Spatial Study Water Collection

Materials

- **2-18g pink needles**
- **1-50 mL syringe**
- **1- labeled sterivex filter with luer**
- **1- unlabeled sterivex filter without luer**
- **1 set of luer lok caps in Whirl-Pak**
- **1-3mL syringe**
- **1- 2 mL epi with RNA later**
- **3-40 mL amber DIC vials**
- **3- 40 mL amber OCN vials**
- **3- 40 mL amber ICR vials**
- **3- 15 mL proteomic friendly white cap ION tubes**
- **3- 2mL amber THA vials**
- **1- 1L amber GRA bottle**
- **1- 2L amber TSS bottle**
- **Gloves**

FILTERED WATER

1. Put on a fresh pair of gloves.
2. Locate the three THA (2mL) vials and the three OCN (40 ml amber) vials, 1-60 mL syringe, the labeled sterivex filter, and 1 needle.
3. Open the filter package that has the labeled filter but **do not touch the inlet to the filter** and attach the pink 18g needle.
4. Open the 60 mL syringe package and remove the syringe. Please **d on't touch the outlet of the syringe and do not fully remove the plunger from the syringe body**. This is important to avoid contamination.
5. While sampling, please **stand downstream of the sampling location and point the opening of the syringe upstream**. This is important to collect a representative sample.
6. Fill the syringe with river water, collecting water from 50% of the water column depth. Expel the syringe contents into the river (downstream of the sampling location) and repeat this two more times. You only need to flush the syringe as described when you first open the syringe package. You do not need to flush repeatedly when collecting additional syringe volumes.
7. After flushing the syringe 3 times, fill the syringe again from 50% of the water column depth; this is the sample water to be collected.
8. Screw the syringe onto the filter that has a needle connected to it. Remove the plastic cover that is protecting the needle while the needle is still in the filter package. Please **don't touch the outlet of the syringe, the inlet of the filter, or any part of the needle**. This is important to avoid contamination. Also, retain the plastic needle cover. It is okay to touch the cylindrical filter housing.
9. Push 5 mL of water through the filter/needle assembly. This water is not collected.
10. Without removing the cap of the rep-1 THA vial, pierce the septa of the vial and fill only up to the 1 mL line.
11. Open the rep-1 OCN vial (keep cap in your hand, do not put it down) and filter water into it until reaching the pre-marked line (¾ full).

12. After collecting rep-1 for THA and OCN, remove the filter+needle from the syringe and pull a fresh volume of water for each of the next two reps for THA and OCN. Push 5ml of water (not collected) through the filter+needle after each syringe re-fill. Be sure to collect samples in order from rep 1, then rep 2, then rep 3. This results in THA and OCN samples being direct paired. Store the amino acid vials first inside a Whirl-Pak and then seal the Whirl-Pak in a Ziploc bag. Be sure the Ziploc bag is completely sealed. Then store all THA and OCN vials on ice. CAUTION: THA vials are small and fragile. Be aware not to smash them when moving things in and out of the cooler.
13. Locate the three DIC (40mL) vials and using the same syringe+filter+needle setup to continue filtering.
14. After refilling the syringe with water (from 50% depth) and reattaching the filter+needle and pushing 5ml of uncollected water, open the rep-1 DIC vial and hold the cap in your hand (do not place it on the ground).
15. Place the tip of the needle inside of the vial angled towards the side wall of the vial and push water through the filter. Only place the needle inside the vial (don't allow anything else inside the vial, only the metal part of the needle). Keep the needle in the vial and push about 160 ml, letting it overflow. This will exchange the vial volume a few times for a robust sample. Do not push too hard because you don't want to create bubbling. It's important to **keep the vial completely full and seal the vial** with the lid so there is no headspace. Ensure that surface tension produces a dome of water above the top of the vial. Then carefully seal the vial tightly and flip upside down to make sure there are no trapped air bubbles.
16. Collect reps 2 and 3 for DIC, each time refilling the syringe and pushing 5ml of uncollected water just like was done for the THA and OCN sample collection. Store the vials on ice.
17. Remove and safely dispose of the needle in the bottle labeled sharps.
18. Continue using the same filter and syringe to fill the rest of the vial types.
19. Locate the 3 ICR vials (40 mL vials). Caution: these vials will be pre-acidified with 10µL of 85% phosphoric acid.
20. Fill the syringe again from 50% of the water column depth and reattach to the filter. Push 5 mL of water through the filter before filling each vial.
21. Uncap the vial and keep the cap in your hand (do not touch inside of cap as doing so will contaminate the sample).
22. Fill the 1 ICR vial to the pre-marked line. If the mark is rubbed off, fill halfway. Store on ice.
23. Detach filter from syringe and push out any remaining volume (one syringe volume per vial; just like for samples above; do NOT use left-over volume to fill the next vial...the only exception to this rule is for the paired THA and OCN samples).
24. Then repeat to collect ICR reps 2 and 3.
25. Locate the 3 ION tubes (15 mL tubes).
26. Filter water into these vials, just like was done for the ICR vials (separate syringe fills and pushing 5ml of uncollected water for each vial). Fill each ION tube to the 10 mL mark line and store on ice.
27. Locate the GRA (1L) bottle and fill using the same syringe and filter as before.
28. After the 7th syringe pull or about 1/3 of the way up the bottle you can preserve the filter as described below, but only if it is slowing down. If the filter is still flowing really well, keep using the same filter to get more microbial biomass on the filter. (The reason for preserving at this step is because we need to push at least 1 L of water through the filter before preserving. Again, if the filter is flowing well, don't preserve it until the flow slows down. If it lasts all the way through the GRA bottle, that is fine, but make sure to preserve the filter once you're done.)

