

Microplastic Sampling, Processing and Detection in Surface Water Using Whole Water Samples (Wiggin and Holland 2019)

SUPPLIES:

Field Sampling (depending on accessible versus non-accessible locations)

1. Pump
2. Adjustable 3-Section Extension Pole (Home Depot)
3. Duct tape/zip ties, screw drivers
4. Transect tape
5. Extra D batteries (pump requires 3D batteries)
6. 20 ft of tubing
7. Clamps for attaching tubing to pump
8. 4L amber bottles (5 per sample, i.e. enough to collect 20L per section at a location of interest)
9. 1.6µm filtered nanopure water

Laboratory – Size Fractionation, Water Extraction and Digestion of Organic Material

1. 8L glass bottle (Note: only needed if you want to detect below 20µm debris)
2. Large ceramic funnel to hold sieves if saving <20 µm fraction
3. Fisherbrand™ U.S. Standard Stainless Steel Sieves, 8 in. dia. x 2 in.D, 500 µm mesh (Cat. No. 04-881-10Q)
4. Fisherbrand™ U.S. Standard Stainless Steel Sieves, 8 in. dia. x 2 in.D, 125 µm mesh (Cat. No. 04-881-10Y)
5. Fisherbrand™ U.S. Standard Stainless Steel Sieves, 8 in. dia. x 2 in.D, 63 µm mesh (Cat. No. 04-881-10C)
6. Gilson 8in diameter Stainless Steel Sieve, #635, 20 µm mesh (Cat. No. V8CF #635 BU)
7. Nalgene Analytical Test Filter Funnels, 250 mL (Cat. No. 09-740-30J)
8. Fisherbrand™ Vacuum Filter Holders, Silicone Rubber Stoppers (Cat. No. 09-753-9)
9. Teflon Tape
10. MilliporeSigma™ Isopore™ Polycarbonate Membrane Filters, 3 µm, 47mm (Cat. No. TSTP04700)
11. Fisherbrand™ Solid Rubber Stoppers, size 3 (Cat. No. 14-130E)
12. 600 mL beakers (need one beaker for each processed filter)
13. Plant Dryer/Incubator able to hold 50°C
14. 2-4L amber bottles filled with 1.6µm filtered nanopure water
15. Hydrogen Peroxide, 30% (Cat. No. H325-100)
16. Prepared 15% Hydrogen Peroxide (diluted with 1.6µm filtered nanopure water)
17. 2-Propanol (Cat. No. A416-4)
18. Fisherbrand™ Reusable Heavy-Wall Filter Flasks, 1000 mL (Cat. No. FB3001000)
19. Fisherbrand™ Reusable Heavy-Wall Filter Flasks, 500 mL (Cat. No. FB300500)
20. Tubing for filter flask

21. MilliporeSigma™ PetriSlide™ for Contamination Analysis (Cat. No. PD1504700)
22. Fine Point Forceps
23. Glass slides

Staining with Nile Red

1. Acetone (Cat. No. A18-4)
2. N-Hexane (Cat. No. AC160780010)
3. Nile Red (Cat. No. AC415711000)
 - a. Create a stock at 1mg/ml in pure acetone
 - b. Create a working solution at 10µg/ml by diluting your stock in n-hexane
 - c. Filter working solution on a 1.6µm filter
4. Foil

Detection of unstained and stained particles:

1. Compound microscope with a 4X and 10X Objective
2. External light source (e.g. Dyna Light #DL-150)
3. CMOS mounted camera (e.g. Olympus Lumenera Infinity-3C)
4. Fluorescence Microscope
 - a. Capable of green wavelength excitation (e.g. excitation 460-500, emission 535LP)
 - b. Or- Crime Light (450-510nm, with orange filter at 529nm) with viewing goggles

PROTOCOL:

Field Sampling:

1. Prior to field sampling, wash required number of amber bottles with 1.6µm filtered nanopure water three times
2. Choose three replicate sections at each sampling location that are ~5 m apart
3. Attach pump with appropriate length of tubing to the extendable pole using clamps
4. If needed add a floating device to pump so pump is not submerged
5. Tape or zip tie the transect tape to the end of the pole near the pump and run the transect tape and the [thin] tubing all the way down to the bottom of the pole
6. Turn on pump before deploying
7. Place pump 3m from shoreline
8. Wash out each amber bottle 3X with sample water
9. Fill 5-4L amber bottles to get 20L of sample water

