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Abstract

The effectiveness of mussel caging for active microplastics (MPs) biomonitoring was investigated for the first time by comparing abundance and characteristics (shape, size, color and type of polymers) of MPs ingested by caged depurated blue mussels with those ingested by native mussels collected at the same sites and with those found in their surrounding environment (surface water and sediments). Mussels were exposed along a pollution gradient originating from a wastewater treatment plant discharge and near an abandoned coastal landfill. After 6 weeks of deployment, the majority (93%) of clean transplanted mussels had ingested MPs with a mean number of items ranging from 0.61 to 1.67 items/g. The occurrence, abundance and properties of MPs ingested by caged mussels were similar to those found in native mussels. Among the debris items detected in caged and native mussels, fragments were the most predominant type, consistent with the MPs found in their surrounding environment. MPs sizes were very similar whether in the water, sediments and both caged and native mussels, with a dominance of items < 150 µm. Although some polymers were under-represented or totally absent in the caged mussels compared to overlying seawater or surrounding sediment, there was a good overlap in polymer types proportion being found between caged mussels and sediments (Morisita's index of similarity = 0.93) or seawater (0.86). Polystyrene dominated all samples in all the different matrices. Our study suggests that blue mussels caging may be a promising tool for MPs biomonitoring making monitoring more reliable with an accurate assessment of the biological effects of MPs over a predetermined exposure period. However, further methodological improvements should be considered to define a uniform protocol for blue mussels caging to allow spatial and temporal microplastics active biomonitoring.

Keywords: Microplastics; Biomonitoring; Caging; Blue mussels; micro-Raman

Introduction

Filter-feeding bivalves such as mussels of the genus *Mytilus* have been widely used as sentinel organisms to monitor coastal water pollution (Beyer et al., 2017; Marigómez et al., 2013; Turja et al., 2014). The popularity of mussels as bioindicator stems from their biological and ecological characteristics which make them virtually ideal for pollution monitoring. These sedentary organisms filter large amounts of water efficiently and use waterborne particles as food and, thus, are able to bioaccumulate high amounts of different contaminants (Beyer et al., 2017; Farrington et al., 2016). Their broad geographical distribution, abundance and ability to tolerate a wide range of environmental parameters make them species of choice in large-scale and long-term marine monitoring programs (Andral et al., 2004; Scott et al., 2019; Turja et al., 2014). Most often mussel biomonitoring studies involve collection of samples from natural populations, but the adoption of an active biomonitoring alternative by using transplanted mussel (caging) has gained considerable popularity in ecotoxicology research and monitoring (Andral et al., 2004; Brooks et al., 2018; Galgani et al., 2011; Lacroix et al., 2015; Marigómez et al., 2013; Tsangaris et al., 2011). Mussel caging is particularly useful when indigenous mussels are scarce or absent at the planned study sites (Li et al., 2019). In addition, using caged mussels from a single population minimizes confounding factors such as the genetic variability and the physiological, age or reproductive status of the organisms that influence both contaminant bioaccumulation (Schøyen et al., 2017; Viarengo et al., 2007). However, several studies have previously reported differences in response between caged and native organisms exposed to environmental pollution. Observed differences were attributed to several factors: absence of cumulative long-term effects of pollutants in caged organisms (Nigro et al., 2006), the existence of adaptive traits or compensatory mechanisms in native mussels chronically exposed to pollution, leading for example to lower bioaccumulation levels than in short-term caged organisms (Greenfield et al., 2014; Lacroix et al., 2015; Marigómez et al., 2013; Schøyen et al., 2017). Others studies found that native mussels accumulated higher concentrations of chemicals (Bolognesi et al., 2004; Piccardo et al., 2001). In some studies, the two monitoring approaches showed similar temporal and spatial changes in chemical contaminant concentrations (Hunt and Slone, 2010; Piccardo et al., 2001). The caging influence on mussels contaminants accumulation is still poorly understood (Schøyen et al.,

2017). Many studies have suggested that an integrated use of monitoring data from both native and transplanted mussels may provide a more accurate assessment of environmental pollution (Bebianno et al., 2007; Brooks et al., 2012; Hunt and Slone, 2010; Marigómez et al., 2013; Serafim et al., 2011).

Microscopic particles and fragments of plastics smaller than 5 mm (commonly called microplastics, MPs) represent a matter of growing concern for the marine environment. Their ingestion has been documented in several marine species worldwide (Cole et al., 2013; Watts et al., 2015) particularly in coastal marine environments acting as recipients for plastics and MPs input sources (Leslie et al., 2017; Kazour et al., 2019). The occurrence and sources of MPs in the marine environment and their potential biological impact are subjects of major scientific and public concern. To monitor spatial and temporal trends of MPs pollution and to understand the risk they pose to the health of marine species, it is important to obtain accurate measurements of MPs abundance in environmental compartments (such as water, sediments and biota). However, in highly variable environment as marine coastal areas, the assessment of MPs abundance in water and sediments is often imprecise and tend to be affected by a variety of environmental factors such as winds, tides, currents and bioturbation (Scott et al., 2019). A key challenge for marine MPs biomonitoring is to develop an appropriate and standardized method with a sentinel organism that can be applied over large spatial and temporal scales to allow cross calibration between studies worldwide. Recently, many studies have focused on the uptake of MPs by mussels, both in laboratory experiments (Browne et al., 2008; Van Cauwenberghe et al., 2015) and in their natural habitats (De Witte et al., 2014; Li et al., 2018; Phuong et al., 2018; Qu et al., 2018; Santana et al., 2016; Zhao et al., 2018). The species faces a strong exposure to microplastics because of its high filtering activity (Riisgård et al., 2014). Microplastic abundance in field collected mussels is closely related to human activity (Kazour et al., 2019; Li et al., 2016), and there is evidence for a positive and quantitative correlation between MPs in mussels and surrounding waters (Qu et al., 2018). Their capacity to integrate and amplify the MPs contamination signal (Karlsson et al., 2017) led to their potential consideration as bioindicator for coastal MPs pollution (Beyer et al., 2017; Li et al., 2019; Wesch et al., 2016)**.** The International Council for the Exploration of the Sea (ICES) has suggested blue mussels as suitable sentinels for monitoring of MPs contamination (OSPAR, 2015; Vandermeersch et al., 2015). Although *Mytilus* spp. controlled deployment has been largely used to study diffuse chemical contaminants and biomarkers, this approach was only recently used to study MPs pollution. To our knowledge, only three

studies caged blue mussels in specific areas to investigate the MPs pollution related to specific anthropogenic activity such as wastewater discharge (Railo et al., 2018), wreck removal (Avio et al., 2017) or to assess seasonal changes in plastic pollution (Catarino et al., 2018). This latter study suggested that the use of cage deployed mussels can be an effective method to quantify and assess MPs pollution in the field whereas the study of Avio et al. (2017) highlighted the limited capability of transplanted mussels to discriminate MPs pollution around the wreck area.