PRESERVE THE FILTER

29. Detach the 50mL syringe from the filter, expel remaining water, and **fill the syringe with air.** Attach the air-filled syringe to the filter and push the air through the filter. The goal is to expel as much water from the filter as possible. Repeat 2 or 3 times if needed.
30. Place the 50 mL syringe back into its plastic package. (it will be used later in the sampling).
31. Take the Whirl-Pak with the luer lok caps and attach the female cap to the filter.
32. Take out the small plastic epi tube filled with RNALater. This is the preservative for the filter. Also take out a 3mL syringe and a new needle.
33. Connect the new needle to the 3mL syringe, carefully open the small epi tube, and fill the syringe with the RNALater by simply putting the needle down into the liquid. Pull the entire volume of the in the epi tube.
34. After filling the syringe with RNALater, invert the syringe so that the needle is facing up. Safely discard the needle.
35. Attach the 3mL syringe to the female side of the filter and rotate so that the syringe is facing down and the filter is below it. Push the plunger to slowly fill the filter with RNALater. Fill until you feel some resistance or until you have used all the RNALater. Cap with the other end of the filter with the remaining luer lock cap.
36. Gently shake the sealed filter to distribute the RNALater.
37. Put the capped filter into a small Whirl-Pak bag, tie up to seal, and place in the cooler.

CONTINUE FILTERING

38. If you still need to fill more volume in the GRA bottle, fill the syringe again from 50% of the water column depth and attach to the new filter that is not labeled and does not have a luer (no purple end).
39. Push 5 mL of sample through the new filter before continuing to fill the rest of the GRA 1L bottle.
40. Fill until you have reached the neck. Don't need to overfill. Place on ice.

UNFILTERED WATER

41. Locate the TSS (2L) bottle.
42. Uncap and dip into the water. Rinse 3 times.
43. To take your sample, again face upstream but point the bottle downstream as to not catch any floating debris from the surface water.
44. Cap underwater. Then store on ice.

NOTES

- **It is possible that your filter clogs before you get to the 1L point for the GRA bottle. This is very normal. If that happens, go ahead and preserve your filter at that point and continue the filtering using the unlabeled non luer sterivex filter.**
- **A filter is clogged when it is hard to push the water through the filter housing. Do not spend too much time trying to get droplets out of the filter. Stop, and grab a new filter. Its not worth spending time on trying to get droplets out of a filter.**
- **In some cases, you might need to use more than the 2 filters in the pre-made bag and that is ok. You can pull more filters from the extra materials bag. DON'T WASTE TIME PUSHING DROPS THROUGH A CLOGGED FILTER!!**
- **If you break any vials grab a blank vial and label it with the appropriate sample ID.**

Storage

- Mini Freezer (hotel folks)/-20 Freezer (Richland): THA, RNA
- -80 freezer (Richland): RNA
- Cooler with ice (hotel folks)/4C fridge (Richland) : DIC, ICR, TSS, OCN, GRA

Protocol for visually-derived metadata and photos/videos

While you will always have two people in the field, one person could be in charge of this part of the overall protocol. Water shoes are highly recommended as some parts of the protocol are best done by getting into shallow water.

