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Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for quantifying synthetic particles in waters and sediments

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Laboratory Methods for the Analysis of Microplastics in the Marine Environment

Recommendations for quantifying synthetic particles in waters and sediments

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Abbreviations

(aq)	aqueous
Fe(II)	iron (II)
NaCl	sodium chloride (a salt)
NOAA	National Oceanic and Atmospheric Administration
NOS	National Ocean Service
OR&R	NOAA Office of Response and Restoration
PVC	polyvinylchloride
WPO*	wet peroxide oxidation
40X	magnification with light microscopy by 40 times

Units

C	Celsius
d	density
g	gram
h	hour
in	inch
M	Molar
mg	milligram
mL	milliliter
mm	millimeter
min	minute

The Wet Peroxide Oxidation (WPO) process results in a highly reactive mixture. Please review your laboratory safety practices and policies before completing this analysis. In this manual, we have highlighted the WPO steps with a* **CAUTION *note.*

Microplastics Background

Over the past decade, microplastic debris in both marine and freshwater systems has become an emerging issue. There is an increased interest to understand the impacts of microplastics on aquatic wildlife, as the impacts still remain poorly understood (Thompson et al. 2004, Browne et al. 2007). Microplastics were first noted in North America as spherules in plankton tows along the coast of New England in the 1970s (Carpenter et al. 1972). Since then, microplastics have been found in most large bodies of water (oceans, seas, lakes, and rivers).

Microplastics are plastic particles smaller than 5.0 mm in size (Arthur et al. 2009). The lower bound (size) of the microplastics is not defined; however, it is common practice to use the mesh size (333µm or 0.33mm) of the neuston nets used to collect the samples (Arthur et al. 2009). There are two main ways microplastics are formed and enter a body of water: primary and secondary microplastics (Arthur et al. 2009). Primary microplastics consist of manufactured raw plastic material, such as virgin plastic pellets, scrubbers, and microbeads (Browne et al. 2007, Arthur et al. 2009) that enter the ocean via runoff from land (Andrady 2011). Secondary microplastic introductions occur when larger plastic items (meso- and macro- plastics) enter a beach or ocean and undergo mechanical, photo (oxidative) and/ or biological degradation (Thompson et al. 2004, Browne et al. 2007, Cooper and Corcoran 2010, Andrady 2011). This degradation breaks the larger pieces into progressively smaller plastic fragments which eventually become undetectable to the naked eye.

There are many uses for microplastics. For example, microbeads are used in personal care products such as exfoliants in face scrubs. Microplastics are also used to deliver drugs in some medical applications (Browne et al. 2007). Further, fibers that shed from synthetic clothing and rope are microplastics (Thompson et al. 2004, Browne et al. 2007), as are particles used in “media blasting” processes to clean boat hulls and large machinery (Browne et al. 2007). Many of these microplastics, microbeads, and fibers are small enough to pass through wastewater treatment plants and enter a watershed (Browne et al. 2007).

Impacts of microplastics on wildlife are not well understood at this time. However, a number of organisms, both vertebrates and invertebrates have been found to ingest microplastics. These examples represent numerous organisms with differing feeding mechanisms, including detritivores, deposit feeders, and filter feeders. Examples include scleractinian corals (Hall et al. 2015), mussels (*Mytilus edulis;* Browne et al. 2007), fish (Carpenter et al. 1972), as well as lugworms, amphipods, and barnacles (Thompson et al. 2004).

Scientists are also concerned that organisms ingesting plastic debris may be exposed to contaminants sorbed to the plastic (Teuten et al. 2007). Plastic debris provides a sink and a source for chemical contaminants. Additives used in the manufacturing of plastics can leach from the plastics into the marine environment (Andrady 2011). On the other hand, hydrophobic contaminants present in the water may sorb to the plastic particles (Carpenter et al. 1972, Teuten et al. 2007, Andrady 2011). Thus, microplastics may provide a mechanism to transport concentrated contaminants to organisms (Browne et al. 2007).

Introduction to Methods Manual

This document is a methods manual for measuring microplastic in the environment. This manual outlines step-by-step instructions for quantifying microplastic in marine environmental samples, including processes to streamline terminology and approaches.

The methods described here were determined after careful study and laboratory work conducted through a grant from the NOAA Marine Debris Program to the University of Washington, Tacoma. Project goals sought to streamline the terminology and techniques used to assess microplastic concentrations in marine environmental samples, and to develop laboratory procedures to quantify microplastic particles in marine surface waters and bed sediments, as well as personal care products. An overarching aim was to provide scientists and educators with simple techniques that are reproducible and robust without requiring extensive equipment, and to describe a method that could be easily adopted by groups around the world.

Depending on the study aims and environmental collection techniques, these techniques can be used to calculate concentrations of microplastics using a variety of metrics, including per piece, per mass, or per volume. Considering metrics is important for comparing results with other researchers. For guidelines and considerations when conducting microplastic analyses, as well as calculations, see the appendices.

1.0 Methods for the Analysis of Microplastics in Water Samples

This method can be used for the analysis of plastic debris as suspended solids in water samples collected by a surface net. Plastics include hard plastics, soft plastics (e.g. foams), films, line, fibers, and sheets. The method involves the filtration of solids obtained in a 0.335 mm surface sampling net (e.g. a manta net for surface water tows) through 5.6-mm and/or 0.3-mm sieves to isolate the solid material of the appropriate size (Figures 1-4). The sieved material is dried to determine the solids mass in the sample. The solids are subjected to wet peroxide oxidation (WPO) in the presence of a Fe(II) catalyst to digest labile organic matter. The plastic debris remains unaltered. The WPO mixture is subjected to density separation in NaCl(aq) to isolate the plastic debris through flotation. The floating solids are separated from the denser undigested mineral components using a density separator. The floating plastic debris is collected in the density separator using a custom 0.3-mm filter, air-dried, and plastic material is removed and weighed to determine the microplastics concentration.

