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# Experimental accumulation of microplastics in acorn barnacle *Amphibalanus amphitrite* and its use in estimating microplastic concentration in coastal waters

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Microplastic (MP) pollution has been an emerging problem in the marine environment over the last few decades. Considering wide-ranging negative impacts of MPs on marine organisms, monitoring MP pollution in marine ecosystems is essential and urgent for developing effective management of the problem. An exposure experiment was conducted to assess the potential of the striped barnacle *Amphibalanus amphitrite* as a bioindicator of MPs. The barnacles were exposed to either polypropylene fibers or fragments for 8 days. A full factorial experiment was conducted, with the shape (fragment, fiber), exposure concentration (10, 100, and 1000 items $L^{-1}$) and exposure duration (2, 4, and 8 days) as independent factors. The highest number of MPs in *A. amphitrite* was $21.04 \pm 15.22$ fragments $g^{-1}$ and $17.60 \pm 13.8$ fibers $g^{-1}$ wet weight recorded after 4 days of exposure, and the MP concentration in the barnacles was positively correlated with the exposure concentration, regardless of the form of MPs. A regression equation relating the MP concentration in the barnacles and that in the water was computed to estimate the MP concentration in the coastal waters of Hong Kong. The predicted values were close to the published data, indicating the potential of *A. amphitrite* as a bioindicator of MP pollution.

KEYWORDS

microplastics, polypropylene, barnacles, fragments and fibers, bioindicator

# 1 Introduction

Since invented in the 1950s, plastics have replaced many traditional materials and join the ranks of the world's most commonly used materials, reaching a global production of 367 million tons in 2020, and an estimated 4.8 - 12.7 million tons were discharged into the ocean (Jambeck et al., 2015). Microplastics (MPs) are commonly defined as plastic particles smaller than 5 mm in diameter. They are either manufactured as micro-sized plastic particles or originated from the fragmentation of macro-plastic debris. Since MPs and prey of many marine organisms have a similar size range, accidental ingestion of MPs by marine organisms is not uncommon. MPs are found in the digestive tracts of more than 100 species, including planktons (Aytan et al., 2022), bivalves (Santana et al., 2016; Rios-fuster et al., 2022), barnacles (Zhang et al., 2022), crabs (Horn et al., 2019; Zhang et al., 2021), gastropods (Courtene-Jones et al., 2019; Doyle et al., 2019), fish (Collard et al., 2019; Ferreira et al., 2019), and marine mammals (Nelms et al., 2018; Zantis et al., 2021).

The ingested MPs could elicit various health effects. These include reduction of feeding capacity and energy intake (Xu et al., 2017), reduction of feeding rate and fecundity (Yu et al., 2020), growth inhibition (Xia et al., 2020; Silva et al., 2021), oxidative stress (Tlili et al., 2020), immunotoxicity (Revel et al., 2020; Tang et al., 2020), neurotoxicity (Barboza et al., 2020; Kim et al., 2020), and transcriptional changes (Limonta et al., 2019).

Considering the ubiquitous distribution of MPs in the marine environment and the negative impacts of MPs on marine organisms, effective marine monitoring programs for MPs are urgently needed (Gallo et al., 2018).Barnacles are commonly used as biomonitors for pollutants, such as trace metals (Rainbow and Blackmore, 2001) and polybrominated diphenyl ethers (PBDEs) (Chen et al., 2015). Barnacles are filter feeders which have cirri carrying a variety of setae (Chan et al., 2008) to capture plankton in the water column ranging from several millimeters to a few microns (Crisp and Southward, 1961). Since food particles of barnacles and MPs fall into a similar size range, there is a potential for barnacles to be bioindicators of MPs. Several studies have reported on the plastic debris or MPs in the field-collected barnacles. Goldstein and Goodwin (2013) found plastics in the gastrointestinal tract of 33.5% (or 385 individuals) of the gooseneck barnacles (*Lepas* spp.) obtained from the North Pacific Subtropical Gyre. Thushari et al. (2017) reported 0.23–0.43 particles $g^{-1}$ tissue in *Balanus amphitrite* (=*Amphibalanus amphitrite*) at three study sites in Thailand. Zhang et al. (2022) examined four species of barnacles on the coast of the Yellow Sea, China. The highest MP abundance was found in *Balanus albicostatus* (ecotype A), with a concentration of 21.59 ± 27.26 items $g^{-1}$. A similar abundance (10.26 ± 9.76 items $g^{-1}$) was reported in *A. amphitrite* in Luk

Keng, Hong Kong (Xu et al., 2020). They also found a strong correlation between the MP abundance in *A. amphitrite* and that in the sediment and concluded that *A. amphitrite* could be a potential bioindicator of MP pollution.

