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Microplastics Quantification in *Meretrix lyrata* through Rapid Screening Method using Nile Red

NGIE-HAO ERIC LAU[1*], AAZANI MUJAHID[1*], CINDY PETER[2] & MORITZ MÜLLER[3]

[1]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia; [2]Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia; [3]Faculty of Engineering, Computing and Science, Swinburne University of Technology Sarawak Campus, Kuching, Sarawak, Malaysia
*Corresponding author: 18020217@siswa.unimas.my; maazani@unimas.my
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ABSTRACT

Microplastics have emerged as a significant form of plastic pollution. Many ingestions in biota were reported worldwide. Filter feeder such as bivalves are prone to microplastics ingestion due to the non-selective feeding behavior. Bivalves are usually consumed as whole, without gut removal, which can pose a threat towards human consumption. This study focused on *Meretrix lyrata* to examine the level of microplastics ingestion under two factors: size class and weather seasons. The rapid screening approach with the fluorescent tagging using Nile red on microplastics for biota samples was used to quantify *M. lyrata* samples (n = 81) in this study. The high lipid content of the clams presented a significant challenge to the effectiveness of the method. As an alternative, high temperatures from acidification were used to disrupt lipid membranes and improve extraction efficiency. A total of 15,867 microplastics were quantified, with the average microplastics ingestion of 195.90±43.6 items/individuals. The statistical analysis indicated that clam size had a significant effect on the rate of microplastics ingestion ($p<0.05$), whereas weather season did not show a significant effect ($p>0.05$). Polyvinylchloride (66.5%), polypropylene with silicate mix (8.5%), resin dispersion (8.5%) and polydimethylsiloxane (16.5%) were from the 12 isolated items. The higher rate of microplastics ingestion observed in smaller clam size *M. lyrata* and similar rate between weather season (dry season and wet season) indicate that these contaminated seafood increases the human exposure through consumption. The type of polymer identified in this study indicate that most of the microplastic may sources from human daily products.

Keywords: Fluorescent tagging, High lipid content samples, Microplastics, Nile red, Rapid screening method

INTRODUCTION

Plastics pollution have become a prominent environmental issue in aquatic environments. The increasing demand for plastic in various industries is due to its low cost, strength, durability, corrosion resistance, and good thermal and electrical insulation properties (Geyer *et al*., 2017). However, plastics have, finite lifespans and are quickly discarded, often ending up in landfills, being burned, or littering the environment. Research indicates that plastic materials constitute 95% of floating marine debris (Lebreton *et al*., 2018). The persistence of plastics in the environment is the primary factor contributing to their dominance as the leading component of marine debris (Auta *et al*., 2017).

Among the various forms of plastic pollution, small plastic particles typically less than 5 mm in size which also commonly known as microplastics have emerged as a critical environmental contaminant. These particles originate from direct source as such personal care products, industrial processes and synthetic textiles (primary microplastics) and from the degradation of larger plastic items (secondary microplastics) (Hartmann *et al*., 2019). Their minute sizes and extensive distribution make them particularly challenging, as they penetrate even the most secluded aquatic ecosystems, from freshwater rivers to deep-sea sediments.

The small size of microplastics leads to increase accumulation of contaminants due to their hydrophobic nature and high surface area (Shahul Hamid *et al*., 2018; Deng *et al*., 2021). Microplastics pollution has been reported in many marine ecosystems worldwide due to massive input from land source, the persistence of microplastics in environment and the fact that they are readily transported by ocean current (Lavers & Bond, 2017; Brach *et al*., 2018; Reed *et al*., 2018). Lebreton *et al*. (2019) suggested that buoyant macroplastic accumulate in offshore surface water should be lesser than predicted as a series of repeated activity like stranding, settling and resurfacing in coastal environment tend to trap most of the marine litters. The intertidal zone, situated between land and ocean, experiences more human activity compared to other marine environments and can be exposed to microplastics from both land-based and marine sources (Cole *et al*., 2011; Zhang *et al*., 2020; Zhang *et al*., 2022).

Rapid screening method using fluorescent tagging is a new alternative to aid in increasing the precision of the microplastics quantification and identification through visual identification. Nile red is recommended by Maes *et al.* (2017) as the dye for fluorescent tagging due to the lipophilic characteristics, which allow selective staining of plastic polymers when appropriate protocols are employed.

Microplastics pose significant risks to aquatic organisms and can enter the food webs, as they can be ingested by organisms (Hasbudin *et al.,* 2022). *M. lyrata* is particularly susceptible to microplastic ingestion due to its filter-feeding behavior (Woods *et al.,* 2018). Chinfak *et al.* (2021) had shown the possibility of higher microplastics ingestion rate of in small-sized shellfish for *Perna viridis* and *M. lyrata*. Similar trends were also reported in *Mythilus edulis* and *Donax cuneatus* (Scott *et al.,* 2019; Sathish *et al*., 2020). Studies reported negative effects of microplastics on bivalves such as reduced filtration activity and altered feeding behavior (Rist *et al.,*2016; Green *et al.,* 2017), potentially causing physical and chemical stress (Woods *et al.,* 2018), impairing feeding efficiency, and acting as vectors for harmful substances such as persistent organic pollutants (POPs) (Zhang *et al.,* 2020) and heavy metals (Fu *et al.,* 2020; Idrus *et al.,* 2022a). This not only impacts the health of *M. lyrata* populations but also raises significant concerns about the transfer of microplastics and associated contaminant to humans through the consumption of contaminated seafood.

Despite increasing research research on microplastics in aquatic ecosystems, there remains a critical need to understand the specific pathways, interactions, and impacts of microplastics on key species such as M. lyrata. Previous studies (Kolandhasamy *et al*.,2018; Li *et al*., 2019; Hasbudin *et al*., 2022) highlight three pathways of microplastic accumulation in mussels including ingestion, adherence and fusion. Tang *et al.* (2022) suggested quantifying amount of microplastics in clams could provide insight of microplastics contamination in intertidal zones and thus suggested their use as a bio-indicator for environmental monitoring of microplastics.

As bivalves have high potential to serve as bioindicators (Ding *et al*., 2021) for microplastics pollution in sediment and water, investigating the prevalence of microplastics in *M. lyrata* and their surrounding habitats is essential for assessing ecosystem health and potential risks to food safety, where these organisms can serve as vectors, transferring microplastics to humans.

Thus, the aims of this study were to quantify microplastics in *M. lyrata* and to address the impact of size classes and weather seasons towards microplastics ingestion in *M. lyrata*.

