Accumulation of microplastic particles in the freshwater shrimp Caridina multidentate

Yoshitaka Nakanishi (y-naka@mech.kumamoto-u.ac.jp)
Kumamoto University https://orcid.org/0000-0002-7975-3595
Hayato Iga
Kumamoto University: Kumamoto Daigaku
Yukio Fujiwara
Kumamoto University: Kumamoto Daigaku
Yuta Nakashima
Kumamoto University: Kumamoto Daigaku

Research

Keywords: Microplastic, Freshwater shrimp, Caridina multidentate , Polystyrene microparticle

Posted Date: March 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-354265/v1

Accumulation of microplastic particles in the freshwater shrimp *Caridina multidentate*

Yoshitaka Nakanishi[1]*, Hayato Iga[2], Yukio Fujiwara[3], Yuta Nakashima[1,4]

[1] Faculty of Advanced Science and Technology, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan

[2] Faculty of Engineering, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan

[3] Faculty of Life Science, Kumamoto University, 1-1-1 Honjo, Cyuo-ku, Kumamoto 860-8556, Japan

[4] International Research Organisation for Advanced Science & Technology, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan

***Corresponding author**

Yoshitaka Nakanishi, Professor, Dr. Eng.

Faculty of Advanced Science and Technology, Kumamoto University, Japan

2-39-1 Kurokami, Cyuo-ku, Kumamoto 860-8555, Japan

Telephone number: +81-96-342-3733

Fax number: +81-96-342-3733

E-mail address: y-naka@mech.kumamoto-u.ac.jp

Abstract

Background: Plastic particles less than 5 mm in size are formed by the fragmentation of plastic materials. The ingestion of microplastic particles by marine organisms is known to cause bioaccumulation, toxicity, and adverse effects on the immune system. We experimentally demonstrated the transfer and accumulation of microplastic particles in the digestive system of the freshwater shrimp *Caridina multidentate*.

Methods: The shrimps were exposed to the spherical fluorescent polystyrene (PS) microparticles of various diameters (1.1, 0.49, 0.20, 0.15 and 0.046 μm). The shrimps were first kept in water without PS particles and fed for 48 h for facilitating the excretion process. Shrimp tissues were fixed in 10% formalin neutral buffer solution, stained with haematoxylin-eosin and observed under a sliding microtome, polarising microscope and inverted fluorescence microscope.

Results: Although the shrimps were not fed for 120 h and exposed to a high concentration of PS particles for 72 h, almost all the shrimps were alive. PS aggregates 0.49 and 0.20 μm in diameter were found in the digestive tract and intestinal tract wall of the shrimps exposed to the PS particles.

Conclusions: We hypothesized that sub-micron microparticles that are accumulated in the intestinal tract wall might activate non-specific immune mechanisms such as phagocytosis by macrophages and produce inflammatory substances. The microparticles in the digestive system might adsorb these inflammatory substances, eventually leading to microparticle aggregation.

Keywords: Microplastic, Freshwater shrimp, *Caridina multidentate*, Polystyrene microparticle

Background

Plastic pollution is a global environmental concern [1-3]. Plastic particles blended with daily supplies and cosmetics, and fibres released from laundered clothes diffuse in the environment. Fragmented plastic particles that are formed by the ultraviolet radiation emitted by the Sun contribute to environmental pollution [4-8]. Spherical, fragmented and fibriform plastic particles have been found in soils, freshwater bodies and oceans [9-12]. Small plastic particles (less than 5 mm in size) are called “microplastics,” whereas those in the micrometre range are called “nanoplastics” [16-22].

Micro- and nanoplastics present in the marine ecosystem are ingested by marine organisms such as whales, fishes, shrimps, jellyfishes and birds [23-28], and their bioaccumulation results in biotoxicity. Plasticising agents eluted from microplastics [29-31] and the harmful substances adsorbed onto microplastics affect material exchange and metabolism, and render the reproductive organs of these organisms dysfunctional [32-37].

Microplastics diffused in the environment remain there for a longer period, and their fragments formed by ultraviolet radiation and ocean waves are further diffused and accumulated in the environment [38,39]. Microplastics ingested by organisms also affect their immune

system [40]. Macrophages identify these particles as foreign substances and activate non-specific immune mechanisms. The morphology of the particles (size and shape) is a key parameter in the activation of non-specific immune mechanisms. Furthermore biodegradable plastic fragments can also adversely affect the immune responses of marine organisms [41-45].

In this study, we experimentally demonstrated the transfer and accumulation of microplastics in the freshwater shrimp *Caridina multidentate* and identified the mechanism by which microplastics affects the immune system of this shrimp species.

Methods

Preparation of microplastics

Spherical fluorescent PS microparticles (Polymer Microspheres, Blue Fluoresent, Fluoro-Max™, Thermo Scientific) of different diameters such as 1.1, 0.49, 0.20, 0.15 and 0.046 μm were prepared. Microparticles were treated to unify the surface characteristics of the particles, and the protocol for the surface treatment has been summarised in Figure 1. Unknown dispersant components adsorbed on the surface of microparticles were removed by the removing process, which was repeated three times. Ultrapure water was added to the particles

and the mixture was ultracentrifuged (OptimaTM MAX-UP Ultracentrifuge, Beckman Coulter) at 150 × $10^3 g$ for 3 h. The supernatant was discarded and water solution of 0.1% polyoxyethylene sorbitan monooleate (Tween 80, TCI Chemicals) was added for the adsorption of surfactant to the particles, followed by dispersing ultrasonic waves through the mixture for 2 min (UCD-250, Bioruptor). The mixture was ultracentrifuged, the supernatant was discarded and ultrapure water was added with dispersing ultrasonic waves. The mixture was ultracentrifuged again, the supernatant was discarded and ethyl alcohol was added with dispersing ultrasonic waves. After ethyl alcohol was evaporated, PS particles were ready for analysis.

