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Advancing mussel-based monitoring: integrating litter and harmful algal bloom data into a multi-stressor assessment of England and Wales

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Effective monitoring is essential for decision makers to make informed choices to address pollution issues, including marine litter and microplastics which are subjects of increasing interest. Monitoring biota is essential for estimating the bioavailable fraction of litter in the environment and is a step towards understanding the risks associated with microplastics. The present study assessed the suitability of mussels as a sentinel species in a multi-stressor context. Mussels are already used as sentinel species for many contaminants, including harmful algal blooms, but to date there is no agreed sentinel species targeting microplastics. Mussels from seven locations on the English and Welsh coast were monitored for both microplastics and harmful algal biotoxins. Fluorescent and non-fluorescent microplastics were quantified. Over half (53%) of mussels contained microplastics. No geographical accumulation zones in microplastic abundance were identified at the sample locations with a mean contamination of 1.33 ± 3.04 (SD) items per individual (95% CI = 0.88–1.79) and 0.33 ± 0.71 items per g (wet weight) (95% CI = 0.23–0.44). Five groups of harmful algal toxins were screened within the study animals, with no quantifiable levels of any being present at the time of sampling for this study. However, four of the six sites were exposed to the Diarrhetic shellfish toxins earlier in the year, representing a prior exposure to harmful algal toxins. Research has shown that microplastic exposure alone does not always negatively impact organisms. But there is mounting evidence that microplastics may increase sensitivity and susceptibility to other stressors in the environment. Given the presence of both microplastics and algal neurotoxins in oceans around the world and the concern for multi-stressor impacts on the marine environment, it is proposed that multi-factor monitoring could provide insight into the true risk of microplastics as a contaminant vector and antagonistic pollutant. This evidence is urgently needed

to support governments globally in creating mitigation strategies and monitoring the success of these interventions. The present study finds that mussels are a suitable sentinel species for this use, but ongoing research is needed to determine spatial and temporal variations.

KEYWORDS

microplastics, *Mytilus edulis*, mussels, sentinel species, plastic pollution, harmful algal blooms, multi-stressor monitoring

1 Introduction

Due to its mass production, transportation and disposal, plastic pollution has become a significant global issue. Demand for plastic has increased annually with 413.8 million metric tonnes produced in 2023 (PlasticsEurope, 2024). Large plastic fragments break up in the environment to make small microplastics (Andrady, 2022; Sipe et al., 2022). These are defined as plastic items between 1 μm and 5 mm (GESAMP, 2019). Primary microplastics, those manufactured at smaller than 5 mm rather than fragmented from larger items, can also enter the environment directly (Van Wesel et al., 2016; Wang et al., 2019). The term ‘microlitter’ refers to all anthropogenic materials including plastic. Recently developed guidance from the regional seas convention OSPAR (OSPAR MicroPlastics Expert Group (MPEG), 2024), however, includes semi-synthetic cellulosic material as a microplastic. The present study, therefore, refers to all items collected as microplastics. Microplastics are more widely bioavailable than larger items and have been ingested by entire food webs (McGoran, 2023) from plankton (Desforages et al., 2015; Zheng et al., 2020) to cetaceans (Besseling et al., 2015; Moore et al., 2020). Once ingested, microplastics have the potential to adversely affect the host organism (Khalid et al., 2021; Castro et al., 2022) sometimes altering gut physiology, immune function, growth and more (Osman et al., 2023). Effects on immune function and metabolism have been observed in mussels when exposed to microplastics smaller than 250 μm (Nardi et al., 2024). A review by Xu et al. (2024) also concluded that exposure to small microplastics (<400 μm) negatively impacted bivalve mollusks including mussels. Mussels have been shown to be pathways for the entry of microplastics to humans through the food chain with some evidence of impacts in humans, though more evidence is needed on this topic (Danopoulos et al., 2022; Walker et al., 2022). Models have also predicted that harmful pollutants can leach from microplastics into the human digestive system (Peters et al., 2022). However, it is not definitive that microplastics cause harm with many studies observing minimal or no negative impacts of microplastic exposure. Indeed, this has been observed in studies of suspension feeding mollusks (Hamm and Lenz, 2021; Opitz et al., 2021; Joyce and Falkenberg, 2022). It is predicted that in the next 70 to 100 years there will be wide-scale environmental harm as a result

of microplastic abundance (Thompson et al., 2024). As such it is essential to develop a sentinel species to gather baseline data prior to widescale negative effects, even if current environmental levels are not sufficient to elicit a response from biota.

Despite their abundance, persistence and potential risk to biota and human health, there is no agreed sentinel species targeted at microplastics monitoring (Matiddi et al., 2021; Ghosh et al., 2023). Whilst sentinel species do not inherently infer risk, once thresholds are determined, monitoring levels of ingestion is important to track changes in the bioavailable fraction of microplastics in the environment. In the meantime, monitoring data can feed into environmentally relevant concentrations for the necessary ecotoxicological tests. But harmonized approaches are needed to ensure that regional and international data can be compared. In March 2022, UN member states agreed to forge the first international legally binding framework to end plastic pollution. Whilst talks eventually failed due to geopolitical complexities and economic challenges, it highlighted a global demand for improved mitigation of marine litter. Therefore, at this pivotal time, monitoring programs are needed to inform governments and decision making for future frameworks. The latest assessments on Good Environmental Status (GES) for the UK Marine Strategy (part one) show that the UK has not met GES for marine litter which includes microplastics. It also highlights the need to continue to build the evidence base for harm that marine litter causes on ecosystems (Defra, 2024). OSPAR uses two sentinel species: fulmars (Van Franeker, 2019) and turtles (Galgani et al., 2022) which capture microplastics larger than 1 mm but are unable to quantify the smaller fraction of microplastics, which are typically more abundant (Lindeque et al., 2020) and may be able to translocate to other tissues and organs, including the circulatory system, organs, placenta and lungs once ingested (Browne et al., 2008; Cattaneo et al., 2023; Yang et al., 2020; Zeytin et al., 2020; Li et al., 2021; McIlwraith et al., 2021). These indicators were selected to quantify the abundance of mesoplastics at the ocean surface and not to monitor microplastic abundance. More suitable species may be available for this purpose.

Mollusks, especially mussels, have often been proposed as a potential sentinel species for microplastics (Bråte et al., 2018; Li et al., 2019; Gerigny et al., 2023; Wu et al., 2024). Mussels, as a suspension feeder, are able to ingest particles between 2 and 500 μm

(Rosa et al., 2018), retaining them for just a few days (Ward and Kach, 2009; Catarino et al., 2017). Several species exist with large populations and wide geographical spreads (e.g., *Mytilus edulis*, blue mussel; Tyler-Walters, 2008). Additionally, mussels are sedentary, easy to sample from the shore and are of economic importance. Through their use as sentinel species for chemical pollutants (e.g., ROCCH, OSPAR, CEMP), mussels are a strong contender for a microplastics sentinel species. Whilst not a perfect sentinel for microplastics (Ward et al., 2019; Mladinich et al., 2022; Shumway et al., 2023), microplastic assessments have recently been added to existing monitoring programs with mussels (Farrington et al., 2016). The Mussel Watch program has been a long-standing initiative by the National Oceanic and Atmospheric Administration (NOAA) to monitor chemical contaminants and more recently microplastics in coastal waterways and Great Lakes (NCCOS, 2020). Following NOAA's initiative, a case study on the suitability of a mussel watch program for the Mediterranean Sea was carried out using *Mytilus galloprovincialis* (Provenza et al., 2022).

Despite several studies investigating microplastic ingestion by mussels (Van Cauwenberghe et al., 2015; Li et al., 2016, 2018; Catarino et al., 2017, 2018; Lusher et al., 2017; Digka et al., 2018; Qu et al., 2018; Reguera et al., 2019), a lack of harmonized and standardized methods limits comparisons (Li et al., 2021) with a need for higher study design and quality requirements (Shumway et al., 2023). Shumway et al. (2023) highlighted that a rapid increase in publications lacking the necessary quality and contamination controls lead to misinformation and incorrect assumptions around bivalve suitability as a sentinel species. Thus, more work is needed to refine analysis and monitoring of microplastics in mussels.

Microplastics can act as a vector of chemical contaminants and biological agents (Tumwesigye et al., 2023) and thus antagonistic effects of their combined presence with other contaminants may be observed. Current monitoring focuses on individual contaminant assessments, siloing analyses and overlooking these multi-stressor interactions. If mussels were proposed as a microplastic sentinel they would align with existing monitoring programs to support these comparisons, for example biotoxins from harmful algal blooms. This contaminant group comprises several distinct classes of toxins, each exhibiting different properties and often containing multiple analogues of a parent compound (Van Dolah, 2000). Many of these microalgal produced toxins can be

accumulated in filter feeding bivalve mollusks, such as mussels. These accumulated marine algal biotoxins are known to negatively affect the health of higher consumers of mussels, including humans (Grattan et al., 2016). Consequently, a robust monitoring program exists for classified shellfish growing areas around the UK coast. The monitoring program is for the presence of the harmful algal groups known to produce the toxins in shellfish growing waters, as well as for the presence of the toxins within the shellfish. The frequency of toxin monitoring undertaken at each site is dependent on a risk assessment, with specified frequencies ranging from monthly sampling through to weekly, phytoplankton sampling occurs fortnightly from April until September and monthly from October until March. There are five groups of toxins specified in legislation and therefore monitored within the UK (EC 853/2004, 2004; EU 786/2013, 2013) and more broadly in many other countries (Table 1). The most common of the toxins present in British waters are the Diarrhetic Shellfish Poisoning toxins caused by Okadaic acid and its analogues (Dhanji-Rapkova et al., 2018; Bresnan et al., 2021). This group of toxins has been found to impact a wide geographic spread around the British coast, from the Shetland Islands in Scotland, to the Southwest of England, as well as causing the longest temporary closures of shellfish production areas, alongside the Azaspiracids (Dhanji-Rapkova et al., 2018, 2019). Whilst having caused lengthy harvesting restrictions, the Azaspiracids are far less common in occurrence in British waters than those compounds found in the Okadaic acid group. Although these toxins have acute impacts in humans (Grattan et al., 2016), their impact on shellfish has been less well explored. There is evidence of deleterious effects within bivalves, such as genotoxicity and DNA fragmentation (Prego-Faraldo et al., 2013; McCarthy et al., 2014) as well as suppression of immune function (Chi et al., 2016). It should be noted, however, that bivalves show a high level of resistance to the impacts of Okadaic acid, indicating that they have internal mechanisms for dealing with the negative effects of exposure (Prego-Faraldo et al., 2013; McCarthy et al., 2014).