The tolerance of *A. amphitrite* was evaluated in laboratory exposure experiments. After *A. amphitrite* was exposed to MPs of environmentally relevant concentrations, the ingestion of MPs did not cause any lethal or sublethal response (Yu and Chan, 2020a). The tolerance of *A. amphitrite* to MPs enables this species to become a biomonitors for MP pollution. However, the ingestion of MPs could cause higher mortality of offspring, which may be attributed to lower energy available for gonad development and offspring production (Yu and Chan, 2020b). If there is an impact from MPs on the population sustainability, the competency of *A. amphitrite* as a long-term bioindicator of MP pollutions has to be considered with caution.

According to Gerhardt (2002), a bioindicator is "a species or group of species that readily reflects the abiotic or biotic state of an environment, represents the impact of environmental change on a habitat, community or ecosystem, or is indicative of the diversity of a subset of taxa or the whole diversity within an area". According to the applications, bioindicators can be classified as environmental, ecological, or biodiversity indicators. Environmental indicators should respond predictably to environmental disturbance or change (e.g., sentinels, detectors, exploiters, accumulators, bioassay organisms) and are used for diagnosing the state of the environment. Ecological indicators are species sensitive to pollution, habitat fragmentation, or other stresses, and their responses are representative of the community. Biodiversity indicators refer to the use of species richness of an indicator taxon to reflect the species richness of a community (Gerhardt, 2002).

This study was a laboratory exposure experiment, trying to quantify the relationship between MP abundance in the water and that accumulated by *A. amphitrite* and assess the potential of *A. amphitrite* to be a bioindicator in predicting environmental concentrations of MPs using regression equations calculated from the exposure experiment. A similar approach was used by Berg and Steinnes (1997) in predicting the absolute deposition rates of heavy metals using concentrations determined in the ground-growing mosses *Hylocomium splendens* and *Pleurozium schreberi*. The experiment included two forms of MPs (fiber and fragment), and three concentrations, including one realistic environmental concentration (10 items $L^{-1}$) (Mak et al., 2020) and two higher concentrations (100 and 1000 items $L^{-1}$), were prepared for each form of MPs. The accumulation of MPs in *A. amphitrite* was examined after 2, 4, and 8 days of exposure. Fibers and fragments were chosen for the study because they were the most common forms of MPs found in field-collected barnacles (Xu et al., 2020; Zhang et al., 2022).

# 2 Materials and methods

## 2.1 Collection and maintenance of experiment organisms

We collected individuals of the acorn barnacle *A. amphitrite* from a pebble beach near the Wu Kai Sha pier (114.23°N, 22.42°E). As barnacles might die when separated from the substrate, they were transferred back to the laboratory with the pebbles, which were scrubbed to remove epifauna. The barnacles were kept in 50 L glass aquaria (35 × 50 × 45 cm), with about 40 pebbles per tank equipped with a filtration system and a protein skimmer. Filtered natural seawater (30 psu) was constantly aerated, and 70% of the seawater was renewed every week to maintain the water quality. Light and dark conditions followed cycles of 12 h. The barnacles were fed daily with the alga *Skeletonema costatum* at $10^4$ - $10^6$ cells $L^{-1}$. Seawater used in this exposure experiment was collected from the eastern waters of Hong Kong and filtered through 5 μm filter papers before use.

## 2.2 Preparation of microplastics

Blue PP fragments (63-125 μm) were prepared from stalks for marker pens by cryogenically ground at –196°C using a cryomilling machine ( CryoMill, Retsch, Haan, Germany). Fragments with a size of between 63 and 125 μm were obtained after sieving. For PP fibers, small bundles of fibers separated from orange ropes made from PP were cut by dissecting scissors under a stereomicroscope. Fibers were sieved to obtain the required length (63-250 μm). The MPs remained on the sieve were transferred on filter papers for size measurement and quantification. Micro-Fourier transform infrared spectroscopy (μ-FTIR, Thermo fisher Nicolet iN5, USA) was used to confirm the chemical composition of fragments and fibers.

For size measurement of MPs, photos of the prepared MPs were taken under a stereomicroscope (Leica 165MC) and the perimeter as well as 2D surface area of MPs were measured using ImageJ. The 2D surface area of fragments and fibers were similar (fragments: 6408 ± 605 μm$^2$; fibers: 6246 ± 535 μm$^2$). The shape factor of the MPs was calculated by the following formula (Zhao et al., 2018):

$$Shape\ factor\ =\ (4\pi \cdot A)/P^2$$

where A represents the 2D surface area (mm$^2$) and P, the perimeter of MPs.

The shape factor of fragments and fibers was 0.31± 0.02 and 0.70 ± 0.02, respectively.

Quantification of MPs was achieved by counting the MPs in a sub-area (1/16) of the filter papers. To remove counting errors caused by uneven distribution of MPs on the filter papers, the suspension of MPs inside the filtering funnel was stirred before turning on the vacuum pump. This suspending, stirring and

filtering procedure might repeat several times by adding more DI water into the filtering funnel until the MPs were distributed evenly. Due to the high concentration of MPs in the suspension, the prepared MPs were usually filtered onto several filter papers, to avoid serious overlapping of MPs and to increase the number of replicates for quantification, both of which could further reduce the counting errors.