MATERIALS AND METHODS

The laboratory benchtop was cleaned, and surfaces were wiped with 70 % ethanol using a cotton cloth. A full cotton lab coat and nitrile gloves were worn during the experiment. All solutions (distilled water, 10 % potassium hydroxide (KOH), zinc chloride ($ZnCl_2$), 10 % hydrochloric acid (HCl) were pre-filtered with 0.5 µm Whatman GC50 filter paper to prevent contamination from other sources. Filter papers used were GF/C (ADVANTEC GC-50, 0.5 µm). Apparatus materials were either glass or stainless steel. Glassware and stainless-steel tweezers were pre-cleaned with filtered deionized water and rinsed with filtered 70 % ethanol.

Sample Processing and Microplastics Extraction

The clams (n = 81) with random sizes were purchased from the local fish market in Kampung Buntal (1° 41' 43.58" N, 110° 22' 19.87" E), from October 2020 to March 2021. The determination of monsoon seasons was based on the dates provided by the Malaysian Meteorological Department (May 2020 – September 2020: Southwest monsoon; November 2020 – Mac 2021: Northeast monsoon). Additionally, Kampung Buntal is one the most active artisanal fishing communities in Kuching Bay where there is an overlap between fisheries activities with cetacean occurrence (Ambie *et al*., 2023).

Upon acquisition, the clams were stored in a freezer at –20 °C after their shells were rinsed with filtered distilled water to remove any surface contaminants. For subsequent processing, the clams were thawed to room temperature, then each clam was dissected, and transferred into a glass bottle for digestion process.

The KOH digestion method was based on the study by Lusher and Hernandez-Milian (2018), where the details of this protocol can be found in their study.

After the digestion process, the microplastics in the clam samples were separated by applying density separation technique based on the study by Maes *et al.* (2017) using $ZnCl_2$ to reduce the possible interference from other non-plastic substances such as shell, wood, sand and clay.

An additional step adapted from Prata *et al.* (2021) is added prior to the density separation steps as clogging and precipitation were observed. High temperature can aid in disrupt lipid membranes and improve extraction efficiency. Acidification is an exothermic process which can achieve the mentioned condition to reduce clogging and prevent precipitation.

Nile Red Staining for Quantification of Microplastics

Nile red stock solutions were prepared in propanol with the ratio of 1:100. Approximately 0.06 g of Nile red powder were measured and mixed with 60 ml of propanol. The stock solution was then stored in a 60 ml Scott bottle pre-wrapped with aluminum foil and kept at 4 ˚C. The stock solution was diluted 100 times in filtered distilled water to make a working solution (1 mg/ml). The filtrate (microplastics) collected from density separation were pre-washed with a small volume of 10 % HCl to remove residual $ZnCl_2$. Subsequently, 10 ml of the Nile red working solution were added to the microplastics and left for 10 – 15 minute to obtain optimum staining results. After staining, the excess Nile red solution was removed by filtering, and the stained microplastics on the filter paper were washed with a small amount of filtered distilled water to eliminate any remaining excess dye.

Lowering the pH of the digested residue extract (10 % HCl) improved the dissolution, thus increased the filtration rate and inhibited precipitation when mixed with $ZnCl_2$. To avoid quenching and false positives (Erni-Cassola *et al*., 2017), additional steps were incorporated.

The filter paper with stained microplastics, was then placed in an automated filter-scanning rig (Maes *et al*., 2017) combines with a commercial micro-milling machine and a DSLR (EOS 90D) camera that was adjusted to create a dim background to enhance the visibility of the fluorescent microplastics. G-code routines controlled the scanning process, dividing the 47 mm filter paper into 24 segments, with each segment being captured individually. This segmentation aimed to produce detailed images with minimal noise, thereby reducing counting errors during the quantification process.

After that, the optimized samples images were illuminated with blue light (Crime Lite: 450 – 510 nm) and viewed through an orange filter (529 nm). The resulting photographs were then processed using ImageJ software, and saved in TIFF format. The camera settings were calibrated individually for each sample. Prata *et al*. (2020) noted that higher ISO settings tend to introduce more noise and defects in images, thus a lower ISO setting was employed in this study in combination with a longer exposure time (2s).

The TIFF images were reviewed and analyzed to quantify the amount of microplastics, which were identified as fluorescent particles.

Verification of Microplastics using ATR-FTIR

The polymers of the microplastic samples were identified by using The Nicolet IN10 ATR-FTIR (Thermo Scientific). Spectra ranges were set at 4000 – 675 cm^{-1}, with resolution of 4 cm^{-1} and 32 scans s^{-1}. The resulting spectra were directly compared with the available open-source database OpenSpecy (www.openspecy.org; Cowger *et al*., 2021).

Statistical Analysis

Morphometric data and quantitative data were compiled and tabulated using Microsoft Excel to compare different size classes and different weather seasons. Statistical test was conducted to analyze the significant differences of microplastics ingestion by difference sizes of bivalve and weather seasons. Normality tests were done to determine data sets were close to normal or skewed data. Student's T test was used as the parametric test if the data sets were normally distributed, while the Mann-Whitney U test was used as the non-parametric test if the data sets were not normally distributed.

RESULTS AND DISCUSSION

Microplastics Quantification in *M. lyrata*

A total of 81 clams with variant of clam size were tested for microplastic counts resulting with 12 to 1047 items/individual. A total of 15,867 items were detected, with an average of 195.90±43.6 items/individual.

The average microplastic counts obtained from current study is higher than other findings nearby countries in the same species (*M. lyrata*) 12.73±4.49 items/individual, 13.20±7.66 items/individual (Tran-Nguyen *et al*., 2023), 3.6±2.1 items/individual (Kieu-Le *et al*., 2022) and 0.67±0.15 items/individual (Chinfak *et al*., 2021).

These differences might be due to the method chosen for digestion and separation (Kieu-Le *et al*., 2022; Tran-Nguyen *et al*., 2023). Quinn *et al*. (2017) suggested to use denser solution (such as $ZnCl_2$) for density separation to get higher recovery rates. Study by Zarlf (2019) found out that extracting lower density microplastics using NaCl solution might fail if the plastic particles were agglomerated with mineral particles. Chinfak *et al*. (2021) and Idrus *et al.* (2022b) used oxidative digestion using 30 % hydrogen peroxide (H_2O_2) to degrade labile organic matters. However, Nuelle *et al*. (2014) advised the usage of oxidative digestion method should be only applied to organic rich samples because of the bleaching effect that might aggravate the visual detection process and cause potential exothermic reaction with other chemicals (NaCl) for density separation.

