UiT The Arctic University of Norway

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Microplastics Accumulation in Recirculating Aquaculture Systems (RAS)

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Abstract

It has in recent years been a shift in the land-based aquaculture production from flow through systems (FTS) to recirculating aquaculture systems (RAS). With emerging use of RAS, it is necessary with knowledge regarding potential issues, whereas one potentially increasing problem is accumulation of microplastics (MP) in the RAS water. The objects of this study were to determine MPs concentration in RAS water, if the MPs originated from within the RAS, and provide a size distribution of the particles. RAS water were sampled in triplicates from three different RAS at three different locations in Norway. The water for each RAS were sampled effluent of the fish tank, effluent of the drum filter, and effluent of the biofilter as well as make-up water and sludge. Bio-media from the corresponding RAS were also sampled. Organic material in the water samples were digested prior to filtration, and filters were stained with Nile Red for quantification. There were found MPs in all the RAS water samples with mean concentration of 61 MP/L at Sunndalsøra RAS, 371 MP/L at the commercial site RAS and 41 MP/L at Kårvika RAS. There were higher concentrations in the RAS water than in the make-up water for all locations, indicating that the MPs originated from within the systems. Sludge samples were only possible to quantify from Kårvika RAS, while sampling were not performed at the commercials site and MPs concentration were too high to perform a count in Sunndalsøra RAS. The sludge samples support that the origin of MPs are within the system, as more MPs leave then enters the RAS. Nuclear magnetic resonance (NMR) spectroscopy analyses gave almost identical signals as their reference, which were the corresponding bio-media. The MPs also showed indistinguishable similarities with polyethylene (PE), which the bio-media were made off. There were found no significant trends regarding concentration at the different sampling sites. All RAS had similar size distribution of the MPs with approximately 75% of the particles being less than 100 μm.

In conclusion, there were high concentrations of MPs in RAS which likely were due to shattering of the bio-media. Future research is necessary to determine an accumulation rate, but the high abundance of MPs proves the emerging issue regarding MPs. And although RAS has the potential to be the environmental winner within aquaculture, there still are opportunities for improvement.

1 Introduction

1.1 Aquaculture

It has been predicted that by year 2050 the human population will consist of over 10 billion people. An increase in mouths to feed which will require an increased food production, where the major limitation is believed to be proteins (FAO, 2022a). Food production in general are entering tougher times, with an increase in food insecurity. Climate changes are both changing how we produce food, and how we should produce food. A lot of the terrestrial farming is unfavourable because of large emission. Crop field are tougher to manage because of larger variations in climate. Marine aquaculture may require changes because of predicted increase of seawater temperatures (FAO, 2022b), and problems for freshwater aquaculture can potential increase due to more rapid events of extreme weather, droughts, increased climate variability, sea level rise and limitations of suitable areas (Galappaththi et al., 2020).

The yearly global consumption of aquatic animals are believed to increase with 24 million tonnes by 2030. Where some of the factors believed to stimulate the increase are rising incomes, urbanization, dietary trends with an increased focus on health, and the sustainability of the industry (FAO, 2022a; Klinger & Naylor, 2012). In 2020 there were cultured a total of 87.5 million tons of aquatic animals, approximately the same as the total amount of wild fishery the same year, at 90.3 million tonnes (FAO, 2022a). Fishery catches has been relatively stable since the 1980´s, and is believed to be surpassed from the aquaculture industry by 2030 (FAO, 2022a). As the wild fish stocks has limited exploitation, the increase must be within aquaculture production.

Norway had the same year a production of 1.5 million tons of aquatic animals, 45% of the European production (FAO, 2022a). The production were mainly consisting of the marine finfish Atlantic salmon (*Salmo salar*), where Norway contributes with almost half of the world production (FAO, 2022a). Norwegian production has had a steady increase with an average annual production growth of 12.5% in the period 1985-2020 (Afewerki et al., 2022), and it is projected with an continuous increase. It is estimated that there will be a production of 500 million smolts in 2030, a large increase from 179 million in 2008 and 344 million in 2018 (Bergheim et al., 2009; Meriac, 2019).

1.1.1 Problems today

Since the beginning of the modern Norwegian aquaculture industry, the most common type of production has been with flow through sea pens in the fjords (Afewerki et al., 2022). In the beginning it was open systems which was rather small and simple. Over time the pens has been modernised, with material shift from wood to steel, and now a day mostly PVC (polyvinyl chloride) and other plastic materials. With shift in material, it came the possibility for extension, and the common pens now a day are often 40 000 m^3, a vastly increase from predecessor of 80 m^3 (Afewerki et al., 2022). Flow through systems (FTS) in fjords assures good exchange of ambient water, natural lightning and the structures are sheltered for the worst weather. There were in 2022 1571 licenses on production of salmonids, with 95.9% of the grow out licenses and 79.7% of juvenile licenses in use (Directorate of Fisheries, 2023a, 2023b). During the last few years, the increase in production has gradually stagnated with an average annually growth of 4.1% in the period 2010-2020 (Afewerki et al., 2022), mainly because of environmental induced restrictions. There are several reasons why there are strict regulations, and why the regulations are necessary. The pens can function as large reservoir for pathogens, viruses, and parasites. With the salmon lice (*Lepeophtheinus salmonis*) as the functional environmental indicator for biomass regulations (Bøhn et al., 2022). The maximum allowable biomass of salmon which is allowed to be farmed are determined by expected lice-induced mortality of post-smolt salmon (Bøhn et al., 2022). Another large issue for the wild fish stocks are escapees, which contributes with competition for breeding in the rivers, which can lead to genetically intermixing. Both reducing the fitness and productivity of the offspring, and genetically polluting the wild salmon stocks (Olaussen, 2018), which in Norway are of cultural importance. Issues as genetically pollution and transmitting of disease re not only a problem for salmonids. Other farmed species as Atlantic halibut (*Hippoglossus hippoglossus*) and Atlantic cod (*Gadus morhua*) are also facing the same problems, even if the production in Norway are much smaller (Olaussen, 2018).

Smolt production, which is the growth of salmonids in freshwater until their seawater phase has commonly been land based in Norway because of presence of rivers close to the sea providing continuous flow through of freshwater for the production. In 2000 about 250 of 260 hatcheries were land based, while the restoring ten were cages in lakes (Bergheim & Brinker, 2003), and in 2006 all smolt production were produced in land based FTS (Bergheim et al., 2009). To be able to increase the production, without increasing the environmental stress, new ways of production must be envisaged and adopted. There are a couple future pathways for

aquaculture which may facilities increased production regarding environmental impact, offshore sites, semi-closed systems in fjords, aquaponics, integrated multitrophic systems and recirculating aquaculture systems (RAS) (Klinger & Naylor, 2012).

1.2 Recirculating aquaculture systems

1.2.1 Description and usage

RAS stands for *Recirculation Aquaculture System* and is often classified as systems with water exchange rate off < 1 m^3/kg feed (Martins et al., 2010). Which is a rate suitable for robust species, as Nile tilapia (*Oreochromis niloticus*) which can have an water exchange as low as 0.030, European eel (*Anguilla anguilla*) 0.3, and African catfish (*Clarias gariepinus*) 0.1 m^3/kg feed (Martins et al., 2009a). For less robust species, as salmonids, the water exchange is commonly higher, 0.37-0.59 (Davidson et al., 2023), 0.77 and 7.69 (Good et al., 2014), 4.75 (Kolarevic et al., 2014) and 0.69–1.58 m^3/kg feed (Mota et al., 2022). It is by many regarded as a potential solution to increase the aquaculture production without increasing environmental stress (Martins et al., 2010). In Norway there has been land based production of smolt for a long time, but with traditional FTS. In the recent years there has been a distinct shift from the traditional FTS to use of RAS (Mota et al., 2022), as it was believed that in 2019 that 70% of the salmon in the sea pens at all times originated from RAS (Meriac, 2019), in contrast to 2008 when 1-2% originated from RAS (Bergheim et al., 2009). Faroe Islands had for comparison 100% of their smolt production in RAS already in 2008 (Bergheim et al., 2009). The increased interested of RAS in Norway started for smolt production, and it was because of temperature control, possible freshwater limitation during draughts, inlet water having low alkalinity (low amount of calcium), development of technology for disinfection and stripping of CO_2 and expected advantages with RAS in the future (Dalsgaard et al., 2013).

A simplified sketch of a standard RAS is shown in Figure 1. RAS is a closed system where the water is recycled and used again through several steps of filtration and treatments (Helfrich & Libey, 1991). It is usually several fish tanks connected to one RAS, but it can also be singular tanks integrated to a smaller RAS. The first step in the water recycling process after the fish tank is the mechanical filter, either drum or belt filter, where large particles as excess feed, mucus, scales, and faeces are filtrated out of the system. Drum filter with a filter cloth of 40 – 100 μm is the most common (Bregnballe, 2015). It is important with sufficient removal of substance, as increased material in the water has been proved to lead to

a higher egg mortality, reduced hatching percentage and reduced larvae length on the common carp (*Cyprinus carpio*) (Martins et al., 2009b). High volumes of suspended solids in the water can also lead to a bad habitat for the biofilters (Xiao et al., 2019). For removal of smaller particles, a protein skimmer or a polishing filter can be present. The cleaned water enters a biofilter consisting of bio-media with nitrifying bacteria which are oxidating ammonia and nitrate. The bio-media, which are plastics structures, are either fixed or free moving in moving bed biofilm reactors (MBBR) (Bregnballe, 2015).

After the biofilter there are degassing, removal of excessive CO_2 produced by both fish and microbiota, as well as oxygenation as both fish and microbiota are using oxygen. It is also common with UV-filters for disinfecting the water. The water then completes the loop and enters the fish tank again. Even though the water is recycled there is still need of new water due to evaporation, spilling, biomass growth and loss during filtration. The new water, make-up water, is usually added into the biofilter. The make-up water undergoes both mechanical and UV filtration before entering the system.

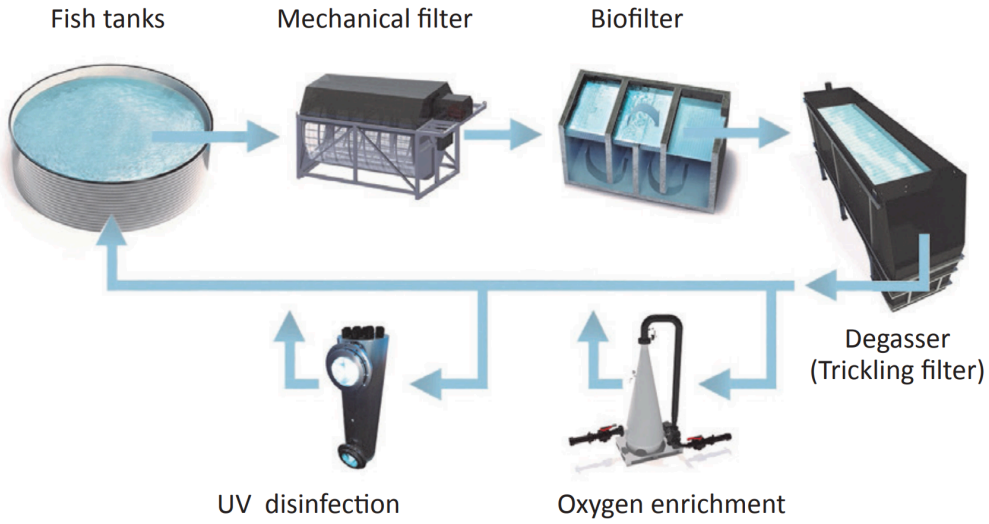

Figure 1 Schematic representation of the basic principles of a RAS. Figure retrieved from Bregnballe (2015).

1.2.2 Advantages

In Norway there are regional restrictions regarding maximum biomass of fish in the sea. With RAS it is therefore possible to increase the total yearly production due to increased time the fish spend on land, or have the entire production on land. The production can be continuous independent of seasons, as the colder winter months in Norway are less ideal for production. Land based facilities are often connected to rivers, and by using flowing well water the need

of domestic tap water is reduced. During draughts or wintertime with less flowing water, production in the traditional land based FTS can be limited to water shortage. With an implemented RAS, the water usage can be as low as 0.016 m^3/kg fish produced (Tal et al., 2009), compared to a flow though systems which may use as much as 30 m^3/kg. This can provide a much more stable platform during less ideal conditions. In Norway the water consumption may not be a large issue, but by reducing the water usage it is possible to create large scale facilities in areas with less water available, where FTS are not possible. By controlling water quality and abiotic factors, it is possible to optimize growth, feed efficiency, reduce accessibility for pathogens, and decrease the mortality rate. Some of the factors which rather easily can be controlled and utilized are temperature, salinity, pH and dissolved O_2 and CO_2 (Dalsgaard et al., 2013). Comparisons of RAS and FTS regarding growth and welfare has shown to be ideal predictors for RAS. With rainbow trout (*Oncorhynchus mykiss*) proved to grow better in RAS compared with FTS (during identical conditions), with a 17% higher weight after 77 days of rearing, while reducing water consumption by 95% (Roque d'Orbcastel et al., 2009). A survey on Atlantic salmon did not show any improvements of growth and fish mortality, neither worse, but had a 98% water reduction in RAS compared to FTS (Kolarevic et al., 2014).

1.2.3 Challenges

In a review by Badiola et al. (2012) it was concluded that the main issues regarding RAS were biofilter, waste of solids and management. Unlike FTS, unwanted particles will not be removed passively with the exchange of water, they must actively be filtrated out through filters. One of the issues by re-using water can be accumulation of particles too small to be filtered out, as for instant microplastics (MP). A lot of the components in RAS are made off plastics as piping, tanks and filters, all potential origin sites of MPs.

1.3 Microplastics

1.3.1 What are they?

Plastics are anthropogenic polymers, and particles 0.001 > 5 mm are often defined as microplastics (Kershaw, 2015). MPs can either be primary or secondary (An et al., 2020; Cole et al., 2011). Primary MPs are small particles produced on purpose. It is often used in cosmetics and personal care products (Fendall & Sewell, 2009), as granulates on sport fields with artificial turf (An et al., 2020) and as virgin plastic pellets (An et al., 2020). Secondary MPs, which are degenerated plastics from larger items, as fibres from fleece clothing which

can enter marine systems through wastewater, or old plastics, as plastic bags disintegrating (An et al., 2020). It is believed all plastics ever produced are still intact, either as macroplastics or debris fragmented into MPs or nanoplastics (Thompson et al., 2005).

The most prominent mariculture derived polymers are polyethylene (PE), polypropylene (PP) and polystyrene (PS). Where the PE is the most distributed polymer on a world basis with almost 30% of total polymer production (Iheanacho et al., 2023).

1.3.2 Their potential effect in RAS – why it is negative

One of the issues with MPs, which is not present with macroplastics are that MPs due to its small size can penetrate the cell membrane, or carry small chemicals which can do it, and enter the endocrine system (Teuten et al., 2009). The chemicals can be divided into two groups. Small molecules with origin from the plastics itself (Teuten et al., 2009), and adsorbed chemicals from the surrounding. MPs can be suitable of adsorbing from the surroundings because they often has a relatively large and hydrophobic surface (Cole et al., 2011).

MPs can be problematic both directly, and indirectly. Indirectly, MPs can provide as a stable colonization platform for microorganisms (Reisser et al., 2014). The MPs platform can serve as a vector for harmful bacteria, as they can grow and make a biofilm on the particles (Zhang et al., 2020). Zhang et al. (2020) investigated occurrence of antibiotic resistant bacteria on MPs in RAS and found 100-5000 times increase compared to the water which the medium were taken from. The MPs consisted of polyethylene terephthalate (PET), and most likely originated from the biofilters.

If MPs are to accumulate in fish, it can lead to malnutrition and starvation. For smaller fish it can also affect buoyancy (Boerger et al., 2010). In a study by Qiao et al. (2019) zebrafish (*Danio rerio*) were exposed for PS-MPs for 21 days. It was observed inflammation and oxidative stress, and a significant alteration within the gut microbiome and the gut tissue (Qiao et al., 2019). Atlantic salmon can also be affected by MPs, immune cells were proved to phagocyte (6% of the cells) both PE and PS in the range of 1-5 µm. The uptake were concentration dependent, but there were determined phagocytoses even during rather low concentrations (Abihssira-García et al., 2020).

There has been documentations of MPs in both RAS and associated with common flow though sea pens (Gomiero & C., 2020; Lu et al., 2019). Some examples of documented MPs

observations in relation to mariculture are listed in Table 1. Since a large proportion of the MPs in marine environments are due to wear and tear from macroplastics, it is naturally to believe RAS can contribute with MPs as its main constructional material are plastics, and the plastics are exposed to a continuous movement of the water. Although bio-media in moving beds can be of different shape, they are all designed to have a large surface area to be able to have an equivalent large biofilm. The structure makes them light and fragile, and the water currents leads to a continuous movement of the large number of bio-media in their enclosed basins. Bio-media in moving beds are therefore very prone to movements, and may release fragments off MPs (Lu et al., 2019).

Table 1 Selection of different MPs concentrations measured in relation to mariculture. The table summarize which medium the samples were taken from, which polymer present, concentration of the polymer, origin of polymer and the paper the data is retrieved from.