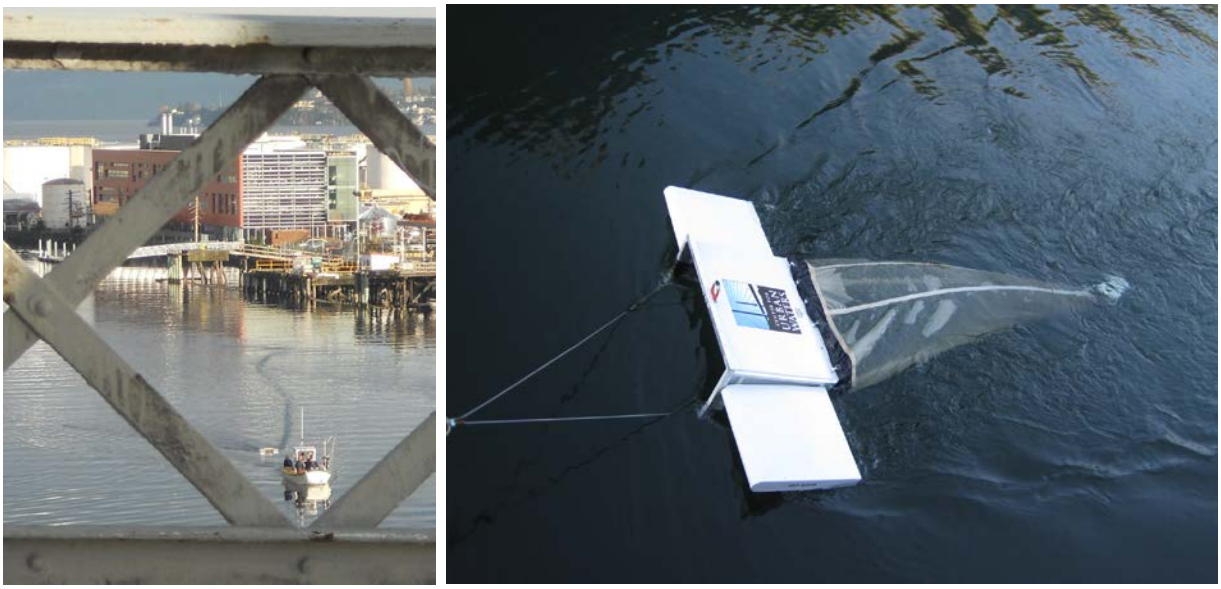

Figure 1. Researchers use a manta net to collect surface water samples in the Thea Foss Waterway, Puget Sound, Washington.

Figure 2. A water sample from Puget Sound is shown before processing.

Figure 3. Note the variety in these field samples, all collected from surface waters.

Figure 4. Sieving and rinsing field samples.

An overview of the analysis of microplastics in water is shown in Figure 5.

This method is applicable for the determination of many common plastics including polyethylene (0.91-0.97 g/mL), polypropylene (0.94 g/mL), polyvinyl chloride (1.4 g/mL), and polystyrene (1.05 g/mL).

The plastic debris analyzed by this method is considered microplastic and ranges in size from 5 mm to 0.3 mm. Microplastic debris is operationally defined by this method as any solid material in the appropriate size range that is resistant to wet peroxide oxidation, exhibits flotation in a 5 M NaCl (d=1.15 g/mL) or ~5.4 M lithium metatungstate (d=1.62 g/mL) solution, and passes positive visual inspection under a microscope at 40X power.

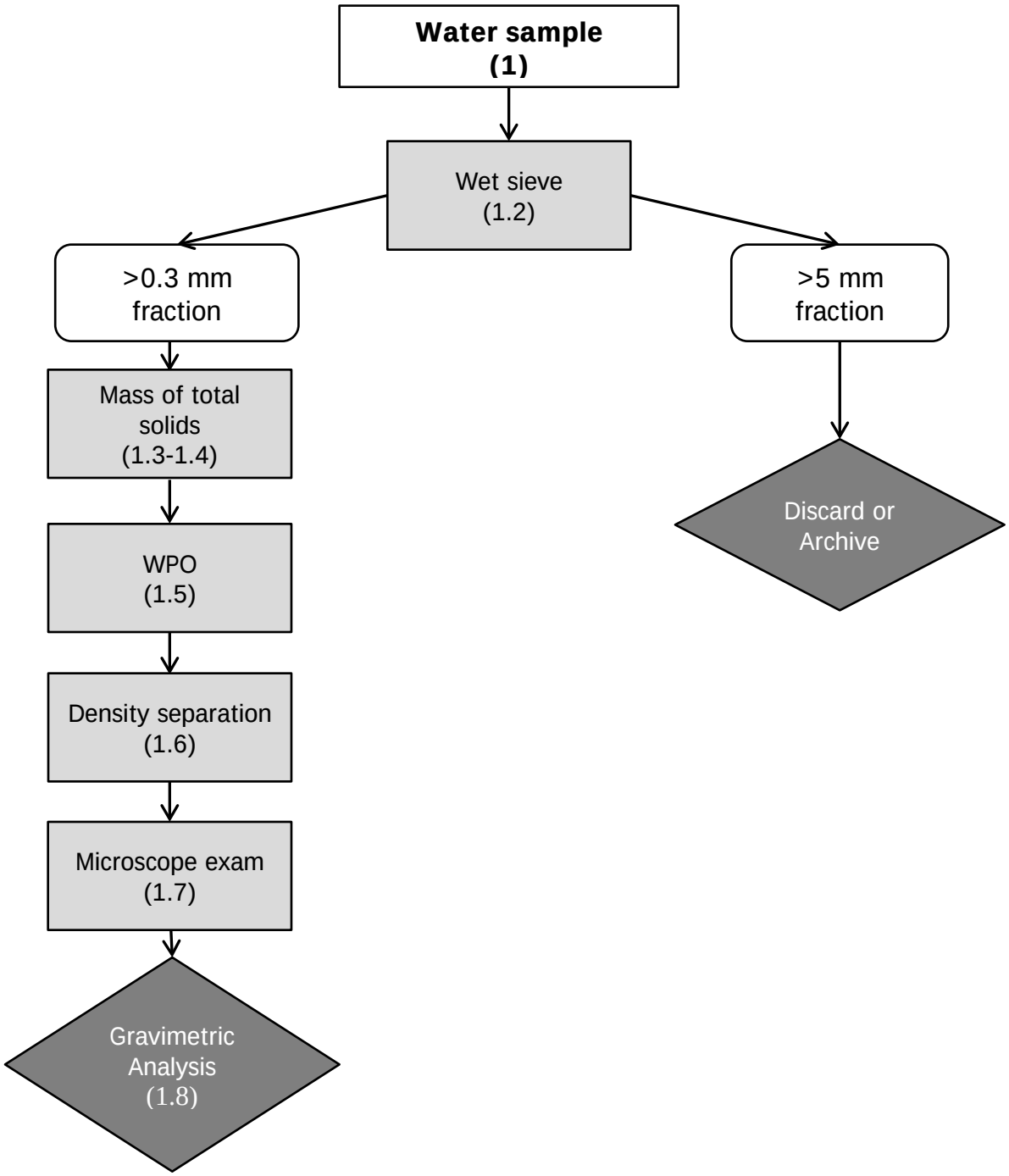

Figure 5. Flow diagram for the analysis of microplastics in water samples. Note numbers refer to the numbers in this section.