Mussels are hardy creatures tolerant to handling that are easy to keep in culture, making them suitable for translocation and caging exposure. In addition, the mussels can be depurated in the laboratory before caging and, thus, allow the use of reference individuals to be deployed in different areas. The objective of this research was to investigate the MPs ingestion in caged blue mussels exposed along a pollution gradient originating from a WWTP discharge and near an abandoned coastal landfill (Kazour et al., 2019). The effectiveness of the mussel caging approach to quantify and assess MPs pollution in the field was assessed for the first time by comparing abundance and characteristics (shape, size, color and type of polymers) of MPs ingested by caged mussels with those ingested by native mussels collected at the same site and MPs found in their surrounding environment (surface water and sediments).

Material and methods

2.1 Mussels field deployment and sampling

a. Mussels depuration

One week prior to field deployment, blue mussels (*Mytilus edulis*) were collected from farmed Bouchot mussels from Camiers near Boulogne-sur-mer, (50°35'43.1"N, 1°34'41.3"E; French Eastern English Channel coast). Mussels were sorted in the laboratory to get 157 individuals of the same size range (5.77 ± 0.5 cm shell length, mean ± standard deviation). Seven of these mussels were immediately frozen at -20°C (reference samples collected prior to the start of the depuration experiment) and the remaining 150 individuals were pre-cleaned to remove any adhering organisms and placed in a 100 L glass aquarium (1 m x 0.45 m x 0.3 m) meticulously pre-rinsed with Milli-Q water. The aquarium was maintained in a thermoregulated room with a constant temperature of 8 ± 1°C and a 12 h light-dark illumination regime. The aquarium was equipped with an air pump and was supplied by filtered sea water (filtered on Polycarbonate filters; 0.1 µm) and the water was changed every

24 hours for 7 consecutive days. During the experiment, the mussels were daily fed (except on Saturday and Sunday) with live microalgae, *Rhodomonas* sp. and *Isochrysis* sp. in order to enhance the mussels' filtration capacity. After 7 days of depuration, seven mussels were frozen at -20°C in order to compare the MPs concentration in both non-depurated and depurated mussels. The rest of the mussels were used for the caging experiment.

b. Transplantation and caging experiment

After the depuration period, mussels were transported to caging sites in a small aquarium (60 L, 0.6 m x 0.35 m x 0.3 m) supplied by oxygenated filtered sea water. The translocation experiment was carried out between the 3rd and the 4th of February 2018 and lasted 42 days (until the 4th and 5th of April 2018). Five marine coastal sites were chosen for the caging experiment: four sites in Le Havre harbor along a pollution gradient originating from a WWTP discharge and one site near an abandoned coastal landfill (Figure 1; see Kazour et al., 2019 for more detailed sampling locations). At each site, a stainless-steel cage (50 cm x 50 cm x 50 cm, length x width x height) with no plastic materials to avoid contamination was deployed. The cage's mesh size was 15 mm allowing water circulation and stopping mussels from falling out of the cages. A stainless-steel grid was placed in the mid-height of the cages as a suitable substrate allowing mussels' attachment and avoiding direct contact with sediments. 17 to 23 mussels were put in each cage. The cages were deployed during low tide in the lower intertidal shore zone and attached to the bottom using four reinforced iron rods. The study area is characterized by semi-diurnal tide with an average tidal range of about 1 m at neap tides and 6 m at spring tides.

After retrieval, each mussel was put separately inside an aluminum foil and frozen at -20°C until analysis. Water temperature, salinity and dissolved oxygen were measured using a multiparameter probe (Mutiparameter HI 982, HANNA instruments) during cages transplantation and retrieval.

2.2 Water, sediment and native mussels sampling

Water, sediments and native mussels were taken on the 3rd and 4th of April as described in Kazour et al. (2019). In short, at each site, 1 to 2 m^3 of the first 10 cm of the water column was pumped and filtered on stainless steel sieves of different mesh sizes (500 µm, 200 µm, 80 µm and 20 µm). Infralittoral sediments were also collected at each site except for site 4 (due

to its rocky substrate). Sea water and sediments analyses were preformed according to Kazour et al. (2019).

Mussels from the French Eastern English Channel coast are dominated by *Mytilus edulis*, but some areas are also populated by hybrids between *M. galloprovincialis and M. edulis.* This is the case of Le Havre Harbor where 70% of the mussels are hybrids (J. Couteau, personal communication). Therefore, for native collected mussels the term *Mytilus* spp. is used in this study. Native mussels (*Mytilus* spp.) were hand collected from the lower intertidal shore zone from the same sites near the deployed cages. At each site, 10 mussels of similar size were collected and immediately conserved inside an aluminum foil and frozen at -20°C until analysis. In the Harbor (sites 1 to 4), mussels were bigger 6.44 ± 0.73 cm (mean shell length ± standard deviation) than at site 5 (3.2 ± 0.4 cm).

2.3 Microplastics analyses

a. Contamination control

Microplastics contamination control is an important step during microplastics analysis. Several precautions were followed. Cotton lab coats were worn throughout the experiments, and all steps (filtration, measurements and digestion) were realized under a laminar flow hood. Only laboratory materials (bottles, petri dishes, Erlenmeyers, filtration system) made of glass were used. All used solutions were filtered three times on glass fibers filters (GF/A) Whatman, (France) in order to remove any unwanted particles and fibers. All surfaces and equipment were cleaned using filtered ethanol 70% and MilliQ water. With every manipulation, a control was made following the same conditions as the samples in order to track the contamination.

b. Samples preparation

Water and sediment samples were prepared as described by Kazour et al. (2019). In short, water samples were rinsed inside a separation funnel and underwent a density separation step using zinc chloride ($ZnCl_2$; 1.8 g/cm^3) and the supernatant was filtered on GF/A filters. Sediment samples were digested using hydrogen peroxide 30% (H_2O_2 30%) then they underwent density separation using $ZnCl_2$.

Mussels were defrosted 4 hours prior to digestion and their total length, width (cm), total and net weight (g) were measured. The condition index (CI) was calculated as follow (AFNOR, 1985):

$$CI = \left({Net\ Weight\ (g)}/{Total\ weight\ (g)}\right) \times 100$$

This index is a good indicator for the physiological condition (tissue growth, energy reserves) of mussels (Orban et al., 2002).

Mussels were digested using a solution of potassium hydroxide (KOH) 10% (m/v, ChimiePlus, France) (Dehaut et al., 2016). Caged mussels were thawed, dissected and put inside graduated Erlenmeyers equipped with glass stopper containing a volume of 150 mL of KOH 10% (the volume 150 mL was used because it was the necessary volume that led to an optimal digestion efficiency percentage). These Erlenmeyers were maintained on a heating magnetic stirrer at 60 °C for 24 h. After digestion, the digestat of each individual was filtered on a GF/A filter (90 mm diameter). The same procedure was done with native mussels but a volume of 250 mL was added instead (see Kazour et al. 2019).

c. Visual and Raman spectroscopy analysis

All filters were observed under 120x magnification using Leica M165 C Stereomicroscope and images of suspected MPs particles were taken with a Leica M170 HD camera and LAS (Leica application suite) software. All suspected MPs particles were counted, categorized by type (fragments, fibers, microbeads or films) and color. The following criteria were taken into consideration while observing the items: (1) absence of cellular or organic structures; (2) a homogenous thickness across the particles; and, (3) homogenous colors (Hidalgo-Ruz et al., 2012). Measurements were done on the suspected items at their longest dimension and they were divided into different size classes of 50 µm.

