

Accumulation of Pd-doped Polystyrene Nanoplastics in the Digestive Tract of *Sebastes Schlegelii*

Xiaohan Wang¹, Hao Wang^{2*} and Xia Liu²

¹Sanya Oceanographic Institution, Ocean University of China, 572000 Sanya, China

²College of Environmental Science and Engineering, Ocean University of China, 266100 Qingdao, China

Abstract. Nanoplastics (NPs) are widely concerned because of their potential adverse effects on marine organisms. However, the detection and quantification of NPs are challenging due to their small size and weak signals. In this study, the accumulation of Pd-doped polystyrene (PS) NPs in the digestive tract of *Sebastes schlegelii* was investigated by an inductively coupled plasma mass spectrometer (ICP-MS). Results showed that NPs could be accumulated in the digestive tract and faeces after 3-day exposure with concentrations of 0.5, 5, and 10 mg/L. The highest accumulation of NPs was in the digestive tract, up to 0.23 ± 0.02 mg/g wet wt after 2 days. However, the highest accumulation of NPs was found in faeces (29.59 ± 0.54 mg/g wet wt) after 3-day exposure. Moreover, the digestive tract and faeces showed a concentration-dependent of NPs, which may lead to the transfer of NPs to higher trophic levels. The present study provides evidence for the bioaccumulation of NPs in organisms and the potential impact of NPs on ecological risk.

1 Introduction

Plastics are widely distributed in the environment. It is estimated that the annual discharge of plastic waste into rivers, lakes and oceans was 9-23 million tons in 2016, which will increase to about 53 million tons by 2030 [1]. Plastic debris in the marine environment degrades into nanoscale plastic debris and particles caused by weathering processes (e.g., ocean currents, wind, mechanical wear) [2]. Micro-plastics (MPs) and nanoplastics (NPs) could be ingested by fish and inhaled through their gills via respiration [3]. Due to their small size, NPs can pass through biological barriers, penetrate tissues, and accumulate in organs [4-5]. Fluorescence spectrophotometers are primarily used in these studies for quantitative analysis of NPs accumulated in biological tissues, however, this detection method is inaccurate because fluorescence is easily leaky [6]. Therefore, more precise quantitative methods are required. Metal-doping NPs are confirmed as an available method to quantify NPs. It enables the simple and accurate quantification of NPs in living organisms [7].

The *Sebastes schlegelii* was chosen as typical marine fish to investigate the accumulation of Pd-doped polystyrene (PS) NPs in the digestive tract through a short-term exposure experiment. The findings will provide helpful information for understanding the accumulation of NPs in marine organisms.

2 Materials and Method

2.1 Synthesis and characterization of Pd-doped nanoplastics

The Pd-doped PS NPs were synthesized by the modified method of Mitrano et al. [8]. Typically, the metal Pd particle core was synthesized using acrylonitrile (AN), surfactants (SDS and KPE), an initiator (KPS), and metal precursor (K_2PdCl_4). The second step is adding more dissolved KPS, and then connecting a feed of the monomer mixture to the reaction vessel to produce Pd-doped PS NPs with a core-shell structure. Following synthesis, anhydrous ethanol was added to the synthesized PS NPs before centrifuging them at 8000 rpm for 10 minutes and removing the supernatant. After three repetitions, ultrasound with ultrapure water for 30 minutes, and dialysis for two to three days were used to remove impurities from the NPs. Then the PS NPs suspension was mixed with a 3:1 mixture of nitric acid and hydrochloric acid for microwave digestion. After that, the concentration of Pd in NPs was determined using an inductively coupled plasma mass spectrometer (ICP-MS, NexION 350X, Perkin Elmer, American).

The morphology of PS NPs was observed using the scanning electron microscopy (SEM, S-4800, Hitachi, Japan) operating at 3.0 kV. Fourier transformation infrared spectroscopy (FTIR, Spectrum Two, Perkin Elmer, American) was examined for ascertaining the chemical groups of PS NPs. The particle hydrodynamic diameter and zeta potential of NPs in ultrapure water and

*wanghaoouc@163.com

filtered seawater (0.45 μm , cellulose acetate membrane) were determined using a Zetasizer (Nano ZS90, Malvern Instruments, UK).

2.2 Subject organism and experimental design

The *S. schlegelii* (purchased from Qingdao Qingyuan Marine Biotechnology Co., LTD.) were randomly placed in 3 tanks (16 individuals in each tank) containing 20 L of seawater and continuously aerated for 14 days at a temperature of $20 \pm 0.5^\circ\text{C}$. The fish were fed commercial feeds at 1% of their body weight for two days before the exposure experiment.