Medium	Polymer	Concentration	Origin	Reference
Sediment	The most abundant polymer was Polyethylene (PE)	Approx. 110 MP/kg sediment (dry weight) close to the pen.	Believed to origin from the pens.	Gomiero and C. (2020)
Water	Polyethylene (PE). Only polymer with significant higher abundance than reference sample.	0.6 – 2.3 MP/L.	Believed to origin from the pens.	Gomiero and C. (2020)
Water retrieved from biofilter, fish-, and recycling-pond.	Polyethylene terephthalate (PET) at 200 – 2000 µm.	0.058 – 0.072 MP/m^3.	Likely from bio-media, as they were made of PET fibres.	Lu et al. (2019)
Sediment	Polyethylene foam (PE) and synthetic cellulose fibres.	1739±2153 MP/m^3.	36.8% of MPs originated from mariculture.	Chen et al. (2018)
Water	PE foam, majority 250 – 2000 µm.	8.9±4.7 MP/m^3 ≥ 333 µm.	55.7% of MPs originated from mariculture.	Chen et al. (2018)
Water	Polyethylene terephthalate (PET). Only fibres (max. 75% proportion of total MP) counted.	44 – 53 MP/m^3 ≥ 300 µm.	Mostly derived from filter materials from the RAS.	Zhang et al. (2020)

1.4 Objectives

The main objective of this study was to identify and quantify MPs (0.001 < 5 mm) in RAS. There is an increase in use of RAS in Norway (Mota et al., 2022), and with increased use it is important to provide knowledge of its potential risks and flaws. The first aim of the study was to identify and quantity MPs through water sampling at different sites in several RAS, filtration of the water, and staining the potential particles with Nile Red to ease the identification of the MPs. By knowing the quantity of MPs in each sample, the total amount

of water in the system and the water exchange rate it gives a possibility to predict the potential number of MPs in other RAS.

The second aim was to identify where in the RAS the potential MPs particles originated, by carry out a compositional analysis of the present MP. It is believed that the bio-media can be an origin for potential MPs (Zhang et al., 2020). In MBBR, they are in constants movement because of the water velocity which can lead to wear and tear and degenerated MPs. The third aim were to look at the size distribution though the systems, to see if MPs concentration varied between sites in each RAS due to filtration and potential origin of the MPs.

Question 1: Are microplastics present in RAS water?

H0: Microplastics are not present in RAS water.

H1: There are microplastics present in RAS water.

Question 2: Which RAS units are microplastics originated?

H0: No specific units.

H1: Much of the MPs originates from the bio-media in the biofilter unit (when moving bed).

Question 3: Is there a microplastics size distribution in RAS units?

H0: No trends to size distribution.

H1: Less larger particles after the drum filter, due to filtration.

2 Material and methods

2.1 Data acquisition

2.1.1 Nofima Sunndalsøra RAS

Sampling at Nofima in Sunndalsøra was performed on 22. and 23.09.2022. Samples were taken from three identical RAS from an ongoing project (FastWell), which examined how Atlantic salmon reacted to feeding pause. The RAS were MicroRAS from Landing Aquaculture, compact RAS with fish tank and RAS integrated in the same mainframe (flow scheme; Figure 2) mainly used for research purpose. The three RAS samples were all from

control groups and had not undergone any abnormal treatment. The sampling consisted of water samples from three different sites, sludge samples, fish samples and bio-media samples. The water was sampled in 10 L plastic canisters with use of one of two identical siphons filled with tap water to start a water flow. The tap water was then used for rinsing the bottom of the canisters and poured out. The three sites of sampling were effluent of the fish tank, effluent of the drum filter and effluent of the biofilter, at the sump. Field blank was an identical canister with unscrewed lid during one sampling. The sludge were sampled into 45 mL falcon tubes.

Fish tank samples were taken at an external opening in the fish tank (Appendix 1 A). The drum filter samples were taken from the water entering the biofilter (Appendix 1 B). The biofilter samples were taken after the biofilters, from sump 1 (Appendix 1 C). Sludge samples were taken from the drum filter backwash pump (Appendix 1 D). It automatically dispose sludge after a while, but during the sampling it was sampled manually by releasing sludge with a button. All RAS used the same make-up water, which was sampled prior entering the RAS (Appendix 1 E). All water samples were stored cold until analyses, while the sludge samples were stored frozen.

The biofilters were divided into two chambers with free water flow, and it was sampled five bio-media from each. Bio-media used at Kårvika and Sunndalsøra were RK BioElements (RK Plast AS, Denmark) produced in polypropylene (PE/PP) with barium sulphate ($BaSO^4$) as filler (Medium density 1.00 g/cm^3, surface area 750 m^2/m^3) (Figure 3). Fish were euthanized with an overdose of anaesthesia (Finquel®) on 23.09.2022 due to the FishWell project being finished. Three fish from each RAS were randomly selected and stored frozen in case of analyses.

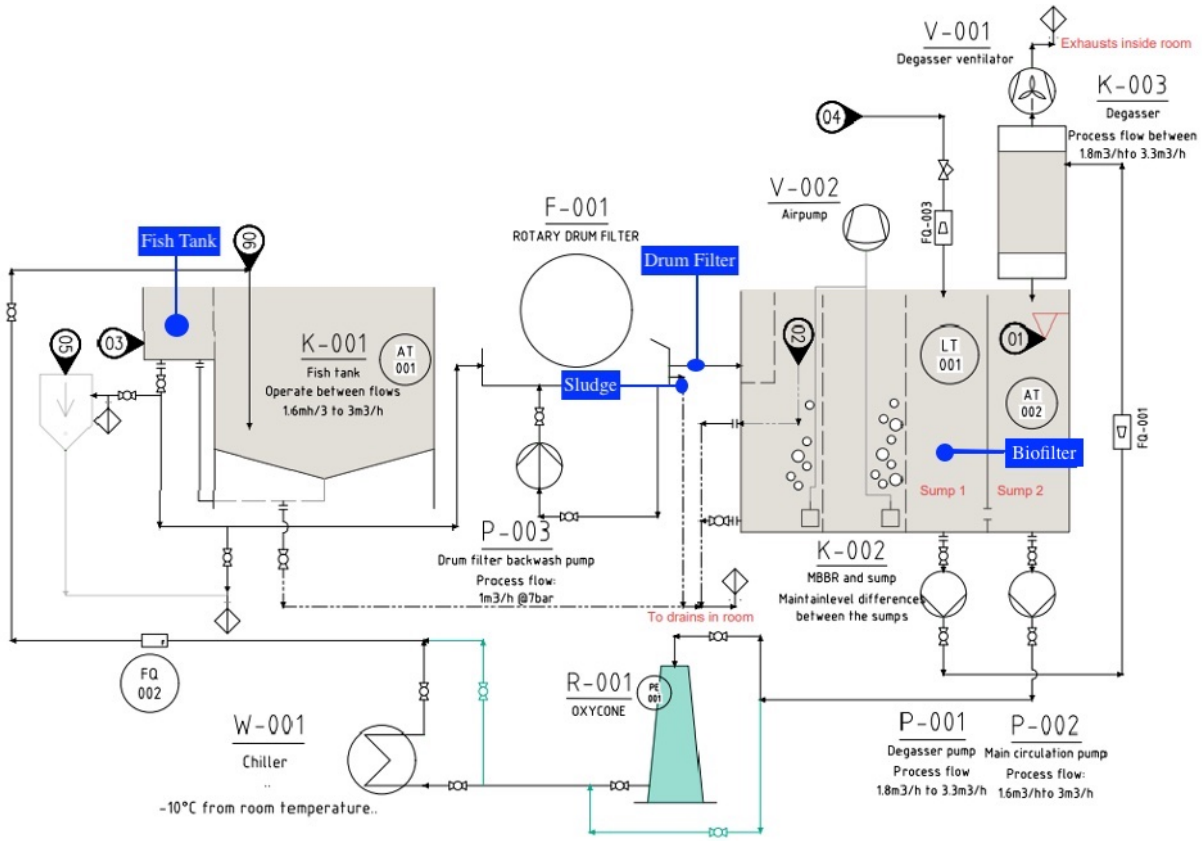

Figure 2 Flow scheme of the RAS at Sunndalsøra, with additional marking of the sampling sites.

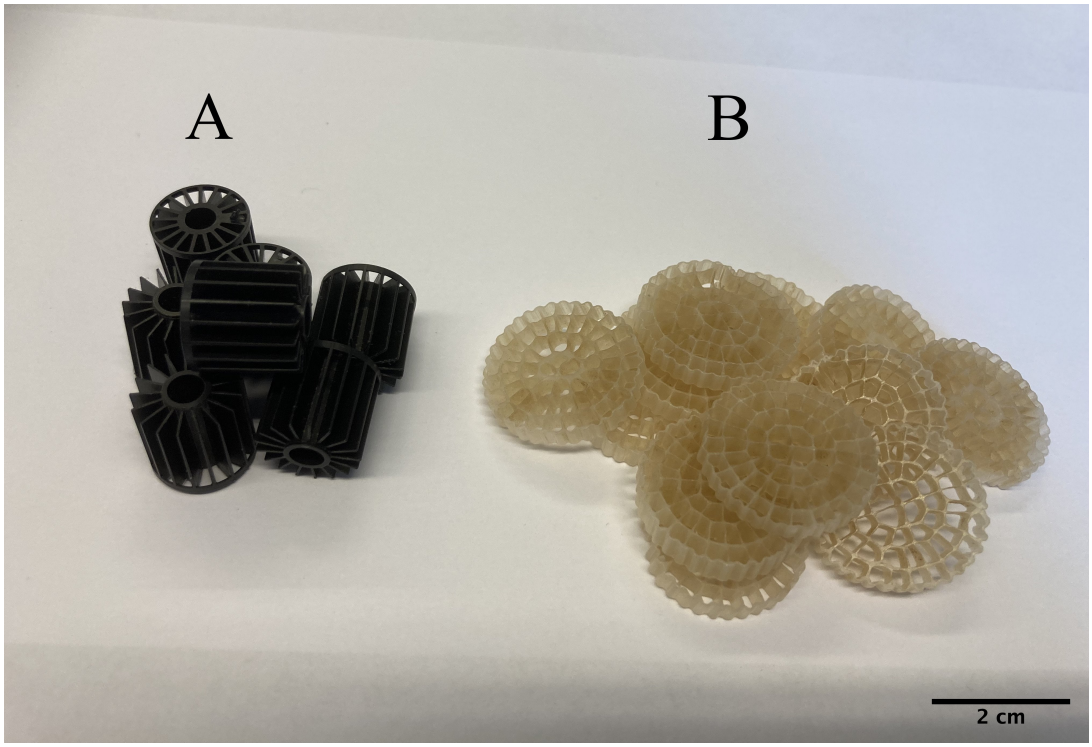

Figure 3 A: Bio-media used at Sunndalsøra RAS and Kårvika RAS. B: Bio-media used at the commercial site.

2.1.2 Commercial site RAS

Sampling at the commercial site was carried out on 08.11.2022, at a rather new facility opened in 2022. The commercial facility contained several RAS, all from hatchery to smolt production. They consisted of several fish tanks connected to each RAS, not integrated tanks within the RAS as in Sunndalsøra and Kårvika (see flow scheme; Figure 4). The water samples were collected in 1 L glass bottles with plastics lids. The bottles and lids were washed, then rinsed with distilled water prior to the sampling. Samples were collected in triplicates, at three different sites and from three different RAS. RAS 1 was start-feeding, RAS 2 was on growing and RAS 3 was smolt production.

Due to high biosecurity, the sampling bottles were not permitted into the RAS facility, the water samples were taken with a measure cup (1 L), poured over to another measure cup (1 L), then transferred to the sampling bottles. Both measure cups were rinsed with water from the sites to prevent possible MPs contamination between the sites. Different measuring cups were used at the different RAS, but they were all made of unknow plastics.

The three RAS had close to similar values regarding water quality and water treatment units (Table 2), but had different rearing tanks regarding size and shape. For the three RAS the water were sampled effluent of the units. The water from the fish tanks met before entering the drum filter (Appendix 2 A), and the samples were taken posterior of the mixing. The drum filter samples were taken at the waterfall entering the MBBR (Appendix 2 B). And the biofilter samples were taken effluent of the MBBR (Appendix 2 C). Make-up water was not possible to sample at the different RAS and were instead sampled at the Hatching 2 facility, which had undergone the same filtration and treatment before entering the system (Appendix 2 D). Sludge from the drum filter was not possible to sample. Because the difference of the RAS the analyses and results from the three RAS were presented individually, and not combined as the data from RAS Sunndalsøra and RAS Kårvika.

Approximately ten bio-media were sampled from each RAS with the same measuring cup as the water samples, but were done last to prevent potential MPs contamination. Approximately three fish were sampled from each RAS, a few more at RAS 1 due to small fish size, and euthanized with overdose of anaesthetic. Field blanks were sampled during sampling at RAS 1, and feed was sampled from a feeder at RAS 3.

The samples were transported back to Tromsø and stored in a cold room. Fish, feed and bio-media were stored frozen. Two samples broke during transport, 3.3.1 and 3.3.2, both from the biofilter at RAS 3.

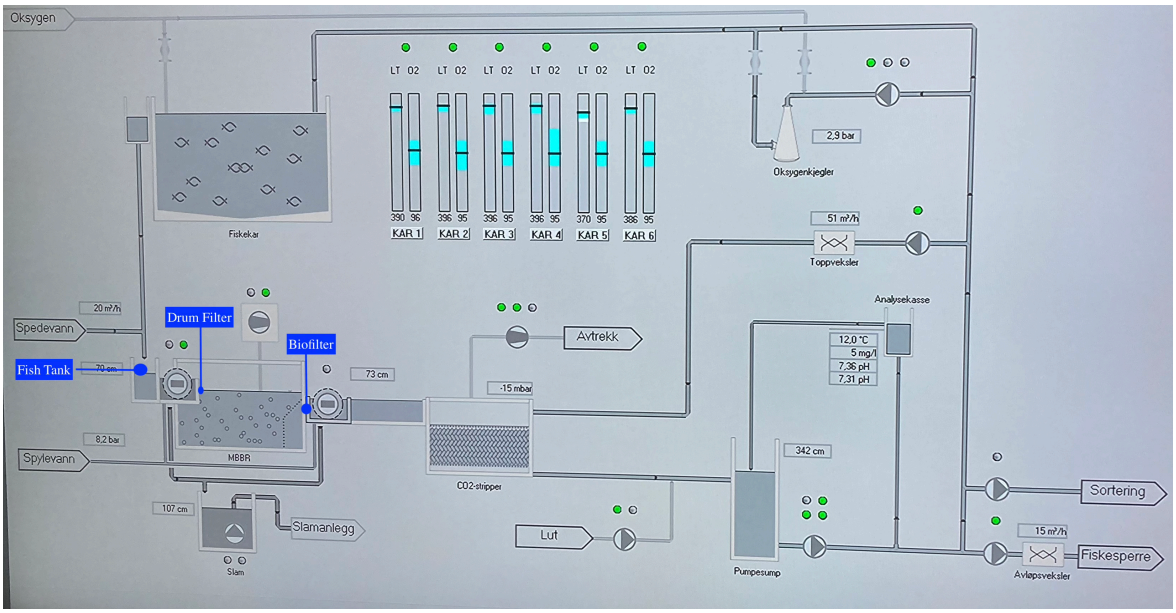

Figure 4 Flow scheme of RAS at the commercial site, with additional marking of the sampling sites.

2.1.3 Havbrukstasjonen in Tromsø RAS

Sampling at Kårvika were performed on 16.12.2022 at the RAS facility in the Fish-Health lab at an ongoing project (RASHealth). The three RAS samples were acquired from control groups and had not undergone any abnormal treatment. The RAS were research units with one fish tank integrated in each RAS (see flow scheme; Figure 5).

Samples were sampled into 1 L glass flasks pre-washed identically as the ones used for sampling at the commercial site. Water from the fish tanks were sampled from an outlet opened manually before entering the drum filter (Appendix 3 A). The water effluent of the drum filter was sampled from the outlet stream before entering the biofilter (Appendix 3 B). And the biofilter water was sampled directly from the pump sump (Appendix 3 B). Sludge water was sampled by unscrewing the piping, which may have led to debris from the pipes, and external pollution of the sampled water (Appendix 3 A). Sludge was not sampled from RAS 2 because of technical issues. All RAS shared inlet of make-up water and was taken prior to entering the RAS. Ten bio-media were collected from each RAS, the bio-media used were the same as from RAS Sunndalsøra (Figure 3). Three fish from each RAS were sampled by Vasco Mota on 28.12.2023, at the end day of the ongoing project. Water samples and sludge were stored cold, while bio-media, feed and fish were stored frozen.

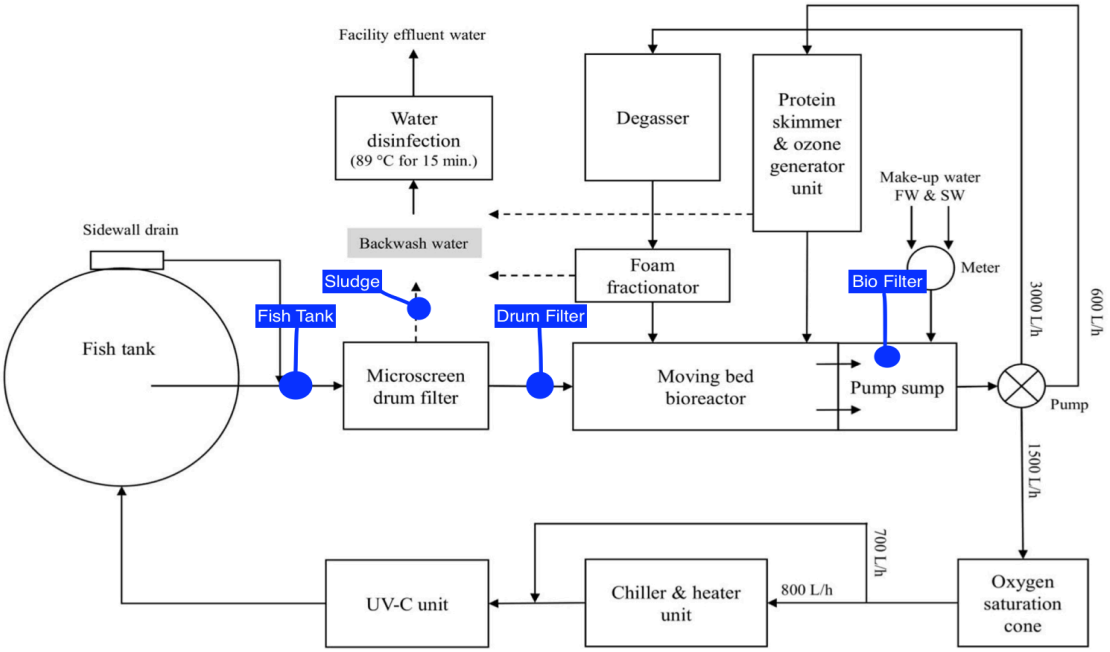

Figure 5 Flow scheme of the RAS at Kårvika, with additional marking of the sampling sites. Figure retrieved from Mota et al. (2022).