Suspected microplastics were identified using Micro-Raman Xplora Plus (HORIBA Scientific®, France). The machine is protected by a door so that the filter placed inside would not be affected by any airborne contamination. Due to the time consumption that takes when analyzing filters under micro-Raman, five filters of caged and native mussels containing the highest number of potential MPs from each sampling site were chosen and analyzed. All sediments items, and subsamples of water items were also taken for Raman analysis. For identification, two lasers with a wavelength of 532 nm and 785 nm and a range of 200-3400

cm^{-1} were used with x10 and x100 objectives (Olympus). Each particle spectrum is compared to a polymer database identification software (KnowItAll, BioRad®) and a personal library made with standard polymers obtained from Goodfellow (France). KnowItAll software gives a resemblance (correlation) between the obtained spectra and the one existing in the database. If the obtained spectrum showed a high fluorescence, several acquisition parameters are changed from one sample to another: whether in the acquisition time (0.2s, 1s, 2s, or 5s), the number of accumulations (2, 5, 10, or 15), the laser wavelength and intensity (0.1%, 1%, 10%, 50% or 100%), the slit and the hole, to obtain a better identifiable spectrum. The identification is considered correct when the HQI (Hit Quality Index) was above 70 (ranging from 0 to 100). Baseline correction was realized on spectra in order to have a cleaner identifiable spectrum. Pigments spectra (Copper phthalocyanine, Hostasol Green, etc…) obtained were identified using an option called "Mixture Analysis" existing in "KnowItAll software" that is capable to decompose a mixed spectrum into two spectra consisting of the dye and its associated polymer (see Supplementary Figure 1 for an example).

2.4 Statistical and data analyses

Microplastics found in water, sediments and mussels were reported in unit volume (per L) and per gram of sample, respectively. Statistics were performed with SPSS software (IBM SPSS STATISTICS 20). The Wilcoxon signed-rank test was used to compare the ingested MPs between depurated and non-depurated mussels and a Student t-test was used to compare the condition index. For inter-site comparisons, if data did not comply with the parametric assumption of normality (Shapiro–Wilk tests) and homogeneity of variance (Levene tests), the non-parametric Kruskall–Wallis test and Mann–Whitney U test for post hoc pairwise comparisons were used. All results are expressed as mean ± SD, and p-value < 0.05 was considered as statistically significant. Morisita's index of similarity (or Morisita overlap index) is a measure of how similar or different two sets of data are**.** This index was calculated to compare size and polymer types similarity found in caged mussels and those found in native mussels, water and sediments samples.

$$\hat{C} = \frac{2\sum_{i}^{n} \hat{p}_{ij}\hat{p}_{ik}}{\sum_{i}^{n} \hat{p}_{ij}\left[\frac{(n_{ij}-1)}{(N_j-1)}\right] + \sum_{i}^{n} \hat{p}_{ik}\left[\frac{(n_{ik}-1)}{(N_k-1)}\right]}$$

$\hat{C}$: Morisita's overlap index between *j* and *k*

j: Caged mussels

k: Native mussels or water or sediments

$\hat{p}_{ij}$: Proportion of a size class or a polymer *i* used by *j*

$\hat{p}_{ik}$: Proportion of a size class or a polymer *i* l used by *k*

n_{ij}: Number of size class or polymer *j* that use *i*

n_{ik}: Number of size class or polymer *k* that use *i*

N_j ; N_k: Total number of size class or polymer in sample

This index ranges from 0 (when no common size or polymer types are shared between the two sample types) and 1 (when all sizes or polymer types are common between the two sample types).

Results

3.1 Mussels caging experiment

Before translocation, mussels were depurated. There was no significant difference in mussel's condition index after seven days of depuration ($p = 0.264 > 0.05$). However, a significant difference in the amount of MPs was observed between non-depurated and depurated mussels ($p = 0.018 < 0.05$). Non-depurated mussels had an average of 2.22 ± 1.35 items/g with fragments constituting 82.3% of the observed items (Figure 2). The number of items significantly decreased by 97.6% after 7 days of depuration reaching 0.05 ± 0.15 items/g consisting only of fibers. The size of observed fibers before depuration ranged from 41.54 µm to 622.04 µm with a mean size of 284 ± 156.8 µm; these fibers were of different colors: black, green, blue and red. Only two blue fibers were observed after depuration with a respective length of 304.06 and 415.73 µm.

Even though the cages were transplanted during the winter period when the temperature had been at its lowest (around 6-7°C), the mean mussels survival rate was 73%. After 42 days of caging experiment, mussels shell length (5.7 ± 0.47 cm) and condition index (54.7 ± 3.8%) were not significantly different from the beginning of the experiment ($p = 0.621 > 0.05$). Also, there was no significant inter-sites difference in the caged mussels condition index ($p = 0.521$).

293

294 ## 3.2 Microplastics abundance, type and color

295

296 The absence of fragments, microbeads and films in our procedural background blanks
297 indicates that contamination was only limited to airborne fibers. The average fiber
298 contamination during caged mussels dissection and digestion had an average of 0.72 ± 0.78
299 fibers (representing the average found for a batch of 9 mussels representing a total one hour
300 constant laboratory work under the laminar flow hood). Similar values were observed for the
301 other three matrices (see Kazour et al. (2019)). The importance of following procedural
302 blanks is that any potential contamination is then eliminated from the analyzed samples.
303 Microplastic contamination of seawater, sediments, and mussels was evident at all of our
304 sampling sites. The number of suspected MPs in the water decreased with the increasing
305 distance from the effluent of the WWTP and the highest amount was found near the landfill
306 (site 5). Such pattern was not observed for MPs found in the sediments (Figure 3 - A).
307 Suspected MPs were found in mussels of all sites; 93% of native mussels and 94.7% of caged
308 mussels had ingested suspected MPs with a mean number of items ranging from 0.41 to 2.76
309 items/g. The spatial pattern of MP abundances in mussels was different from that observed for
310 water or sediment samples (Figure 3). Caged mussels ingested the same or higher amount of
311 MPs than native mussels at all sites except for site 5; however for the same site, the amount of
312 ingested MPs by caged and native mussels were not significantly different ($p > 0.05$) (Figure
313 3).

314

315 Both fragments and fibers were found in the water, sediment and in both caged and native
316 mussels. In all the matrices analyzed, fragments dominated the type of MPs except at sites 1,
317 3 and 4 for water samples (Figure 3). Primary microplastics (polystyrene raw microbeads)
318 were found only at site 5 in the water but were not detected in mussels nor in sediment
319 samples. Films were observed only in native mussels. Blue fragments dominated the water
320 (45%) and sediment (69%) samples whereas red and blue fragments dominated in both caged
321 (76%) and native mussels (42% each). For the fibers, black (51%) and blue and green color
322 (71.4%) dominated the water and sediment samples respectively whereas blue and black
323 fibers dominated in caged (85%) and native (80%) mussels. At each site, the proportion of
324 MPs items type or color was quite similar among caged and native mussels. For fragments,
325 the order of dominant colors was blue, red, green, white and transparent; whereas for fibers, it
326 was blue, black, green and red.