The aim of the present study was to build on the CleanAtlantic Project, which developed a harmonized extraction method for mussels (Gerigny et al., 2023), by adding analysis of non-fluorescent litter items and integrating additional environmental stressors. The present study aims to progress beyond single pollutant monitoring towards a more comprehensive and

TABLE 1 The five groups of toxins monitored in the UK under specific legislation (EC 853/2004, 2004; EU 786/2013, 2013).

Syndrome	Parent toxin	Number of known analogues	Regulatory maximum permitted levels in classified shellfish, wet weight	Reference
Paralytic Shellfish Poisoning (PSP)	Saxitoxin	57	800 µg STX di-HCl eq.kg ⁻¹	Wiese et al., 2010
Amnesic Shellfish Poisoning (ASP)	Domoic Acid	9	20 mg kg ⁻¹	Saeed et al., 2017
Diarrhetic Shellfish Poisoning (DSP)	Okadaic Acid	11	160 µg OA eq.kg ⁻¹	Dominguez et al., 2010
Azaspiracid Shellfish Poisoning (AZP)	Azaspiracid	>60	160 µg AZA1 eq.kg ⁻¹	Krock et al., 2019; Twiner et al., 2008
Yessotoxins (YTX)	Yessotoxin	36	3.75 mg YTX eq.kg ⁻¹	Paz et al., 2008

systems-based approach that includes harmful algal bloom monitoring data to enable a multi-stressor assessment. This approach aims to better capture the combined and potentially detrimental effects of multiple contaminants on organisms.

2 Materials and methods

2.1 Sample collection

To maximize efficiency, existing monitoring networks were utilized for the collection of samples. As such, selection of sample locations was limited to those already established. The Food Standards Agency monitors harmful algal bloom levels utilizing monthly collections of mussels (*Mytilus edulis*) and other shellfish from around England and Wales (Food Standards Agency (FSA), 2025). Within the biotoxin official control program mussels are collected by Local Authority Environmental Health Officers (EHO). In the present study, surplus mussels were collected from seven stations (3052: Taw/Torridge, 3077: Morecambe Bay, 3083: Brixham, 3156: Lyme Bay, 3221: Menai Strait West, 3222: Menai Strait East, 3223: Blakeney) monitored within the biotoxin official control program (Figure 1) in November 2022 for microplastic

analysis. Coordinates of sample locations are available in the [Supplementary Material](#). Biotoxin analysis was completed at the Cefas laboratory in Weymouth, UK. As recommended by Bakir et al. (2020b), a minimum sample of 25 surplus individuals were sampled. Of the available locations, the seven selected were chosen to ensure enough material could be analyzed and to give the best spread around England and Wales. Mussels were frozen (-20°C) and stored at the Cefas laboratory in Lowestoft, UK until microplastic extraction.

2.2 Microplastic extraction and analysis

2.2.1 Chemicals

The chemicals used in microplastic extraction in the present study are listed in Table 2. All chemicals were prepared as per Gerigny et al. (2023) and Bakir et al. (2023). Details are available in the [Supplementary Materials](#).

2.2.2 Contamination control procedures

Samples were processed in a designated microplastic laboratory with an anteroom so that two doors separated the laboratory from the corridor. This reduced airflow to minimize airborne

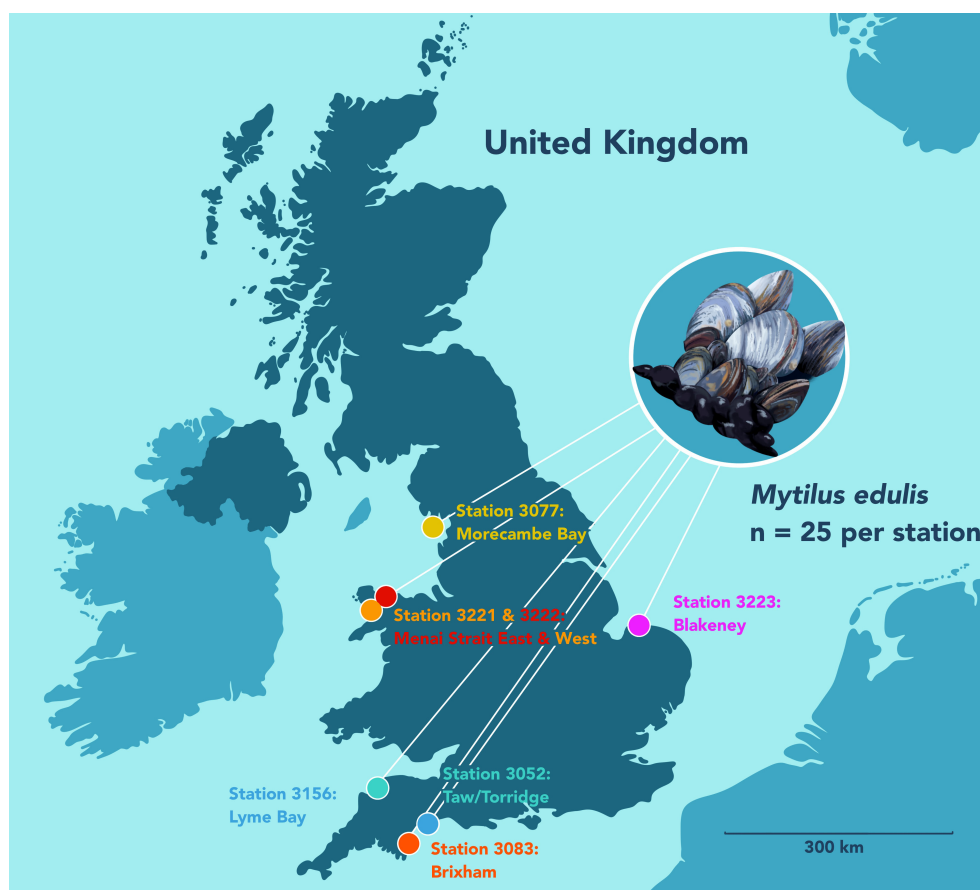


FIGURE 1
Sampling locations of mussels on the English and Welsh coastline.

TABLE 2 List of chemicals, manufacturers, and suppliers.

Chemicals	Molecular formula	Manufacturer/Supplier	Purity (%)
Potassium hydroxide	KOH	VWR/VWR	-
Sodium hypochlorite	NaClO	VWR/VWR	14% active chlorine
Ethanol	C ₂ H ₆ O	Acros organics/ThermoFisher scientific	95% purity
Nile Red	C ₂₀ H ₁₈ N ₂ O ₂	Acros organics/ThermoFisher scientific	99% purity

contamination into the laboratory, which was also restricted access for the same reason. In the anteroom, a sticky contamination mat collected contamination from the soles of shoes. Mats were changed at a minimum every two weeks to ensure they remained sticky. Cotton lab coats dyed purple (Dylon dye) for easy contamination recognition were put on in this space prior to entering the laboratory. Before work began, the floor and surfaces were cleaned with a vacuum cleaner to remove any dust. Surfaces were also wiped with damp cotton cloths to remove any microplastics that may have settled on them after vacuuming. All glassware and equipment were triple rinsed with reverse osmosis (RO) water. A RO dispenser was used to first rinse the surfaces of the glassware. Following this, the glassware was allowed to fill with RO, which was then poured out. This was repeated twice more and the glassware wrapped in RO rinsed foil. The plastic handle of the shucking knife was wrapped in RO rinsed aluminum foil to prevent any contamination during shucking. All reagents were rinsed through a regenerated cellulose filter (Whatman, ϕ 45 mm, 0.2 μ m pore size) prior to use and all work was conducted in a biological safety cabinet (Monmouth Guardian MSC T1200). Samples were kept covered whenever possible. In addition, laboratory controls (procedural blanks or negative controls) were collected. Three controls were collected for each station ($n=7$) for a total of 21 blanks. Controls were collected throughout sample processing to reflect potential changes in the laboratory environment during processing. A control beaker was opened at the start of the dissection and remained open until the mussel was sealed in a second beaker. The first control was collected during the dissection of one of the first three mussels. The second was collected at around mussel 12 and the third was collected during one of the last three mussels. Thereafter, lab controls were processed at the same time as the paired mussel.

2.2.3 Sample preparation

Mussels were defrosted and measured in their longest dimension. The tissue was removed from the shell with a blunt shucking knife. The byssal threads were removed and the tissue was then rinsed with filtered (0.2 μ m regenerated cellulose filter) RO water in a red wash bottle, as recommended by Kolandhasamy et al. (2018). For stations 3052 (Taw/Torridge) and 3083 (Brixham), the filtrate was reserved and examined to quantify microplastic removal during this stage. The filtrate was only analyzed for two stations as it

was not the main focus of the study. The data is available in the open access dataset (McGoran and Barry, 2025a). The wet weight mass of the tissue was recorded.