After quantification of MPs, stock solutions of MPs (10, 100, and 1000 items mL$^{-1}$) were prepared by transferring the MPs on the filter papers into 50 ml centrifuge tubes with a calculated amount of DI water. To achieve the final exposure concentration (10, 100, and 1000 items $L^{-1}$), a calculated amount of stock solution was added into the exposure aquarium.

## 2.3 Bioaccumulation of MPs in barnacles

An 8-day exposure experiment was conducted to investigate the bioaccumulation of MPs in *A. amphitrite*. A full factorial experiment was conducted, with the shape (fragment, fiber), exposure concentration (10, 100, and 1000 items $L^{-1}$) and exposure duration (2, 4, and 8 days) as independent factors. This study included 18 treatment groups in total, each containing ten replicates. In addition, ten replicates of the procedural blank were prepared without microplastics in the seawater.

The rostro carinal diameter of barnacles was measured before the exposure, and similar-sized individuals were selected. As a pooled replicate, five barnacles were placed in a 500 mL glass bottle with constant aeration to keep the MPs in suspension and create water flow, which stimulated the barnacles to extend their cirri and filter particles in the water. When more than 5 barnacles were attached to one pebble, which was very common, extra barnacles were removed carefully. The bottles were emptied and washed with filtered seawater during the exposure experiment every 24 h. Then the water was renewed with the subsequent addition of MPs. The barnacles were fed daily with a 5 mL algal solution of *S. costatum* at $10^7$ cells $L^{-1}$, immediately after adding MPs.

To determine the MP ingestion, the barnacles were sampled on Day 2, 4, and 8, with their rostro carinal diameter measured. Chemical digestion was used to extract MPs in the body tissue of barnacles, according to Karami et al. (2017). The soft tissue of five barnacles in each pooled replicate was dissected, and the wet weight was measured separately for each barnacle. The tissue was placed in a 100 mL beaker containing 60 mL of 10% potassium hydroxide (KOH). The digestion procedure (~48-96h) was conducted at 40°C in an incubator, with shaking at 200 rpm by an orbital shaker until complete digestion. After filtering the digested solution onto cellulose nitrate filter papers (Membrane Solution, pore size = 5 μm), the filter papers were checked under a stereomicroscope (Leica M165C) at a magnification of 40× - 120×, to identify and quantify the MPs.

The MP abundance in the barnacles was estimated as the number of MPs divided by the tissue mass of five barnacles.

## 2.4 Quality assurance/quality control

The seawater was pre-filtered with 5 μm filter papers and all the glassware and tools were pre-rinsed with DI water for three times. The background contamination was also checked, by conducting control experiments (n = 10), which shared the same procedure except for the addition of MPs, together with the exposure experiments. None of the control groups was contaminated by MPs.

Procedural controls (n=3) for the KOH digestion were conducted daily but without animals. As the digesting procedure might cause loss of MPs, the recovery rate of MPs after the KOH digestion was also evaluated by spiking 10 fragments or fibers into the body of the barnacles and counting the number of recovered MPs after the barnacles going through the KOH digestion.

## 2.5 Statistical analysis

Even after data transformation, the data failed the normality test (Shapira-Wilk method). Scheirer-Ray-Hare test was conducted for both the fragment and fiber exposure experiments, with the MP concentration and exposure duration being independent factors. To compare the abundance of different forms of MPs in barnacles, Mann Whitney tests were conducted between pairs of the fragment and fiber under the same exposure durations and concentrations. The relationship between the MP abundance in the barnacles and that in the water was analyzed by the linear regression following Berg and Steinnes (1997) and Kono et al. (2012). Berg and Steinnes (1997) used the original data for the linear regression analysis, whereas Kono et al. (2012) used log-transformed data. In this study, both original and log-transformed data were used in the linear regression analysis and the best fit model was reported. Linear regression equations were used to predict the MP concentration in Hong Kong waters based on the MP abundance in field-collected barnacles reported in a previous study (Xu et al., 2020). The Scheirer-Ray-Hare test was conducted by RStudio, and the linear regression analysis by Prism.

## 3 Results

### 3.1 MP abundance in barnacles

The highest number of MPs in *A. amphitrite* was 21.04 ± 15.22 fragments g$^{-1}$ and 17.60 ± 13.8 fibers g$^{-1}$ after being exposed to a concentration of 1000 items L$^{-1}$ for 4 days (Figure 1). The MP abundance in barnacles increased with the MP concentration for both fiber and fragment. The abundance of fragments in the barnacles exposed to 1000 items L$^{-1}$ was significantly higher than those exposed to 100 items L$^{-1}$ and 10 items L$^{-1}$, but there was no difference between 10 items L$^{-1}$ and 100 items L$^{-1}$. Differences in the abundance of fibers among all the concentrations were statistically significant (Table 1). The abundance of fragments in the barnacles did not vary with the duration of exposure, but that of fibers was significantly higher after 4 days of exposure than on Day 2 and Day 8 (Table 1). There was no significant difference between the abundance of two forms of MPs for all the exposure durations and concentrations (Table 2).

