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Effects of food presence on microplastic ingestion and egestion in *Mytilus galloprovincialis*

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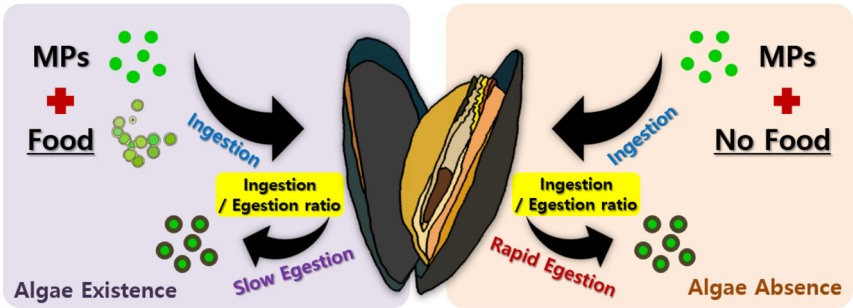
HIGHLIGHTS

- Effects of food on ingestion and egestion of MPs in mussels were examined.
- MPs egestion rates of mussels were investigated for 24 h after exposure.
- MPs exposed with algal food persisted in the mussels longer.
- Food existence can affect the cycle or fate of MPs in marine environments.

GRAPHICAL ABSTRACT



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ABSTRACT

Plastic wastes are widespread pollutants in marine environments and several studies have focused on their impacts on different ecosystems. Microplastics (MPs, < 5 mm) have been the focus of a particularly extensive investigation because of their ubiquity, large surface area, interactions with organisms, and the challenges they present in terms of disposal and management. However, studies regarding their fates and life cycle in ecosystems are still limited. This study examined the effects of presence of food (the green microalga *Dunaliella salina*) on egestion rate of polyethylene MPs in the mussel *Mytilus galloprovincialis*. Ingestion and egestion rates were calculated after 6, 12, 18, and 24 h of depuration. The results suggest that MPs exposed to algal food persisted in the mussels. A single exposure of MPs without food induced relatively rapid excretion by the mussels compared to MPs exposure with food. This could be attributed to the ability of mussels to distinguish between nutritive foods and unusable suspended particles. Thus, environmental factors, such as food abundance, can affect the cycle or fate of MPs in marine environments.

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1. Introduction

Research on plastic pollution in marine environments has increased exponentially in the recent decades (Strungaru et al., 2018; Alimba and Faggio, 2019). In particular, small particles of manufactured or weathered plastic (microplastics (MPs), <5 mm), characterized by global abundance, have been studied extensively. MPs are widely distributed and have been monitored in polar regions (Lusher et al., 2015; Waller et al., 2017), tropical regions (Lima et al., 2014; Do Sul et al., 2014), the East (Ng and Obbard, 2006; Isobe et al., 2015), the West (Claessens et al., 2011; Collignon et al., 2012; Alomar et al., 2016; Bellas et al., 2016), coastal areas (de Lucia et al., 2014; Stolte et al., 2015), open seas (Reisser et al.,

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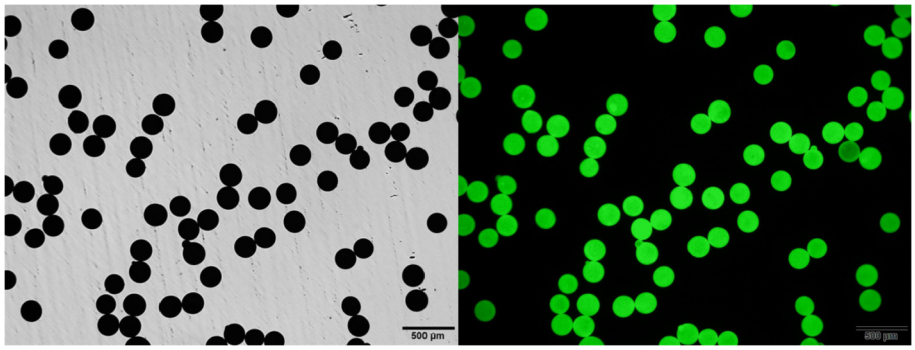

Fig. 1. Images of microplastics (MPs) in bright field (left) and fluorescent (right) modes of an optical microscope. Scale bar = 500 μm.