Each individual mussel was placed in a clean 100 mL glass beaker and covered with 40 mL of potassium hydroxide/sodium hypochlorite (15% KOH/2% chlorine) solution following the protocols of Gerigny et al. (2023) and Bakir et al. (2023). A glass Petri dish was used as a lid for the beaker to prevent atmospheric contamination. Beakers were placed in an ultrasonic bath (VWR USC200T) for 5 minutes and then transferred to a shaker incubator for 72 hours (VWR 980151UK, 40 °C, 120 rpm). After 72 hours, 40 mL of filtered degreaser (Elbow Grease, <https://elbowgreasecleans.com/>) was added to each sample. Beakers were returned to the incubator with the same settings for a further 24 hours. Following these steps, all tissue and fatty residues were removed and samples could be vacuum filtered over Whatman GF/D filters (45 mm ϕ , 2.75 μ m pore size) in a six-sample manifold with 300 mL glass filtration units. Filters were flushed with 100 mL RO water to remove chemicals and the sides of the funnels were rinsed using a red wash bottle. Nile red (0.01 g L⁻¹ in ethanol), stored in amber glass to prevent UV degradation, was added to each filter so that a small layer formed above visible material. The solution was left for 30 minutes and then filtered off. The filter was flushed and rinsed with reverse osmosis water for a second time. Filters were frozen (-20 °C) in glass petri dishes until further analysis could be completed.

Using a binocular microscope (Leica MZ10F), filters were examined under blue light (Fluo III Cool LED) followed by white light. A USB camera attachment was used to image and measure all suspected microplastic items (GXCAM-U3PRO-20, GX Capture-T). Items were then transferred to an Anodisc (VWR, ϕ 25 mm, 0.2 μ m pore size) and dried at 40 °C for 24 hours prior to FTIR analysis. A Lumos II μ FTIR (Bruker) was used to identify polymer type in a subset of picked items. Analysis utilized the MCT detector and ATR FTIR (32 scans in reflectance mode, 4000–500 cm⁻¹, 4 cm⁻¹). Identification was only accepted with a minimum match of 60% against the library spectra (ATR-FTIR-library complete, vol. 1-4; Bruker Optics ATR-Polymer library; IR-Spectra of Polymers, Diamond-ATR, Geranium-AT & IR-Spectra of Additives, Diamond-ATR) as recommended by Leistenschneider et al. (2021).

The results presented below have been corrected for contamination. For each station the mean contamination was calculated and removed from each sample where items (e.g., white filaments) were present in both controls and samples. Corrected and uncorrected data is available in the open access dataset (McGoran and Barry, 2025a).

2.3 Harmful algal biotoxin extraction and analysis

2.3.1 Reagents, chemicals and analytical equipment

The reagents which were used for the toxin extractions and High-Performance Liquid Chromatography (HPLC) analysis were

of HPLC grade or higher. Chemicals of Liquid Chromatography with Mass Spectrometry (LC-MS) grade were utilized for the preparation of reagents for the Lipophilic toxins, where mass spectrometry was used for analysis. The chemicals used were sourced either from Fisher Scientific (Loughborough, UK) or VWR (Lutterworth, UK). All solid phase extraction (SPE) processes were automated and performed using a Gilson (Dunstable, UK) ASPEC, running Trilution software. A full list of chemicals (>30) is not provided here.

2.3.2 Marine biotoxin extractions

For each sample, a minimum of 10 individual animals were shucked to yield a minimum of 50 g (wet weight) of flesh, these criteria are considered representative of the sample within the official control framework. This flesh was collected and allowed to drain before being homogenized. Once the sample was homogenous, the required quantity of shellfish homogenate was weighed into a 50 mL centrifuge tube, 2 ± 0.01 g each for ASP and LT analysis and 5 ± 0.1 g for PSP analysis. A solvent with a high affinity for the respective toxin was added to each. These were methanol for the lipophilic toxins, a 50:50 mix of methanol and water for the ASP toxins and weak (1%) acetic acid for the PSP toxins.

Lipophilic toxins (LTs), including Okadaic acid toxins (OAs), Azasparacids (AZAs) and Yessotoxins (YTXs) were extracted and analyzed using a refined version of the [Gerssen et al. \(2009\)](#) method. Specifically, 6 mL of methanol was added to the shellfish tissue, which was mixed thoroughly and separated via centrifugation (8 minutes at 3500 rpm). The supernatant was then collected, and the process was repeated two further times, with supernatants from the same sample combined after each centrifugation step, until 18 mL of supernatant was recovered. This was then topped up to 20 mL with methanol. At this stage 1 mL was filtered into an LCMS vial for analysis and a further 1 mL was aliquoted into a separate LCMS vial and underwent hydrolysis by the addition of 125 μ L of 2.5M sodium hydroxide and incubation at 76 °C for 40 minutes. This reaction was then neutralized by the addition of 125 μ L of 2.5 M hydrochloric acid. Both vials were then ready for analysis by LC-MS/MS. For the analysis of the OA, AZA and YTX toxins, LC-MS/MS analysis was utilized, it was performed using a Waters (Milford, MA, USA) Acquity I class UPLC coupled with either a Waters Xevo TQ or Xevo TQ-S triple quadrupole mass spectrometer as described in [Gerssen et al. \(2009\)](#).

The ASP extraction was performed using a refined version of [Quilliam et al. \(1995\)](#). Specifically, 18 mL of 50:50 methanol:water was added to the 2 ± 0.01 g of shellfish homogenate. This was thoroughly mixed before separation via centrifugation (10 minutes at 4500 rpm). Following this, 1 mL of the supernatant was then passed through a 0.45 μ m filter into an HPLC vial, ready for analysis. No selective cleanup or pre-concentration solid phase extraction steps were performed on any samples. For the analysis of ASP toxins, HPLC-DAD analysis was utilized, it was performed using an Agilent 1100/1200 series HPLC with ultraviolet detection as per [Quilliam et al. \(1995\)](#).

PSP analysis was undertaken using a refined [AOAC, 2005.06](#) pre-column oxidation method ([AOAC, 2005](#)) described by [Hatfield et al. \(2016\)](#). Specifically, the addition of 3 mL of 1% HAC to 5 ± 0.1 g of shellfish homogenate. This was then mixed, boiled at 100 °C in a water bath for 5 minutes, cooled for 5 minutes, mixed again and then separated by centrifugation (10 minutes at 4500 rpm). The supernatant was collected and a further 3 mL of 1% Acetic acid was added to the remaining shellfish pellet, this was subsequently mixed and separated again by centrifugation, with the supernatant being combined with that from the first step. This mixture was topped up to 10 mL with deionized water. Following this, 1 mL of the extract underwent automated solid phase extraction. The cleaned sample was then pH adjusted to between 6.0 and 7.0 using either sodium hydroxide or acetic acid. At this stage, 500 μ L of this adjusted sample was then added to an HPLC vial and derivitized by oxidation with 100 μ L of a periodate reagent (1:1:1 periodic acid: ammonium formate:sodium phosphate), in the presence of 100 μ L of matrix modifier (blank pacific oyster extract). After 1 minute this reaction was quenched with 5 μ L of glacial acetic acid. After resting on the bench this derivitized sample was ready for analysis. For the analysis of PSP toxins, HPLC with fluorescence detection (FLD) analysis was utilized; it was performed using an Agilent 1100/1200 series HPLC-FLD. No samples were fully quantified, instead samples were analysed using a semi-quantitative approach as described in [Hatfield et al. \(2016\)](#).

2.4 Statistical analysis

The statistical analysis mainly consisted of comparisons between stations. This approach was taken to determine whether there was evidence of potential accumulation zones on the British coast. This could potentially be linked to plastic inputs or hydrodynamics reducing plastic flushing. Three different comparisons were made. These were:

- i. Comparison of microplastic mean abundances between stations. These were done in two ways: per individual and per gram (wet weight). A non-parametric Wilcoxon procedure was used because Shapiro-Wilk tests suggested that both abundance measures were not normally distributed.
- ii. Whether the proportion of individuals (p) containing microplastics differed between stations. This was achieved by fitting a binomial generalized linear model. The full model was of the form ([Equation 1](#)):

$$\log(p/1-p) = a + s_j \quad (1)$$

where a is an intercept term and s_j represents the effect of the j th station ($j = 1, \dots, 7$). The reduced model was the same as (1) but without the station effect s_j . The statistical significance of stations was assessed with a likelihood ratio test between the full and reduced models ([Faraway, 2006](#)).

- iii. A comparison of the abundance of rayon polymers between stations. Because no replicate batches of samples were analyzed at each station, a standard binomial generalized linear model could not be used. Instead, to achieve this comparison, a randomization test was run (Manly and Navarro Alberto, 2021). Essentially testing whether the rayon proportion for each station is consistent with some overall probability of rayon under the null hypothesis that the proportion of rayon is the same at each station.

Formally, the test estimated the overall probability p (over all stations) that a fiber is rayon by the total number of rayon fibers divided by the total number of fibers. Assuming the null hypothesis of no significant difference between stations and assuming independence between fibers in a station, the number of rayon fibers N_j per station is distributed (Equation 2):

$$\text{binomial}(p, N_j) \quad j = 1, \dots, 7 \quad (2)$$

A summary statistic of the variation of rayon counts over stations is the variance. Thus, to perform the randomization procedure, we first calculated the variance of the observed counts over stations. Then the model in (3) was simulated 999 times and the variance of the counts on each simulation calculated. If there were differences between stations in the proportions of rayon, it would be expected that the variance of the observed counts would be larger than the variance of the simulated counts. The p -value for statistical significance was calculated as: $p\text{-value} = (g + 1)/1000$, where g is the number of simulated variances that are greater than or equal to the observed variance (Manly and Navarro Alberto, 2021).

