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Efficacy of microplastic depuration on two commercial oyster species from the west coast of Ireland

Ann Tracy Paul[1] ⬤ | **Colin Hannon**[1] ⬤ | **Mateja Švonja**[1] ⬤ |
Iarfhlaith Connellan[2] | **João Frias**[1] ⬤

[1]Marine & Freshwater Research Centre, Atlantic Technological University, Galway, Ireland

[2]Cartron Point Shellfish Ltd, Co. Clare, Ireland

Correspondence
Ann Tracy Paul, Marine & Freshwater Research Centre, Atlantic Technological University, Dublin Road, H91 T8NW, Galway, Ireland.
Email: anntracypaul@gmail.com

Abstract

Studies investigating microplastics (MPs) in marine species have been published over recent decades, including studies on depuration efficacy on aquaculture products. This preliminary study investigates the depuration efficacy of MPs in two commercial oyster species from Ireland. The innovative aspects are the sampling size ($n = 50$ per species) and the experiment duration (up to 96 h). The case study organisms are the Pacific oyster (*Magallana gigas*) and the European flat oyster (*Ostrea edulis*). Prior to depuration, the mean MP concentration on *M. gigas* edible tissue was 0.6 MP g^{-1} while for *O. edulis* was 0.4 MP g^{-1}. Significant differences in mean MP concentrations were identified after 96-h for *M. gigas* 0.2 MP g^{-1} ($p = 0.014$) and *O. edulis*, 0.1 MP g^{-1} ($p = 0.003$). Additionally, no significant correlation was established between MP concentrations and edible tissue weight. Polymer identification revealed that 51.6% were fibers of natural origin. Preliminary results show that increasing depuration times beyond 72-h can significantly reduce MPs in selected oyster species, which is what is being recommended with this baseline study. Further investigation on commercial conditions at adequate depuration facilities is required. Given the relevance of low-trophic aquaculture species for local economies, this preliminary study provides important baseline information for stakeholders.

1 | INTRODUCTION

Global projections by the United Nations and the Food and Agriculture Organization (FAO) on population growth and food production, respectively, estimate that by 2050 there will be approximately 10 billion people (United Nations, 2019), which will require a 70% food production increase to keep pace with growing population (FAO, 2022). Seafood consumption, both from freshwater and marine sources, has been rising at a 1.5% yearly rate, reaching 20.5 kg per capita in 2018 (FAO, 2020). Considering that seafood consumption represents about 17% of the world's intake of animal protein (OEDC-FAO, 2020) and that shellfish aquaculture, particularly for bivalve filter feeders, has a low environmental impact per gram of protein produced (Hilborn et al., 2018), then the potential for sustainable global expansion of food production, when compared to capture fisheries or finfish aquaculture (Gentry et al., 2017), is immense. In this preliminary study, the authors focussed on filter-feeding sessile marine mollusks, particularly oysters, to target a scientific literature and policy recommendation knowledge gap when it comes to microplastics (MPs; da Costa et al., 2020; STECF, 2021b; Wu et al., 2023). Oyster production, because of its nature, is known to have a low environmental impact when compared to intensive and extensive finfish and/or shrimp aquaculture (EC, 2017; Tacon et al., 2009).

During 2018, European shellfish production reached approximately 675,000 metrics, representing approximately €1.3 billion in revenue (STECF, 2021b), of which oyster production corresponded to 21% (143,600 metric tonnes) with a revenue of €626.9 million, respectively (STECF, 2021b). France (86% of total volume and 85% value) and Ireland (7.3% of volume and value) (STECF, 2021b) are the leading European oyster producers.

Suspension filter feeder bivalves are bioindicator organisms and were selected as a case study for depuration experiments in Ireland because of their economic relevance as well as their ability to extract nutrients and particles from the surrounding environment, including MPs (STECF, 2021a). Bivalves are more likely to be exposed to MPs than higher trophic predators because of their sedentary living and filter-feeding capacities (Walkinshaw et al., 2020). Moreover, the depuration of bivalves provides a reliable reduction exposure proxy prior to human consumption (Wu et al., 2023).

Microplastics are emerging contaminants that can be defined as "synthetic solid particle, or polymeric matrix, having a regular or irregular shape, with size ranging from 1 μm to 5 mm and can be either of primary manufacturing origin or secondary when they result from degradation of larger plastic items, insoluble in water" (Frias & Nash, 2019). Evidence of MP ingestion in marine biota has been extensively documented in several taxa including zooplankton (Frias et al., 2014, 2020), wild-caught fish (cod, dab, flounder, herring and mackerel) (Rummel et al., 2016), farmed estuarine fish (sea bass, sea bream, flounder) (Bessa et al., 2018), gastropods—*Littorina littorea* (Doyle et al., 2019), farmed sea cucumber—*Apostichopus japonicus* (Mohsen et al., 2019) and wild-caught crustaceans—*Neprhops norvergicus* (Joyce et al., 2022). MPs have also been detected in wild and commercially important shellfish species such as oysters, mussels, and clams (Birnstiel et al., 2019; Baechler et al., 2020; Ding et al., 2021). The Descriptor 10 of the Marine Strategy Framework Directive (MSFD; 2008/56/EC), targets Marine Litter including plastics and MPs, and over the last couple of years, assessment of presence/absence and concentrations in species have been taking place across Europe and the globe. Early studies like the ones just mentioned provide baseline information on the prevalence of MPs in the environment and biota within.

Depuration, according to the FAO, is the process by which "shellfish are held in tanks of clean seawater under conditions which maximise the natural filtering activity which results in the expulsion of intestinal contents, which enhances separation of the expelled contaminants from the shellfish, and which prevents their recontamination"

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(Lee et al., 2008). This is a common post-harvest practice in shellfish aquaculture aimed at minimizing contamination risks (e.g., coliforms). Experimental depuration treatments across Europe have been shown to reduce the MP concentrations in soft tissues in oysters (Birnstiel et al., 2019; Van Cauwenberghe & Janssen, 2014). Retention time and depuration efficiency are species-specific and depend on several factors such as MP size, shape, surface properties, and length of depuration (Birnstiel et al., 2019; Ward et al., 2019). Nonetheless, laboratory depuration assays targeting MPs in aquaculture products often have limitations such as small sampling sizes or short experiment duration, which does not allow for robust information gathering or recommendations to be provided to policymakers or developed for the aquaculture industry.

