



Density and transport of anthropogenic particles in the Maldonado Stream basin (Maldonado, Uruguay).

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Abstract: It is well known that seas are polluted by anthropogenic particles (APs), but little is known about freshwater ecosystems. Rivers and streams transport these pollutants from land to marine areas, so generating baseline data in these systems is essential. The main objective of this project is to conduct the first detailed analysis of the presence, distribution, and transport of APs in Maldonado stream basin, considering the stream and its tributary, the San Carlos Stream, as a transport route for these pollutants from the basin to the coastal area. We analysed APs in surface water at five sites along the basin, sampling seasonally in 2019 with a pump and 100 µm filter. Sites included the upper watershed, urban areas, and near the estuary. APs were found at all sites, with densities increasing with water flow, suggesting diffuse rather than point-source contamination. July showed the highest APs transport due to peak discharge, though no seasonal density differences were observed. These findings provide the first baseline on APs at the basin scale for Uruguayan streams and are valuable for monitoring and policy-making.

Key words: Freshwater ecosystems, streams, anthropogenic and microplastic pollution, human impact.

Densidad y transporte de partículas antropogénicas en la cuenca del Arroyo Maldonado (Maldonado, Uruguay). Resumen: Los ríos y arroyos son las principales vías de entrada de partículas antropogénicas (PAs) a mares y océanos; sin embargo, poco se sabe sobre estos ecosistemas, y por ello generar líneas de base es prioritario. El objetivo de este proyecto es realizar el primer análisis detallado de la presencia, distribución y transporte de PAs en la cuenca del arroyo Maldonado, considerando al arroyo y su tributario, el arroyo San Carlos, como rutas de transporte de estos contaminantes hacia la zona costera. Este trabajo analiza la presencia de PAs en agua superficial de 5 sitios de la cuenca del arroyo Maldonado, incluyendo la cuenca alta, áreas urbanas y antes de la desembocadura del arroyo. Las muestras se tomaron estacionalmente en 2019 con una bomba y fueron filtradas a través de una malla de 100 µm. Se encontraron PAs en todas las muestras sin diferencias en densidades aguas arriba o abajo de los sitios urbanos, aunque estas aumentaron con el caudal en todos los sitios, indicando contaminación difusa. Julio presentó el mayor transporte de PAs debido a descargas máximas, sin variaciones estacionales de densidad. Este estudio, pionero en Uruguay, es útil para políticas de monitoreo y reducción de contaminantes.

Palabras clave: ecosistemas de agua dulce, arroyos, contaminación por microplásticos, impacto humano.

Introduction

Anthropogenic particles (APs) comprise a wide array of synthetic, semi-synthetic, and non-synthetic particles or fibres produced directly or indirectly by human activities, including textile fibres, dyed particles, and most noticeably microplastics (MPs) (Collard et al. 2018, Athey et al. 2020, Mattsson et al. 2021). From their continental sources, APs have easily dispersed through fluvial systems to coastal areas and oceans worldwide (Moore et al. 2011, Lebreton et al. 2017, Tanentzap et al. 2021). For instance, it is estimated that between 1.15 and 2.41 million tons of plastic enter the oceans through the global river system each year, with the main polluting rivers representing 86% of the world's annual contribution (Lebreton et al. 2017). APs can have harmful effects on both marine and freshwater biota at various trophic levels and are susceptible to bioaccumulation and biomagnification processes (Farrell & Nelson 2013, Browne et al. 2015, Lozoya et al. 2016, Vidal et al. 2021).