In addition to comparisons between stations A standard linear regression model was used to determine the relationship between mussel length (mm) and microplastic load.

Plots were created with ggplot (Wickham, 2016), ggpubr (Kassambara, 2023) and ggbreak (Xu et al., 2021). The R script for all analysis is available in the [Supplementary Material S1](#).

No statistical analysis could be conducted on the harmful algal biotoxin data due to the large number of results below the limit of detection.

3 Results

Between 26 and 55 individuals were collected from each of seven locations. As described above, a random subsample of 25 individuals was analyzed from each location. Shell lengths of all collected mussels ranged between 25–75 mm. Individuals subsampled for microplastics analysis measured between 40–70 mm long (mean $\pm$ SD: 56.5 ± 5.9 mm). The wet weight of the tissue was between 1.24–12.89 g (4.3 ± 1.9 g).

Mean contamination was calculated from the three blanks per station and used for data correction. The overall mean contamination ($\pm$ SD) across all 21 blanks was 3.05 ± 2.22 items per sample. Corrected and uncorrected microplastic concentrations are reported in the [Supplementary Material S3](#), where contamination correction refers to the removal of the average contamination at each station. After correction, between 42% (STN 3052) and 67% (STN 3156) of mussels contained microplastics at each station. The binomial model (1) indicated there was no statistically significant difference between the proportion of individuals containing microplastics between stations ($p = 0.139$). The likelihood ratio test confirmed this conclusion ($p = 0.137$).

The Shapiro-Wilk identified the data was skewed (MP ind⁻¹, $p = 2.2 \times 10^{-16}$; MP g⁻¹, $p = 2.2 \times 10^{-16}$). As the data was positively skewed count data, a non-parametric Wilcoxon test was used to compare microplastic load between stations. The mean contamination per station ranged from 0.60 ± 0.71 (STN 3083) to 2.84 ± 7.31 (STN 3222) items per individual (95% CI = 0.88–1.79)

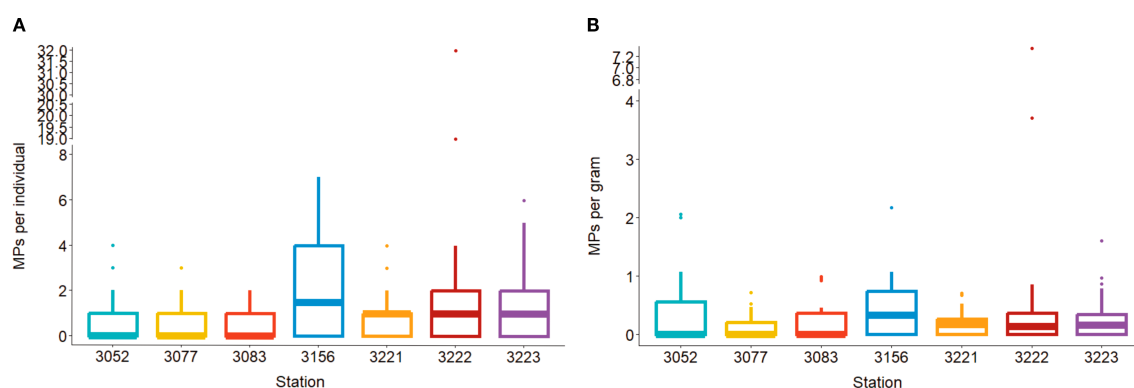


FIGURE 2

Microplastic abundance (corrected) in mussels from seven stations (3052: Taw/Torridge, 3077: Morecombe Bay, 3083: Brixham, 3156: Lyme Bay, 3221: Menai Strait – West, 3222: Menai Strait – East, 3223: Blakeney) around the coastline of England and Wales per individual (A) and per gram wet weight (B).

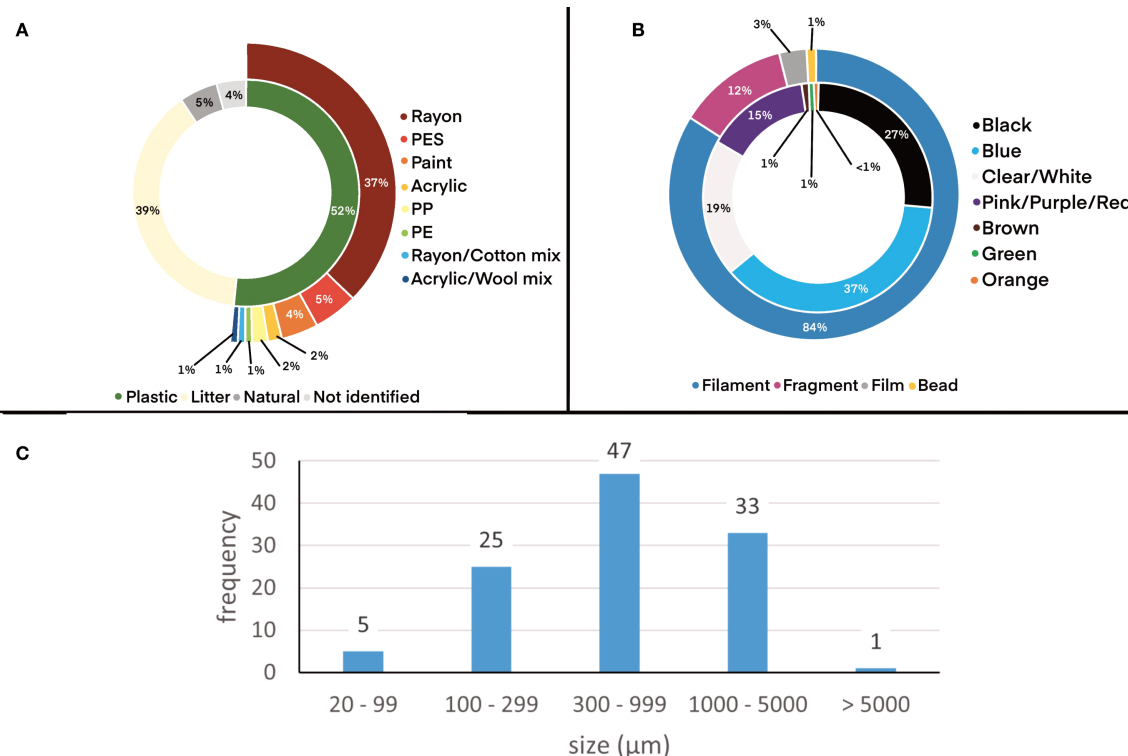


FIGURE 3

Descriptive analysis of microplastics present in mussels from the coast of England and Wales (combined across stations and corrected for contamination). (A) Polymer identification including plastics (rayon, polyester, paint, acrylic, polypropylene and polyethylene) and other litter (cotton and cellulose); (B) Microplastic shape (filament, fragment, film, bead) and color (black, blue, clear/white, pink/purple/red, brown, green, orange); (C) Length (longest dimension) of meso- and microplastics.

and 0.15 ± 0.23 (STN 3077) to 0.59 ± 1.56 (STN 3222) items gram^{-1} (wet weight) (95% CI = 0.23–0.44), but there was no statistically significant difference in microplastic abundance between stations for either microplastic load per gram or individual (all pairwise p -values were below 0.05; range: 0.13–0.99) (Figure 2).

Shell length of mussels and microplastic load (model 2) did not correlate ($MP = (0.05 \times \text{length}) - 1.01$, $p = 0.402$). The mean microplastic loads recorded in the present study are in agreement with global concentrations (Supplementary Material S4).

Plastic was the dominant material (52%) with cellulose the second most common litter item (28%). Of the plastics, rayon was most common (37%). Other materials present included polyester, paint, acrylic, polypropylene and polyethylene (Figure 3A). A randomized test was used to determine whether polymer abundance varied between stations. Rayon was selected as the polymer was present in all stations and the proportion of rayon in samples varied from 21% to 56%. The randomization test estimated the overall probability that a particle was rayon by the total number of rayon particle divided by the total number of particles. This was $p = 47/114 = 0.41$. When comparing rayon across the stations, the p -value from the randomization test was 0.66 (model 3, $p > 0.05$). Thus, there is no evidence of differences in the proportion of rayon particles between stations (Figure 4). Items were primarily blue or black filaments (84%) with some fragments,

films and microbeads present (Figure 3B). One mesoplastic item was recovered (Figure 3C) but was removed from analysis as the study focused on microplastics. The majority (42%) of items were between 300–999 μm long (mean $889 \pm 934 \mu\text{m}$).

