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Fusion of microplastics into the mussel byssus

Qipei Li [a], Chengjun Sun [b], Youji Wang [c], Huiwen Cai [a], Lingyun Li [a], Jiana Li [a], Huahong Shi [a, *]

[a] State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai, 200241, China
[b] Key Laboratory of Marine Bioactive Substances, The First Institute of Oceanography, Ministry of Natural Resources, Qingdao, 266061, China
[c] National Demonstration Center for Experimental Fisheries Science Education, College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, 201306, China

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ABSTRACT:

Microplastics have been found to adhere to the surface of specific tissues or organs other than being ingested by the organisms. To further test the hypothesis that microplastics might get into specific body parts of organisms, mussel byssus was chosen as a target subject in the present study. In the field investigation, microplastics were found in mussel byssus, and the abundance of microplastics was 0.85−1.02 items/individual mussel and 3.69−9.16 items/g byssus, but the location of microplastics in byssus was not easily determined. Therefore, we simulated environmental conditions in the laboratory for mussels to form fresh byssus in the presence of microplastics. Three types of man-made microplastics (Polystyrene beads, Polyamide fragments, and Polyester fibers) were found in newly formed byssus of mussels after exposure to these test materials. We observed that microplastics not only adhered to the surface but also fused into the byssus of mussels. Since byssus is important for the well-being of mussels, the incorporation of microplastics into the byssus might impair the function of byssus. To the authors' best knowledge, this is the first study to show that microplastics can contact and fuse with the byssus of mussels during their formation, suggesting possible alternations for mussels to grip and interact with microplastics in the aquatic environments.

1. Introduction

Microplastic pollution has become a potential threat to living organisms and ecosystems around the world (Galloway et al., 2017; Law and Thompson, 2014). Generally, microplastics are defined as plastic particles or fibers that are smaller than 5 mm. There are various sources of two types of microplastics: primary microplastics, such as plastic pellets released from plastic industrial/manufacturing uses and microbeads used in personal care and cosmetic products; and secondary microplastics, such as plastic debris formed by fragmentation of larger plastic litter items in the ocean (Rocha-Santos and Duarte, 2015). Microplastic has a higher surface-to-volume ratio compared to the large plastic item and can adsorb chemical pollutants from the surrounding water, which can increase the bioavailability of pollutants (Barnes et al., 2009; Hidalgo-Ruz et al., 2012; Mato et al., 2001). Consequently, microplastics have more adverse effects on marine biota compared to large plastics.

Since mussels are easily accessible, have a wide geographical distribution, and can tolerate a range of salinities, they have been used as biomonitoring species for marine environmental pollutants (Avio et al., 2015; Browne et al., 2008; Li et al., 2016; von Moos et al., 2012). Most recently, mussels have also been suggested as biomonitoring species for investigating marine microplastic pollution globally (Li et al., 2019a). In previous studies, microplastic pollution research has been conducted in the soft tissues of mussels, such as stomach, intestine, gill, and foot (De Witte et al., 2014; Kolandhasamy et al., 2018; Li et al., 2016). Interestingly, microplastics were found to adhere to the surface of specific body parts of mussels that are not participating in the ingestion process (Kolandhasamy et al., 2018). Hence, we postulate that microplastics might get into specific body parts of organisms via a possible alternative pathway.

In order to inhabit various marine environments with intense currents, waves, and tides, mussels have developed byssal threads to attach to the substrate by adhesive plaques. The byssus enables

* Corresponding author.
E-mail address: hhshi@des.ecnu.edu.cn (H. Shi).

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mussels to form huge colonies in habitats (Hagenau et al., 2014; He et al., 2018; Seguin-Heine et al., 2014). The byssal threads are made up of fibrous, elastin-like and histidine-rich collagens, such as preCols, which are imbedded in a microfibrillar matrix (Sun et al., 2002). The adhesive plaques contain a series of mussel adhesive proteins, such as mfp-3, which works as the "glue" that binds mussels to the surface of the substrate (Lin et al., 2007). Anything affecting the integrity of the byssus will potentially impair the performance of the byssal thread and affect the survival of mussels. Therefore, it is very meaningful to use mussel byssus as a model system to study the interactions between the organism and exogenous environmental pollutants, such as microplastics.

Byssal thread is produced through an injection and extrusion molding like process (Waite, 1992). The formation of new and older byssal threads are illustrated in Fig. 1. Fresh byssal threads and adhesive plaques are produced by the mussel when it protrudes its foot outside the shell and finds footholds on the substratum (Fig. 1A). The mussel can produce the first thread within minutes to hours. Usually after two days, sufficient byssal threads are formed. Old threads tend to have darker yellowish to brownish color than the fresh ones due to oxidation (Sun et al., 2001)(Fig. 1B).

In this study, we first investigated microplastic pollution in wild and farmed mussel byssus, and then characterized microplastics in the field samples. Meanwhile, we tested the hypothesis that microplastics can accumulate and fuse into mussel byssus during its secretion by 3 days of microplastic exposure experiment in the laboratory. Ultimately, we clarified how microplastics contact and fuse with the byssus.