2014; Romeo et al., 2015), sea surfaces (Song et al., 2014, 2015), and the deep sea (Van Cauwenberghe et al., 2013; Taylor et al., 2016).

The distributions of MPs, and their effects on marine ecosystems in particular, are also being researched extensively (Hidalgo-Ruz et al., 2012; Lusher, 2015). Several studies have focused on the impacts of small plastics on marine organisms such as fish (Cedervall et al., 2012; Ferreira et al., 2016; Alomar et al., 2017; Brandts et al., 2018a; Ory et al., 2018; Savoca et al., 2019), invertebrates (Canesi and Corsi, 2016; Setälä et al., 2016; Messinetti et al., 2018; Weber et al., 2018), bivalves (Canesi et al., 2016; Bour et al., 2018; Brandts et al., 2018b; Oliveira et al., 2018), and microorganisms (Sjollema et al., 2016; Zhang et al., 2017; Okubo et al., 2018; Prata et al., 2018). The representative model test *Mytilus sp.* is one of the most studied genera in this regard. Several studies have focused on the relationships and interactions between MPs and *Mytilus sp.*, because these filter feeders play an important role in marine ecosystems (Browne et al., 2008; Farrell and Nelson, 2013; Van Cauwenberghe and Janssen, 2014; Faggio et al., 2018). Previous studies have investigated the impacts of MPs on the enzyme activities (Von Moos et al., 2012), tissues and cells (Von Moos et al., 2012; Van Cauwenberghe et al., 2015; Canesi et al., 2016; Kolandhasamy et al., 2018), behavior (Ward and Kach, 2009; Wegner et al., 2012; Woods et al., 2018), and physiology (Browne et al., 2008) of *Mytilus sp*. However, no research has been conducted on the impacts of environmental conditions on the ingestion and egestion processes in *Mytilus sp*. Ingestion and egestion in marine organisms are significant processes that can potentially influence the cycling and transformation of, and secondary pollution by, MPs (Browne et al., 2011). Additionally, these processes can influence exposure routes for other organisms. Understanding the ingestion and egestion of MPs in marine organisms can thus elucidate their fate in various environments under different environmental conditions.

Therefore, this study focused on the relationships between MP ingestion and egestion rates and environmental conditions, especially food abundance. It was assumed that the presence of food (algae) would affect the ingestion and egestion of MPs in mussels. Therefore, these processes were investigated in mussels as food alga presence (AP) or food alga absence (AA). The quantities of MPs ingested or egested were evaluated after several depuration periods. In this study, the Mediterranean mussel (*Mytilus galloprovincialis*) was used as the test species. This marine bivalve is widely used as a test species and bioindicator due to its wide availability and global distribution (Al-Sabti and Kurelec, 1985; Amiard et al., 1986; Sanjuan et al., 1997; Laffon et al., 2006; Capillo et al., 2018).

2. Materials and methods

2.1. Test materials and organisms

Polyethylene MPs (fluorescent polyethylene microspheres UVPMS-BG-1.025) were procured from Cospheric Inc. (Santa Barbara, CA, USA) in dry powder form. These spheres were