3.3 Microplastics size and polymer types

Suspected MPs sizes were very similar whether in the water, sediments and both caged and native mussels, with a dominance of items < 150 µm (Figure 4). This was confirmed by the very high overlaps between caged mussels vs native mussels (0.98), vs sediments (0.9) or vs water samples (0.84). Smaller size classes (< 200 µm) were more abundant in native mussels compared to caged mussels (Figure 4 - A). Caged mussels showed greater number of items with a size class > 200 µm compared to native mussels. Sediments and water samples showed higher proportion of items of size class < 150 µm compared to caged mussels (Figure 4 - B-C).

Of the 503 items analyzed with the micro Raman, 422 (83.9%) were positively identified as a known polymer (examples of various parameters used to identify samples are indicated in Supplementary Table 2). Among the non-polymers items, only 2% were identified as cellulose-based fibers. The rest of the spectra were highly fluorescent and non-identifiable (for example if a sample is too thin, Raman tends to detect the underlying substrate instead of the sample (Käppler et al., 2016)).

Five polymers were identified in caged mussels, by order of dominance: polystyrene (PS), acrylonitrile butadiene styrene (ABS), polyurethane (PUR) and polyethylene (PE),. A higher number of polymers were identified in native mussels (9), water (11) and sediments (8) (Figure 5). PS dominated all samples in all the different matrices with ABS and PA the second most abundant polymers. The highest overlap in polymer types was found between caged mussels and sediments (0.93) followed with native mussels (0.88) and water samples (0.86).

Discussion

The key advantage of using blue mussels for microplastics biomonitoring, is that they can be depurated in the laboratory, reducing initial contamination bias, and thus, clean reference individuals will reflect the MPs contamination of their specific deployment sites. In mussels, MPs may be retained for extended periods of time, for example, complete clearance of MPs was not achieved after a seven-days depuration period under laboratory conditions with microbeads (2,6 µm) being retained within the digestive tracts (Paul-Pont et al., 2016). Our results showed that after 7 days of depuration, 97.6% of the MPs were eliminated by the

mussels. Similar depuration rate (85%) was observed in the Mediterranean mussel, *M. galloprovincialis*, after seven days depuration period (Fernández and Albentosa, 2019). Lower depuration period seems however to be insufficient for total depuration; in a three days depuration experiment of farmed *M. edulis*, Van Cauwenberghe and Janssen (2014) found a reduction of ca. 33% in the MPs quantity. Since depuration significantly decreased the quantity of MP in blue mussels, the eliminated particles were probably located in the digestive system as suggested for the brown mussels (*Perna perna*) by Birnstiel et al. (2019).

The main objective of the study was to test effectiveness of the mussel caging for active microplastics biomonitoring, and this was investigated by comparing MPs ingested by caged blue mussels with those ingested by native mussels collected at the same site and MPs found in their surrounding environment (surface water and sediments). Two aspects can be compared: the quantitative aspect (MPs concentrations in the different matrices) and the qualitative aspect (shape, size, color and type of polymers) of MPs.

4.1 Quantitative MPs comparison

A key question for microplastics caging studies is how long the blue mussels should stay deployed to be equalized with native mussels and to reflect MPs concentrations of their surrounding environment. For chemical pollution, the reported caging periods lasted generally between 3 to 6 weeks but may last 18 - 24 weeks or up to 2 years (see review in Beyer et al., 2017). Short term mussel deployments, such as one or two months, has been shown to be suitable for chemical pollutant monitoring in coastal waters, e.g. metals (Greenfield et al., 2014; Lacroix et al., 2015; Piccardo et al., 2001). To date, only three studies used caged blue mussels to investigate the MPs pollution (Avio et al., 2017; Catarino et al., 2018; Railo et al., 2018). These studies showed that mussels transplanted for 4 weeks period have accumulated MPs in their tissues. However, they have not compared the ingested MPs with those found in native mussels or in their surrounding environment. In the present study, even though mussels were not transplanted during their optimal condition (the seawater temperature has been at its lowest: around 6-9°C), mussels' condition and growth were maintained after 6 weeks, and their survival rate was high (73%). More importantly, the majority (93%) of transplanted mussels had ingested MPs. This percentage was similar to that of native mussels (94,7%) collected in the same areas. In addition, the spatial pattern in the amount of ingested MPs by native and caged was quite the same except in site 5 where the native mussels ingested higher

number of MPs. This difference in ingested MPs can be due to the significantly lower body weight of native mussels from the site 5 compared to that of transplanted mussels (1.07 ± 0.35 g and 5.15 ± 1.11 g, respectively). The increase on the retention time of plastics particles with decreasing size in mussel has been reported (Fernández and Albentosa, 2019; Van Cauwenberghe and Janssen, 2014). Using caged mussels from a single population avoids bias related to the age, the size or the reproductive status of the organisms that can influence ingestion, retention or clearance rates.

Among the detected debris items in native and caged mussels, fragments were the most predominant type observed, consistent with the MPs found in mussels and cockles (Hermabessiere et al., 2019) and juvenile fish (Kazour et al., 2018) collected along the Eastern English Channel coast. This high occurrence and abundance of fragments in mussels reflect those found in their surrounding environment (seawater and sediments). A recent study showed a positive and quantitative correlation of MPs in mussels (*M. edulis* and *Perna viridis*) and their surrounding waters (Qu et al., 2018). The authors suggested that the amount of MPs in mussels can reflect the real abundance of MPs in the coastal water but that mussels were more likely to ingest smaller MPs. In our study, the spatial pattern of MPs concentrations in mussels (both caged and native) was different from that observed for water or sediment samples. Similar results were observed along the coastal waters of the U.K., with no quantitative correlation between MPs in mussels and their ambient seawaters (Li et al., 2018; Scott et al., 2019). Such absence of correlation may be due to the limited sampling sites (only five in our study and seven in Li et al. (2018) study) which are insufficient for robust correlation analyses. In addition, it is likely that a range of factors influence mussel encounter rates with particles within their immediate environment, including hydrodynamics processes in the study area, particle behavior in the water column and the mussels or seawater sampling location in the water column (Avio et al., 2017; Scott et al., 2019). In the study of Avio et al. (2017) mussels were caged for 4 weeks at 2 different depths, approximately at 1.5 m from the surface and from the bottom (30-45 m). They found some differences in the occurrence of ingested MPs as a function of caging depth, with higher ingested particles (32%) in surface deployed mussels compared to those caged in proximity of the bottom (12%). In addition, some variations were observed in terms of typology and size of particles between surface and bottom-caged mussels highlighting the influence of a different distribution of MPs along the water column. The density of the plastic particles will determine their position in the water column, and, therefore, the likelihood of encounter by an organism (Desforges et al., 2014). In

our study, we sampled surface water whereas mussels were near the bottom. When comparing different matrices or compartments, a more adapted sampling strategy should take into account numerous parameters such as the sampling time according to the tide, the time lag necessary for MPs to sink in the water column, to be ingested by mussels and to be sedimented at the bottom (Schmidt et al., 2018). As these conditions have not been implemented in the present study, it is therefore difficult to compare the MP concentrations found in mussels, water and sediments. Thus, the present data should be considered as semi-quantitative until the influence of environmental factors on the measured MP concentrations found in the seawater and sediments and those ingested by blue mussels have been further investigated. In addition, an understanding of MPs bioaccumulation is a prerequisite for the use of mussel as biomonitoring organisms in the aquatic environment.