In this study, the *M. lyrata* samples were divided into 6 size classes in which (0 – 29.99 mm, 30.0 – 39.99 mm, 40.0 – 49 mm) representing small size classes and (50.0 – 59.99 mm, 60.0 – 69.99 mm, 70.0 – 79.99 mm) representing big size classes. The microplastics ingestion in smaller sized class clam (0 – 49.9 mm) with the average of 218.63±54.78 items/individual compared to size class (50.0 – 99.9 mm) with the average of 166±68.86 items/individual. Figure 1 shows the number of microplastics ingestion according to size classes.

Prior to analysis, the data set were identified as not normally distributed, non-parametric analysis, Mann-Whitney U test was used. The Mann-Whitney U test revealed a statistically significant difference in the number of microplastics ($p<0.05$), ($U= 553$, $|z| = 2.40$, $p= 0.018$) between the clam size less than 50 mm and the clam size more than 50 mm.

Chinfak *et al.* (2021), obtained about a similar trend where smaller size of Mytilus edulis and M. lyrata recorded higher microplastic particles compared to the larger sizes. Scott *et al*. (2019) and Sathish *et al.* (2020) also reported similar patterns in M. edulis and Donax cuneatus. Woods *et al.* (2018) reported certain bivalves are selective filter feeders with the ability to reject some of the ingested particles such as microplastics through excretion. The capacity of ingestion, accumulation and digestion may differ according to habitat, size, surface characteristics, and concentration microplastics.

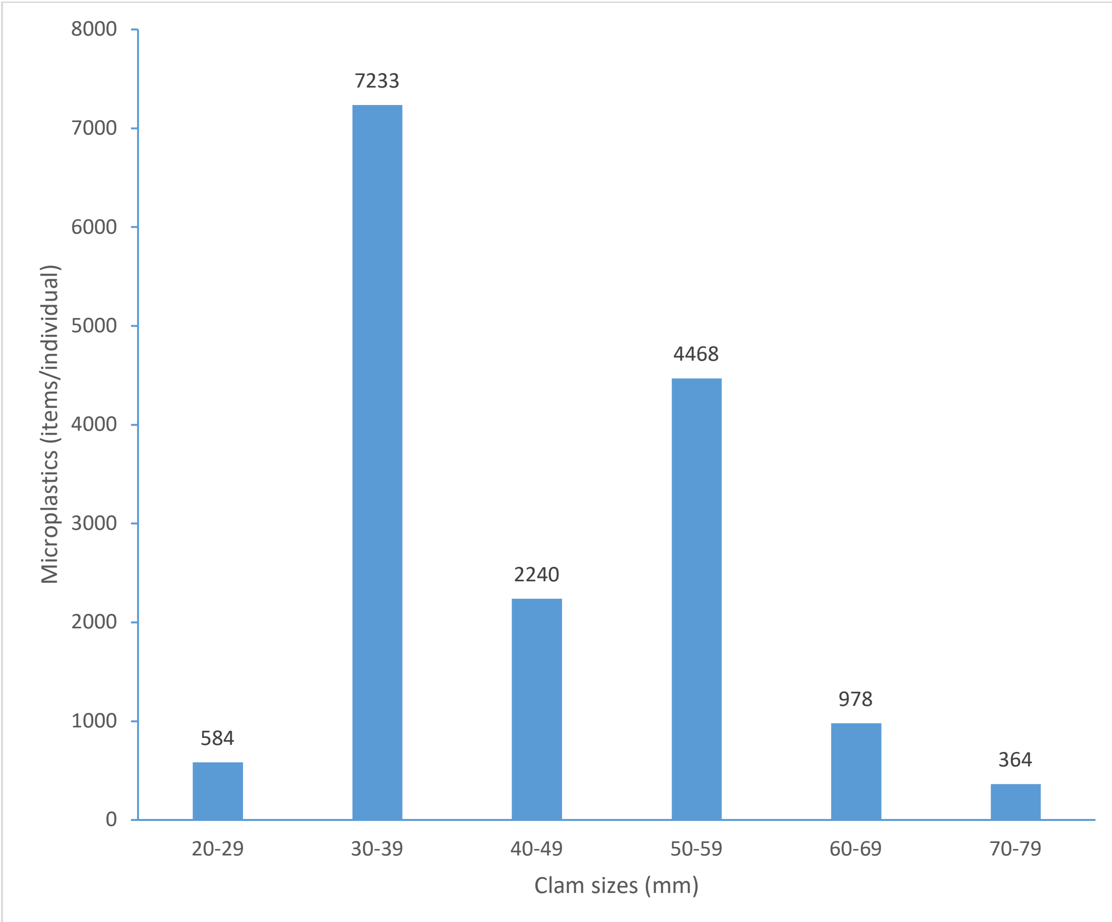

Figure 1. The amount of microplastic ingested by *M. lyrata* according to different size classes which further categorised into small size (<50mm) and large size (>50mm)

The samples were also tested microplastics ingestion according to weather season. The average microplastics ingestion of *M. lyrata* during the dry season is 174.86±21.2 items/individual and is 212.71±71.14 items/individual during the wet season. Figure 2 shows the number of microplastics ingested according to weather seasons. Mann-Whitney U test revealed no significant difference in the number of microplastics ($p<0.05$), ($U= 752$, $|z| = 0.55$, $p= 0.581$) between the dry season and the wet season.