1.1 Apparatus and Materials

- Stainless steel sieves, each measuring 8 in (diameter) and 2 in (depth)
  - 5.6 mm mesh (Number 3.5)
  - 1 mm mesh (Number 18)
  - 0.3 mm mesh (Number 50)
- Squirt bottle containing distilled water
- 500-mL glass beaker
- Analytical balance (precise to 0.1 mg)
- Metal spatula
- Drying oven (90^{o}C)
- Iron (Fe(II)) solution (0.05 M)
  - Prepared by adding 7.5 g of $FeSO_4 \cdot 7H_20$ (= 278.02 g/mol) to 500 mL of water and 3 mL of concentrated sulfuric acid
- 30% Hydrogen peroxide
- Stir bar
- Laboratory hot plate
- Watchglass
- Sodium chloride (commercial table salt is sufficient)
- Standard Metal Forceps
- Density separator, which is assembled using the following method:
  - A glass funnel (122-mm in diameter) is fitted with a 50-mm segment of latex tubing on the bottom of the stem and a pinch clamp is attached to control liquid flow from the funnel.
- Retort stand
- O-ring
- Spring clamp (2-inch)
- Aluminum foil
- Customized small sieves, each measuring 59 mm (diameter), which may be fabricated using either of two methods:
  - Use polypropylene Büchner funnels and nylon mesh. The funnel bottoms are removed and nylon mesh is glued to the modified funnel. Three funnels, with varying nylon mesh size (5 mm, 1 mm, and 0.3 mm), are needed.
  - Cut sections of polyvinylchloride (PVC) pipe, approximately 75 mm (diameter) and 25 mm (length), and use gel-type superglue to attach nylon mesh to one end of the pipe. Three funnels, with varying nylon mesh size (5 mm, 1 mm, and 0.3 mm), are needed.
- 4-mL glass vials
- Dissecting microscope (40X magnification)

1.2 Wet Sieving

- Pour the sample through a stacked arrangement of 5.6-mm (No. 3.5) and 0.3-mm (No. 50) stainless steel mesh sieves (Figure 4).
- Rinse sample with squirt bottle filled with distilled water to transfer all residual solids to the sieves. This also removes salts from the field sample. Repeat as necessary.
- Rinse sieves thoroughly using distilled water. Ensure all material has been well washed, drained, and sorted.
- Discard or archive material retained on 5-mm sieve, as appropriate, depending on individual study objectives.

1.3 Transfer Sieved Solids

- Weigh a clean and dry 500-mL beaker to the nearest 0.1 mg. (*a*)
- Transfer solids collected in the 0.3-mm sieve into the tared beaker using a spatula and minimal rinsing with a squirt bottle containing distilled water.
- Ensure all solids are transferred into the beaker.
- Place beaker in 90^{o}C drying oven for 24 hours or longer to sample dryness (Figure 6).

Figure 6. Sieved samples are dried in an oven to determine dry weight.

1.4 Determine the Mass of Total Solids

- Determine the mass of the beaker with dried solids using an analytical balance to the nearest 0.1 mg. (*b*)
- Subtract the mass of the tared beaker (*a*) to provide the mass of total solids (*c*) collected on the sieve. (Formula: $b - a = c$)* See Appendix 5.1.1 for formulae. This is the *mass of all microplastics and natural materials*.

1.5 Wet Peroxide Oxidation (WPO)

CAUTION: This mixture is highly reactive. Please review and follow your laboratory safety practices and policies for handling this mixture before completing this analysis.

- Add 20 mL of aqueous 0.05 M Fe(II) solution to the beaker containing the 0.3 mm size fraction of collected solids (Figure 7).

Figure 7. Addition of iron sulfate solution catalyzes the reaction.

- Add 20 mL of 30% hydrogen peroxide. **CAUTION**: this solution can boil violently if heated >75^{o}C (Figure 8).

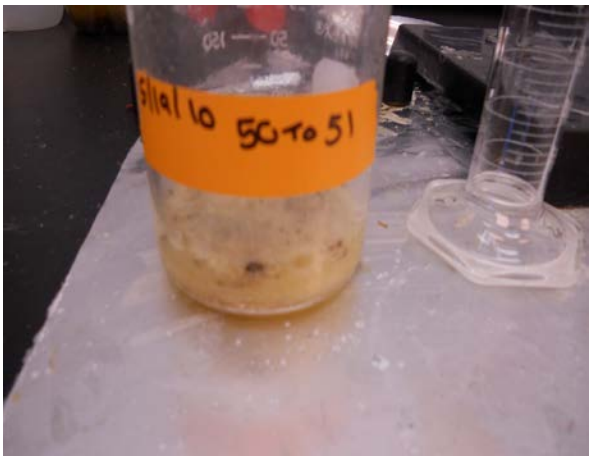

Figure 8. Addition of hydrogen peroxide oxidizes natural organic material.

- Let mixture stand on lab bench at room temperature for five minutes prior to proceeding to the next step.
- Add a stir bar to the beaker and cover with a watchglass.
- Heat to 75^{o}C on a hotplate.

- As soon as gas bubbles are observed at the surface, remove the beaker from the hotplate and place it in the fume hood until boiling subsides. If reaction appears to have the potential to overflow the beaker, add distilled water to slow the reaction.
- Heat to 75°C for an additional 30 minutes.
- If natural organic material is visible, add another 20 mL of 30% hydrogen peroxide.
- Repeat until no natural organic material is visible.
- Add ~6 g of salt (NaCl) per 20 mL of sample to increase the density of the aqueous solution (~5 M NaCl).
- Heat mixture to 75°C until the salt dissolves.

1.6 Density Separation

- Transfer the WPO solution from step 1.5 to the density separator (Figure 9).

Figure 9. A sample is depicted in a glass funnel to separate plastic.

- Rinse the WPO beaker with distilled water to transfer all remaining solids to the density separator.
- Cover loosely with aluminum foil.
- Allow solids to settle overnight.
- Visually inspect settled solids for any microplastics. If any are present, drain the settled solids from the separator and remove microplastics using forceps. Archive or discard.
- Drain settled solids from the separator and discard.
- Collect floating solids in a clean 0.3-mm custom sieve (Figure 10).

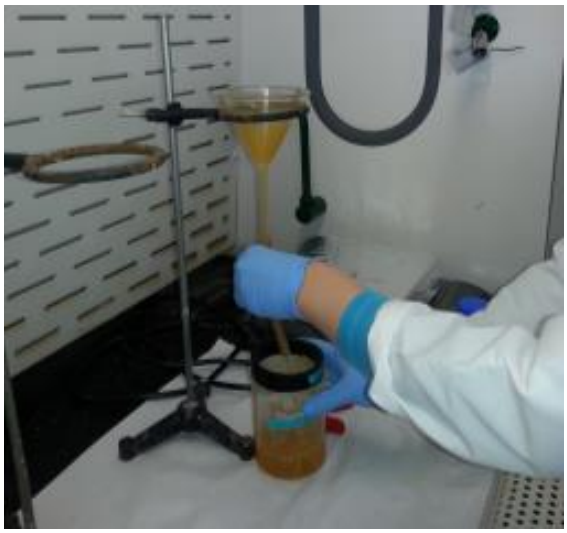

Figure 10. Floating material is drained into a custom sieve.