2.1.4 Water quality & RAS details

Water quality, information on rearing units and water treatment units present in the respective RAS are summarized in Table 2.

*Table 2 Rearing units, water treatment units, and water quality for the different RAS. For pH and redox, * are automatically measured values, and ´ are manually measured values. Turbidity measurements in FNU at commercial RAS, and NTU at Kårvika RAS. Make-up (l/kg/feed) for RAS Kårvika is a range, due to sampling during the project.*

	Sunndalsøra RAS			**Commercial RAS**			**Kårvika RAS**		
	RAS 1	RAS 2	RAS 3	RAS 3	RAS 2	RAS 3	RAS 1	RAS 2	RAS 3
Fish tanks in use	1	1	1	3	6	2	1	1	1
Rearing Units									
Species	*Salmo salar*	*Salmo salar*	*Salmo salar*	*Salmo salar*	*Salmo salar*	*Salmo salar*	*Salmo salar*	*Salmo salar*	*Salmo salar*
Fish tanks	Circular	Circular	Circular	Circular	Octagon	Octagon	Circular	Circular	Circular
Standing stock (kg)	2.79	3.14	2.78	4427	46 527	22 461	-	-	-
Stock density (kg/m³)	2.54	2.85	2.53	13.2	21.9	29.1	-	-	-
Feed load (kg/d)	0.226	0.226	0.226	16	98	246	0.302	0.302	0.302
Weight g.	199.23	209.33	213.84	6.09	34.01	194.75	-	-	-
Fish tank (m³)	0.5	0.5	0.5	333	2123	589	0.5	0.5	0.5
Fish tank water flow (l/h)	-	-	-	-	-	-	1590	1510	1630
Population	14	15	13	727 079	1 368 227	88 194	-	-	-
Skimmer flow (l/h)	-	-	-	-	-	-	740	780	700
Water treatment units									
Loop water flow (l/h)	1020	1020	1020	-	-	-	1500	1500	1500
Weeks in operation	17	17	17	13	7	14	7	7	7
Mechanical filtration	Drum	Drum	Drum	Drum	Drum	Drum	Drum	Drum	Drum
Bio-reactor	MBBR	MBBR	MBBR	MBBR	MBBR	MBBR	MBBR	MBBR	MBBR
Co2-degasser	Present	Present	Present	Present	Present	Present	-	-	-
Ozone	Not active	Not active	Not active	Present	Present	Present	Not active	Not active	Not active
UV	Not active	Not active	Not active	Present	Present	Present	-	-	-
Make-up (l/kg/feed)	1062	1062	1062	8100	4898	3610	500	500	500
Make-up (m³/d)	0.24	0.24	0.24	129.6	480	888	0.02	0.02	0.02
Water quality									
Temperature (°C)	12	12.3	12.3	12	12	10.3	11.5	11.1	11.4
Salinity (ppt.)	0.3	0.3	0.3	0.88	5.4	15.5	0	0	0
pH	8.14	8.14	8.07	7.33	7.34	7.41	7.42* / 7.68´	7.35* / 7.50´	7.52* / 7.58´
TAN (mg/l)	0.07	0.07	0.06	0.189	0.731	0.430	-	-	-
Nitrite (mg/l)	0.07	0.07	0.04	0.114	0.374	0.001	-	-	-
Nitrate (mg/l)	-	-	-	12	37.2	11.74	< 100	< 100	< 100
Turbidity	-	-	-	1.118	3.97	2.55	9.37	4.66	14.0
Lye (l/h)	-	-	-	0.6	7.3	1.6	-	-	-
CO_2 (mg/l)	-	-	-	-	-	-	3	3	3
NO4 (mg/l)	-	-	-	-	-	-	0.39	0.33	0.23
NO3 (mg/l)	-	-	-	-	-	-	32.8	29.6	31.6
NO2 (mg/l)	-	-	-	-	-	-	0.27	0.24	0.20
Redox (mv)	-	-	-	-	-	-	238.2* / 188´	232.8* / 234´	230.2* / 262´
O_2 saturation	-	-	-	-	-	-	107.1	105.7	97.6
Conductivity (us/cm)	-	-	-	-	-	-	715	556	567

2.2 Microplastics analysis

2.2.1 Sample preparation - digestion and filtration

To minimalize background contamination, all equipment were frequently washed and rinsed with distilled water (DW). Sample water were stored in containers with either lids or aluminium foil to prevent air exposure, and 100% cotton lab and nitril gloves were used. Water from the 10 L canisters from Sunndalsøra were mixed well before poured into three 1 L glass flasks, which previously had been washed and rinsed with DW. All DW used has been produced through a Milli-Q Direct 8/16 system, with Milipak Express 40 0.22 µm. For digestions of organic material, it was added 100 g of potassium hydroxide (KOH) to receive a 10% KOH solution (Sigma-Aldrich Potassium hydroxide, ASC reagent, 85% pellets & Merck Potassium hydroxide pellets > 85%). The solution were mixed well over a magnet mixer to dissolve the KOH and stored at 60°C for 24 hours as in protocol 1B from Dehaut et al. (2016) with glass lid at top to prevent contamination. Protocol 1B was proved to have high digestion rate with minimal plastics degradation (Dehaut et al., 2016). Contents were mixed before each vacuum filtration through filters (Whatman 47mm diameter glass filters, GF/F with 0.7 µm pores and GF/C with 1.2 µm). The samples were divided into four filters (250 mL each), two for quantity identification and two for composition analyses. The edges of the funnel were rinsed with DW in case of attached MPs. The two filters for quantification were then covered in Nile Red (1 mg Nile Red powder to 1 mL 70% ethanol) and stored dark and with a lid for minimum one hour. Nile Red is likely the most common staining protocol for MPs (Prata et al., 2019), as it is low-cost and simple to use (Shruti et al., 2022). Microscopical identification of MPs were used with microscope Leica MZ125 with 1x magnification and lens with 10x magnification. There were used red led lights and red filter, and pictures were photographed with a Leica DFC295, and with computer program LAS V4.7. The two other filters were stored in either glass or porcelain petri dishes covered with tinfoil awaiting composition analyses.

Samples from the commercial RAS and Kårvika RAS were sampled directly into 1 L glass bottles, and digestion was performed in the same bottles. Not all bottles had entirely 1 L of water, KOH were therefore measured out to be approximately 10%. Samples from commercial RAS 2 and RAS 3 contained much more organic material, samples 1:4 and 1:5 were therefor diluted with DW (50 mL sample and 200-250 mL DW). Control filters of DW

from the same apparatus (Milli-Q) were taken. Samples from RAS 1 and make-up water were filtrated without being diluted.

Due to suspected errors regarding filtration of make-up water from Sunndalsøra RAS it were done twice, and concentration of second filtration were used for further analyses. Since sampling at Sunndalsøra were done in plastic containers, the MP concentration were adjusted against a control filtration with distilled water stored in identical container for 24 h. The mean concentration from the control filters were then subtracted from all Sunndalsøra samples. If concentration became < 0, it was set to zero.

2.2.2 Microplastics composition

Three filters were picked to be analysed for MPs composition. One filter from the biofilter outlet from the three locations. Filters were picked based on numbers of MPs particles ≥ 300 µm. The filters used for composition analyses were 2.3.1 from RAS 2 at Sunndalsøra, filter 1.3.1-1 from RAS 1 at the commercial site and filter 3.3.2-2 from RAS 3 at Kårvika. The data acquisition was performed by Johan Mattias Isaksson at the Department of Pharmacy, UiT. The filters were analysed with Nuclear magnetic resonance (NMR) spectroscopy to determine the composition of the MPs, and a comparison with the bio-media (reference material) from the corresponding RAS.

The NMR spectroscopy was performed on samples dissolved in acetone for several days. Acetone works as a deuterated solvent, with a chemical shift which significantly differ from the suspected MPs and reference. Hence possible to separate the signals originating from the acetone and the MPs (Peez et al., 2019). In ^{1}H NMR spectroscopy tetramethylsilane (TMS) is established as an internal reference, and signals from the spectroscopy are given as chemical shift, which are proton absorption relative to the TMS (0 ppm.).

2.2.3 Data analysis and statistics

Pictures were opened in ImageJ (1.52q), scale was set after a reference picture taken with the equipment with the same settings. Colour threshold was adjusted to maximum the colour contrast in the pictures, and size of the particles were measured by manually drawing one line for length and one for width using a control photo for scale. MPs were sorted into four different size ranges based on the longest measurement of the particles. Size 1 ≤ 100, size 2 100-200, size 3 200-300 and size 4 ≥ 300 µm. During the quantification particles less than approximately 25 µm were generally not counted since they had low visibility with the 10x

magnification, but there were still some exceptions and those were included when calculating the concentrations. Data were logged in Microsoft Excel (Microsoft Corporation, 2023) and all data analyses and graphs were done in the statistical software program R (R Development Core Team, 2023) with the additional packages tidyverse, cowplot and dplyr (Wickham H et al., 2019; Wickham H, 2023; Wilke, 2020). Homogeneity was assessed with Bartlett´s test, and normality with Shapiro-Wilk test. When data were both normally distributed and showed homogeneity of variance, ANOVA was employed to look for significance, and if not the above conditions the non-parametric Kruskal-Wallis test was used instead. Differences were considered significant when $p < 0.05$.

3 Results

3.1 Microplastics abundance

MPs quantification with staining of particles with Nile Red showed presence of MPs on all subsamples, including RAS water, sludge, make-up water, and distilled water. The abundance varied greatly with MPs concentration in RAS water in the range of 0–557 MP/L at RAS Sunndalsøra (Figure 6 A), the highest concentration in the range of 60–1923 MP/L at the commercial RAS (Figure 6 B), and the lowest concentration within 16–96 MP/L at RAS Kårvika. For MPs > 300 µm, RAS Sunndalsøra was within the range of 0–20 MP/L (Figure 7 A), highest concentration at the commercial RAS with 0–90 MP/L (Figure 7 B), and concentration at RAS Kårvika with 0–20 MP/L (Figure 7 C). Figure 8 shows a subsample with staining of a single particle, with and without colour enhancing.

3.1.1 Contamination

Distilled water had a concentration of 4 and 16 MP/L, mean of 10 MP/L. Distilled water from the plastic containers used at sampling in RAS Sunndalsøra (36, 40, 48 and 16 MP/L) had a mean concentration of 35 MP/L.

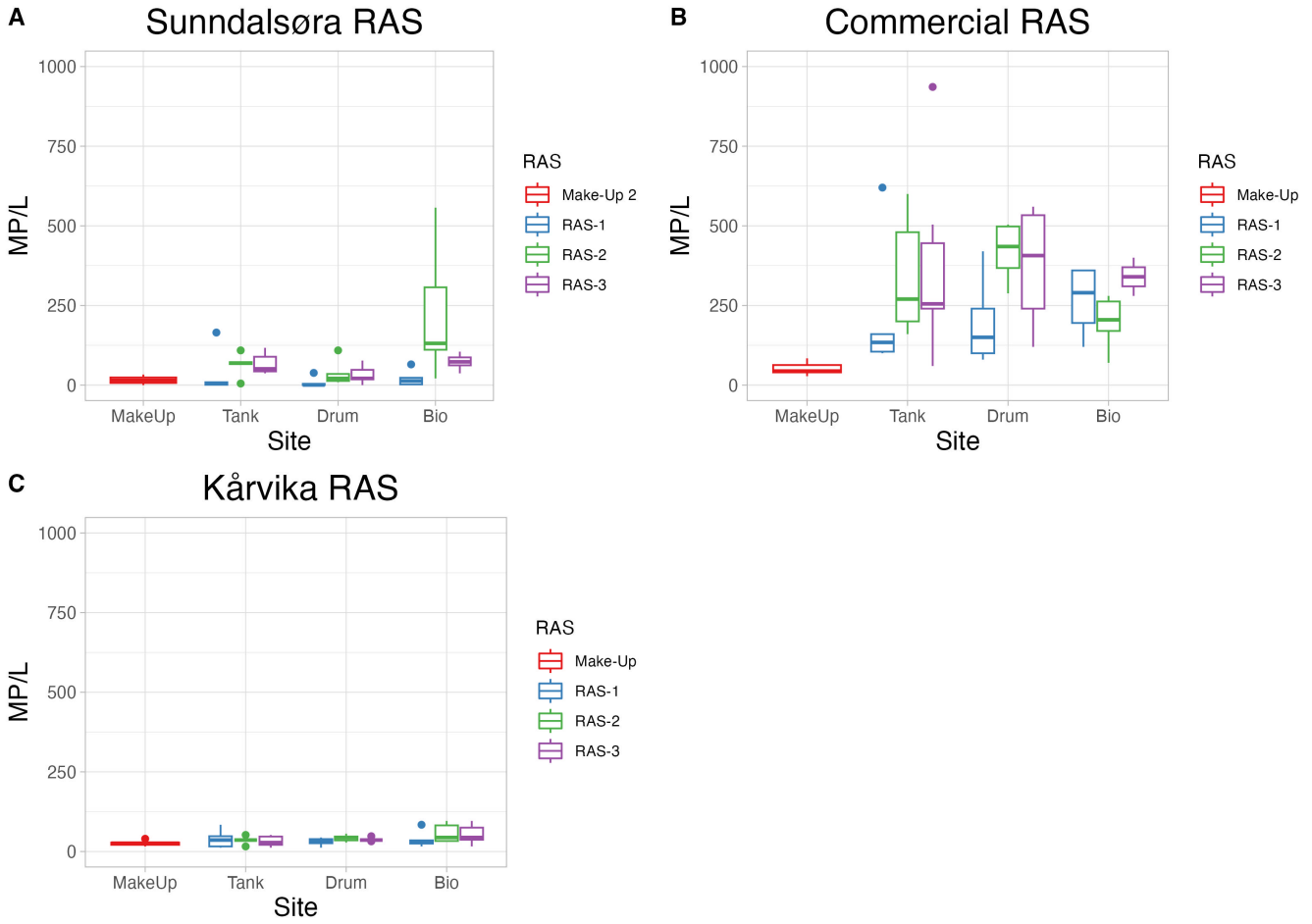

Figure 6 MP/L RAS water for all RAS at all locations. A: Sunndalsøra RAS, B: Commercial RAS & C: Kårvika RAS. The lower end of the vertical line up to the lower edge of the box is the first quartile (25% of the data), the box is the second quartile (50%) and the upper edge of the box to the upper end of the vertical line is the third quartile (25%). The dots are outliers.

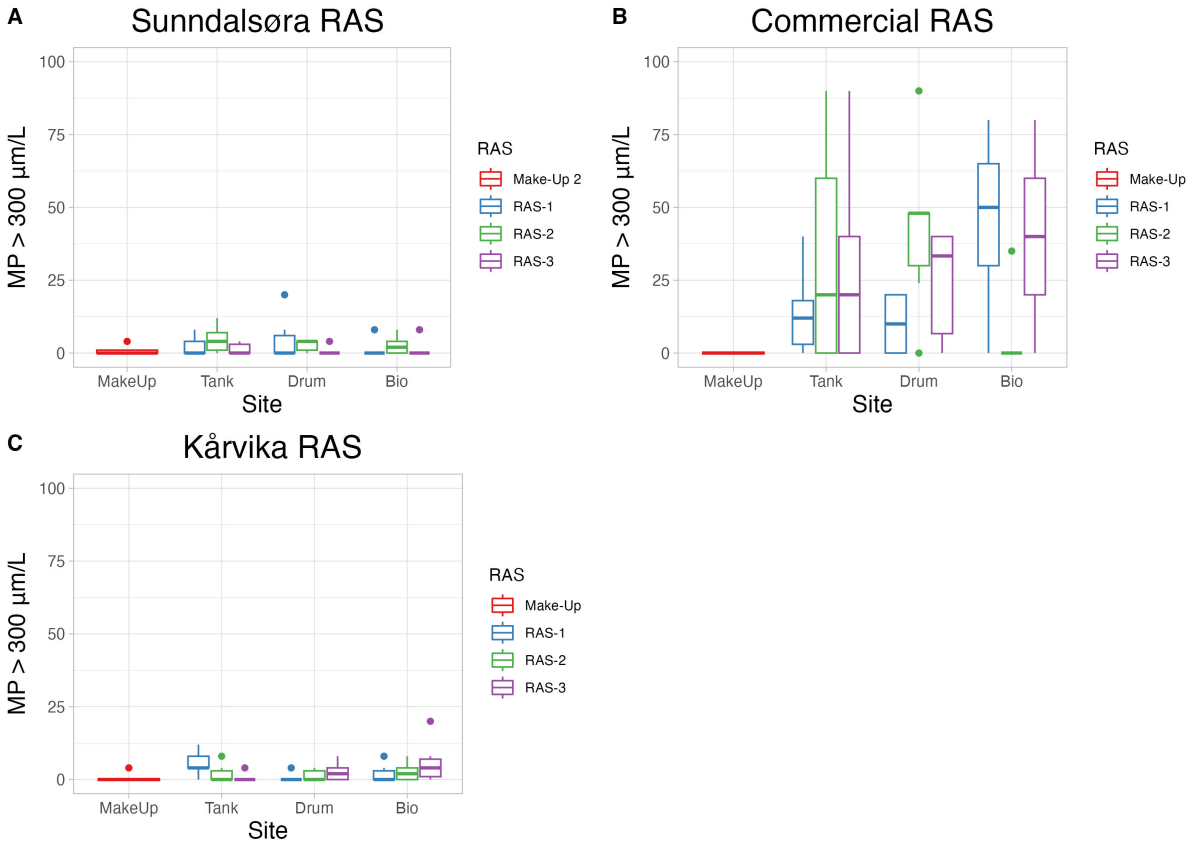

Figure 7 MPs > 300 µm/L for RAS water for all RAS at all locations. A: Sunndalsøra RAS, B: Commercial RAS & C: Kårvika RAS. The lower end of the vertical line up to the lower edge of the box is the first quartile (25% of the data), the box is the second quartile (50%) and the upper edge of the box to the upper end of the vertical line is the third quartile (25%). The dots are outliers.

