Marine Pollution Bulletin 208 (2024) 117018

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

A hidden lethal effect of long microplastic fibres on the coastal copepod *Acartia erythraea*

Kazutaka Takahashi [a,*], Jun Chin Teh [b]

[a] *Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1, Yayoi, Bunkyo, Tokyo, Japan*
[b] *International Institute of Aquaculture and Aquatic Sciences (I-AQUAS), Universiti Putra Malaysia, Port Dickson, Negeri Sembilan, Malaysia*

ARTICLE INFO

Keywords:
Calanoid copepod
Microfibre
Microplastic pollution
Synthetic fibre

ABSTRACT

We investigated the lethal effects of a long fibrous microplastic (the range of lengths, widths, and thicknesses were 1.5–3.6 mm, 10–16 μm, and 7–8.5 μm, respectively) made of polyethylene terephthalate textile, on the marine copepod *Acartia erythraea*. In laboratory, starved copepods were observed to take in a piece of fibrous microplastic sedimented on the bottom. While no individual ingested the entire fibre, the tip of the ingested fibre reached deep into the gut of the copepods. This suggests that ingestion was not accidental but purposeful behaviour to take in non-living organic matter as a supplementary food source. All copepods that had the fibre in their mouths eventually died within 24-h because the fibre penetrated deep into the gut, preventing feeding and potentially causing stress. Our finding implies that a single piece of microplastic fibre remaining at the bottom of coastal zones could continue to kill copepods owing to its non-degradability.

1. Introduction

Plastic debris has been accumulating in the oceans over the past few decades and is considered one of the main threats to marine environments and associated biodiversity (Agathokleous et al., 2021; Hale et al., 2020). In particular, microplastics (plastic particles or fibres <5 mm in size) have been discovered in every ocean region, from the surface to the deepest trenches, and their contamination of the food chain is a significant concern (Cole et al., 2011). Among the microplastics, fibrous particles released from textiles have been identified as a dominant component transported by rivers to marine systems (Periyasamy and Tehrani-Bagha, 2022). Several studies from the field investigating microplastic ingestion in zooplankton have found that microfibres were most commonly ingested (Desforges et al., 2015; Sun et al., 2017; Sun et al., 2018; Zheng et al., 2021; Aytan et al., 2022; Gunaalan et al., 2023). Furthermore, the ingestion of microfibres by zooplankton has been studied, with evidence of reduced feeding, energy depletion, injury, and mortality (Jemec et al., 2016; Cole et al., 2019; Iwalaye and Maldonado, 2024), although some studies did not detect a substantial effect on their ecophysiology and survival (Köster and Paffenhöfer, 2022).

To date, most of these studies regarding the effects of microfibres have been largely limited to those of edible size for zooplankton (<1 mm). However, microfibres are often flexible and possess a uniquely asymmetric geometry; they are polydisperse in size, from the μm to the mm range, and thus, their characteristics may present novel risks. For instance, Kang et al. (2020) showed that zooplankton may become entangled in long microfibres, which poses a potential risk to these organisms. During an experimental study using coastal copepods (Rahman et al., 2022), we occasionally found individuals holding a long microplastic fibre, which had contaminated to the experimental bottle, in their mouth (Supplementary material). Thus, we sought to understand the potential risk of exposure to a single long fibrous microplastic on the survival of the coastal planktonic copepod *Acartia erythraea*.

2. Materials and methods

2.1. Preparation of microplastic fibres from PET fabric

PET (polyethylene terephthalate) fibres were chosen since they are most abundant polymer in aquatic environments (Coppock et al., 2019; Mahara et al., 2022; Zhang et al., 2022; Zheng et al., 2020) and denser (1.23–2.30 g.cm^{-3}) than seawater (1.02–1.03 g.cm^{-3}) (Uddin et al., 2020), enhancing the encounter rates with marine organisms in the water column. PET microfibres were collected from a washing machine where a pullover shirt made of PET fabric was laundered at a laboratory

---

* Corresponding author.
E-mail address: kazutakahashi@g.ecc.u-tokyo.ac.jp (K. Takahashi).

https://doi.org/10.1016/j.marpolbul.2024.117018
Received 24 July 2024; Received in revised form 14 September 2024; Accepted 15 September 2024

Fig. 1. Fibrous microplastic used in this study: a) pullover shirt from which fibrous microplastic was taken, b) fibrous microplastic taken from the shirts, and c) enlarged view of b), showing the thickness of the fibres.

of BSCI Shin-Kamigoto Co., Ltd., Nagasaki (Fig. 1a, b). These microfibres were washed three times with distilled water and then three times with 90 % ethanol. The cleaned microfibres were dried at 50 °C overnight and stored in a laboratory dessicator until the experiments. Stainless tweezers cleaned with 90 % ethanol were used to handle the microfibre throughout the experiments. The ranges of microfibre lengths, widths, and thicknesses were 1.5–3.6 mm, 10–16 μm, and 7–8.5 μm, respectively (Fig. 1c).