Assessing and monitoring MPs in aquaculture products is still a voluntary bottom-up approach. The lack of European guidelines and uncertainty of sources and pathways of plastic pollution has been identified as reasons to monitor MP in aquaculture products (FAO, 2017). At this moment, Ireland does not have guidelines to assess MP in water or shellfish, particularly in aquaculture products destined for human consumption. However, the Shellfish Water Quality Directive (2006/113/EC), differentiates between water quality categories (A, B, or C), across Europe. This directive has been transposed to Irish legislation under the European Communities (Quality of Shellfish Waters) Regulations 2006 (S.I No. 268 of 2006) and is one of the regulations focussing on bivalve production. The depuration of bivalves in Ireland is dictated by the water quality and classification of the harvesting site. For Class A, depuration is not mandatory, however, for Class B, there is a 42-h depuration requirement (SFPA, 2017 & Regulation [EC] No 854/2004). Ireland bivalve production only occurs in either Class A or Class B waters (SFPA, 2023).

Examples of EU environmental regulations pertaining to aquaculture fall under the Water Framework Directive (2000/60/EC), the MSFD (2008/56/EC), and the Shellfish Water Quality Directive. Nonetheless, there are yet no guidelines that have been established regarding MP levels as contaminants in Ireland or the EU. Given the importance of this emergent topic and ongoing work in the European Commission (STECF, 2021b) providing guidelines to the industry can facilitate future decision-making processes.

This preliminary pilot research investigates the presence, concentrations, and polymer composition of MP from two farmed oysters, the Pacific oyster *Crassostrea gigas or Magallana gigas*, in the new nomenclature, which is still under debate to reach academic consensus (Bayne et al., 2017, 2019); and the European flat oyster *Ostrea edulis*. These species were collected on the west coast of Ireland and depurated under laboratory conditions over a 96-h period. The preliminary results provided here contribute to establishing a baseline on MP contamination in oysters in Ireland while contributing to providing information to policymakers and the industry regarding the food safety of seafood products.

2 | MATERIALS AND METHODS

2.1 | Sampling site

A total of $N = 100$ oysters ($n = 50$ European flat oysters and $n = 50$ Pacific oysters) were obtained from Cartron Point Shellfish Ltd, located in Aughinish Bay, New Quay, The Burrin, Co. Clare, Ireland (Figure 1), which is a designated shellfish production area classified as Class A shellfish harvesting waters (STECF, 2021b). The collection of Pacific oysters was carried out in the intertidal zone from suspended grow-out baskets, while European oysters were collected from the natural oyster beds in the same production area at low water. After collection, oysters were held at the farm facilities, where their shells were gently scrubbed with a metal brush to remove epibionts and rinsed with 1 μm filtered seawater before being transported in a cooler box with chiller blocks to the Marine and Freshwater Research Centre (MFRC) laboratories at the Atlantic Technological University (ATU).
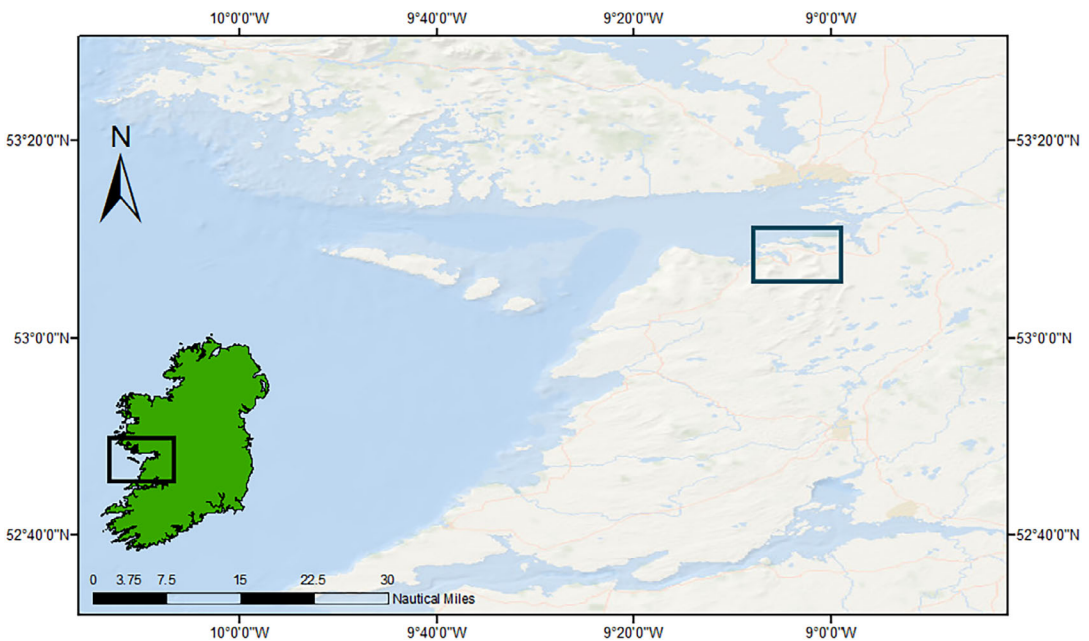
FIGURE 1 Oyster sampling site in Co. Clare (black box), west coast of Ireland.

2.2 | Experimental design

A pilot-scale depuration system was set up in a temperature-controlled facility ($\sim$10°C) at the ATU. The experiment was carried out during Winter (February 2022) and oysters were acclimatized for a 24-h period, prior to the experiment. Four depuration treatments based on time were assessed at 24, 48, 72, and 96 h (with 10 individuals [$n = 10$] per treatment), per species (see Supplementary Material 1). An additional 10 individuals per species were used as controls (not depurated). Approximately 500 mL of 1 μm filtered seawater was replaced daily in each test beaker, to prevent reingestion of egested MPs.

2.3 | Biometrics

Total shell length (in centimeters), total body wet weight (shell + edible tissue, in grams), and total edible tissue w.w. (wet weight, in grams), were recorded for each individual prior to the experiment started. The total shell length was measured using a digital vernier caliper (Digitronic Caliper), while the weight was recorded using a digital scale (Explorer™ Pro Precision EP 4101C Ohaus). The total shell length is the maximum dimension from the hinge to the posterior growth edge of the oyster (Thomas et al., 2020).

2.4 | Tissue digestion and MP isolation

A total of 10 control oysters were euthanized (opened and shucked) at t_0, and the edible tissue was immediately transferred to a labeled pre-decontaminated glass jar (300 mL) with a metallic lid. A 10% potassium hydroxide (KOH) solution was added to each glass jar at a ratio of 3:1 volume (mL) to tissue wet weight, and jars were kept in a drying oven (BinderTM) at a constant temperature of 40°C for 24 h to allow for complete digestion, as proposed by

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Bessa et al. (2019). A similar process was conducted at the end of each depuration treatment, where oysters were collected, wrapped into labeled aluminum foil, and transported to a plastic-free laboratory, and all the steps above were repeated.