The surface of the particles was observed by using a scanning electron microscope (SEM, JSM-6390LV, JEOL) and fluorescence microscope (TS2-FL, Nicon) before and after surface treatment. Particle size distribution was measured to determine the effect of surface treatment on PS particles.

Exposure of freshwater shrimps to PS particles

Experimental protocol has been summarised in Figure 2. All the procedures were performed in a clean room environment (MCV-710ATS, SANYO). *Caridina multidentata*, a freshwater

shrimp that is 20–30 mm long and weighs 0.7–0.8 g, was used in the study. Each shrimp was isolated in a plastic container in 100 mL of water. Water with and without PS particles was prepared 24 h before feeding for stabilising the water quality (dissolved oxygen, etc.). The number of PS particles in 100 mL of water was adjusted to 1.0×10^8 particles for each particle size (1.1, 0.49, 0.20, 0.15 and 0.046 μm). All the shrimps were first kept in water without PS particles and feeding for 48 h for facilitating excretion. Eight shrimps were then moved into containers with water and PS particles of a single size, and maintained for 72 h without feeding to determine the effect of PS particle exposure.

Shrimp tissues were fixed in 10% formalin neutral buffer solution (WAKO) and stained with haematoxylin-eosin. The stained tissue sections were processed into paraffin-embedded samples by using a vacuum infiltration processor (Tissue-TekTM VIP Jr., SAKURA) and embedding console system (Tissue-TekTM TEC 6, SAKURA). The tissue sections were observed under a sliding microtome (SM2000R, LEICA), polarising microscope (BX51, OLYMPUS) and an invert fluorescence microscope (TS2-FL, Nicon). An image-editing software (ImageJ-MosaicJ) for processing the images.

Results and Discussion

Figure 3 shows SEM and fluorescence microscope images and particle size distribution of PS particles before and after surface treatment. The spherical shape and size of PS particles were not affected by ultracentrifugation. The fluorescence properties of PS particles were not affected by the adsorption of surfactant on their surfaces. These results showed that the processed PS particles can be detected and their properties can be studied after their transfer and accumulation in the tissue sections.

Table 1 summarises the survival fraction of shrimps after treatment with PS particles. Although the shrimps were not fed for 120 h and exposed to a high concentration of PS particles for 72 h, almost all the shrimps were alive. However, some shrimps exposed to 0.49–0.15 μm particles died.

Table 1 Exposure of freshwater shrimps to microplastics

	Number of shrimps alive / Number of shrimps tested
Control (without PS particles)	8 / 8
Post-exposure of 1.1 μm particles	8 / 8
Post-exposure of 0.49 μm particles	7 / 8
Post-exposure of 0.20 μm particles	6 / 8
Post-exposure of 0.15 μm particles	7 / 8
Post-exposure of 0.046 μm particles	8 / 8

The representative histological images captured after exposure to PS particles are shown in Figure 4. PS aggregates were found in the digestive system of shrimps exposed to 0.49 and 0.20 μm PS particles (Figure 5). Furthermore, PS particles were also detected in the intestinal tract wall. However, the transfer and accumulation of PS particles outside the digestive system were not confirmed (Figure 6). We believe that sub-micron microparticles that accumulate in the intestinal tract wall might activate non-specific immune mechanisms such as phagocytosis by macrophages and produce inflammatory substances. The microparticles in the digestive system might adsorb the inflammatory substances and eventually form aggregates.

Microparticles accumulated in the intestinal tract wall might be transferred to a vascular system, tissues and material exchange organs such as branchia. A possible reason that these phenomena were not observed is because of the limitation of the fluorescence technique or the duration of PS microparticle exposure.

4. Conclusions

The transfer and accumulation of microplastic particles to the digestive system of freshwater shrimps were experimentally demonstrated. Microparticles transferred and accumulated in the intestinal tract wall might activate non-specific immune mechanisms such as phagocytosis by macrophages and produce inflammatory substances. The microparticles in the digestive system might adsorb these substances, resulting in the production of microparticle aggregates.

Abbreviations

PS: Polystyrene; SEM: Scanning electron microscope; H&E: Haematoxylin-eosin

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Environment Research and Technology Development Fund of the Environmental Restoration and Conservation Agency of Japan [grant number 1–1908] and JSPS KAKENHI [grant number 19KK0096].

Authors' contributions

YN: Conceptualization, Formal analysis, Data Curation, Writing – Original Draft, Supervision, Project administration, Funding acquisition; HI: Software, Investigation; YF: Methodology, Resources, Writing – Review & Editing ; YN: Validation, Visualization

Acknowledgements

Not applicable.

References

[1] Gallo F, Fossi C, Weber R, Santillo D, Sousa J, Ingram I, Nadal A, Romano D (2018) Marine litter plastics and microplastics and their toxic chemicals components: the need for urgent preventive measures. Environ Sci Eur 30:13. doi:10.1186/s12302-018-0139-z.

[2] Wong SL, Nyakuma BB, Wong KY, Lee CT, Lee TH, Lee CH (2020) Microplastics and nanoplastics in global food webs: a bibliometric analysis (2009–2019). Mar Pollut Bull 158: 111432. doi:10.1016/j.marpolbul.2020.111432.

[3] Sendra M, Sparaventi E, Novoa B, Figueras A (2021) An overview of the internalization and effects of microplastics and nanoplastics as pollutants of emerging concern in bivalves. Sci Total Environ 753:142024. doi:10.1016/j.scitotenv.2020.142024.

[4] Song YK, Hong SH, Jang M, Han GM, Jung SW, Shim WJ (2017) Combined effects of UV exposure duration and mechanical abrasion on microplastic fragmentation by polymer type. Environ Sci Technol 51:4368-4376. doi:10.1021/acs.est.6b06155.

[5] Andrady AL (2011) Microplastics in the marine environment. Mar Pollut Bull 62:1596-1605. doi:10.1016/j.marpolbul.2011.05.030.