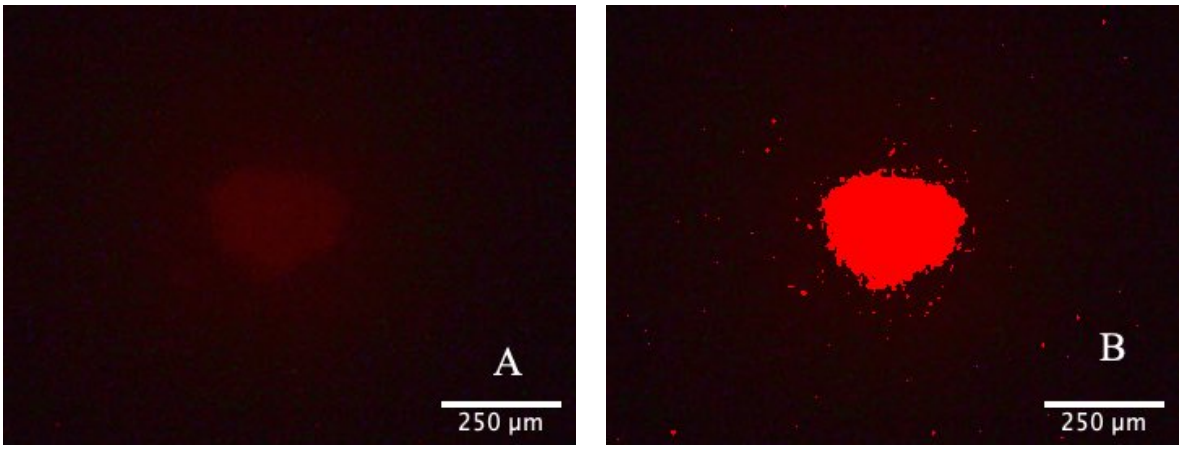

Figure 8 MP without (A) and with (B) colour enhancing for simplifying quantification. Sample 3.1.2-2 from fish tank, RAS 3, Kårvika.

3.1.2 Microplastics distribution RAS Sunndalsøra

For the analyses, samples from all three RAS were combined. Samples were tested for significant difference with the non-parametric Kruskal-Wallis test due to lack of normal distribution, and heterogenous distribution of variance. The test outputs were chi squared = 0.753 and p-value = 0.686. Indicating there were no significant difference between MPs concentration between the sites at RAS Sunndalsøra (Figure 9).

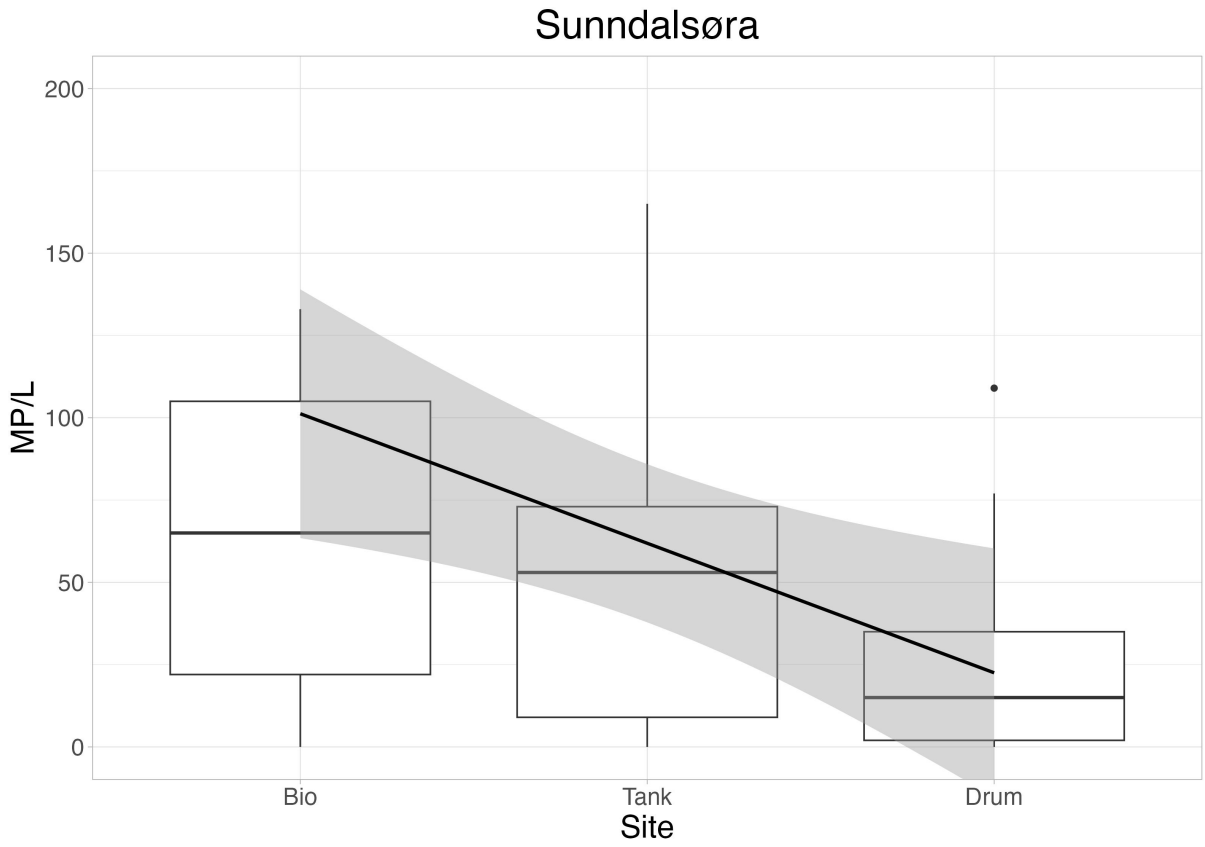

Figure 9 MP/L in RAS water from the three different sites from all three RAS at Sunndalsøra combined, adjusted against control filter. The black horizontal line in the boxes represent the median. The lower end of the vertical line up to the lower edge of the box is the first quartile (25% of the data), the box is the second quartile (50%) and the upper edge of the box to the upper end of the vertical line is the third quartile (25%). The black dots are outliers. The black diagonal line is a linear regression line based on the mean value from each site, while the grey area around is the equivalent 95% confidence interval.

3.1.3 Microplastics distribution RAS Kårvika

For the analyses, samples from all three RAS were combined. Samples were tested for significant difference with the non-parametric Kruskal-Wallis test due to lack of normal distribution, and heterogenous distribution of variance. The test outputs were chi squared = 0.525 and p-value = 0.769. Indicating there were no significant difference between MPs concentration between the sites at RAS Kårvika (Figure 10).

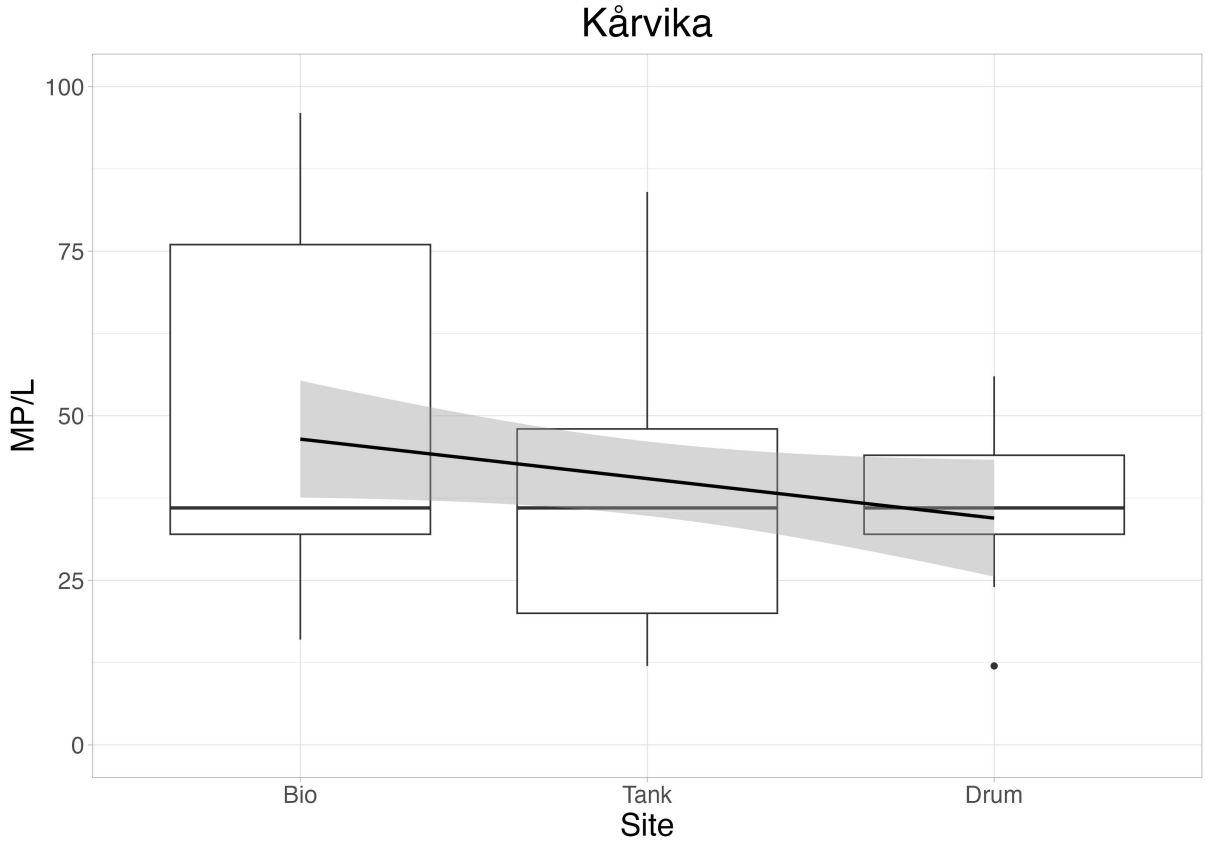

Figure 10 MP/L in RAS water from the three different sites from all three RAS at Kårvika combined. The black horizontal line in the boxes represent the median. The lower end of the vertical line up to the lower edge of the box is the first quartile (25% of the data), the box is the second quartile (50%) and the upper edge of the box to the upper end of the vertical line is the third quartile (25%). The black dots are outliers. The black diagonal line is a linear regression line based on the mean value from each site, while the grey area around is the equivalent 95% confidence interval.

3.1.4 Microplastics distribution Commercial RAS 1

Samples were tested for significant difference with the non-parametric Kruskal-Wallis test due to lack of normal distribution. The test outputs were chi squared = 1.391 and p-value = 0.499. Indicating there were no significant difference between MPs concentration between the sites at RAS 1 at the commercial RAS (Figure 11 A).

3.1.5 Microplastics distribution Commercial RAS 2

Samples were tested for significant difference with the non-parametric Kruskal-Wallis test due to lack of normal distribution, and heterogenous distribution of variance. The test outputs were chi squared = 3.668 and p-value = 0.160. Indicating there were no significant difference between MPs concentration between the sites at RAS 2 at the commercial RAS (Figure 11 B).

3.1.6 Microplastics distribution Commercial RAS 3

Samples from biofilter were not possible to analyse because *n*=2, due to broken containers during shipping. Data had normal distribution, and homogenous distribution of variance. ANOVA could therefore be used, which gave F-value 0.288 and p-value = 0.775, Indicating there was no significant difference between MPs concentrations between the sites at RAS 3 at the commercial RAS (Figure 11 C).

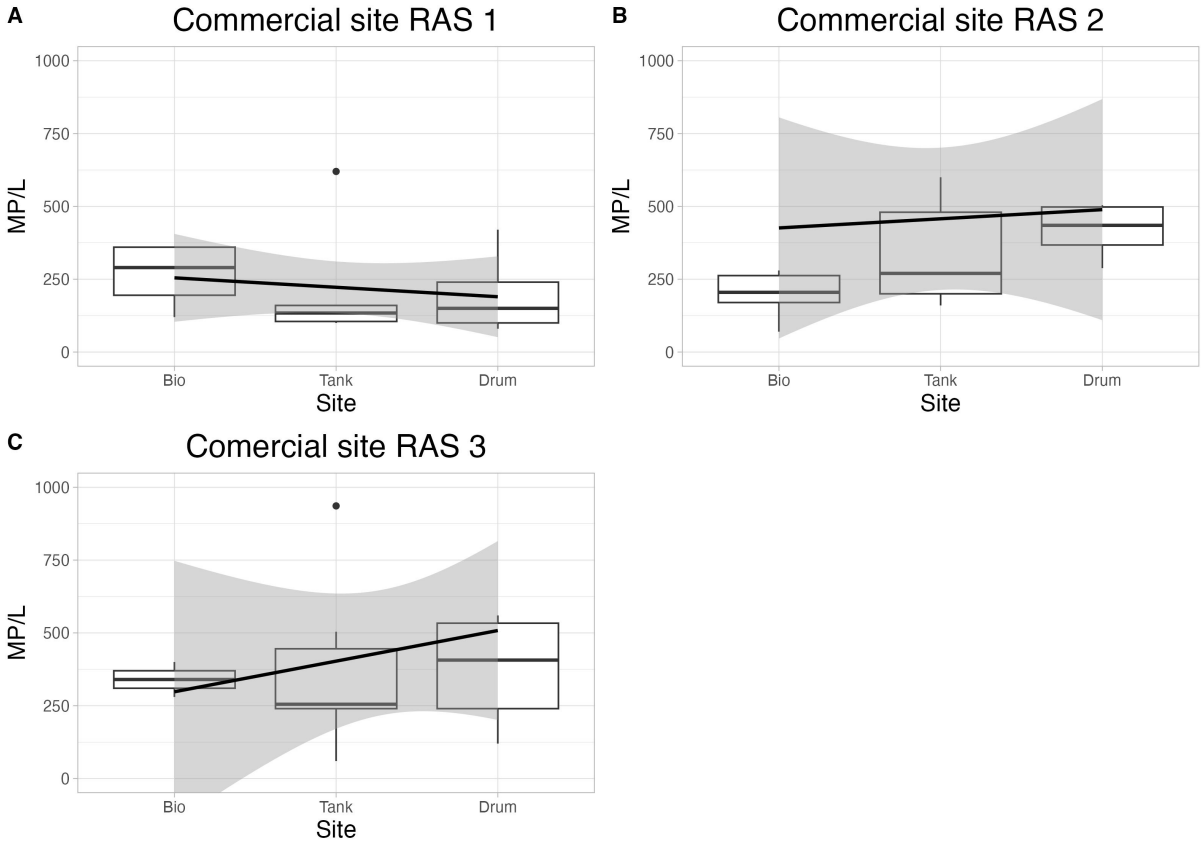

Figure 11 MP/L in RAS water from the three different sites from the three RAS at the commercial site. RAS not combined due to difference in size and structures. The black horizontal line in the boxes represent the median. The lower end of the vertical line up to the lower edge of the box is the first quartile (25% of the data), the box is the second quartile (50%) and the upper edge of the box to the upper end of the vertical line is the third quartile (25%). The black dots are outliers. The black diagonal line is a linear regression line based on the mean value from each site, while the grey area around is the equivalent 95% confidence interval. A: RAS 1, B: RAS 2 and C: RAS 3.

3.1.7 Sludge samples

Not all subsamples of sludge were possible to measure accurately due to high concentration of MP. For Kårvika RAS, five out of six samples were measured, while one sample had much higher concentration (Figure 12 and Figure 13). Sludge samples from Sunndalsøra RAS all had too high concentration for making an estimate. Sludge sample from Kårvika RAS had a mean concentration of 1968 MP/L (Figure 14), with 1207 MP/L at RAS 1 (Figure 15) RAS 3 3110 MP/L (Figure 16).