- Rinse the density separator several times with distilled water to transfer all solids to the 0.3-mm sieve.
- Allow the sieve to air dry while loosely covered with aluminum foil for 24 hours (Figure 11).

Figure 11. A prepared sample is ready for microscope examination.

1.7 Microscope Exam

- Weigh a clean and dry 4-mL vial. Include the label and cap. (*d*)
- Under a dissecting microscope at 40X magnification (Figure 12), use forceps to collect identifiable microplastics from the 0.3-mm sieve and transfer them to the tared vial (Figure 13). See Appendix 5.2 for more guidance.

Figure 12. Microplastic fragments are extracted from the prepared sample during a microscope examination.

Figure 13. A final microplastic sample is preserved in a vial.

1.8 Gravimetric Analysis

- Weigh the mass of the vial and microplastics to the nearest 0.1 mg. (*e*)
- Subtract the mass of the tared vial (*d*) to provide the mass of microplastics (*f*) collected on the sieve. (Formula: *e-d=f*)* See Appendix 5.1.1 for formulae. This is the *mass of all microplastics*.

2.0 Methods for the Analysis of Microplastics in Beach Samples

This method can be used for the analysis of plastic debris in beach sand collected by shovel or spade. Plastics include hard plastics, soft plastics (e.g., foams), films, line, fibers, and sheets. The method involves sieving dry beach samples to 5 mm to remove large macroscopic debris.

An overview of the analysis of microplastics in beach sand is shown in Figure 14.

This method is applicable for the determination of many common plastics including polyethylene (0.91-0.97 g/mL), polypropylene (0.94 g/mL), polyvinyl chloride (1.4 g/mL), and polystyrene (1.05 g/mL).

The plastic debris analyzed by this method is considered microplastic and ranges in size from 5 mm to 0.3 mm. Microplastic debris is operationally defined by this method as any solid material in the appropriate size range that is resistant to wet peroxide oxidation, exhibits flotation in a 5 M NaCl (d=1.15 g/mL) or ~5.4 M lithium metatungstate (d=1.62 g/mL) solution, and passes positive visual inspection under a microscope at 40X power.

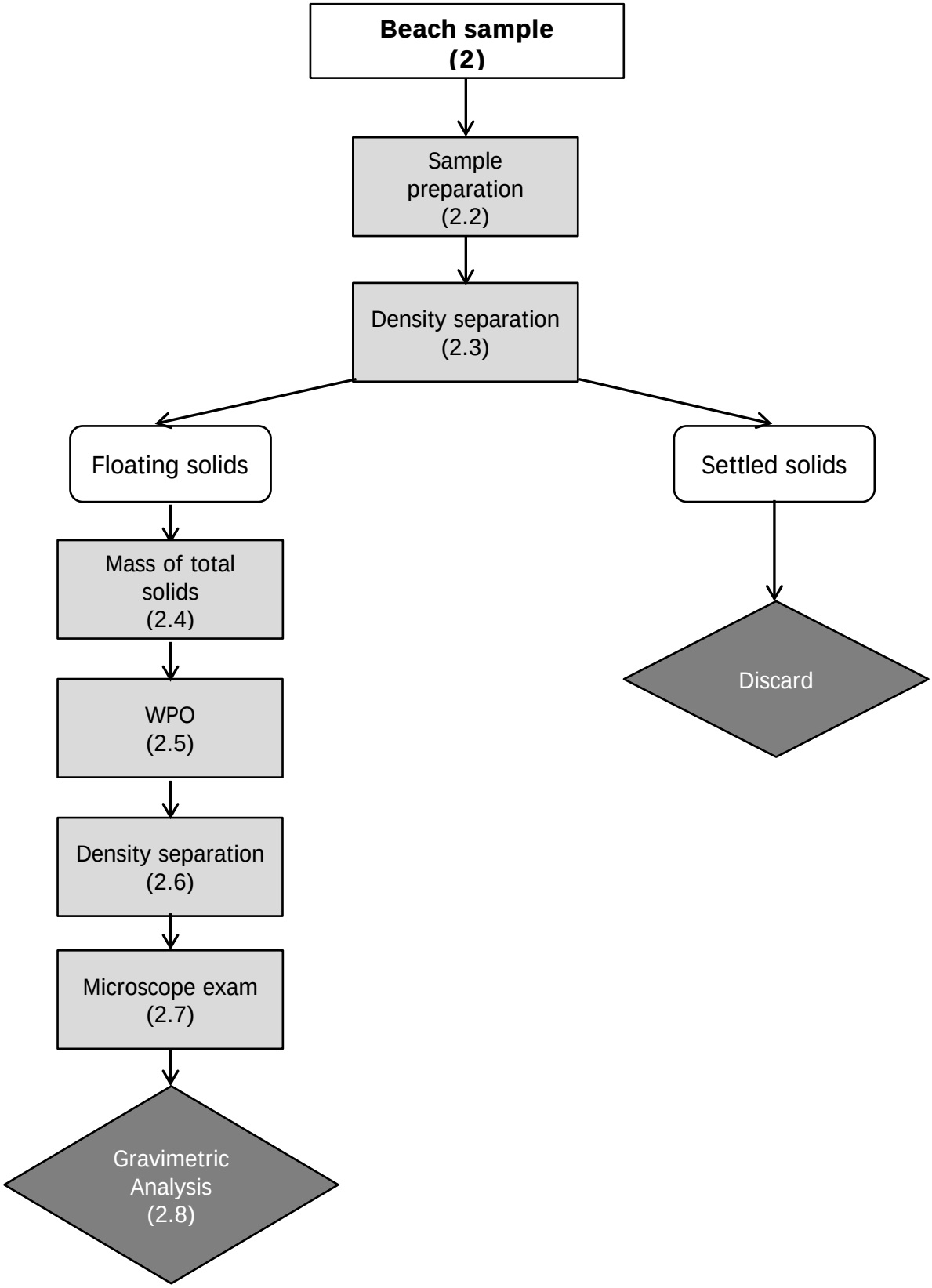

Figure 14. Flow diagram for the analysis of microplastics in beach samples. Note numbers refer to the numbers in this section.

