, despite having been passed through the 100 μm and 63 μm meshes; this phenomenon can occur because: (a) particles with a large axial ratio may permit them to pass through coarse meshes when orientated in a certain position (in a similar way to fibres); and (b) owing to inconsistency in mesh pore size across a filter that may be exacerbated by pressure from the vacuum pump pulling fragments through mesh pores during filtration.

3.4. Lifetime exposure of salmon to anthropogenic particles through aquaculture feed

Using our results, we estimated anthropogenic particle exposure via aquaculture feed for farmed Atlantic Salmon. Atlantic Salmon have a feed conversion ratio (FCR) of approximately 1.1, meaning that they require 1.1 kg feed for 1 kg biomass gain. Aquaculture feeds are variable in biomass content, with fishmeal, fish oil, plant-based meal and meal from other origins (e.g. poultry) all used in different proportions by different producers for different species. The latest figures from some producers show fishmeal making up 15 % of Atlantic salmon feed (Mowi, 2021), and though soybean meal content in aquaculture feed is also highly variable, prior research has shown up to 20 % soybean content within feed caused no observable difference in Atlantic salmon health (Olli et al., 1995). Atlantic Salmon are frequently grown to a size of 4–5 kg before harvest (Cohen et al., 2016; Davidson et al., 2016). With a diet comprising 15 % fishmeal (0.66–0.83 kg) and 20 % soybean meal (0.88–1.1 kg), we calculate Atlantic Salmon will be exposed to 1788–3013 anthropogenic particles throughout their commercial lifespan from aquaculture feed, with 706–1660 particles from fishmeal and 1082–1353 particles from soybean meal.

4. Discussion

Anthropogenic particles, including microplastics and cellulosic microfibres, were identified in all aquaculture feeds tested, with an average of 1070–2000 anthropogenic particles kg^{-1} across fishmeals and soybean meal. In other studies, mean microplastic content in fishmeal ranges from 0 to 10,000 particles kg^{-1} (Gündoğdu et al., 2021; Karbalaei et al., 2020; Thiele et al., 2021; Wang et al., 2022; Yao et al., 2021). The orders of magnitude difference in microplastic and anthropogenic particle concentrations may stem from high variability in the source material and heterogenous particle distributions within aquaculture feeds; this is evident within our study, where anthropogenic particle concentrations from the same type of fishmeal sourced from two