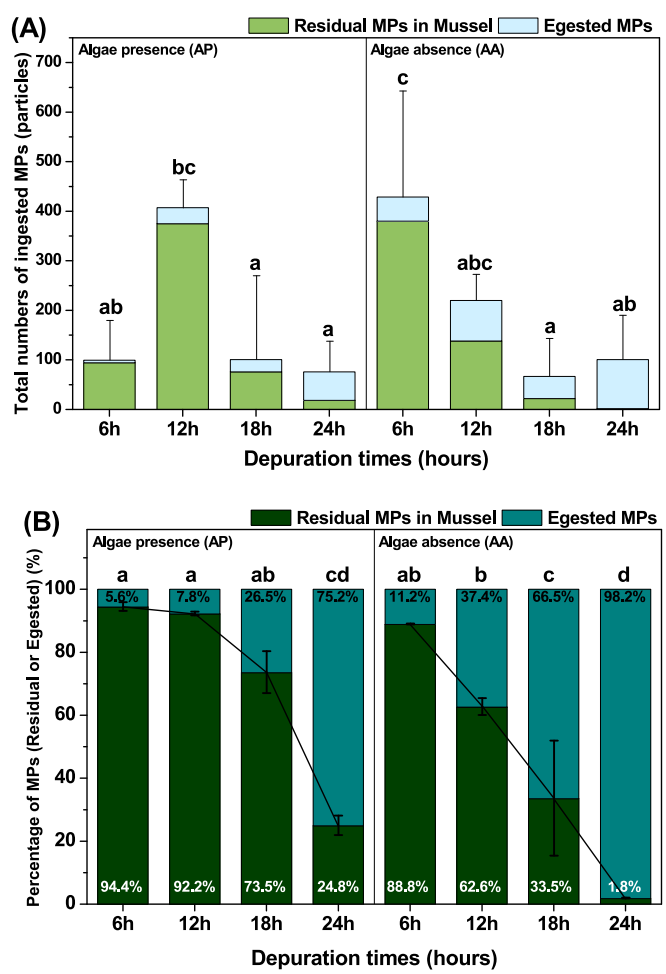

Fig. 2. (A) Total number of ingested MPs in the presence or absence of algae (AP and AA, respectively). (B) Percentages of residual and egested MPs for AP or AA treatments after 6, 12, 18, and 24 h of depuration. Error bars represent standard deviation of the mean of five replicates. Lowercase letters (a, b, c, and d) indicate significant differences between treatment groups at $p < 0.05$.

characterized as having excitation and emission wavelengths of 414 nm and 515 nm, respectively, and a density of 1.026 g/cc. The nominal size of the MPs was 180–212 μm; the measured size (analyzed for over 200 particles in the laboratory using a microscope and image analyzing program) was 203.84 ± 13.76 μm. Previous studies reported that microplastics with these sizes are dominant in real environmental conditions (Desforges et al., 2014; Fischer et al., 2016; Tanaka and Takada, 2016; La Daana et al., 2017; Di and Wang, 2018; Zhu et al., 2018). To quantify their weight, the number of particles in 2 mg was replicated five times with a microbalance. The number of MP particles per mg was 200. Images of the MPs obtained from the bright-field and fluorescent modes of an optical microscope are given in Fig. 1.

The *Mytilus galloprovincialis* used in this study was procured commercially. Individuals that were 5 ± 0.5 cm in size were collected and acclimated in artificial seawater (ASW, 30 ± 1 PSU) and dark conditions at 10 ± 0.1 °C for a week. No food was supplied during the acclimation and the ASW was continuously aerated and changed daily. The food alga, *Dunaliella salina*, was provided by the Library of Marine Samples, Korea Institute of Ocean Science & Technology (Geoje, Korea) and acclimated in the laboratory for more than 10 months. The algae was incubated in the F/2 medium at 20 °C in light:dark (16:8 h) conditions.

2.2. Test design

To maximize buoyancy and ensure that the MPs stayed in the water column for as long as possible, a 500-mL mass cylinder with a depth of 230 mm and a diameter of 60 mm was used as the test vessel. First, it was filled with 10 ± 0.1 °C and 500 mL ASW and one mussel was placed at the bottom. Subsequently, 1 mL of *D. salina* (1×10^7 cells mL^{-1}) was added to the AP treatment vessels. No algal food was added to the AA treatment vessels. Test vessels with the ASW and the mussels were placed in an incubator at 10 °C 30 min prior to the start of the test. A stock solution of MPs with a concentration of 5 g L^{-1} was prepared with the ASW and 0.1% of a surfactant, Tween 20 (Duksan Chemical, Daejeon, Korea); the later suspended the test material in the ASW. After an acclimation period of 30 min, each mussel was examined for an opened shell and 1 mL of the MP stock solution was injected into the water column. The final concentrations of MPs and the surfactant in the test column were 10 mg L^{-1} (1000 particles per 500 mL ASW) and 0.0002%, respectively (the showed no toxicity surfactant of this concentration in preliminary experiments). The exposed mussels were transferred to clean ASW after an hour of exposure which is a long enough exposure time for mussels to filter MPs in water column. Previous studies reported that the average filtration rate of *Mytilus* sp. was about 0.5–4 L h^{-1} ind^{-1} (Schulte, 1975; Clausen and Riisgård, 1996; Riisgård et al., 2003). Subsequently, the depuration process was conducted for 6, 12, 18, and 24 h at 10 °C under dark conditions. Additionally, to clarify the validity of this test, a negative control (without food or MPs) were also set up; there were no abnormalities on the mussels). Each treatment (negative control, AP, and AA, respectively) of the experiment used five individuals.

