



ASTRAL

All Atlantic Ocean Sustainable, ProfiTable and Resilient AquacuLture

GA no **863034**

Research and Innovation Action (RIA)

Start date: 1st September 2020. End date: 30th August 2024

Title

Microplastics in IMTA labs



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 863034

Disclaimer: This material reflects only the author's view and the Commission is not responsible for any use that may be made of the information it contains.

**Document administration**

Deliverable number	D2.9
Deliverable name	Microplastics at IMTA labs
WP number	WP2
Deliverable due date	End of June 2024
Submission date	28/06/2024
Dissemination levels	Public
Lead beneficiary	NORCE
Responsible	Alessio Gomiero (NORCE)
Contributors	Brett Marc Macey (DFFE/UCT), John Bolton (UCT), Marissa Brink-Hull (UCT), Mark Cyrus (UCT) Louis Poersch (FURG)
Internal reviewers	Elena Torralba (LEITAT), Marie Smith (CSIR), Elisa Ravagnan (NORCE)

Document history and changes

Version	Date	Author	Description
1.0	29/06/23	NORCE	Table of Contents to WP Leaders (WPL), to Reviewers and Project Coordinator (PC)
1.0	1/09/23	NORCE	Advanced draft to contributors
1.1	1/02/24	NORCE, DFFE, UCT, FURG	Input collection and document update
1.2	20/04/24	NORCE, FURG	Last IMTA samples collection, analysis and reporting
1.3	20/06/24	NORCE, LEITAT, CISR	Internal review process completed.

Evidence of accomplishment

Report



Contents

1. Summary.....	4
2. Objectives of the deliverable.....	4
3. Background.....	4
4. Case studies description	5
4.1 IMTA lab South Africa.....	5
4.2 IMTA lab Brazil.....	7
5. Materials and methods	8
5.1 Sampling strategy during the ASTRAL project.....	8
5.2 Instrument description.....	9
5.3 Sampling & samples preparation	10
5.3.1 Sampling at IMTA South Africa.....	11
5.3.2 Sampling at IMTA Brazil.....	13
5.3.2.1 Inputs and sampling for microplastic analyses	14
5.4 Analysis.....	16
5.4.1 Identification of MPs by vibrational spectroscopy: μ FTIR.....	16
5.4.2 Thermal degradation analysis: pyr-GCMS.....	16
5.5 Data treatment.....	17
5.5.1 QA/QC -Exclusion of potential contamination	17
5.5.2 Microplastic particle concentration - μ -FTIR.....	17
5.5.3 Microplastic particle concentration – pyr-GCMS	17
6. Results	18
6.1 IMTA South Africa.....	18
6.1.1 Contamination monitoring.....	18
6.1.2 Plastic materials used within the SA IMTA facility	18
6.1.3 Microplastic fragments in the recirculating system	18
6.1.4 Back confirmation of microplastics occurrence by pyr-GCMS	20
6.2 IMTA Brazil	21
6.2.1 Contamination monitoring.....	21
6.2.2 Microplastic fragments in the recirculating system	21
6.2.4 Back confirmation of microplastics occurrence by pyr-GCMS	25
7. Literature comparison and conclusions	26
8. References	29

1. Summary

This document was created as part of the H2020 All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture (ASTRAL) project. One of the specific objectives of the ASTRAL project is to map the distribution of pollutants of emerging concern in the selected integrated multi-trophic aquaculture (IMTA) systems/labs, focusing on the application of robust and reliable sampling, sample preparation and analysis methods to be used to establish routine monitoring plans in such types of aquaculture production facilities. The deliverable's scope and objectives include the development of an *ad hoc* large volume water microplastics sampling device for application in the ASTRAL selected IMTA systems, and development of the application of the best available sample preparation and analysis technologies. Furthermore, this deliverable will provide recommendations for further improvement of the sampling, preparation and analysis and will define a routine monitoring plan for assessing aquaculture related microplastic pollution fingerprints.

2. Objectives of the deliverable

ASTRAL will analyse the presence of microplastics, an emerging pollutant that could affect the production as well as the quality, health and safety of the products, the environment around the IMTA facilities, as well as the closed or semi-closed systems themselves. To mitigate risks related to these threats, ASTRAL will suggest specific monitoring programmes for each IMTA lab, considering the regional specificities. The most appropriate technologies and sampling strategies will be considered for addressing specifically the identified risks. The focus in the present deliverable represents RAS and partially recirculating land-based pump-ashore systems, and not all IMTA systems.

The ultimate purpose of the deliverable is to:

- Identify areas for technology improvement: Based on the assessment, we will identify areas where the technology could be improved to meet the needs of IMTA producers and policy makers more effectively.
- Suggest an implementation plan: We will outline a plan for implementing the recommended improvements, including timelines, resource allocation, and any necessary changes to processes or systems.
- Map the distribution of pollutants of emerging concern in the selected IMTA labs systems.

3. Background

Microplastics (MPs; plastic fragments, fibers, sheets, films or beads in the size range 0.1 μm to 5 mm in their widest dimension) are widespread in the environment. MP pollution has been reported in marine, freshwater, and terrestrial ecosystems, from the sea surface to sediments, from beaches to the deep sea, from lakes to rivers, and from the tropics to the poles, and in a wide range of organisms representing different trophic levels, including human tissue and biological samples.

The release of MP from aquaculture operations has gained attention in recent years as a potential contributor to pollution in the marine environment, but also as a concern in terms of food safety. Until now, the focus has been on MP releases from open net cages. Recirculating aquaculture systems (RAS), as well as partially recirculating land-based pump-ashore systems, are increasingly



used for aquaculture production. RAS and other land-based aquaculture systems are complex built environments, where several key components are made of different plastics. These can include tanks and tank coatings, tubes for transportation of water, feed, fish and gases, protein skimmers and so forth. These systems are increasing in size, production volume and technical complexity, which leads to the need for efficient management of accumulating and released particles and chemicals from these systems.

4. Case studies description

4.1 IMTA lab South Africa

Buffeljags Abalone Farm is a partially recirculating land-based commercial aquafarm run by Viking Aquaculture (<https://www.vikingaquaculture.co.za/abalone/>) and forms part of the ASTRAL IMTA lab South Africa in collaboration with Viking, the University of Cape Town (UCT) and the Department of Forestry, Fisheries and the Environment (DFFE). It is ca. 200 km east of Cape Town, situated on a pristine stretch of coastline near the remote settlement of Buffeljags on the Cape south coast (34°45'14.7"S; 19°36'51.9"E). The farm is modern, efficient and environmentally sustainable and is one of the first large commercial abalone farms in South Africa to consistently recirculate ca. 50% of their seawater by making use of the bio-remediation capacity of the green seaweed *Ulva*. The farm currently has seven modular abalone-*Ulva* IMTA systems, called platforms, which are each composed of four clusters that each consist of one *Ulva* paddle-raceway and several (42) abalone tanks (six rows each made of seven abalone raceway tanks; Fig. 1).

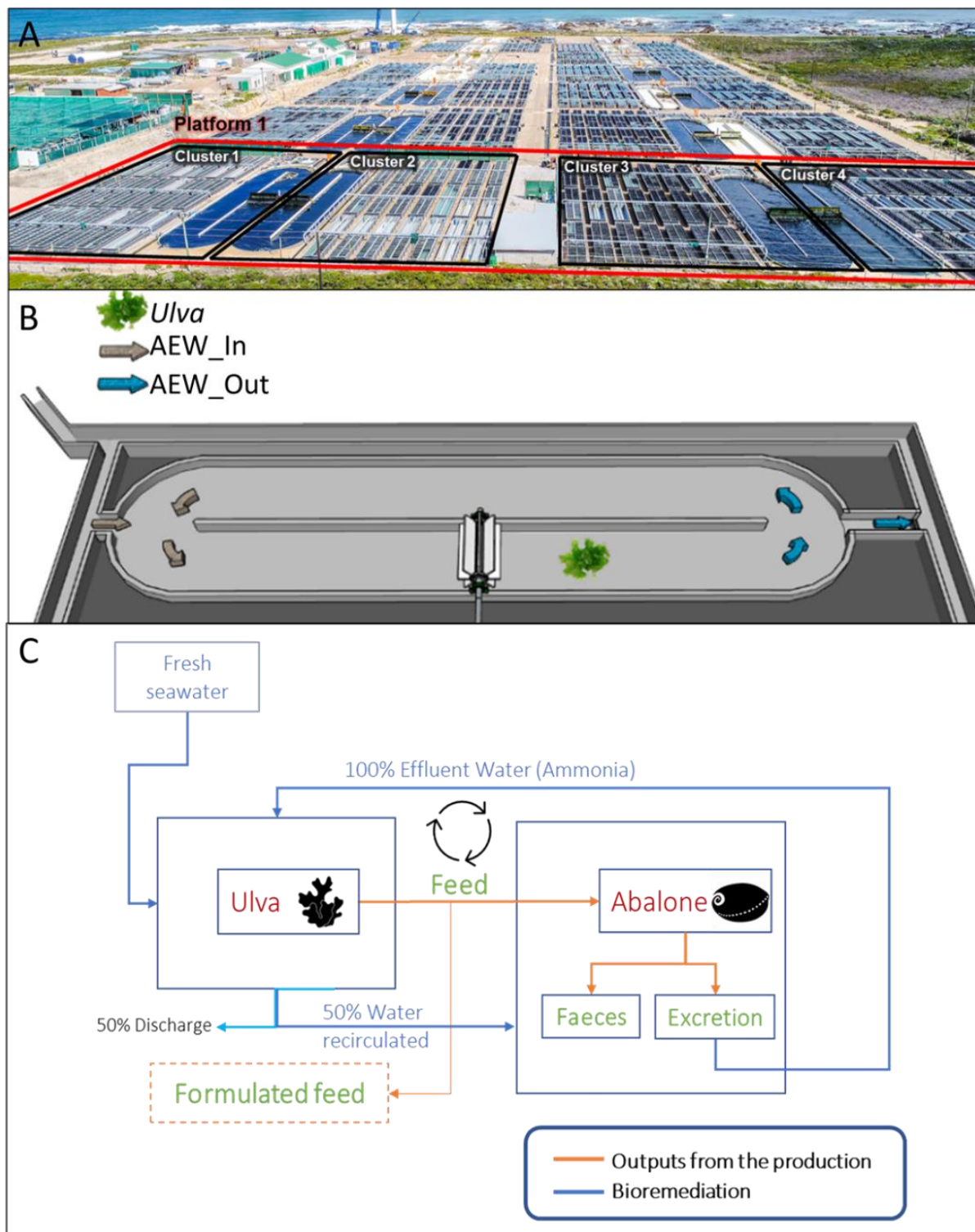


Figure 1 - (A) Land-based pump-ashore IMTA system to produce abalone (*Haliotis midae*) and seaweed (*Ulva lacunculata*) at Buffeljags abalone. The photograph shows the modular abalone-*Ulva* systems, arranged as seven platforms on the farm, with each platform composed of four clusters each consisting of one *Ulva* paddle-raceway and several abalone tanks. (B) Schematic of *Ulva* raceway receiving effluent water from the abalone tanks and bioremediated water leaving these tanks. (C) Schematic of the IMTA process at Buffeljags Abalone, where the effluent water leaving the abalone tanks is bioremediated by the *Ulva* before being mixed with 50% fresh seawater and returned to the abalone raceways.