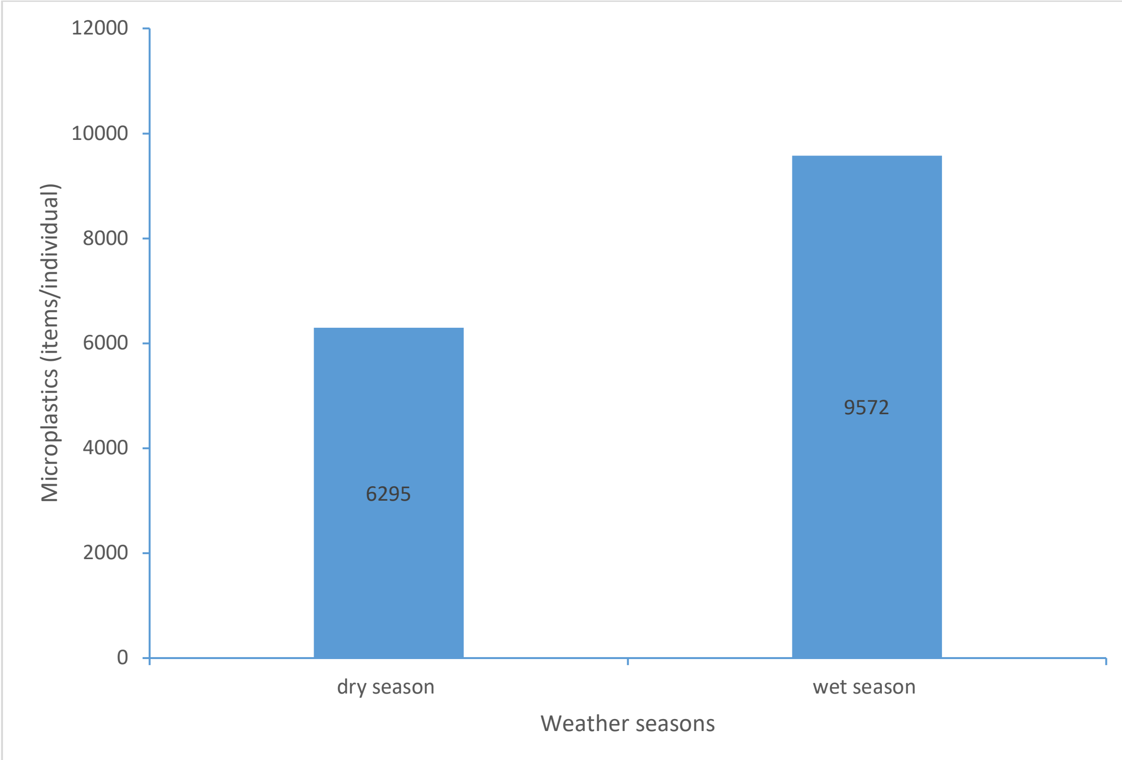

Figure 2. Amount of microplastics ingested by *M. lyrata* according to different weather seasons

The possible microplastics input for the study site is through both land input (human settlement area), input through river systems (oil palm plantation, aquaculture farm) and input through tidal change as well as wave activities. Figure 3 shows the map of the study sites with different land use zonation identified.

Figure 3. Map of the study site (Kampung Buntal) with the land use surrounding the study site identified based on outcome from informal interviews with the local clam collectors and spatial data from Google Earth.

The zonation indicated in the map were derived from informal interviews with the local bivalve collectors and interpretations based on satellite imagery through Google Earth.

Lebreton *et al*. (2019) hypothesized that most of the plastic marine debris should be settling in the coastal environment through a series of processes such as stranding, settling and resurfacing process. The mentioned process can also break down the bigger plastic marine debris into smaller microplastics (through physical abrasion), therefore the plastic marine debris and the byproduct (microplastics) will be highly polluting the habitat of the *M. lyrata*. Wu *et al*. (2020) reported higher microplastics abundance in surface sediment of intertidal zone during neap tide cycles compared to spring tide cycles. The microplastics settled at surface sediment during the neap tide cycle can either be brought away by strong tidal actions during spring tide cycles or buried in the sediment column. *M. lyrata* as one of the common bivalves inhabits at intertidal zone were highly at risk of microplastics contamination due to the high bioavailability of microplastics within the region.

Polymer Identification Result

About 12 items were isolated for polymer identification due to limitation. 4 plastic polymers were identified in which polyvinyl chloride (PVC) (8 samples), polydimethylsiloxane (PDMS) (2 samples), resin dispersion (1 sample), and polypropylene with silicate mix (PP with silicate mix) (1 sample). Figure 4a shows the type of polymer identified from the isolated items and Figure 4b shows the result of spectral comparison with OpenSpecy database of the polymers. Figure 4c shows some photos of the isolated items to be process with polymer identification using Nicolet IN10 ATR-FTIR (Thermo Scientific).

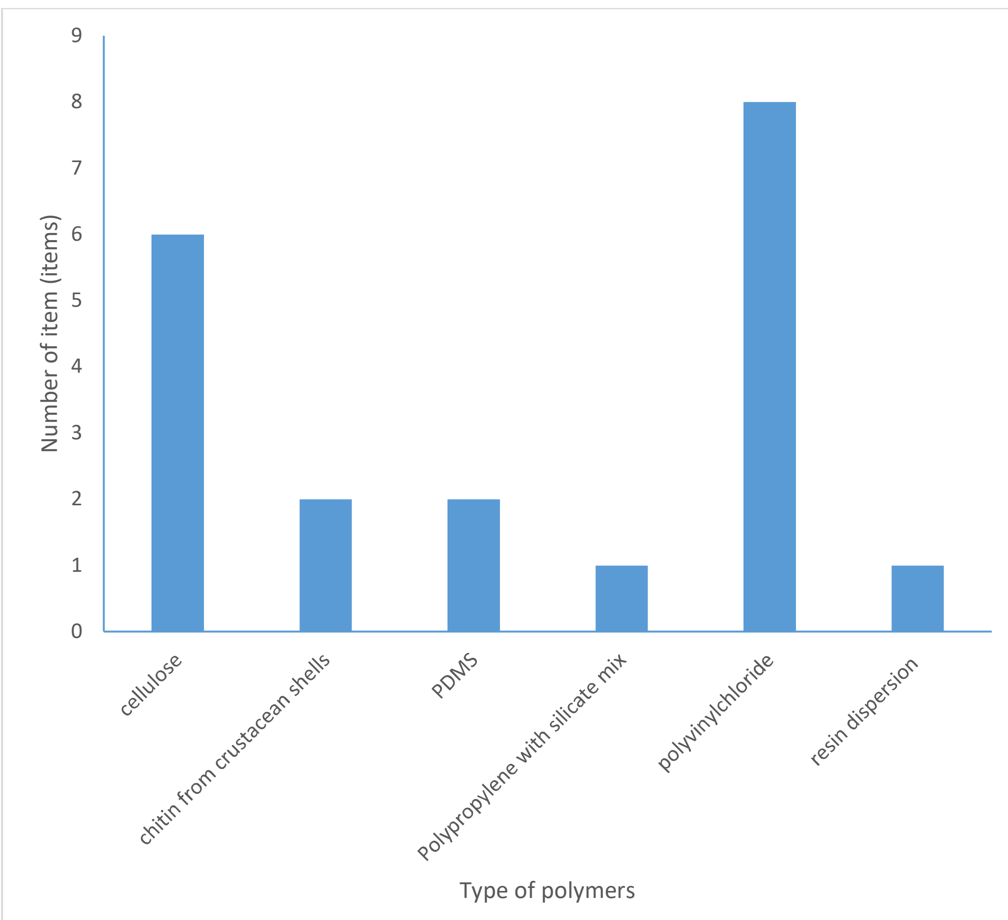

Figure 4(a). Four (4) types of identified polymers (polydimethylsiloxane, polypropylene with silicate mix, polyvinylchloride and resin dispersion) sorted according to the size class and harvesting season of the clam in which the microplastics were isolated for polymer identification.