2.2. *Experimental setting*

Copepods used in the experiments were collected at night (ca. 19:00) on the day before the experiments from a pier at Azetsu, Shin-Kamigoto, Nagasaki (32°57′09.5″N 129°06′31.7″E) using a conical plankton net (200-μm mesh) with a closed cod-end on 14–16 October 2021. Collected individuals of *Acartia erythraea* were immediately transferred to a container (20*L*) filled with surface seawater taken from the sampling site and pre-incubated for 24 h with gentle aeration at 25 °C. Only adult copepods with a body length range of 1100–1500 μm were used for experiments. As food, a diatom strain—TW P105 (*Thalassiosira weissflogii*)—known to be effective in ameliorating the adverse effects of high-density cultivation owing to its highly anti-oxidative capacity (Rahman et al., 2022) was added at 1500 μg C L^{-1}.

Two types of experiments were conducted in triplicate to examine the effects of PET fibres on copepods in either sedimented or suspended conditions, assuming the different encounter situations through diel vertical migration, which is characteristic behaviour of coastal *Acartia* species including *A. erythraea* (Emery, 1968, Hamner and Carleton, 1979, Ueda et al. 1983). All incubation experiments started at night (ca. 20:00–22:00). For the sedimented condition, five females and two pieces of microfibre were placed in a 10-mL chamber filled with filtered (0.2 μm) seawater collected using a bucket from the same site at which the copepods were sampled. A total of 11 chambers each were prepared for treatments with food (TW P105 at 1500 μg C L^{-1}) and without food and incubated under static conditions for 24 h at 25 °C in darkness. Mean mortality rates after the 24-h incubations were 22 and 23 % for fed and non-fed conditions, respectively. As for the suspended conditions, 30 adult females with 12 microfibres were introduced to a 50-mL jar filled with filtered seawater. Triplicate bottles were prepared with food (TW P105 at 1500 μg C L^{-1}) and without food, respectively. The jars were mounted on a slowly rotating (<0.3 rpm) plankton wheel and incubated for 24 h at 25 °C under dark conditions. All bottles were sealed with

Table 1
Results of incubation experiments to examine the possible ingestion of fibrous microplastic by copepods under suspended and sedimented conditions.

			% copepods with fibre in moutth	
Experimental conditions	Exp ID	n	With food	No food
Suspended condition (30 copepods +12 fibres)	R-1	3	0	0
	R-2	3	0	0
	R-3	3	0	0
	Mean		0	0
Sedimented condition (5 copepods +2 fibres)	S-1	11	0	3.6
	S-2	11	0	5.5
	S-3	11	0	1.8
	Mean		0	3.6

cling film to exclude air bubbles. Mean mortality rates after the 24-h incubations were 15 and 29 % for the fed and non-fed conditions, respectively.

For all experiments, after the 24-h incubations, any interaction with microfibres was recorded under a dissecting microscope (MZ12, Leica, Wetzlar, Germany). Any individuals associated with microfibres were transferred to the well (ca. 10 mL) of a micro-plate with filtered seawater for an extra 24 h of observation. TW P105 was added at 1500 μg C L^{-1} to each well. After that, all individuals in a given well were fixed with 2 % formalin for later observation with higher magnification using a digital microscope (VHX-8000, Keyence, Osaka, Japan).

3. Results

Under the suspended condition, no interaction between microfibres and copepods was observed after 24 h of incubation (Table 1). In the static condition, in which the microfibres were sedimented on the bottom of the experimental chamber, 3.6 % of *A. erythraea* on average held a piece of fibre in their mouths in the non-fed treatment; meanwhile, no such occurrences were observed in the fed treatments (Table 1). Although all the individuals that held a piece of microfibre in their mouths were alive just after the incubation (Supplementary material), subsequent observation showed that they all died within the following 24 h. Detailed observations revealed that the fibre was tightly held in the mouth of the copepod (Fig. 2). In most cases, copepods appeared to be trying to take in a microfibre from the end, and the tip of the ingested fibre reached deep into its gut (Fig. 2a–d). One individual was observed trying to take in a microfibre at the middle (Fig. 2e).