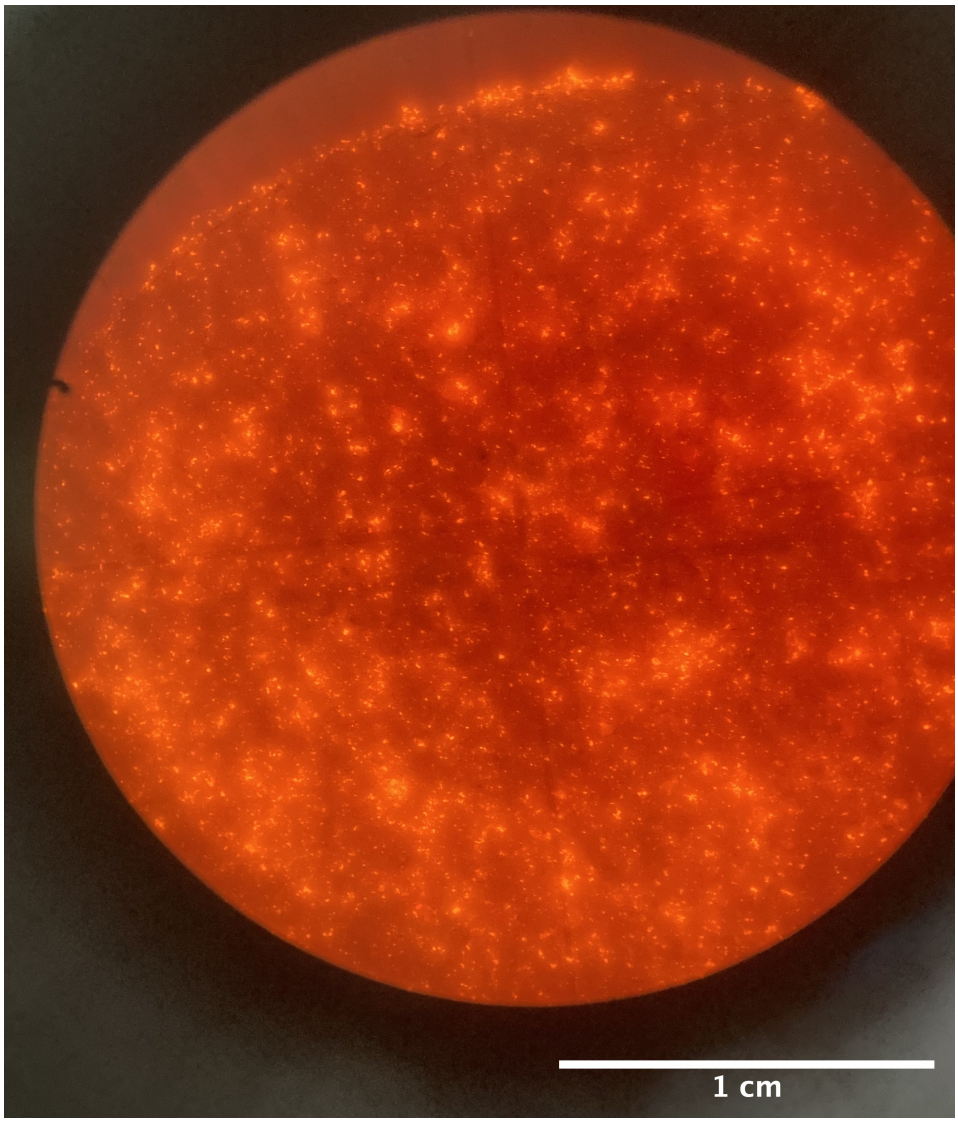

Figure 12 Filter with sludge sample from Kårvika RAS with very high concentration of MPs..

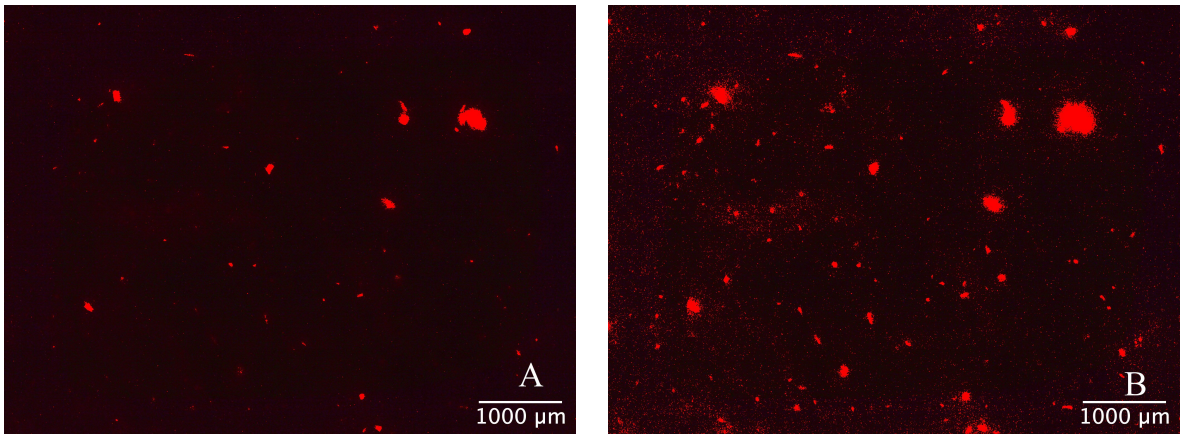

Figure 13 Sludge sample from Kårvika RAS with high abundance of MPs with different levels of colour enhancing. A: Low amount of colour enhancing, B: More enhanced to show the smaller particles.

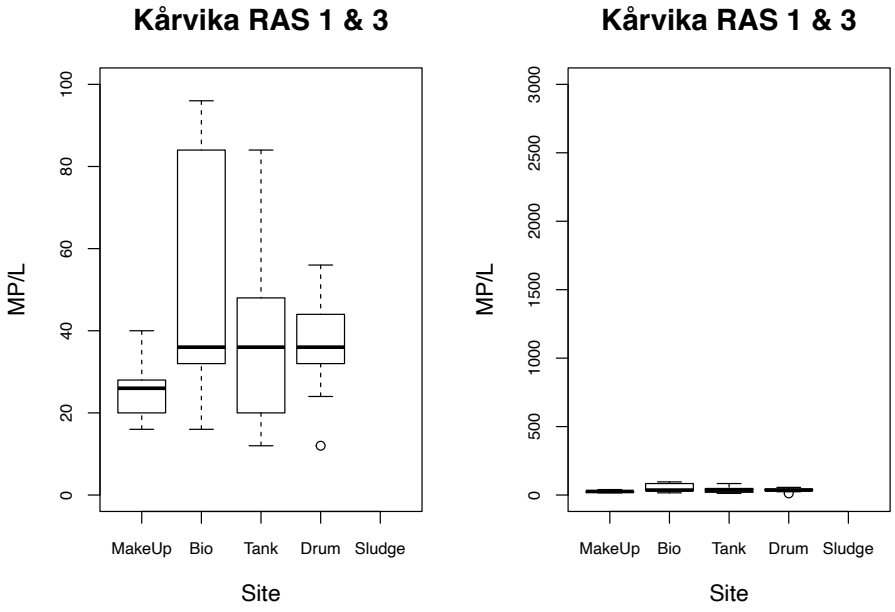

Figure 14 MP/L in Kårvika from all RAS. Y-axis extended on the figure to the right to include sludge sample. The black horizontal line in the boxes represent the median. The lower end of the vertical line up to the lower edge of the box is the first quartile (25% of the data), the box is the second quartile (50%) and the upper edge of the box to the upper end of the vertical line is the third quartile (25%).

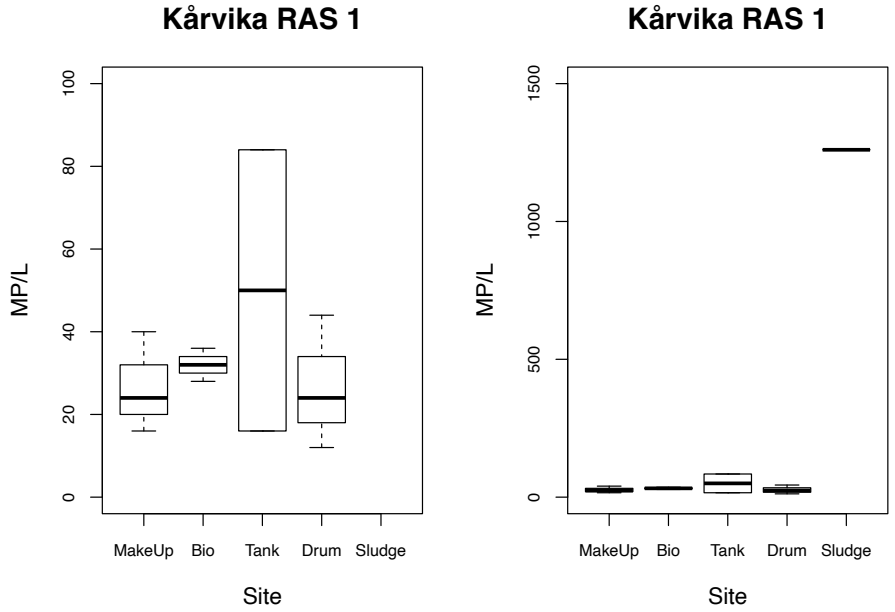

Figure 15 MP/L in RAS 1 from Kårvika. Y-axis extended on the figure to the right to include sludge samples (n=2). The black horizontal line in the boxes represent the median. The lower end of the vertical line up to the lower edge of the box is the first quartile (25% of the data), the box is the second quartile (50%) and the upper edge of the box to the upper end of the vertical line is the third quartile (25%).

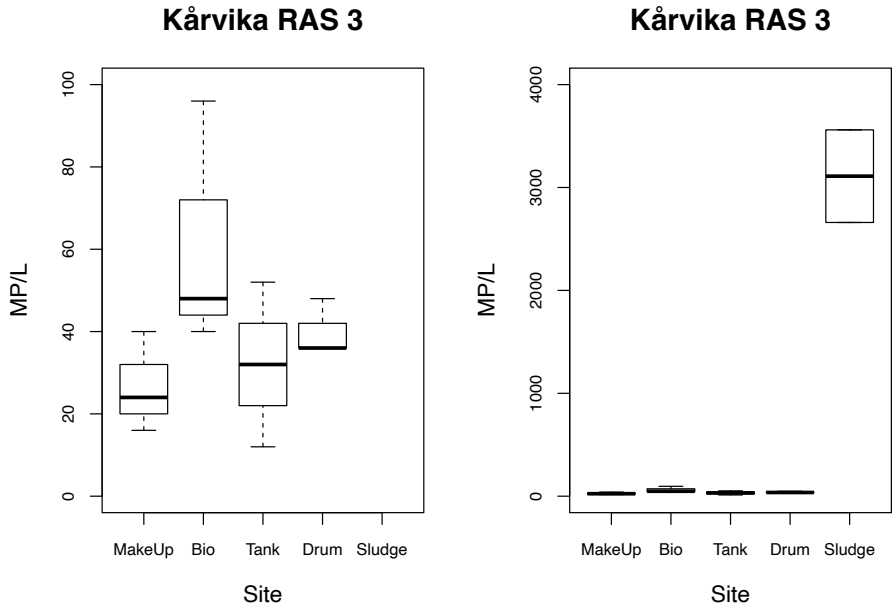

Figure 16 MP/L in RAS 3 from Kårvika. Y-axis extended on figure to the right to include sludge samples (n=2). The black horizontal line in the boxes represent the median. The lower end of the vertical line up to the lower edge of the box is the first quartile (25% of the data), the box is the second quartile (50%) and the upper edge of the box to the upper end of the vertical line is the third quartile (25%).

Table 3 is a mass balance table of MPs from all RAS at the three locations. With no data on evaporation, spill, and body mass growth it is assumed that the make-up rate and sludge rate are identical. Which in that case, indicates that there are more MPs leaving than entering for Kårvika RAS. MP/kg biomass for Kårvika RAS were based on the estimated biomass at the end of the RASHealth project, which was not adjusted against unpredicted fish mortality, or unexpected growth. 520 MPs are entering each RAS daily (20 L make-up water), while 24 140 and 62 200 MPs are filtered out through the drum filter (20 L sludge).

Table 3 MP data from all RAS at all locations.

	Sunndalsøra RAS			**Commercial RAS**			**Kårvika RAS**		
	RAS 1	RAS 2	RAS 3	RAS 1	RAS 2	RAS 3	RAS 1	RAS 2	RAS 3
Make-up water/d (m^3)	0.24	0.24	0.24	130	480	888	0.02	0.02	0.02
MP/L/make-up water	13	13	13	51	51	51	26	26	26
MP into the system/d (10^3)	3.12	3.12	3.12	6 630	24 480	45 288	0.52	0.52	0.52
MP/L RAS water	20	106	57	220	478	433	36	45	41
Total system volume (m^3)	1.2	1.2	1.2	333	2 123	589	0.8	0.8	0.8
Total MP abundance (10^3)	24	127	68	73 260	1 014 794	255 037	28	36	32
MP 300 µm/L RAS water	2.8	3.3	1.1	20.9	27.2	28.3	2.6	2.0	3.1
MP/L/sludge	-	-	-	-	-	-	1207	-	3110
MP/sludge/make-up/d	-	-	-	-	-	-	24 140	-	62 200
MP/standing stock (kg)	8.6	40.5	24.6	16.5	32.1	11.4	2.0	2.5	2.3

3.2 Microplastics composition

As seen in Figure 17, Figure 18 and Figure 19 the samples and references (bio-media) showed very similar signals from the NMR spectroscopy, both between the samples and corresponding references, and between the RAS from the different locations. The strongest peak for all three figures at approximately 1.33 ppm corresponds with protons of methylene groups (CH_2), while the weaker peak at around 0.90 ppm corresponds with protons of methyl groups (CH_3) (Peez et al., 2019). The results indicates that the samples and reference are all made of PE.

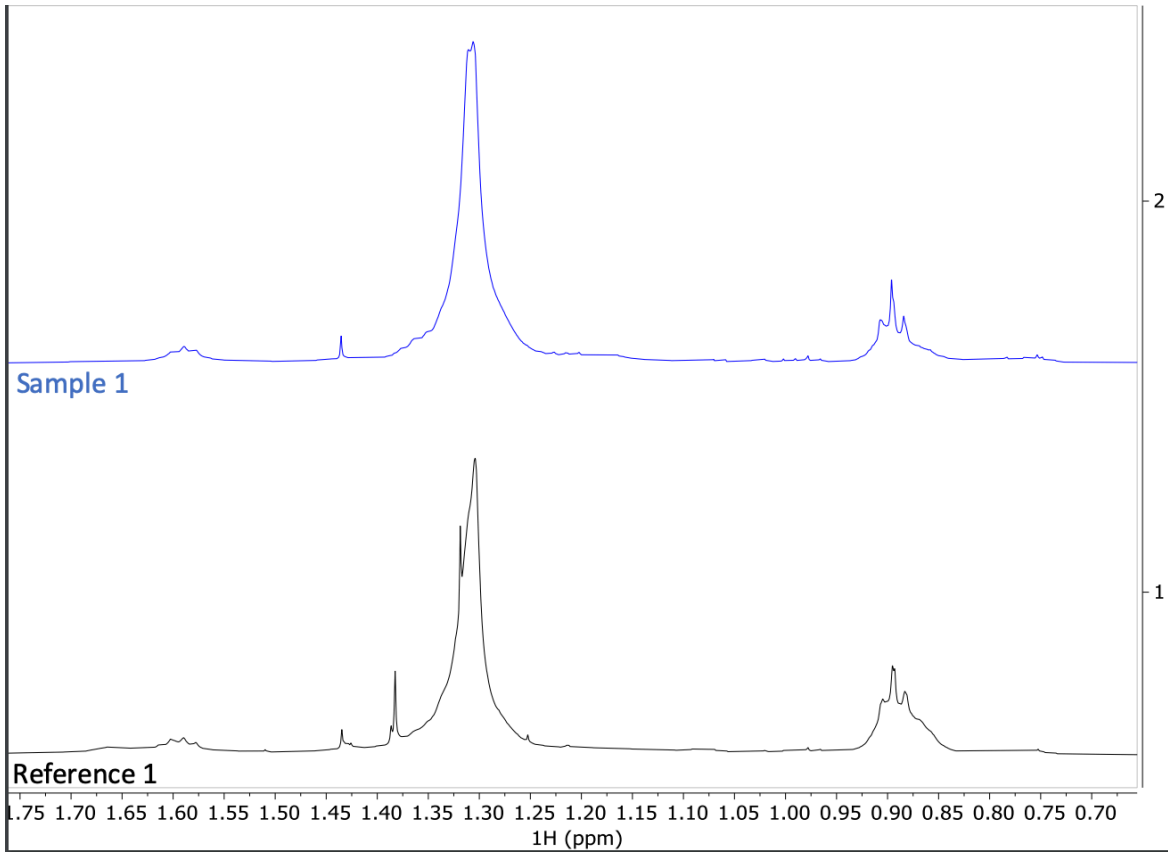

Figure 17 NMR spectroscopy results of water from Sunndalsøra RAS. Sample 1 are filter (2.3.1-2) with water sample from the biofilter in RAS 2. Reference 1 is the bio-media from the same RAS. X-axis is the chemical shift, absorbed protons, relative to the absorption of TMS (0 ppm).

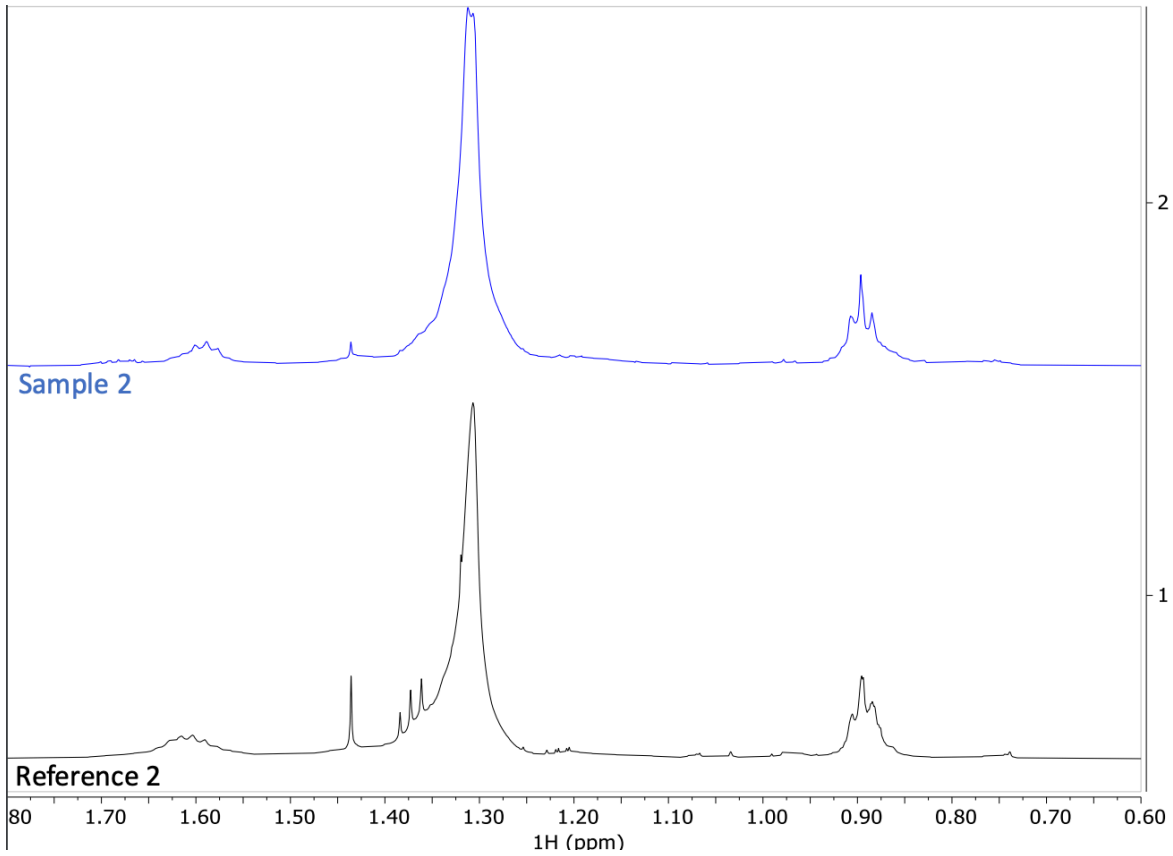

Figure 18 NMR spectroscopy results of water from the commercial RAS. Sample 2 are filter (1.3.1-1) with water sample from the biofilter in RAS 2. Reference 1 is the bio-media from the same RAS. X-axis is the chemical shift, absorbed protons, relative to the absorption of TMS (0 ppm).