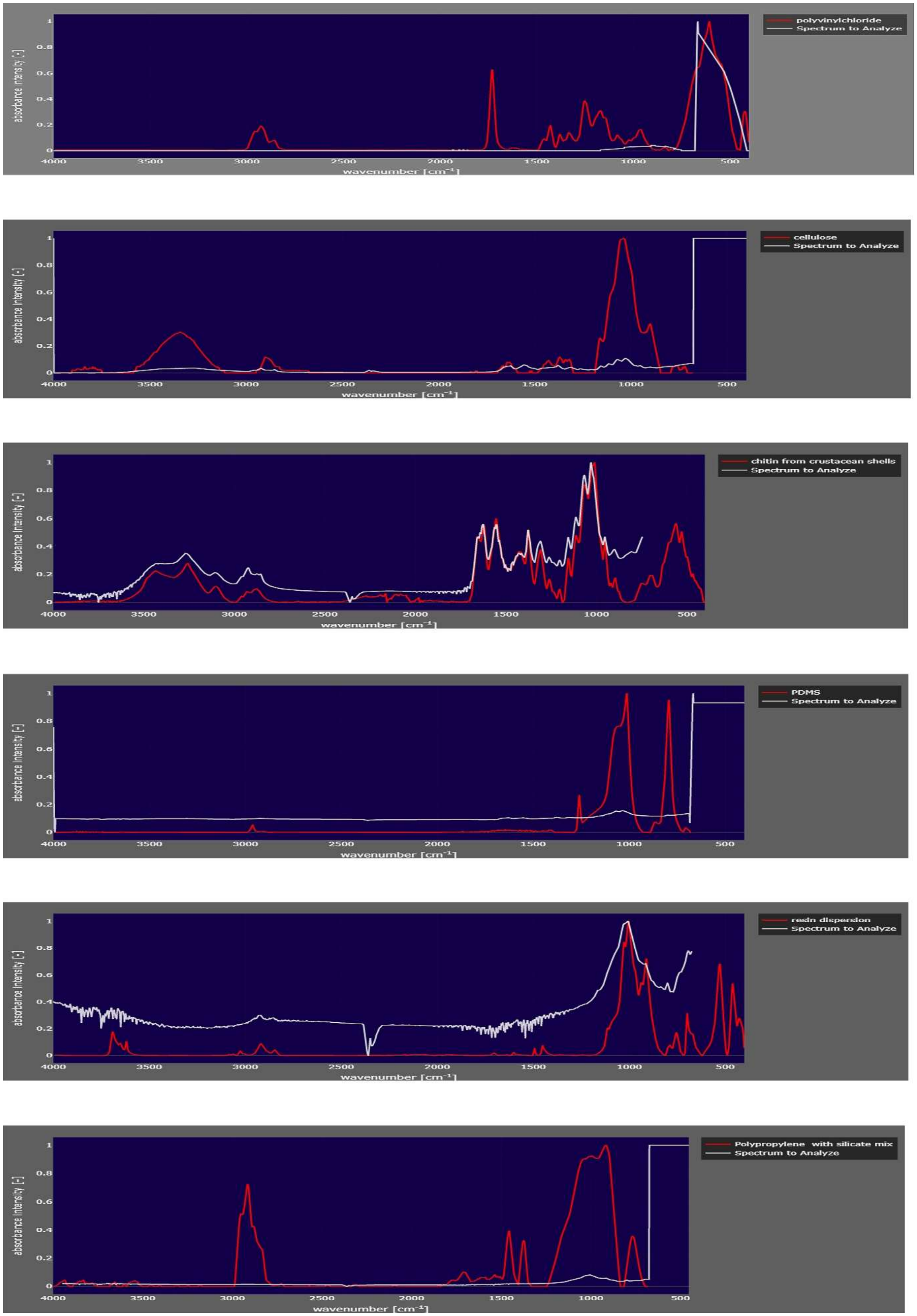

Figure 4(b). Comparison of the spectral data from the isolated items spectral comparison of the isolated particles with OpenSpecy database.