[6] Ter Halle A, Ladirat L, Gendre X, Goudouneche D, Pusineri C, Routaboul C, Tenailleau C, Duployer B, Perez E (2016) Understanding the fragmentation pattern of marine plastic debris. Environ Sci Technol 50:5668-5675. doi:10.1021/acs.est.6b00594.

[7] Tiwari N, Santhiya D, Sharma JG (2020) Microbial remediation of micro-nano plastics: current knowledge and future trends. Environ Pollut 265:115044. doi:10.1016/j.envpol.2020.115044.

[8] Cole M, Lindeque P, Halsband C, Galloway TS (2011) Microplastics as contaminants in the marine environment: a review. Mar Pollut Bull 62:2588-2597. doi:10.1016/j.marpolbul.2011.09.025.

[9] Duis K, Coors A (2016) Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ Sci Eur 28:1-25. doi:10.1186/s12302-015-0069-y.

[10] Li Y, Lu Z, Zheng H, Wang J, Chen C (2020) Microplastics in surface water and sediments of Chongming Island in the Yangtze Estuary, China. Environ Sci Eur 32. doi:10.1186/s12302-020-0297-7.

[11] Deng H, He J, Feng D, Zhao Y, Sun W, Yu H, Ge C (2021) Microplastics pollution in mangrove ecosystems: a critical review of current knowledge and future directions. Sci Total Environ 753:142041. doi:10.1016/j.scitotenv.2020.142041.

[12] Barboza LGA, Gimenez BCG (2015) Microplastics in the marine environment: current trends and future perspectives. Mar Pollut Bull 97:5-12. doi:10.1016/j.marpolbul.2015.06.008.

[13] Wagner M, Scherer C, Alvarez-Muñoz D, Brennholt N, Bourrain X, Buchinger S, Fries E, Grosbois C, Klasmeier J, Marti T, Rodriguez-Mozaz S, Urbatzka R, Vethaak AD, Winther-Nielsen M, Reifferscheid G (2014) Microplastics in freshwater ecosystems: what we know and what we need to know. Environ Sci Eur 26:12. doi:10.1186/s12302-014-0012-7.

[14] Shahul Hamid F, Bhatti MS, Anuar N, Anuar N, Mohan P, Periathamby A (2018) Worldwide distribution and abundance of microplastic: how dire is the situation?. Waste Manag Res 36:873-897. doi:10.1177/0734242X18785730.

[15] Kutralam-Muniasamy G, Pérez-Guevara F, Elizalde-Martínez I, Shruti VC (2020) Review of current trends, advances and analytical challenges for microplastics contamination in Latin America. Environ Pollut 267:115463. doi:10.1016/j.envpol.2020.115463.

[16] Lusher AL, Hernandez-Milian G, O'Brien J, Berrow S, O'Connor I, Officer R (2015) Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: the True's beaked whale Mesoplodon mirus. Environ Pollut 199:185-191. doi:10.1016/j.envpol.2015.01.023.

[17] Betts K (2008) Why small plastic particles may pose a big problem in the oceans. Environ Sci Technol 42:8996. doi:10.1021/es802970v.

[18] Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, Rist S, Karlsson T, Brennholt N, Cole M, Herrling MP, Hess MC, Ivleva NP, Lusher AL, Wagner M (2019) Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ Sci Technol 53:1039-1047. doi:10.1021/acs.est.8b05297.

[19] Kor K, Ghazilou A, Ershadifar H (2020) Microplastic pollution in the littoral sediments of the northern part of the Oman Sea. Mar Pollut Bull 155:111166. doi:10.1016/j.marpolbul.2020.111166.

[20] Corradini F, Casado F, Leiva V, Huerta-Lwanga E, Geissen V (2021) Microplastics occurrence and frequency in soils under different land uses on a regional scale. Sci Total Environ 752:141917. doi:10.1016/j.scitotenv.2020.141917.

[21] Yang L, Zhang Y, Kang S, Wang Z, Wu C (2021) Microplastics in freshwater sediment: a review on methods, occurrence, and sources. Sci Total Environ 754:141948. doi:10.1016/j.scitotenv.2020.141948.

[22] Pan Z, Sun Y, Liu Q, Lin C, Sun X, He Q, Zhou K, Lin H (2020) Riverine microplastic pollution matters: a case study in the Zhangjiang River of Southeastern China. Mar Pollut Bull 159:111516. doi:10.1016/j.marpolbul.2020.111516.

[23] Wang Q, Zhu X, Hou C, Wu Y, Teng J, Zhang C, Tan H, Shan E, Zhang W, Zhao J (2021) Microplastic uptake in commercial fishes from the Bohai Sea, China. Chemosphere 263:127962. doi:10.1016/j.chemosphere.2020.127962.

[24] Zhang C, Wang S, Pan Z, Sun D, Xie S, Zhou A, Wang J, Zou J (2020) Occurrence and distribution of microplastics in commercial fishes from estuarine areas of Guangdong, South China. Chemosphere 260:127656. doi:10.1016/j.chemosphere.2020.127656.

[25] Nan B, Su L, Kellar C, Craig NJ, Keough MJ, Pettigrove V (2020) Identification of microplastics in surface water and Australian freshwater shrimp Paratya australiensis in Victoria, Australia. Environ Pollut 259:113865. doi:10.1016/j.envpol.2019.113865.

[26] Daniel DB, Ashraf PM, Thomas SN (2020) Abundance, characteristics and seasonal variation of microplastics in Indian white shrimps (Fenneropenaeus indicus) from coastal waters off Cochin, Kerala, India. Sci Total Environ 737:139839. doi:10.1016/j.scitotenv.2020.139839.

[27] Iliff SM, Wilczek ER, Harris RJ, Bouldin R, Stoner EW (2020) Evidence of microplastics from benthic jellyfish (Cassiopea xamachana) in Florida estuaries. Mar Pollut Bull 159:111521. doi:10.1016/j.marpolbul.2020.111521.