MATERIALS

- Hardcopy metadata form
- Pencil (mechanical is preferred, or sharp normal pencil)
- 80x80cm PVC quadrat with grid strings for photos
- SFA tablet (for taking photos/videos and acquiring GPS coordinates)

COLLECT METADATA ON THE HARDCOPY FORM

1. Check that the site ID *AND* your vial ID are recorded on the form. The site ID is the code the identifies the location (e.g., S25, T55R, or similar, without an underscore). The vial ID starts with SPS_ followed by a four digit number (e.g., SPS_0001) and that's on all the sample tubes used for the site.
2. Record latitude and longitude in decimal degrees from the app on the tablet (e.g., 'My GPS Coordinates').
3. Fill out the rest of the metadata fields associated with field site characteristics, such as sediment texture, hydromorphic setting, and weather. These variables are not quantitative assessments, they are *quick* subjective visual assessments to provide low-resolution information. It should take ~5 minutes to fill out these fields.

TAKE GENERAL PHOTOS AND VIDEO OF THE SITE

1. Clearly write the site name (e.g., S02) and date (e.g., 30 Aug 2021) on the white board and take a photo of the white board so we know which site you're at. Then take the following.
2. Photo looking across the river to give a sense of how broad the river is. Put a measuring tape with 30cm of 'tape' showing somewhere in the picture frame for scale.
3. Photo looking upstream, showing the river surface, shoreline sediments, and vegetation. Put a measuring tape with 30cm of 'tape' showing somewhere in the picture frame for scale.
4. Photo looking downstream, showing the river surface, shoreline sediments, and vegetation. Put a measuring tape with 30cm of 'tape' showing somewhere in the picture frame for scale.

5. Video that starts by looking directly upstream with riparian vegetation in view and rotates towards the river downstream until looking directly down the shoreline with riparian vegetation in view
6. Photo of the hardcopy metadata sheet. Please confirm that all fields on the paper metadata sheet are filled out prior to leaving the field, and take a picture of the data sheet. This picture will also be uploaded. You will fill in the rest of the metadata via an online form.

TAKE PHOTOS FOR GRAIN SIZE ANALYSIS

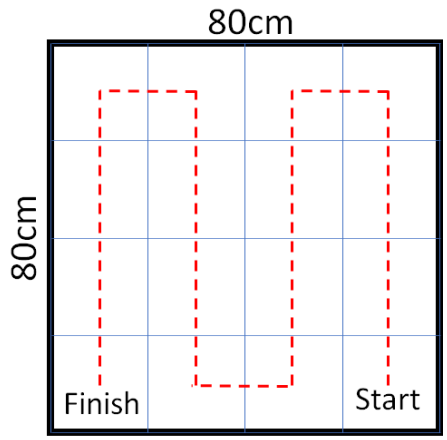
The goal of these photos is to estimate grain size of the surficial sediments at each spatial study site. This protocol needs to be done once at each site. If there is a return visit to a given site, this protocol does not need to be repeated. The assumption is that grain size won't change much between trips. There are three types of photos to take using the SFA tablet. For all these the tablet should be parallel with the riverbed being imaged. This is important to avoid distortion in the images, which is needed to generate robust estimates of grain size. For the photo taken through the water it may be impossible to have the tablet parallel to the riverbed, just do your best. For each photo use the PVC quadrat for scale. If the quadrat can't be used for any reason, use a tape measure with 30cm of 'tape' showing. If any other method is used for scale, record details in the metadata notes. Do your best to avoid creating a shadow with your body as you take the photos.

Photos of exposed riverbed sediments

For these photos we are focused on materials that are obviously riverbed sediments (i.e., those that would normally be underwater during normal/seasonally high water, not the shoreline soils that would go underwater during a large flood). The sediments should be between the water's edge and the upper "scour line." The scour line can be a little subjective, but there are obvious soils above it and little to no soil below it. Below the scour line you'll see sediments exposed due to water removing (I.e., scouring) soil. Take the photos at 3-5 locations at each site. Image more than 3 locations only if time permits. Imaging more locations will be useful if time permits, but don't allow this to be the only task keeping you at a site.