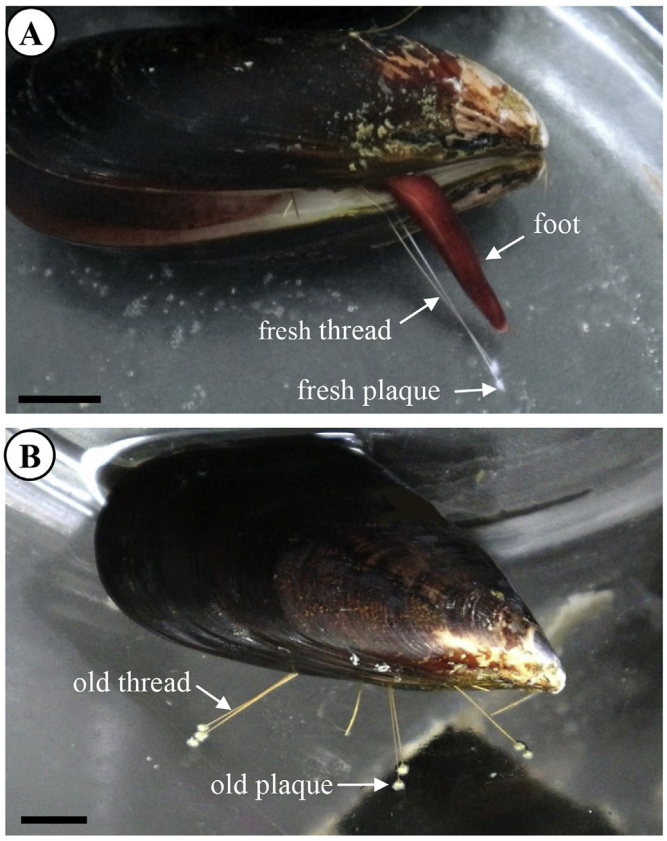

Fig. 1. The process of mussel byssus secretion in the laboratory. (A) One mussel (*Mytilus coruscus*, about 7 cm in shell length) was placed on the substratum and protruded its foot to form several fresh byssal threads and adhesive plaques. (B) After a few days, the mussel was strongly supported by a number of byssal threads. Scale bar = 1 cm.

2. Materials and methods

2.1. Sample collection

Mussels (*Mytilus* spp.) were obtained from a fishery farm (122°44′25″E, 30°44′22″N) near the Gouqi Island in the East China Sea in April 2018. The mussels were divided into two groups: wild and farmed mussels. Six replicate samples of mussels were collected for each group (n = 6). Each replicate consisted of 8 living mussels with similar sizes (Supplementary Table 1). They were individually put into aluminum foil bags and kept on ice immediately in the field, then transferred and frozen at −20 °C in the laboratory for further analysis of microplastics (Li et al., 2016). In addition, about 100 farmed individuals were collected and acclimated for laboratory exposure experiment.

2.2. Laboratory exposure experiment

The acclimated mussels were cultured with enriched oxygen aeration in artificial seawater (Red Sea Salt) at 28 ± 2‰ salinity, 18 ± 1 °C, and alternating light/dark cycles (12 h/12 h) for 2 weeks. During this period, the seawater was replaced 4–6 times depending on water turbidity (Qu et al., 2018). Twenty-four mussels were randomly selected and cleaned by carefully removing the associated epiphytes on the shell and old byssus outside the shell. Two mussels were arbitrarily placed in a glass tank with 2 L artificial seawater. Three treatments were set by adding three types of man-made microplastics and one control group without microplastics. Each treatment included three tanks. Mussels were fed with *Chlorella vulgaris* (2×10^6 cells/L) every day.

Three types of microplastics were used in this exposure experiment, including microbeads of polystyrene (PS), fragments of polyamide (PA) and fibers of polyester (PES) (Supplementary Fig. S1). The 10 μm green fluorescent PS microbeads (Fluoro-Max G1000, 1% solids), with a density of 1.05 g/cm^3, were bought from Thermo-Fisher. The white PA fragments were obtained from Sigma-Aldrich, which were stained using Nile Red based on the methods of Shim et al. (2016). The long and soft PES fibers were purchased from a local market and cut into tiny pieces using a dissecting scissor (Kolandhasamy et al., 2018). Processed PA fragments and PES fibers were passed through a metal sieve with 5 mm mesh size, and then collected on a nylon membrane filter (Millipore NY0504700) with 5 μm pore size. Aqueous stocks of PA fragments and PES fibers (approximately 1000 items/mL) were prepared by using a Sedgewick-Rafter counting chamber (Li et al., 2019b).

During the 3 days of exposure, the artificial seawater containing a single type of microplastics (approximately 1000 items/L) and the microalgae (*Chlorella vulgaris*, 2×10^6 cells/L) was replaced for each tank every day. In addition, all of the tanks were set up on a motor-driven oscillating cart with maximum displacement distance of 100 mm and reciprocated frequency of 33 rounds/min for 2 h/day to facilitate byssus production and keep the microplastics in suspension. The secretion of fresh mussel byssus using this method was recorded in a video (Supplementary Video 1). Meanwhile, the number and length of all secreted byssal threads were also recorded (Supplementary Table 1).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.envpol.2019.05.093.