Numerous studies have focused on the state and consequences of APs pollution in marine ecosystems, with little information available from freshwater ecosystems (Li et al. 2020, Wong et al. 2020, Sarijan et al. 2021), particularly in South America (Kutralam-Muniasamy et al. 2020). To better understand the state of APs pollution in fluvial systems and its relevance for the transport of APs to the sea, it is necessary to know the magnitude of APs transport from river systems to coastal areas, and how they relate to human activities occurring in their drainage basins (Yonkos et al. 2014, Baldwin et al. 2016). Like other pollutants (e.g. nutrients), APs reach watercourses through point and diffuse sources. In the case of MPs, diffuse sources typically consist of dust and civil runoff from cities as well as vehicle tyres and road wear particles (Campanale et al. 2022). Soils can also be a diffuse source, acting as long-term MPs sinks (Rillig et al. 2017) from agricultural (Ouyang et al. 2020) and livestock (Ramachandraiah et al. 2022) activities, as well as from atmospheric deposition (Sun et al. 2022). On the other hand, point sources are associated with effluents from industries or cities, such as treatment plants, which can be an important source of fibres (Sierra et al. 2020). Increased precipitation can elevate diffuse inputs via runoff, resulting in heightened concentrations at elevated flow rates, contrasting with point sources, which dilute with increased flow (Bowes et al. 2008, Goyenola et al. 2015).

In Uruguay, no studies have evaluated APs contamination in fluvial systems, except for one

study of MPs presence in freshwater fishes (Vidal et al. 2021). Hence, our objective is to evaluate, for the first time, the presence, seasonal and spatial distribution, and transport of APs in a stream from the eastern region of Uruguay, considering the influence of urban areas located in its basin. Considering the higher incidence of APs near cities (e.g. MPs, Derraik 2002), and the common reception of untreated sewage water by Uruguayan streams (Alvareda et al. 2020, Teixeira de Mello et al. 2024), we expect to find a higher incidence of APs pollution downstream from urban areas, especially during rainy periods.

Methodology

Sampling area: Maldonado Stream has a basin of 1,354 km² and discharges into the Atlantic Ocean. It is a free-flowing basin with no dams in its main tributaries. The basin has a variety of land uses, including agricultural (92.3 km²), forestry (50.5 km²), and extensive livestock production (1,119.5 km²) (Ministerio de Ambiente 2022). The stream runs alongside the city of San Carlos (27,241 inhabitants; INE 2011) and then through an extensive wetland. The lower basin has two major cities in its margin: Maldonado (86,782 inhabitants; INE 2011), Punta del Este, and La Barra balneary (population on and off summer season increasing from 250,000 to 300,000 inhabitants; Intendencia de Maldonado 2024).

Two sampling sites were in the upper basin, upstream from San Carlos City. Site 1 in Matajojo Stream and Site 2 in Carapé Stream, with a drainage area of 136 km² and 74 km², respectively (Fig. 1). Both sites are characterised by low population and land use dedicated mainly to extensive livestock production, *Eucalyptus* spp. afforestation, and olive groves for oil. Two more sites were located in the lower-middle basin, downstream of San Carlos City: Site 3 in Maldonado Stream (drainage area 488 km²) and Site 4 in San Carlos Stream (drainage area 866 km²), both in Alameda Park (Fig. 1). This is an urban park with recreational activities, vehicle circulation, and a small racecourse, and has been regarded as a priority area for fish conservation (Teixeira de Mello et al. 2021). These two sites downstream of the City of San Carlos are expected to be influenced differently by urbanization. In particular, Site 4, located downstream of the area receiving direct effluent from the urban wastewater treatment plant, differs from Site 3. Finally, Site 5 was located after merging Maldonado and San Carlos streams (drainage area 1,490 km²) and near the mouth of the Atlantic

Ocean, and therefore susceptible to a marine influence (Fig. 1).

Sample collection: Sampling was conducted throughout 2019 with seasonal frequency (26th March/autumn, 15th June/winter, 29th October/spring, and 19th December/summer; Fig. 1). This sampling strategy allowed us to cover the seasonal variability in rainfall, and therefore in water discharge. This is regarded as one of the most influential variables in the dynamics of APs in river systems (Baki et al. 2014, Rech et al. 2014, 2015). Surface water samples (for each site in triplicate) were taken with a 1.5 hp motor pump and a 2.5 inch and 20 m long highly durable pipe combined with a skimmer, ensuring only surface water was collected. Water was pumped for 10 minutes and filtered through a 100 μ m mesh. Filtered volume was estimated (at each site and for each sampling) by measuring the volume of water pumped for 1 minute, in three separate measurements (Supplementary section 1).