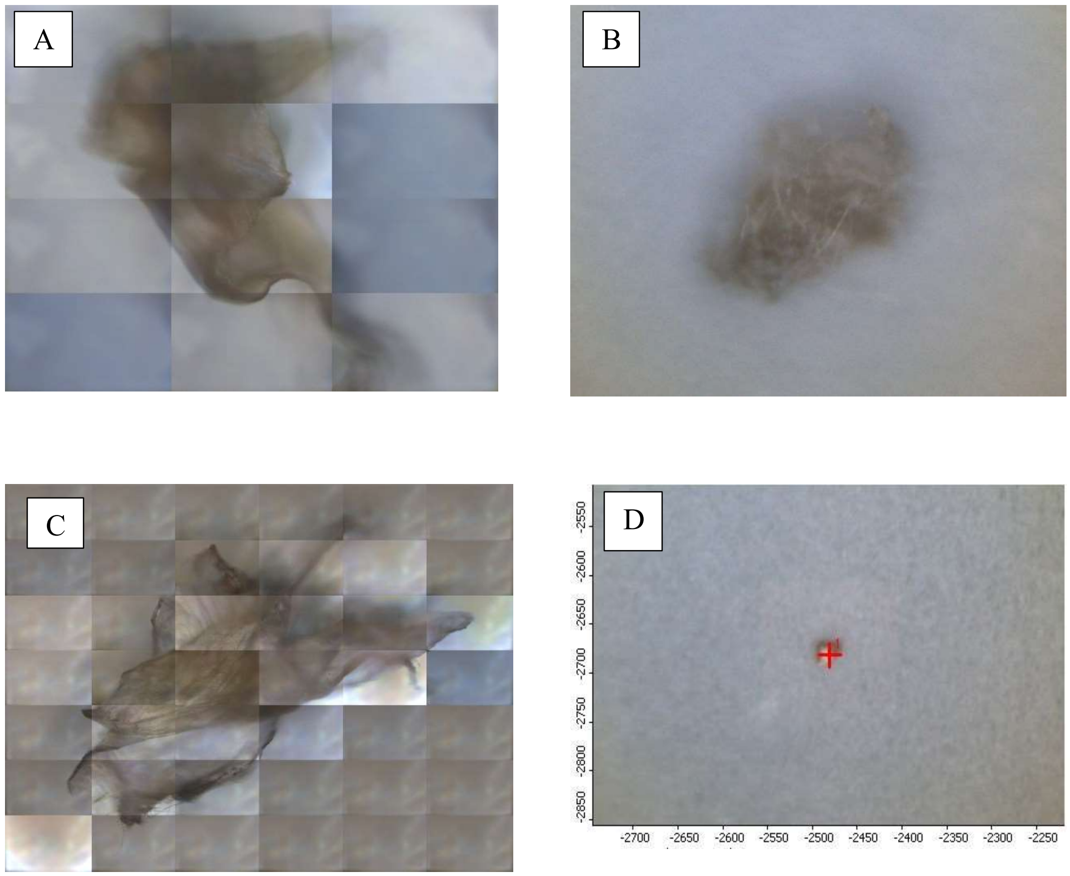

Figure 4(c). Photos of the isolated items to be process with polymer identification using Nicolet IN10 ATR-FTIR (Thermo Scientific). A, B and C are fragment and D particles.

Local publications also reported similar detection of plastic polymer, polyvinyl chloride and polypropylene in both *Corbicula fluminea* and *Polymesoda expansa* (Karim *et al*., 2023) and polypropylene in *Perna veridis* (Mazni *et al*., 2022).

All four types of detected plastic polymers (PVC, PDMS, PP and resin) were either common additives or building materials and easily entered the environment if there were human settlements around the area.

PVC is commonly used in the building and construction sector. It is a thermoplastic having a good resistance to alkalis, salt and highly polar solvents. Despite the relatively high density of PVC in nature, formation of biofilm may also alter the density through increasing the density and decreasing the buoyancy of the particles. (Rummel *et al*., 2017). PDMS is an elastomeric polymer used as fuel additives in car waxes, cleaners and polishers, and biomedical application such as implants for cosmetic surgery. One of the routes that PDMS can enter the aquatic environment is through wastewater treatment plants or directly introduced into the aquatic environment as wastewater (Álvarez-Muñoz *et al*., 2016). PDMS adsorb strongly to sludge which reduces the volatility of PDMS (Whelan *et al*., 2009). The combination of PDMS with sludge will settle down in the environment, therefore PDMS is usually found in high concentration near the wastewater discharge areas (Sparham *et al*., 2011). PP with silicate mix is one of the common plastics used in human daily life. Single use plastic bags are a good example of polypropylene products. Fishing lines and nets were mostly composite of PP and polyethylene (PE) Improper discard of broken nets may break down into smaller pieces and form microplastics. Resin dispersion according to Yoshida (2014) is formed from epoxy resin after mixing with different component such as basic epoxy resin, curing agents, accelerators and additives to produce epoxy with specific performance, therefore it's hard to determine the density of the dispersed resin. Current fishing boats materials are shifting from wood to glass fiber boats due to its durability and lightweight. Resins were commonly used as the main adhesion agent in repairing fiber boats. This might explain the availability of this polymer within the study site.

Ke *et al*. (2019) and Luan *et al*. (2019) reported that the exposure of microplastics (PP and PS) to clams (*Meretrix meretrix*) can lead to reduction in total oocyte number, oocyte diameter peroxidation damage of embryo membrane and death of *M. meretrix* larvae.

The possibility of microplastics entering humans usually through consumption of seafood for example, bivalves are usually consumed as whole organisms and rarely with gut removal (Cho *et al*., 2019). The toxicity of the plastic additives and chemicals adhered to the surface of microplastics during exposure in the environment may affect humans upon consumption of bivalves or other seafood that are contaminated with microplastics (Karbalaei *et al*., 2018).

CONCLUSION

This study provides compelling evidence of widespread microplastic contamination in *Meretrix lyrata* from an intertidal coastal habitat, with 100% of individuals found to contain microplastics. The average ingestion was 195.90±43.6 items per individual and a total count of 15,867 items across all samples. These concentrations are significantly higher than those reported in similar species from neighboring countries, suggesting local environmental and methodological factors may influence microplastic accumulation. Notably, smaller clams (<50 mm) exhibited significantly higher microplastic ingestion than larger individuals, a pattern consistent with previous studies and potentially linked to size-related differences in filtration capacity or feeding behavior. Although no significant seasonal variation was observed, the overall high prevalence of microplastics across seasons underscores the persistent nature of contamination in the study area. The identification of four distinct polymer types—polyvinyl chloride (PVC), polydimethylsiloxane (PDMS), polypropylene with silicate mix (PP), and resin dispersion—points to multiple anthropogenic sources, including urban runoff, aquaculture, and maritime activities. Given that bivalves are commonly consumed whole, the detection of high microplastic loads in *M. lyrata* raises potential concerns regarding food safety and human health risks.

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REFERENCES

Álvarez-Muñoz, D., Llorca, M., Blasco, J. & Barceló, D. (2016). Contaminants in the Marine Environment. In Blasco. J., Chapman, P, M., Campana, O. & Hampel, M. (eds.) *Marine Ecotoxicology: Current knowledge and future issue,* Cambridge: Academic Press. pp. 1-34.

Ambie, S., Peter, C., Minton, G., Ngeian, J., Zulkifli Poh, A. N., Mujahid, A. & Tuen, A. A. (2023). Utilizing interview-based data to measure interactions of artisanal fishing communities and cetacean populations in Kuching Bay, Sarawak, East Malaysia. *Ocean & Coastal Management*, 239: 106592. https://doi.org/10.1016/j.ocecoaman.2023.106592

Auta, H. S., Emenike, C. U. & Fauziah, S. H. (2017). Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environment International*, 102: 165-176. 10.1016/j.envint.2017.02.013

Brach, L., Deixonne, P., Bernard, M. F., Durand, E., Desjean, M. C., Perez, E., Sebilie, E. V. & Ter Halle, A. (2018). Anticyclonic eddies increase accumulation of microplastics in the North Atlantic subtropical gyre. *Marine Pollution Bulletin,* 126: 191-196. 10.1016/j.marpolbul.2017.10.077

Chinfak, N., Sompongchaiyakul, P., Charoenpong, C., Shi, H., Yeemin, T. & Zhang, J. (2021). Abundance, composition, and fate of microplastics in water, sediment, and shellfish in the Tapi Phumduang river system and Bandon Bay, Thailand. *Science of The Total Environment,* 781: 146700. 10.1016/j.scitotenv.2021.146700