Once all soft tissues were fully digested, the resulting digestate was filtered using a vacuum pump filtration unit (VWR™ VCP 130) through a clean glass microfibers filter membrane (Whatman GF/C, $\emptyset = 47$ mm, pore size 1.2 μm), under a laminar flow, to minimize airborne contamination. The Büchner/Kitasato flask (2000 mL) and the cylinder funnel were rinsed with filtered ultra-pure Mili-Q water 12–18 MΩ prior to filtering different samples. Filters were then left to dry inside a desiccator with copper (II) sulfate anhydrous ($CuSO_4$).

2.5 | Visual sorting and polymer identification of suspected particles

Visual identification of plastic particles was conducted on the dried filter membranes under a stereomicroscope (Olympus SZX7). Suspected particles found on the filters were numbered, photographed, and measured using Olympus CellSens® software. Particles were sorted and classified based on their morphotypes (Bessa et al., 2019), size (Frias & Nash, 2019), and color (Bessa et al., 2019).

Polymer identification was carried out on a sub-sample ($n = 109$ out of $N = 596$ isolated microparticles) under a Bruker Hyperion 2000 series micro-Fourier Transformed Infrared Spectroscopic (μ-FTIR) microscope. The μ-FTIR microscope has an MCT (mercury cadmium-telluride) detector and was used in transmission mode, using 128 scans per sample, with a wavenumber range of 4000–400 cm^{-1}, and spectral resolution of 4 cm^{-1}. A background spectrum was acquired, using the same parameters, prior to scanning individual samples. The polymer identification was compared to the JPI Oceans Baseman Project (Frias et al., 2018) FTIR Polymer reference database, and only matches >80% in similarity were accepted.

2.6 | Prevention of cross-contamination

A forensic approach to reducing cross-contamination was employed here, following the works of Frias et al. (2020), Pagter et al. (2020), and Joyce et al. (2022). A review of these processes is described here: all glass materials used were decontaminated by soaking them into 1 M 0.1% nitric acid (HNO_3) bath solution and triple rinsed with filtered ultra-pure Mili-Q water 12–18 MΩ prior to initial use, and triple rinsed in ultrapure water between filtrations. During the shucking of the oysters, 1 L of ultra-pure Mili-Q water 12–18 MΩ was used to clean the knives between the oysters. Operators were required to wear 100% cotton lab coats and to avoid wearing synthetic clothing underneath. Moreover, the color of clothes worn by the operator during analysis was recorded. Three types of blanks and/or controls were performed: (1) seawater blanks on each treatment; (2) airborne controls during microscope observations; and (3) KOH controls run in parallel with digestion procedures. For each depuration treatment, a one-liter glass container was filled with filtered seawater to act as a control (seawater blank) to account for potential air-borne MPs cross-contamination during the running of the experiment (see Figure S1). For each digestion batch of oysters, three procedural blanks containing only 10% KOH were run in parallel and subjected to the same treatments as the oysters. Filtration was conducted in a laminar flow to avoid cross-contamination. All surfaces were cleaned using filtered ultra-pure Mili-Q water 12–18 MΩ and 70% ethanol. During filtration, airborne contamination was further minimized by covering the Büchner/Kitasato flask and cylinder funnel with aluminum foil before pouring the samples to be filtered. Airborne controls were carried out as an open petri dish containing a sterile filter pad to account for airborne cross-contamination during microscope analysis of the filters. To account for airborne cross-contamination, MPs of the same type (color, shape, and thickness) found within the water or procedural blanks were subtracted from the final count. Similar methods were developed for the other blank and control.

2.7 | Statistical analysis

All statistical analysis was performed in *R* version 2021.09.0 (R Core Team, 2021). Descriptive statistics such as histograms and normal Q-Q plots (Visual interpretation plots for normality testing) and the Shapiro–Wilk's test were used to verify the normality of variables. The Levene's test was conducted to check the equality of variances. Since assumptions for normality and homogeneity were not satisfied, non-parametric tests using Kruskal-Wallis (instead of ANOVA) followed by the Pairwise Wilcoxon test for pairwise comparisons. Pearson's Chi-squared test was used to test significant associations between MP color, size classes, and polymer composition between the two species and across depuration treatments. A generalized linear model (Poisson distribution) was used to perform regression analysis between MP abundance and biological parameters (weight of edible tissue). Results are reported as the number of MPs per individual or per gram wet weight (w.w.) edible tissue. The significance level was set at $\alpha = 0.05$ for all statistical analyses.

3 | RESULTS

3.1 | Biometrics

The total body wet weight (shell + edible tissue) for *O. edulis* ranged between 35.7 g and 70.0 g while for *M. gigas* it ranged between 24.9 g and 123.1 g. Results will be expressed in (Mean ± SD). The mean total w.w. for *O. edulis* was 67.6 ± 22.1 g, while for *M. gigas* was 46.6 ± 7.9 g.

Regarding edible tissue, *O. edulis* ranged between 2.91 g and 21.9 g, with a mean weight of 9.7 ± 22.1 g, while for *M. gigas*, the edible tissue weight ranged between 6.04 g and 18.21 g, with an average of 11.1 ± 7.9 g. Shell length ranged between 5.3 and 8.7 cm with a mean shell length of 7.2 ± 0.8 for *O. edulis*, while *M. gigas* was slightly larger with lengths ranging between 6.8 and 9.3 cm and a mean shell length of 7.8 ± 0.6 cm.

3.2 | Cross-contamination

Cross-contamination was assessed using three types of controls during laboratory processing: (1) procedure seawater blanks; (2) airborne controls; and (3) KOH controls (Table 1 and Supplementary Materials 3 and 4, respectively). Controls and blanks were run separately for each species and Table 1 summarizes the mean MP count in each blank for each species, as well as the mean length of the particles identified as cross-contamination.

TABLE 1 Mean MP count and length of microplastics identified under forensic approach to prevent cross-contamination.

		Magallana gigas	*Ostrea edulis*
Procedure blank	Mean MP count (#)	4	2
	Mean length (μm)	1328.5	1431.9
Airborne controls	Mean MP count (#)	12	40
	Mean length (μm)	2429.9	1276.1
KOH controls	Mean MP count (#)	1.4	0.6
	Mean length (μm)	532.9	149.3

3.3 | Abundance of MPs

A total of 596 micro-particles were isolated from all organisms in this experiment ($N = 100$), of which 540 were considered as potential MPs ($n = 56$ were identified as microparticles of natural origin). Micro-particles of natural origin were identified as such based on more than 80% match with the JPI Oceans BASEMAN project FTIR Polymer reference database.