[28] Perold V, Schoombie S, Ryan PG (2020) Decadal changes in plastic litter regurgitated by albatrosses and giant petrels at sub-Antarctic Marion Island. Mar Pollut Bull 159:111471. doi:10.1016/j.marpolbul.2020.111471.

[29] Guimarães ATB, Estrela FN, Rodrigues ASL, Chagas TQ, Pereira PS, Silva FG, Malafaia G (2021) Nanopolystyrene particles at environmentally relevant concentrations causes behavioral and biochemical changes in juvenile grass carp (Ctenopharyngodon idella). J Hazard Mater 403:123864. doi:10.1016/j.jhazmat.2020.123864.

[30] Brandts I, Barría C, Martins MA, Franco-Martínez L, Barreto A, Tvarijonaviciute A, Tort L, Oliveira M, Teles M (2021) Waterborne exposure of gilthead seabream (Sparus aurata) to

polymethylmethacrylate nanoplastics causes effects at cellular and molecular levels. J Hazard Mater 403:123590. doi:10.1016/j.jhazmat.2020.123590.

[31] Wright SL, Kelly FJ (2017) Plastic and human health: a micro issue?. Environ Sci Technol 51:6634-6647. doi:10.1021/acs.est.7b00423.

[32] Ma Y, Huang A, Cao S, Sun F, Wang L, Guo H, Ji R (2016) Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water. Environ Pollut 219:166-173. doi:10.1016/j.envpol.2016.10.061.

[33] Lee H, Shim WJ, Kwon JH (2014) Sorption capacity of plastic debris for hydrophobic organic chemicals. Sci Total Environ 470-471:1545-1552. doi:10.1016/j.scitotenv.2013.08.023.

[34] Wurl O, Obbard JP (2004) A review of pollutants in the sea-surface microlayer (SML): a unique habitat for marine organisms. Mar Pollut Bull 48:1016-1030. doi:10.1016/j.marpolbul.2004.03.016.

[35] Zhao Y, Qiao R, Zhang S, Wang G (2021) Metabolomic profiling reveals the intestinal toxicity of different length of microplastic fibers on zebrafish (Danio rerio). J Hazard Mater 403:123663. doi:10.1016/j.jhazmat.2020.123663.

[36] Sendra M, Pereiro P, Yeste MP, Mercado L, Figueras A, Novoa B (2021) Size matters: Zebrafish (Danio rerio) as a model to study toxicity of nanoplastics from cells to the whole organism. Environ Pollut 268:115769. doi:10.1016/j.envpol.2020.115769.

[37] Kashiwada S (2006) Distribution of nanoparticles in the see-through Medaka (Oryzias latipes). Environ Health Perspect 114:1697-1702. doi:10.1289/ehp.9209.

[38] Laist SW (1987) Overview of the biological effects of lost and discarded plastic debris in the marine environment. Mar Pollut Bull 18:319-326. doi:10.1016/S0025-326X(87)80019-X.

[39] Ter Halle A, Jeanneau L, Martignac M, Jardé E, Pedrono B, Brach L, Gigault J (2017) Nanoplastic in the North Atlantic Subtropical Gyre. Environ Sci Technol 51:13689-13697. doi:10.1021/acs.est.7b03667.

[40] Prietl B, Meindl C, Roblegg E, Pieber TR, Lanzer G, Fröhlich E (2014) Nano-sized and micro-sized polystyrene particles affect phagocyte function. Cell Biol Toxicol 30:1-16. doi:10.1007/s10565-013-9265-y.

[41] Zimmermann L, Göttlich S, Oehlmann J, Wagner M, Völker C (2020) What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to Daphnia magna. Environ Pollut 267:115392. doi:10.1016/j.envpol.2020.115392.

[42] Nakanishi Y, Nakashima Y, Fujiwara Y, Komohara Y, Hino K, Miura H, Higaki H (2020) Microfluidic device used for the secretion of inflammatory cytokines from human monocyte-derived macrophages stimulated by ultra-high molecular weight polyethylene particles. Biotribology 23:100137. doi:10.1016/j.biotri.2020.100137.

[43] Nakanishi Y, Nakashima Y, Fujiwara Y, Komohara Y, Takeya M, Miura H, Higaki H (2018) Influence of surface profile of Co-28Cr-6Mo alloy on wear behaviour of ultra-high molecular weight polyethylene used in artificial joint. Tribol Int 118:538-546. doi:10.1016/j.triboint.2017.06.030.

[44] Yoshioka R, Nakashima Y, Fujiwara Y, Komohara Y, Takeya M, Nakanishi Y (2016) The biological response of macrophages to PMMA particles with different morphology and size. Biosurf Biotribol 2:114-120. doi:10.1016/j.bsbt.2016.09.003.

[45] Chikaura H, Nakashima Y, Fujiwara Y, Komohara Y, Takeya M, Nakanishi Y (2016) Effect of particle size on biological response by human monocyte-derived macrophages. Biosurf Biotribol 2:18-25. doi:10.1016/j.bsbt.2016.02.003.