Laboratory – Size Fractionation, Water Extraction and Digestion of Organic Material

Size Fractionation and Water Extraction for 1- 20L sample:

1. Pour contents of one 4 L amber bottle through stack of stainless-steel sieves set up on the 8 L collection bottle (**See Figure X**)
2. Rinse amber bottle with ~250ml of 1.6 µm filtered nanopure, each time pouring through the stack of sieves

3. Repeat step 2 for a total of three times to adequately wash the bottle
4. Remove stainless steel stack
5. If detecting sizes below 20 μm save the contents collected in the 8L collection bottle
 - a. Place ceramic funnel into original 4 L amber bottle
 - b. Fill with volume collected in the 8 L bottle
 - c. If extra water remains, place in an empty pre-washed 4L amber bottle (“6th” bottle)
6. Repeat until all five amber bottles have been size fractionated, washed and refilled if required
7. Rinse 8 L bottle with nanopure water 3x into 6th amber bottle
8. Rinse ceramic funnel with nanopure water 3x into 6th amber bottle

Digestion of organic material in a filtering unit:

For 500-20 μm size fractions:

1. Setup a 1-L vacuum flask with the 500 ml secondary collection flask for potential overflow
2. Use 1.6 μm filtered nanopure to backwash each sieve into a separate filtering unit that already contains a 3 μm polycarbonate filter (**See Figure 2A**)
3. Vacuum filter the remaining liquid in the filtering units
4. Place rubber stopper in the bottom of the filtering unit
5. Place filtering unit, with stopper, into 600 mL beaker (**See Figure 2B**)
6. Add 30 mL 15% H_2O_2 that has been filtered to 1.6 μm
7. Place lid on top of filtering unit
8. Place beaker into incubator at 50°C for 16h (overnight)
9. After incubation, vacuum filter the remaining liquid
10. Rinse sides of filtering unit with 20% 2-Propanol
11. Remove the filtering unit from the blue base and gently rinse sides and edges with minimal volume onto filter
12. Using 2 forceps, remove filter from filtering unit and place into PetriSlide

Microplastic detection using a light microscope and subsequent staining with Nile Red for detection on a fluorescent microscope

Visual Examination using Light Microscope

1. Remove lid of petrislide and replace with clean glass slide
2. Turn on and position external light to shine directly under objective lens
3. Use 4x objective lens to count all microplastics sized above 63 μm
4. Use 10x objective lens to count all microplastics sized below 63 μm
5. Take a picture of each particle of interest in using an attached camera
6. Size class each particle using imaging software

Staining debris with Nile Red

1. Place a drop of nanopure water onto the filter in the PetriSlide to loosen filter
2. Using 2 forceps, remove filter from PetriSlide and place onto filtering unit
3. Plug filtering unit with rubber stopper
4. Place filtering unit into foil covered 600 mL beaker
5. Add 5 mL 10 µg/mL Nile Red working solution onto filter
6. Place lid onto filtering unit and place foil lid onto beaker
7. Incubate at room temperature for 30 min
8. Vacuum filter remaining liquid
9. Rinse filter with 5 mL N-hexane
10. Rinse sides of filtering unit with 20% 2-Propanol
11. Take off the top clear portion of the filtering unit and rinse sides and edges onto filter
12. Using 2 forceps, remove filter from filtering unit and place into PetriSlide

Detection of stained particles on a fluorescent microscope

1. Remove PetriSlide lid and replace with clean glass slide
2. Set fluorescent microscope to FITC (excitation 460-500, emission 535LP) filter
3. Use 4x objective lens to count fluorescent items sized above 20 µm
4. Use 10x objective lens to fluorescent items sized below 20 µm
5. Take picture of each particle of interest
6. Size class each particle using an imaging software such as Image J

This Protocol was based off of the methods outlined by: Wiggin, K. J. and E. B. Holland (2019). "Validation and application of cost and time effective methods for the detection of 3–500 µm sized microplastics in the urban marine and estuarine environments surrounding Long Beach, California." Marine Pollution Bulletin **143**: 152-162.

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