The experiments were carried out in 5 L tanks containing 2 L of clean seawater as a control and NPs suspension (0.5, 5, 10 mg/L) as a treatment. The whole experiment lasted for 3 days. Every day, three fish from every tank were removed, washed with ultrapure water, and anesthetized with ice. Then, the fish were dissected to harvest the digestive tract. Moreover, the faeces from the bottom of the tank were collected and rinsed with ultrapure water three times. The digestive tract of *S. schlegelii* and its faeces were weighed and stored at a -20°C refrigerator for later analysis.

2.3 Quantification of NPs by ICP-MS

Nitric acid and hydrochloric acid with a 3:1 mixture was added to the digestive tract or faeces sample for digestion. The acid was removed by evaporation to about 1 mL from the samples after they were digested using a microwave-assisted digestion system (MARS 6, GEM, USA). The

concentrated solution was then diluted to 10mL with ultrapure water. The Pd content in the digestion tract and faeces sample solution was then measured using ICP-MS to quantify NPs.

2.4 Statistical analysis

All data was were analysed using SPSS 26.0. Analysis of Variance (ANOVA) and a least significant difference (LSD) test ($p < 0.05$ for the statistical significance) were used to test for significant differences in digestion tract and faeces between treatment groups.

3 Results and Discussions

3.1 Characterization

The SEM images showed that the particle size of PS NPs was 102.1 ± 4.7 nm with a typical spherical shape (Fig. 1A). Then the polymer types are proved to PS NPs according to the typical functional groups in FTIR (Fig. 1B). Specifically, the peaks appear at 786 cm^{-1} and 677 cm^{-1} for monosubstituted benzene ring=CH deformation vibration, stretching vibration, 1074 cm^{-1} for aromatic C-H deformation vibration, 1250 cm^{-1} for C-O stretching, 1358 cm^{-1} for C-OH stretching, 1454 cm^{-1} for aromatic C-H tensile vibration, 1656 cm^{-1} for C=O stretching vibrations, 2243 cm^{-1} for C $\equiv$ N tension and 2940 cm^{-1} for asymmetric tension of C-H of CH₂, respectively [9]. We speculated that PS NPs were made of polystyrene from the peak signals of FTIR.

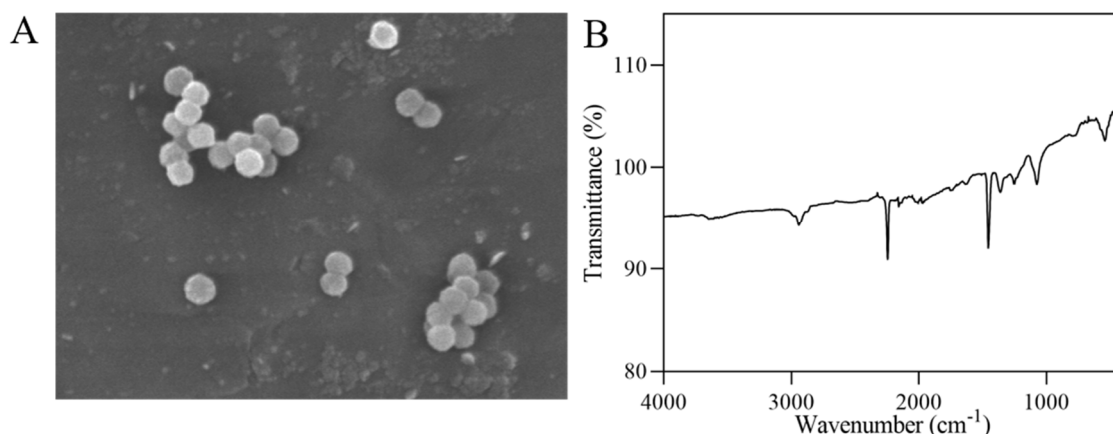


Figure 1. Characterization of PS NPs. (A) SEM images of PS NPs; (B) FTIR spectra of PS NPs

The hydrodynamic diameters of PS NPs in ultrapure water and seawater were 175.2 ± 5.5 nm and 230.4 ± 3.8 nm, respectively. It was observed that PS NPs could easily aggregate in seawater (Fig. 2B). For the zeta potential of

NPs (Fig. 2A), it was observed that the potential of PS NPs in ultrapure water (-26.3 ± 1.8) was more negative than that in seawater (-10.7 ± 0.7 mV), which may be due to the stronger ionic strength.