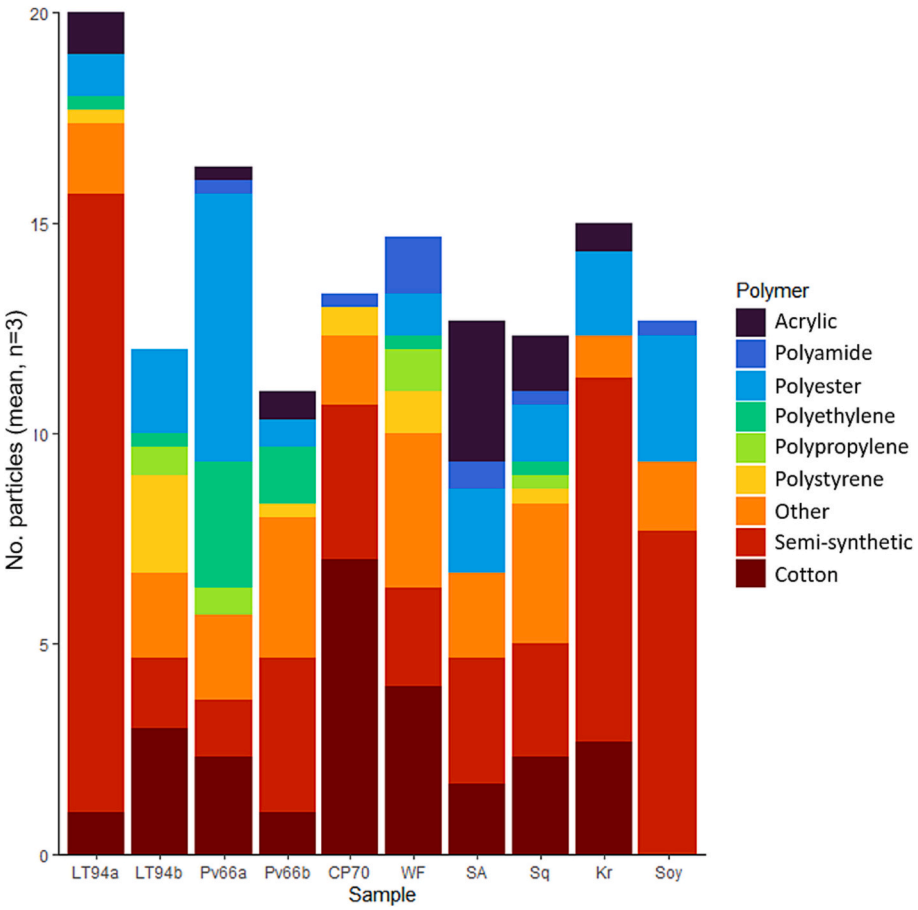

Fig. 1. Polymer composition of identified anthropogenic particles within each sample (mean per 10 g replicate, n = 3). Refer to Table 1 for detail on aquaculture feeds.

different suppliers (LT94a/b) contained the lowest and highest particle concentrations observed. Inter-laboratory comparisons may be further compounded by methodological differences in extracting and enumerating anthropogenic particles in complex organic substrates (Lusher et al., 2017). For example, the use of larger pore size filters can preclude the capture of microfibres (Athey and Erdle, 2022; Lindeque et al., 2020). This is illustrated in the difference in microfibre prevalence between our study (82.5 % total particles) which utilises a minimum filter pore size of 25 μm, and that of Hanachi et al. (2019) (6 % total particles), who used a filter pore size of 149 μm. Particle capture rates depend upon the shape of the particle and the shape of the filter pore (Lees, 1964a, 1964b; Lees and Sherigold, 1965). The smallest cross-sectional area is the most important determining factor, and in the case of sediment grains the longest dimension of the particle usually has little effect on whether the particle will pass through any given hole (e.g. Fernlund, 1998; Fig. 1). This means that prolate and rod shaped particles tend to pass through the holes in a sieve according to their intermediate diameter. Our results demonstrate a similar process occurs during filtration of anthropogenic particles; fibres of over 1000 μm length and fragments with highly heterogeneous morphologies were able to pass through filters of 63–100 μm pore size. This has important implications for the extraction of different shaped microplastics from the environment, and demonstrates the importance of using small pore size filters and sequential filtration to improve microfibre capture rates. Due to these methodological limitations we surmise that the number of anthropogenic particles identified in studies such as this will almost always be conservative.

We identified microplastics in all feeds tested; conversely, Gündoğdu et al. (2021) identified no microplastics within fishmeal derived from Antarctic Krill, and Hanachi et al. (2019) identified no microplastics in soybean meal. In this study, semi-synthetic and/or cellulosic microfibres were also identified in all types of aquaculture feed, making up >50 % of the anthropogenic particles in krill and soybean meals. However, cellulosic microfibres were not investigated in detail in other studies examining aquaculture feed. For example, Gündoğdu et al. (2021) characterised particles using Raman spectroscopy and compared results with the spectra of 13 commercially-available materials including cellulose, but did not include any cellulosic particles in their results; while Hanachi et al. (2019) identified low levels of rayon within salmon, sardine and kilka meal (4 % total particles) but not in soybean meal. Numerous studies describe challenges in the identification of semi-synthetic particles owing to difficulties in differentiating naturally-occurring and anthropogenic cellulosic fibres using spectroscopy (Dris et al., 2017) and issues of contamination (Halstead et al., 2018). There is often a perception that semi-synthetic plastics may pose less of a risk to the natural environment, compared with synthetic plastic, given their comparatively faster degradation times (Henry et al., 2019; Ladewig et al., 2015; Zambrano et al., 2020, 2019). However, their prevalence in aquaculture feed demonstrates that semi-synthetic polymers and cellulosic microfibres (e.g. cotton) may enter marine food webs irrespective of their biodegradability. We advocate that where feasible, microplastics research should also consider the prevalence, fate and biological effects of these anthropogenic particles.