Sample analysis: We classified and quantified the APs according to type and size in 5 different sites. Each sample was filtered through a 100 μ m mesh and the retained material was deposited in a glass Petri dish using milli-Q water. Samples were not digested as they had very little organic matter and were observed under a stereomicroscope (SMZ745, 2X, Nikon, Tokyo, Japan). All APs found were photographed, measured, counted, and classified by

shape according to Viršek et al. (2016) (i.e. fibre, fragment, foam, pellet, film, and granule). Only fibres greater than 1.0 mm and fragments greater than 0.5 mm were included in this study. All particles were also analysed under an Eclipse 50i microscope (type 104, Nikon, Tokyo, Japan) equipped with a polarised light filter (Acer 2015, Nikon, Tokyo, Japan). This light allows the identification of APs such as polyethylene, polypropylene, and polyethylene terephthalate, because they are anisotropic materials that appear bright while other materials appear completely dark (Sierra et al. 2020; Supplementary section 2). Another technique used to confirm APs, it's the method called "hot needle", where we evaluated the heat response of the object (e.g. melting, deformations; Sierra et al. 2020, Lusher et al. 2017a; Supplementary section 3).

To prevent potential airborne contamination of the samples during sampling and analysis, all work surfaces and materials were previously cleaned with distilled water and kept covered before use. During each sample analysis, a glass Petri dish filled with milli-Q water was placed near the stereomicroscope and opened simultaneously with the Petri dish containing the sample. Afterwards, each control was inspected, and any particles resembling APs that matched the shape, colour, and approximate size of particles in the samples were discarded.

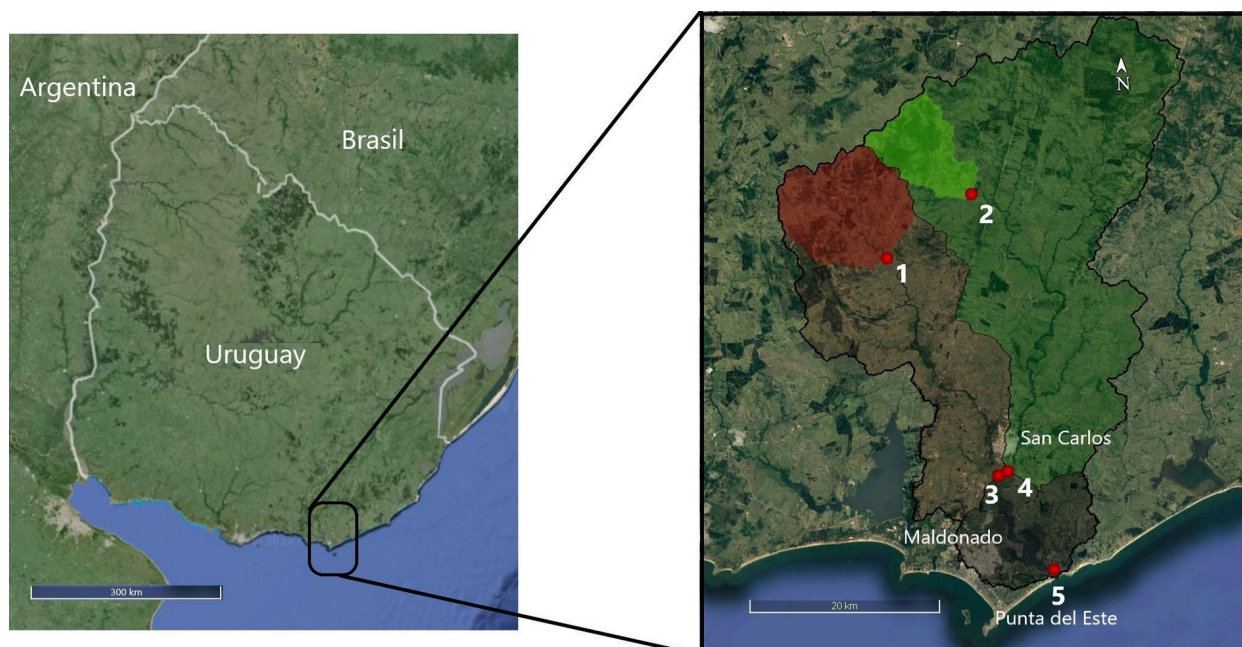


Figure 1. Location of the Maldonado stream basin (black line) and sub-basins San Carlos (green) and Maldonado (red). Sites 1 and 2 are located upstream of San Carlos City. Sites 3, and 4 are located downstream of San Carlos City, and Site 5 is near the mouth of the Atlantic Ocean.