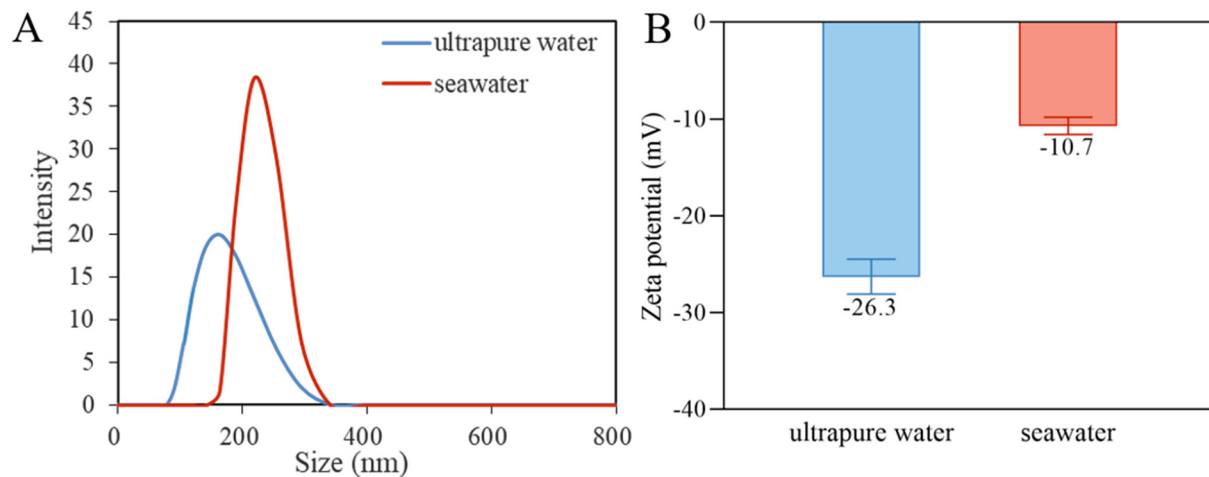


Figure 2. Hydrodynamic diameters (A) and Zeta potentials (B) of PS NPs in ultrapure water and seawater.

3.2 Accumulation and excretion of NPs by *S. schlegelii*

After three days of exposure to PS NPs (0.5, 5 and 10 mg/L), it was observed that the NPs in the digestive tract (Fig. 3) of *S. schlegelii* were highest after 2 days, with the concentrations were 0.06 ± 0.02 , 0.10 ± 0.03 , 0.23 ± 0.02 mg/g wet wt, respectively.

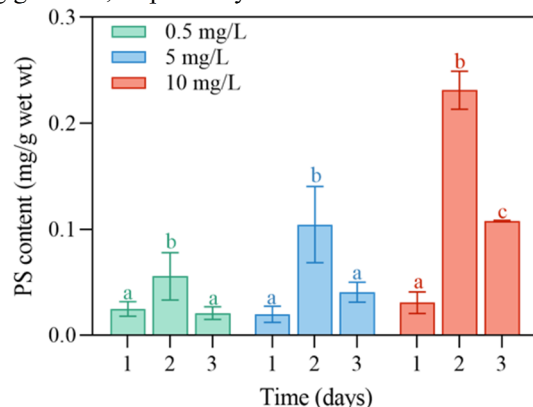


Figure 3. The content of PS NPs in digestive tract.

The accumulation in faeces of *S. schlegelii* was different from the digestive tract. The accumulation of NPs in faeces (Fig. 4) was highest after 3 days of exposure, with the concentration were 0.74 ± 0.01 , 12.72 ± 0.33 , 29.59 ± 0.54 mg/g wet wt. It should be noted that the accumulation of NPs in the digestive tract was about 4 times higher in the high-concentration group (10 mg/L) than in the low-concentration group (0.5 mg/L). These findings suggest that NPs can accumulate in the digestive tract and cause health risks.

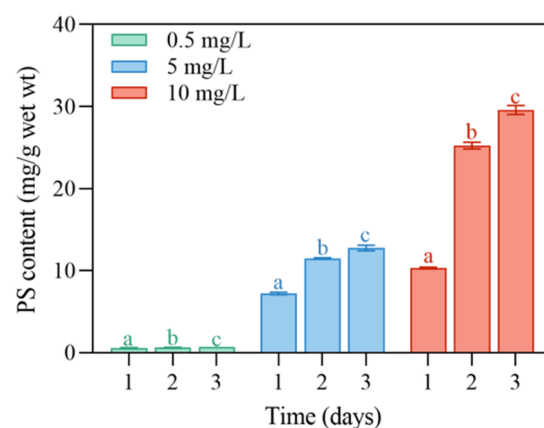


Figure 4. The content of PS NPs in faeces.

The digestive tract is an essential organ for fish to absorb substances, and pollutants or nutrients may be absorbed and transferred to other organs or muscles via small intestinal epithelial cells. It is necessary to study the accumulation of the digestive tract [10]. There have been few quantitative assessments of NPs uptake by fish, and one study found that absorption of 44 nm PS NPs was not detected in goldfish (*Carassius auratus*) exposed to NPs for 30 days using dimensional exclusion chromatography in the gastrointestinal tract in combination with high-resolution mass spectrometry [11]. This result contrasts with our findings, revealing a high accumulation of NPs in the digestive tract, with the majority of it excreted in faeces. Other tissues and organs play an essential role in the accumulation of NPs, which will investigate in the future work.

