WHONDRS Continental Model-Sample Study
Sediment and Surface Water Sampling Protocol

Note: A video protocol is available at https://tinyurl.com/CM-video-protocol. The last page of the document has a one page summary of the protocol.

This protocol will describe sensor deployment and sediment and river water sampling (~1.5hrs) for the ICON-ModEx Campaign. Please collect samples as close as possible to the agreed-upon coordinates.

NOTE: Sampling must be completed on **Monday** and samples shipped the following **Tuesday**. If that is not possible, please contact WHONDRS@pnnl.gov for an alternate arrangement. Please contact WHONDRS@pnnl.gov with any questions or concerns. Also note that it is the responsibility of the sampler to obtain any required access and sampling agreements, permits, etc. for the site(s) they collect samples from. PNNL has no responsibility for access, agreements, permits, etc.

MATERIALS

In addition to the WHONDRS sampling kit, you will need the following:	**The WHONDRS sampling kit should contain the following:**
1) Ideally two people to do the sampling 2) Cooler with wet or blue ice for keeping samples cold in the field 3) Method for collecting latitude and longitude in decimal degrees in the field (smart phone is sufficient) 4) Method for taking pictures of the field site (smart phone is sufficient) 5) -20 C freezer for freezing the ice packs in advance of shipping (do not freeze the samples but be sure to freeze the ice packs at least 48 hours prior to shipping) 6) Refrigerator to store samples prior to shipping 7) Access to a FedEx office for shipping samples back overnight (WHONDRS pays for shipping) 8) A method to safely dispose of used needles 9) Thermometer to measure temperature in the water column 10) Safety glasses (some vials are pre-acidified and should be treated with care) 11) Metric measuring tape for estimating water depth in cm 12) If needed, use a rock, brick, stake, etc. to tie the dissolved oxygen sensor to, so it stays in place	1) Pencil 2) Hard copy metadata form 3) Shipping label that is pre-filled for sending samples back via FedEx overnight 4) Freezer "blue ice" packs (place these in a -20C freezer for at least 48 hours prior to shipping) 5) Four pairs of nitrile gloves to minimize contamination 6) One sterile/clean sediment scoops 7) One foil-wrapped metal scoopula to assist in distributing sediment from the sediment scoop 8) Three 18 gauge needles 9) One 3mL and one 60mL syringe 10) Three opaque brown plastic bottles (one for sediment, two for filtered water). 11) Two sterile 50mL white capped tubes pre-filled with RNAlater 12) Three **pre-acidified** 60mL amber vials for filtered water 13) Three 40mL amber vials for filtered water 14) Three 15 mL white cap tubes for ions and SpC from filtered water 15) One labeled sterivex filter and multiple unlabeled sterivex filter in case the labeled one clogs 16) One epi tube with 3 mL of RNAlater 17) Two luer-lok caps to seal filter after filled with RNALater 18) One Whirl-Pak bag to store preserved filter 19) Small bag with two pH strips 20) Dissolved oxygen sensor 21) Some kits: 2mm sieve with or without a catch pan

Note: The sampling kit does not need to be refrigerated when first received. The ice pack should be frozen prior to sampling. The samples need to be refrigerated after collection.

IDENTIFY SAMPLING LOCATIONS

After confirming that you have all of the needed gear, identify a wadeable (i.e., **in water**) sampling location for sediment collection in a depositional zone, as defined in the NEON protocol (NEON.DOC.001193; Jensen, 2019): "A depositional zone is defined as the area within a river where the energy regime is low and typically are found at the inside bend of a stream, riffle, pool lip, downstream from obstacles or simply shallow waters near the shore (USGS, 1994) (Figure 1)". As in the NEON protocol (NEON.DOC.001193; Jensen, 2019), "The sample strategy for sediment analysis focuses on fine-grained surficial sediments from natural depositional zones...(USGS, 1994). Surface sediment is considered to range from 1 to 3 cm in depth (Golterman et al., 1983; Keith, 1991)." Sampling should avoid large debris greater than 4 mm in size if possible.