Table I. Sampling data, watershed area draining to each sampling site, specific discharge estimated for each sampling site, and sampling data and discharge. * indicate the estimated discharge

Sites	Data	Watershed area (Km ²)	Specific discharge (m ³ /km ²)	Discharge (m ³ /s)
1	Autumn (26/03/2019)	136	0.00022	0.03*
2	Autumn (26/03/2019)	74	0.00085	0.06*
3	Autumn (26/03/2019)	488	0.00022	0.11
4	Autumn (26/03/2019)	866	0.00085	0.74
5	Autumn (26/03/2019)	1487	0.00063	0.93*
1	Winter (15/07/2019)	136	0.01697	2.31*
2	Winter (15/07/2019)	74	0.01597	1.18*
3	Winter (15/07/2019)	488	0.01697	8.28
4	Winter (15/07/2019)	866	0.01597	13.83
5	Winter (15/07/2019)	1487	0.01633	24.29*
1	Spring (29/10/2019)	136	0.00444	0.60*
2	Spring (29/10/2019)	74	0.00278	0.21*
3	Spring (29/10/2019)	488	0.00444	2.17
4	Spring (29/10/2019)	866	0.00278	2.41
5	Spring (29/10/2019)	1487	0.00338	5.03*
1	Summer (19/12/2019)	136	0.00021	0.03*
2	Summer (19/12/2019)	74	0.00032	0.02*
3	Summer (19/12/2019)	488	0.00021	0.1
4	Summer (19/12/2019)	866	0.00032	0.28
5	Summer (19/12/2019)	1487	0.00028	0.42*

Data analysis: We estimated the density of APs in items/m³ for each site and season. Then, we extrapolated the densities for the stream by using the stream discharge at each site (m³/s), which was then used to estimate the total load of APs at each sampling site (total load APs= APs/m³ * discharge).

Stream discharge values for sites 3 and 4 were obtained from the daily information generated by the national hydrometric network of DINAGUA (Direc-

ción Nacional de Aguas https://www.ambiente.gub.uy/informacion_hidrica/index.php). For the other sites (1, 2, and 5), discharge was estimated by the watershed area draining upstream to each sampling site considering the same information of specific discharge (m³/km²) obtained from observed data for sites 3 and 4. Discharge was estimated for each sampling period to account for seasonal and basin area differences. For site 1, the specific dis-

charge of site 3 was used for the estimation, as site 1 belongs to the same sub-basin (Maldonado). For site 2, the specific discharge for site 4 was used. In the case of site 5, it was estimated as a weighted average of the input area of sites 3 and 4 for each sampling time (Table I).

Differences among sites were evaluated with a Two-way ANOVA with post-hoc comparison by Tukey's test. Density normality was tested with Shapiro-Wilk, and homoscedasticity was tested using the Bartlett Test. Results were considered significant at $p < 0.05$. We evaluated the relationship between the density (APs/m^3) and the river discharge (m^3/s) using a log-log linear regression, to assess the importance of the diffuse component of this contamination. All analyses were conducted in the software Past 4.2 (Hammer et al. 2001).

Results

We analysed 60 samples from the 5 sites, identifying 2,918 APs, catalogued as fibres and fragments (Supplementary section 4). We detected APs in all samples, with fibers being the most prevalent shape, representing an average of 88%, while fragments accounted for 12% (Fig. 2). Colour proportion for all APs was 30.3% blue, 22.2% transparent, 13.8% grey, 12.1% black, 10.2% sky blue, 6.3% red, 3.4% white, 0.6% violet, 0.5% green, 0.3% pink, 0.2% orange and 0.1% yellow.