### 3.2 Regression between MP abundance and exposure concentration

To validate *A. amphitrite* as a bioindicator of MPs, linear regression equations were computed to relate the mean MP abundance in the barnacles and that in the water for both the 4-day and 8-day exposure groups. Since the relationships were not linear for the 4-day exposure groups (Figure 2), the exposure concentrations in the water were log-transformed. Two regression lines are computed (Figure 2), and the equations are as follows:

$Fragment: MP_{barnacle}$

$= 8.854 \times log(MP_{water}) - 6.346 \left(R^2 = 0.9747, p = 0.1017\right)$

(Equation 1)

$Fiber: MP_{barnacle}$

$= 7.943 \times log(MP_{water}) - 6.265 \left(R^2 = 1, p < 0.005\right)$

(Equation 2)
where
$MP_{barnacle}$ is the MP abundance in barnacles (items g$^{-1}$), and
$MP_{water}$ is the exposure concentration in the water (items L$^{-1}$)

Regression equations relating MPs (fragment and fiber) in the barnacles and that in the water were also computed for the 8-day exposure group (Figure 2):

$Fragment: MP_{barnacle}$

$= 0.01630 \times MP_{water} + 4.64 \left(R^2 = 0.9974, p < 0.005\right)$

(Equation 3)

$Fiber: MP_{barnacle} = 0.007881 \times MP_{water} + 0.5923 \left(R^2 = 0.9833, p = 0.0826\right)$
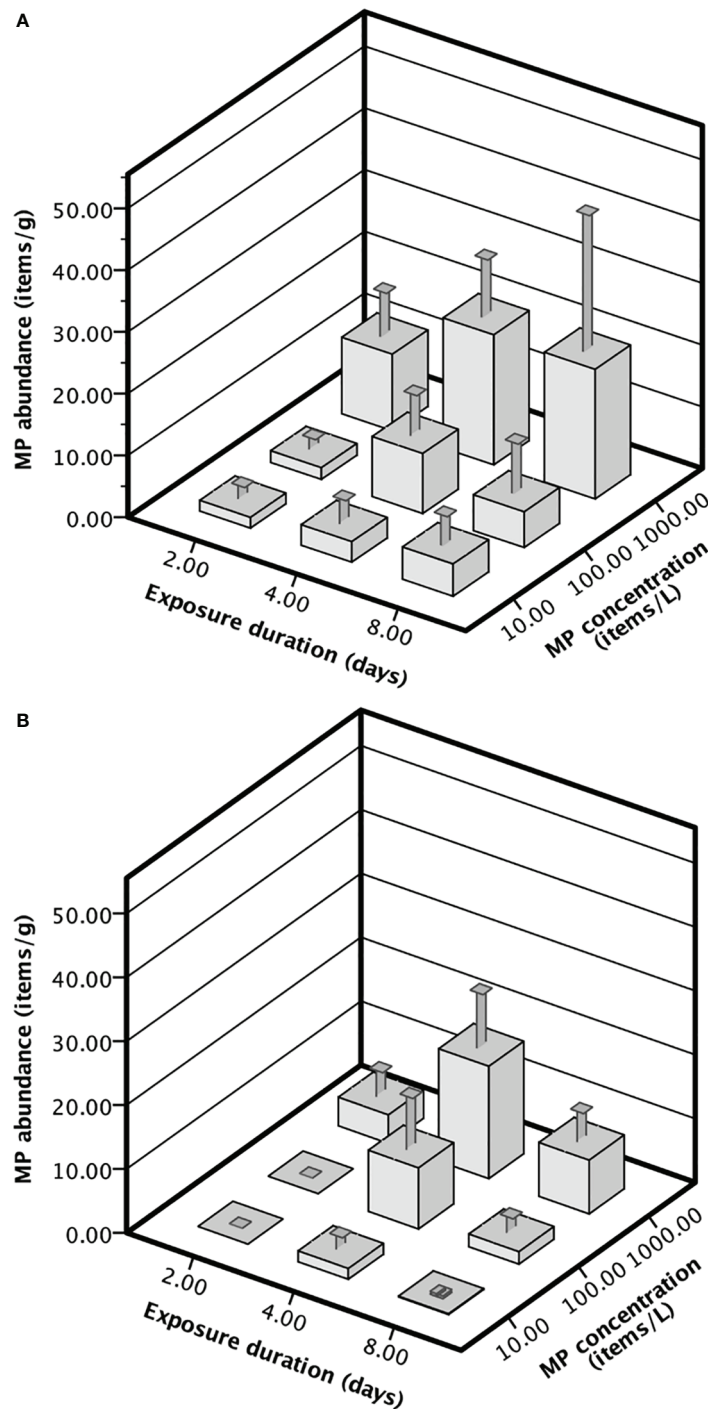
**FIGURE 1**
The MP abundance (mean ± SE, n = 10) in *A amphitrite* exposed to different concentrations of **(A)** fragment, or **(B)** fiber for 2, 4, or 8 days.