Effluent water from the abalone tanks flows into the *Ulva* paddle-raceway and ca. 50% of this bio-remediated water is then recirculated back to the abalone tanks after mixing with ca. 50% fresh seawater, resulting in 50% recirculation. The fresh seawater is pumped directly from the adjacent coastline into a settlement pond prior to entering the abalone clusters. Abalone are grown in baskets suspended in sturdy fiberglass tanks (20 baskets per tank with ca. 10 kg abalone per basket). Seawater is circulated through the ponds, ensuring a constant supply of cool, aerated water for growing abalone. Animals are fed on a combination diet consisting of freshly harvested kelp (*Ecklonia maxima*), farm produced *Ulva lacinulata* and formulated feed. The farm produces ca. 450 tons abalone and ca. 600 tons of *Ulva* annually.

4.2 IMTA lab Brazil

The IMTA lab in Brazil is located in Rio Grande do Sul State, Southern Brazil, and operated by the Federal University of Rio Grande (FURG) at the Marine Aquaculture Centre in the Rio Grande do Sul. Each of the six independent systems consists of 3 components: a 16 m³ raceway containing the biofloc community and shrimp (Fig. 2); a 4 m³ raceway stocked with oysters suspended in bags (5/8” mesh); and a 4 m³ circular tank housing the fish (Fig. 3). Each system (composed of a shrimp raceway, macro algae raceway, and fish/oyster tank) is maintained independently of the other five systems, resulting in six distinct replicate systems constructed within two adjacent greenhouses. The shrimp raceway is stocked at 400 individuals per m³, an established density that ensures a rich biofloc community. Water from the shrimp raceway is analysed (biochemical oxygen demand-BOD; Total Suspended Solids – TSS; alkalinity, oxygen concentration – DO; pH and nitrogen compounds). Water is then flown to the raceway where the oysters and fish will filter out suspended particles that were not removed by the system. Water is then directed from the oyster raceway into the fish tank whereafter it leaves through a central bottom drain, designed to entrain solids, before being pumped back into the shrimp raceway. Solids in the shrimp effluent is eaten by the fish and oyster and toxic ammonia in the effluent is converted to nitrate by the microbial community associated with the biofloc. The proposed project effectively integrates the expertise of the Federal University of Rio Grande (FURG) research and their collaborators to develop a new technology that incorporates distinct species. The resulting, integrated, multi-trophic system is potentially more biologically efficient, more environmentally sustainable, and more economically competitive than the four cultivation systems maintained separately.

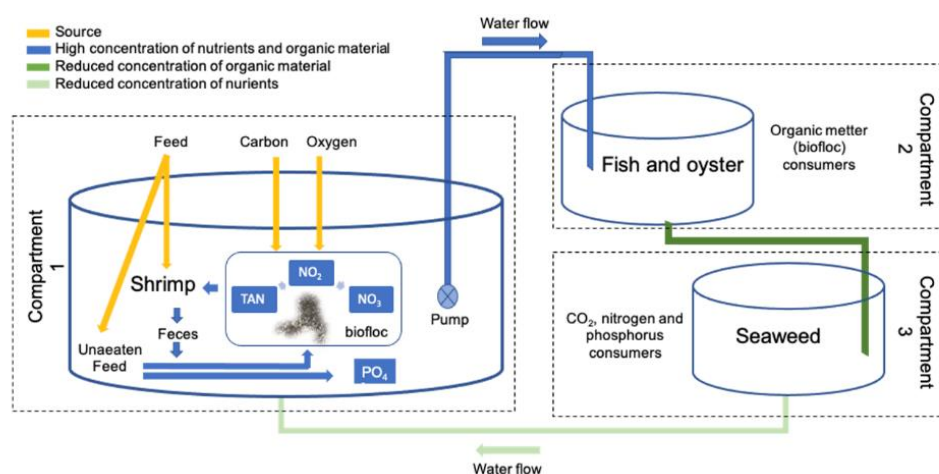


Figure 2 -Schematic design of IMTA FURG system.



Figure 3 - Picture of multitrophic greenhouse at FURG IMTA facility.

5. Materials and methods

5.1 Sampling strategy during the ASTRAL project

Microplastic sampling can be conducted using pumps, a strategy already used by scientists around the world, which enables the development of sampling methods that yield comparable results. Pump sampling can be conducted both from commercial and research vessels as well as from stationary and mobile vehicles inland. In the ASTRAL project a compact, large volume microplastics sampling device was developed by NORCE staff (Fig. 4), delivered to the IMTA sites and locally operated by assigned personnel.

In contrast to South Africa, Brazil used a small volume of water during the filtration. The high TSS concentration (biofloc) quickly saturated the filters, and it was no longer possible to continue with filtration (Fig. 5). After each sampling session the stainless-steel filters were released from the filter holder unit and transferred to pre-cleaned glass petri dishes of an appropriate size (\varnothing 180 mm), sealed, kept in dark conditions and stored in a freezer at -20°C . Stored samples were grouped and shipped to the lab at NORCE for the sample preparation and analysis by post or courier.

Furthermore, samples of the plastic material used within each IMTA facility have been requested to map the use of different polymers within the different aquaculture production sites.



Figure 4 – Large Volumes Microplastics Sampling device engineered by NORCE for ASTRAL project. (Credit: Alessio Gomiero).



Figure 5 – Microplastics Sampling device (left) and clogged filter after 5 litres filtration at FURG IMTA, Brasil

5.2 Instrument description

The sampling system consisted of a pumping unit and a filtration unit, containing two filter holders. Fig. 6 shows the schematic drawing of the sampling system.

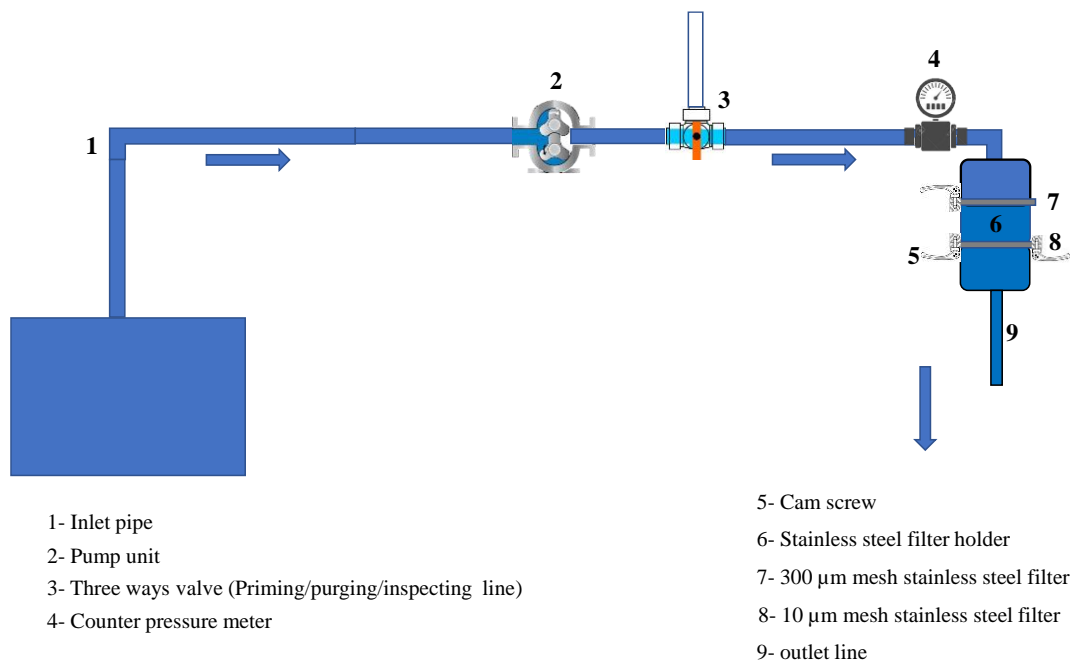


Figure 6 - Schematic drawing of the microplastics sampling device.

The filtration unit consists of two interconnected chambers where the top chamber holds the single-use 300 µm mesh stainless steel filter while the bottom chamber holds the single-use 10 µm mesh stainless steel filter. The preparation of the filter holders before the sampling session starts with the opening of the filtration chambers.