2.1 Apparatus and Materials

- 800-mL and 500-mL glass beakers
- Analytical balance (precise to 0.1 mg)
- Drying oven (90°C)
- Lithium metatungstate solution
    - Prepared from commercial lithium metatungstate solution (=2.95 g/mL) and diluted as needed with deionized water to 1.6 g/mL
- Metal spatula
- Customized small sieves, each measuring 59 mm (diameter), which may be fabricated using either of two methods:
    - Use polypropylene Büchner funnels and nylon mesh. The funnel bottoms are removed and nylon mesh is glued to the modified funnel. Three funnels, with varying nylon mesh size (5 mm, 1 mm, and 0.3 mm), are needed.
    - Cut sections of polyvinylchloride (PVC) pipe, approximately 75 mm (diameter) and 25 mm (length), and use gel-type superglue to attach nylon mesh to one end of the pipe. Three funnels, with varying nylon mesh size (5 mm, 1 mm, and 0.3 mm), are needed.
- Standard Metal Forceps
- Iron (Fe(II)) solution (0.05 M)
    - Prepared by adding 7.5 g of $FeSO_4 \cdot 7H_20$ (= 278.02 g/mol) to 500 mL of water and 3 mL of concentrated sulfuric acid
- 30% Hydrogen peroxide
- Stir bar
- Watchglass
- Laboratory hot plate
- Squirt bottle containing distilled water
- Sodium chloride (commercial table salt is sufficient)
- Density separator, which is assembled using the following method:
    - A glass funnel (122-mm in diameter) is fitted with a 50-mm segment of latex tubing on the bottom of the stem and a pinch clamp is attached to control liquid flow from the funnel.
- Retort stand
- O-ring
- Spring clamp (2-inch)
- Aluminum foil
- 4-mL glass vials
- Dissecting microscope (40X magnification)

2.2 Beach Sediment Sample Preparation

- Weigh and label a clean and dry 800-mL beaker to the nearest 0.1 mg. (*a*)
- Weigh 400 g of wet sediment to the nearest 0.1 mg and add to the beaker (Figure 15).

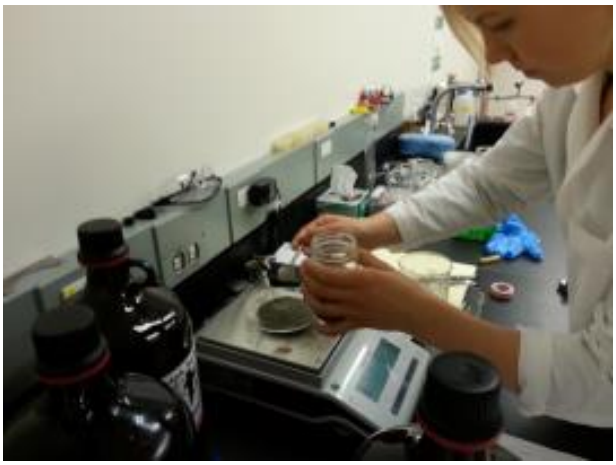

Figure 15. A beach sand (or sediment) sample is weighed.

- Dry in a drying oven at 90°C overnight or until sample dryness.
- Weigh the dried beaker and material to determine the dry sample weight. (*b*)
- Subtract the mass of the tared beaker (*a*) to provide the mass of total solids (*c*). (Formula: $b - a = c$)* See Appendix 5.1.2 for formulae. This is the *mass of the sample matrix*.

2.3 Density Separation

- Add 300 mL of aqueous lithium metatungstate (d=1.6 g/mL) solution to the dried sediments in the 800-mL beaker (Figure 16). Be sure to adjust density to 1.6 g/mL. See Section 2.1 (Apparatus and Materials) for preparation instructions.

Figure 16. Lithium metatungstate is added to a sediment sample.

- Vigorously stir the sand-water mixture in the beaker for several minutes with a spatula to float out the microplastics.
- Transfer all floating solids in the beaker to the custom 0.3 mm sieve. Rinse beaker with distilled water to transfer all residual solids to the sieves.
- Remove any visible material > 5 mm with forceps. Either discard or archive, depending on individual study objectives.
- Discard or archive settled solids left in the beaker.
- Weigh and label a clean and dry 500-mL beaker to the nearest 0.1 mg. (*a*)
- Transfer the solids collected on the 0.3-mm sieve into the tared 500-mL beaker.
- Repeat until all of the floating debris is collected.
- Dry the beaker and solids in a drying oven at 90^{o}C for 24 hours or longer to sample dryness (Figure 6).
- Recover and filter the metatungstate solution in a separate bottle to recycle for future use (Figure 17).

Figure 17. The lithium metatungstate solution is sieved and saved.

2.4 Determine the Mass of Total Solids

- Determine the mass of the beaker with dried solids using an analytical balance to the nearest 0.1 mg. (*b*)
- Subtract the mass of the tared beaker (*a*) to provide the mass of total solids (*c*). (Formula: $b - a = c$)* See Appendix 5.1.2 for formulae. This is the *mass of all microplastics and natural materials*.

2.5 Wet Peroxide Oxidation (WPO)

CAUTION: This mixture is highly reactive. Please review and follow your laboratory safety practices and policies for handling this mixture before completing this analysis.

- Add 20 mL of aqueous 0.05 M Fe(II) solution to the beaker containing the 0.3 mm size fraction of collected solids (Figure 7).
- Add 20 mL of 30% hydrogen peroxide. **CAUTION**: this solution can boil violently if heated >75^{o}C (Figure 8).
- Let mixture stand on lab bench at room temperature for five minutes prior to proceeding to the next step.
- Add a stir bar to the beaker and cover with a watchglass.
- Heat to 75^{o}C on a hotplate.
- As soon as gas bubbles are observed at the surface, remove the beaker from the hotplate and place it in the fume hood until boiling subsides. If the reaction appears to have the potential to overflow the beaker, add distilled water to slow the reaction.
- Heat to 75^{o}C for an additional 30 minutes.
- If natural organic material is visible, add another 20 mL of 30% hydrogen peroxide.
- Repeat until no natural organic material is visible.
- Add ~6 g of salt (NaCl) per 20 mL of sample to increase the density of the aqueous solution (~5 M NaCl).
- Heat mixture to 75^{o}C until the salt dissolves.

2.6 Density Separation

- Transfer the WPO solution from step 2.5 to the density separator (Figure 9).
- Rinse the WPO beaker with distilled water to transfer all remaining solids to the density separator.
- Cover loosely with aluminum foil.
- Allow solids to settle overnight.
- Visually inspect settled solids for any microplastics. If any are present, drain the settled solids from the separator and remove microplastics using forceps. Archive or discard.
- Drain settled solids from the separator and discard.
- Collect floating solids in a clean 0.3-mm custom sieve (Figure 10).
- Rinse the density separator several times with distilled water to transfer all solids to the 0.3-mm sieve.
- Allow the sieve to air dry while loosely covered with aluminum foil for 24 hours (Figure 11).

2.7 Microscope Exam

- Weigh a clean and dry 4-mL vial. Include the label and cap. (*g*)
- Under a dissecting microscope at 40X magnification (Figure 12), use forceps to collect identifiable microplastics from the 0.3-mm sieve and transfer them to the tared vial (Figure 13). See Appendix 5.2 for more guidance.