Results will be expressed in (mean ± SD). Mean MP concentration per individual ranged between 0.6 ± 0.7 and 8.2 ± 6.7 MP/Individual between depuration treatments for *O. edulis* and between 1.7 ± 2.1 and 7.3 ± 7.0 MP/Individual for *M. gigas*.

The mean MP concentration of the edible tissue varied between 0.1 ± 0.1 and 0.9 ± 0.8 MP/g and 0.2 ± 0.2 to 0.6 ± 0.3 MP/g, respectively.

The initial (t_0) mean MP abundance in *O. edulis* was 5.6 ± 4.5 MP/Individual or 0.4 ± 0.4 MP/g w.w and for *M. gigas* was 6.4 ± 3.0 MP/Individual or 0.6 ± 0.3 MP/g w.w.

3.4 | Efficacy of depuration on reducing MP abundance in oysters

MP concentrations per individual after 96-h of depuration were reduced by 76.7% in *M. gigas* (88.9% in the edible tissue) and 92.7% in *O. edulis* (66.7% in the edible tissue).

The depuration treatments had a significant effect on MP abundance in *M. gigas* (x^2Kruskal-Wallis = 12.574, df = 4, *p*-value = 0.01356, $p < 0.05$) and Pairwise Wilcoxon rank sum test with continuity correction indicating a significant difference between MP abundance for 96-h|No depuration: $p = 0.014$ and 96-h|24-h: $p = 0.049$ but was not significant for 96-h|48-h: $p = 0.095$ or 96-h|72-h: $p = 0.442$.

A significant effect was also reported in *O. edulis* (x^2Kruskal-Wallis = 20.602, df = 4, *p*-value = 0.0003797, $p < 0.05$) and Pairwise Wilcoxon test: (96-h|No depuration: $p = 0.0033$; 96-h|24-h: $p = 0.0032$; 96-h|48-h: 0.0182; 96-h|72-h: 0.0020). No significant variation in MP concentration was explained to vary by species (x^2Kruskal-Wallis = 0.13226, df = 1, *p*-value = 0.7161, $p > 0.05$).

Figure 2 shows the relationship between the mean number of MPs per individual over the depuration period. The mean MP abundance in non-depurated *M. gigas* (6.4 ± 3.0 MP/Ind. or 0.6 ± 0.3 MP/g) and those subjected to 96-h depuration showed a significant reduction (1.7 ± 2.1 MP/Ind. or 0.2 ± 0.2 MP/g) (*p*-value = 0.01356). Similarly, non-depurated *O. edulis* (5.6 ± 4.5 MP/Ind. or 0.4 ± 0.4 MP/g) significantly declined (0.6 ± 0.7 MP/Ind. or 0.1 ± 0.1 MP/g) after 96-h depuration period (*p*-value = 0.0003797) (Figure 2).
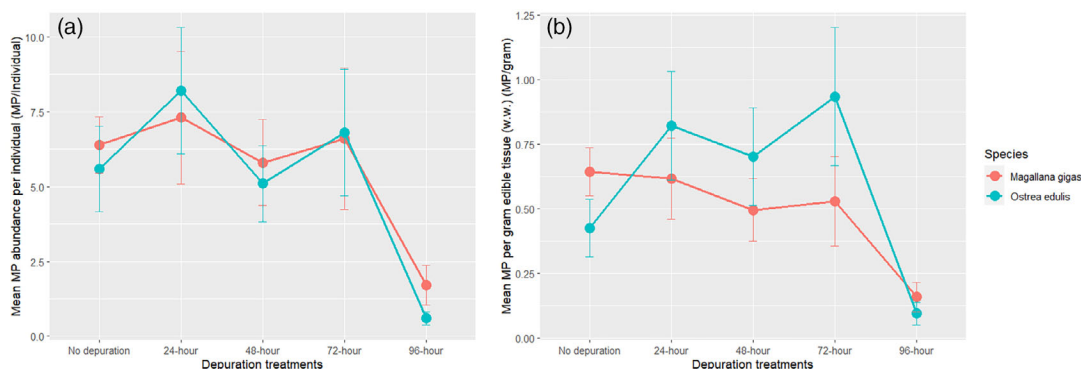

FIGURE 2 (a) Represents the mean abundance of MPs per individual (Mean ± 95% CI) per depuration treatment ($N = 10$ per treatment) and (b) the mean abundance of MPs per gram (w.w.) edible tissue (Mean ± 95% CI) per depuration treatment ($N = 10$ per treatment).

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3.5 | Concentrations of MPs in edible tissue weight wet of oysters