5.3 Sampling & samples preparation

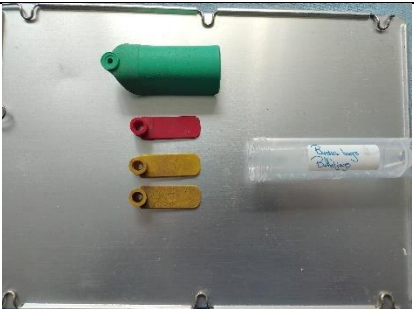
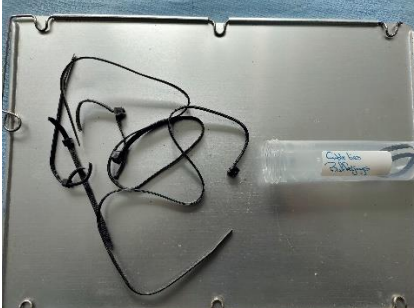


The sample preparation method applies a gentle and efficient purification step before quantitative chemical identification. The main interferences for a reliable quantification are the residual organic components that may create a high background signal during analysis, hampering a correct MP quantification. The principle used is a sequential enzymatic degradation and removal of proteins and fats followed by a strong oxidation treatment and enhanced dissolution of fats in an alkaline environment. Once in the ultraclean plastic lab the stainless-steel filters were removed from the container and placed on a large crystallizer filled with 5% Sodium Dodecyl Sulphate (SDS) and placed on orbital shaker (40 rpm) and incubated at 50°C for a minimum of 6 hours before further enzymatic treatments. After incubation in SDS, the sample was sonicated to release the particles from the filtering surface and transferred quantitatively to a vacuum filtration assembly and passed through a 10 µm stainless steel filter. The funnel was rinsed with a 50% ethanol/water solution from a Polytetrafluoroethylene (PTFE) squeeze bottle to ensure complete transfer. The filter and sample were placed back into the beaker (inverted), and glycine buffer was added. Ultrasonication was performed on the sample beaker for 5 minutes to release any particles attached to the filter. Following ultrasonication, the filter was rinsed with 1 mL of GF/A filtered Milli-Q water to transfer any remaining microplastics to the sample beaker. Then, 1 mL of protease enzyme was added, and the beaker was incubated at 50°C for 48 hours. For the final purification step, a strong oxidative digestion was performed using 30% hydrogen peroxide at 50°C for 6 hours reducing the sample into a clear liquid. The filtration and sonication steps were repeated, and after sonication, the filter was thoroughly rinsed on both sides with 50% ethanol/water. Any remaining ethanol/water solution in the sample beaker


was evaporated at 50°C until 1 mL of solution remained. The 1 mL of sample was transferred to a glass vial for storage, and the tube was washed with 4 x 1 mL of 50% ethanol, resulting in a final volume of 5 mL in the sample vial. Prior to analysis, the contents were filtered onto a 0.1 µm Anodisc filter (Whatman, Φ 13 mm) and dried at room temperature in a glass petri dish with a lid until further examination.

5.3.1 Sampling at IMTA South Africa

The sampling session took place in August 2022. Five samples of the plastic material used within the IMTA facility to produce abalone and *Ulva* were submitted for chemical characterization (Tab. 1).

Table 1 - Sample of the plastic material collected from the South Africa IMTA production site.

Sample #	Description	Sample picture
1	Basket tags	
2	Cable ties	
3	Water pipe	
4	Basket dividers	

5	Piece of abalone basket	
---	-------------------------	--

For the water sampling, approximately 500 L of water was collected from two sampling sites (n = 3 per site) located in the IMTA’s facility (Tab. 2).

Table 2 - Recorded volumes of seawater for each of the collected replicates in the two sampling sites (settlement pond for seawater entering the farm and effluent water leaving an abalone-*Ulva* cluster) at the South Africa IMTA.

Sample code	Location description	Collected volume (L)
SA_MP_#01	Settlement pond – Inlet water	985
SA_MP_#02	Settlement pond – Inlet water	590
SA_MP_#03	Settlement pond – Inlet water	520
SA_MP_#04	Abalone cluster2.1 – Outlet water	518
SA_MP_#05	Abalone cluster2.1 – Outlet water	522
SA_MP_#06	Abalone cluster2.1 – Outlet water	520

After each filtration session each filter was placed in pre-cleaned large glass petri dishes, sealed with tinfoil, and stored in cold and dark conditions prior to shipment to NORCE partner Plastlab.

5.3.2 Sampling at IMTA Brazil

The study was conducted for 52 days at the Laboratório de Avaliação de Impactos da Aquicultura and at the Laboratório de Ecologia de Microorganismos Aplicada à Aquicultura. Both laboratories are part of the Marine Aquaculture Center – Federal University of Rio Grande - FURG.

The experiment was performed in a completely randomized design involving two treatments with three replicates, integrating Pacific white shrimp (*Penaeus vannamei*), seaweed (*Ulva lactuca*), and Nile tilapia (*Oreochromis niloticus*) using biofloc technology. The treatments were analyzed, one two types of culture systems were the different feeding management, where fish were subjected to two feeding rates (1 and 3% of body weight per day; BW/d; Table 3).

The experimental units consisted of 16, 4, and 4 m³ circular tanks (Polyvinyl chloride vinitank, Sansuy®) of useful volume to culture the shrimp, the fish, and the macroalgae, respectively. All were placed in a greenhouse and covered with 80% shade cloth. Each experimental units had an aeration system composed of micro-perforated hoses (Aerotube®) with 15 cm at the bottom of the tank connected to a 7.5 HP blower (Ibram®) via polyvinyl chloride pipes to keep the biofloc in suspension and dissolved oxygen above 5 mg/L. Macroalgae were placed in floats made of polyvinyl chloride pipe and a 5 mm polyethylene mesh.

Both treatments were filled with 13,014 L of seawater and 8,986 L of freshwater from a well to adjust the salinity to 20 g/L and, subsequently, were chlorinated at 30 ppm and dechlorinated with aeration for three days. Then, the system remained in constant recirculation whereby water circulated from the shrimp tank to the fish tank through a Boyu submersible pump (SPA 4000 L/hr, BOYU®), with a flow rate adjusted daily to 400 L/hr, and then passed to the algal tank by gravity and returned to the shrimp tank by gravity. Throughout the experiment, no water exchange occurred in the experimental units. Freshwater from the well was added only to replace water lost through evaporation to maintain salinity of 20 g/L.

Before the beginning of the experiment, based on water quality analyses, ammonia control was performed aimed only at preventing the values from surpassing 0.8 mg/L, with the intention of leaving substrate for the nitrifying bacteria. The organic carbon source used was sugar cane molasses at a ratio of 6 g carbon to 1 g of total ammonia nitrogen. Throughout the experiment, no sugar cane molasses was added because the bacteria community and macroalgae controlled the concentrations of nitrogen that tend to increase over time during the production cycle. Before the beginning of the experiment, agricultural hydrated lime was also added to increase alkalinity and pH to 150 mg/L and 7.3, respectively. Throughout the experiment, when pH and alkalinity values were respectively lower than 7.3 and 150 mg/L, they were corrected with the addition of agricultural hydrated lime.

After the biofloc system stabilization, shrimp tanks were stocked with 230 shrimp/m², fish tanks with 30 tilapia/m³, and algal tanks with 0.7 kg of macroalgae/m³ equivalent to 0.13 kg of macroalgae/m³ considering 22 m³ of the whole system.

Shrimp were fed two times a day with a commercial feed (Guabitech 1.6 mm, Guabi®), with daily amounts adjusted weekly after biometrics. Fish individuals were fed twice a day with a commercial feed (Pirá Evolution 2-3 mm, Guabi®), corresponding to two feeding rates (1 and 3% of BW/d) to stimulate the biofloc consumption.



5.3.2.1 Inputs and sampling for microplastic analyses

Table 3. Inputs and sampling for microplastic analyses at the beginning and end of the study in a multi-trophic system with biofloc applied to *Penaeus vannamei*, *Ulva lactuca*, and *Oreochromis niloticus* fed with two feeding rates (1 and 3% of body weight per day), with three replicates.

Inputs and samples	Sample Amount	Dilution ^(a)	Total input used in the experiment
Fresh water (from the well)	5 L	No	Tank 1: 11,590 L Tank 2: 11,594 L Tank 3: 11,822 L Tank 4: 12,595 L Tank 5: 12,261 L Tank 6: 11,716 L
Salt Water (from the sea)	5 L	No	Tank 1: 13,014 L Tank 2: 13,014 L Tank 3: 13,014 L Tank 4: 13,014 L Tank 5: 13,014 L Tank 6: 13,014 L
Sugar cane molasses	1 kg	1 L	Tank 1: 1.39 kg Tank 2: 1.13 kg Tank 3: 0.92 kg Tank 4: 0.25 kg Tank 5: 0.29 kg Tank 6: 1.10 kg
Agricultural hydrated lime	1 kg	1 L	Tank 1: 3.45 kg Tank 2: 3.92 kg Tank 3: 3.45 kg Tank 4: 4.23 kg Tank 5: 3.29 kg Tank 6: 3.76 kg
Shrimp feed	1 kg	1 L	Tank 1: 52.70 kg Tank 2: 52.52 kg Tank 3: 52.70 kg Tank 4: 52.52 kg Tank 5: 52.52 kg Tank 6: 52.52 kg
Tilapia feed ^(b)	No	No	Tank 1: 6.89 kg Tank 2: 1.84 kg Tank 3: 7.68 kg Tank 4: 1.86 kg Tank 5: 7.13 kg Tank 6: 1.74 kg
Culture water initial (from the integrated multitrophic aquaculture system) ^(c)	Tank 1: 5 L Tank 2: 5 L Tank 3: 5 L Tank 4: 3 L Tank 5: 2 L Tank 6: 2 L	No	Tank 1: 24,604 L Tank 2: 24,608 L Tank 3: 24,836 L Tank 4: 25,609 L Tank 5: 25,275 L Tank 6: 24,730 L
Culture water final (from the integrated multitrophic aquaculture system) ^(c)	Tank 1: 2 L Tank 2: 2 L Tank 3: 2 L Tank 4: 2 L Tank 5: 2 L Tank 6: 2 L	No	

Note: ^(a) The dilution was made using one liter of distilled water in a glass beaker; ^(b) Nile tilapia feed was not sent for microplastic analyses; ^(c) The volume of the initial and final samples of the culture water was different due to the total



suspended solids concentration in the biofloc system in each tank. Thus, it was defined as 2 liters as the standard for sampling. Treatment 1 (1% of body weight per day): tanks 2, 4, and 6; Treatment 2 (3% of body weight per day): tanks 1, 3, and 5.

Sampling was conducted on days 1, 26, and 52 (Table 3- 4). On day 1, samples of all elements entering the experimental system were collected. Seawater and freshwater were filtered directly through the equipment, while sugar cane molasses, lime, and feed were dissolved in 1 liter of water before being filtered.

Table 4 – Samples type and associated volumes (L) collected at FURG IMTA facility in Brazil.