4 Conclusion

This study focused on the accumulation of NPs in the digestive tract and faeces of *S. schlegelii*. The main results are as follows:

- (1) The synthetic PS NPs are about 100 nm with a negative charge in seawater.
- (2) Accumulation of NPs in the gastrointestinal tract and faeces showed a concentration-dependent, and most were excreted in faeces, up to 29.6 mg/g wet wt (10 mg/L).

Acknowledgments

This work was financially supported by Natural Science Foundation of Shandong Province (ZR2020QD128), China Postdoctoral Science Foundation (2019M660168) and Qingdao Postdoctoral Applied Research Project.

References

1. Borrelle, S. B., Ringma, J., Law, K. L., Monnahan, C. C., Lebreton, L., McGivern, A., Murphy, E., Jambeck, J., Leonard, G. H., Hilleary, M. A., Eriksen, M., Possingham, H. P., Frond, H. D., Gerber, L. R., Polidoro, B., Tahir, A., Bernard, M., Mallos, N., Barnes, M., Rochman, C. M. (2020) Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. *Science*, 369(6510), 1515-1518. <https://doi.org/10.1126/science.aba3656>.
2. Kim, S. W., Chae, Y., Kim, D., An, Y. J. (2019) Zebrafish can recognize microplastics as inedible materials: Quantitative evidence of ingestion behavior. *Sci. Total Environ.*, 649: 156-162. <https://doi.org/10.1016/j.scitotenv.2018.08.310>.
3. Jaafar, N., Azfaralariff, A., Musa, S. M., Mohamed, M., Yusoff, A. H., Lazim, A. M. (2021). Occurrence, distribution and characteristics of microplastics in gastrointestinal tract and gills of commercial marine fish from Malaysia. *Sci. Total Environ.*, 799, 149457. <https://doi.org/10.1016/j.scitotenv.2021.149457>.
4. Alimi, O. S., Farner, B. J., Hernandez, L. M., Tufenkji, N. (2018) Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport. *Environ. Sci. Technol.*, 52(4): 1704-1724. <https://doi.org/10.1021/acs.est.7b05559>.
5. Huang, D., Tao, J., Cheng, M., Deng, R., Chen, S., Yin, L., Li, R. (2021) Microplastics and nanoplastics in the environment: Macroscopic transport and effects on creatures. *J. Hazard. Mater.*, 407: 124399. <https://doi.org/10.1016/j.jhazmat.2020.124399>.
6. Nguyen, B., Tufenkji, N. (2022) Single-particle resolution fluorescence microscopy of nanoplastics. *Environ. Sci. Technol.*, 56(10), 6426-6435. <https://doi.org/10.1021/acs.est.1c08480>.
7. Cai, H., Xu, E. G., Du, F., Li, R., Liu, J., Shi, H. (2021) Analysis of environmental nano-plastics: Progress and challenges. *Chem. Eng. J.*, 410: 128208. <https://doi.org/10.1016/j.cej.2020.128208>.
8. Mitrano, D. M., Beltzung, A., Frehland, S., Schmiedgruber, M., Cingolani, A., Schmidt, F. (2019) Synthesis of metal-doped nanoplastics and their utility to investigate fate and behaviour in complex environmental systems. *Nat. Nanotechnol.*, 14(4): 362-368. <https://doi.org/10.1038/s41565-018-0360-3>.
9. Baig, N., Alghunaimi, F. I., Saleh, T. A. (2019) Hydrophobic and oleophilic carbon nanofiber impregnated styrofoam for oil and water separation: A green technology. *Chem. Eng. J.*, 360, 1613-1622. <https://doi.org/10.1016/j.cej.2018.10.042>.
10. Zazouli, M., Nejati, H., Hashempour, Y., Dehbandi, R., Fakhri, Y. (2022) Occurrence of microplastics (MPs) in the gastrointestinal tract of fishes: A global systematic review and meta-analysis and meta-regression. *Sci. Total Environ.*, 815, 152743. <https://doi.org/10.1016/j.scitotenv.2021.152743>.
11. Brandts, I., Cánovas, M., Tvarijonaviciute, A., Llorca, M., Vega, A., Farré, M., Pas-tor, J., Roher, N., Teles, M. (2022) Nanoplastics are bioaccumulated in fish liver and muscle and cause DNA damage after a chronic exposure. *Environ. Res.*, 212, 113433. <https://doi.org/10.1016/j.envres.2022.113433>.