Sediments will be collected from one stream reach, and potentially a single depositional zone if there is sufficient fine-grained sediment. The first step is identifying the reach/site. Then deploy the oxygen sensor, collect metadata, conduct water sampling, and then collect sediments (**collect water before sediments**). Once the site is selected, follow the steps below to deploy the oxygen sensor and collect the metadata and samples.

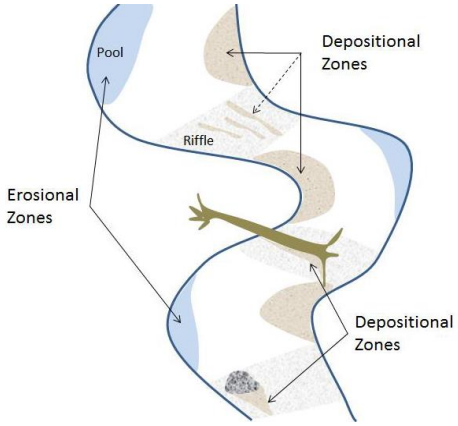

Figure 1. Examples of depositional zones. Collect sediments from a depositional zone and avoid erosional zones, to the degree it is possible. Image from NEON protocol (NEON.DOC.001193; Jensen 2019).

DEPLOY THE OXYGEN SENSOR

1) Prior to deploying the sensor, please make sure to keep your sensor in similar temperature conditions to the stream you are sampling. We want to ensure the sensor is not a completely different temperature from the stream being sampled (i.e., do not leave the sensor in a hot car prior to deploying.) If deploying in a very cold stream, the sensor can be placed in a cooler, but must be wrapped in a towel to keep off the ice.
2) Deploy the sensor immediately after selecting the sampling site. The sensor needs at least 60 minutes in the water to provide good data. The sensor is already launched and is logging data. You do not need to do anything to start the sensor. The black part is where the sensor is. Avoid scratching the little black window.
3) On the provided metadata sheet, record the large number written on the sensor housing. This is to identify which sensor you deployed.
4) Remove the soft black protective cap from the end of the sensor. This is NOT the part that unscrews, it will simply slide off (Figure 2).

Figure 2. Oxygen sensor with cap on (left) and with cap removed (right).

5) Place the sensor **upstream** of your sampling location. This avoids contaminating the water going past the sensor as you sample sediment. Do your best to keep the sensor away from the water's surface and away from the sediment surface. The goal is to measure the water column oxygen. Ideally, elevate the sensor off the bed of the river at least 10cm if possible (e.g., set it on a rock, stick the white base vertically into soft sediment, etc.), but **don't allow it to disturb the water's surface (find a deeper pool if required)**. If you have no choice, it's okay to lay the sensor on the stream bed. Position of the sensor window within the water column is more important than orientation. If needed, secure the sensor to a rock, brick, stake, etc. so it doesn't move downstream or become lost in shifting sediments. Consider placing a flag next to the sensor if needed.
6) On the provided metadata sheet, record the date and time the sensor is deployed. Also record the time zone so we can translate your recorded time to the time logged by the sensor.
7) At the end of the protocol, you will find steps to remove the sensor and record the end time. You will leave it in place while you fill out your metadata sheet and collect your samples. **Note**: Without your start/end times, time zone, and the sensor housing number, the sensor data cannot be used.

COLLECT METADATA ON THE PROVIDED METADATA SHEET

8) Record your name, the stream name, and the sample kit ID. The kit ID is the set of four digits at the start of every vial label. They should all be identical and formatted as "CDX_####."
9) Record the general weather conditions, river intermittency, vegetation type, sediment type, hydrogeomorphology, and coverages of algae, in-stream plants, and overhead canopy using the checklists/categories on the metadata form.
10) Record the latitude and longitude of the **sensor deployment** location in **decimal degrees**. You can use a GPS or a smartphone app such as 'My GPS Coordinates' or Google maps.
11) Measure the water depth where sediments will be collected and record it on the datasheet in centimeters.
12) With a smartphone or camera held horizontally, take a photo looking straight down through the water of the undisturbed sediment so that about $1m^2$ can be seen. Hold the camera/phone **parallel** to the riverbed so that the image is taken looking straight down to avoid distortion. If possible, place a measuring tape extended to 30 cm on the bed of the river so it is visible in the photo. Note that this will be uploaded along with the metadata.
13) In addition, take the following photos (note these will be uploaded along with metadata). For the last three pictures (b, c, d), lay out a **measuring tape to 30cm** as a reference for scale and make sure it can be seen in the photos. The first picture (looking across the river) doesn't need

to contain the measuring tape. Try to hold the phone/camera in a **horizontal** orientation if possible such that the picture should be wider than it is tall.