Day 1	Day 26	Day 52
- Freshwater: - Petri Dish: 2 - Volume: 5 liters	- Tank 1: - Petri Dish: 1 - Volume: 5 liters	- Tank 1: - Petri Dish: 15 - Volume: 2 liters
- Seawater: - Petri Dish: 9 - Volume: 5 liters	- Tank 2: - Petri Dish: 8 - Volume: 5 liters	- Tank 2: - Petri Dish: 7 - Volume: 2 liters
- Molasses: - Petri Dish: 10 - Volume: 1 liter	- Tank 3: - Petri Dish: 17 - Volume: 5 liters	- Tank 3: - Petri Dish: 6 - Volume: 2 liters
Lime: - Petri Dish: 12 - Volume: 1 liter	- Tank 4: - Petri Dish: 16 - Volume: 3 liters	- Tank 4: - Petri Dish: 5 - Volume: 2 liters
- Feed: - Petri Dish: 11 - Volume: 1 liter	- Tank 5: - Petri Dish: 14 - Volume: 2 liters	- Tank 5: - Petri Dish: 4 - Volume: 2 liters
	- Tank 6: - Petri Dish: 13 - Volume: 2 liters	- Tank 6: - Petri Dish: 3 - Volume: 2 liters

Samples origin description

Seawater – Represents the inlet saline water used to start the aquaculture production.

Freshwater – Represents the inlet freshwater used to adjust the running RAS system water to its final optimal salinity.

Molasses – is a viscous byproduct, principally obtained from the refining of sugarcane or sugar beet juice into sugar. Molasses is rich in vitamins and minerals, including vitamin B6, iron, calcium, magnesium, and potassium. Different carbon sources have been used to promote microbial development in biofloc, including molasses, glycerol and glucose. However, researchers have noted that using organic sources of carbon promotes the growth of beneficial heterotrophic bacterial communities. Even though it is classified as a by-product, molasses has considerable benefits for shrimp cultivation. Start by encouraging good bacteria growth, improving pond water quality, preventing pathogenic bacteria from growing, and reducing the feed conversion rate.

Lime - The application of lime in the biofloc system aims to correct the pH of the water, which tends to be consumed by chemoautotrophic bacteria in the process of oxidizing nitrite to nitrate, mainly. The carbonate concentration must be between 130 and 180 mg/L for the system to function fully and without compromising the performance of the shrimp.

Feed - Commercial foods formulated for *Penaeus vannamei* and *Oreochromis niloticus* were used throughout the experiment. The shrimp food was formulated based on fish meal and the tilapia food was formulated based on vegetable protein.

5.4 Analysis

5.4.1 Identification of MPs by vibrational spectroscopy: μ FTIR

Identification of plastic fragments $> 500 \mu\text{m}$ was performed using an Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy using a Thermo Fisher Nicolet iN10 MX microscope. ATR-FTIR spectroscopy is utilized for identifying chemical compounds, studying molecular structures, examining surface properties by contact and without further preparation. It utilizes total internal reflection to generate an evanescent wave that penetrates the sample, providing valuable molecular information.

Identification of polymer types and measurement of particle sizes $10 < X < 300 \mu\text{m}$ were performed using Micro Fourier Transform Infrared Spectroscopy (Thermo Fisher Nicolet iN10 MX Infrared Imaging Microscope). The instrument is equipped with a N₂-cooled 64x64 line array mapping detector and a quantum MCT (mercury cadmium telluride) detector. The linear array detector collected 4 scans/acquisition point per time, and the Infrared (IR) spectra of each microplastic particle was recorded in the mid-IR range of $4000\text{--}850 \text{ cm}^{-1}$, with a spectral resolution of 4 cm^{-1} in transmission mode. Polymer identification was performed by comparing the spectral match of the particles with a reference library (SiMPle, v1.3.1 β). Polymers with a spectral match greater than 70% were considered positively identified. The software used for identification and grouping of polymer types also facilitated the theoretical mass calculations per particle. Four morphotypes (fiber, pellet, film and fragment) of MPs were classified. The relevant polymer groups identified in this study were Polypropylene (PP), Polyethylene (PE), Polyester (PES), Polystyrene (PS), Polyurethane (PU), Nylon or Polyamide (PA), and Polyethylene terephthalate (PET), ethylene vinyl acetate (EVA), acrylic paint, Polyvinyl alcohol (PVOH), Cellulose ester (CE) and Polyether sulfones (PES). Due to the high content of black carbon, a material that adsorbs completely in the Infra-Red spectral range, the occurrence of tyre wear particles in the samples cannot be detected by this technique but through pyrolysis mass spectrometry (pyr-GCMS).

5.4.2 Thermal degradation analysis: pyr-GCMS

Pyrolysis Gas Chromatography Mass Spectrometry (pyr-GCMS) is a destructive method that uses thermal decomposition of materials at elevated temperatures in an inert (low-oxygen) atmosphere. Large molecules break at their weakest bonds, producing smaller, more volatile fragments. These fragments can be separated by gas chromatography and detected by a mass spectrometer and the output data can be used as a fingerprint to identify material. The obtained pyrograms, with peaks of ions appearing at different retention times, are compared with a customized database and cross-checked with literature to identify the chemical composition of the material using recommendations and selection criteria from Fischer and Scholz-Böttcher (2017) and Gomiero et al. (2019).

Nine of the most used plastic polymers are quantified: (PE, PP, PS, PVC, PA6, PMMA), PC, PET and PU. Additionally, differently from the FTIR technique, pyr-GCMS can estimate the content of car tyre particles in the samples by characterizing the occurrence of styrene-butadiene rubber.

Standard curves with known concentrations are used to calculate the concentrations of target materials in the sample. Pyr-GCMS analyses were performed with a Shimadzu Nexis GCMS QP2020NX

equipped with a Rxi-5ms column (RESTEC, Bellefonte, PA) and coupled with Frontier Laboratories Multi-Shot Pyrolizer EGA/PY-3030D with auto-shot sampler. In this analysis the Limits of Quantification (LoQ) and Detection (LoD) per each of the addressed polymers is presented on Tab. 5.

For the μ -FTIR analysis. The lower size of the analysed plastic particles depends on the filter size used in the sample preparation steps. For the present study stainless steel filters of 10 μ m size were used. Aliquots of MP stored in the ethanol/water solution were filtered on pre-burned GF/C 25 mm fiberglass filters. The obtained filters were folded in a pyrolysis cup for chemical analysis. During the pyr-GCMS analyses dust trap collectors were used to evaluate possible contamination from airborne particles. Furthermore, the steel cups used for the pyrolysis analysis were cleaned before use with a butane blow torch at 1400°C to remove any plastic.

Table 5 - Pyrolysis -GCMS: calculated Limits of Detection (LoD) and Quantification (LoQ) of for the addressed polymer types.

	LOD	LOQ
N66	0.48	1.61
PS	0.02	0.06
PMMA	0.01	0.03
PE	0.28	0.93
PET	0.37	1.23
ABS	0.01	0.03
PP	1.34	4.47
N6	0.02	0.06
PVC	0.35	1.18
PC	2.69	8.95
PU	2.08	6.93
SBR	0.82	2.72

5.5 Data treatment

5.5.1 QA/QC -Exclusion of potential contamination

To assess and address potential contamination, we examined and evaluated wet traps corresponding to samples with positive microplastic identifications.

5.5.2 Microplastic particle concentration - μ -FTIR

The concentration of microplastic particles (MP) was calculated in number of plastic particles (MPS) per cubic meter of samples water using the SiMPLe software output. Data are stored in Microsoft Excel based environment. The number of detected MP particles is standardized to 1 m³ of water.

5.5.3 Microplastic particle concentration – pyr-GCMS

The concentration of microplastic particles (MP) was calculated in number of plastic particles (MPS) per cubic meter of samples water using the estimated mass per particle obtained from the MPS 2.0 (Lab Frontiers) software output, and the wet weight of the sample at the time of dissection.

$$\text{MP Concentration} = (\text{Particle mass (mg)}) / (\text{Sample volume (m}^3\text{)})$$

6. Results

6.1 IMTA South Africa

6.1.1 Contamination monitoring

Three procedural blanks and six samples from the wet dust trap collectors were analyzed to monitor potential sources of contamination affecting the sample preparation and the analysis. The contamination was related to handling the sampling equipment, preparing the sample for analysis, and finally the analysis itself. The results showed a contamination of 6.0 ± 1.0 MPs per procedural blank sample. The polymeric composition of the contaminating MPs was $\approx 50\%$ PE, $\approx 30\%$ PES and $\approx 20\%$ PP. The measured contamination of non-synthetic materials (approximately 22 ± 5 particles) was protein-based material and cellulose fibres. Furthermore, the result of the wet trap collectors showed a contamination of 2.9 ± 2.0 MPs per trap located in the clean lab where the samples were processed and analysed by μ -FTIR imaging. The polymeric composition of the wet trap collectors were $\approx 25\%$ PES, $\approx 25\%$ PE and $\approx 50\%$ PS.

6.1.2 Plastic materials used within the SA IMTA facility

The chemical characterization of samples of the main plastic material used within the IMTA showed the occurrence of five different polymer types: PVC, PA, PU, PS and PE (Tab. 6).

Table 6 – Results of the chemical characterization of the plastic material collected from the South Africa IMTA production site.

Sample #	Description	Chemical identification	Accuracy
1	Basket tags	PU	79 %
2	Cable ties	PA	81 %
3	Water pipe	PVC	88 %
4	Basket dividers	PS	79 %
5	Piece of abalone basket	PE	88 %

6.1.3 Microplastic fragments in the recirculating system

Sample numbers 1-3 were collected from the settlement pond in the IMTA (Water entering the farm), whereas samples 4-6 were collected from one of the abalone clusters, cluster 2.1 (abalone water that has passed through the *Ulva* raceway). Overall, 128 MP fragments/m³ were observed in sample #1; 143 MP fragments/m³ were observed in sample #2; 222 MP fragments/m³ were observed in sample #3; 355 MP fragments/m³ were observed in sample #4; 409 MP fragments/m³ were observed in sample #5; 429 MP fragments/m³ were observed in sample #6 (Fig. 7). The total amount of MPS significantly increases through the system however only few polymer types such as PVC, PLY, Acrylic paint, and PP show a significant increment while the abundance of others like PE fluctuates within the two sampling sites of the IMTA. Overall, PE ($\approx 25\%$) followed by PP ($\approx 24\%$) and PVC ($\approx 19\%$) are the three most distributed polymer types in the partially recirculating aquaculture system while the remaining detected polymer types (PS, PU, EVA, PA, acrylic paint, PET, PVOH, cellulose ester and PES) accounted for a total of $\approx 30\%$ (Fig. 8).