Figure legends

Figure 1 Surface treatments of spherical fluorescent microparticles made of a polystyrene (PS)

Figure 2 Freshwater shrimps exposed to microplastics

Figure 3 Scanning electron microscopic images, fluorescence microscope images and particle size distribution before and after the surface treatment of PS particles

Figure 4 Histological images after exposure to PS particles

Figure 5 Detailed observations of the shrimps exposed to 0.20 μm particles (digestive system)

Figure 6 Detailed observations of the shrimps exposed to 0.20 μm particles (outside the digestive system)

Figures

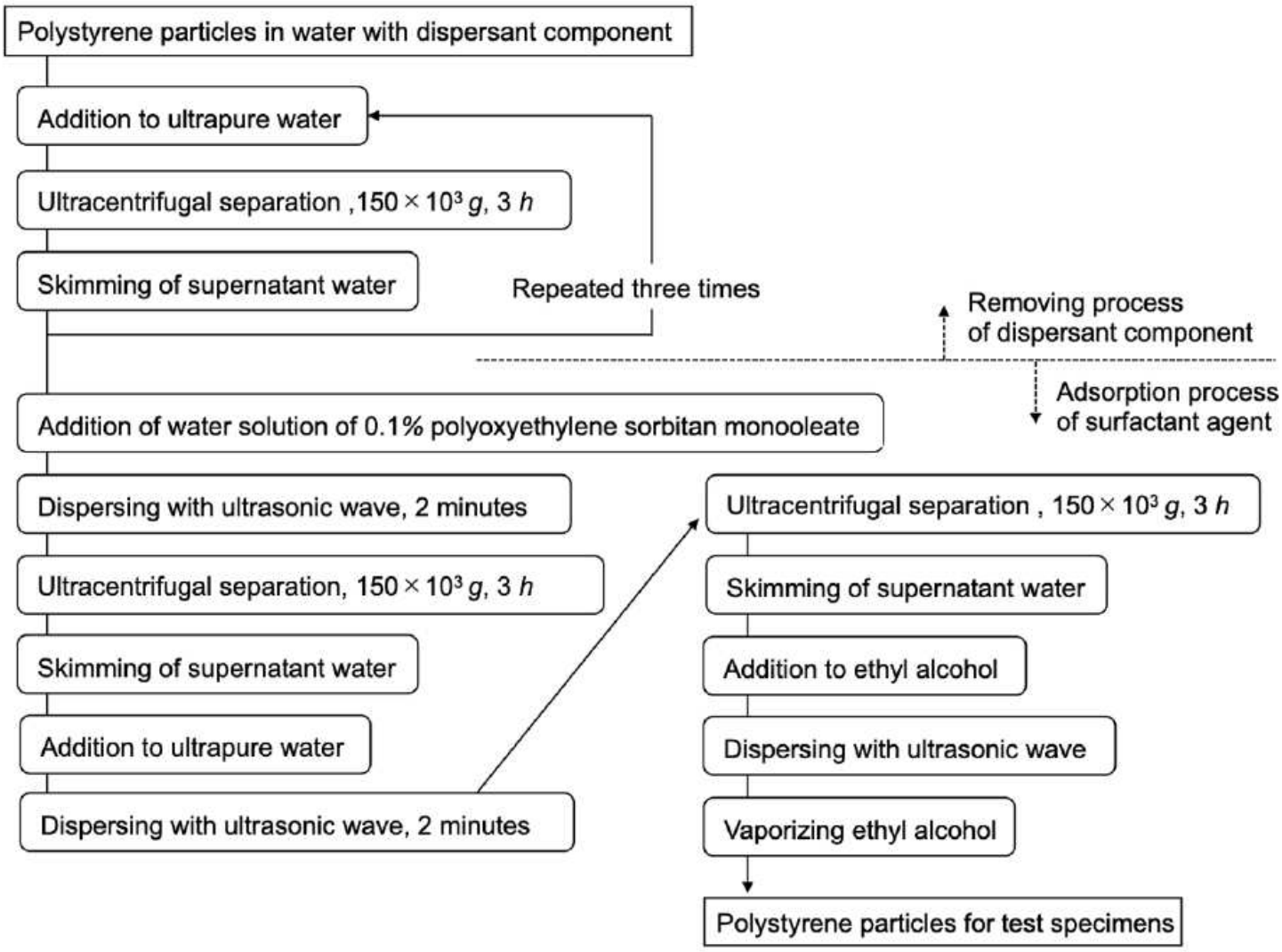

Figure 1

Surface treatments of spherical fluorescent microparticles made of a polystyrene (PS)