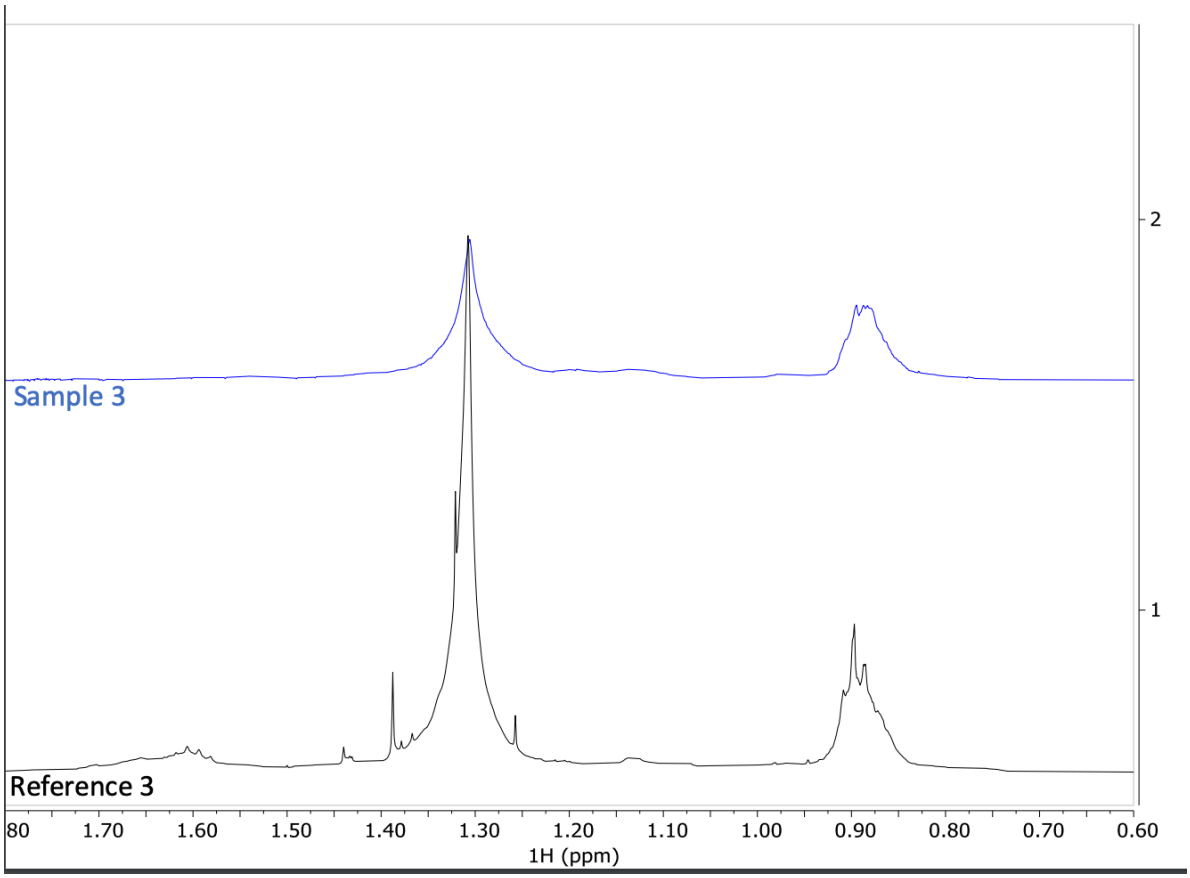

Figure 19 NMR spectroscopy results of water from Kårvika RAS. Sample 3 are filter (3.3.2-2) with water sample from the biofilter in RAS 3. Reference 3 is the bio-media from the same RAS. X-axis is the chemical shift, absorbed protons, relative to the absorption of TMS (0 ppm).

3.3 Microplastics size

The trends for MPs size are similar for all locations, with clearly most small particles and fewest large particles. The size distribution of the MPs for all location combined and each location separately are shown in Figure 20. Approximately 75% of the MPs were ≤ 100 µm, and MPs ≥ 300 µm accounted for 0–15%, with some exceptions where it accounted for up to 50%.

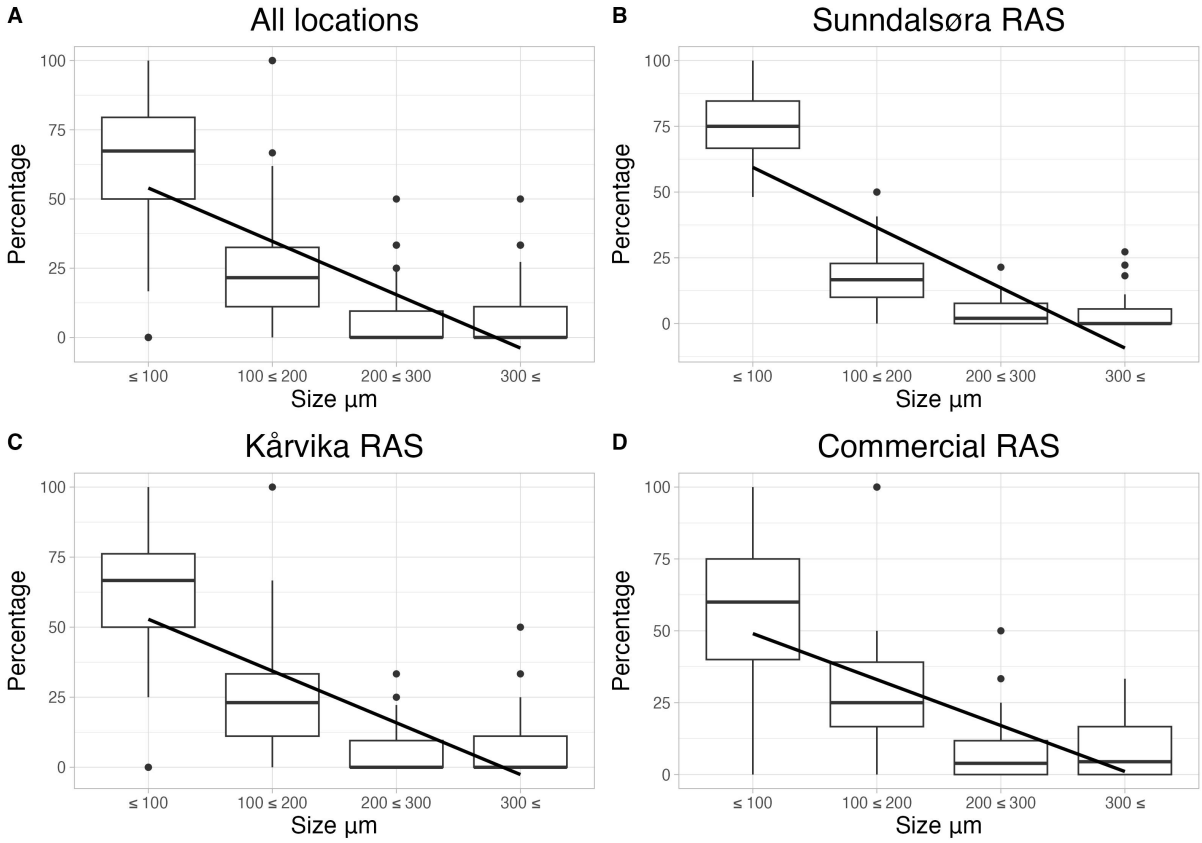

Figure 20 Size distribution of MPs in percentage combined from the fish tank, drum filter and biofilter. A: Combined for all locations. B: All three RAS from Sunndalsøra. C: All three RAS from Kårvika. D: All three RAS from the commercial site. The black horizontal line in the boxes represent the median. The lower end of the vertical line up to the lower edge of the box is the first quartile (25% of the data), the box is the second quartile (50%) and the upper edge of the box to the upper end of the vertical line is the third quartile (25%). The black dots are outliers. The black diagonal lines are linear regression lines based on the mean value from each site.

Figure 21 shows the size distribution of MPs divided into < 100 µm and >100 µm for all sites at the three locations. More detailed figures for each RAS are presented in Appendix 6, Appendix 7 and Appendix 8. The commercial site RAS and Kårvika RAS had the largest proportion of MPs > 100 µm, while Sunndalsøra RAS had the lowest. But there were no trends of MPs > 100 µm and < 100 µm distribution regarding sites within the different RAS.

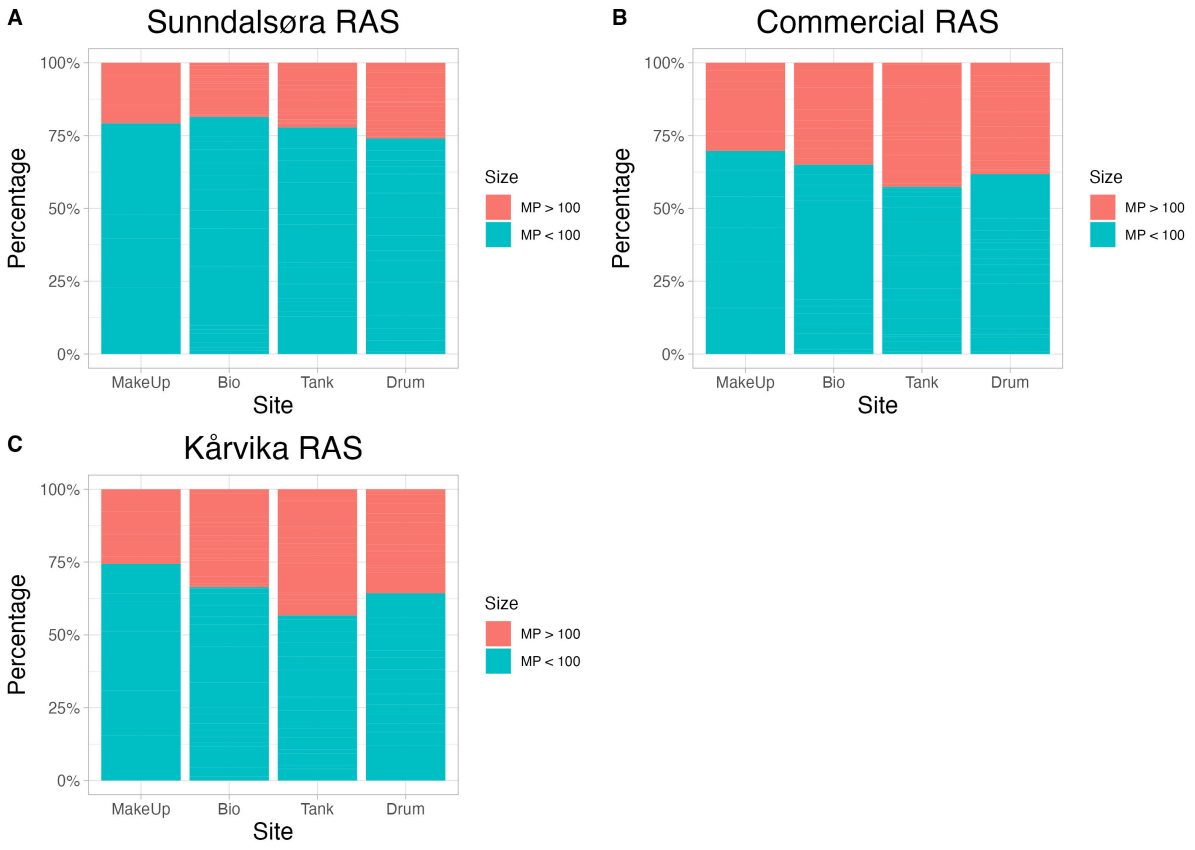

Figure 21 Size distribution of MPs in percentage for each location. MPs divided into two groups, larger and smaller than 100 µm. A: Kårvika RAS. B: Sunndalsøra RAS. C: Commercial RAS.

4 Discussion

4.1 Interpretation of results

4.1.1 Quantification and composition of microplastics

The results highlight presence of MPs in all samples, RAS water, make-up water, sludge and control water. Control samples with distilled water (DW) showed presence of 10 MP/L, which indicates a low contribution, but still shows the general problem of MPs related lab work. That contamination through air, distilled water and from equipment can be a problem, as documented by Su et al. (2016) which had a mean background contamination consisting of 6.8%, of a total concentration of 3.4-28.8 MP/L, when filtering RAS water. The control (10 MP/L) were only used for adjusting samples which were diluted with DW, which were some samples from RAS 2 and RAS 3 from the commercial site due to high concentration of organic material. Since it is unknow if the contamination origin from the DW, or from the air during the filtration, other samples were not adjusted. Although the samples from

Sunndalsøra RAS were adjusted against a control, the values can still be misleading, and can explain some of the high variance observed in the sampling from Sunndalsøra RAS.

There were a much higher concentration of MPs in the commercial site than the two research units. The research units, Sunndalsøra and Kårvika, had comparable results with means of 61 and 41 MP/L respectively, while the commercial site had a mean of 371 MP/L. Looking at MP/kg standing stock RAS Sunndalsøra and the commercial RAS were more similar with means of 24.6 and 20.0 respectively, while RAS Kårvika had a much lower concentration of 2.3 MP/kg standing stock. The RAS Kårvika had been the shortest time in operation (7 weeks), and compared to RAS Sunndalsøra (17 weeks), and commercial RAS (13, 7 and 14 weeks) would have had a much shorter duration of possible accumulation. It is worth to note that one should be careful comparing the results from commercial units and research units, as system size, purpose of production and activity may be very different. The commercial units were much larger, and with a water flow containing much more water. There were visually differences of the movement in the biofilters, as the bio-media from the commercial site were exposed to more force from the waterfall of water entering the basin, than the research units. On the other side, the low concentration of biomass from the research units may induce a higher MP/kg standing stock, since the stock density were much lower there.

Compared with existing literature regarding MPs in RAS, the concentration of MPs in this study were generally higher. In literature, concentration varies greatly, with observed concentrations for MPs >300 µm as low as 0.065 MP/L from a RAS in the Fujian province in China (Lu et al., 2019) and 0.048 MP/L (only the fibres, thereof only 75% of total MPs) from a mariculture system with a recirculating unit in Yantai City in China (Zhang et al., 2020). Huang et al. (2022) found a concentration of 1.67 MP/L for MPs > 20 µm, but it were assumed most of the particles originated from the make-up water. Matias et al. (2023) found 39.1 MP/L (undefined size) in RAS containing European seabass (*Dicentrarchus labrax*) which are very similar to the research units in the present study. But it were suggested through composition analyses that RAS components had a limited contribution to the concentration.

4.1.2 Origin of the microplastics

There were no significant difference of MPs concentration between the sites, and this is supported by Lu et al. (2019) which also found no significant difference between fish tanks, biofilter and recycled water. However, in the present study there were trends for the research

units to have highest concentration of MPs at the sampling site right after the biofilter, which is sensible as it is believed the MPs originate from the biofilter. Lower concentration in the fish tank, which may be because the concentration is diluted as the fish tanks contains large proportion of the total water in the system (RAS Kårvika 63%, RAS Sunndalsøra 42%). And lowest concentration after the drum filter, which likely is due to the filtration. The water, regarding MPs concentration, is found to be more homogeneous than expected throughout the system.

Since there were more MPs leaving the systems than entering it at Kårvika RAS, it is plausible to indicate that a major part of the MPs found in the RAS water has originated from within the system. The same would apply for Sunndalsøra RAS, although there are no numbers on the concentration in the sludge due to the excessive concentration and inability to perform a count. The NMR spectroscopy gave signals (approximately 1.33 and 0.90 ppm) which are corresponding with low density PE (1.33 and 0.93 ppm) (Peez et al., 2019). The bio-media employed in the different studied RAS are made of PE, pointing towards that the MPs present in the RAS water mainly origin from the biofilters' media. The density of the bio-media used at RAS Sunndalsøra and RAS Kårvika had a density of 1.00 g/cm^3 due to filler. Which makes it heavier than normal PE which has a density of 0.92–0.966 g/cm^3 (Hidalgo-Ruz et al., 2012). The density of the fragmented MPs are unknow, but due to its low weight there can be an accumulation at the surface, where most of the samples were taken. PVC for instant has a much higher density at 1.16–1.58 g/cm^3 (Hidalgo-Ruz et al., 2012), and therefore may accumulate deeper in the water column.