2.3. Microplastic recovery and quantification

The mussels were collected after the depuration process and their shells were washed with tap water to prevent contamination and remove MPs adsorbed on the shell surfaces. After then, the shell was removed and inner soft tissue was carefully isolated. The soft tissue was dried overnight in an oven at 65 °C followed by digestion with a mixed solution of 35% H_2O_2 and 1 N KOH (1:10) at 80 °C. The dissolved liquid samples were passed through a Whatman® No. 2 filter (pore size: 8 μm) after 48 h of digestion and the filters were dried at room temperature overnight. The clean ASW used during depuration was also filtered and dried using the same process. Finally, the fluorescent MPs in the filtered tissue samples, filtered ASW, and all glassware used during the experiment were observed using a medical loupe on the UV transilluminator (DUT-260, Core Bio System Co. Ltd., Seoul, Korea). Before the experiments, all glassware and instruments were washed with distilled water to prevent contamination. The number of MP particles was counted, and the residual or egestion rates were calculated as follows:

$$\text{Residual Rate } (\%) = \frac{\textit{Residual MPs in dissolved samples}}{(\textit{Residual MPs in dissolved samples} + \textit{Egested MPs in clean ASW and secretions})} \times 100$$

$$\text{Egestion Rate } (\%) = \frac{\textit{Egested MPs in clean ASW}}{(\textit{Residual MPs in dissolved samples} + \textit{Egested MPs in clean ASW and secretions})} \times 100$$

Additionally, the secretions (agglomerated feces or pseudofeces with MPs from mussels) were collected with a Pasteur pipette and observed under an optical microscope (Olympus BX 51, Olympus, Japan) equipped with a fluorescent filter (excitation wavelength: 460–495 nm; emission wavelength: 510 nm; U-MWIB3; Olympus Cooperation, Tokyo, Japan) to examine 1) the presence of MPs in the mussel excrement, 2) morphological changes in the MPs after the digestion process, and 3) the possibility of secondary exposure by MPs present in the excrement.

2.4. Statistical analyses

Data were analyzed using the SPSS statistical software (version 24.0 for Windows, SPSS Inc., Chicago, IL, USA). The reliability of data was confirmed by checking for normality and homoscedasticity of variance using the Shapiro–Wilk and Levene variance homogeneity tests. One-way analysis of variance (ANOVA) and Bonferroni tests were conducted to determine the significance of multiple comparisons and identify significant differences.

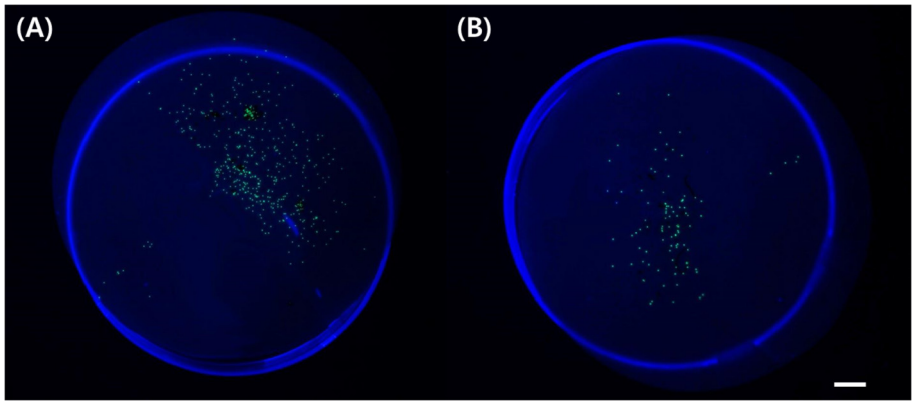

Fig. 3. Representative UV transilluminator images of tissue samples and MPs after digestion and filtration. Residual fluorescent MPs (green fluorescent spheres) in mussel samples exposed to MPs (A) with algal food (AP) and (B) without algal food (AA) after the 12 h depuration period. Scale bar = 1 cm.