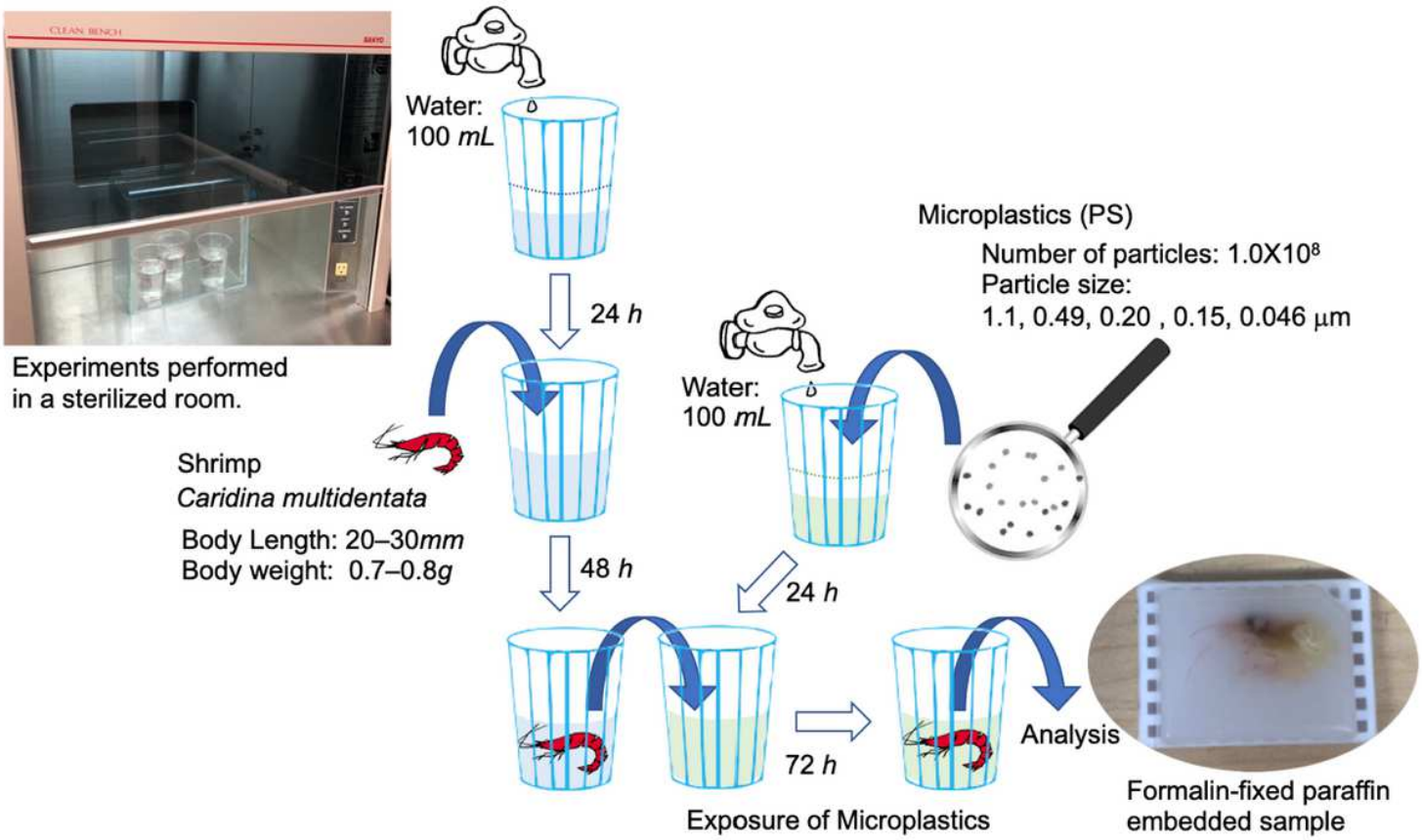

Figure 2

Freshwater shrimps exposed to microplastics

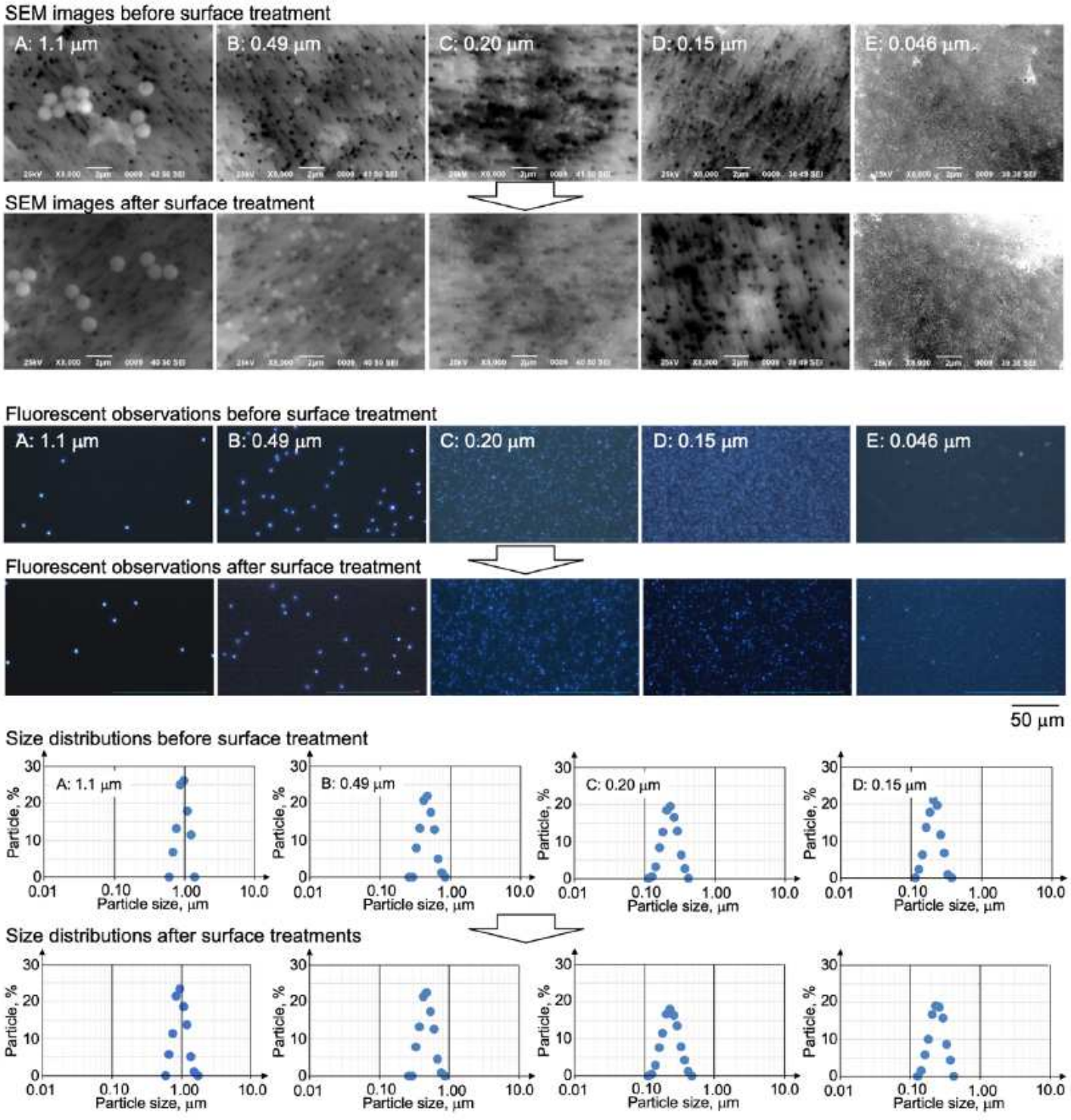

Figure 3

Scanning electron microscopic images, fluorescence microscope images and particle size distribution before and after the surface treatment of PS particles