2.3. Extraction of microplastics from the byssus of mussels

The byssus of specimens from both the field and exposure experiments were cut and collected in clean Petri dishes with glass covers prior to extraction of microplastics. The byssus from eight mussels was pooled together as one replicate and six replicates

were used for each field group. Concurrently, one blank control group without any tissue was carried out for correction of potential procedural contamination. In addition, the byssus from two mussels collected as one replicate and three replicates used for each exposure group due to the maximum capacity of cultured tanks at one time.

The extraction of microplastics from mussel byssus was performed as previously described (Li et al., 2015; Su et al., 2016). In brief, mussel shell length, total body weight and byssus wet weight were recorded (Supplementary Table 1). The pooled byssus of one replicate was transferred to a clean glass bottle and roughly 100 mL of hydrogen peroxide (30%, v/v) solution was added to break down the byssus. Then the bottles were sealed and shaken in a thermostatic oscillator at 80 rpm at 65 °C for 24–48 h until digestion is completed. After digestion, the supernatant liquid was filtered directly onto nitrocellulose membrane filters (47 mm diameter, 5 μm pore size; Millipore SMWP04700) by a vacuum-pumping system. Then the filters were placed in labeled and sealed dishes for microplastic observation and identification.

2.4. Visual observation and identification of microplastics

Fibers and fragments were observed under a Zeiss Discovery V8 stereo optical microscope, and the images were captured using an AxioCam Icc3 camera. Fluorescent microbeads were detected using an Olympus BX53 epifluorescence microscope equipped with an Olympus DP 80 camera (Ex. 460–500 nm, Em. 510–560 nm). For SEM observation, some man-made microplastic particles on the byssus after exposure were selected and dried by a freeze dryer. Then the morphology of these specimens was further examined by a Hitachi S-4800 Scanning Electron Microscope (SEM) in accordance with the methods of Su et al. (2016).

From the 151 isolated and visually identified particles in field samples, 102 common and suspected microplastics were randomly picked for validation with a micro-Fourier Transform Infrared (μ-FT-IR) spectroscope (Nicolet iN10 MX, Thermo-Fisher Scientific). Data acquisition of all test samples was performed with a database in the transmission mode with a 4 cm^{-1} resolution and a 3 s collection time (Yang et al., 2015). Comparison of all infrared spectra was done with the database provided by Thermo-Fisher and our own library of semi-synthetic celluloses. The spectrum matches were no less than 60% for most of the identified particles, and the characteristic band of known polymer types was also used for identification (Woodall et al., 2014).

2.5. Data statistical analysis

Data statistical analysis was done using software SPSS 22.0, Origin 9.0, and GraphPad Prism 6.0. Normality of data and homogeneity of variance were checked through the Kolmogorov-Smirnov's and Levene's test, respectively. A parametric method was employed in the samples of field investigations and the Kruskal-Wallis nonparametric tests were applied in the samples of laboratory exposures. A one-way analysis of variance (ANOVA) was used to determine the quantity difference of microplastics for more than two groups. Dunnett's test was used for post-hoc multiple comparisons. Statistical significance was accepted at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3. Results

3.1. Abundance and composition of microplastics in mussel byssus in the field investigation

In mussel byssus samples collected from the field, the average abundance of microplastics ranged from 3.69 to 9.16 items/g. byssus (wet weight) and 0.85–1.02 items/individual mussel (Fig. 2). Significantly higher numbers of microplastics were detected in both wild and farmed samples compared with the blank control by items/individual mussel ($p < 0.001$ for Wild, $p < 0.01$ for Farmed) (Fig. 2A). However, only wild samples had significantly more plastic items compared with the blank control by items/g. byssus ($p < 0.001$) (Fig. 2B). In addition, a large size difference between wild and farmed mussels results in a significant byssal mass difference between two groups, which accounted for the average abundance of microplastics in wild samples significantly greater than the latter based on the unit of weight ($p < 0.05$) (Fig. 2B, Supplementary Table 1).

The size, shape, and color of microplastics were similar in all tested field samples. The size range of 1.0–5.0 mm microplastics was more often observed than other size sections, accounting for 47–66% of the total microplastics detected (Supplementary Fig. S2A). Fibers were the most prominent shape (55–68%) followed by fragments and pellets (Supplementary Fig. S2B). Colorless particles (transparent and white) were prevalent in all samples, accounting for 54–58% of the total microplastics identified (Supplementary Fig. S2C). Generally, farmed mussel byssus contained a relatively higher proportion of fibrous microplastics compared with the wild samples.

Out of the 102 randomly selected particles, 80 particles were identified with a spectrum match over 60% according to μ-FT-IR analysis. Overall, 50% of these measured particles were verified to