4.2 Qualitative (size, color and polymers) MPs comparison

Comparison between native and caged mussels showed that, at each sampling site, the proportion of MPs items type, size, color and to some extent their composition (polymer types) were quite the same with a high overlap index. Both native and caged mussels from all sites have mainly ingested small sized MPs (< 200 µm). This was similar to other studies that demonstrated that MPs below 300 µm were the most found in mussels (Leslie et al., 2017; Naji et al., 2018; Phuong et al., 2018). Blue fragments were the dominant items found in all the analyzed compartments. Although small sized MPs dominated the water and sediments samples, it is currently unknown whether their abundance in mussels are due to mussels size preferences in regards to prey, or because the majority of MPs in the environment were typically in this size range (Bråte et al., 2018; Scott et al., 2019).

Concerning polymer types, a good overlap was found between caged mussel and the others compartment analyzed. The highest overlap in polymer types being found between caged mussels and sediments (0.93). Polystyrene (PS) dominated all samples in all the different matrices. This polymer was among the most common plastics used worldwide whether in packaging or other household materials. We found that some polymers were under-represented or totally absent in the caged mussels compared to overlying seawater or surrounding sediment. More polymer types (N=13) were identified in the surface water compared to sediments (N=9) or caged mussels (N=6). In the South West of England, Scott et

al. (2019) found significant differences in the relative abundance of polymer types and particle sizes between seawater, sediment, and mussels. However, they found that particles within mussels reflected more closely those found in the intertidal surface sediment compared to those found in the surface seawater. This is also the case in our study where particles found within caged mussels presented the highest similarity with those found in the sediments. The bioavailability of MPs to aquatic organism depends on their characteristics, such as polymer type, chemical composition, size, shape, density, etc. (Carbery et al., 2018). The shape of MPs may also influences their accumulation (persistence inside mussels) or elimination (Browne et al., 2011; Thompson et al., 2004). As suggested by Scott et al. (2019), it is likely that both environmental and biological partitioning of microplastic particles and the selective feeding ecology of this species is responsible for the under-representation of certain polymer types and particle sizes within the mussels. This should be taken into consideration when using mussels for MPs pollution biomonitoring.

4.3 Mussels biomonitoring as a standardized tool for microplastics assessment

In this work, we were able to demonstrate the potential use of mussels as a MPs biomonitoring tool. But a lot of factors should be considered to optimize and standardize this tool. The first issue is that mussels represent different filtration rates depending on the region and genotype, for example (Lüskow and Riisgård, 2018; Riisgård et al., 2013). The difference in the uptake rate for analogous species needs to be further studied to appropriately adjust interspecific difference. Algal abundance, temperature and salinity of water, as well as the depth of the transplanted cages could alter mussel's filtration and clearance rates (Comeau et al., 2008; Riisgård et al., 2014, 2013) and, therefore, their MPs uptake. For example, differences in MPs ingestion have been observed between caged mussel's transplanted at 1.5m and 30-45m depth (Avio et al., 2017). In addition, seasonal variation for MPs intake has been observed in caged mussels but it has been more linked to an important water flow from a river rather than the different seasons (Catarino et al., 2018). More progression in this area is highly needed to obtain a standardized protocol. Such tool would constitute plenty of advantages for MPs evaluation such as allowing a microplastics assessment while using similar population (genetic and physiological) in areas lacking mussel beds, controlling exposition period and to potentially study the toxicological impact of microplastics on mussels exposed in the natural environment.

Conclusion

In summary, the present study demonstrated for the first time the effectiveness of the mussel caging approach. The abundance and properties of MPs found in caged and native mussels were quite similar. If we rely on the assumption that MPs concentrations in native mussels have reached steady-state (balance between intake and defecation/egestion), the results of this study indicate that short term mussel deployments (i.e. 6 weeks) appear to be suitable to reflect native mussels MPs ingestion. Although microplastics monitoring is a complex task that it is in its initial phases (Carbery et al., 2018), caging presents many advantages including the selection of well-characterized homogenous organisms (number, age, size, weight, sex) and the control of exposure (location, time, season). To the best of our knowledge, mussels caging as tool to study microplastics pollution has not been investigated before. Though the abundance and properties of microplastics in mussels does not exactly match those in their environment (seawater and sediments), our study suggests that blue mussels caging may be a promising tool for MPs biomonitoring in marine coastal environments. The caging approach makes monitoring more reliable and may allow accurate assessment of the biological effects of MPs over a predetermined exposure period. However, further methodological improvements should be considered, such as the choice of the deployment time and the caging location (with respect to the shore and the depth of their implantation in the water column), to define a uniform protocol for blue mussels caging allowing an accurate spatial and temporal microplastics biomonitoring.

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Authors Contributions

R.A. conceived of the presented idea. R.A. and M.K. planned and carried out the experiment. M.K. performed the samples analyses. All authors discussed the results and contributed to the final manuscript.

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Declaration of interests: None

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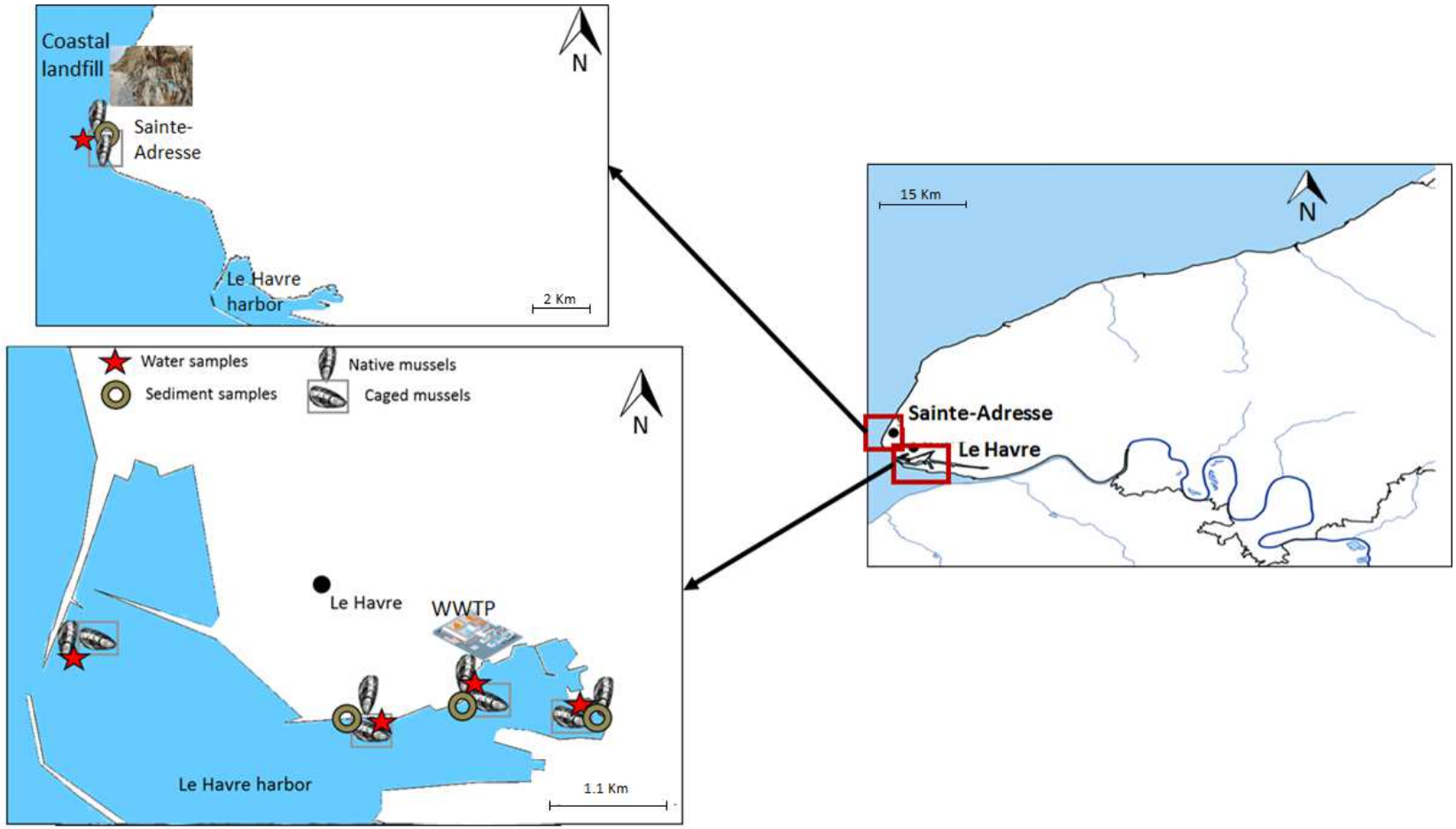