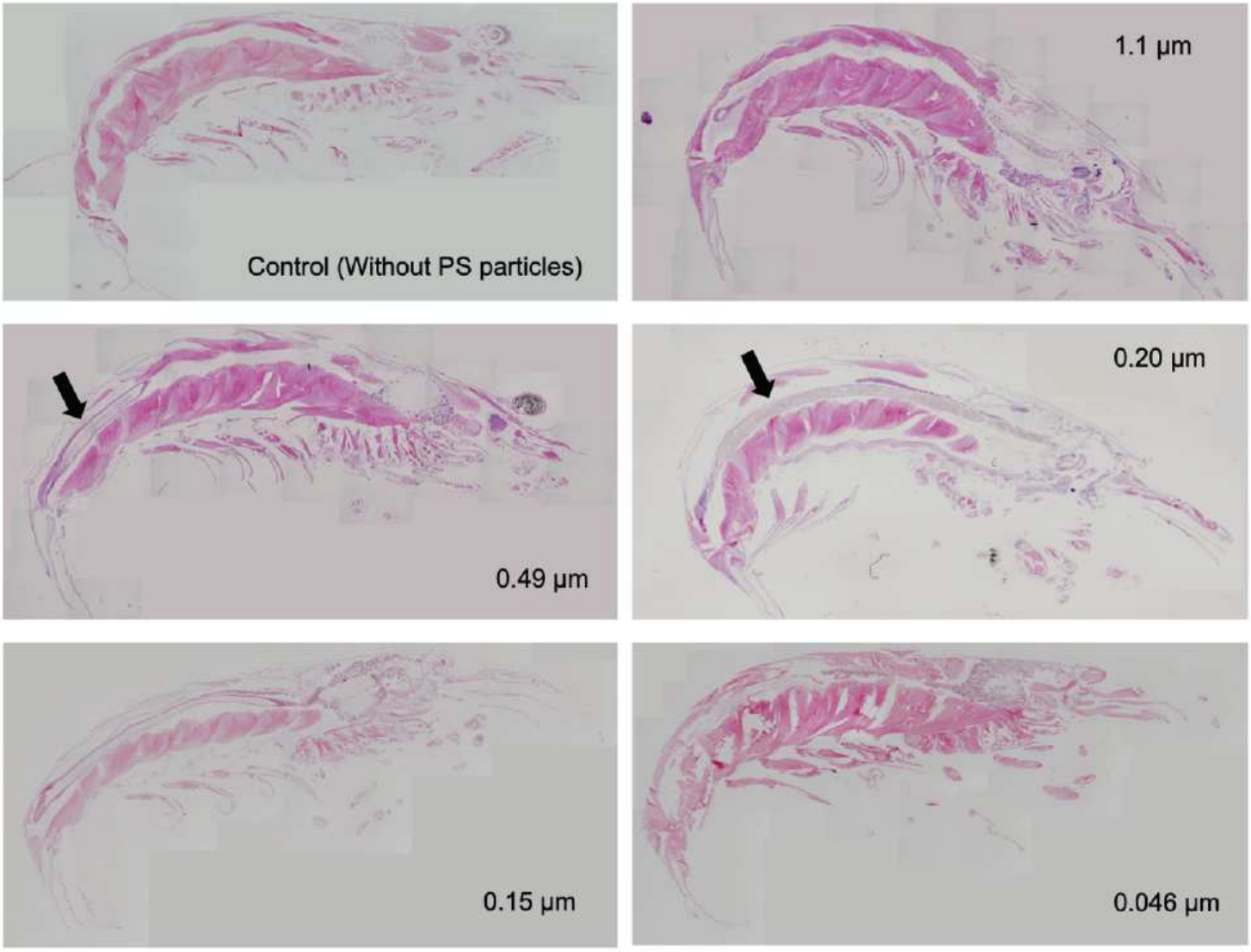

Figure 4

Histological images after exposure to PS particles

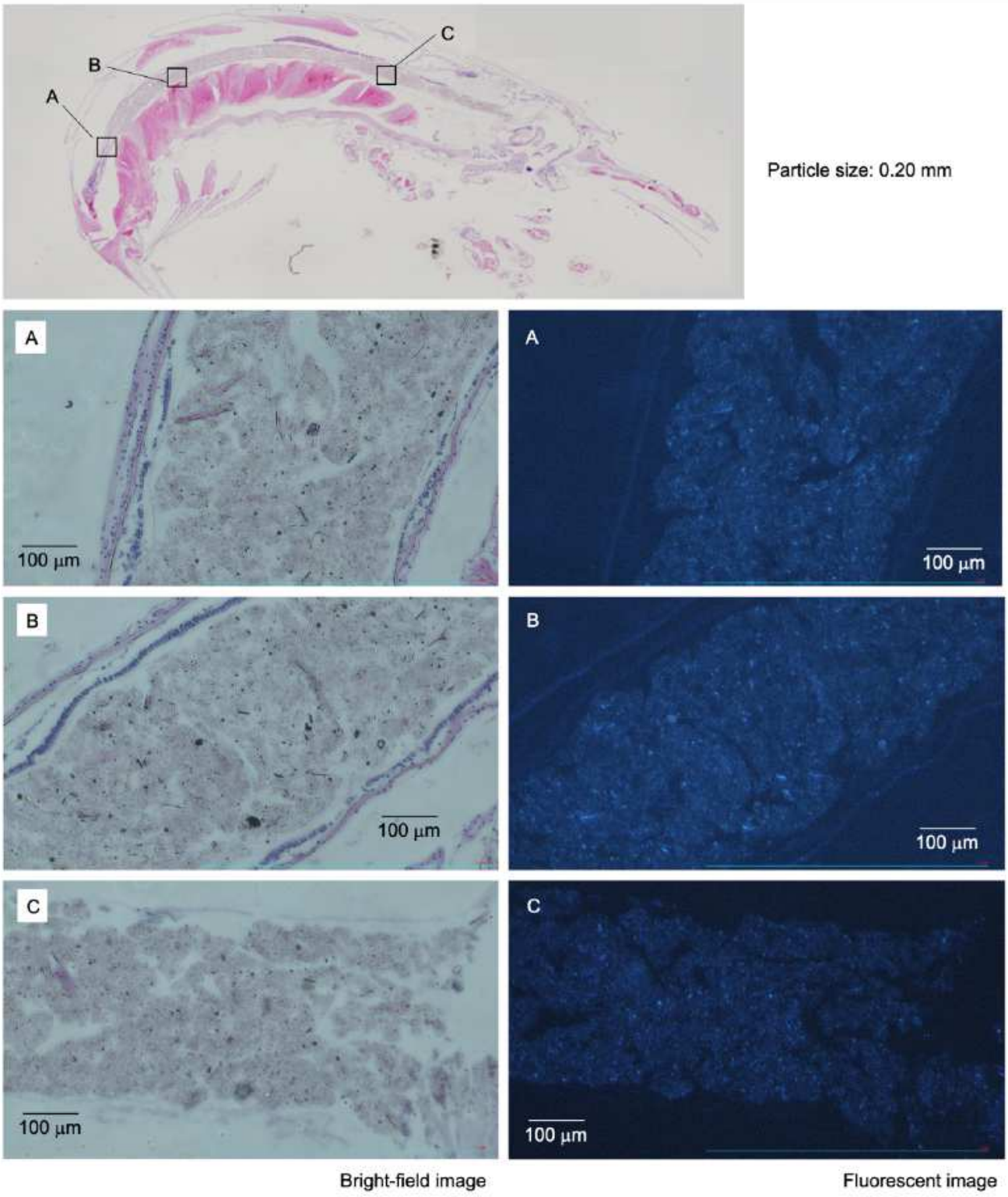

Figure 5

Detailed observations of the shrimps