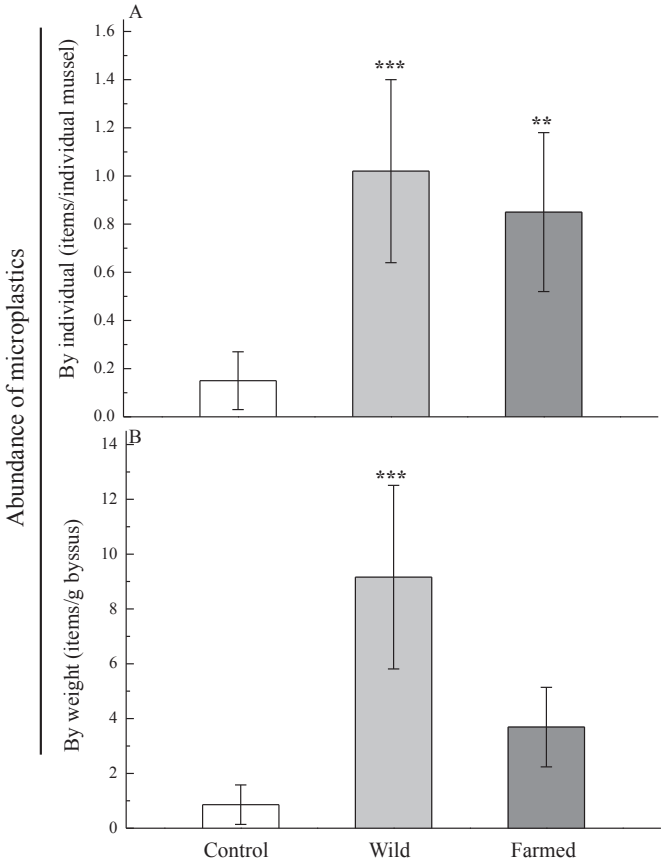

Fig. 2. The abundance of microplastics in the field samples by the terms of items/individual mussel (A) and items/g byssus (B). Each value represents as mean ± standard deviation of the six replicates (n = 6), and the byssus from eight mussels was pooled as one replicate. Asterisks represent the significant difference to the controls (One-way ANOVA followed by a Dunnett post hoc test, $^{**}p < 0.01$, $^{***}p < 0.001$).

be microplastics including polyester, polyethylene, polypropylene and rayon (Fig. 3, Supplementary Table 2). Among them, the most prevalent polymer type was polyester in both wild and farmed samples but followed by rayon in wild and polypropylene in farmed byssus. Additionally, the other 50% of particles were identified as non-microplastics and mainly made up of natural cotton and cellulose fibers as well as other inorganic substances (Supplementary Table 2).

3.2. Abundance and morphology of microplastics in mussel byssus in laboratory exposure experiment

In the laboratory exposure experiment, the accumulation of three types of man-made microplastics was observed in all harvested mussel byssus. After three days of microplastic exposure, the average abundance of microplastics in byssus altered from 0.25 to 1.96 items/individual byssus (Fig. 4). The number of each type of microplastics accumulated in byssus was significantly greater than that in the matching controls ($p < 0.05$). The highest average abundance of microplastics was found in the PES fiber treated group, while the lowest was detected in the PS beads treated group. In addition, no significant changes were found in the number and average length of byssal threads among all treatments ($p > 0.05$).

The morphology of man-made microplastics on the surface of mussel byssus was also observed in the exposure experiment (Fig. 5). It showed that three types of microplastics adhere to and fuse to newly formed byssus in different ways. For instance, some small particles such as 10 μm fluorescent PS microbeads could infiltrate the structure of byssus individually (Fig. 5$A_{1\text{-}2}$). PA fragments were liable to aggregate in the waters, thereby forming clumps that wrapped the byssal thread (Fig. 5$B_{1\text{-}2}$). PES fibers were soft and slender, so they were easy to form bundles twining around and adhere to the byssal thread (Fig. 5$C_{1\text{-}2}$).

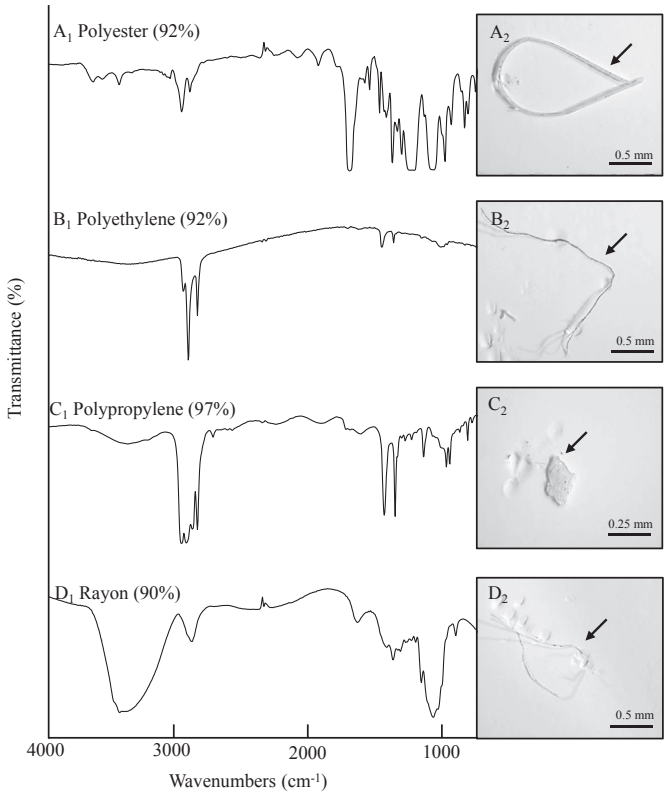

Fig. 3. Analysis with micro-Fourier Transform Infrared (μ-FT-IR) spectroscope (A_1-D_1) and microscopic images (A_2 –D_2) of selected types of microplastics found in the field samples. They are consisting of (A_1-A_2) Polyester; (B_1–B_2) Polyethylene; (C_1–C_2) Polypropylene; (D_1-D_2) Rayon.