The increment of the frequencies of some identified polymers in the IMTA flow stream may be related to the shape and design of the tanks, the orientation and the strength of the water flow, high pressure cleaning routine procedures as well as to chemical and physical processes such as photodegradation which turn plastic material brittle. For PVC the observed trend may be explained by the release of microplastics from friction of water moving through pipes supplying water to tanks, while the observed levels of acrylic paint particles can be linked to the paint abrasion due to the harsh saline environment. Polypropylene is the major component of the bags used to store formulated feeds. From the perspective of MPs characteristics, only morphotypes of fiber and fragment were visualized (Fig. 9). In all systems, fiber was the most abundant shape of MPs and similar proportion of the two forms in both inlet and outlet of the IMTA’s water.

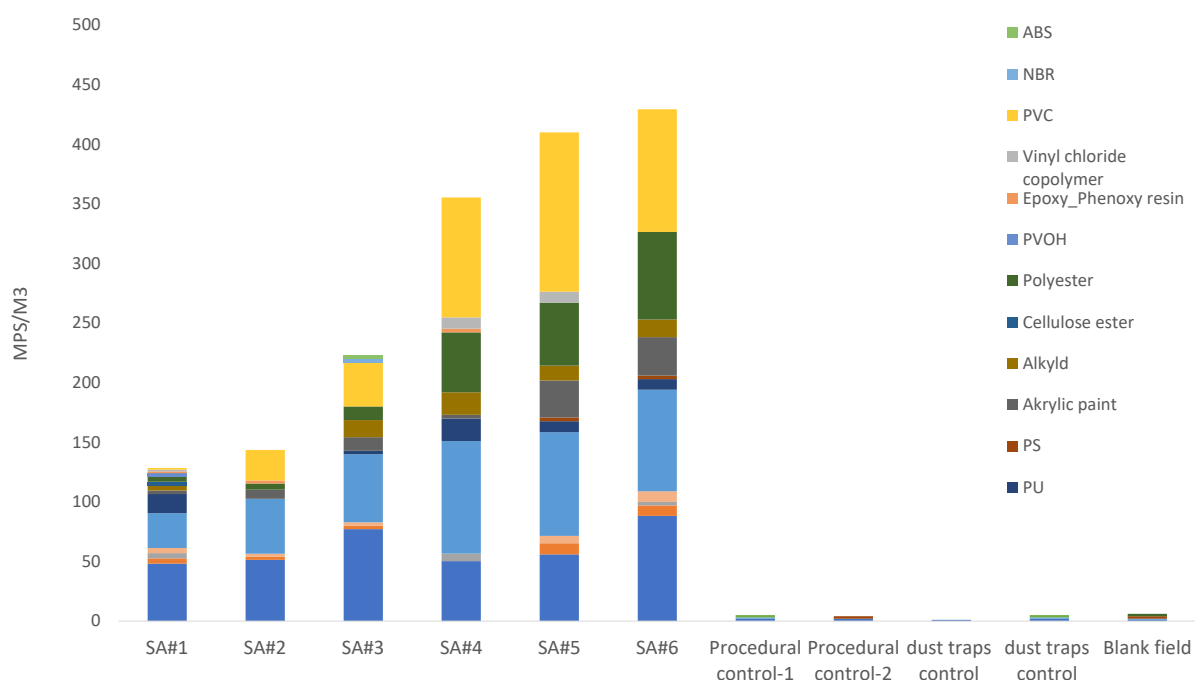


Figure 7 – MPs distribution in the in the settlement pond (SA #1-3) and in the abalone-*U/ua* aquaculture systems (SA #4-6) of the South African IMTA.

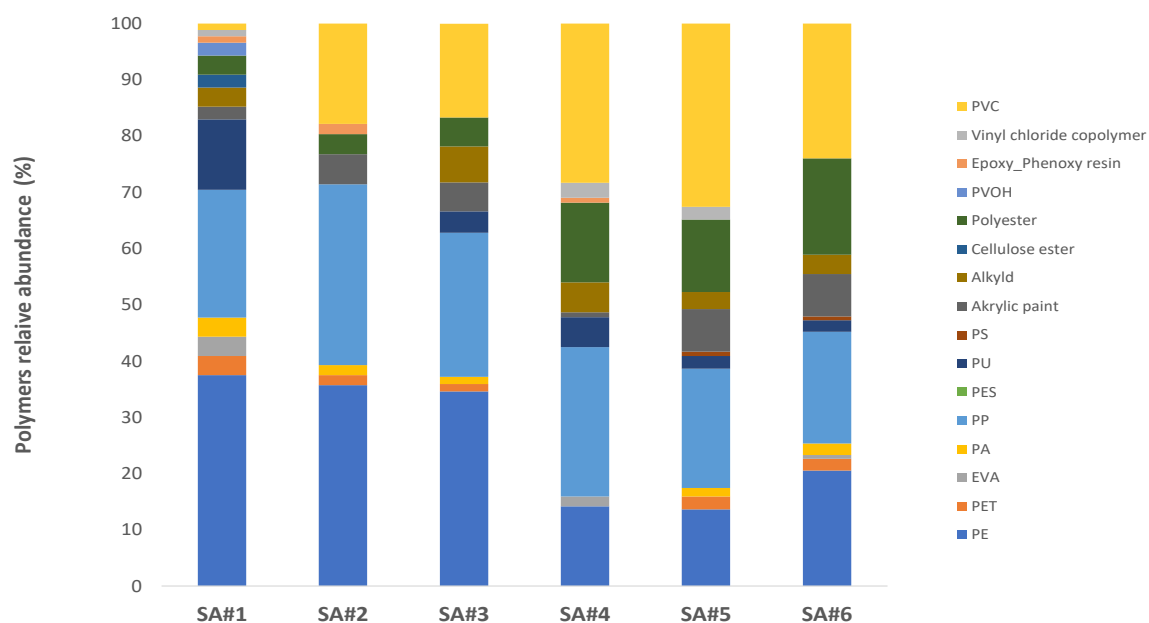


Figure 8 – Polymers’ relative abundance in the settlement pond (SA #1-3) and in the abalone-*Ulva* aquaculture system (SA #4-6).

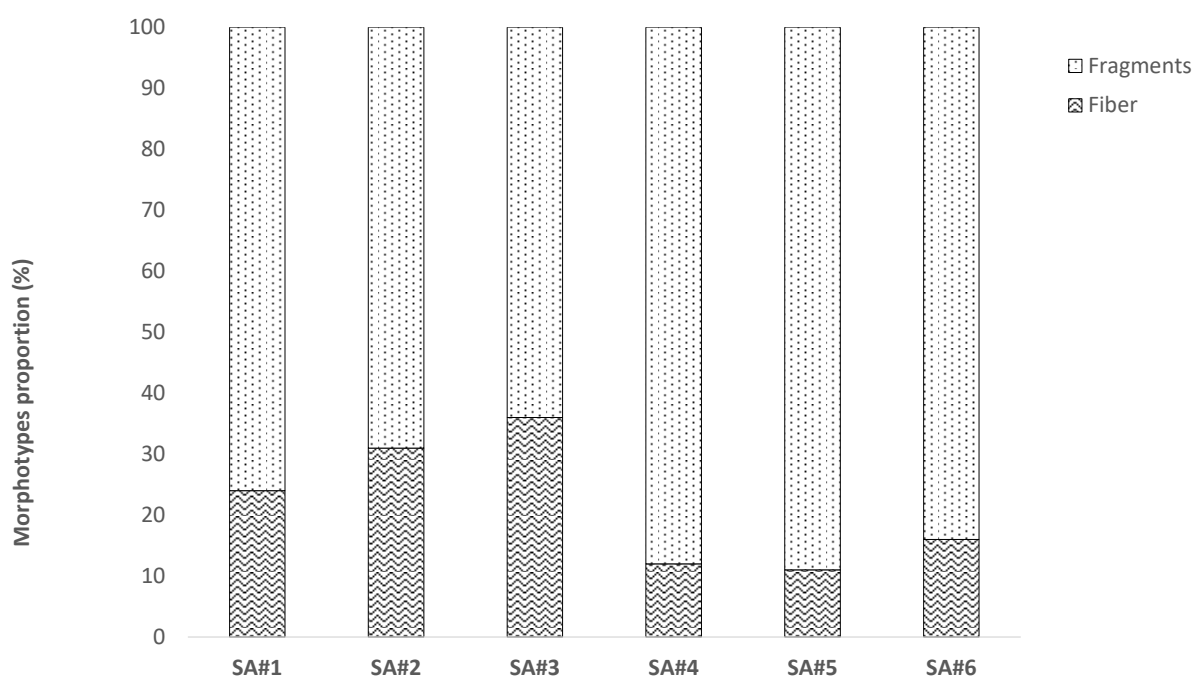


Figure 9 - Morphotypes presentation and proportion of MPs in the settlement pond (SA#1-3) and in the abalone-*Ulva* aquaculture system (SA #4-6).

6.1.4 Back confirmation of microplastics occurrence by pyr-GCMS

The results of the vibrational spectroscopy oriented technique (μ -FTIR) are confirmed by the thermoanalytical analysis as all detectable polymers in the pyr-GCMS method were back detected. Total

amount of polymers varied from 105 (SA#1) to 194 (SA#6) $\mu\text{g}/\text{m}^3$ of PE, from 81 (SA#1) to 264 (SA#4) $\mu\text{g}/\text{m}^3$ of PP, from 5 (SA#1) to 480 (SA#6) $\mu\text{g}/\text{Kg}$ of PVC, from < 1 (SA#4) to 31 (SA#6) $\mu\text{g}/\text{Kg}$ of PA, from 13 (SA#1) to 227 (SA#6) $\mu\text{g}/\text{Kg}$ of Pls (Polyester), from < 1 (SA#2) to 67 (SA#1) $\mu\text{g}/\text{Kg}$ of PU and finally from 26 to 42 $\mu\text{g}/\text{Kg}$ of SBR in SA#1 and SA#5, respectively; Tab. 7).