Irrespective of source material, aquaculture feeds contained similar levels of anthropogenic particles, with fishmeal containing an average of 1070–2000 anthropogenic particles kg^{-1} and soybean meals containing an average of 1230 anthropogenic particles kg^{-1}. Nanoplastics and very small microplastics ≤2 μm could potentially contaminate plant vascular

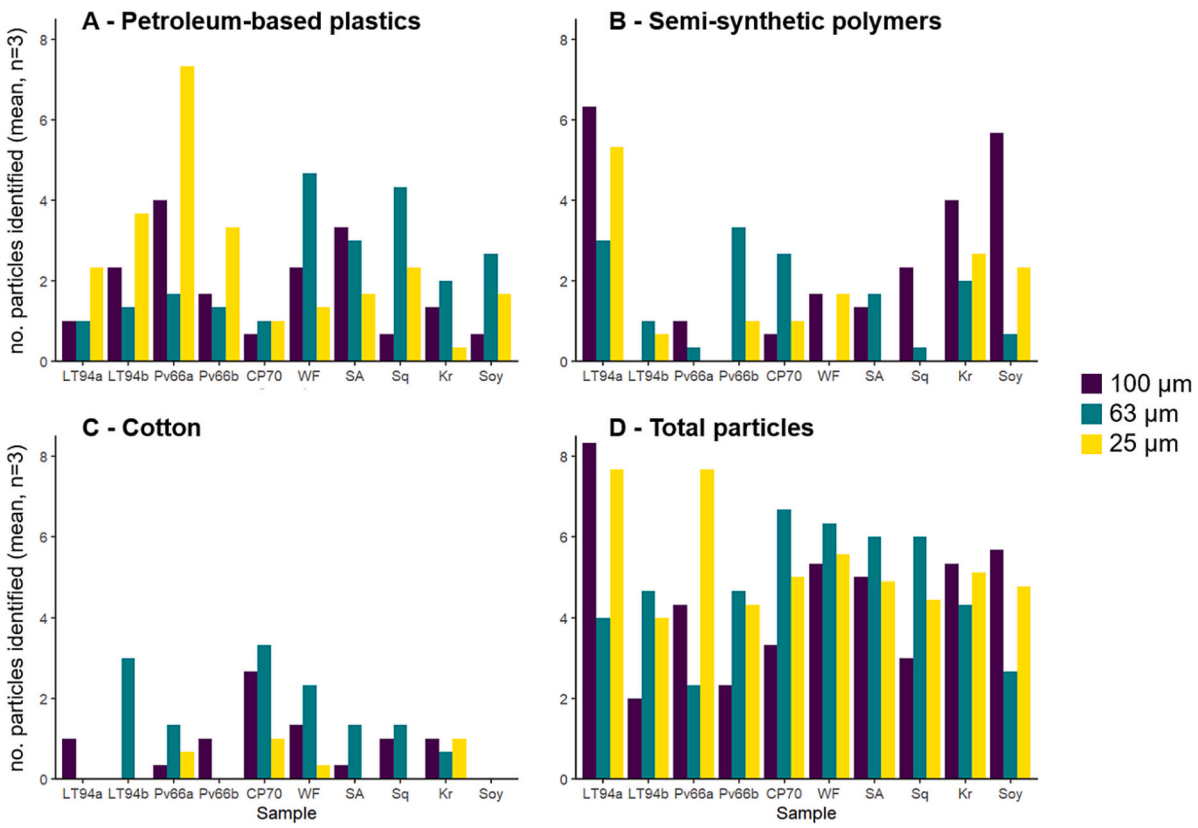

Fig. 2. Mean number of particles identified of each category (petroleum-based plastics, semi-synthetic polymers, cotton, and total of all particles) on 100, 63, and 25 µm pore size filters for each sample (n = 3). Refer to Table 1 for detail on aquaculture feeds.