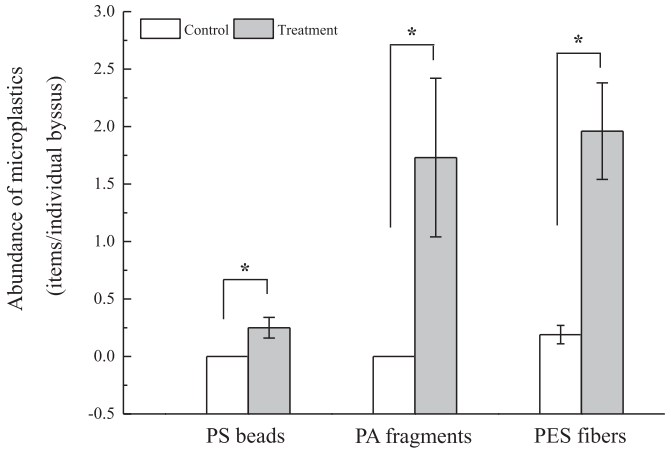

Fig. 4. The abundance of man-made microplastics between different exposure groups and blank control by the term of items/individual byssus. Each value represents as mean ± standard deviation of the three replicates (n = 3), and the byssus from two mussels was pooled as one replicate. Asterisks represent significant difference to the controls (The Mann-Whitney U test, $^*p < 0.05$).

4. Discussion

4.1. Microplastics in byssus between wild and farmed mussels

Microplastic abundance in mussels has been reported closely related to microplastic concentration in their ambient seawaters and human population density in many countries (Browne et al., 2011; Karlsson et al., 2017; Li et al., 2018; Li et al., 2016; Qu et al., 2018; Van Cauwenberghe et al., 2015). Particularly, the microplastic levels in soft tissue differed significantly between wild and farmed mussels from different investigated coastal regions (Li et al., 2016; Li et al., 2018). In China, Li et al. (2016) stated that farmed mussels are usually suspension-cultured in the cleaner water areas where are less affected by human activities compared with wild mussels. In our present study, our results suggested that the abundance of microplastics by the term of items/g. wet weight in the byssus of wild mussels was significantly higher than that of farmed mussels. However, no significant differences in the abundance of microplastics by the term of items/individual mussel were found between wild and farmed mussels. This is caused by large size differences between the two groups as described in our results. Taken together, our results further confirmed the positive correlation between microplastic pollution levels in body parts of mussels and that in their living environments.

4.2. Fusion of microplastics into the byssus of mussels

In our previous studies, even though we found that nearly half of the total detected microplastics adhere to the soft tissues of mussels (Kolandhasamy et al., 2018), there is still a chance for some particles to be incorporated into specific body parts during their biological process. When the byssus is being formed, all of the adhesive proteins are injected into the ventral groove where they mix and form a pre-matured thread (Waite, 1992; Sun and Waite, 2005). A functional thread is only made after further processing. During the formation process, the ventral groove is always partially exposed to seawater. Hence, the adhesive proteins do have the opportunity to interact with the microplastics in the environment