In total, 119 shellfish samples were tested in 2022 for the presence of marine algal biotoxins from the same sites at which microplastic sampling occurred. Of these 10 were from Blakeney, 11 from Menai Strait – East, 27 from Lyme Bay, 23 from Brixham, 21 from Morecombe Bay and 27 from Taw/Torridge. Of these samples 118 were tested for LTs, 75 screened for PSP and 74 tested for ASP. None of the samples tested contained any PSP toxins above the limit of detection. None of the samples tested contained any ASP toxins above the limit of quantitation. None of the samples tested contained AZAs or YTXs above the LT reporting limit of $16 \mu\text{g AZA eq kg}^{-1}$ and $100 \mu\text{g YTX eq kg}^{-1}$ respectively. Results are summarized in the Supplementary Materials S5. OA group toxins were present at Taw/Torridge (48% $> 16 \mu\text{g OA eq kg}^{-1}$ with a maximum $60 \mu\text{g OA eq kg}^{-1}$), Morecombe Bay (62% $> 16 \mu\text{g OA eq kg}^{-1}$ with a maximum of $181 \mu\text{g OA eq kg}^{-1}$), Brixham (48% $> 16 \mu\text{g OA eq kg}^{-1}$ with a maximum of $131 \mu\text{g OA eq kg}^{-1}$) and Lyme Bay (37% $> 16 \mu\text{g OA eq kg}^{-1}$ with a maximum of $173 \mu\text{g OA eq kg}^{-1}$) (Figure 5). OA group toxins were present at variable times at each site from April 2022 in Brixham to October 2022 in Morecombe Bay. No OA group toxins $> 16 \mu\text{g OA eq kg}^{-1}$ were detected in

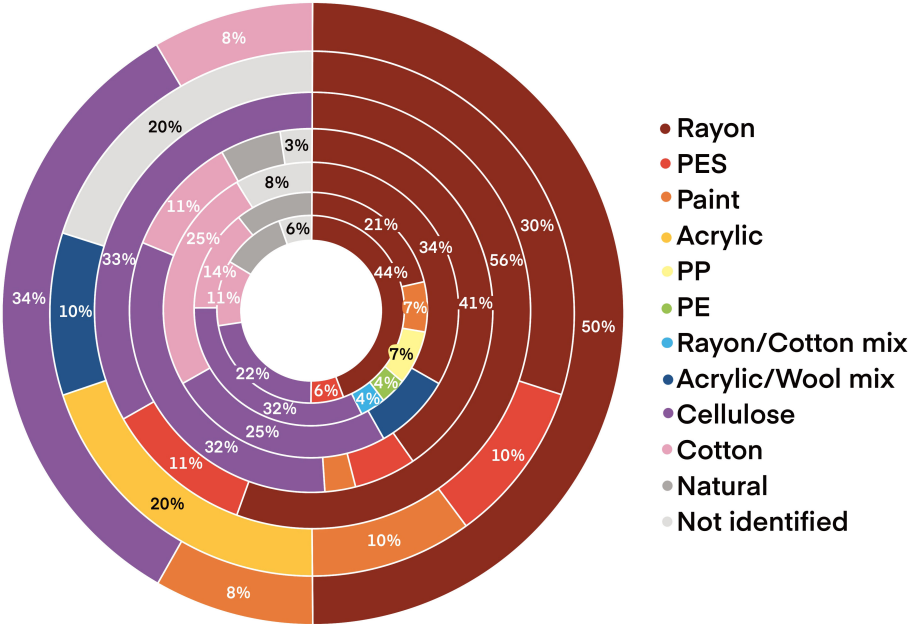


FIGURE 4
Polymer abundance of microplastic contamination in mussels between stations (outside to center: 3052: Taw/Torridge, 3077: Morecombe Bay, 3083: Brixham, 3156: Lyme Bay, 3221: Menai Strait - West, 3222: Menai Strait - East, 3223: Blakeney).

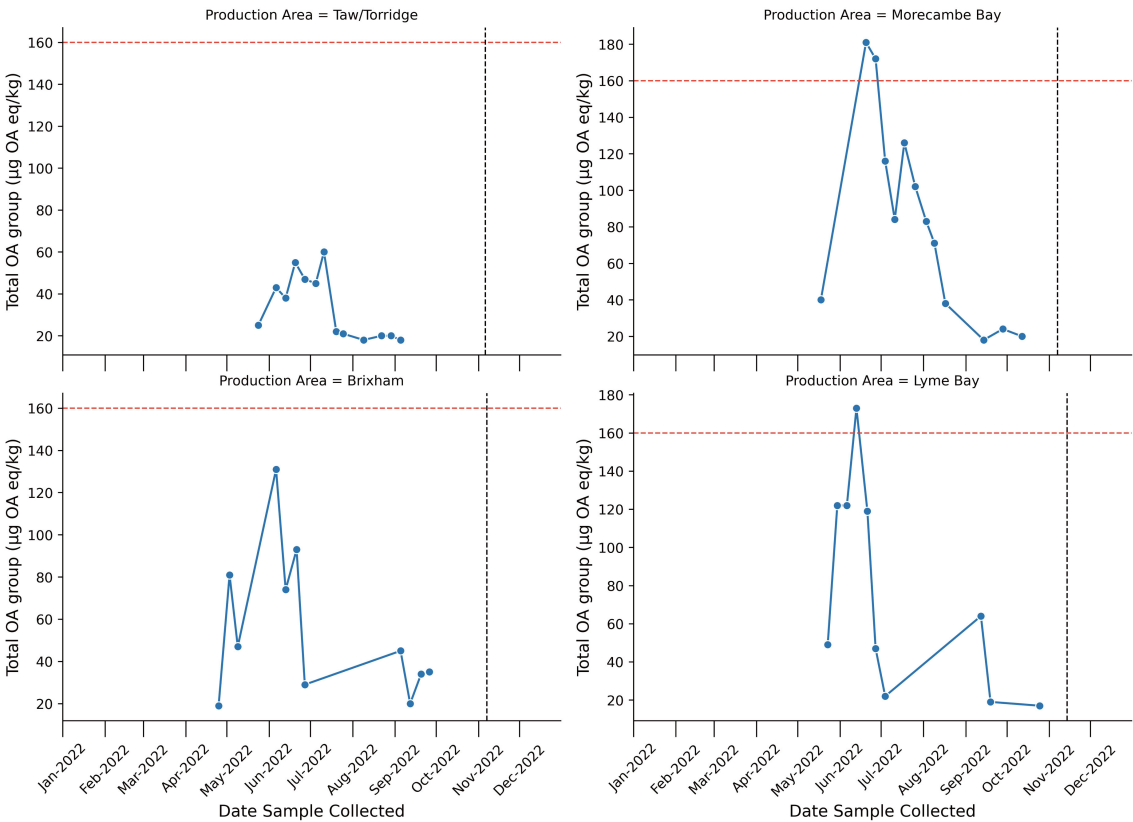


FIGURE 5
Line plot describing Total Okadaic acid toxins (OA) group concentrations across 2022 at each sampling location. Red horizontal hashed line represents the maximum permitted level of OA group toxins (160 µg OA eq/kg) in shellfish destined for human consumption. Black vertical line represents the sampling time of microplastics samples. Only points which exceeded 16 µg OA eq/kg are plotted.

Menai Strait – East or Blakeney in any sample. At time of sampling for microplastics, none of the sites contained any OA group toxins $>16 \mu\text{g OA eq kg}^{-1}$.

4 Discussion

4.1 Occurrence and abundance of microplastics and harmful algal blooms in UK mussels

Microplastics, including cellulosic items, were recovered from 53% of mussels with a mean load of 1.33 ± 3.04 items per individual (95% CI = 0.88–1.79) and 0.33 ± 0.71 items per g (wet weight) (95% CI = 0.23–0.44). Mussels from all seven stations were contaminated, most commonly with rayon, a semi-synthetic material categorized as microplastics by OSPAR (OSPAR MicroPlastics Expert Group (MPEG), 2024). No harmful algal biotoxins were found at quantifiable levels in the shellfish samples tested for microplastics. However, of the seven sites analyzed for microplastics within this study, four (3052: Taw/Torridge, 3077: Morecambe Bay, 3082: Brixham, 3156: Lyme Bay) showed presence of the DSP group toxins in shellfish within the preceding six months. For two of the sites assessed, the levels of the okadaic acid group toxins exceeded the regulatory threshold, whereas in the other two sites with quantifiable levels of DSP toxins, this food safety limit was not reached. The final two sites assessed showed no presence of DSP toxins within the year 2022. All other testing for marine biotoxins indicated that there were no detectable levels present within the study year. This means that those animals assessed for microplastics would have had a prior exposure to DSP toxins in four of the six samples analyzed, with higher levels previously present in shellfish from Morecambe Bay and Lyme Bay.

4.2 Risks from harmful algal blooms in the UK

The primary sources of algal biotoxins within shellfish growing in British waters are planktonic species of microalgae. Several species within varying genera of Dinoflagellates are responsible for PSP, DSP, AZP and YTX, with several species of the Diatom genus *Pseudonitzschia* being the producers of the toxins responsible for ASP. The blooms or cell abundances causing the contamination of mollusks can occur naturally within the spring and summer months, occasionally continuing into the Autumn. The frequency and scale of algal blooms can be exacerbated by anthropogenic impacts, with some factors such as nutrient-enriched coastal runoff having the potential to stimulate algal growth (Heisler et al., 2008) whilst also acting as an input for microplastic pollution. The most common impacts in British waters are those from *Dinophysis acuta* and *Dinophysis acuminata*, both known to be producers of DSP toxins (Bresnan et al., 2021; Dhanji-Rapkova et al., 2018). This leads to the shellfish growing in some areas being exposed to recurrent levels of DSP toxins; these will vary between years but can reach

high levels or prolonged exposures in some localities (Dhanji-Rapkova et al., 2018). As the okadaic acid group of compound has been shown to have potential negative impacts on bivalve molluscan health, albeit in limited studies (Prego-Faraldo et al., 2013; McCarthy et al., 2014; Chi et al., 2016; Lassudrie et al., 2020), this group of compounds could adversely affect the ability of bivalves to deal with the additional stressor of microplastic contamination. It is also known that several harmful algal species produce a wide range of bioactive extracellular compounds, which may also have negative effects on bivalve health, although these are often much less well described (Lassudrie et al., 2020). As such, the confirmed presence of okadaic acid producers at four of the sites assessed in this study represents an additional environmental stress factor impacting the study animals from those sites.