Table 7 – Occurrence of plastic polymer in the in the settlement pond (SA #1-3) and in the aquaculture systems (SA #4-6) of the South African IMTA as detected by the pyr-GCMS technique.

Sample	Polymer type ($\mu\text{g}/\text{m}^3$)										Sum
	PE	PP	PS	PVC	PA	PMMA	PC	Pls	PU	SBR	
SA#1	105	81	< 1	5	15	< 2	< 1	13	67	26	316
SA#2	112	129	< 1	92	9	< 2	< 1	15	< 1	31	390
SA#3	169	160	< 1	133	10	< 2	< 1	35	8	34	552
SA#4	110	264	< 1	362	< 1	< 2	< 1	155	58	40	992
SA#5	122	243	8	480	22	< 2	< 1	163	28	42	1113
SA#6	194	238	8	370	31	< 2	< 1	227	27	36	1135
	Polymer type ($\mu\text{g}/\text{l}$)										
	PE	PP	PS	PVC	PA	PMMA	PC	PET	PU	SBR	
Procedural control	< 1	< 1	< 1	< 1	< 2	< 2	< 1	< 1	< 1	< 1	
Dust trap control	< 1	< 1	< 1	< 1	< 2	< 2	< 1	< 1	< 1	< 1	
GCMS room control	< 1	< 1	< 1	< 1	< 2	< 2	< 1	< 1	< 1	< 1	

6.2 IMTA Brazil

6.2.1 Contamination monitoring

Three procedural blanks and six samples from the wet dust trap collectors were analyzed to monitor potential sources of contamination affecting the sample preparation and the analysis. The contamination was related to handling the sampling equipment, preparing the sample for analysis, and finally the analysis itself. The results showed a contamination of 9.1 ± 2.0 MPs per procedural blank sample. The polymeric composition of the contaminating MPs was $\approx 50\%$ PE, $\approx 30\%$ PES and $\approx 20\%$ PP. The measured contamination of non-synthetic materials (approximately 42 ± 7 particles) was protein-based material and cellulose fibres. Furthermore, the result of the wet trap collectors showed a contamination of 5.5 ± 1.0 MPs per trap located in the clean lab where the samples were processed and analysed by μ -FTIR imaging. The polymeric composition of the wet trap collectors were $\approx 25\%$ PES, $\approx 50\%$ PE and $\approx 50\%$ PP.

6.2.2 Microplastic fragments in the recirculating system

Freshwater inlet - the total initial MPs input from the freshwater source scored 800 MPs/ m^3 (Fig. 10) where PE, PP and PS dominate the polymers composition of the detected plastic fragments (Fig. 11).

Seawater inlet - the total initial MPs input from the freshwater source scored 1200 particles/ m^3 together with PE, PP and PS also PVC dominate the polymers composition of the analyzed sample.

Molasses input - the total number of MPs in the molasses food source was of 2000 particles/ m^3 . In the analyzed samples the most recurring polymer type are PE and PS.



Lime - 5000 particles/m³ were detected in the lime source; the most recurring polymer type is PE followed PP and PVC.

Feed – 4000 particles/m³ were scored in the feed source. PS was the most recurring polymer type followed by PA and PE.

IMTA production water – the levels of MPs in the six production tanks ranged from 600 to 5500 particles/m³ during the first monitoring period after 26 days since the start of the production (T26) and from 1000 to 6000 particles/m³ at the end of the monitoring period (T=52 days). Overall, the levels of MPs tend to increase in time in production tanks nos. 1, 2, 3, 5 and 6 while an opposite trend is observed in production tank no. 4.

From the perspective of MPs characteristics, only morphotypes of fibre and fragment were visualized (Fig. 12). In all samples belonging to seawater, freshwater and Lime treatment fibre was the most abundant shape of MPs while particles for was most dominant in all other samples.

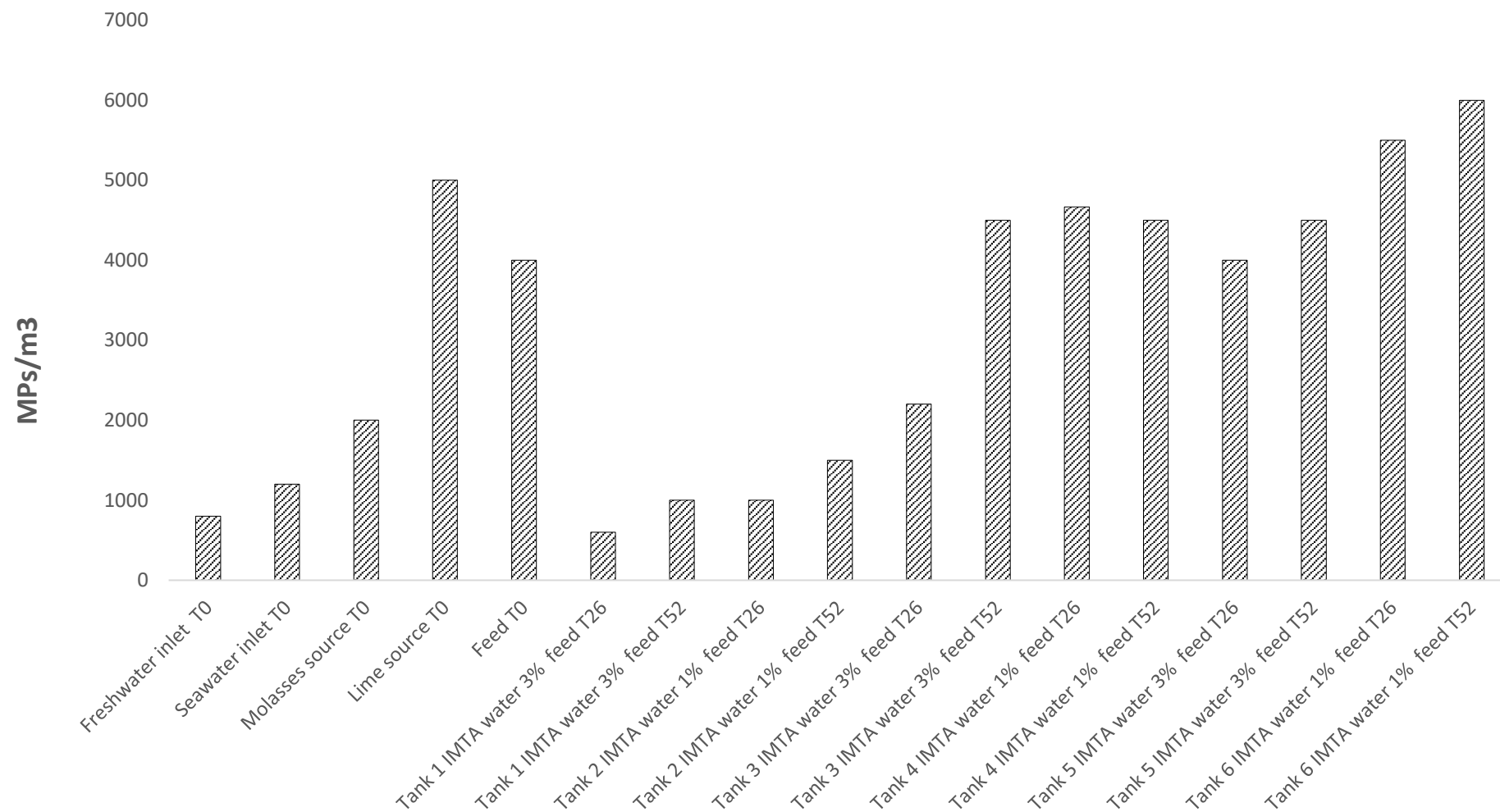


Figure 10 – MPs distribution in the FURG IMTA, Brasil.

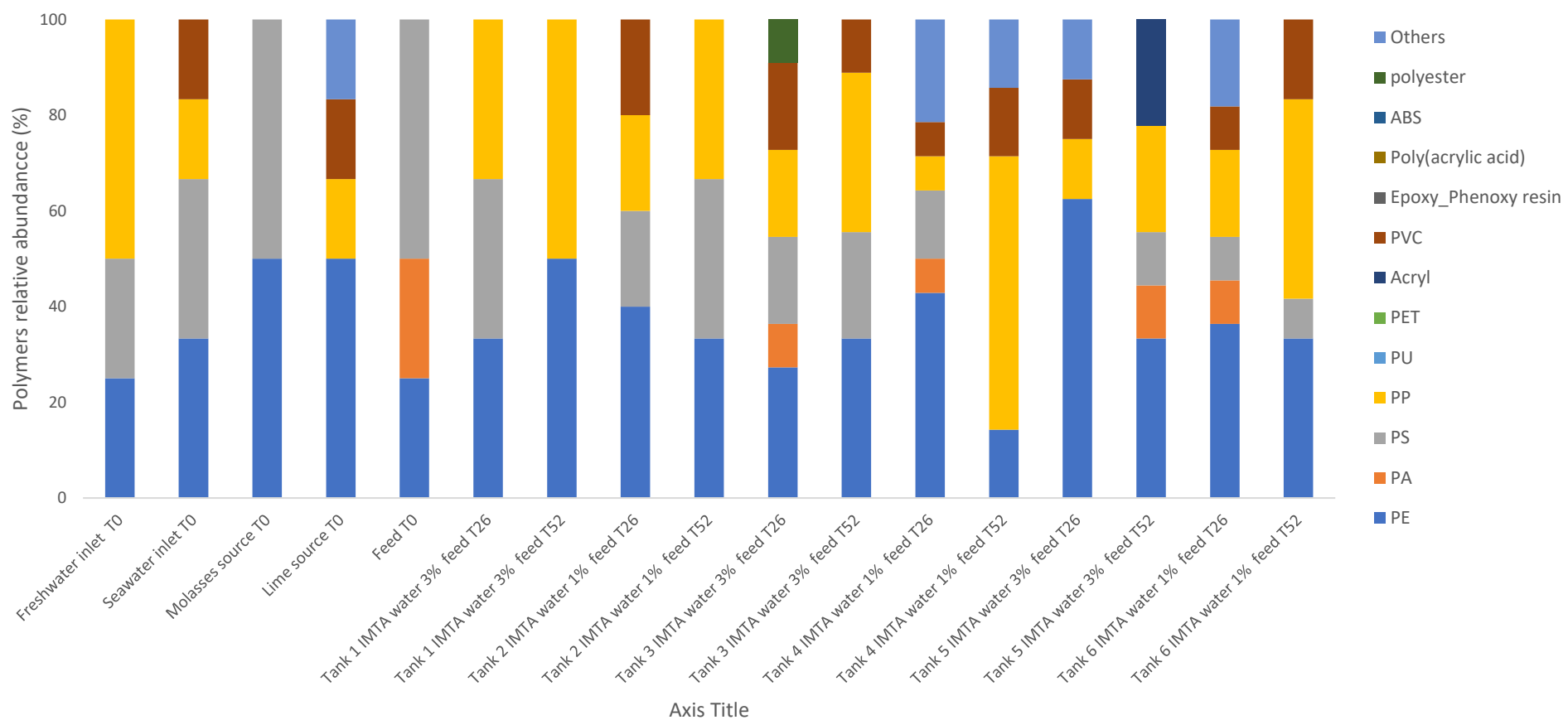


Figure 11 – Polymers’ relative abundance in the FURG IMTA, Brasil.