(Equation 4)

Only the regression equations computed using 4-day exposure data for fibers and 8-day exposure for fragments were significant. Since the fiber is the most dominant form of microplastics in the field-collected barnacles, the MP concentration in the water of each study site in Xu et al. (2020) was estimated by converting Equation 2, which becomes:

$$MPenvironment = 10 \ (0.1260 \times \ MP_{barnacle} + \ 0.7887)$$

TABLE 1 The effect of exposure duration and MP concentration on the abundance of fragments and fibers in *A. amphitrite*.

| Exposure to MP fragments | | | | Exposure to MP fibers | | | |
|---|---|---|---|---|---|---|---|
| | Df | H value | p value | | Df | H value | *p* value |
| Concentration | 2 | 19.853 | **<0.001** | Concentration | 2 | 23.885 | **<0.001** |
| Day | 2 | 4.515 | 0.105 | Day | 2 | 13.368 | **<0.005** |
| Interaction | 4 | 2.344 | 0.673 | Interaction | 4 | 2.793 | 0.593 |
| *Post hoc* test among different concentrations | | | | *Post hoc* test among different concentration | | | |
| | Z | | p value | | Z | | p value |
| 100 - 10 | 0.646 | | 0.518 | 100 - 10 | 1.487 | | **<0.05** |
| 1000 - 10 | 4.141 | | **<0.001** | 1000 - 10 | 4.775 | | **<0.001** |
| 1000 - 100 | 3.595 | | **<0.001** | 1000 - 100 | 3.288 | | **<0.005** |
| *Post hoc* test among different exposure durations | | | | *Post hoc* test among different exposure durations | | | |
| | Z | | p value | | Z | | p value |
| 4 - 2 | 2.097 | | 0.108 | 4 - 2 | 3.627 | | **<0.001** |
| 8 - 2 | 0.752 | | 0.452 | 8 - 2 | 1.411 | | 0.158 |
| 8 - 4 | -1.345 | | 0.268 | 8 - 4 | -2.216 | | **<0.05** |

The results were tested by the Scheirer-Ray-Hare test followed by *post hoc* test. *p* value< 0.0.5 are shown in bold letters.

(Equation 5)

where

$MP_{environment}$ is the MP concentration in the water at each study site (items $L^{-1}$), and $MP_{barnacle}$ is the MP concentration in field-collected barnacles reported upon by Xu et al. (2020).

Using Equation 5 and the MP abundance in the field-collected barnacles (Xu et al., 2020), the mean and medium predicted MP concentration in the coastal waters was 31.26 items $L^{-1}$, and 14.10 items $L^{-1}$, respectively (Table 3, Figure 3).
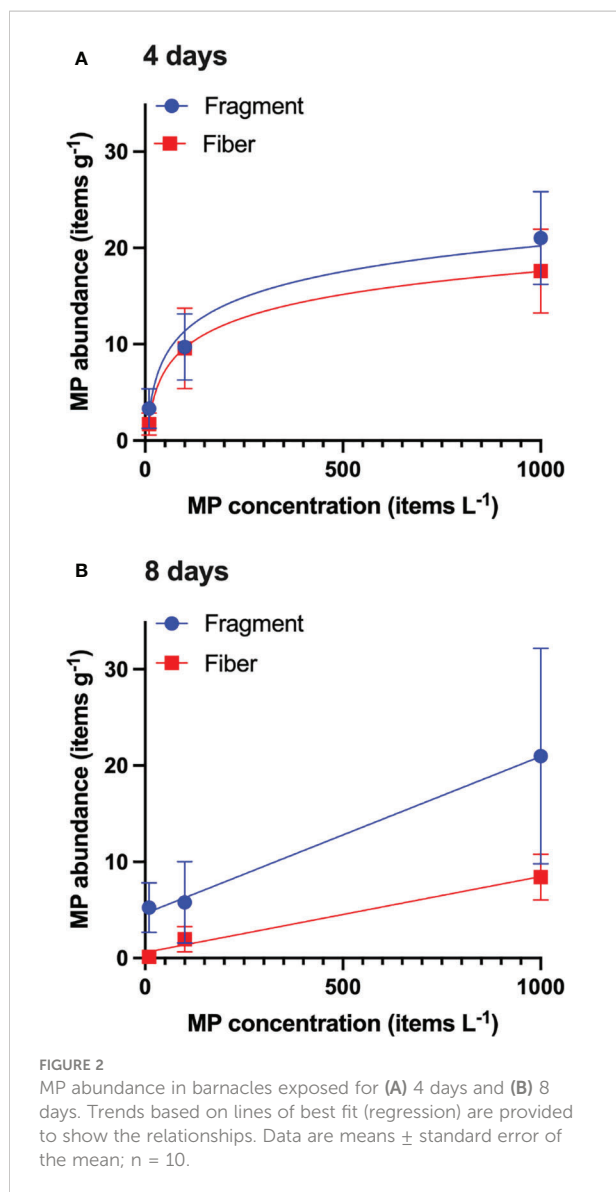
# 4 Discussion