Cho, Y., Shim, W. J., Jang, M., Han, G. M. & Hong, S. H. (2019). Abundance and characteristics of microplastics in market bivalves from South Korea. *Environmental Pollution*, 245: 1107-1116. 10.1016/j.envpol.2018.11.091

Cole, M., Lindeque, P., Halsband, C. & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin.* 62: 2588–2597. 10.1016/j.marpolbul.2011.09.025

Cowger, W., Steinmetz, Z., Gray, A., Munno, K., Lynch, J., Hapich, H. & Herodotou, O. (2021). Microplastics spectral classification needs an open source community: Open specy to the rescue! *Analytical Chemistry*, 93(21): 7543–7548. 10.1021/acs.analchem.1c00123

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. *Science of The Total Environment,* 753: 142041. 10.1016/j.scitotenv.2020.142041

Ding, J. F., Sun, C. J., He, C. F., Li, J. X., Ju, Peng., & Li, F. M. (2021). Microplastics in four bivalve species and basis for using bivalves as bioindicators of microplastic pollution. *Science of The Total Environment*, 782: 146830. https://doi.org/10.1016/j.scitotenv.2021.146830

Erni-Cassola, G., Gibson, M. I., Thompson, R. C. & Christie-Oleza, J. A. (2017). Lost, but found with Nile Red: A novel method for detecting and quantifying small microplastics (1 mm to 20 µm) in environmental samples. *Environmental Science & Technology,* 51(23): 13641–13648. 10.1021/acs.est.7b04512

Fu, Z., Chen, G., Wang, W. & Wang, J. (2020). Microplastics pollution research methodologies, abundance, characteristics and risk assessments for aquatic biota in China. *Environmental Pollution*, 266: 115098. 10.1016/j.envpol.2020.115098

Geyer, R., Jambeck, J. R. & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances,* 3(7): e1700782. 10.1126/sciadv.1700782

Green, D. S., Boots, B., O'Connor, N. E. & Thompson, R. (2017). Microplastics affect the ecological functioning of an important biogenic habitat. *Environmental Science & Technology*, 51(1): 68-77. 10.1021/acs.est.6b04496

Hartmann, N. B., Hüffer, T., Thompson, R. C., Hassellöv, M., Verschoor, A., Daugaard, A. E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M. P., Hess, M. C., Ivleva, N. P., Lusher, A. L. & Wagner, M. (2019). Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. *Environmental Science & Technology,* 53(3): 1039–1047. 10.1021/acs.est.8b05297

Hasbudin, H., Harith, M. N. & Idrus, F. A. (2022). A review on microplastic ingestion in marine invertebrates from Southeast Asia. *Songklanakarin Journal of Science and Technology* 44(3): 609–618. 10.14456/sjst-psu.2022.84

Idrus, F. A., Fadhli, N. M. & Harith, M. N. (2022a). Occurrence of microplastics in the Asian freshwater environments: A Review. *Applied Environmental Research,* 44(2), 1–17. https://doi.org/10.35762/AER.2022.44.2.1

Idrus, F. A., Roslan, N. S. & Harith, M. N. (2022b). Occurrence of macro- and micro-plastics on Pasir Pandak Beach, Sarawak, Malaysia. *Jurnal Ilmiah Perikanan Dan Kelautan* 14(2). https://doi.org/10.20473/jipk.v14i2.34034

Karbalaei, S., Hanachi, P., Walker, T. R. & Cole, M. (2018). Occurrence, sources, human health impacts and mitigation of microplastics pollution. *Environmental Science and Pollution Research,* 25(36): 36046–36063. 10.1007/s11356-018-3508-7

Karim, N., Musa, S. & Mat Lazim, A. (2023). *Microplastics physicochemical properties in commercial bivalves from Malaysia.* SSRN preprint. 10.2139/ssrn.4366421

Ke, A.Y., Chen, J., Zhu, J., Wang, Y.-H., Hu, Y., Fan, Z. L., Chen, M., Peng, P., Jiang, S. W., Xu, X. R. & Li, H. X. (2019). Impacts of leachates from single-use polyethylene plastic bags on the early development of clam *Meretrix Meretrix* (Bivalvia: Veneridae). *Marine Pollution Bulletin,* 142: 54–57. 10.1016/j.marpolbul.2019.03.029

Kieu-Le, T.-C., Tran, Q.-V., Truong, T.-N.-S. & Strady, E. (2022). Anthropogenic fibres in white clams, *Meretrix lyrata*, cultivated downstream a developing megacity, Ho Chi Minh City, Viet Nam. *Marine Pollution Bulletin,* 174. 113302. 10.1016/j.marpolbul.2021.113302

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. *Science of the Total Environment*, 610: 635-640. 10.1016/j.scitotenv.2017.08.053

Lavers, J. L. & Bond, A. L. (2017). Exceptional and rapid accumulation of anthropogenic debris on one of the world's most remote and pristine islands. *Proceedings of the National Academy of Sciences,* 114(23): 6052-6055. 10.1073/pnas.1619818114

Lebreton, L., Slat, B., Ferrari, F., Sainte-Rose, B., Aitken, J., Marthouse, R., Hajibane, S., Cunsolo, S., Schwarz, A., Levivier, A., Noble, K., Debelijak, P., Maral, H., Schoeneich-Argent, R., Brambini, R. & Reisser, J. (2018). Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. *Scientific Reports,* 8(1): 1-15. 10.1038/s41598-018-22939-w

Lebreton, L., Egger, M. & Slat, B. (2019). A global mass budget for positively buoyant macroplastic debris in the ocean. *Scientific Reports*, 9(1): 1–10. 10.1038/s41598-019-49413-5

Li, Q., Sun, C., Wang, Y., Cai, H., Li, L., Li, J. & Shi, H. (2019). Fusion of microplastics into the mussel byssus. *Environmental Pollution*, 252: 420–426g. 10.1016/j.envpol.2019.05.093

Luan, L., Wang, X., Zheng, H., Liu, L., Luo, X. & Li, F. (2019). Differential toxicity of functionalized polystyrene microplastics to clams (*Meretrix meretrix*) at three key development stages of life history. *Marine Pollution Bulletin,* 139: 346–354. 10.1016/j.marpolbul.2019.01.003

Lusher, A. L. & Hernandez-Milian, G. (2018). Microplastic extraction from marine vertebrate digestive tracts, regurgitates and scats: A protocol for researchers from all experience levels. *Bio Protocol,* 8(22): 10.21769/BioProtoc.3087.