Fibre size oscillated between 0.5 and 23.0 mm (mean 2.1 ± 1.8 SE mm), while fragments ranged between 0.1 and 17 mm (mean 1.1 ± 1.5 SE mm). Also, the most abundant sizes between items were between 1 and 2 mm (Supplementary section 5).

Total average densities of APs/m^3 varied between 77.8 (Site 2 in spring) to 15.4 (Site 4 in summer, Fig. 3). No significant differences were found between seasons ($F=1.301$, $p=0.29$), but differences were observed between sites ($F=3.986$, $p=0.008$), with Site 5 showing significantly higher APs density than sites 3 and 4 (site 5 vs. site 3, $p=0.03$; site 5 vs. site 4, $p=0.04$).

Sites 1 and 2 had a higher variability in total APs densities (Fig 3). Considering only fibres densities, no significant differences were found between seasons ($F=0.511$; $p=0.677$), but significant differences were observed between sites ($F=3.136$; $p=0.02$), with Site 5 showing slightly higher density compared to Site 3 ($p=0.08$). In the case of fragments, and unlike fibres and total APs, significant differences were found between seasons ($F=6.493$;

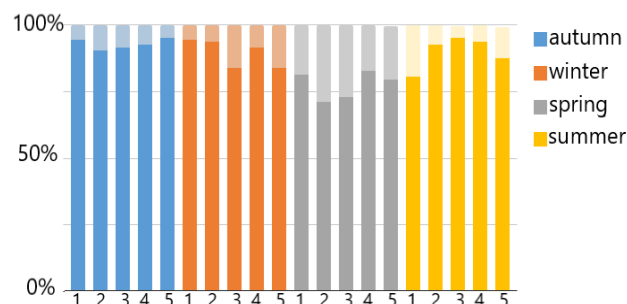


Figure 2. The proportion of fibres (dark shade) and fragments (light shade) observed at each of the 5 sites analysed, in the four sampling seasons.

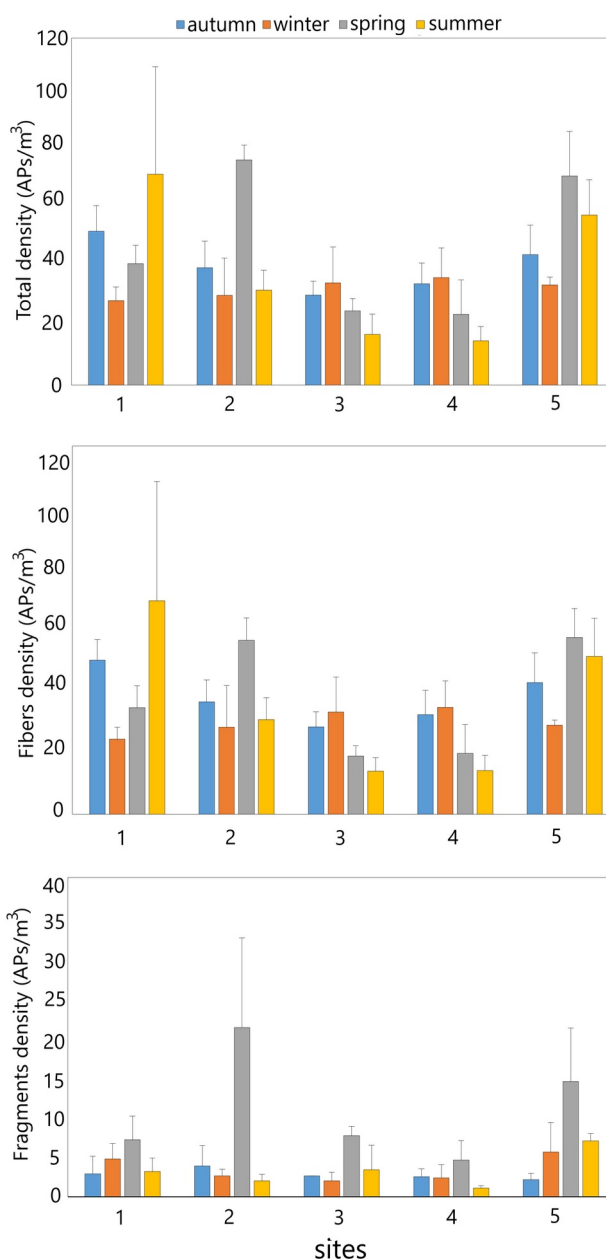


Figure 3. The total density of APs, fibres and fragments (APs/m^3) found in each site (1, 2, 3, 4, and 5) for the four sampling seasons. Note the differential scale in fragments.