Figure 1: Mussels caging site location with respect to the wastewater treatment plant (WWTP, Le Havre harbor) and the coastal landfill (Sainte-Adresse).

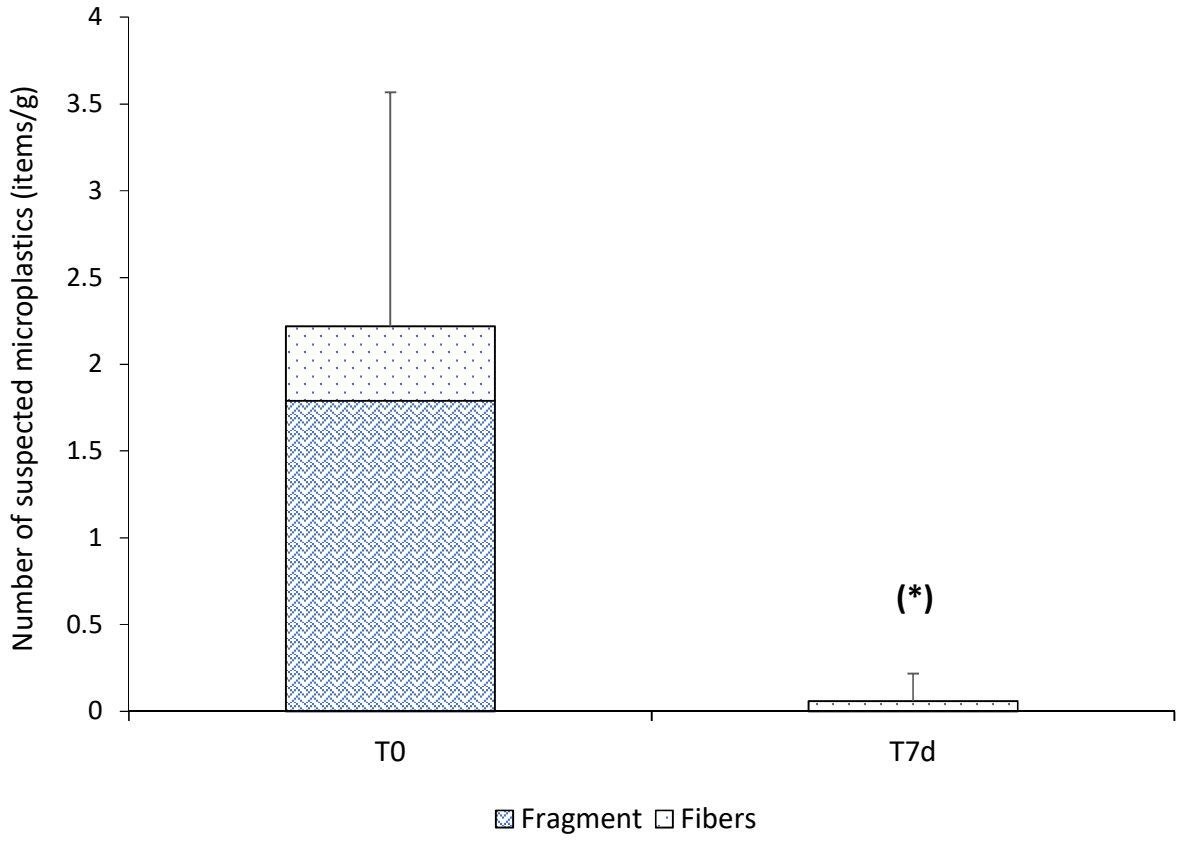


Figure 2: Average (+SD) number of suspected microplastics (items/g of mussels' wet weight) by shape categories found in the non-depurated mussels (T0: farmed mussels) and the 7 days depurated mussels (T7d).