Fish feed was not analysed in the present work, but other studies have shown that it may contain up to 123.9 MP/kg feed (Thiele et al., 2021). For example, given a daily feeding of 268 kg (Commercial RAS 3), the feed can in theory contribute with 32 860 MPs to the water daily. Which in relation to the present abundance of 255 037 000 MPs in the same RAS are only 0.013% of the total MPs. It is therefore not likely that the feed is a large contributor of MPs.

4.1.3 Microplastics size distribution

There were clearly a highest presence of the smallest particles for all three sites (Figure 20) and fewest of the largest particles. The size distribution appeared quite similar for all RAS. The same applied to the size distribution within the RAS at the different locations, which were somewhat similar too. There were no clear trends of change in size distribution, even

after the drum filter outlet, which in theory should only consist of MPs of size 1, due to the mesh size of the filter (Sunndalsøra 50 µm, Kårvika 40 µm, commercial site 60 µm). This may indicate that the present MPs from the mechanical filter outlet originated from the mesh itself, or that particles above mesh size are not filtered out through the drum filter. This can be because the filters are constructed to separate out fish related waste, as faeces and uneaten feed, not necessarily MPs (Rito et al., 2022). A review by Li et al. (2023) looked at the removal efficiency of different particulate matters, and found drum filters (40–100 µm) in RAS to have a removal efficiency of 31-94%. The MPs were categorized into size based on the longest measurement out of length and width. This can to some parts give a false suspicion towards the efficiency of the mesh, as it is likely that some of the large particles which were observed effluent of the filter can have had a dimension shorter than the mesh openings.

4.2 Today's microplastics issues

As seen in this study there are MPs present in the fish production systems, and due to perhaps not sufficient filtrating, the MPs are also distributed in the fish rearing water. The most common understanding of MPs transfer from environment to animals are trough digestion, where the MPs ends up in the gut. Seafood where human consumes the entire animal or the gut, as shellfish and small fish, the presence of MPs in the gut will be transferred to the consumer (Smith et al., 2018). Whereas there are studies showing translocation of MPs from gut to fillet through phagocytoses, there are also studies showing no translocation from gut to either fish fillets, liver or gonads (Kim et al., 2020). There is no general understanding on how consumers could potentially be affected by fish exposed to MPs. In a recent study Kim et al. (2020) rainbow trout were exposed to different concentrations of spherical PE-MPs (10–300 µm) through their diet. After two weeks there were observed MPs in the guts, but no MPs outside of the gut, where the fish exposed to higher quantity of MPs had digested the most particles. A study on European seabass reared for eight months in seawater RAS, fish were found to have accumulations of MPs in the gastrointestinal tract, gills, liver and fillet. The concentration were lowest in the fillet with 400 MP/kg fillet in the range of 30-5038 µm, which following the EFSA´s recommendation of weekly fish consumption would expose the consumer to 5616 MPs yearly (Matias et al., 2022). More research are needed in the study of MPs translocation, but there are a general understanding that smaller particles are more likely to translocate, and that not all experimental designs captures the smaller MPs (Kim et al., 2020).

4.2.1 Microplastics environmental impact

Due to the high concentration of MPs in the sludge, a direct release into nature is not ideal, and not common either due to risk of release of unwanted contamination. There are several used pathways of the sludge from RAS. One pathway is incineration, where the end product of bio-media from RK Bioelements, used at RAS Sunndalsøra and RAS Kårvika, are exclusively water and CO_2 (RK BioElements). To utilize the nutrients in the sludge, there are several other pathways. It can be used for making biogas with anaerobic digestion, it can be used as agricultural fertilizer, nutrients for kelp farming, and it can be used as ingredient for fish feed (Del Campo et al., 2010). Due to its high concentration of MPs, usage of sludge from RAS should be utilized with caution, and sufficient removal of MPs would be necessary before being released into the nature. Compared to other water sources, the relative contribution of MPs from RAS are high. The organisation Norwegian Water concluded with a survey on MPs concentration, that only one out of 24 waterwork in Norway samples had a concentration above 4.1 MP/L (60-5000 μm). Which were set as the limit of quantification, with a 99.99% probability to be distinguished from belonging blanks. But the sample were believed to have been exposed to air contamination, and it was therefore concluded that it is likely that no MPs can be detected in Norwegian tap water (Uhl et al., 2018). A large contributor to MPs into the marine environment are domestic wastewater (Sun et al., 2019). Treatment plants can be inefficient in removal of MPs less than 100 μm, as influent and effluent sampling proved to have similar concentrations of MPs < 100 μm (Freeman et al., 2020). A review by Sun et al. (2019) found concentrations of water effluent of treatment plants to have a concentration of 0-447 MP/L, which is a much lower concentration than the sludge from RAS. Indicating that although the amount of wastewater from RAS in not comparable with other sources as domestic wastewater, the relative MPs contribution to the environmental can be very high.

4.2.2 Biodegradable alternatives

Biodegradable alternatives for everyday items are generally becoming more and more common due to their possible reduced climate footprint, or reduced environmental impact, as in the transition from plastics to paper in disposable cutlery and shopping bags. Regarding RAS, there has been tested biodegradable alternatives as wood chips and wheat straws compared to traditional bio-media in plastics. This provided adequate denitrification, but had time limitations due to loss of masses (Saliling et al., 2007). Mnyoro et al. (2022) looked at performance of using bio-media of biodegradable materials regarding total ammonia nitrogen

(TAN), nitrite and oxygen conversion rate, and concluded that bio-media made of coconut shell might have the potential to be used instead of plastic in simple RAS, although further studies were recommended.

4.3 Further research

Two of the locations in this thesis were research facilities with small RAS with integrated fish tanks, purposely made for research, and not commercial use. The data of MP concentration obtained from the research units can be transferred into a much larger system, but it is possible the size, water pressure and other factors can affect the concentration in different ways. An analysis on a commercial site with sludge samples would be ideal to estimate a more credible estimate on MPs concentration originating from within the RAS. Another aspect would be to analyse water from a RAS over time, to calculate an accumulation-rate of MPs.

5 Conclusion

In this study there were found MPs concentration at all nine RAS from the three different locations. RAS Kårvika had the lowest concentration within the range of 36–45 MP/L, RAS Sunndalsøra 20–106 MP/L and highest concentration at the commercial RAS 220–478 MP/L. For the research units there were tendencies of highest concentration effluent of the biofilter, and lowest effluent of the drum filter, but no significant difference. A NMR spectroscopy indicated the MPs originated from the bio-media, and the concentration in the sludge from RAS Kårvika and RAS Sunndalsøra support the fact that more MPs leaves than enter the systems. There were found similar size distribution for MPs at all RAS with approximately 75% of the MPs being ≤ 100 µm.

Sampling from RAS Sunndalsøra were performed in plastic containers. All though adjusted against a control, using plastics is not recommended but were preferred to glass bottles due to shipping and uncertainties of necessary amount of water prior to the study. The filtration were as well not conducted in a laminar flow hood, both against the recommendation to avoid MPs contamination (Prata et al., 2021). Another issue with unsure affect is the incubation time of Nile Red. Organic material can in presence of MPs interact with Nile Red, which can make overestimation. Incubation time for the staining were set at 60 min, but a review of literature recommended 30 min to avoid background fluorescence (Shruti et al., 2022). For the sludge samples and RAS water samples from the commercial RAS 2 and RAS 3, which contained

most organic material, there can therefore be overestimations which can explain some of the high variance.

The results from this study can be used as basis for comparison for future studies of MPs in RAS water. And as justification for further research regarding the effect of the present MPs in relation to fish health, accumulation in fish fillets and human consumption. Because of the high concentration of MPs in the wastewater from the mechanical filters, the waste must be treated with care. And as important as nutrient utilizing are, it must not be on the expanse of potential MPs spread to the environment.

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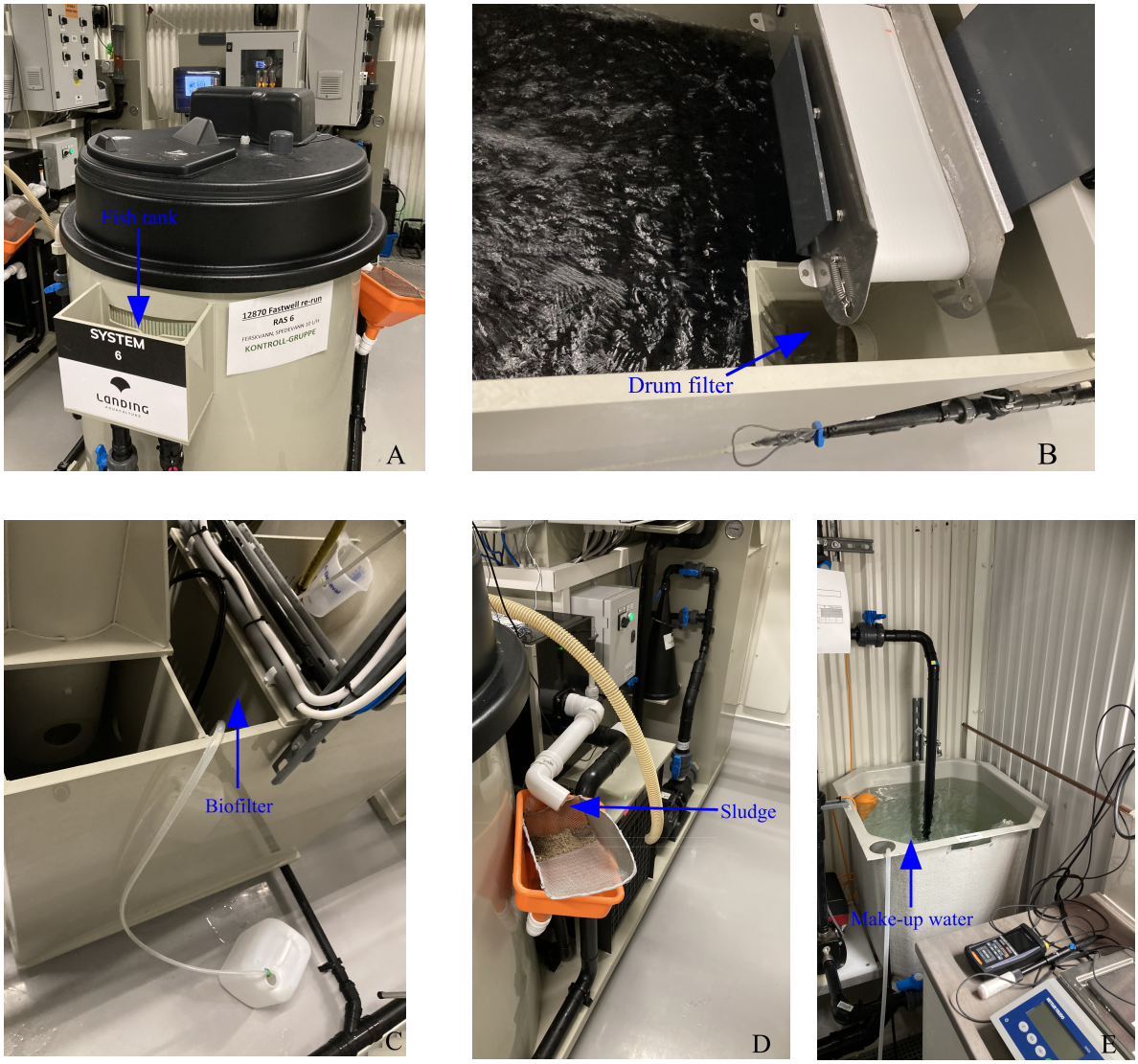
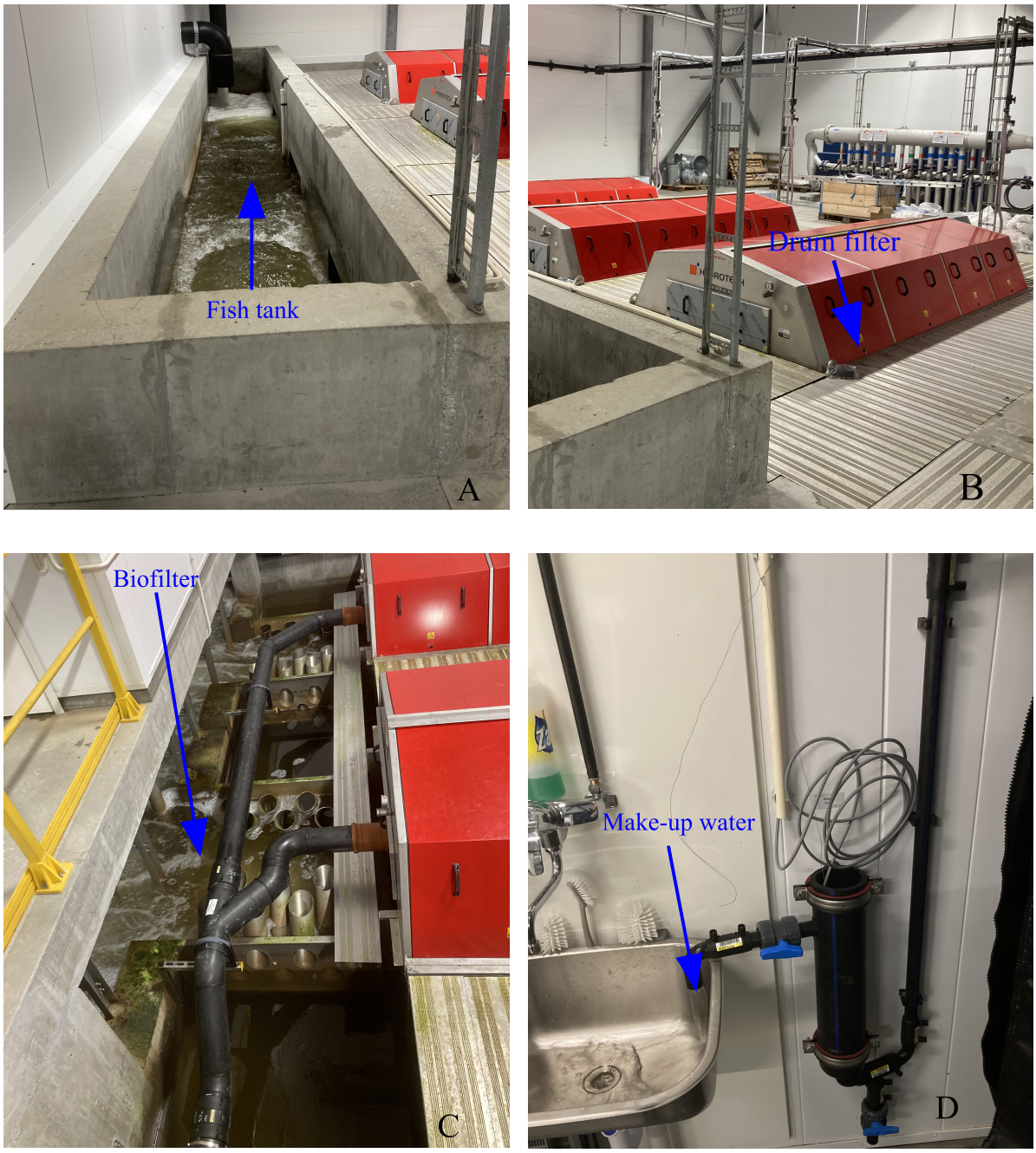
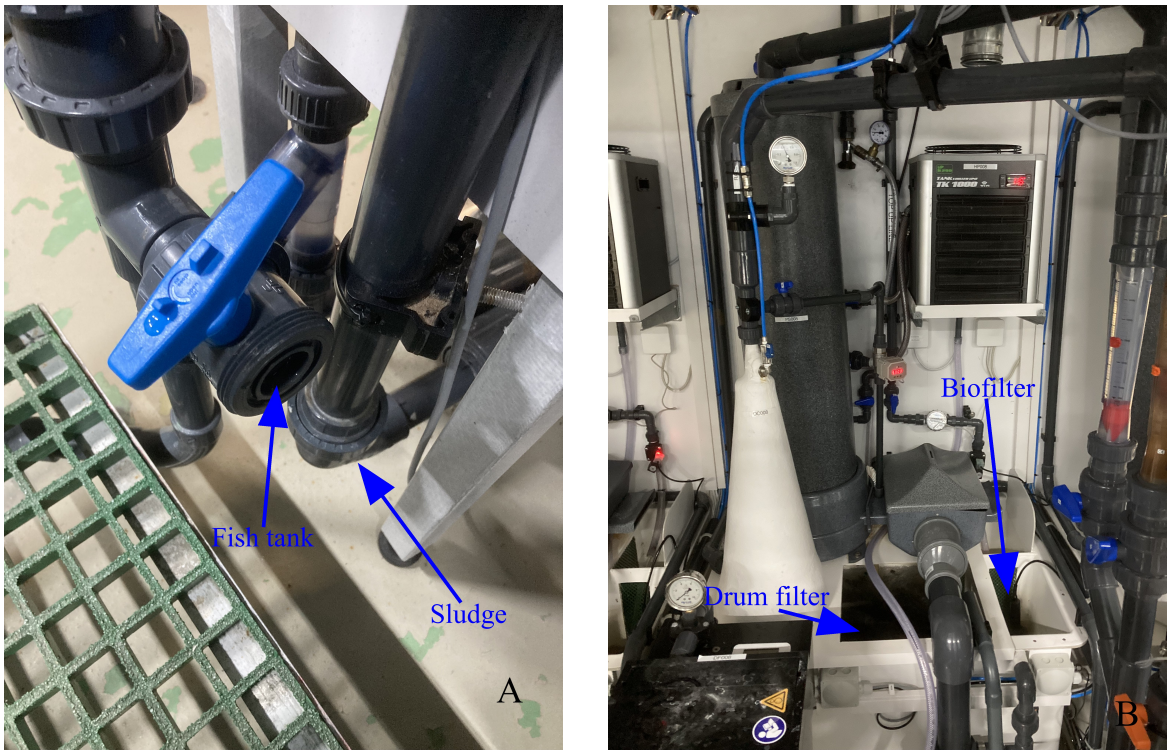
Appendix