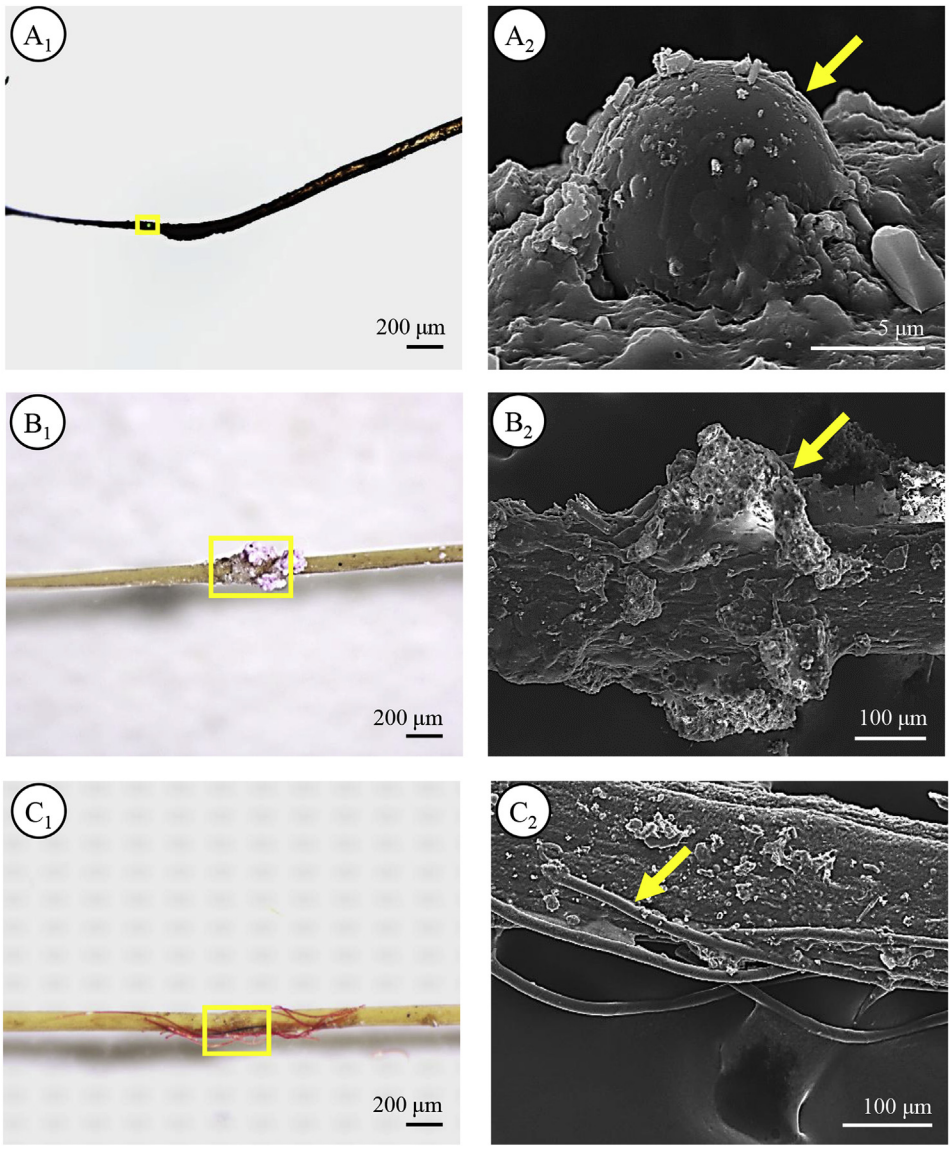

Fig. 5. Adhesion and fusion of microplastics in mussel byssus. The left images were taken under optical microscopes, and the right images were taken under SEM for the yellow box areas as indicated in the left ones. Some particles were PS microbeads (A_1-A_2), some were PA fragments (B_1–B_2), and some were PES fibers (C_1–C_2). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and therefore bind to them. Nonetheless, to our best knowledge, there has been no report on sand grains being incorporated into the byssus. In addition, microplastics and sand grains have different interactive mechanisms with the byssal proteins, the former is more easily imbedded. Therefore, no negative effects of sand grain or other substance on the byssal structures have been found, this issue deserves further investigation in our future studies.

In the present study, exposure of mussels with newly secreted byssus in microplastics-supplemented seawater resulted in a significant increase in microplastics accumulation in all of the byssal threads tested. Small 10 μm fluorescent PS microbeads were confirmed to be partially or completely incorporated into the cuticle of the byssal threads, and thin PES fiber was found to bind to the outside of the byssal thread. It is very probable that other microplastics with similar size acts in an analogous way but could not be observed due to technical limitations. These results suggest that fusion of microplastics into mussel byssus is based on not merely the availability of microplastics but on the size and shape of microplastics.

4.3. Approaches of microplastics to enter aquatic organisms

Microplastics are ubiquitous in aquatic environments and have multiple potential ways to be taken up by aquatic organisms. In field studies, microplastics are ingested as food by zooplankton and transferred to different trophic classes of animals through the food webs (Ory et al., 2017; Wright et al., 2013). Hence, ingestion has been commonly recognized as a pathway for uptake of microplastics into aquatic organisms. Additionally, microplastics have also been found in other specific organs other than stomach and intestine in laboratory exposure experiments. For instance, microbeads not only occurred on the surface of the foot of copepods and mussels but also in the gills of crabs and mussels (Cole et al., 2015; Kolandhasamy et al., 2018; Watts et al., 2016; Wegner et al., 2012). Therefore, adherence has been demonstrated as another important pathway for accumulation of microplastics to aquatic organisms beyond ingestion. In the present study, microplastics are proved to be embedded into mussel byssus during their secretion process. Taken together, three pathways of accumulating

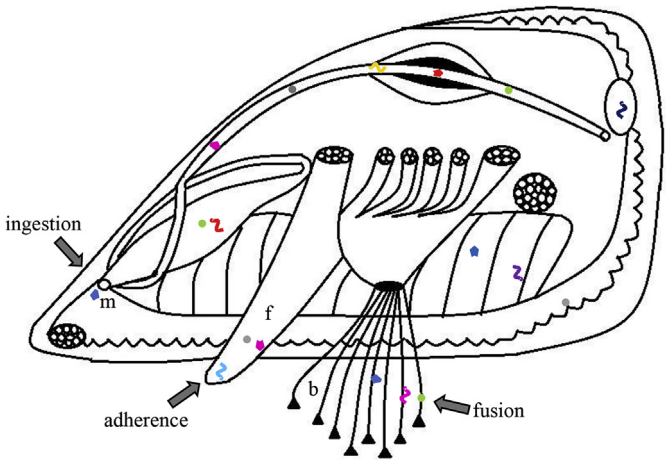

Fig. 6. Three pathways of accumulating microplastics in mussels. Colorful symbols represent different types of microplastics. Arrows indicate the approaches by which microplastics enter the mussels. Abbreviations: b, byssus; f, foot; m, mouth.

microplastics in mussels are summarized in Fig. 6.