3. Results and discussion

3.1. Effects of food presence and depuration duration on MP egestion rates

As shown in Fig. 2A, the total number of ingested MPs did not indicate a significant difference between the AP and AA treatments. Therefore, the presence of algal food did not have a significant effect on the total number of ingested MPs depending on the depuration time. However, the ratios of egested to total ingested MPs varied with food present at several depuration time points (Fig. 2B). The average ratios of egested MP particles increased as the depuration time increased from 6 to 24 h. MPs ingested with the AP treatment, in particular, were excreted slowly; this was in contrast to the situation with the AA treatment. In the AP treatment, 5.6, 7.8, 26.5, and 75.2% of the MPs were excreted after 6, 12, 18, and 24 h of depuration, respectively. Therefore, nearly 75% of the ingested MPs were excreted after 24 h of depuration. In contrast, 11.2, 37.4, 66.5, and 98.2% of the MPs were excreted after 6, 12, 18, and 24 h of depuration, respectively, during the AA treatment. Thus, nearly all ingested MPs were excreted after 24 h. Additionally, there were significant differences between the two treatments in terms of both the egestion rate and absolute quantity of residual MPs in the mussels for all durations except the 6 h depuration groups (Figs. 2A and 3).

Consequentially, egestion rates for the AP and AA treatments were also significantly different for the 12 and 24 h depuration groups (Fig. 2B). Although slight differences in egestion rates were observed for the 6 and 24 h depuration groups, no statistical significance was established. Nevertheless, a declining trend of residual MPs in the mussel bodies and an increasing tendency of MP egestion were observed. Additionally, AP treatments indicated that egestion rates for the 6, 12, and 18 h depurations showed no statistical significance; however, the 24 h depuration was statistically significant. There was no statistical significance in the egestion rates observed for the 6 and 12 h depuration groups during the AA treatment; however, there were significant differences for the 12, 18, and 24 h depuration groups. This result suggests a significant increase in the egestion rate from 12 to 24 h for the AA treatment, in contrast to the AP treatment where no such increase is seen. Additionally, excreted MP was observed in both pseudofeces and feces (Fig. 4); however, there was no difference observed in the quantity of MPs excreted in the two ways. Furthermore, no breakdown or fragmentation of MPs was observed. Additionally, we could calculate the MP depuration rate in the mussels depending on either the PA or AA conditions for 24 h. In the case of AP and AA, MPs egestion by mussels can be described with the following equations (where t = depuration time):

$$\text{(a) Egestion (AP)} = 2.2718t^3 - 5.4222t^2 + 2.5821t + 6.1816$$

$$\text{(b) Egestion (AA)} = 1.3649t^2 + 22.205t - 12.422$$

3.2. Environmental implications

The present study is the first to investigate the relationships

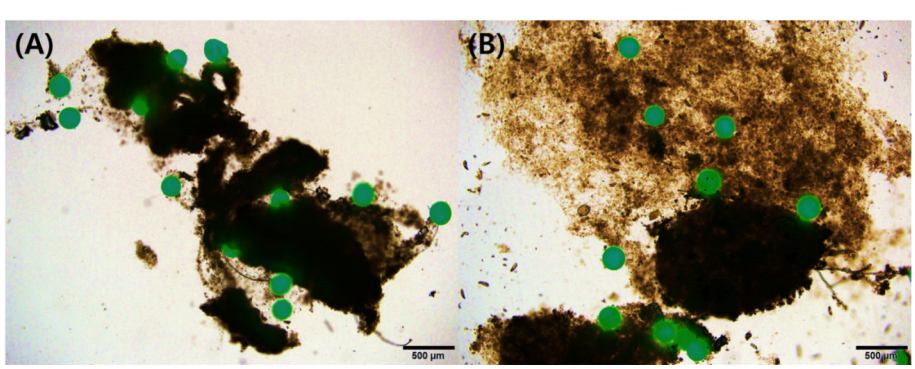

Fig. 4. Merged images of (A) pseudofeces and (B) feces containing egested MPs (green spheres) from mussels exposed to microplastics after a 24 h depuration period. Scale bar = 500 μm.