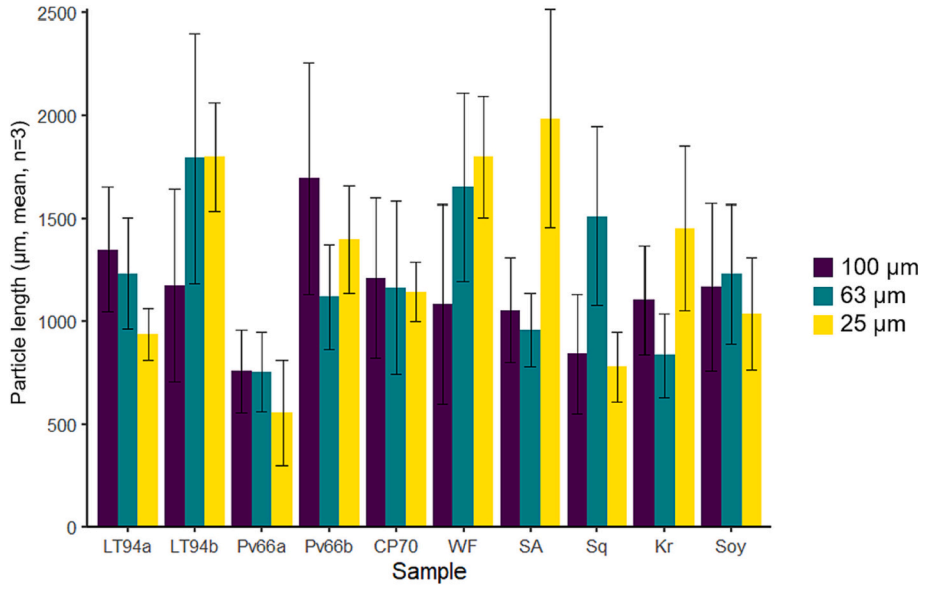

Fig. 3. Mean length of particles (calculated as largest dimension in µm) identified on 100, 63 and 25 µm pore size filters for each sample (n = 3, error bars = standard error). Refer to Table 1 for detail on aquaculture feeds.

systems via the apoplastic space in plant root cells (Azeem et al., 2021; Li et al., 2020). However, while particles could adhere to the external surfaces of plants (Mateos-Cárdenas et al., 2019), there is no indication that anthropogenic particles in the size range observed in soybean meal (24–11,400 µm) can directly contaminate vascular plants. Therefore, we surmise that the anthropogenic particles identified in soybean meal will have stemmed from post-harvest contamination. Given microplastics are widely evidenced in marine organisms (Collard et al., 2017; Lusher et al., 2013; Tanaka and Takada, 2016; Welden et al., 2018), we had anticipated fishmeals would contain higher levels of anthropogenic particles compared with soybean meals. While we demonstrated the types of anthropogenic particles differed between fishmeal and soybean meal, there was no significant difference in anthropogenic particle concentrations between feeds of different origin. It is possible that

anthropogenic particles present within source material (i.e. fish tissues) were broken-down or destroyed during manufacture, either through mechanical abrasion or combustion owing to the use of high temperatures (up to 500 °C in direct air drying) during desiccation (Hertrampf and Piedad-Pascual, 2000). The melting points of plastics, including polyamides, polyethylene and polystyrene, range 170–290 °C, meaning they would be subject to degradation during processing. However, some fishmeals (e.g. LT94), are cooked at temperatures of 90–100 °C, and yet did not display significantly higher levels of anthropogenic particles compared with other types of fishmeal. Future studies may wish to consider the prevalence of toxic by-products, including higher ringed PAHs, free radicals and toxic heavy metals, that are emitted by anthropogenic particles during combustion (Simoneit et al., 2005; Valavanidis et al., 2008). Based on our data, we conclude that post-harvest contamination is the predominant source of anthropogenic particles in aquaculture feed.