## 4.1 Accumulation and ejection of MPs by barnacles

The concentration of both the fragment and fiber in the barnacles increased from Day 2 to Day 4. No further increase, however, was observed from Day 4 to Day 8 for the fragment, while a considerable decrease was found for the fiber. The MP concentration in an organism is a balance between the rates of

TABLE 2 Comparisons between the concentration of fragments and fibers in barnacles under the same exposure conditions.

| Exposure duration (days) | MP concentration in water (items $L^{-1}$) | MP abundance in barnacles (items $g^{-1}$) | | |
|---|---|---|---|---|
| | | Fragment | Fiber | *p* value |
| 2 | 10 | 1.75 ± 3.69 | 0 | 0.474 |
| | 100 | 1.90 ± 3.22 | 0 | 0.211 |
| | 1000 | 12.39 ± 11.64 | 4.47 ± 6.48 | 0.110 |
| 4 | 10 | 3.33 ± 6.50 | 1.71 ± 3.64 | 0.721 |
| | 100 | 9.71 ± 10.85 | 9.57 ± 13.22 | 0.899 |
| | 1000 | 21.04 ± 15.22 | 17.59 ± 13.81 | 0.528 |
| 8 | 10 | 5.24 ± 8.13 | 0.14 ± 0.43 | 0.087 |
| | 100 | 5.79 ± 13.40 | 1.97 ± 4.18 | 0.721 |
| | 1000 | 20.99 ± 35.41 | 8.42 ± 7.56 | 0.535 |

*Mann-Whitney test

MP abundance in barnacles exposed for **(A)** 4 days and **(B)** 8 days. Trends based on lines of best fit (regression) are provided to show the relationships. Data are means ± standard error of the mean; n = 10.

MP ingestion and removal. Since the accumulation of MPs was the highest on Day 4, this indicates the efficiency of MP removal was improved in the latter part of the experiment. Crisp and Southward (1961) reported that compared with those feeding on animal food, barnacles feeding on dinoflagellate with a thick cell wall produced larger numbers of fecal pellets containing undamaged plant cells. Since MPs are undigestible, they should be treated the same as dinoflagellates, and were eliminated by the barnacles through fecal production. When more and more MPs were accumulated in the intestine, this may trigger a faster removal of MPs, as shown in the latter part of the experiment. When the amount of inorganic particulates in the water increases, marine mussels reduce the gut retention time of ingested particles. This strategy allows the mussels to remove the non-nutritious particles efficiently (Galimany et al., 2013). Laboratory experiments that monitor the gut retention time of

barnacles upon exposure to different MP concentrations can clarify whether barnacles possess a similar adaptation for handling MPs.

Fragments and fibers used in this experiment have a similar surface area, but the former has a smaller shape factor. Although there was no significant difference between the abundance of fibers and fragments in the barnacles for all the exposure concentrations and durations, the removal of fibers was more efficient than fragments after 8 days of exposure, resulting in an insignificant difference in the fiber concentration between Day 2 and Day 8. The results agree with Zhao et al. (2018), who reported that plastics in the feces of mussels had larger shape factors, while plastics with smaller shape factors tend to retain in digestive glands/guts. The irregular shape of fragments could also lengthen their retention time in the digestive system of barnacles and might induce physical damage to the gut by their sharp edges, as demonstrated in other marine organisms (Wright et al., 2013).