4. Discussion

The experiments revealed that *A. erythraea* potentially takes in a piece of fibrous microplastic in the sedimented condition under starvation. As the length of microplastic used in this study was longer than 1 mm, no individual ingested the entire fibre. However, the tip of the ingested fibre reached deep into the gut of copepods, suggesting that ingestion was purposeful. A congeneric species, *Acartia tonsa*, also reported showing a preference for fresh-cut virgin nylon fibres (20 μm × 10 μm), particularly those infused with the algal-derived infochemicals, dimethyl sulfide (DMS) or dimethylsulfoniopropionate (DMSP) (Botterell et al., 2020). Furthermore, plastic fibres were commonly ingested by the marine copepod *Neocalanus cristatus* (556 ± 149-μm-long fibres), the euphausiid *Euphausia pacifica* (816 ± 108-μm-long fibres) (Desforges et al., 2015), and the freshwater daphnid (300–1400-μm-long fibres) (Jemec et al., 2016). This indicates that zooplankton, including copepods, readily ingest long synthetic fibres from the environment.

Why does *A. erythraea* attempt to ingest such long synthetic fibres, even though it inevitably causes mortality? Because the fed copepods in the static condition did not appear to ingest the microfibre, it is

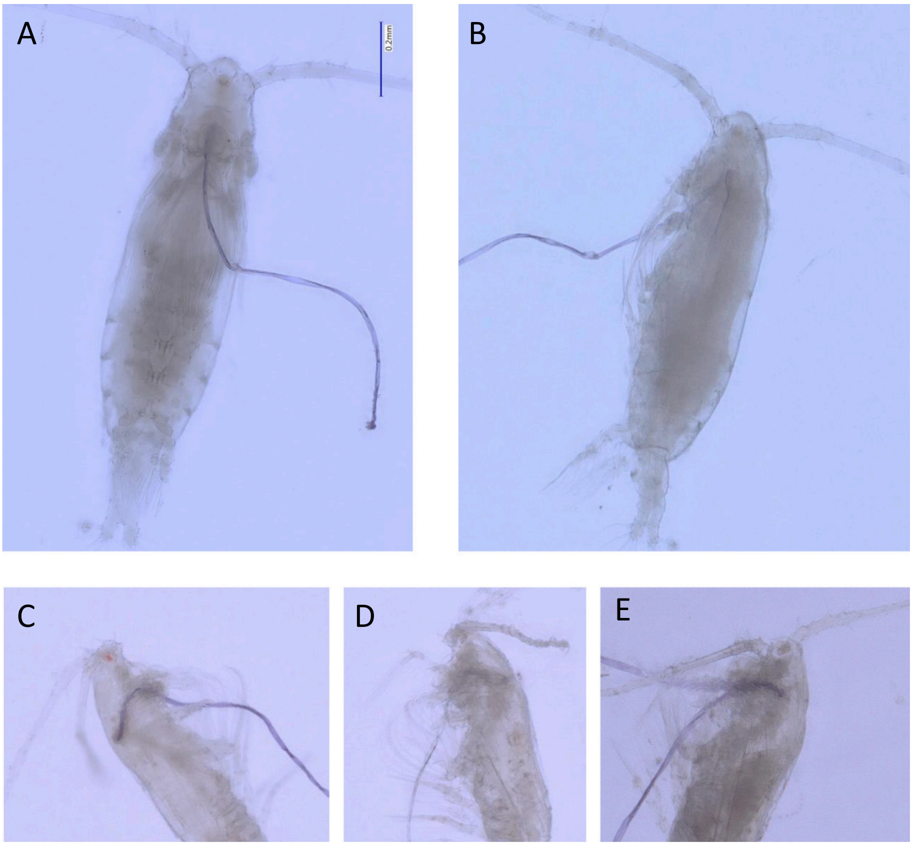

Fig. 2. *Acartia erythraea* with a long microplastic fibre in its mouth obtained from incubation experiments under the static condition without food. Note: a) and b) show the same individual but from ventral and dorsal sides, respectively, while c), d), and e) show different individuals.

reasonable to assume that *A. erythraea* consumed the plastic fibre as the result of a hunger-induced response. Generally, coastal *Acartia* species including *A. erythraea* are known to form swarms and are closely associated with the bottom during the daytime (Emery, 1968; Hamner and Carleton, 1979; Ueda et al., 1983). Therefore, it is likely that *Acartia* species can forage on non-living organic matter (detritus) in the near-bottom environment, particularly when they are starved. Hu et al. (2018) reported that land plants mostly dominate the diet of *A. erythraea* at 29 °C, and they attributed this to food limitation at the study site, which likely resulted in the consumption of large amounts of land-plant detritus as a supplementary food source. Natural fibres derived from land plants or seagrasses are known to be rich in associated bacteria and microbiota (Blum et al., 1988). Because synthetic fibres are as soft and flexible as natural fibres, starved copepods may mistakenly ingest them.