As an external polymeric structure, byssus is regularly applied as an environmental indicator to study impacts of environmental pollutants and factors. For instance, impaired attachment strength of byssus has been found in blue mussel, *Mytilus edulis*, upon microplastic exposure (Green et al., 2018). In addition, reductions in the tenacity of thick shell mussel, *Mytilus coruscus*, and green-lipped mussel, *Perna viridis*, have also been reported in response to environmental stressors, such as low salinity, hypoxia and sea acidification(Sui et al., 2015; Wang et al., 2012). Reduced attachment strength and tenacity are very likely a direct result from mussels being stressed. In our laboratory study, we observed microplastics in byssus. This might be a direct impairment to the biophysical function of the byssal thread and can therefore reduce the tenacity of the mussel directly. Furthermore, there have been several reports that show effects of microplastics on other physiological endpoints in mussels, including histological changes, inflammatory responses, induced apoptotic process, decrease in lysosomal membrane stability, phagocytic activity, and filtering activity, increase in elimination, haemocytic infiltration, genotoxicity and transcriptional responses (Avio et al., 2015; Canesi et al., 2015; Detree and Gallardo-Escarate, 2017; Gandara et al., 2016; Goncalves et al., 2018; Goncalves et al., 2019; Magni et al., 2018; Paul-Pont et al., 2016; von Moos et al., 2012; Wegner et al., 2012). Overall, the microplastics used in many laboratory studies are at unrealistically high concentrations with uniform size or shape, in virgin condition, and for a short exposure time. Microplastics with more environmentally relevant concentrations, mixed type, size and shape, in weathered conditions, representing different scenarios are required to be further investigated to assess the potential ecological risks of microplastics to aquatic organisms.

Conclusion

In the current study, our results suggest that microplastics, at least with regard to commercial PS microbeads with small size and smooth surface, can be incorporated into the byssus of mussels. Our findings indicate that we need to determine if microplastics can get into the tissues of organisms in the future.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.05.093.

References

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222.
Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. T. R. Soc. B. 364, 1985–1998.
Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ. Sci. Technol. 45, 9175–9179.
Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L). Environ. Sci. Technol. 42, 5026–5031.
Canesi, L., Ciacci, C., Bergami, E., Monopoli, M.P., Dawson, K.A., Papa, S., Canonico, B., Corsi, I., 2015. Evidence for immunomodulation and apoptotic processes induced by cationic polystyrene nanoparticles in the hemocytes of the marine bivalve *Mytilus*. Mar. Environ. Res. 111, 34–40.
Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. Environ. Sci. Technol. 49, 1130–1137.
De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., Robbens, J., 2014. Quality assessment of the blue mussel (*Mytilus edulis*): comparison between commercial and wild types. Mar. Pollut. Bull. 85, 146–155.
Detree, C., Gallardo-Escarate, C., 2017. Polyethylene microbeads induce transcriptional responses with tissue-dependent patterns in the mussel *Mytilus galloprovincialis*. J. Molluscan Stud. 83, 220–225.
Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat. Ecol. Evol. 1, 0116.
Gandara, E.S.P.P., Nobre, C.R., Resaffe, P., Pereira, C.D., Gusmao, F., 2016. Leachate from microplastics impairs larval development in brown mussels. Water Res. 106, 364–370.
Goncalves, C., Martins, M., Costa, M.H., Costa, P.M., 2018. Development of a method for the detection of polystyrene microplastics in paraffin-embedded histological sections. Histochem. Cell Biol. 149, 187–191.
Goncalves, C., Martins, M., Sobral, P., Costa, P.M., Costa, M.H., 2019. An assessment of the ability to ingest and excrete microplastics by filter-feeders: a case study with the Mediterranean mussel. Environ. Pollut. 245, 600–606.
Green, D.S., Colgan, T.J., Thompson, R.C., Carolan, J.C., 2018. Exposure to microplastics reduces attachment strength and alters the haemolymph proteome of blue mussels (*Mytilus edulis*). Environ. Pollut. 246, 423–434.
Hagenau, A., Suhre, M.H., Scheibel, T.R., 2014. Nature as a blueprint for polymer material concepts: protein fiber-reinforced composites as holdfasts of mussels. Prog. Polym. Sci. 39, 1564–1583.
He, Y., Sun, C., Jiang, F., Yang, B., Li, J., Zhong, C., Zheng, L., Ding, H., 2018. Lipids as integral components in mussel adhesion. Soft Matter 14 (35), 7145–7154.
Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075.
Karlsson, T.M., Vethaak, A.D., Almroth, B.C., Ariese, F., van Velzen, M., Hassellov, M., Leslie, H.A., 2017. Screening for microplastics in sediment, water, marine invertebrates and fish: method development and microplastic accumulation. Mar. Pollut. Bull. 122, 403–408.
Kolandhasamy, P., Su, L., Li, J., Qu, X., Jabeen, K., Shi, H., 2018. Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion. Sci. Total Environ. 610–611, 635–640.
Law, K.L., Thompson, R.C., 2014. Oceans. Microplastics in the seas. Science 345, 144–145.
Li, J., Green, C., Reynolds, A., Shi, H., Rotchell, J.M., 2018. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. Environ. Pollut. 241, 35–44.
Li, J., Lusher, A.L., Rotchell, J.M., Deudero, S., Turra, A., Brate, I.L.N., Sun, C., Shahadat Hossain, M., Li, Q., Kolandhasamy, P., Shi, H., 2019a. Using mussel as a global bioindicator of coastal microplastic pollution. Environ. Pollut. 244, 522–533.
Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in mussels along the coastal waters of China. Environ. Pollut. 214, 177–184.
Li, J., Yang, D., Li, L., Jabeen, K., Shi, H., 2015. Microplastics in commercial bivalves from China. Environ. Pollut. 207, 190–195.
Li, L., Su, L., Cai, H., Rochman, C.M., Li, Q., Kolandhasamy, P., Peng, J., Shi, H., 2019b. The uptake of microfibers by freshwater Asian clams (*Corbicula fluminea*) varies based upon physicochemical properties. Chemosphere 221, 107–114.
Lin, Q., Gourdon, D., Sun, C.J., Holten-Andersen, N., Anderson, T.H., Waite, J.H., Israelachvili, J.N., 2007. Adhesion mechanisms of the mussel foot proteins mfp-