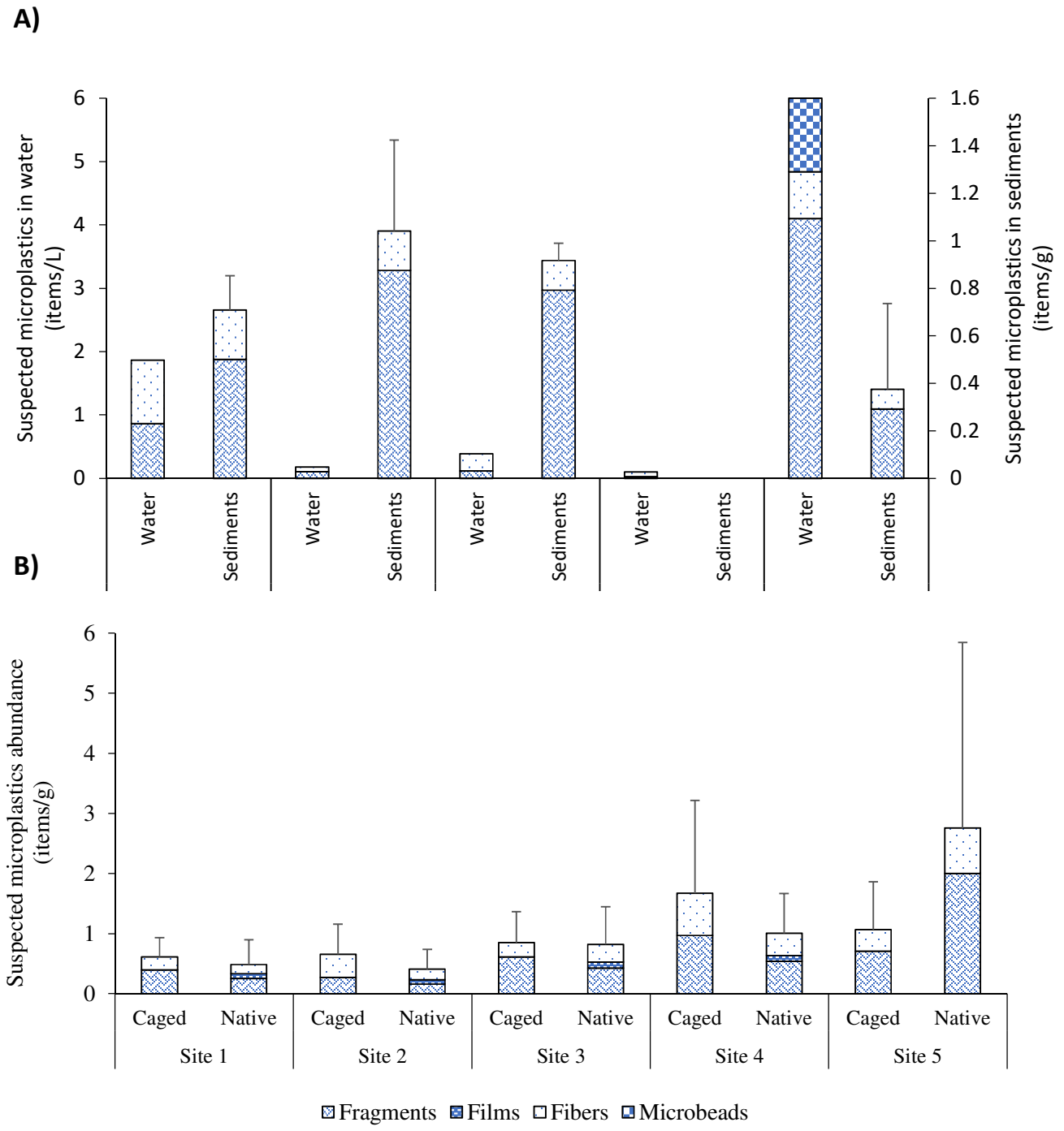


Figure 3: Average (+SD) number of suspected microplastics by shape categories found in (A) the surface water (items/L) and sediments (items/g), and in (B) the caged and native mussels (items/g of mussels' wet weight).

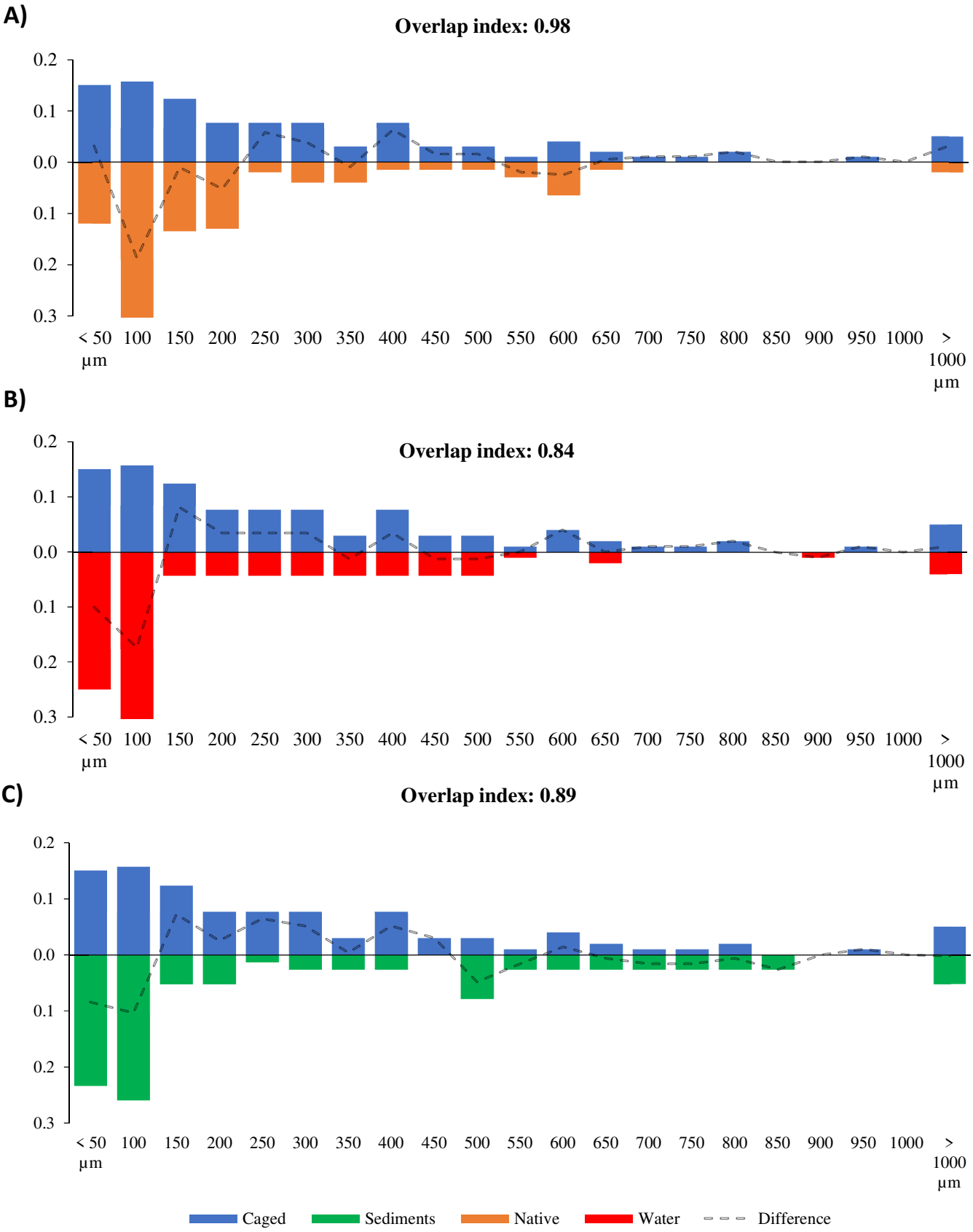


Figure 4: Back to back histograms of size classes of suspected microplastic items found between A) caged mussels (in blue) and native mussels (orange), B) caged mussels and the surface water (in red) and C) caged mussels and sediments (in green). The difference in size classes frequency between samples is shown with the dotted black lines, while the overlap index (Morisita's index) is indicated within the graph.