2.8 Gravimetric Analysis

- Weigh the mass of the vial and microplastics to the nearest 0.1 mg. (*h*)
- Subtract the mass of the tared vial (*g*) to provide the mass of microplastics (*i*) collected on the sieve. (Formula: $h - g = i$)* See Appendix 5.1.2 for formulae. This is the *mass of all microplastics*.

3.0 Methods for the Analysis of Microplastics in Bed Samples

This method can be used for the analysis of plastic debris in bed sediments collected by corer or grab sampler (e.g. Ponar sampler). Plastics include hard plastics, soft plastics (e.g., foams), films, line, fibers, and sheets. The method involves an initial disaggregation of dried sediments. The disaggregated sediments are sieved using stacked 5-mm and 0.3-mm sieves. Microplastics collected on the 0.3-mm sieve are subjected to wet peroxide oxidation (WPO) in the presence of a Fe(II) catalyst to digest labile organic matter. The plastic debris remains unaltered. The WPO mixture is subjected to density separation in NaCl(aq) to isolate the plastic debris through flotation. The floating solids are separated from the denser undigested mineral components using a density separator. The floating plastic debris is collected in the density separator using a custom 0.3-mm filter, air-dried, and plastic material is removed and weighed to determine the microplastics concentration.

An overview of the analysis of microplastics in bed sediments is shown in Figure 18.

This method is applicable for the determination of many common plastics including polyethylene (0.91-0.97 g/mL), polypropylene (0.94 g/mL), polyvinyl chloride (1.4 g/mL), and polystyrene (1.05 g/mL).

The plastic debris analyzed by this method is considered microplastics and ranges in size from 5 mm to 0.3 mm. Microplastic debris is operationally defined by this method as any solid material in the appropriate size range that is resistant to wet peroxide oxidation, exhibits flotation in 5 M NaCl (d=1.15g/mL) or ~5.4 M lithium metatungstate (d=1.62 g/mL) solution, and passes positive visual inspection under a microscope at 40X power.

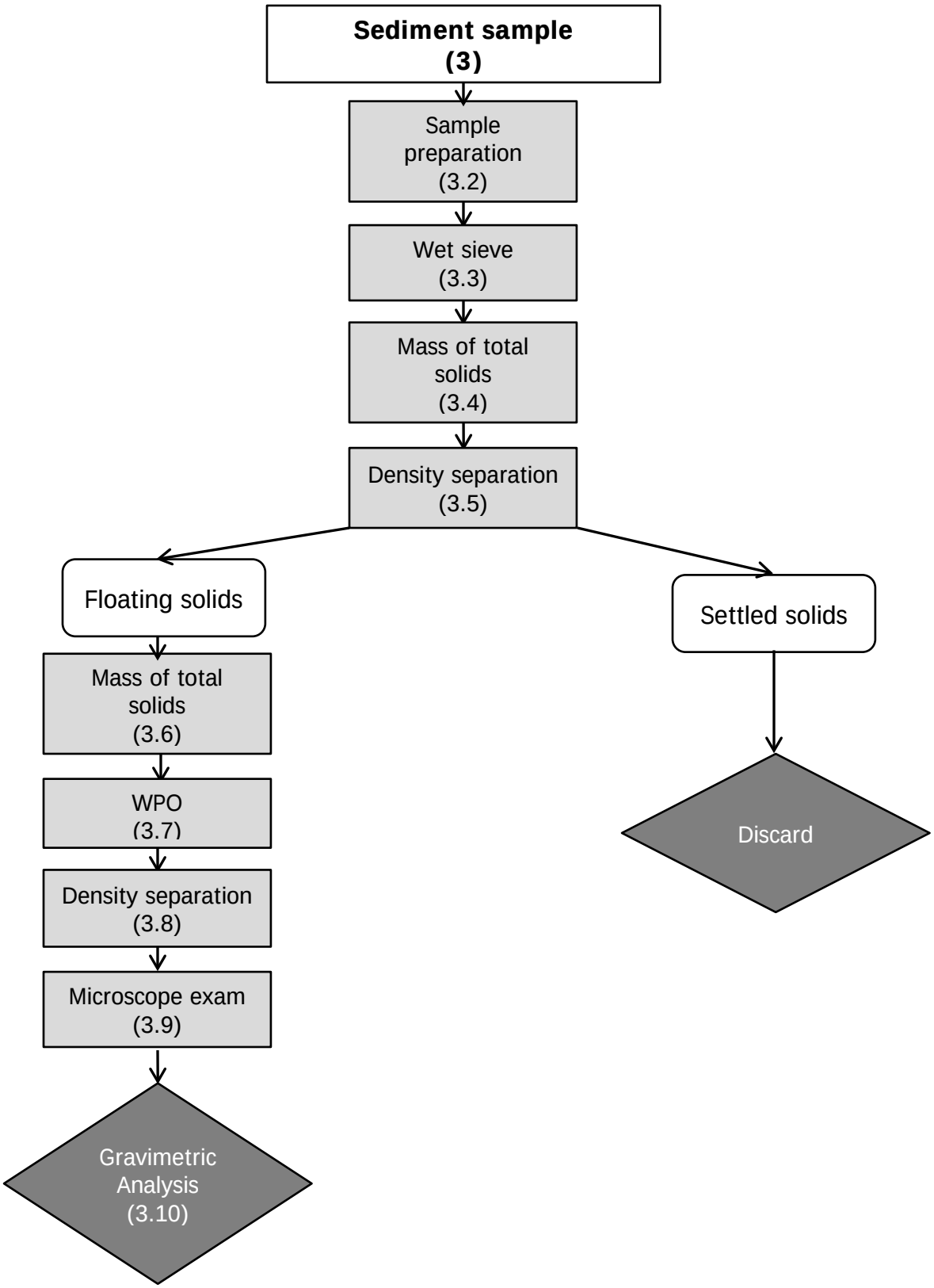

Figure 18. Flow diagram for the analysis of microplastics in bed samples. Note numbers refer to the numbers in this section.