$p=0.001$), but not between sites ($F=1.538$; $p=0.21$). Fragments were significantly more abundant in spring than in the other seasons ($p=0.003$, $p=0.007$, $p=0.006$, for autumn, winter, and summer, respectively; Fig 3).

The average total load of APs (APs/s) varied between 1.6 APs/s (Site 1 in autumn) and 841.1 APs/s (Site 5 in winter) (Fig. 4), with significant differences between seasons ($F=60.300$; $p<0.0001$) and sites ($F=28.850$; $p<0.0001$). The post-hoc Tukey's test identified significant differences between all seasons (winter vs spring, autumn and summer ($p<0.00001$); spring vs autumn ($p=0.02$), winter ($p<0.00001$) and summer ($p<0.01$)), except between summer and autumn ($p=0.09$) (winter>spring>autumn and summer). Site 5 had a significantly higher value of APs load than all the other sites (site 5 vs. site 4, site 3, site 2 and site 1; $p<0.00001$), and Site 4 had a significantly higher load than sites 1 ($p=0.003$) and 2 ($p=0.001$).

The increase in APs load (APs/s) corresponded not only to a higher discharge (m^3/s) but also to an increase in APs density (APs/m^3) with increasing stream discharge (Fig. 5).

Discussion

This is the first study to analyse the presence of APs in Uruguayan water samples and assess the potential impact of land use. As the first study of its kind in Uruguay, the results provide an overview of anthropogenic debris in the water column of the country's marine and freshwater ecosystems. Notably, the APs densities found in the Maldonado

Stream basin are similar to those reported in previous studies in lotic ecosystems with significantly larger population densities such as the River Thames in the UK (e.g. MPs averages 24.8 and 14.2 items/ m^3 , Rowley et al. 2020), and higher (e.g. MPs means 1.93 to 5.67 items/ m^3 , Lenaker et al. 2019) or lower (e.g. APs means 2970 items/ $m^3 \pm 326,7$ (SE), Berg et al. 2024) than others such as in the Milwaukee River Basin (United States). Although this study was not able to analyse polymers, although the literature shows significant variants, a percentage is always usually MPs. For example, studies with similar sampling methods showed that plastics as the most common polymers (95%; e.g. polyethylene 25.4%) and only 5% of cellulose (Sulistyowati & Cordova, 2022), other studies showed cellulose as the most common polymer (66.7 %, Berg et al. 2024; 76.8%, Baraza & Hasenmueller 2023), with only 8.6% of plastics (Berg et al. 2024) or 23.2% of synthetic materials (Baraza & Hasenmueller 2023).

Fibres were the most common shape found in the study. Their higher density, along with their predominant colours of blue and black, aligns with findings from other Uruguayan microplastics studies (in stomach and intestinal contents, Vidal et al. 2021; and in sedimentary cores of coastal lagoons, Alves et al. 2022) and studies with comparable sites (Vardar et al. 2021). These fibres may be linked to the textile industry, entering ecosystems primarily through wastewater laundry from residential areas (Boucher et al. 2017, Falco et al. 2020) but also from atmospheric transport (Sun et al. 2022).

As stated in our hypothesis we classified the sampled sites downstream of San Carlos city (3, 4, and 5) as urbanised, while those upstream (sites 1 and 2) as non-urbanised. This suggests that higher levels of urbanisation lead to increased anthropogenic pollution, as the urban land cover is positively correlated with the presence of APs such as MPs (Grbić et al. 2020). Nevertheless, this relationship might not be entirely linear, as various other factors likely influence the contribution of micro and meso litter to the water column throughout the watershed. In our study, we found that Site 5 had higher concentrations of APs compared to sites 3 and 4. This aligns with the fact that Site 5 is downstream of the city of Maldonado, which has a population three times larger than San Carlos and experiences a marked increase during the summer months due to tourism activity. Nevertheless, we found no significant differences with sites 1 and 2, which were expected to have the lowest densities for being in a rural