Our findings imply that a single piece of non-degradable microplastic fibre remaining at the bottom of coastal water poses an ongoing and lethal threat to copepods that aggregate near the sea bottom. Furthermore, a copepod with a fibre in its mouth would be susceptible to predation owing to disruption of its escape response (see Supplementary material). This also suggests that the ingestion of long microfibres by copepods may enhance microplastic contamination in the marine food web (Gunaalan et al., 2023). Fibres are identified as the most common form of microplastic in marine sediment (Harris, 2020; Fagiano et al., 2023). Additionally, the genus *Acartia* is diverse and distributed throughout the coastal waters of the world's oceans. It is also a dominant member of the zooplankton community. Therefore, the risk of *Acartia* encountering synthetic fibres is currently increasing on a global scale. Yet how *Acartia* or other planktonic copepods may react to these particles, other than directly feeding on them, has not been adequately investigated. Given that microplastics are diverse in shape, texture, and size, their influence on aquatic organisms is also likely to differ. Various toxicological outcomes—for example, blocking of the digestive tract, or irritation or laceration of tissues—have been suggested (Hale et al., 2020), in addition to the disruption of key behaviour owing to microfibre entanglement (Kang et al., 2020). These possibilities suggest that microplastic fibres may have a detrimental impact on the survival of marine zooplankton, due to their distinctive asymmetric geometry and flexibility. Further research on the impact of microplastic fibres on zooplankton is thus required.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.117018.

CRediT authorship contribution statement

Kazutaka Takahashi: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Funding acquisition, Conceptualization. **Jun Chin Teh:** Writing – review & editing, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank Norio Nagao, BSCI Shin-Kamigoto Co., Ltd., Nagasaki, Japan for providing facilities for laboratory experiments. Kentaro Miyazono helped photograph samples with a digital microscope. T. Katayma provided diatom culture. This research was performed with the help of the Environment Research and Technology Development Fund (JPMEERF20221001) of the Environmental Restoration and Conservation Agency of Japan and the University of Tokyo FSI (Nippon Foundation Research Project on Marine Plastics). We thank Nattalie Kim, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

References

Agathokleous, E., Iavicoli, I., Barceló, D., Calabrese, E.J., 2021. Ecological risks in a 'plastic' world: a threat to biological diversity? J. Hazardous Materials 417, 126035.

Aytan, U., Esensoy, F.B., Senturk, Y., 2022. Microplastic ingestion and egestion by copepods in the Black Sea. Sci. Total Environ. 806, 150921.

Blum, L.K., Mills, A.L., Zieman, J.C., Zieman, R.T., 1988. Abundance of bacteria and fungi in seagrass and mangrove detritus. Mar. Ecol. Prog. Ser. 42, 73–78.

Botterell, Z.L.R., Beaumont, N., Cole, M., Hopkins, F.E., Steinke, M., Thompson, R.C., Lindeque, P.K., 2020. Bioavailability of microplastics to marine zooplankton: effect of shape and infochemicals. Environ. Sci. Tech. 54, 12024–12033.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597.

Cole, M., Coppock, R., Lindeque, P.K., Altin, D., Reed, S., Pond, D.W., Sørensen, L., Galloway, T.S., Booth, A.M., 2019. Effects of nylon microplastic on feeding, lipid accumulation, and moulting in a Coldwater copepod. Environ. Sci. Tech. 53, 7075–7082.

Coppock, R.L., Galloway, T.S., Cole, M., Fileman, E.S., Queirós, A.M., Lindeque, P.K., 2019. Microplastics alter feeding selectivity and faecal density in the copepod. *Calanus helgolandicus*. Sci. Total Environ. 687, 780–789.

Desforges, J.P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. Arch. Environ. Contam. Toxicol. 69, 320–330.

Emery, A.R., 1968. Preliminary observations on coral reef plankton. Limnol. Oceanogr. 13, 293–303.

Fagiano, V., Compa, M., Alomar, C., Rios-Fuster, B., Morató, M., Capó, X., Deudero, S., 2023. Breaking the paradigm: marine sediments hold two-fold microplastics than sea surface waters and are dominated by fibers. Sci. Total Environ. 858, 159722.