3.1 Apparatus and Materials

- 800-mL and 500-mL glass beakers
- Analytical balance (precise to 0.1 mg)
- Drying oven (90°C)
- Potassium metaphosphate
    - Prepared by dissolving the salt in deionized water to 5.5 g/L
- Stir bar
- Customized small sieves, each measuring 59 mm (diameter), which may be fabricated using either of two methods:
    - Use polypropylene Büchner funnels and nylon mesh. The funnel bottoms are removed and nylon mesh is glued to the modified funnel. Three funnels, with varying nylon mesh size (5 mm, 1 mm, and 0.3 mm), are needed.
    - Cut sections of polyvinylchloride (PVC) pipe, approximately 75 mm (diameter) and 25 mm (length), and use gel-type superglue to attach nylon mesh to one end of the pipe. Three funnels, with varying nylon mesh size (5 mm, 1 mm, and 0.3 mm), are needed.
- Standard Metal Forceps
- Plastic squirt bottle containing distilled water
- Lithium metatungstate solution
    - Prepared from commercial lithium metatungstate solution (=2.95 g/mL) and diluted as needed with deionized water to 1.6 g/mL
- Metal spatula
- Iron (Fe(II)) solution (0.05 M)
    - Prepared by adding 7.5 g of $FeSO_4 \cdot 7H_20$ (= 278.02 g/mol) to 500 mL of water and 3 mL of concentrated sulfuric acid
- 30% Hydrogen peroxide
- Watchglass
- Laboratory hot plate
- Sodium chloride (commercial table salt is sufficient)
- Density separator, which is assembled using the following method:
    - A glass funnel (122-mm in diameter) is fitted with a 50-mm segment of latex tubing on the bottom of the stem and a pinch clamp is attached to control liquid flow from the funnel.
- Retort stand
- O-ring
- Spring clamp (2-inch)
- Aluminum foil
- 4-mL glass vials
- Dissecting microscope (40X magnification)

3.2 Bed Sediment Sample Preparation

3.2.1 Dry Bed Sediments

- Weigh and label a clean and dry 800-mL beaker to the nearest 0.1 mg. (*a*)
- Weigh 400 g of wet sediment to the nearest 0.1 mg and add to the beaker (Figure 15).
- Dry in a drying oven at 90°C overnight or until sample dryness.
- Weigh the dried beaker and material to determine the dry sample weight. (*b*)
- Subtract the mass of the tared beaker (*a*) to provide the mass of total solids (*c*). (Formula: $b - a = c$)* See Appendix 5.1.2 for formulae. This is the *mass of the sample matrix*.

3.2.2 Disaggregate Dried Bed Sediments

- Add 400 mL of potassium metaphosphate (5.5 g per liter of water) to the sediment sample.
- Add a stir bar to the beaker and mix for 1 hour at high rpm.

3.3 Wet Sieving and Transfer of Sieved Solids

- Pour the sample through 0.3-mm sieve.
- Rinse the sample beaker with distilled water to transfer all residual solids to the sieve.
- Repeat as necessary.
- Ensure all material has been well washed, drained, and sorted.
- Remove any visible material > 5 mm with forceps. Either discard or archive, depending on individual study objectives.
- Weigh and label a clean and dry 500-mL beaker to the nearest 0.1 mg. (*a*)
- Transfer the solids retained on 0.3-mm sieves to tared 500-mL beakers using a spatula and minimal rinsing with a squirt bottle containing distilled water.
- Ensure all solids are transferred to the beaker.
- Dry the beakers and solids in a drying oven at 90^{o}C for 24 hours or longer to sample dryness (Figure 6).

3.4 Determine the Mass of Total Solids (Sample Matrix)

- Determine the mass of beaker with dried solids using an analytical balance to nearest 0.1 mg. (*b*)
- Subtract the mass of the tared beaker (*a*) to provide the mass of total solids (*c*). (Formula: $b - a = c$)* See Appendix 5.1.2 for formulae. This is the *mass of the filtered sample matrix*.

3.5 Density Separation

- Add 300 mL of aqueous lithium metatungstate (d=1.6 g/mL) solution to the dried sediments in the beaker (Figure 16). Be sure to adjust density to1.6 g/mL. See Section 3.1 (Apparatus and Materials) for preparation instructions.
- Vigorously stir the sand-water mixture in the beaker for several minutes with a spatula to float out the microplastics.
- Transfer all floating solids in the beaker to the custom 0.3 mm sieve. Rinse beaker with distilled water to transfer all residual solids to the sieves.
- Weigh and label a clean and dry 500-mL beaker to the nearest 0.1 mg. (*a*)
- Transfer the solids collected on the 0.3-mm sieve into tared 500-mL beaker.
- Dry the beaker and solids in a drying oven at 90^{o}C for 24 hours or longer to sample dryness (Figure 6).
- Recover and filter the metatungstate solution in a separate bottle to recycle for future use (Figure 17).

3.6 Determine the Mass of Total Solids (Microplastics and Natural Materials)

- Determine the mass of beaker with dried solids using an analytical balance to nearest 0.1 mg. (*b*)
- Subtract the mass of the tared beaker (*a*) to provide the mass of total solids (*c*). (Formula: $b - a = c$)* See Appendix 5.1.2 for formulae. This is the *mass of all microplastics and natural materials*.

3.7 Wet Peroxide Oxidation (WPO)

CAUTION: This mixture is highly reactive. Please review and follow your laboratory safety practices and policies for handling this mixture before completing this analysis.

- Add 20 mL of aqueous 0.05 M Fe(II) solution to the beaker containing the 0.3 mm size fraction of collected solids (Figure 7).
- Add 20 mL of 30% hydrogen peroxide. **CAUTION**: this solution can boil violently if heated >75^{o}C (Figure 8).
- Let mixture stand on lab bench at room temperature for five minutes prior to proceeding to the next step.
- Add a stir bar to the beaker and cover with a watchglass.
- Heat to 75^{o}C on a hotplate.
- As soon as gas bubbles are observed at the surface, remove the beaker from the hotplate and place it in the fume hood until boiling subsides. If reaction appears to have the potential to overflow the beaker, add distilled water to slow the reaction.
- Heat to 75^{o}C for an additional 30 minutes.
- If natural organic material is visible, add another 20 mL of 30% hydrogen peroxide.
- Repeat until no natural organic material is visible.

- Add ~6 g of salt (NaCl) per 20 mL of sample to increase the density of the aqueous solution (~5 M NaCl).
- Heat mixture to 75^{o}C until the salt dissolves.

3.8 Density Separation

- Transfer the WPO solution from step 3.7 to the density separator (Figure 9).
- Rinse the WPO beaker with distilled water to transfer all remaining solids to the density separator.
- Loosely cover with aluminum foil.
- Allow solids to settle overnight.
- Visually inspect settled solids for any microplastics. If any are present, drain the settled solids from the separator and remove microplastics using forceps. Archive or discard.
- Drain settled solids from separator and discard.
- Collect floating solids in a clean 0.3-mm custom sieve (Figure 10).
- Rinse the density separator several times with distilled water to transfer all solids to the 0.3-mm sieve.
- Allow the sieve to air dry while loosely covered with aluminum foil for 24 hours (Figure 11).

3.9 Microscope Exam

- Weigh a clean and dry 4-mL vial. Include the label and cap. (*j*)
- Under a dissecting microscope at 40X magnification (Figure 12), use forceps to collect identifiable microplastics from the 0.3-mm sieve and transfer them to the tared vial (Figure 13). See Appendix 5.2 for more guidance.