4.3 UK microplastics data in a regional and global context

The microplastic contamination reported in the present study is in line with most previous studies in the UK. But they are low compared to some studies in the country, which reported mean concentrations as high as 7.64 items per individual (Scott et al., 2019) and 12.6 items per individual (Catarino et al., 2017). The concentrations in the present study are also in line with estimates from Europe (De Witte et al., 2014; Van Cauwenberghe et al., 2015; Vandermeersch et al., 2015; Bråte et al., 2018; Phuong et al., 2018; Railo et al., 2018; Hermabessiere et al., 2019; Nalbone et al., 2021; Ferreira et al., 2023; Gerigny et al., 2023; Digka et al., 2024). This suggests that the high concentrations reported by Scott et al. (2019) and Catarino et al. (2017) may be outliers rather than indicative of accumulation zones of contamination. However, a greater number of sites would better capture regional differences.

Globally, all continents and regions find that most mussels consume less than one microplastic per individual or gram on average (Supplementary Material S4). On the American continents, mean contamination ranges from <1 – 8.7 particles per individual (Zhao et al., 2018; Klasios et al., 2021) or <1 –40 particles per gram (Zhao et al., 2018; Migliarini et al., 2025). Oceania is represented by relatively few published studies. Webb et al. (2019) reported that in New Zealand mean contamination per individual and per gram were both less than one. Most African studies originate from South Africa, reporting contamination of mussels at ca. 4 particles per individual (Sparks, 2020; Sparks et al., 2021) and between <1 – 2.3 particles per gram (Sparks, 2020; Ferguson et al., 2024). In Asia mussels were contaminated with <1 – 22.5 particles per individual (Dowarah et al., 2020; Do et al., 2024) or <1 – 9.2 particles per gram (Kolandhasamy et al., 2018; Naidu, 2019). Few studies are available for mussel contamination in the Middle East, with Bagheri et al. (2020) reporting concentrations of up to 19.8 particles per gram in Iran. Whilst some records of contamination are high, the majority are similar to the results of the present study.

Comparison between studies is hindered by a lack of standardized protocols and reporting (Li et al., 2021; Shumway et al., 2023). Best practice is to report microplastic abundance per

gram (wet weight) as well as per individual. Yet, several studies only report a single unit (e.g., MPs g⁻¹ Courtene-Jones et al., 2017; MPs individual⁻¹ McCoy et al., 2020). In the UK, only six studies have estimated microplastic abundance in mussels (S3), with a further two studies on clams (McCoy et al., 2020) and scallops (Akoueson et al., 2020). Some potential local accumulation zones have been identified (Li et al., 2018; Scott et al., 2019) but long-term data is needed to confirm whether spatial variation in abundance persists. According to Li et al. (2018), mussels from Plymouth contained more microplastics than those from Brighton (6.4 to 1.1 MPs individual⁻¹). Similarly high concentrations were reported at Whitsand Bay compared to Torquay Bay (mean 7.64 to 1.43 items individual⁻¹, Scott et al., 2019). With no statistical difference in abundance between stations and a mean abundance of between 0.60 and 2.84 items individual⁻¹, the present study identifies no accumulation zones relevant for the UK. Plymouth and Whitsand Bay are located near the mouth of the River Tamar, previously shown to be contaminated with plastic debris (Sadri and Thompson, 2014), for which more than 80% consisted of microplastics. It was, however, unclear whether the River Tamar acted as a net source or sink for plastic debris (Sadri and Thompson, 2014). Three stations in the present study are located in the southwest of the UK (3052: Taw/Torridge, 3083: Brixham, 3156: Lyme Bay). Mussels at these stations primarily contained cellulosic fibers, which is in line with the reports of Scott et al. (2019). For both Scott et al. and Sadri and Thompson (2014), polypropylene and polyethylene were the most abundant plastics. However, the present study recovered polyester and paint at the Southwest stations (3052: Taw/Torridge, 3083: Brixham, 3156: Lyme Bay). In the present study, only the station at Lyme Bay (3156) is located near a major river (River Exe). This station had the highest proportion of contaminated mussels and the second highest mean contamination per individual. High abundances of microplastics were previously reported for seafloor sediments collected from Lyme Bay and Off Tamar; both of these sites are considered as accumulation zones for microplastics (Bakir et al., 2023). Regular repeat monitoring is necessary to detect persistent accumulation zones. This is useful to identify whether river mouths are accumulation zones that may lead to a greater rate of microplastic ingestion due to the increased abundance of particles.

4.4 Addressing the need for a microplastics sentinel species and the requirements for success

In future years, microplastics are likely to become much more abundant through increased and varied use, as well as through fragmentation of large litter already in the environment. Inconsistent methods for sampling and processing, a lack of robust controls in some early studies and advances in particle detection hinder comparisons between studies. Plastic abundance in the ocean is expected to double by 2040 (Thompson et al., 2024). Litter already negatively impacts the marine environment, with calls for policy interventions to make drastic changes (Roman et al.,

2020) and evidence that the cost of inaction is greater than cost of implementing these changes (Watkins et al., 2017). As microplastic abundance increases, so does the likelihood of negative environmental impacts. Many ecotoxicological studies, especially early studies, utilized concentrations far higher than those observed in the environment. Whilst less useful for determining current risks from microplastic exposure, these studies demonstrate that eventually microplastic loads will be high enough to cause severe negative impacts (Foley et al., 2018). Indeed, microplastic toxicity is positively correlated to microplastic concentration (e.g., in oysters Teng et al., 2021). As particles continue to fragment, small microplastics and nanoplastics are able to translocate to organs other than the gills and digestive system (Cattaneo et al., 2023; Yang et al., 2020; Zeytin et al., 2020; Li et al., 2021; McIlwraith et al., 2021). This increases retention time (Ward and Shumway, 2004) and likelihood of negative impacts. At high concentrations, microplastics can move from the digestive gland to the circulatory system of mussels (Browne et al., 2008).

As suspension feeders, mussels are susceptible to the negative impacts from microplastic ingestion (Moore, 2008; Kühn and van Franeker, 2020), albeit at often high concentrations. Ingestion of microplastics has been linked to negative health impacts. For instance, reduced feeding rate in mussels (Pedersen et al., 2020; Hatzonikolakis et al., 2024), which reduced mussel growth. Furthermore, the gut microbiome biodiversity can decline in mussels that ingested polystyrene; with damage also observed in the gut tissues (Ferguson et al., 2022). Although, Collins et al. (2023) observed no impact on gut microbiome. The shape of the plastic is likely to impact the severity of the reaction, with fibers and filaments being more toxic than microbeads (waterflea *Ceriodaphnia dubia* Ziajahromi et al., 2017a; zebrafish *Danio rerio* Qiao et al., 2019; Rebelein et al., 2021). Mussels are potentially less selective when consuming filaments compared to beads (Ward et al., 2019) making them vulnerable to these negative effects. The abundance of cellulosic filaments, and associated dyes, in the environment has raised concerns over their health impact as well as plastic filaments (Remy et al., 2015; Mateos-Cárdenas et al., 2021). Walkinshaw et al. (2023) demonstrated that at exposures associated with heavily polluted environments (80 filaments L⁻¹) cotton and polyester filaments both reduced growth rate of juvenile mussels, with polyester reducing growth more than cotton.

At low exposures (0.1 g L⁻¹), 70% of ingested microplastics are excreted by mussels within 24 hours (Pedersen et al., 2020). Similarly, oysters exposed to 0.33 g L⁻¹ daily for 10 days retained less than 0.5% of particles; though more were retained when particles were biofouled (Fabra et al., 2021). In contrast, at higher concentrations (0.4–0.8 g L⁻¹) over 94% of microplastics were retained after 24 hours (Pedersen et al., 2020). Woods et al. (2018) noted that even at 3,000 filaments per L⁻¹, 71% of particles are ejected in pseudofaeces, but those ingested were retained after 72 hours. Increased retention time is necessary for the chemical to leach into the digestive tract and to increase the chance of negative impacts on the organism. Jang et al. (2021) demonstrated that expanded polystyrene could increase mussel exposure to plastic additives but noted that direct exposure to leachates posed a more

significant threat. Microplastics and their associated chemicals, such as adhered persistent organic pollutants (Bakir et al., 2014), are linked to negative health effects when ingested. The chemicals within plastic can be carcinogenic and endocrine disrupting (Zimmermann et al., 2019). Mussels exposed to polyethylene and polystyrene accumulated pyrene, a polyaromatic hydrocarbon (PAH). This resulted in an immunological response and a change in gene expression affecting apoptosis (Avio et al., 2015). More recent studies, however, suggest that microplastic exposure is not correlated to PAH uptake in environmental conditions (Klasios et al., 2021). Whilst physically possible to transfer chemicals from plastics upon ingestion, this is not the primary route of exposure in the marine environment. However, at environmental concentrations evidence of risk is limited. Some studies suggest a negligible toxicological impact (Koelmans et al., 2016; Daniel et al., 2024), including on mussels (Santana et al., 2018). Though with concentrations set to increase, harm is likely to intensify in coming years (Thompson et al., 2024). Thus, collecting baseline data through monitoring is essential to have an early warning system of harm before impacts become severe. Additionally, sub-lethal and population level effects are important to monitor, especially for commercially important species such as mussels (Beaumont et al., 2019).