exposed to 0.20 μm particles (digestive system)

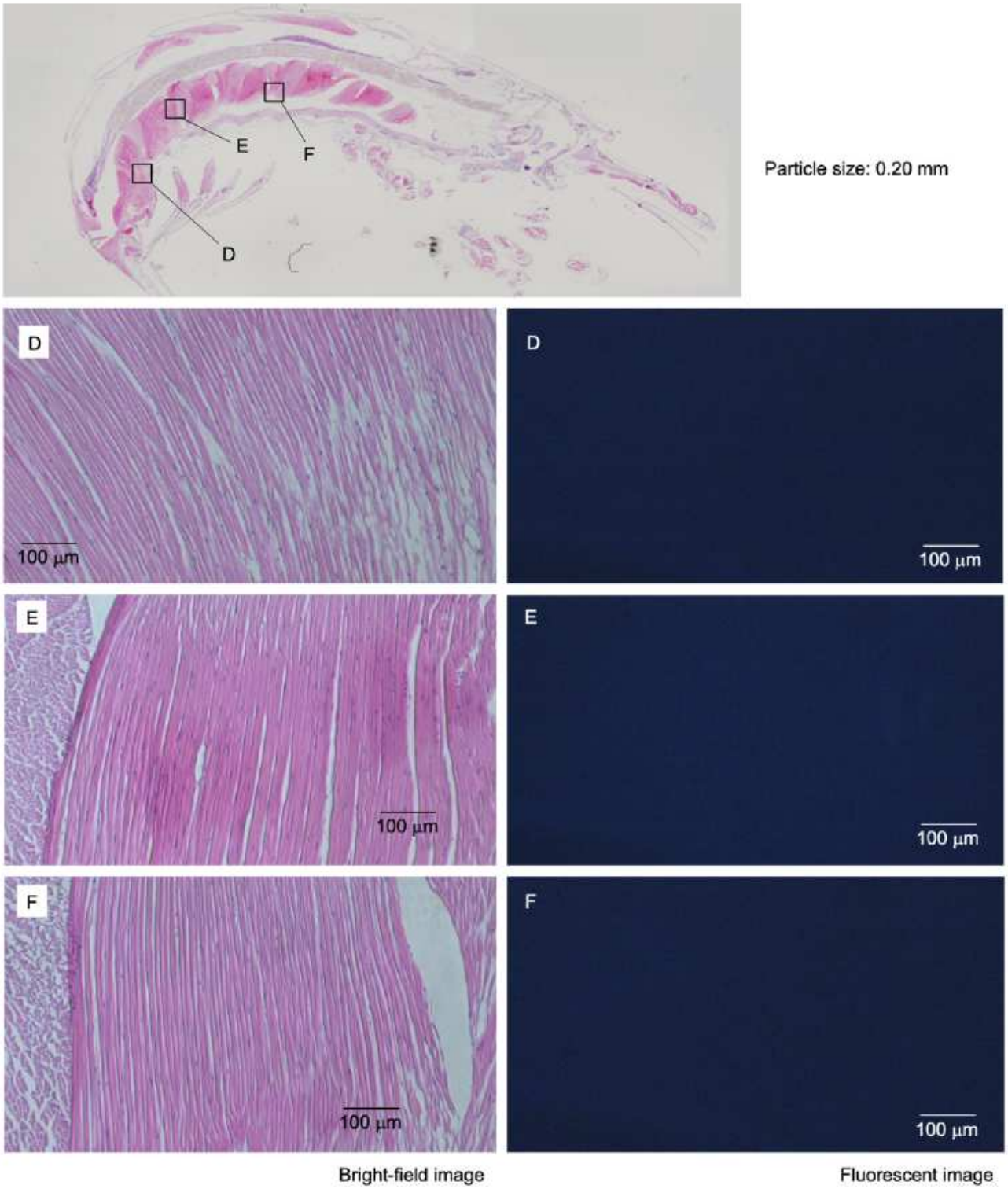

Figure 6

Detailed observations of the shrimps exposed to 0.20 μm particles (outside the digestive system)