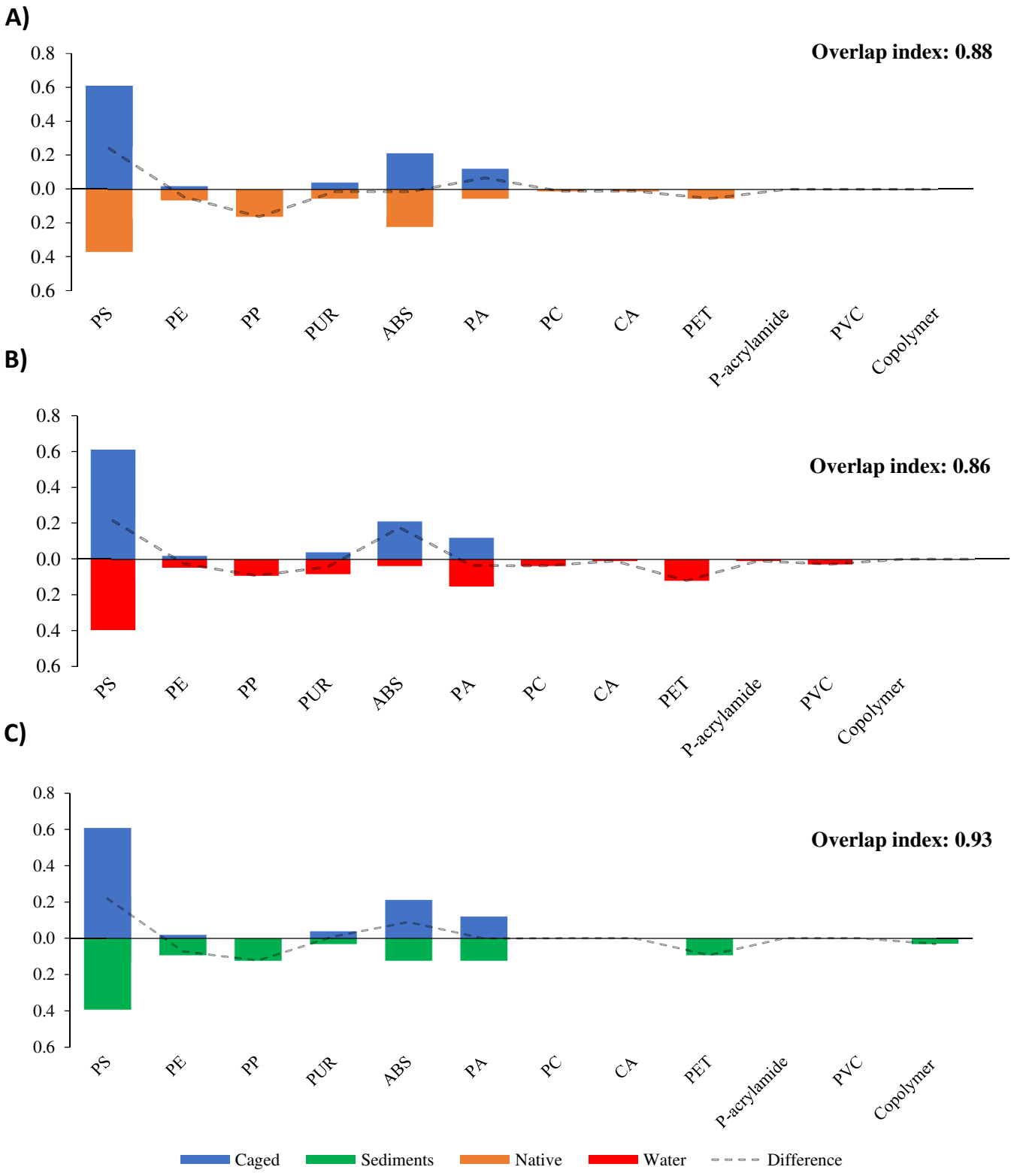
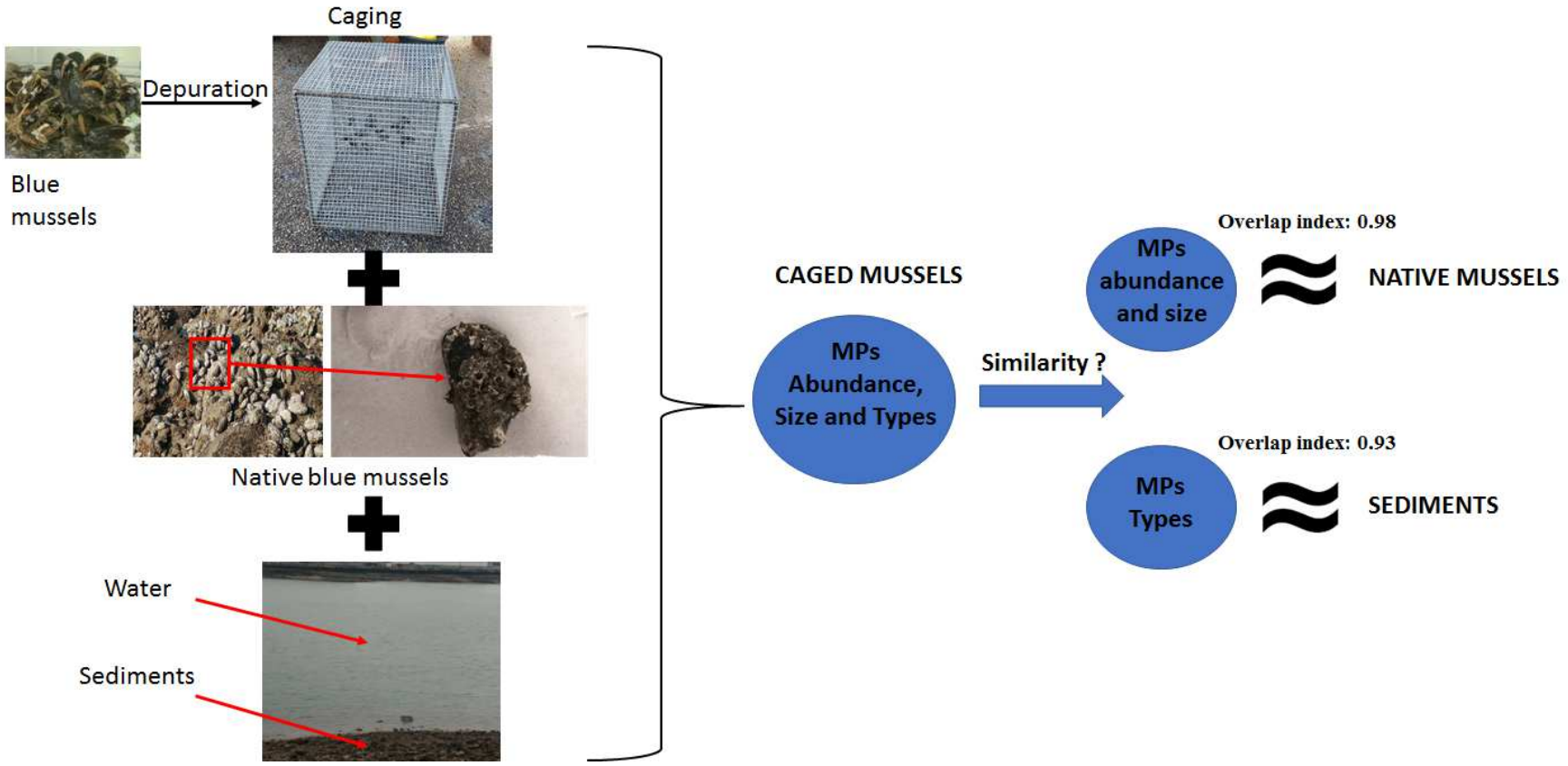


Figure 5: Back to back histograms of polymer types found between A) caged mussels (in blue) and native mussels (orange), B) caged mussels and the surface water (in red), C) caged mussels and sediments (in green). The difference in polymer frequency between samples is shown with the dotted black lines, while the overlap index (Morisita's index) is indicated within the graph.


Caging
Depuration
Blue mussels
Native blue mussels
Water
Sediments
CAGED MUSSELS
MPs Abundance, Size and Types
Similarity ?
Overlap index: 0.98
MPs abundance and size
NATIVE MUSSELS
Overlap index: 0.93
MPs Types
SEDIMENTS