1 and mfp-3. P. Natl. Acad. Sci. USA. 104, 3782–3786.
Magni, S., Gagne, F., Andre, C., Della Torre, C., Auclair, J., Hanana, H., Parenti, C.C., Bonasoro, F., Binelli, A., 2018. Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia). Sci. Total Environ. 631–632, 778–788.
Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ. Sci. Technol. 35, 318–324.
Ory, N.C., Sobral, P., Ferreira, J.L., Thiel, M., 2017. Amberstripe scad *Decapterus muroadsi* (*Carangidae*) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci. Total Environ. 586, 430–437.
Paul-Pont, I., Lacroix, C., Gonzalez Fernandez, C., Hegaret, H., Lambert, C., Le Goic, N., Frere, L., Cassone, A.L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A., Soudant, P., 2016. Exposure of marine mussels *Mytilus* spp. to polystyrene microplastics: toxicity and influence on fluoranthene bioaccumulation. Environ. Pollut. 216, 724–737.
Qu, X., Su, L., Li, H., Liang, M., Shi, H., 2018. Assessing the relationship between the abundance and properties of microplastics in water and in mussels. Sci. Total Environ. 621, 679–686.
Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. Trac. Trends Anal. Chem. 65, 47–53.
Seguin-Heine, M.O., Lachance, A.A., Genard, B., Myrand, B., Pellerin, C., Marcotte, I., Tremblay, R., 2014. Impact of open sea habitat on byssus attachment of suspension-cultured blue mussels (*Mytilus edulis*). Aquaculture 426, 189–196.
Shim, W.J., Song, Y.K., Hong, S.H., Jang, M., 2016. Identification and quantification of microplastics using Nile Red staining. Mar. Pollut. Bull. 113, 469–476.
Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in taihu lake, China. Environ. Pollut. 216, 711–719.
Sui, Y.M., Hu, M.H., Huang, X.Z., Wang, Y.J., Lu, W.Q., 2015. Anti-predatory responses of the thick shell mussel *Mytilus coruscus* exposed to seawater acidification and hypoxia. Mar. Environ. Res. 109, 159–167.
Sun, C., Lucas, J.M., Waite, J.H., 2002. Collagen-binding matrix proteins from elastomeric extraorganismic byssal fibers. Biomacromolecules 3, 1240–1248.
Sun, C., Waite, J.H., 2005. Mapping chemical gradients within and along a fibrous structural tissue, mussel byssal threads. J. Biol. Chem. 280, 39332–39336.
Sun, C.J., Vaccaro, E., Waite, J.H., 2001. Oxidative stress and the mechanical properties of naturally occurring chimeric collagen-containing fibers. Biophys. J. 81, 3590–3595.
Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. Environ. Pollut. 199, 10–17.
von Moos, N., Burkhardt-Holm, P., Kohler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46, 11327–11335.
Waite, J.H., 1992. The formation of mussel byssus: anatomy of a natural manufacturing process. In: Case, S.T. (Ed.), Biopolymers: Results and Problems in Cell Differentiation, vol. 19. Springer-Verlag, Berlin, pp. 27–54.
Wang, Y.J., Hu, M.H., Cheung, S.G., Shin, P.K.S., Lu, W.Q., Li, J.L., 2012. Chronic hypoxia and low salinity impair anti-predatory responses of the green-lipped mussel *Perna viridis*. Mar. Environ. Res. 77, 84–89.
Watts, A.J., Urbina, M.A., Goodhead, R., Moger, J., Lewis, C., Galloway, T.S., 2016. Effect of microplastic on the gills of the shore crab *Carcinus maenas*. Environ. Sci. Technol. 50, 5364–5369.
Wegner, A., Besseling, E., Foekema, E.M., Kamermans, P., Koelmans, A.A., 2012. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ. Toxicol. Chem. 31, 2490–2497.
Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. Royal Soc. Open Sci. 1 (4), 140317.
Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23, R1031–R1033.
Yang, D.Q., Shi, H.H., Li, L., Li, J.N., Jabeen, K., Kolandhasamy, P., 2015. Microplastic pollution in table salts from China. Environ. Sci. Technol. 49, 13622–13627.