We estimate that farmed Atlantic Salmon will be exposed to 1788–3013 anthropogenic particles via fishmeal and soybean meal over their commercial lifespan. However, farmed finish may also be exposed to anthropogenic particles through other feed ingredients, for example fish oil and other vegetable- and animal-based products, as well as their natural environment. Wang et al. (2022) estimated that farmed Atlantic Salmon consume 9361 microplastic items over their commercial lifespan; differences in exposure data can be explained by Wang using a higher feed conversion ratio (1.2 compared with 1.1 used here) and assuming a higher proportion of fishmeal used in the salmon's diet (42 % compared with 15 % used here). In recent years, the proportion of fishmeal used in aquaculture diets has been decreasing in response to limited supply (Olsen and Hasan, 2012), concerns about ecosystem health and overfishing (Brunner et al., 2009; Deutsch et al., 2007), increasing costs (Tacon and Metian, 2008), and the development of alternative feeds (Bandara, 2018; Ferrer Llagostera et al., 2019; Hemaiswarya et al., 2011; Lock et al., 2018; Rust et al., 2011). In 2020, up to 70 % of the diet of farmed salmon may be composed of plant-based meals (Mowi, 2021); as other plant-based materials are likely to have undergone similar processing steps as soybean meal, we hypothesise that these feeds will also contain anthropogenic particles. In addition to exposure through their feed, farmed fish are exposed to anthropogenic particles present in seawater (Auta et al., 2017; Luo et al., 2019), and stemming from airborne deposition (Roblin et al., 2020; Szewc et al., 2021), workers' clothing (De Falco et al., 2020) and aquaculture equipment (Chen et al., 2021; Floerl et al., 2016). The consumption of anthropogenic particles by finfish may have profound consequences for farmed populations; for example, there is growing evidence that microplastics can negatively affect growth and reproductive output (Galloway et al., 2017), which in commercially-exploited species could result in longer time-to-market and decreased commercial and nutritional value (Walkinshaw et al., 2020). Following consumption, anthropogenic particles such as microplastics are often passed through the gastrointestinal tract and excreted through faeces (Ory et al., 2018; Spanjer et al., 2020), in which they will sink through the water column (Cole et al., 2016). In open cage aquaculture facilities, this may lead to hotspots of anthropogenic particles in the benthos directly beneath aquaculture facilities, which may results in environmental perturbations for underlying benthic communities (Coppock et al., 2021).

Currently, studies show conflicting results regarding whether farmed or wild marine organisms contain more microplastics (Digka et al., 2018; Ding et al., 2018; Gomiero et al., 2020; Li et al., 2018, 2016, 2015; Phuong et al., 2018). It is clear from the study presented here that aquaculture feeds contain anthropogenic particle contaminants, however we do not yet know the additional risk this presents to farmed organisms. Farmed salmon would not only be exposed to anthropogenic particles within their feeds, but also from their surrounding environment, and from contamination during harvest and processing. Further research is required to investigate whether the number of anthropogenic particles ingested by farmed animals through their feed has an effect on apical endpoints which may pose a risk to food security. The sampling of feed material and farmed fish from the same aquaculture sites may shed light on the additional exposure risk from contaminated aquaculture feeds, enabling us to consider the effects of contaminated feed on not only farmed fish health, but on nutritional value and human health.

5. Conclusion

All aquaculture feeds tested contained microplastic and semi-synthetic particles, with 90 % of the samples also containing cotton microfibres. As both animal- and plant-based feeds contained high concentrations of anthropogenic particles regardless of feed origin, we consider it likely that the majority of particles and fibres stem from post-harvest contamination. Contamination of aquaculture feed with anthropogenic particles adds an additional exposure route for farmed species with potential consequences for fish health, and risks to nutritional value, profitability and ultimately food security.

CRediT authorship contribution statement

Chris Walkinshaw: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Trevor Tolhurst:** Supervision, Writing – review & editing. **Pennie Lindeque:** Supervision, Writing – review & editing. **Richard Thompson:** Supervision, Writing – review & editing. **Matthew Cole:** Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.114189.

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