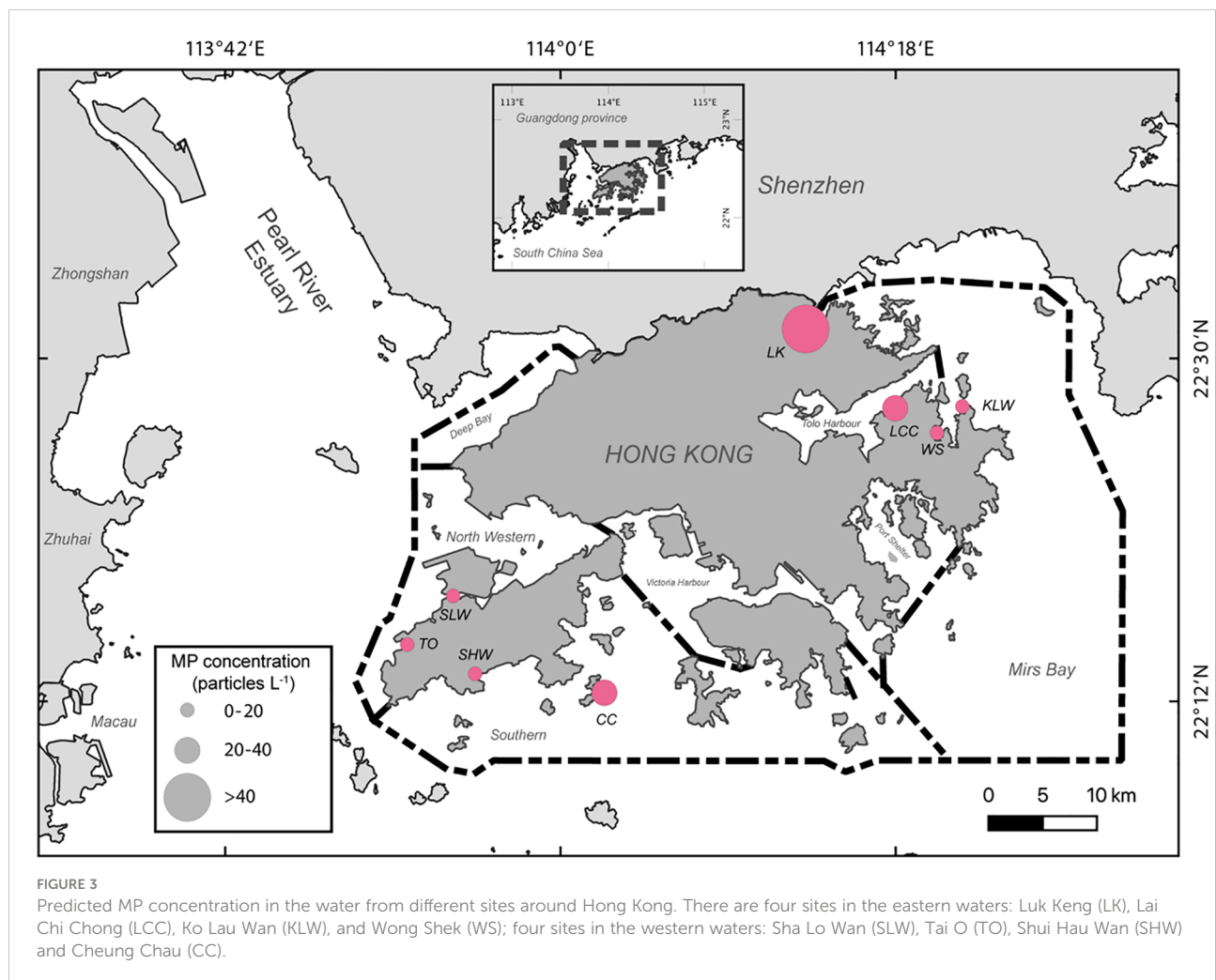## 4.2 Evaluation of *A. amphitrite* as a bioindicator for MP pollution

Using marine organisms, such as barnacles, as bioindicators of pollutants have pros and cons compared with direct water sampling. MPs could accumulate in barnacles during a period. Therefore, the concentration of MPs represents time-averaged pollution. By contrast, direct water sampling only reflects the MP concentration at the time of sampling, resulting in large temporal variations in the MP concentration in water due to fast water movement in the sea. The daily tidal movement further complicates the distribution of MPs in the coastal waters (Malli et al., 2022). Although the disadvantage of direct water sampling could be overcome by repeated sampling, this inevitably increases both the labor and time cost of the monitoring program. On the other hand, the MP abundance in barnacles could be several orders of magnitude smaller than the corresponding MP concentration in the water column, and the rapid removal of MPs aggravates the problem through fecal production.

The present study has demonstrated a positive correlation between the MP concentration in barnacles and the exposure concentration, regardless of the form of MPs and the exposure duration. The results indicate that *A. amphitrite* could be a potential bioindicator of MP pollution. To validate the accuracy of the predicted MP concentrations in the coastal waters, results were compared with published data. In a study of MPs from the effluents of sewage treatment works and stormwater discharging into Victoria Harbor, Hong Kong, the average concentration of MPs ranged from 0.59 to 10.816 items $L^{-1}$, with the highest concentration recorded as 11.927 items $L^{-1}$ (Mak et al., 2020). Sewage and water samples were collected from sewage treatment works and stormwater drains, respectively, in Hong Kong in

TABLE 3 MP concentrations in the water predicted from the measured MP abundance in barnacles in our previous study (Xu et al., 2020), using the equation computed for the 4-day exposure group.