A generalized linear model (GLM) (Poisson distribution) was carried out to test whether MP concentrations varied according to the weight of edible tissue. There were no significant differences between MP with the weight of edible tissue in both *M. gigas*: F1, 46 = 10.817, p = 0.07401 at α = 0.05 and *O. edulis*: F1, 46 = 3.4259, p = 0.3325 at α = 0.05 (Figure 3).
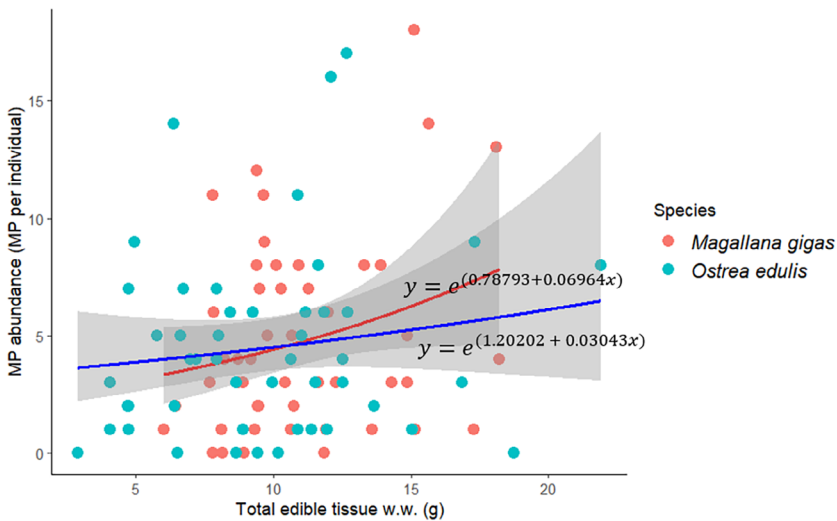
FIGURE 3 Relationship between total edible tissue w.w. and MPs abundance with 95% CI.
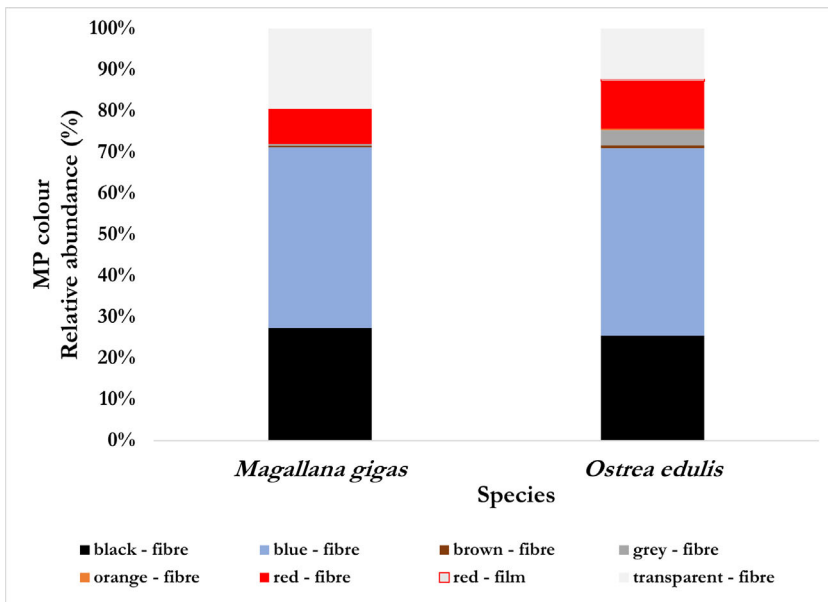
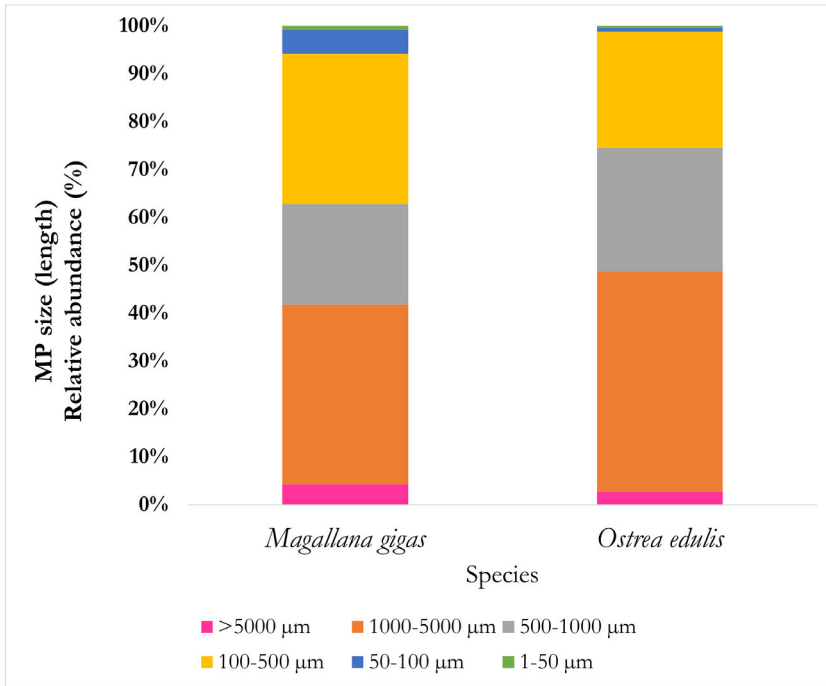**FIGURE 4** MP color composition for each species.

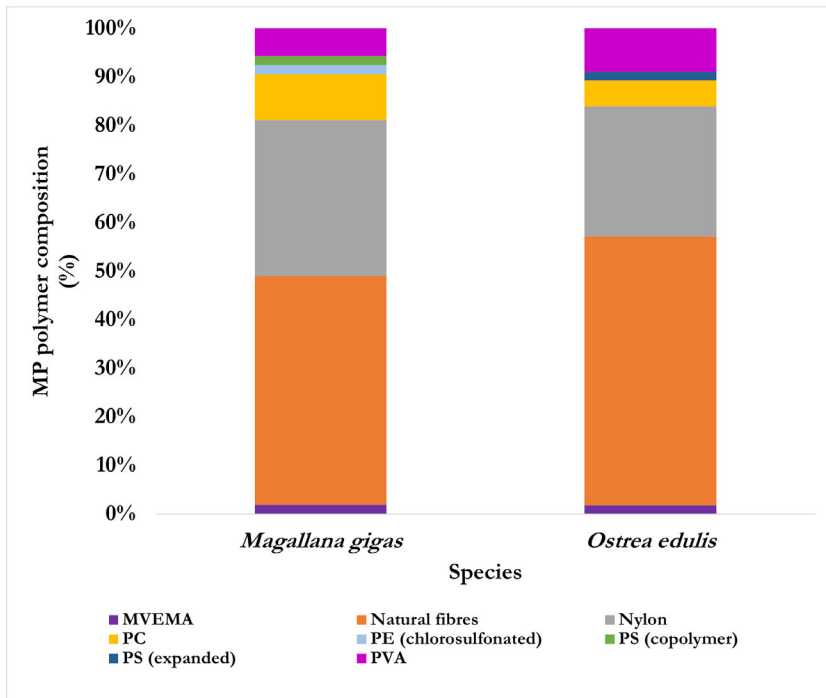**FIGURE 5**   MP fiber size class for both species.

FIGURE 6   Polymer composition for both species.

3.6 | MP types, colors, size range, and polymer identification

The MP fibers were dominant with over 99% (539 out of 540 potential MP particles) of occurrence with a single microparticle identified as "film". Seven different MP colors were identified with the most common MP color being blue (44.6%), followed by black (26.5%), transparent (16.1%), and red (10.6%). The remaining colors identified were gray, brown, and orange, which combined accounted for 2.2%. For *M. gigas*, MP colors in order of decreasing abundance are blue (43.9%), followed by black (27.3%), transparent (19.4%), red (8.6%), gray (0.4%), and brown (0.4%). For *O. edulis*, MP colors in order of decreasing abundance are blue (45.4%), black (25.5%), transparent (12.4%), red (11.6%), gray (3.6%), brown (0.8%) and orange (0.4%) (Figure 4).

TABLE 2 Oyster consumption scenarios and potential microplastic ingestion per species: *M.gigas* (red) and *O. edulis* (blue).