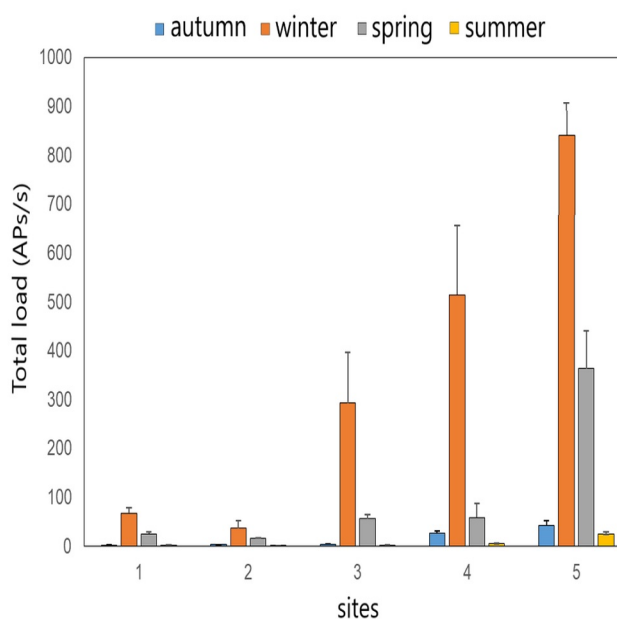


Figure 4. Seasonal total load of APs per second for each sampling site.

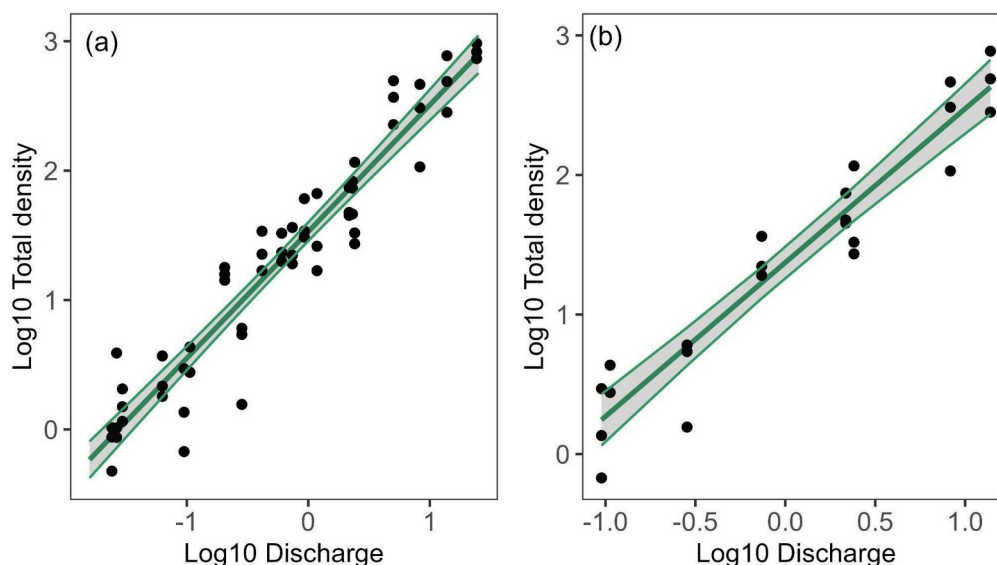


Figure 5. Regression analysis between total APs density (APs/m³) and stream discharge (m³/s). a) combining measured and estimated discharge data ($R^2=0.915$; $p<0.0001$). b) using only the measured discharge data ($R^2=0.921$; $p<0.0001$). Both regressions include data from the four stations sampled. Note the log scale.