| Site | MP abundance in barnacles (items g$^{-1}$) | Predicted MP concentration in water (items L$^{-1}$) [a] |
|---|---|---|
| Shui Hau Wan | 1.416 | 9.27 |
| Wong Shek | 1.425 | 9.29 |
| Sha Lo Wan | 2.307 | 12.00 |
| Ko Lau Wan | 2.564 | 12.93 |
| Tai O | 3.134 | 15.26 |
| Cheung Chau | 5.834 | 33.40 |
| Lai Chi Chong | 6.226 | 37.43 |
| Luk Keng | 10.256 | 120.49 |
| Mean: | | 31.26 |
| Median: | | 14.10 |
| [a]Equation for prediction: MP $_{environment}$ = 10 ^ (0.1260 × MP $_{barnacle}$ + 0.7887) | | |



FIGURE 3
Predicted MP concentration in the water from different sites around Hong Kong. There are four sites in the eastern waters: Luk Keng (LK), Lai Chi Chong (LCC), Ko Lau Wan (KLW), and Wong Shek (WS); four sites in the western waters: Sha Lo Wan (SLW), Tai O (TO), Shui Hau Wan (SHW) and Cheung Chau (CC).

2021, and the MP abundance ranged from 0.07 to 91.9 items L$^{-1}$ in the sewage samples and from 0.4 to 36.48 items L$^{-1}$ in the water samples (Zhang et al., 2022). The MP concentration in the surface water of small-scale estuaries in Shanghai, China, ranged from 13.53 to 44.94 items L$^{-1}$ (Zhang et al., 2019), the highest MP concentration recorded in China (Wang et al., 2022). The predicted concentrations of MPs in the coastal waters of Hong Kong using 4-day exposure data (predicted mean: 31.26 items L$^{-1}$, medium: 14.10 items L$^{-1}$), therefore, were close to the published data but on the high side.

Since the accumulation of MPs was the highest on Day 4, using the 4-day exposure data to estimate the environmental concentrations of MPs in the coastal waters could improve the prediction accuracy, mainly because the MP concentration in the barnacles is generally very low. However, the removal of MPs by *A. amphitrite* was more efficient after 8 days of exposure, which probably better reflects the long-term accumulation of MPs in the barnacles. The predictive power of the model can be improved by increasing the frequency of sampling during the exposure duration. The simplification of the exposure conditions in the experiment further limits the use of the experimental results in estimating the environmental concentration of MPs in the coastal waters (Herricks and Schaeffer, 1985). In the field environment, the accumulation of pollutants can be influenced by multiple uncontrollable factors, such as disease, weather, and other environmental stresses (Su et al., 2019). For example, the seasonal variability in metal and metalloid burdens was reported for mussels (Knopf et al., 2020). In the ocean quahog (*Arctica islandica*), copper, zinc, and cadmium concentrations were generally lower in winter when the lead concentration was the highest (Swaileh, 1996). These variations were attributed to the seasonality of the reproductive cycle and food availability, resulting in changes in body weight and composition (Sheehan and Power, 1999). In contrast to a relatively stable laboratory experiment, field populations of barnacles are constrained by various environmental stresses. For example, barnacles are protected by the closure of opercular plates and stop feeding during low tides when they are exposed to air. Seasonal fluctuations in temperature also influence the feeding behavior of barnacles. Nishizaki and Carrington (2014) reported that *Balanus glandula* showed a clear thermal optimum between 10 and 15°C, and feeding was reduced at high (25°C) and low (5°C) temperatures. A better understanding of the seasonal variations in the physiology of barnacles is essential to the development of more realistic models in predicting the environmental concentrations of MPs.

The gut retention time and egestion of MPs could influence the accumulation of MPs and further influence the prediction accuracy. According to the results from an exposure experiment with barnacle larvae, the retention time of MPs was inversely proportional to the size of MPs. Larger MPs were expelled in a shorter time, while MPs with a smaller size were retained for a longer time (Yu et al., 2021). If adult barnacles could also expel larger MPs quickly and retain smaller ones, the absence of larger MPs in barnacles could introduce bias to the estimation of MPs concentration in the environment. In addition, the water quality could also influence the depuration of MPs by barnacles. Barnacle species in the estuarine area can depurate MPs faster than species in pristine waters (Yu et al., 2021). Aging and biofouling could also influence the accumulation of MPs in organisms (Vroom et al., 2017). To further explore the potential of barnacles as bioindicators, aged MPs or MPs with biofilms could be used. All in all, factors that can influence the accumulation of MPs in barnacles could be included in the prediction equation, based on further experiments with more independent variables.

The accuracy of the prediction can further be improved by increasing the number of MP concentrations in the exposure experiment and incorporating more environmental factors, e.g., seasonal change in temperature, in the experimental design.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

S-GC - Supervision, Funding acquisition, Resources, Methodology, Writing - review and editing XX - Methodology, Data curation, Software, Writing - original draft C-YW - Funding acquisition, Resources, Methodology JF - Funding acquisition, Methodology, Resources. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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

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