Maes, T., Jessop, R., Wellner, N., Haupt, K. & Mayes, A. G. (2017). A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Scientific Reports,* 7(1): 1–10. 10.1038/srep44501

Mazni, M. Z., Azman, S., Mohd Ismail, M. I. & Muhammad, M. K. (2022). Microplastics contents in natural and maricultured shellfish from Pasir Putih estuary in Johor, Malaysia. *Journal of Sustainability Science and Management,* 17(10): 121–135. 10.46754/jssm.2022.10.009

Nuelle, M.-T., Dekiff, J. H., Remy, D. & Fries, E. (2014). A new analytical approach for monitoring microplastics in marine sediments. *Environmental Pollution,* 184: 161–169. 10.1016/j.envpol.2013.07.027

Prata, J. C., Alves, J. R., da Costa, J. P., Duarte, A. C. & Rocha-Santos, T. (2020). Major factors influencing the quantification of Nile red stained microplastics and improved automatic quantification (MP-VAT 2.0*). Science of The Total Environment,* 719: 137498. 10.1016/j.scitotenv.2020.137498

Prata, J. C., Sequeira, I. F., Monteiro, S. S., Silva, A. L. P., da Costa, J. P., Dais Pereira, P., Fernandes, A. J. S., da Costa, F. M., Duarte, A. C. & Rocha Santos, T. (2021). Preparation of biological samples for microplastic identification by Nile Red. *Science of The Total Environment,* 783: 147065. https://doi.org/10.1016/j.scitotenv.2021.147065

Quinn, B., Murphy, F. & Ewins, C. (2017). Validation of density separation for the rapid recovery of microplastics from sediment. *Analytical Methods*, 9(9): 1491–1498. 10.1039/C6AY02542K

Reed, S., Clark, M., Thompson, R. & Hughes, K. A. (2018). Microplastics in marine sediments near Rothera research station, Antarctica. *Marine Pollution Bulletin,* 133: 460–463. 10.1016/j.marpolbul.2018.05.068

Rist, S. E., Assidqi, K., Zamani, N. P., Appel, D., Perschke, M., Huhn, M. & Lenz, M. (2016). Suspended micro-sized PVC particles impair the performance and decrease survival in the asian green mussel *Perna viridis*. *Marine Pollution Bulletin*, 111(1-2): 213–220. 10.1016/j.marpolbul.2016.07.006

Rummel, C. D., Jahnke, A., Gorokhova, E., Kühnel, D. & Schmitt-Jansen, M. (2017). Impacts of biofilm formation on the fate and potential effects of microplastics in the aquatic environment. *Environmental Science & Technology Letters*, 4(7): 258–267. 10.1021/acs.estlett.7b00164

Sathish, M. N., Jeyasanta, K. I. & Patterson, J. (2020). Monitoring of microplastics in the clam *Donax Cuneatus* and its habitat in Tuticorin coast of Gulf of Mannar (GoM), India. *Environmental*

Pollution, 266: 115219. 10.1016/j.envpol.2020.115219

Scott, N., Porter, A., Santillo, D., Simpson, H., Lloyd-Williams, S. & Lewis, C. (2019). Particle characteristics of microplastics contaminating the mussel *Mytilus edulis* and their surrounding environments. *Marine Pollution Bulletin,* 146: 125–133. 10.1016/j.marpolbul.2019.05.041

Shahul Hamid, F., Bhatti, M. S., Anuar, N., Anuar, N., Mohan, P. & Periathamby, A. (2018). Worldwide distribution and abundance of microplastics: How dire is the situation? *Waste Management and Research,* 36(10): 873–897. 10.1177/0734242X18785730

Sparham, C., Van Egmond, R., Hastie, C., O'Connor, S., Gore, D. & Chowdhury, N. (2011). Determination of decamethylcyclopentasiloxane in river and estuarine sediments in the UK. *Journal of Chromatography A*, 1218(6): 817–823. 10.1016/j.chroma.2010.12.030

Tang, R., Zhang, T., Song, K., Sun, Y., Chen, Y., Huang, W. & Feng, Z. (2022) Microplastics in commercial clams from the intertidal zone of the South Yellow Sea, China. *Frontiers in Marine Science,* 9: 905923. 10.3389/fmars.2022.905923

Tran-Nguyen, Q. A., Nguyen, T. Q., Phan, T. L., Vo, M. V. & Trinh-Dang, M. (2023). Abundance of microplastics in two venus clams (*Meretrix Lyrata* and *Paratapes Undulatus*) from estuaries in central Vietnam. *Water,* 15(7): 1312. 10.3390/w15071312

Whelan, M. J., Sanders, D. & Van Egmond, R. (2009). Effect of aldrich humic acid on water–atmosphere transfer of decamethylcyclopentasiloxane. *Chemosphere*, 74 (8): 1111–1116. 10.1016/j.chemosphere.2008.10.037

Woods, M. N., Stack, M. E., Fields, D. M., Shaw, S. D. & Matrai, P. A. (2018). Microplastics fiber uptake, ingestion, and egestion rates in the blue mussel (*Mytilus edulis*). *Marine Pollution Bulletin,* 137: 638–645. 10.1016/j.marpolbul.2018.10.061

Wu, F., Pennings, S. C., Tong, C. & Xu, Y. (2020). Variation in microplastics composition at small spatial and temporal scales in a tidal flat of the Yangtze Estuary, China. *Science of The Total Environment,* 699. 134252. 10.1016/j.scitotenv.2019.134252

Yoshida, S. (2014). Quantitative evaluation of an epoxy resin dispersion by infrared spectroscopy. *Polymer Journal*, 46(7): 430–434. 10.1038/pj.2014.15

Zarfl, C. (2019). Promising techniques and open challenges for microplastics identification and quantification in environmental matrices. *Analytical and Bioanalytical Chemistry,* 411(17): 3743–3756. 10.1007/s00216-019-01763-9

Zhang, F., Man, Y. B., Mo, W. Y., Man, K. Y. & Wong, M. H. (2020). Direct and indirect effects of microplastics on bivalves, with a focus on edible species: A mini-review. *Critical Reviews in Environmental Science and Technology*, 50(20): 2109–2143. 10.1080/10643389.2019.1700752

Zhang, T., Song, K., Meng, L., Tang, R., Song, T., Huang, W. & Feng, Z. (2022). Distribution and characteristics of microplastics in barnacles and wild bivalves on the coast of the Yellow Sea, China. *Frontiers in Marine Science,* 8: 78961. 10.3389/fmars.2021.78961