1. Visually survey the riverbed sediments that are above/out of the water and below the upper scour line.
2. Select a location that is representative of the site's exposed sediments and that is relatively flat/even.
3. Place the quadrat on the sediments. If there is a narrow area between the scour line and the water, put the quadrat partially in the water and take photos as best you can. If you have a steep cut bank, try to place the quadrat vertically and take photos while standing in the water (if safe).
4. Clearly write the site name (e.g., S02) and date (e.g., 30 Aug 2021) on the white board and place it just outside the lower left corner of the picture frame, but not on the quadrat, so it is visible in the photo.
5. Take a photo of the quadrat with tablet as close to parallel as possible and as close to the quadrat as possible while still being able to see all inner edges of the quadrat. It is important to be as close as possible so sediment grains can be resolved in the image, but <u>don't cutoff any edges of the quadrat</u>.

6. After taking the photo confirm that it was taken/saved and that the image quality is good, the whiteboard is visible, the writing can be read, and no edges are cut off.
7. Without moving the quadrat and take photos of each 20x20cm square defined by the strings within the quadrat as follows:
    a. For all these photos keep the tablet in the same orientation that was used for the photo of the whole quadrat (stand in the same place, don't rotate the tablet). That way its easier to know where each photo is located. And follow the path indicated below (see the quadrat figure) to make it even easier to know where each photo is located within the quadrat.
    b. Starting in the lower right corner, move the tablet as close as possible to the sediments while being able to see all edges of the lower right 20x20cm square. Here, 'lower right' is in reference to the orientation of the photo taken of the whole quadrat. You should have string on two sides and PVC on two sides, and at least the inner edge of all should be visible in the image. Keep the tablet parallel to the sediments and take a photo.
    c. Then move 'up' the quadrat along the right hand edge. Take a photo of the 20x20cm square. You should have string on three sides and PVC on one side.
    d. Keep moving up until you've reached the top right corner, then move to the left and take photos moving 'down' the quadrat until at the bottom and move left and then take photos going up the quadrat, etc. (see the quadrat figure).
    e. You should end at the lower left corner and you should have taken 16 photos. At that point confirm that the photos look okay and were saved.
8. As time permits, repeat steps 1-7 at additional representative locations with exposed bed sediment along the shoreline of the field site.



Quadrat Figure: Take a photo of the whole quadrat laid over sediments, then take photos of each 20x20cm square within the quadrat, following the path of the dashed red line. Each photo should be taken with the tablet parallel to the sediments and as close to the sediments as possible while still being able to see all four edges of the full quadrat or 20x20cm squares, which are delineated with string.

Photos of sediments through the water

For these photos we are focused on materials that are underwater at time of imaging. The goal is to get a more complete sense of the sediment texture for the whole channel (and not just exposed sediments). Take these photos at 3-5 locations at each site. Image more than 3 locations only if time permits.

Imaging more locations will be useful if time permits but prioritize photos of exposed sediments and don't allow this to be the only task keeping you at a site.

1. Visually survey the riverbed sediments that are underwater.
2. Select a location that is representative of the site's underwater sediments.
3. Place the quadrat underwater so it sits on the sediments. This will require air to escape through the drilled holes.
4. Take a photo of the white board (out of the water) with the site name and date.
5. Then take a separate photo (after the white board photo) of the quadrat with tablet as close to parallel as possible and as close to the quadrat as possible while still being able to see all edges of the quadrat. It is important to be as close as possible so sediment grains can be resolved in the image, but don't cutoff any edges of the quadrat, **and don't submerge the tablet**!
6. After taking the photo confirm that it was taken/saved and that the image quality is good. If there is a bad reflection so you can't see through the water, try having your partner create a shadow over the quadrat.
7. If time permits repeat steps 1-6 at additional representative locations with underwater bed sediments. We will not take photos of the individual 20x20cm squares for the photos of underwater sediments (only take the photo of the whole quadrat).

Photo upload

Each day put your photos into the folder for the correct date and catchment. For each day, within each catchment-specific folder, please create new folders for the specific sites you have photos from. Then simply dump all photos from each site into the correct folder. No need to rename files if you do it this way. Each folder should have the photo looking across, up-river, and down-river, and the video, plus 1 quadrat photo and 16 sub-grid photos for each 'replicate' location you photographed for sediment texture.

##########