	No. of oysters consumed per meal	No depuration	48-h	96-h
M. gigas MPs/Ind	1–2	6.4–12.8	5.8–11.6	1.7–3.4
	3–4	19.2–32.0	17.4–29.0	5.1–8.5
	5–9	38.4–57.6	34.8–52.2	10.2–15.3
	10–15	64.0–96.0	58.0–87.0	17.0–25.5
O. edulis MPs/Ind	1–2	5.6–11.2	5.1–10.2	0.6–1.2
	3–4	16.8–28.0	15.3–25.5	1.8–3.0
	5–9	33.6–50.4	30.6–45.9	3.6–5.4
	10–15	56.0–84.0	51.0–76.5	6.0–9.0
M. gigas MPs/g w.w.	1–2	0.6–1.2	0.5–1.0	0.2–0.4
	3–4	1.8–3.0	1.5–2.5	0.6–1.0
	5–9	3.6–5.4	3.0–4.5	1.2–1.8
	10–15	6.0–9.0	5.0–7.5	2.0–3.0
O. edulis MPs/g w.w.	1–2	0.4–0.8	0.7–1.4	0.1–0.2
	3–4	1.2–2.0	2.1–3.5	0.3–0.5
	5–9	2.4–3.6	4.2–6.3	0.6–0.9
	10–15	4.0–6.0	7.0–10.5	1.0–1.5

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The MP thickness detected in *O. edulis* ranged from 3.1 to 1548.3 μm with length varying between 15.7 and 9494.6 μm and thickness in *M. gigas* ranged between 1.4 and 1071.0 μm while the length varied between 27.8 and 16,523.1 μm. MPs were classified into six size classes based on length (Figure 5). In both species, most MP fibers found within the edible tissues were within the size range of 1000–5000 μm (37.5% in *M. gigas* and 46.0% in *O. edulis*).

A subsample of 109 microfibers ($N = 596$ isolated microparticles) was analyzed under μ-FTIR for polymer identification. Polymer identification revealed the presence of 51.4% natural fibers while the remaining (48.6%) was identified as being of synthetic origin. Seven different types of polymers were detected including natural microparticles (Figure 6). The analysis revealed a high abundance of natural fibers in both *M. gigas* (47.2%) and *O. edulis* (55.4%) followed by nylon, 20.9% in *M. gigas* and 25.8% in *O. edulis*.

A higher proportion of polycarbonate (PC—9.4%) was detected in *M. gigas* than in *O. edulis* (5.4%) while *O. edulis* had a higher abundance of polyvinyl alcohol (PVA—8.9%) than in *M. gigas* (5.7%). Both species had almost similar relative abundance of polystyrene (PS) (1.8%) while *M. gigas* showed a small abundance of polyethylene (PE) (1.9%).

3.7 | Dietary intake scenarios of MPs in Irish oysters

Based on the mean MP abundance from *M. gigas* and *O. edulis* following depuration treatment (No depuration|48-h|96-h), MP exposure levels were calculated based on four different consumption scenarios, depending on the number of oysters consumer per meal. These estimates were inferred from the number of oysters consumed by locals and tourists in Galway city. The basis of this consumption scenario was the results of this preliminary study.

These scenarios reflect the consumption of 1–2; 3–4; 5–9; and 10–15 oysters per meal and include the potential number of MPs ingested with no depuration, 48 and 96-h depuration periods. Table 2 includes scenarios both in MP per Individual and MP per gram edible tissue.

4 | DISCUSSION

4.1 | Depuration efficiency

In the present study, a significant reduction in MP concentrations was observed after 96-h of depuration, in both *M. gigas* and *O. edulis* (Figure 2a,b). This result is in accordance with other depuration studies showing a significant reduction of MPs after depuration treatments in shellfish species (Birnstiel et al., 2019; Van Cauwenberghe & Janssen, 2014). Our results are significant when compared to a 25.5% reduction in MPs in farmed *M. gigas* and 33.3% for farmed Mytilus *edulis* (Van Cauwenberghe & Janssen, 2014) and 29.0% in farmed *Perna Perna* (Birnstiel et al., 2019) following only a 72-h depuration. There is evidence that filter-feeding bivalves can accumulate MPs in their edible tissue from natural sources (Ding et al., 2021; Martinelli et al., 2020; Phuong et al., 2018) and in laboratory assays investigating MP capture (Rosa et al., 2018; Ward et al., 2019). However, there is evidence that filter-feeding bivalves can eliminate a high proportion of MPs after capturing through rejection via pseudo-faces and egestion as fecal pellets (Thomas et al., 2020; Ward et al., 2019) which can be achieved via natural elimination known as depuration (Lee et al., 2008).

In both species of oysters, a slight increase in MP concentration at 24 and 72-h depuration treatments was observed. This unexpected observation might be related to a short acclimatization period in the laboratory facilities associated with the digestive systems of case study oysters. Observations show that *O. edulis* might require a longer depuration period to reduce MP concentrations when compared to *M. gigas*, because of its digestive system structure (Pers. Comms. Mr. Iarfhlaith Connellan, 2022). *O. edulis* contains two different digestive systems (Yonge, 1926), which

slows the elimination of MPs when compared to *M. gigas*, as *O. edulis* accumulates critical mass biodeposits before egestion/rejection while *M. gigas* expels its biodeposits at it feeds (Pers. Comms. Mr. Iarfhlaith Connellan, 2022).

It has been shown that oysters accumulate MP destined for egestion in mucous balls of various sizes, expelling them from the mantle cavity (Ward et al., 2019). It may be possible that the fragmentation of the mucous balls leads to some extent to the fragmentation of fibers (Ward et al., 2019). These could be resuspended in the water column and re-ingested thereby leading to an increased number of fragmented fibers to be re-ingested by the oysters at time points 24 and 72-h, as observed from Figures 2a,b. This conclusion remains speculative but given the extreme care taken to avoid contamination and correction from the blanks, this could be a plausible theory. The MP concentrations recorded in non-depurated oysters and those allowed to depurate for 24 h and those depurated for 72 h for both *M. gigas* and *O. edulis* were not significant meaning that the level of MPs at these specific time points was similar. Therefore, this does not influence the overall result of this experiment, showing a significant net decrease in MP concentration after 96-h of depuration, for both species.

The current depuration period in Ireland is based on a minimum depuration period of at least 42-h (SFPA, 2017, Figure 2), and it is only required for Class B category waters. This means that a corresponding depuration period of 48-h depuration in this study would result in an approximate reduction of 88.9% of MP per gram of edible tissue in *M. gigas* and 66.7% in *O. edulis*.

The present study demonstrated that the current depuration period (~42-h) as a minimum threshold for the elimination of biotoxins and coliforms, may not be sufficient to allow for the optimal elimination of accumulated MPs in farmed oysters in Ireland. For shellfish growing in Class A waters, depuration is not a mandatory process, but shellfish harvested from Class B waters are sent to depuration facilities to eliminate fecal contamination risks for a minimum of 42-h. A depuration period larger than 72-h, ideally 96-h, is recommended as a safe depuration time to allow oysters to eliminate biotoxins, coliforms, and MPs. Although results showed a significant reduction in efficiency in MPs after 96-h, a 100% reduction in efficiency of MPs by depuration has not been proven yet, even after a longer depuration period (10-days) (Fernández & Albentosa, 2019), highlighting the need for further research in this topic, including safe levels of exposure.