Gunaalan, K., Nielsen, T.G., Torres, R.R., Lorenz, C., Vianello, A., Andersen, C.A., Vollertsen, J., Almeda, R., 2023. Is zooplankton an entry point of microplastics into the marine food web? Environ. Sci. Technol. 57, 11643–11655.

Hale, R.C., Seeley, M.E., La Guardia, M.J., Mai, L., Zeng, E.Y., 2020. A global perspective on microplastics. J. Geophys. Res. Oceans 125, e2018JC014719.

Hamner, W.M., Carleton, J.H., 1979. Copepod swarms: attributes and role in coral reef ecosystems. Limnol. Oceanogr. 24, 1–14.

Harris, P.T., 2020. The fate of microplastic in marine sedimentary environments: a review and synthesis. Mar. Pollut. Bull. 158, 111398.

Hu, S., Liu, S., Wang, L., Li, T., Huang, H., 2018. Feeding response of the tropical copepod *Acartia erythraea* to short-term thermal stress: more animal-derived food was consumed. PeerJ 6, e6129.

Iwalaye, O.A., Maldonado, M.T., 2024. Microcosm study of the effects of polyester microfibers on the indigenous marine amphipod (*Cyphocaris challengeri)* in the strait of Georgia (BC, Canada). Sci. Total Environ. 906, 167301.

Jemec, A., Horvat, P., Kunej, U., Bele, M., Krzan, A., 2016. Uptake and effects of microplastic textile fibers on freshwater crustacean *Daphnia magna*. Environ. Pollut. 219, 201–209.

Kang, J.-H., Kwon, O.-Y., Hong, S.H., Shim, W.J., 2020. Can zooplankton be entangled by microfibers in the marine environment?: laboratory studies. Water 12, 3302.

Köster, M., Paffenhöfer, G.-A., 2022. Feeding of marine zooplankton on microplastic fibers. Arch. Environ. Contam. Toxicol. 83, 129–141.

Mahara, N., Alava, J.J., Kowal, M., Grant, E., Boldt, J.L., Kwong, L.E., Hunt, B.P.V., 2022. Assessing size-based exposure to microplastic particles and ingestion pathways in zooplankton and herring in a coastal pelagic ecosystem of British Columbia. Canada. Mar. Ecol. Prog. Ser. 683, 139–155.

Periyasamy, A.P., Tehrani-Bagha, A., 2022. A review on microplastic emission from textile materials and its reduction techniques. Polym. Degrad. Stab. 199, 109901.

Rahman, N.A., Teh, J.C., Katayama, T., Effendy, A.W.M., Nagao, N., Takahashi, K., 2022. Effect of newly isolated high antioxidant diatom on the reproduction of the marine copepod, *Acartia erythraea* under crowding stress. Aquacult. Res. 53, 5365–5374.

Sun, X., Li, Q., Zhu, M., Liang, J., Zheng, S., Zhao, Y., 2017. Ingestion of microplastics by natural zooplankton groups in the northern South China Sea. Mar. Pollut. Bull. 2017 (115), 217–224.

Sun, X., Liang, J., Zhu, M., Zhao, Y., Zhang, B., 2018. Microplastics in seawater and zooplankton from the Yellow Sea. Environ. Pollut. 242, 585–595.

Uddin, S., Fowler, S.W., Saeed, T., 2020. Microplastic particles in the Persian/Arabian gulf–a review on sampling and identification. Mar. Pollut. Bull. 154, 111100.

Ueda, H., Kuwahara, A., Tanaka, M., Azeta, M., 1983. Underwater observations on copepod swarms in temperate and subtropical waters. Mar. Ecol. Prog. Ser. 11, 165–171.

Zhang, S., Zhang, W., Ju, M., Qu, L., Chu, X., Huo, C., Wang, J., 2022. Distribution characteristics of microplastics in surface and subsurface Antarctic seawater. Sci. Total Environ. 838, 156051.

Zheng, S., Zhao, Y., Liangwei, W., Liang, J., Liu, T., Zhu, M., Sun, X., 2020. Characteristics of microplastics ingested by zooplankton from the Bohai Sea. China. Sci. Total Environ. 713, 136357.

Zheng, S., Zhao, Y., Liu, T., Liang, J., Zhu, M., Sun, X., 2021. Seasonal characteristics of microplastics ingested by copepods in Jiaozhou Bay, the Yellow Sea. Sci. Total Environ. 2021 (776), 145936.