Sessile sentinel species not only allow for the development of risk assessments but allow for static points to monitor pathways and sources of microplastics in parallel to environmental matrices. Microplastics in the marine environment can originate from various sources, including, but not limited to, landfill runoff, agricultural runoff (Hurley et al., 2018; Koutnik et al., 2021), road runoff (Sundt et al., 2014; Kole et al., 2017; Tamis et al., 2021; Worek

et al., 2022), riverine inputs (Meijek et al., 2021), wastewater (Ziajahromi et al., 2017b), abrasives, paint from vessels (Sundt et al., 2014), textile fibers shed during use, washing and drying (Napper and Thompson, 2016; Reed et al., 2018; O'Brien et al., 2020), and atmospheric fallout (Dris et al., 2016, 2017). Microplastics may be shed from apparatus used in aquaculture, with farmed mussels often more contaminated than wild mussels (Ding et al., 2022; Vandermeersch et al., 2015). The use of mussels as a sentinel species for microplastics could be used for monitoring at source or in regions of interest to capture changes in inputs and quantify mitigation strategy success. Caged mussels can be utilized to monitor specific inputs of interest, especially those associated with mitigation strategies and policy changes. These would allow governments to rapidly assess the effectiveness of the interventions. Additionally, caged mussels could be deployed on buoys to provide estimates of microplastic contamination offshore. Klasios et al. (2021) compared caged and resident mussels and found similar concentrations in both. Thus, this is a suitable strategy to extend the reach of monitoring programs.

Whilst there is an agreed need for a sentinel species, there is some disagreement around the requirements of sentinel species. According to Ward et al. (2019), who investigated limitations of mussels as a sentinel species, a microplastic indicator should “ingest, without bias, the majority of plastic particles”. However, monitoring with sentinel species may not always be designed to act as a proxy for other environmental matrices. Biota can provide insight into the bioavailable fraction of litter in the environment and can be used to evidence risk, or the lack thereof. Shumway et al. (2023) noted that future studies utilizing bivalves as a sentinel for microplastics need to address specific questions. The authors of the

TABLE 3 Pro-Con list for proposed sentinel species.

Suggested Sentinel	Bivalves	Benthic/Demersal Fish	Small Pelagic Fish	Decapods
Pros	- Wide geographical range - Not endangered - Already a sentinel species - Can ingest particles <1 mm - Filter large quantities of water and are important for water remediation, linking to risk to ecosystem services (e.g., carbon sequestration, food provision, water remediation and habitat provision; Van der Schatte Olivier et al., 2018) - Easy and cost-effective sampling - No seasonal variation in MP uptake (Rosa et al., 2024)	- Wide geographical range - Not endangered - Already a sentinel species - Can ingest all sizes of microplastics - Used in existing monitoring programs	- Wide geographical range - Not endangered - Can ingest all sizes of microplastics	- Wide geographical range - Not endangered - Can ingest all sizes of microplastics - Plastic accumulation can act as an early warning system
Cons	- Cannot ingest >1 mm - Populations oversampled - Selective feeding - Particle capture and ingestion highly dependent on size which will bias retention to microplastics >2-3 µm in size and bias ingestion to particles 2-400 µm in size (Rosa et al., 2018, 2024)	- Mobile - Stomach not consumed, reduced link to human health - Need access to a vessel to sample - Locations may vary seasonally - Seasonal variation in MP uptake (McGoran et al., 2025b)	- Highly migratory (Van der Kooij et al., 2024) - Stomach not consumed, reduced link to human health - Need access to a vessel to sample - Locations may vary seasonally	- Some decapods shred plastic, some form tangles - Mobile - Stomach not consumed, reduced link to human health - Accumulation of plastic for long periods may be less likely to show fluctuations in plastic levels - Need access to a vessel to sample - Locations may vary seasonally

present study agree with this sentiment and argue that the development of a sentinel species-based monitoring program should tackle policy needs, such as how microplastics in the environment link to risk.

The present study utilized an existing mollusk monitoring scheme to complete a multi-stressor analysis. However, other monitoring programs can be used and other sentinel species for microplastics should be explored. Table 3 explores the positives and negatives associated with other proposed groups for microplastic monitoring. Limitations of using suspension feeding bivalves include particle rejection and species-specific differences (Mladinich et al., 2022; Shumway et al., 2023). The latter, however, applies to any indicator that is not species specific, which is challenging on a global scale.

Owing to their selective ingestion and rejection of particles (Ward et al., 2019; Mladinich et al., 2022), mussels generally contain low numbers of particles (Shumway et al., 2023). Selection depends on physiochemical properties and size, with a higher proportion larger particles rejected (Ward et al., 2019; Mladinich et al., 2022). Ward et al. (2019) noted with tests using microbeads (19–1000 µm) only 10–30% of small microbeads were rejected but 98% of 1000 µm beads were rejected. The authors also noted that similar proportions of filaments were ingested regardless of size (75–1075 µm). Mladinich et al. (2022), however, observed a stricter size-based selection with more 65 µm filaments consumed than 500 µm filaments, and similarly more 500 µm filaments ingested compared to those 970 µm long. The study concluded that overall, more particles were ingested than rejected. Less selective suspension feeders have been recommended for use as microplastic sentinels (Mladinich et al., 2025).

At the OSPAR Working Group on Monitoring and on Trends and Effects of Substances in the Marine Environment (MIME), several countries have raised concerns over mussel population levels and the increased impact of using them as a sentinel species (Jon Barber, Cefas, Personal communication). Other mollusks have been suggested as alternatives (Ribeiro et al., 2024). Commercial species are useful to understand risks to human health and socio-economic factors but offer a challenge to collect as there is competition with commercial fisherfolk (Manuel Nicolaus, Cefas, Personal communications). As a result, commercial and non-commercial species have been proposed for monitoring. Alternative sentinel species for microplastic have been suggested, including seabirds, fish and crustaceans (Biamis et al., 2021; Truchet et al., 2022; Bruschi et al., 2023; Taurozzi and Scalici, 2024). Dab (*Limanda limanda*) were selected as a chemical contaminant sentinel species for the Clean Seas Environmental Monitoring Programme, a UK multi-agency program organized by the Clean, Safe Seas Evidence Group who report to the Department for Environment, Food and Rural Affairs (Defra) (National Oceanographic Centre, 2025). The species was chosen due to its close association with the sediment where chemical contaminants accumulate (Jon Barber, Cefas, Personal communication). At Cefas, fish are being explored as another potential sentinel species for microplastics, utilizing small pelagic fishes as an estimate of floating bioavailable microplastics and dab for the benthic habitat. *Nephrops norvegicus* have been

suggested as an indicator (Hara et al., 2020). Decapods could pose a useful indicator for risk as they can accumulate large quantities of microplastics (Murray and Cowie, 2011; Welden and Cowie, 2016a, b; Cau et al., 2019; McGoran et al., 2020). Whilst contamination is higher than that generally observed in fish, the group may act as an early warning system for wider environmental impacts. A combination of sentinel species may be needed to address all knowledge gaps. Indeed, Biamis et al. (2021) recommend multiple species with disparate ingestion levels to help determine risk, with a focus on high-trophic level species, such as seabirds, when considering human health risks.

A holistic approach to research and monitoring would allow a better understanding of microplastic present in all compartments and the pathway between them, improving our understanding of sources, and highlighting the area's most at risk and in need of further monitoring. Indeed, there are calls for a “One Health”, multidisciplinary approach to plastic monitoring (Biamis et al., 2021; Multisanti et al., 2022). But given Government budgets, this is not possible. Indicators should be selected to address major knowledge gaps, which include sources and transport of microplastics, and the risks associated with plastic in the environment. Mobile species, such as fish or decapod crustaceans, may be less helpful when addressing spatial patterns, identifying sources and hotspots. Certainly, small pelagic fish are highly migratory, with anchovies migrating from the North Sea and Biscay to overwinter in the English Channel (Van der Kooij et al., 2024). However, some species such as sprat have a far smaller range (Jeroen van der Kooij, Cefas, Personal communications). But sized-based particle selection in mussels may result in less plastic being consumed and inhibiting conclusions around risk. They can, however, be collected along a transect or stored in cages to better understand microplastic sources. Shumway et al. (2023) noted that bivalves contain very low concentrations of microplastics and Mladinich et al. (2023) observed that the polymers present in bivalves did not align with those in water, marine snow and sediment. However, Ferguson et al. (2024) found that suspension feeding mussels ingested a greater diversity of microplastics than grazing and scavenging invertebrates, which suggests they may be the least selective of researched invertebrates.

4.5 Future recommendations

Whilst isolated risk from plastic exposure might be minimal, in the environment mussels and other biota are exposed to multiple stressors and pollutants simultaneously. Indeed, as plastics fragment in the environment the release of additives and impurities increases, creating a “toxicity debt” for the large items presently in the environment (Rillig et al., 2021). Certainly, mussels have been recovered containing trace metals and microplastics (Alomar et al., 2025). When exposed to microplastics in the environment, plastic additives and hydrophobic organic compounds persist in the tissue of mussels and cockles (Hermabessiere et al., 2019). Additionally, exposure to pesticides in combination with microplastics leads to cumulative negative

effects compared to single exposure (Shi et al., 2024). In laboratory conditions, oysters have been found to exhibit no physiological responses to virgin microbead ingestion, but oxygen consumption and respiration rate increased, likely due to a triggered immune response, when exposed to microplastics coated in *Escherichia coli* bacteria (Fabra et al., 2021). It should be noted that Fabra et al. (2021) used a high dose not environmentally relevant for their study.