Oysters in this study were collected from Class A Shellfish harvesting waters, which are not subjected to mandatory depuration before reaching the consumers or end users. Existing legislation such as the Shellfish Growing Waters Directive provides for the protection of the water quality of areas designated as shellfish production areas in Europe, as is the case of S.I. No. 268 of 2006. This regulation provides for the protection of the water quality of shellfish growing waters and ensures compliance with the directive by measuring water quality parameters and ensuring compliance with values outlined in Annex I of the Shellfish Waters Directive (2006/113/EC) and Schedules 2 and 4 of the Quality of Shellfish Waters Regulations (S.I. No. 268 of 2006). Based upon compliance with these water quality guidelines, shellfish production areas are categorized as either Class A, B, or C waters, depending on the *E. coli* concentrations present. In Europe, depuration is a common post-harvest practice in bivalve aquaculture to minimize fecal contamination risks and comply with European food safety legislation (European Commission Regulation 853 of 2004, 852 of 2004, and 2073 of 2005) regarding specific hygiene rules for food of animal origin. For shellfish growing in Class A waters, depuration is not a mandatory process, but shellfish harvested from Class B waters are sent to depuration facilities to eliminate fecal contamination risks for a minimum of 42-h.

4.2 | Qualitative analysis of microparticles

Microfibres were the most common MP type found in both study species, with similar findings in oysters and other bivalves (Craig et al., 2022; Ding et al., 2021). Microfibres contribute to almost 60% of MPs released into the environment, which significantly pollutes rivers and oceans (Reineccius et al., 2020). In contrast, some studies reported higher proportions of MP fragments in similar species of oysters (Liao et al., 2021; Phuong et al., 2018) where geography, population, density and local sources of MPs might explain this difference (FAO, 2017). MP studies carried out in Ireland

reported MP types and MP colors similar to those observed in gastropods and decapod crustaceans (Doyle et al., 2019; Joyce et al., 2022), as well as surface seawater (Frias et al., 2020), and sediment (Pagter et al., 2020).

Qualitative analysis of microfibres showed that microfibres in the size class 1000–5000 μm were the most common followed by the size class 500–1000 μm. The majority of MP studies in oysters reported microfibres smaller than 300 μm (Patterson et al., 2019; Phuong et al., 2018; Van Cauwenberghe & Janssen, 2014) although some other studies reported MP fibers up to 8720 μm long (Baechler et al., 2020; Craig et al., 2022). This study also indicates that *O. edulis* had lower proportions of microfibres with the smallest size classes in comparison to *M. gigas* which is consistent with a study that reported *O. edulis* to have a lower ability to retain smaller particle sizes (Nielsen et al., 2017). Oysters are selective particle feeders where a selection of particles to be captured, rejected, or ingested depends on the particle size, shape, and surface properties (Rosa et al., 2018). The morphological characteristics of the mouth, labial palps, and gills in oysters will also determine the size of the particle to be ingested (Ward et al., 2019). The siphon of oysters is flexible enough to stretch and engulf larger particles up to a size limit between 600 and 1000 μm (Ward et al., 2019).

In this study, the length of the microfibers is longer than what is commonly reported in oysters but the thickness of most fibers (88.5%—*M. gigas* and 97%—*O. edulis*) are within the 1–50 μm thickness size range indicating that oysters could have potentially ingested unusually longer microfibres as long as the thickness remains within this range.

Qualitative analysis of polymer composition revealed that over half of the subsamples analyzed under μ-FTIR were natural polymers while the remaining was of synthetic origin. Both species had a higher abundance of natural fibers (cellulose-based material such as wood beech, wool, and zein) followed by synthetic fibers identified as nylon, with moderate abundances of polycarbonate (PC) and polyvinyl alcohol (PVA) and low relative abundances of polystyrene (PS), and polyethylene (PE). Nylon is commonly found in materials originating from fishing gears and aquaculture floats and ropes while PC can originate from electronics and construction materials (polycarbonate sheets) (Coyle et al., 2020). PVA is commonly found in plastic wrapping of laundry products and PS (expanded) originates from cool boxes, floats, and cups while PE comes from single-use plastic bags, storage containers, and personal care products (Coyle et al., 2020). The sources of MPs are difficult to track but qualitative analysis of MPs provides baseline data for identification of potential sources of MPs in surrounding waters and associated biota (FAO, 2017).

The availability of MP particles also depends on the density of the polymer (Coyle et al., 2020). Low-density polymers such as PS (0.01–1.05 gcm^{-3}) and PE (0.94–0.95 $g\,cm^{-3}$) have lower specific gravity than seawater ($\sim$1.025 $g\,cm^{-3}$) and float on seawater increasing their availability to oysters. They will eventually make their way to bottom sediment because of incorporation into fecal pellets, marine snow, and biofouling (Coyle et al., 2020; Patterson et al., 2019).

However, both nylon (1.13–1.15 $g\,cm^{-3}$) and PC (1.19–$1.25gcm^{-3}$) will naturally sink, as they are denser than seawater, increasing their availability to mostly the infauna but may become another intake source during bottom deposition or during bioturbation (Wright et al., 2013).

Although Aughinis Bay is located in a rural area with relatively low coastal habitation and industrial activities, it is a designated shellfish production area and is a licensed shellfish aquaculture site with nearby aquaculture infrastructure (hatcheries) and equipment (nets, cages, baskets, etc.) which could potentially be a source of MPs to nearby shellfish growing waters (FAO, 2017). Future MP studies should consider water analysis for MPs in conjunction with biota and sediment analysis to further collect baseline data on MPs' presence within the shellfish production area within Aughinis Bay.

Moreover, agricultural lands are common along the coastal road, which could be an additional potential source of MPs in Aughinis Bay (FAO, 2017) particularly after rainfall events and strong winds, which are prevalent climatic factors in Ireland (Devoy et al., 2021).

4.3 | Effects of body size on MP loads in oysters

Abundance of MPs in relation to total edible weight was investigated, where no correlation was found for total edible tissue w.w. in both *M. gigas* and *O. edulis*. Similarly to other studies, no detectable correlation between the level of MP

with the organisms' edible tissues w.w (Birnstiel et al., 2019; *M. gigas*—Martinelli et al., 2020). Based on this result, the consumption of oysters with higher tissue wet weight is unlikely to predict higher levels of MP in *M. gigas* or *O. edulis*.