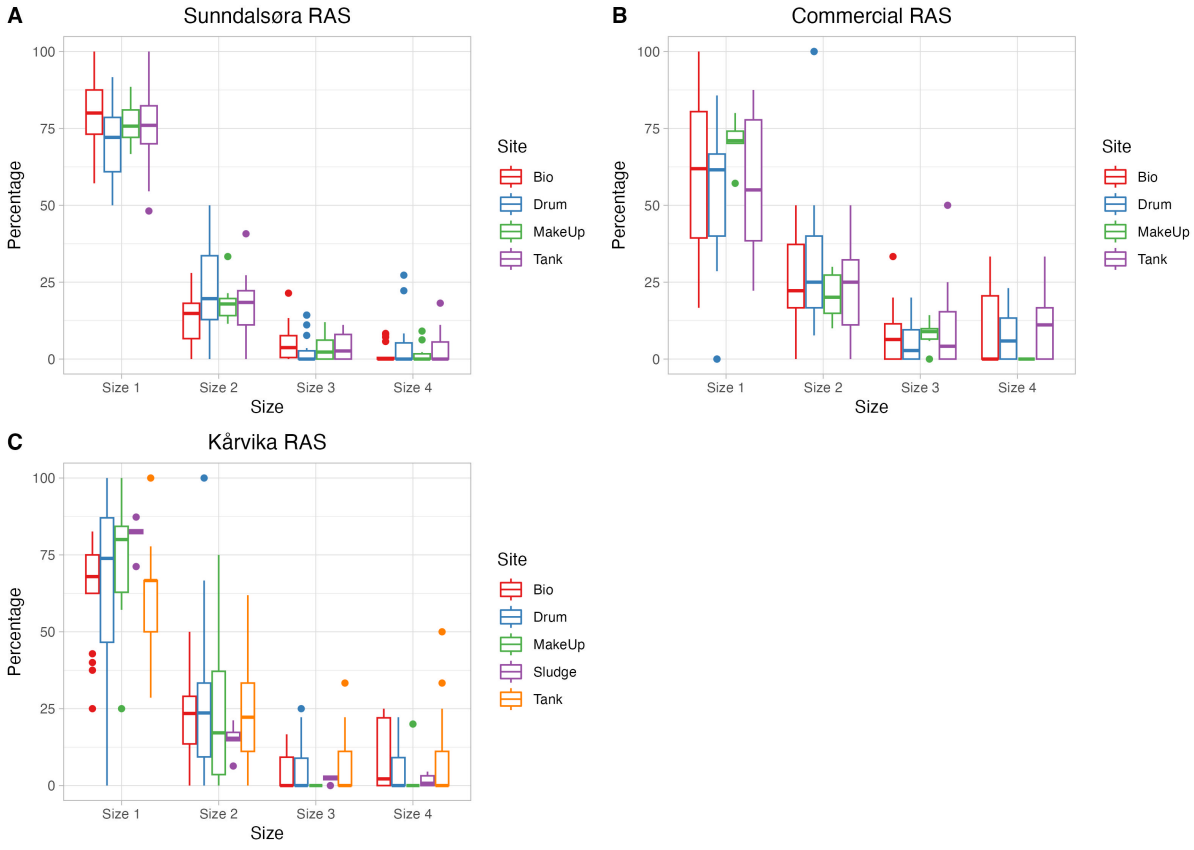
Appendix 1 Overview of the different sampling points from Sunndalsøra RAS. A: fish tank, B: outlet from drum filter, C: outlet from biofilter, D: sludge & E: make-up water.



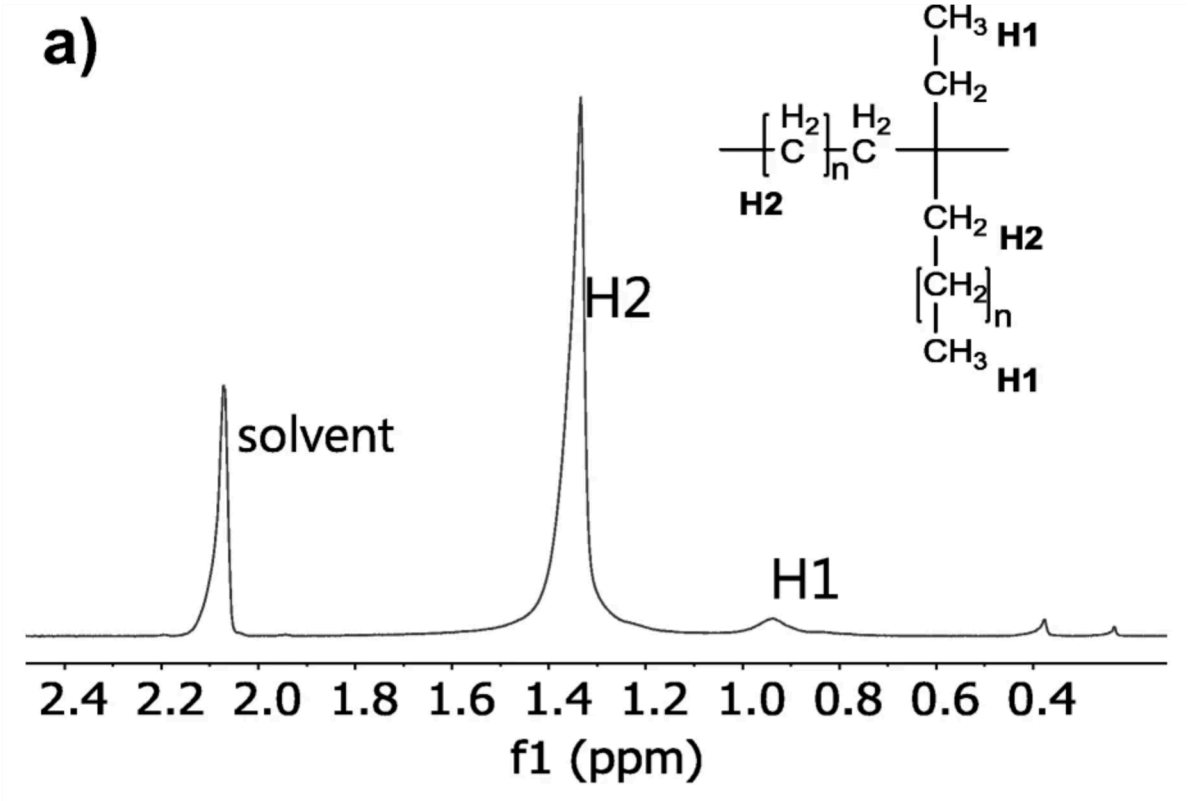
Appendix 2 Overview of the sampling points at the commercial RAS. A: fish tank, B: outlet from drum filter, C: outlet from biofilter & D: make-up water.



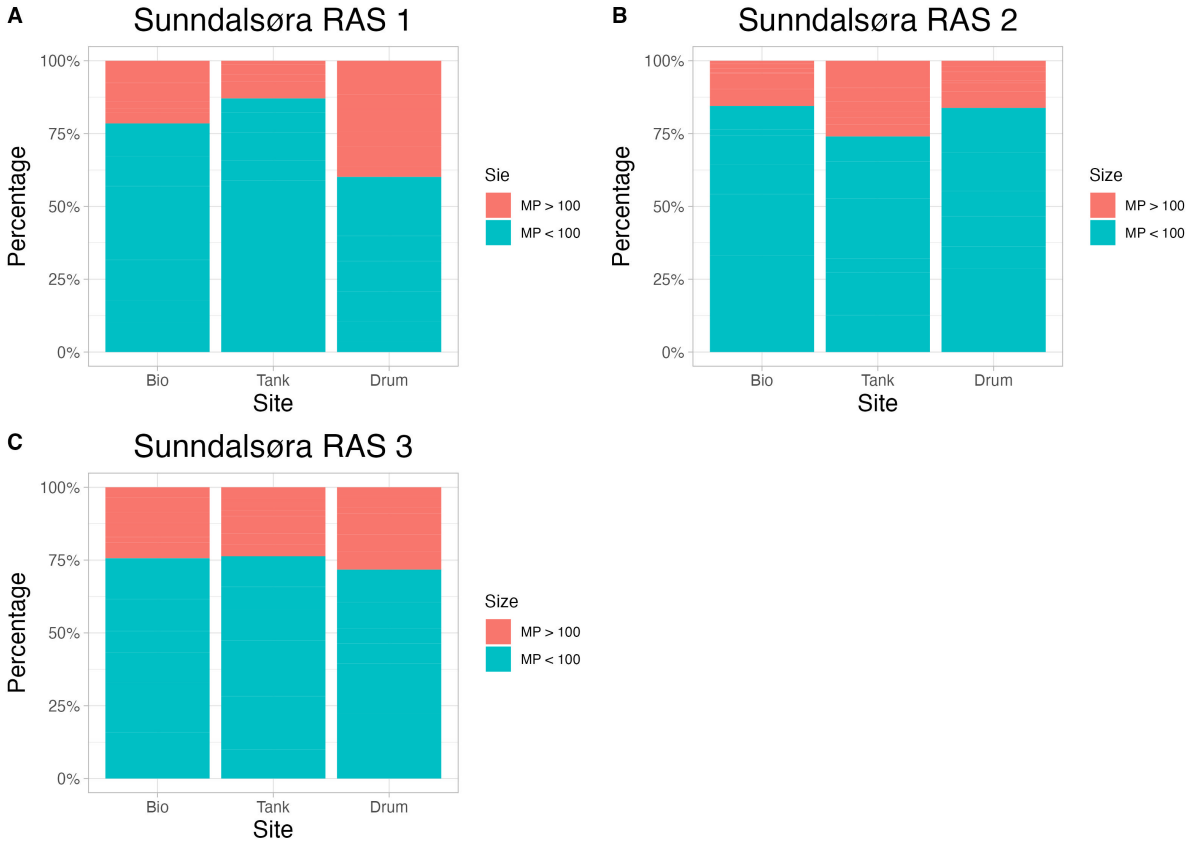
Appendix 3 Overview of the sampling points at RAS Kårvika. A: fish tank and sludge, B: outlet from drum filter and outlet of the biofilter sampled in the pump sump.



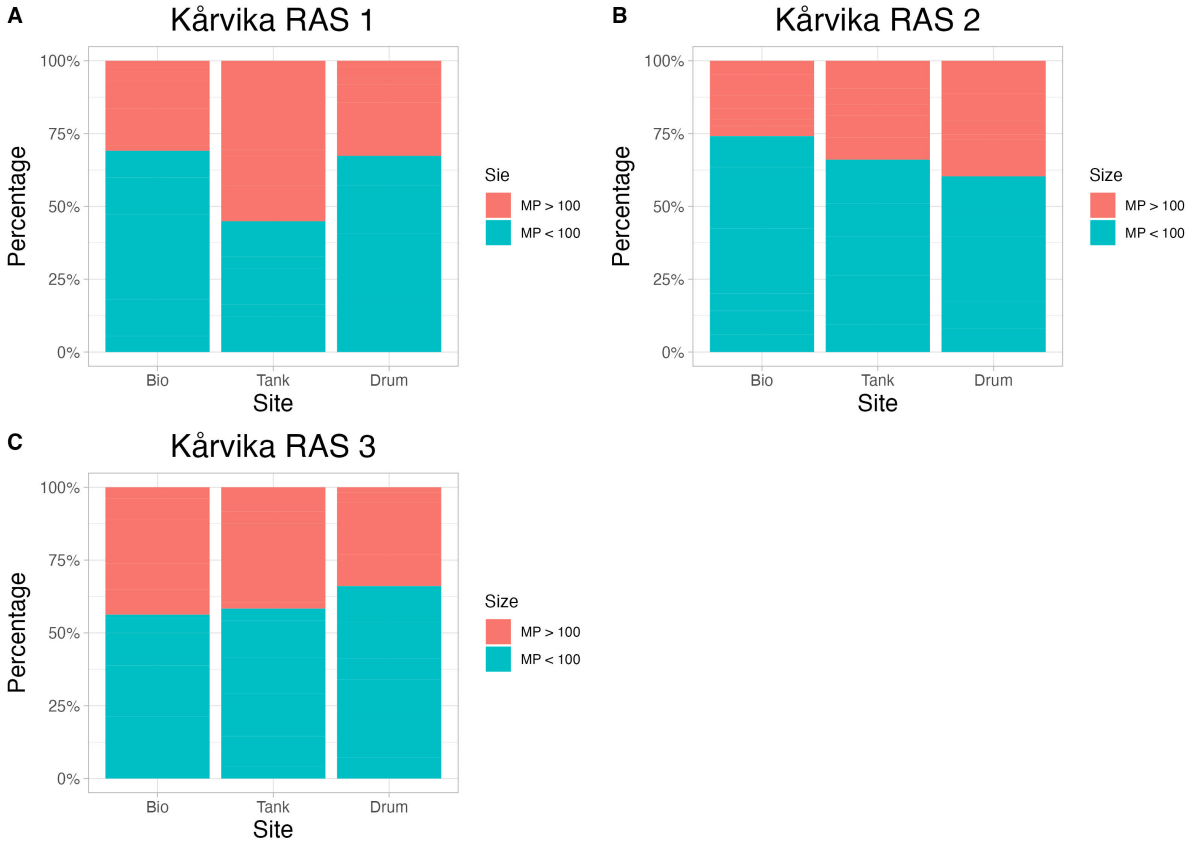
Appendix 4 Size distribution of MPs A: RAS Sunndalsøra, B: RAS Commercial site and C: RAS Kårvika. All including samples from the fish tank, drum filter, biofilter and make-up water, with sludge samples for Kårvika in addition. The MPs are divided into 4 groups regarding size, size 1: ≤100 µm, Size 2: 100-200 µm, Size 3: 200-300 and Size 4 ≥300 µm. The horizontal line within the boxes represents the median. The lower end of the vertical line up to the lower edge of the box is the first quartile (25% of the data), the box is the second quartile (50%) and the upper edge of the box to the upper end of the vertical line is the third quartile (25%). The dots are outliers.



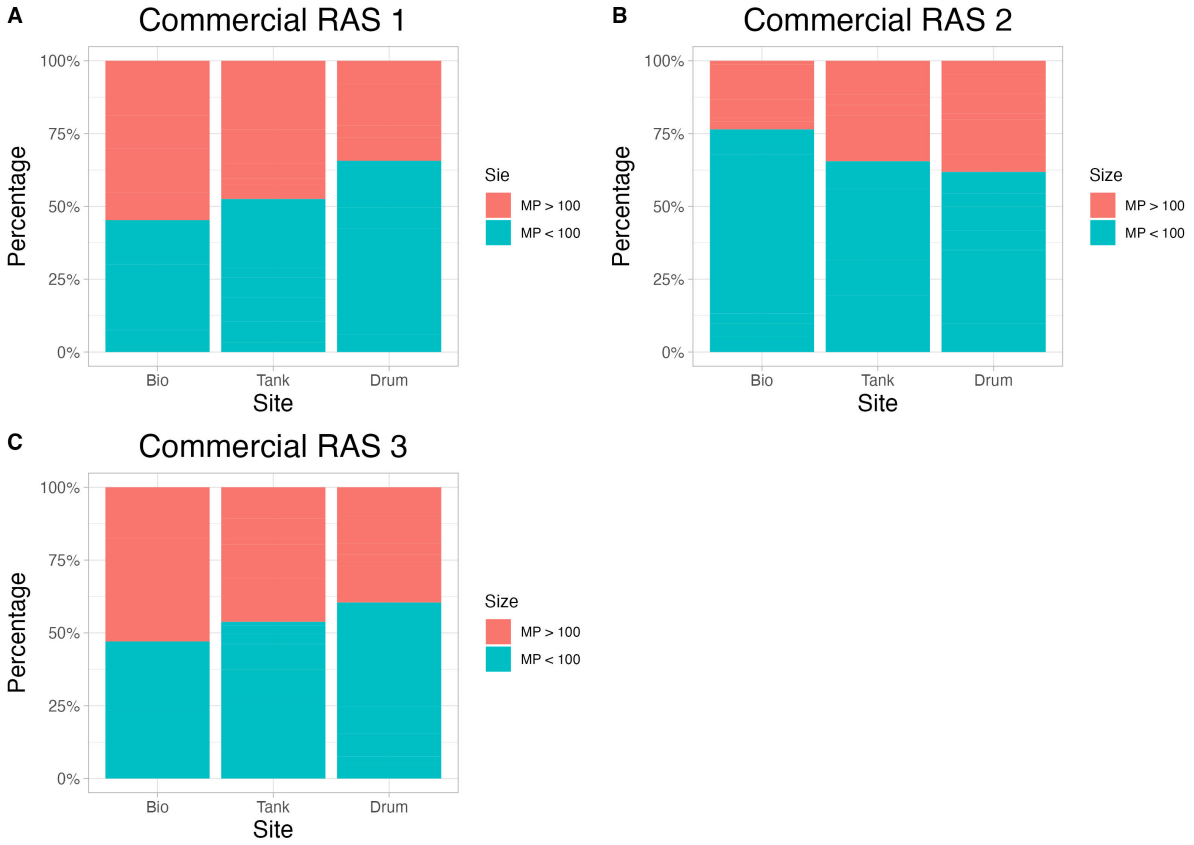
Appendix 5 NMR spectra of PE. Figure retrieved from Peez et al. (2019). X-axis is the chemical shift, absorbed protons, relative to the absorption of TMS (0 ppm).



Appendix 6 Percentage of MPs > 100 and < 100 µm for all three RAS from Sunndalsøra.



Appendix 7 Percentage of MPs > 100 and < 100 µm for all three RAS from Kårvika.



Appendix 8 Percentage of MPs > 100 and < 100 µm for all three RAS from the commercial site.