Harmful algal bloom monitoring was selected for the present study as a cost-effective way of collecting samples for microplastics, piggybacking off an existing program. The presence of harmful algal blooms has increased significantly worldwide in recent decades (Do Prado Leite et al., 2022). This increase has been recently attributed to the increase in monitoring efforts around the world, but with some regions including Europe, appearing to see an increase in harmful algal bloom impacts (Hallegraeff et al., 2021). In the coastal waters of Great Britain, the lipophilic toxin groups and specifically those compounds responsible for Diarrhetic Shellfish Poisoning are the most prevalent in shellfish, annually (Bresnan et al., 2021). Plastics can be colonized by several species of microalgae and can act as a vector for harmful algal species and can increase exposure to benthic pathogens or their toxic excretions which are limited by the amount of substrate available for colonization (Do Prado Leite et al., 2022). Indeed, polyethylene has been shown to accumulate neurotoxins from dinoflagellate *Karenia brevis* on its surface (Shea et al., 2006). Additionally, plastic covered in biofilm can be up to ten times more likely to be ingested by suspension feeders (e.g., oysters) than virgin plastic (Fabra et al., 2021). Plastic can make these benthic threats available on suspended microplastics, increasing their bioavailability. UK waters are, however, predicted to be at less risk of this transport of harmful bacteria or algae than other regions including the Mediterranean Sea and the coasts of North America and East Asia (Do Prado Leite et al., 2022). Ingesting microplastics makes mussels more susceptible to toxic algae (Yuan et al., 2024). In addition to oxidative stress, the mussels experienced aggravated hemocyte apoptosis, reduced hemocyte viability and a reduction in cellular energy. As a result, these mussels experienced more intense deleterious effects. Susceptibility to toxic algae could lead to an outbreak of infection disease or a mass fatality in mussels (Zannella et al., 2017; Lassudrie et al., 2020) and is a potential human health risk. Thus, multi-stressor monitoring is vital to understand and mitigate risk. Thus, it is vital that pollutants are not monitored and assessed in isolation, with sentinel species selected for suitability in multi-stressor risk analysis.

Beyond neurotoxins, to truly assess risk in an environmental context, more cross-field studies are required. Collaboration between microplastic researchers, ecotoxicologists, chemists, climate scientists and more is lacking and vital for establishing risk thresholds and to make the best use of monitoring data. Threshold values are mandatory within the EU (Werner et al., 2020; Van Loon et al., 2020) for marine litter monitoring and enable monitoring programs to determine a set of measurable characteristics for good environmental status (GES) and allow governments to assess whether policy is progressing towards this

achievement. Whilst independent of the EU, the UK Marine Strategy outlines commitments to achieve GES across the four devolved governments. Additionally, it is key for monitoring to be harmonized regionally and globally where possible. The risks associated with microplastics, especially in combination with other stressors, highlighted above demonstrate the need for monitoring. Whilst some OSPAR indicators cover large microplastics (>1 mm), the abundance of smaller items justifies a designated sentinel species. In the present study, microplastic concentrations were unknown and, as such, sample sites were selected to provide a wide geographical spread. Coincidentally, these sites had a low frequency of algal blooms. Future studies should amend site selection to allow comparison between sites more frequently or intensely affected by harmful algal blooms whilst maintaining a broad spread around the coast of England and Wales, which are included in the survey. A similar strategy should be considered by other microplastic monitoring schemes globally.

With the above discussions in mind, mussels could be employed as a sentinel species for microplastics and multi-stressor assessments. Given the uncertainty surrounding microplastic associated risk, an indicator already established for chemical pollutant and harmful algal bloom monitoring is the logical choice. Mussels can be used to indicate potential risk to the ecosystem, potential transfer to humans from contaminated seafood and as a proxy for water contamination. The latter is especially significant given the temporal and spatial variability of microplastics in surface water and the lack of defined sampling volumes to ensure samples are representative of the environment (Danopoulos et al., 2023). Mussels are widely distributed and as suspension feeders are highly susceptible to microplastic ingestion. Of all bivalves, mussels are the ideal group for use as an indicator. There is a clear distinction in contamination levels between bivalves with clams containing on average more microplastics than mussels, scallops and oysters (Danopoulos et al., 2020; Ding et al., 2022). Greenwood et al. (2025) noted that, in these review studies, contamination in mussels was closest to the mean concentration across all bivalve species, making them the most suitable representative. In addition, their long-standing as an indicator for other hazards (Beyer et al., 2017; Afbi, 2023; Environment Agency, 2023; Leung et al., 2024) makes harvesting easier as new monitoring programs do not need to be developed from scratch. It also enables multi-stressor risk assessments to understand the cumulative effects of anthropogenic factors on biota. Additionally, as sessile organisms, mussels provide an estimate of local contamination unlike mobile organisms which could cover a large range. It has been suggested that, to improve the distribution of monitoring locations, caged mussels be utilized (Digka et al., 2024; Kazour and Amara, 2024; Weir et al., 2024). This powerful tool could be used to fill knowledge gaps, such as addressing riverine inputs to the marine environment by placing caged mussels along a transect (Greenwood et al., 2025).

4.6 Validation of method and limitations

In this study, the Nile red screening method for microplastics was applied for a fast and cost-effective assessment of the

occurrence of microplastics in biota. Nile red has been previously applied for the detection and quantification of microplastics in biota (Catarino et al., 2018; Bakir et al., 2020a, 2020b; Nalbone et al., 2021; Shruti et al., 2022). The presence of false positives has been previously identified as a source of error when applying the Nile red screening method (Maes et al., 2017). The introduction of an optimized chemical digestion step (i.e., a mixture of KOH and NaClO) did limit the fluorescence of biological and natural items (Bakir et al., 2023). Previous work carried out by Wang et al. (2021) also reported a lower fluorescence intensity of biogenic materials following a digestion step using hydrogen peroxide. Thus, reducing these false positives. Additionally, the Nile red dye is not effective on all polymers at all wavelengths (Wang et al., 2021) and fails to detect semi-synthetic materials, such as rayon. In the present study rayon was the most common material (37% of items), but when non-fluorescent items are excluded, only 17% of items are rayon (Gerigny et al., 2023). It can also fail to affect materials already containing dark dye. For this reason, a search under white light was also included. This prevented an underestimation of microplastics in the samples.

There is a lack of standardized methods for microplastic analysis. To combat this challenge, Gerigny et al. (2023) quantified microplastic abundance in locations from three countries (UK, France, Spain) in the North Atlantic using harmonized techniques between laboratories (KOH digestion and Nile red staining followed by single particle analysis using μ -FTIR). The assessment found similar levels of contamination across all sites and between the three countries, with some evidence of potential spatial accumulation zones for microplastics. The reported concentrations were, however, relatively low in a global context, possibly due to the exclusion of non-fluorescent items. The present study is an extension of the UK data presented in Gerigny et al. (2023) with the addition of non-fluorescent items otherwise missed by Nile red analysis. Our work demonstrates the importance of including these non-fluorescent items, as concentrations increased from 0.00–0.68 to 0.60–2.84 (mean) items individual⁻¹. The present study and Gerigny et al. (2023) utilized strong contamination controls including rinsing tissue prior to digestion, as recommended by Kolandhasamy et al. (2018). Many studies globally do not record whether mussels were washed prior to microplastic extraction to remove external adhered particles (Greenwood et al., 2025). As a result, the low values presented in the present study may be a more accurate representation of environmental loading in mussels.

Translocation of microplastics is considered one of the major risks of microplastic ingestion but primarily occurs with particles smaller than 10 μ m (Li et al., 2021). This is currently below the detection limit of commonly available analytical tools, such as micro FTIR and LDIR. It is hoped that as technology advances, identifying these smaller microplastics will become more accurate and reliable. Currently monitoring is limited to a minimum size of 20 μ m, but reporting is only mandatory down to 100 μ m (OSPAR MicroPlastics Expert Group (MPEG), 2024).

The present study is a proof of concept for multi-stressor assessments. Whilst harmful algae toxins are monitored throughout the year at frequencies between monthly and weekly dependent on local risk, microplastic analysis could only be conducted as a one off. Thus, further spatial and temporal analysis could not be conducted. A wider range of sites and data collected over several months or years would allow for more powerful statistical analysis. Additionally, further analysis of samples would allow for the selection of sites known to be high in neurotoxins as a comparison to the sites in the present study which exhibited concentrations below the limit of detection.

For neurotoxin analysis, mussels were homogenized, as is the standard approach. This meant it was not possible to compare per individual contamination of microplastics and neurotoxins, only per gram. This highlights the importance of reporting both units for microplastics analysis.

5 Conclusion

Sentinel species are vital to assessing the quality of the environment and how it changes over time. This directly relates to human health and socioeconomic factors, with sentinel species acting as an early warning sign for risks to consumers and environmental hazards. They should be selected based on their abundance and distribution but also their ecology and how they might feed into our ecotoxicological understanding. It is essential to understand how the animal feeds and may selectively accept or reject microplastics when interpreting the data. There is a knowledge gap between observed environmental microplastic contamination of biota and ecotoxicological studies that needs to be filled to ensure that effective risk assessments can be conducted. The authors recommend that a sentinel species for microplastic be developed in conjunction with multi-stressor evaluations, which are essential for understanding real world complexities. These evaluations can then further be developed into effective risk assessments for risk management and avoidance, an area that can be improved upon in the field of marine litter. We suggest that: 1) as microplastic abundance in biota varies, investigation is needed to inform the frequency of sample collection. This could be done at a few sites of interest before implementing a wider monitoring scheme; 2) environmental scientists, ecologists, ecotoxicologists and chemists collaborate on a systems approach to better inform risk management of microplastics and allowing for the development of multi-stressor monitoring. This should compare sites of low and high stress.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://doi.org/10.14466/CefasDataHub.174>.

Author contributions

AM: Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing - original draft, Writing - review & editing. SP: Formal Analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. AL: Writing - original draft, Writing - review & editing. AB: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Writing - original draft, Writing - review & editing. JB: Formal Analysis, Writing - original draft, Writing - review & editing. KD: Writing - original draft, Writing - review & editing. JR: Conceptualization, Funding acquisition, Writing - original draft, Writing - review & editing.

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Supplementary material

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