area at the headwaters. These sites exhibited high variability in APs density, with intermediate values compared to sites downstream of urban areas. These findings suggest the presence of diffuse sources of APs that are challenging to identify, such as agricultural and livestock activities. Soil, for instance, can serve as a major sink for APs while acting as a diffuse source (Strokal et al. 2023). Furthermore, precipitation may contribute to the influx of MPs from diffuse sources, thereby increasing their abundance in freshwater environments (Cheung et al. 2019, Brahney et al. 2020, Alfonso et al. 2021). This argument is further supported by the strong positive relationship between APs density and increasing discharge, which further points to a strong diffuse pollution component (Strokal et al. 2023). If the inputs of APs in our system were solely from point sources, an increase in river flow would lead to a "dilution" of APs and thus a negative relationship between density (items/ m³) and discharge. The diffuse input of APs to the streams aligns with previous findings in Uruguay, where the consumption of MPs by fish was similar in streams with low production or urban activities in their watersheds compared to urban streams (Vidal et al. 2021). Additionally, Strokal et al. (2023) noted that MPs inputs from point sources are fewer than those originating from diffuse sources in sub-basins across South America. Other factors, such as wind, which was not considered in our study, may also influence the dispersion of APs, particularly MPs (González-Pleiter et al. 2021a,b, Edo et al. 2023).

However, in addition to the clear effect of this diffuse contamination, and focusing on APs load, Site 5 had a significantly higher value than all the other sites, and Site 4 was significantly higher than Sites 1 and 2. These findings also suggest the possibility of point source contributions, such as from the water treatment plant of San Carlos located upstream of Site 4. In this sense, while determining the origin of APs and MPs can be challenging, glitter -a hexagonal type of MPs- was identified at sites 3 and 4 downstream of San Carlos. Several studies suggest that glitters used in cosmetics, art, hobby, and craft materials could be considered primary MPs (Yurtsever 2019). Moreover, most studies that have found the presence of glitter are associated with wastewater treatment plants (WWTPs, Lusher et al. 2017b, Magni et al. 2019, Raju et al. 2020, Vardar et al. 2021), which serve as significant pathways for MPs to enter aquatic environments (Iyare & Bond 2020, Schell et al. 2021).

Estimating total APs load highlights the significant discharge potential of a river like the Maldonado to the Atlantic Ocean. The highest APs discharge in this study was observed at Site 5, where from an estimated discharge of 24.3 m³/s for this sampling day a total contribution of 800 APs/s was estimated. However, we would like to point out that this river can experience much higher discharges, with records even quadrupling the value used in this estimate (Supplementary section 6); in lower discharges, site 5 can be affected by de APs concentrations of marine waters. This relevance of rivers as

the main pathway of APs and MPs input from land to the seas and oceans has been extensively documented and established (e.g. Alfonso et al. 2021, He et al. 2021, Stokal et al. 2023). Considering the anticipated rise in extreme precipitation events for the region where Uruguay is situated (IPCC 2021), it is foreseeable that future contributions of anthropogenic particles (APs) to the ocean could escalate even further, with potential impacts on these ecosystems.

Conclusions and perspectives

This study suggests that the Maldonado stream basin is a source of APs to the Atlantic Ocean, specifically secondary MPs related to textile fibres. Differences in the load of APs in the Maldonado stream that reach the ocean can be influenced not only by the human activities developed in the catchment area of the basin that led to punctual or diffuse contamination but also by the precipitation that increases the discharge of freshwater to the marine environment. It is worth noting that in the lower reaches of the Maldonado stream basin, there is a saline wetland of great importance for the conservation of migratory seabirds, as well as one of the largest crab populations in the Uruguayan coast where said biodiversity heritage could be affected by the interaction of fauna with APs (e.g. ingestion) (Muñoz et al. 2023, Compa et al. 2023). For future studies, implementing the polymeric analysis of the APs with techniques such as micro and ATR-FTIR would reinforce the confidence level in identifying microplastics. Furthermore, it is essential to continue analysing the presence of this pollutant in other Uruguayan freshwater ecosystems, such as lagoons, rivers, other urban streams, and even marine-coastal zones, where these systems could mean an entrance of microplastics. Exhaustive monitoring, particularly at a finer temporal scale, is required to better understand the variations in APs' contributions between sites with different land uses.

Ethical statement

The present investigation did not involve regulated animals and did not require approval by an Ethical Committee.

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