between food abundance and MP ingestion/egestion rates in a marine bivalve. The results of this study indicate that food presence did not significantly affect the total number of ingested MPs. However, the ratios of egested MPs after 6, 12, 18, and 24 h of depuration varied in response to the presence and absence of algal food. Previous studies have indicated that ingestion and assimilation in marine organisms are affected by several environmental factors. The factors that have been found to affect food intake and assimilate nutrients or contaminants include seasonal and site-based variations (Bayne et al., 1987; Cranford and Hill, 1999; Roditi and Fisher, 1999), flow rates and vertical positions (Tuchman et al., 2004), food quality (Camacho et al., 2000), and surface properties of suspended particles (Rosa et al., 2013). In this study, all environmental factors except food abundance were controlled. The results indicated no significant differences in the ingestion rates of MPs for the AP and AA treatments (Fig. 2). This could be attributed to the short exposure time (1 h). However, significant differences were observed between the AP and AA treatments with regard to egestion rates, especially for the 12 and 24 h depuration groups (Fig. 2B). These results also indicate that the MP egestion rate in mussels is affected by environmental factors (here, food abundance). Furthermore, environmental factors can affect the fate of MPs in other organisms that are not in marine environments. Previous studies have suggested that organisms such as lamellibranchs or bivalves can sort fine and coarse particles in their complex alimentary tracts (Purchon, 1977) and selectively reject particles through pseudofeces (Kiorboe and Mohlenberg, 1981; Newell and Jordan, 1983). Bricelj et al. (1984) concluded that the clam *Mercenaria mercenaria* could sort different algal species in its gut passage and rapidly eliminate algal species with lower utility. Those results support the findings of this study, which indicate that the mussels eliminated the MPs slowly when the MPs were ingested with useful and nutritious algal cells. On the other hand, MPs without algal food were rapidly excreted because of lack of nutrients for the mussels to absorb or utilize. Other previous studies also support the idea that bivalves can select the foods or other particles to save energy and ingest high-value food (Bricelj and Malouf, 1984; Brillant and MacDonald, 2000; Beninger et al., 2008). In a review, Ward and Shumway (2004) summarized many previous studies and concluded that bivalves can passively select food because of increasing uptake of organics and energy. Overall, bivalves can select only valuable food among ingested substances or particles and excrete poor quality materials in the form of pseudofeces. Therefore, MPs ingested with nutrient-rich food were excreted slowly because the valuable algal food and remained in the mussel bodies for relatively longer durations.

4. Conclusions

In this study, the effects of food abundance on the fate of MPs in marine ecosystems, was investigated via the ingestion and egestion of MPs in mussels. No significant differences in ingestion rates were observed for the AP and AA treatments. However, there were significant differences in terms of the egestion of MPs during the two treatment conditions. This could be attributed to the ability of mussels to sort their gut contents into useful food or useless suspended particles. The results of this study indicate that MPs ingested with algal food remained in the mussel bodies for relatively longer durations. These results suggest that ingestion and egestion of MPs by organisms is affected by food abundance. Furthermore, environmental factors can influence the fate of MPs in marine environments. However, in this study, the mussels were exposed to MPs for a short duration and other environmental factors or uncovered interactions that could potentially affect the egestion rates of MPs were excluded. For instance, the relationships between MP particles and algae in the bodies of mussels could induce the differences of depuration rates between AP and AA treatments. Therefore, future research should focus on the effects of various real environmental conditions on ingestion and egestion of MPs in marine organisms. Additionally, the importance of filter feeders in marine ecosystems and the interactions between filter feeders and MPs should also be studied.

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