Future studies investigating the correlation between edible tissue weight and MP loads in both *M. gigas* and *O. edulis* should thus include larger sampling (a minimum of 50 individuals per treatment based on a power analysis of 0.8) and be conducted in parallel with large depuration facilities to assess results. A very important aspect of this study that needs future consideration is sex differentiation prior to the experiment as wet weight differs between male and female oysters which also significantly depends on environmental factors (Bayne et al., 2017).

MPs have been reported in farmed shellfishes across various regions (Birnstiel et al., 2019; Martinelli et al., 2020; Teng et al., 2019). To our knowledge, this study is the first to investigate the environmental MP abundance in farmed Pacific oysters and European flat oysters in Ireland. MP concentrations in both species did not vary significantly indicating that ingestion rates are similar.

The MP concentrations detected in *M. gigas* (6.4 ± 3.0 MP/Ind. or 0.6 ± 0.3 MP/g) correspond to the environmental level of MP recorded in other studies on *M. gigas* ranging from 1 to 10.95 MP/Ind. (see Table S1). The MP abundance in *O. edulis* (5.6 ± 4.5 MP/Ind. or 0.4 ± 0.4 MP/g w.w.) can be compared with those of *M. gigas* as studies on this species and MP concentrations are limited.

The MP abundance in both species is slightly higher than those presented in other studies in Europe, 0.47 ± 0.16 MP/g (Van Cauwenberghe & Janssen, 2014) and 0.18 ± 0.16 MP/g (Phuong et al., 2018). However, comparison in MP abundance with similar studies (see Supplementary Material 5) was considered with caution as different protocols, sample sizes, and analytical methods differed across studies (Thiele et al., 2019).

The MP density in the surface water of Galway Bay has been documented to be 0.46 ± 0.16 MP m^{-3} within the Inner Bay and 0.62 ± 0.40 m^{-3} at the Outer Bay (Frias et al., 2020). Although concentrations in the inner bay are lower, ocean circulation patterns influence accumulation areas, particularly around Kinvara (Frias et al., 2020; Pagter et al., 2020). In this study, the MP abundance in oysters before depuration was 6.4 ± 3.0 MP Ind.$^{-1}$ and 5.6 ± 4.5 MP Ind.$^{-1}$ for *M. gigas* and *O. edulis*, respectively, reflecting a higher abundance when compared to the surrounding environment.

4.4 | Policy and industry recommendations

Taking into consideration the data presented in this preliminary study, alongside the scenarios developed in 3.6, there are key points to emphasize. First and foremost, this preliminary laboratory assay builds upon other similar research conducted in Europe (e.g., Van Cauwenberghe & Janssen, 2014). Despite being a laboratory study, it can be upscaled to a full commercial testing depuration system to assess the efficiency of depuration at a larger scale. This is particularly relevant because bivalves were destined for human consumption. It has been highlighted that those oysters from Shellfish harvesting water Class B are subjected to depuration (42 h; SFPA, 2017), which may not be sufficient to allow for the egestion/elimination of MPs in farmed or wild harvested oysters. Therefore, while the full commercial testing facility depuration system is not carried out, the industry recommendation would be to increase the depuration time beyond 72-h or to increase it to 96-h. Another important aspect, to ensure Good Environmental Status, as described in the MSFD, is to assess the concentrations of MPs in the sites where oysters are being produced. This assessment will ensure the monitoring of environmental concentrations of MPs in the aquaculture sector and will serve as a site-specific baseline. One of the limitations of this current study is the lack of environmental concentration assessment. Water controls were carried out for MP analysis only in the filtered seawater from the facility (Cartron Point Shellfish Ltd) and not from the licensed oyster bed at Aughinish Bay, where the oysters for this study were collected.

Not only do all these measures will ensure that MPs are virtually absent from the final product, but also they could potentially lead to the creation of a labeling system that can provide consumers with a safer and better-

informed decision while acquiring such products. From a food safety perspective, such a label would meet a sustainable development goal for this sector not only at a local or national scale but at a global scale.

Based on the lack of guidelines and/or regulations to assess MPs in aquaculture products, a valuable and suitable option would be the creation/establishment of a standard based on best practices to ensure MPs are not present in bivalves from aquaculture. Such an endeavor requires a multi-stakeholder commitment and must include policy makers, researchers, and the industry.

As highlighted by the UN, food safety is a vital part of sustainable development, particularly with the rising population globally. It is important to understand that MPs have been identified throughout the environment (Cunningham et al., 2020; Kelly et al., 2020; Napper et al., 2020; Peeken et al., 2018), in various matrices, including biota (Doyle et al., 2019; Joyce et al., 2022; Mohsen et al., 2019). Plastic is an extremely versatile material, that not only allows scientific advances to be made every year but also requires management. Despite being found in organisms, and in humans, it is not clear what are the impacts, if any.

As such, and as far as the data from this article refers, it is safe to consume bivalves from aquaculture in Ireland, particularly after a 96-h depuration period.

5 | CONCLUSION

To our knowledge, this is the first study investigating the depuration efficiency of MPs in farmed oysters in Ireland. Concentrations of MPs in their edible tissue can significantly decrease with increasing depuration times (greater than 72 h). A depuration of at least 96 h can remove more than 90% of MPs. This baseline provides valuable information for decision-makers both at the industry and policy levels, however, an assessment at an industrial-relevant scale is required to substantiate full results.

AUTHOR CONTRIBUTIONS
Ann Tracy Paul: Conceptualization, Methodology, Investigation, Formal analysis, Writing—original draft. **Colin Hannon:** Conceptualization, Writing—review and editing, Supervision. **Iarfhlaith Connellan:** Methodology, Writing—review and editing. **Mateja Švonja**: Investigation. **João Frias:** Conceptualization, Methodology, Writing—review and editing, Supervision.

CONFLICT OF INTEREST STATEMENT
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This preliminary study was conducted with 100 oysters collected from Aughinish Bay, New Quay, Co. Clare, Ireland (53°9.548′N; 9°2.859′W). The industry partner (Cartron Point Shellfish Ltd) contributed to the research team by providing oyster samples and other essential materials needed. No specific permissions were required for this sampling site/activity, and the study did not involve endangered or protected species. An institutional ethical review was not required as the study was conducted with invertebrate species.

ORCID

Ann Tracy Paul 🆔 https://orcid.org/0000-0001-7382-7701

Colin Hannon 🆔 https://orcid.org/0000-0002-9448-627X

Mateja Švonja 🆔 https://orcid.org/0000-0003-1532-0608

João Frias 🆔 https://orcid.org/0000-0001-9943-1035

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.