3.10 Gravimetric Analysis

- Weigh the mass of the vial and microplastics to the nearest 0.1 mg. (*k*)
- Subtract the mass of the tared vial (*j*) to provide the mass of microplastics (*l*) collected on the sieve. (Formula: $k - j = l$)* See Appendix 5.1.2 for formulae. This is the *mass of all microplastics*.

4.0 Cited References

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5.0 Appendices

5.1 Appendix of Calculations

5.1.1 Water Samples

Mass of Total Solids

To calculate mass of total solids in a sample, which includes both natural material and microplastics, use the following formula (see Section 1.4):

$$mass_{(total\ solids)} = mass_{(beaker\ with\ dried\ solids)} - mass_{(tared\ beaker)}$$

$$(c = b - a)$$

Mass of Microplastics

To calculate mass of microplastics in a sample, use the following formula (see Sections 1.8):

$$mass_{(microplastic)} = mass_{(vial\ with\ particles)} - mass_{(tared\ vial)}$$

$$(f = e - d)$$

5.1.2 Beach and Bed Samples

Mass of Total Solids

To calculate mass of total solids in a sample, which includes both natural material and microplastics, use the following formula (see Sections 2.2 and 2.4; Sections 3.4 and 3.6):

$$mass_{(total\ solids)} = (mass_{(beaker\ with\ dried\ solids)} - mass_{(tared\ beaker)})$$

$$(c = b - a)$$

*Note: if you have samples with a lot of organic material or debris, you can sieve the samples using a 5mm, 1mm and 0.3mm sieve during the beginning steps. You will sort and weigh the 1mm and 0.3mm samples separately and add them together to calculate the total mass of solids, using the following formula (see Appendix 5.2 for more information):

$$mass_{(total\ solids)} = (mass_{(beaker\ with\ dried\ 1\text{-}mm\ solids)} - mass_{(tared\ beaker)}) + (mass_{(beaker\ with\ dried\ 0.3\text{-}mm\ solids)} - mass_{(tared\ beaker)})$$

Mass of Microplastics

To calculate mass of microplastics in a sample, use the following formula (see Sections 2.8 and 3.10):

$$mass_{(microplastic)} = (mass_{(vial\ with\ particles)} - mass_{(tared\ vial)})$$

(i = h - g) ---> for beach samples

(l = k - j) ---> for bed samples

*Note: if you have samples with a lot of organic material or debris, you can sieve the samples using a 5mm, 1mm and 0.3mm sieve during the beginning steps. You will sort and weigh the 1mm and 0.3mm samples separately and add them together to calculate the total mass of microplastics, using the following formula (see Appendix 5.2 for more information):

$$mass_{(microplastic)} = (mass_{(vial\ with\ 1\text{-}mm\ particles)} - mass_{(tared\ vial)}) + (mass_{(vial\ with\ 0.3\text{-}mm\ particles)} - mass_{(tared\ vial)})$$

5.2 Appendix of Guidelines and Considerations

Technique

Contamination of Samples
When collecting samples in the field and processing samples in the laboratory, avoid wearing polyester-type clothing (fleece jackets, polyester lab coats, etc.), because they may contaminate your samples. For more information on the contamination of samples from clothing, see Woodall et al. (2015). It is also good practice to inspect all of the equipment made from plastic (sieves, squirt bottle, etc.) before use to ensure that there is no contamination from of these materials to the environmental samples. Sieves should be washed and sonicated before and after use.

Guidelines for Microscope Inspection
When identifying environmental samples, there is natural material that may be mistaken for plastics. Natural material remaining at the picking stage of each process may look like plastic, including grass sheaths, pine needles, diatom tests, and salt crystals. To rule out these particles, drag your forceps across the particles. If they powder or fall apart, then the pieces are not plastic. If the particles retain their shape, then they are properly identified as plastic. Another approach is to become familiar with reference plastic materials.

High Mass Samples
To avoid error in samples that have more organic carbon (*e.g.*, lots of natural grass, pine needles, wood, etc.), sieve samples by 5 mm, 1 mm, and 330 µm at the beginning steps. Archive or discard material > 5 mm. Process the 1 mm and 330 µm fractions separately. Combine results to be used in an area or basin analysis. See Appendix 5.1.2.

Time of Water Sample Collection
Water sample collection is standardized to a 15-minute manta tow. The tow time is shortened if the net becomes clogged with material (typically a plankton bloom) or a large amount of material enters the net (typically flotsam or sea grass). Essentially, if the net loses its ability to sieve or begins to sink, end the tow and recover the sample collected.

Spike and Recovery Rates
1. Water Samples
    a. Water samples were spiked with two sizes of polyethylene microplastic pellets. 95% of the 1000 µm pellets and 60-85% of the 300 µm pellets were recovered.

    b. Water samples were spiked with 3 types of polymer microplastic pellets. 88% of the polyethylene pellets, 80% polypropylene pellets, and 92% polyvinyl chloride pellets were recovered.

2. Bed Sediments:
    a. Bed sediments were spiked with a given mass of polyethylene microplastic pellets with an 81% recovery.

FT-IR Work
Two environmental samples were analyzed using Fourier-Transform Infrared Spectroscopy. Sample 1 contained 219 pieces and Sample 2 contained 63 pieces. Sample 1 contained 72% polyethylene, 18% polypropylene, 2% polyvinyl chloride/polyethylene blend, 5% polystyrene, 1% high dentistry polyethylene, 1% low density polyethylene, and 1% unknown. Sample 2 contained 64% polyethylene, 15% polypropylene, 3% polyvinyl chloride/polyethylene blend, 10% polyvinyl chloride, 2% polyethylene, and 6% unknown. Overall, 8 of the total 282 pieces were not identified as a standard polymer using FTIR analysis.

Reporting Units
The end result of these techniques is an estimated mass of microplastic in a given matrix. Depending on study objectives, a number of reporting units might be useful.

You may want to understand how the microplastic concentration compares to the other natural materials within the specified size range. Compare the amount of microplastic to the total amount of dried material within the specific size range by using:

$$[(g_{\text{microplastic}}) / (g_{\text{total solids}})]$$

You may want to understand regional or temporal trends and make comparisons among sampling sites. Compare the amount of microplastic across samples by using:

$$[(g_{\text{microplastic}}) / (mL_{\text{matrix}})]$$

where matrix is the volume of water, sand, or sediment processed to obtain a sample. For example, use the volume of water filtered during a single surface water trawl or the amount of sediment captured in a Ponar grab.

Lastly, you may want to understand how your samples compare to historic samples, or how the microplastic particles are distributed over a certain area. For these comparisons, use:

$$[(g_{\text{microplastic}}) / (m^2_{\text{matrix}})].$$

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