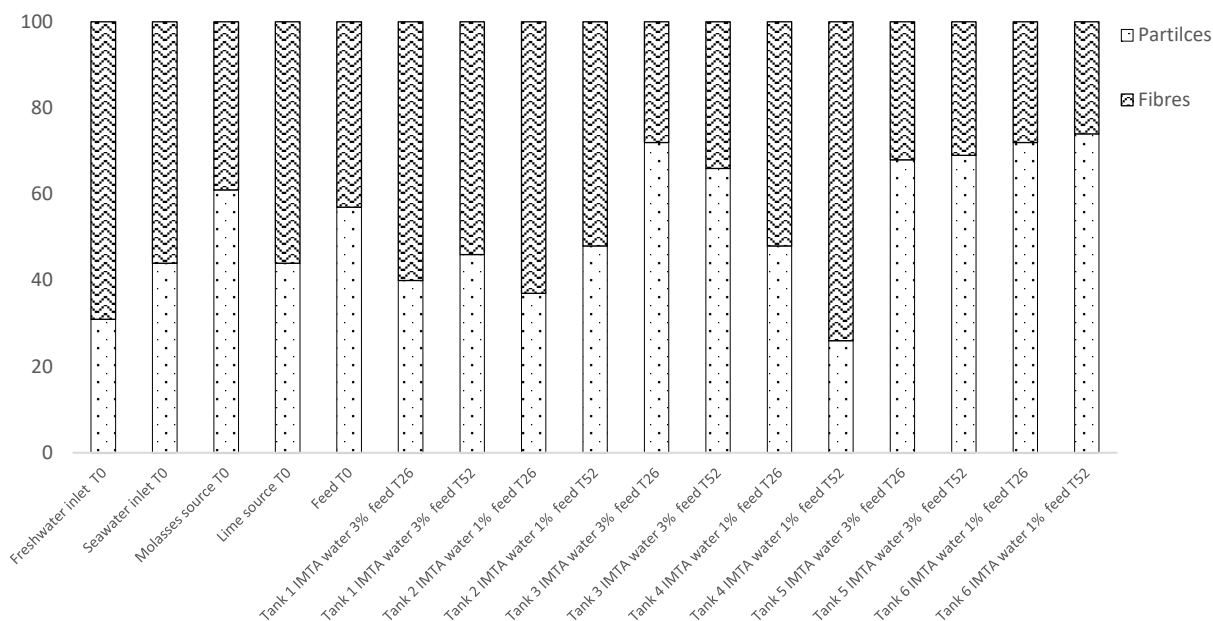


Figure 12 - Morphotypes presentation and proportion of microplastic fragments in the FURG IMTA, Brasil.

6.2.4 Back confirmation of microplastics occurrence by pyr-GCMS

The results of the vibrational spectroscopy oriented technique (μ -FTIR) are confirmed by the thermoanalytical analysis as all detectable polymers in the pyr-GCMS method were back detected (Table 8). During the raw materials sampling (T0) the total amount of polymers varied from 9.4 to 61 mg/m³. While during the production the amounts ranged from 6.7 to 73 mg/m³ respectively from the Tank no. 4 sampled at T26 days and the tank no. 6 sampled after 52 days since the start of the production.

Table 8 – Occurrence of plastic polymer in samples collected within FURG operated IMTA RAS system as detected by the pyr-GCMS technique.

Sample	Polymer type (mg/m ³)											
	PE	PMMA	PS	ABS	N66	PET	N6	PVC	PP	PC	SBR	PU
Freshwater inlet T0	2.3	0,3	0.8	0,01	<LOD	<LOD	<LOD	<LOD	7.4	<LOD	<LOD	<LOD
Seawater inlet T0	1.8	4.3	0.7	<LOD	<LOD	<LOD	<LOD	<LOD	11.8	<LOD	<LOD	<LOD
Lime source T0	1.7	1.5	0.3	<LOD	<LOD	<LOD	<LOD	<LOD	5.9	<LOD	<LOD	<LOD
Molasses source T0	12.1	7.4	1.9	0.098	<LOD	<LOD	<LOD	<LOD	40.3	<LOD	<LOD	<LOD
Feed T0	2.4	2.1	1.3	0.025	<LOD	<LOD	<LOD	<LOD	11	<LOD	<LOD	<LOD
Tank 1 IMTA water 3% feed T26	9.6	4.9	1.4	<LOD	<LOD	<LOD	<LOD	<LOD	19.7	<LOD	<LOD	<LOD
Tank 1 IMTA water 3% feed T52	4.4	1.7	1.3	0.007	<LOD	<LOD	<LOD	<LOD	8.6	<LOD	<LOD	<LOD
Tank 2 IMTA water 1% feed T26	5.5	1.2	0.7	0.008	<LOD	<LOD	0,017	1.7	8.2	<LOD	<LOD	<LOD
Tank 2 IMTA water 1% feed T52	2	0.8	0.2	0.008	<LOD	<LOD	<LOD	0.6	4.0	<LOD	<LOD	<LOD
Tank 3 IMTA water 3% feed T26	10.3	5.3	2.5	0.027	<LOD	<LOD	<LOD	<LOD	24.3	<LOD	<LOD	<LOD
Tank 3 IMTA water 3% feed T52	4.3	2.3	1.2	0.011	<LOD	<LOD	<LOD	<LOD	10.3	<LOD	<LOD	<LOD
Tank 4 IMTA water 1% feed T26	3.4	3.0	0.7	<LOD	<LOD	<LOD	<LOD	<LOD	10.8	<LOD	<LOD	<LOD
Tank 4 IMTA water 1% feed T52	1.4	1.2	0.5	0.018	<LOD	<LOD	<LOD	<LOD	3.6	<LOD	<LOD	<LOD
Tank 5 IMTA water 3% feed T26	1.4	6.3	0.7	<LOD	<LOD	<LOD	<LOD	<LOD	13.5	<LOD	<LOD	<LOD



Tank 5 IMTA water 3% feed T52	10.0	5.8	2.1	0.058	<LOD	<LOD	<LOD	<LOD	32.3	<LOD	<LOD	<LOD
Tank 6 IMTA water 1% feed T26	4.5	2.9	1	<LOD	<LOD	<LOD	<LOD	<LOD	14.4	<LOD	<LOD	<LOD
Tank 6 IMTA water 1% feed T52	17.3	7.7	3.7	0.084	<LOD	<LOD	<LOD	8.4	35.9	<LOD	<LOD	<LOD

7. Literature comparison and conclusions

Several processes in recirculating systems (RAS) may contribute to the release of microplastics into the water. In the present study, we have identified the following areas for technological improvement, and we suggest the following implementation plan:

Feed: Fish feed often contains microplastics either as contaminants or due to the inclusion of certain additives. During feeding, these microplastics can enter the water column as uneaten feed or through fish excretion. The results obtained from the FURG fully recirculating IMTA points out the food as one of the significant input sources of MPs into the system.

Water Sources: Incoming water used to replenish the system can introduce microplastics if the source is contaminated. This is particularly common when using water from natural sources like rivers or lakes that are already polluted with microplastics.

Equipment and Infrastructure Degradation: The degradation of plastic components used in the construction and operation of RAS, such as pipes, tanks, and liners, can release microplastics over time. Wear and tear, as well as routine maintenance activities, can contribute to this issue.

Filtration Systems: While filtration systems are designed to remove contaminants, they can also act as sources of microplastics if they themselves are made from plastic materials that degrade. For instance, plastic filter media and membranes can deteriorate and contribute microplastics to the system.

Biofouling and Cleaning Practices: The buildup of biofouling organisms on surfaces within the RAS can lead to the use of abrasive cleaning techniques, which can degrade plastic materials and release microplastics. Regular cleaning and maintenance activities that involve scrubbing or pressure washing can exacerbate this issue.

Chemical Use: The use of certain chemicals for disinfection and water treatment can contribute to the breakdown of plastic materials. For example, chlorine and other oxidizing agents can degrade plastics, leading to the release of microplastic particles.

Temperature and UV Exposure: Elevated temperatures and UV exposure within an IMTA can accelerate the degradation of plastic components. These conditions are common in greenhouses used in the production process, or part of the process, of the organisms studied here. This is particularly relevant in outdoor systems or those with parts exposed to direct sunlight, where UV radiation can break down plastics more quickly.

Microplastics in partially and fully recirculating aquaculture systems (RAS) and associated production areas have been increasingly scrutinized due to their environmental and health implications (Table 9). RAS are designed to reduce water usage and environmental footprint, but unfortunately these systems may become hotspots for microplastic accumulation. Research indicates that microplastic levels in RAS can vary significantly, with concentrations ranging from 100 to 10,000 particles/m³ depending on

factors such as filtration efficiency and maintenance practices (Pham et al., 2021). In our study MPs ranged from 128 to 429 MPs/m³ in the partially recirculating IMTA located in South Africa and from 800 to 6000 MPs/m³ in the fully recirculating IMTA located in Brazil. RAS systems often harbour microplastics originating from sources like fish feed, water inputs, and the degradation of plastic components within the system itself. Mathias et al. (2023) found 9500 and 37,300 MPs/m³ respectively in the inlet and production water of a seabass RAS system located in Spain while the feed contained 3900 MPs/m³. Black, blue, and transparent fibres made of man-made cellulose/rayon and polyethylene terephthalate were the most common MPs in water and seabass, while black fragments of phenoxy resin were the most common in feed.

The levels of polymers linked to RAS components (polyethylene, polypropylene, and polyvinyl chloride) were low, suggesting a limited contribution to the overall PL levels found in water and/or fish. A study conducted by Zhou et al. (2024) investigated the occurrence and distribution of microplastics in feed, source water and RAS in China. Results showed that microplastics occurred in all samples with the average concentration of 3.53 ± 1.39 particles/g, 700 ± 170 particles/m³ and 1530 ± 210 particles/m³ for feed, source water and RAS, respectively. Microplastics were mainly fiber in shape and 20–500 µm in size. Additionally, Blonç et al. (2023) utilized high-resolution mass spectrometry to identify five different polymers in RAS water and fish tissues. Both polyisoprene (PI) and polysiloxane were detected in water samples, while fish tissues contained polysiloxane in muscle, PE in the gut and stomach, and perhydropolysilazane (PHPS) in the brain. Comparatively, open aquaculture systems and surrounding aquatic environments often exhibit different microplastic profiles. For example, Li et al. (2020) observed that open aquaculture systems in coastal regions frequently register higher microplastic concentrations, sometimes exceeding 50,000 particles/m³, due to greater exposure to external pollution sources such as urban runoff and maritime activities.

The type and size distribution of microplastics also differ; RAS tend to have smaller and more fragmented microplastics, whereas larger and more diverse microplastic types are common in open systems (Lusher et al., 2017). Additionally, the bioavailability of microplastics to aquatic organisms is influenced by these environmental contexts, with higher ingestion rates noted in more polluted open water systems (Wright et al., 2013). These differences underscore the importance of tailored management strategies. Based on the identification of polymer types, MPs pollution within the RAS can be attributed to multiple sources. In two distinct studies conducted in Portugal and China, over 60% of MPs in inlet water are fibers, with their main components being manmade cellulose/rayon, indicating a potential contamination from aquatic environments, such as agricultural water and domestic sewage (Matias et al., 2023; Huang et al., 2023). Fishmeal contains MPs, and the detection of MPs in the feed indicates another route for MPs to enter RAS (Hanachi et al., 2019). Such evidence is further confirmed by the present study where both the lime and feed sources in the FURG IMTA introduced significant number of MPs in the system.

Furthermore, the occurrence of MPs within RAS does not seem to be exclusively related to RAS components, as the prevalence of polymers associated with RAS components (e.g., PE and PP from pipes and tanks, PET fibers from the filtration system) is relatively low (Blonç et al., 2023; Dris et al., 2017). Matias et al. (2023) indicated the potential contribution of airborne and waterborne contamination in the influent reservoir considering the high fiber content in RAS systems.

Overall, the two investigated IMTAs show the occurrence of low levels of MPs if compared to other studies. A limited release of plastic polymers from the infrastructure during production is observed as consequence of the meteorological conditions (latitude dependent temperature and UV light irradiation) in the area where the IMTAs are operating.



Table 9- Occurrence, characteristics, and sources of MPs in RAS environments.

Author	Levels	Note
Pham, M. et al. (2021).	MPS from 100 - 10000 MPs/m ³ in various RAS setups.	This study explores the sources of microplastics in RAS and provides management recommendations to mitigate pollution.
Pittura, L. et al. (2022).	1000 - 15000 MPs/m ³ (Water)	This research identifies key factors affecting microplastic contamination in RAS, including water exchange rates and filtration methods.
Li, J. et al. (2020).	This comprehensive review did not report specific concentrations but highlighted that microplastic contamination in aquaculture systems can be significant, referencing various studies with concentrations up to several dozen particles per liter in contaminated systems.	A comprehensive review of microplastic occurrence in various aquaculture systems, with a section dedicated to RAS.
Lusher, A. L. et al. (2017).	This technical paper reviewed numerous studies indicating levels from a 2000 - 50000 MPs/m ³	This technical paper discusses the presence of microplastics in aquaculture, including RAS, and the potential food safety implications.
De Witte, B. et al. (2021).	500 - 12000 MPs/m ³ (Water)	The study investigates microplastic contamination sources and dynamics within aquaculture systems, including RAS, and proposes potential remediation strategies.
Sun, X. et al. (2022).	500 - 10000 MPs/m ³ (Water)	An assessment of microplastic pollution specifically in RAS, evaluating concentrations and impacts on aquatic organisms.
Rochman, C. M. et al. (2019).	2000 - 48000 MPs/m ³ (Water)	While broader in scope, this review includes valuable information on the occurrence of microplastics in RAS and related methodologies for their detection.
Kutralam-Muniasamy, G. et al. (2021).	1000 - 20000 MPs/m ³ (Water)	This review synthesizes data on microplastic pollution in aquaculture, with specific insights into RAS environments.
Matias et al., 2023	9500-37500 MPs/m ³ (Water) 3700 MPs/m ³ (feed)	An assessment of microplastic pollution specifically in RAS, evaluating concentrations, polymers distribution and impacts on aquatic organisms.
Zhou et al., 2024	3.53 ± 1.39 MPs/g (feed) 700 ± 170 MPs/m ³ (source water) 1530 ± 210 MPs/m ³ (RAS water)	The study discusses about a theoretical basis for the management of MP in RAS.
Huang et al., 2023	1670 MPs/m ³ (Water)	

In RAS systems, enhancing filtration and regularly monitoring microplastic levels can mitigate accumulation, whereas open systems might require broader regulatory measures to control external pollution sources. Overall, while RAS offer more controlled environments, they are not immune to microplastic contamination, necessitating comprehensive approaches to safeguard aquaculture sustainability and food safety.

8. References

- Blonç, M., Husson, F., Llorca, M., Farré, M., Tort, L., Brandts, I., & Teles, M. (2023). Occurrence of micro-nanoplastics in a commercial recirculated aquaculture system and their translocation to cultured fish organs: A baseline study. *Journal of Hazardous Materials Advances*, 12, 100381.
- De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., & Robbens, J. (2021). Microplastic contamination in aquaculture systems: Sources, dynamics and remediation. *Science of The Total Environment*, 757, 143888.
- Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., & Tassin, B. (2017). A first overview of textile fibers, including microplastics, in indoor and outdoor environments. *Environmental pollution*, 221, 453-458.
- Fischer, M., & Scholz-Böttcher, B. M. (2017). Simultaneous trace identification and quantification of common types of microplastics in environmental samples by pyrolysis-gas chromatography–mass spectrometry. *Environmental science & technology*, 51(9), 5052-5060.
- Gomiero, A., Øysæd, K. B., Agustsson, T., van Hoytema, N., van Thiel, T., & Grati, F. (2019). First record of characterization, concentration and distribution of microplastics in coastal sediments of an urban fjord in south west Norway using a thermal degradation method. *Chemosphere*, 227, 705-714.
- Hanachi, P., Karbalaee, S., Walker, T. R., Cole, M., & Hosseini, S. V. (2019). Abundance and properties of microplastics found in commercial fish meal and cultured common carp (*Cyprinus carpio*). *Environmental Science and Pollution Research*, 26, 23777-23787.
- Huang, J. N., Yang, B. T., Wen, B., Gao, J. Z., & Chen, Z. Z. (2023). Occurrence and characteristics of microplastics contamination in different intensive aquaculture systems nearby the Yangtze estuary, China. *Bulletin of Environmental Contamination and Toxicology*, 110(1), 1.
- Kutralam-Muniasamy, G., Pérez-Guevara, F., Roy, P. D., & Elizalde-Martínez, I. (2021). Microplastic pollution in aquaculture: A review and synthesis of data. *Marine Pollution Bulletin*, 169, 112531.
- Li, J., Liu, H., & Chen, J. P. (2020). Microplastics in aquaculture systems and their environmental impact: A review. *Environmental Pollution*, 260, 113811.
<https://doi.org/10.1016/j.envpol.2020.113811>
- Lusher, A. L., Hollman, P. C. H., & Mendoza-Hill, J. J. (2017). Microplastics in fisheries and aquaculture: Implications for food safety. *FAO Fisheries and Aquaculture Technical Paper*, 615, 1-122.
<http://www.fao.org/3/i7677e/i7677e.pdf>
- Matias, R. S., Gomes, S., Barboza, L. G. A., Salazar-Gutierrez, D., Guilhermino, L., & Valente, L. M. (2023). Microplastics in water, feed and tissues of European seabass reared in a recirculation aquaculture system (RAS). *Chemosphere*, 335, 139055.
- Pham, M., Rahman, M. S., & Sarkar, A. (2021). Microplastic pollution in recirculating aquaculture systems: Sources and management. *Journal of Environmental Management*, 289, 112462.
<https://doi.org/10.1016/j.jenvman.2021.112462>



Pittura, L., Avio, C. G., Giuliani, M. E., d'Errico, G., Keiter, S. H., Cormier, B., Gorbi, S., & Regoli, F. (2022). Factors influencing microplastic levels in recirculating aquaculture systems. *Environmental Pollution*, 306, 119349. <https://doi.org/10.1016/j.envpol.2022.119349>

Rochman, C. M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., ... & Box, C. (2019). Microplastics in aquatic systems: A review of methodology, occurrence, and effects. *Environmental Science & Technology*, 53(13), 7087-7094.

Systematic Identification of MicroPLastics in the Environment - SIMPLE 2024, <https://simple-plastics.eu/>

Sun, X., Liang, J., Zhu, M., Zhao, Y., & Guo, D. (2022). Assessment of microplastic pollution in aquaculture: A focus on recirculating aquaculture systems. *Water Research*, 204, 117645. <https://doi.org/10.1016/j.watres.2021.117645>

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: a review. *Environmental pollution*, 178, 483-492.

Zhou, Z., Song, X., Dong, D., Li, X., Sun, Y., Wang, L., ... & Li, M. (2024). Occurrence, distribution and sources of microplastics in typical marine recirculating aquaculture system (RAS) in China: The critical role of RAS operating time and microfilter. *Water Research*, 255, 121476.