a) Looking across the river to give a sense of how broad the river is
b) Looking upstream, showing the river surface, shoreline sediments, and vegetation
c) Looking downstream, showing the river surface, shoreline sediments, and vegetation
d) Looking straight down at exposed shoreline sediments (not under water) so that about $1m^2$ can be seen in the image. Hold the camera **parallel** to the sediments to minimize distortion. (If the sediment is sloped, hold the camera at a matching slope to keep it parallel.)

14) Measure the pH of the surface water.
    a) Remove the pH strip from its protective plastic bag, making sure to keep the paper dry.
    b) Dip the strip into the river water upstream of where you are standing.

c) Wait 15 seconds after wetting the pH strip and then compare the color to the color chart provided with the pH strip. Record the pH value on the metadata sheet.

15) If you have a device for measuring temperature, measure the temperature of the water at 50% of the water column depth; record it on the metadata sheet in degrees C.
16) Measure the height of the water column at the place you plan to sample by extending a measuring tape from the riverbed vertically to the water surface. Record on the metadata sheet in centimeters.
17) 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. You will need to fill out a few fields after collecting the samples.

NOTE ABOUT FILTERED WATER SAMPLE COLLECTION: We have provided multipleSterivex filters, one labeled and several unlabeled. **Use the labeled filter first and replace it only if it clogs**. When you finish collecting water or when the filter clogs (whichever happens first), preserve the filter following that portion of the protocol below. Then you can continue filtering water with the unlabeled filters. Do not preserve or ship back unlabeled filters if they were used. **Preserve and ship the labeled filter**. Only ship back the unlabeled filters if they are unused. Also, it is recommended to do the filter-based sampling with 2 people. One person can operate the syringe and the other can hold the filter/needle assembly (while the syringe is being (re)filled) and sample vials. This will keep everything clean and save a lot of time.

Note that you may change the needle attached to the filter if you believe it has been contaminated or if it touches the acidified water in the amber vial. If an extra filter or needle is needed, please do not touch the inlet or outlet of the filter, and please don't touch the inlet of the needle or any part of the metal needle (it is okay to touch the plastic needle cover). If extra filters or needles are needed, please record the reason on the paper metadata sheet.

COLLECT FILTERED WATER SAMPLES

Follow the instructions below to collect your water samples from the water column above the depositional zone you will collect sediment from. Collect all the water samples before beginning sediment collection.

1) Put on nitrile gloves.
2) Locate all the labeled materials (vials, tubes, filter). Put the 500ml amber bottle marked as "BSD" to the side. Put the two 50ml falcon tubes marked as "MIC" to the side. These are for sediment. The other supplies are for water.
3) After collection, vials, tubes, bottles, and the filter should go into a cooler with ice.

SAMPLE PHASE 1: FILTERED WATER SAMPLES - Three pre-acidified 60mL AMBER GLASS vials (Wear safety glasses) *These vials are for FTICR-MS. The label suffix after the kit ID is "ICR" and a replicate number (-1, -2, -3)*

4) From the non-sediment materials, locate the three 60mL amber vials labeled as "ICR". These contain a very small volume of phosphoric **acid** (safety glasses recommended). **Do not unscrew the vial lids**.
5) Also locate the labeled filter, which is already attached to a needle.
6) Open the filter package that has a needle attached to the filter but **do not touch the inlet to the filter** and leave the needle/filter assembly in the package

7) Open the 60 mL syringe package and remove the syringe. Please **don't touch the outlet of the syringe and do not fully remove the plunger from the syringe body**. This is important to avoid contamination.
8) 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 and to avoid getting disturbed sediment into the water sample.
9) 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.
10) 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.
11) 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.
12) Push 5 mL of water through the filter/needle assembly. This water is not collected.
13) Remove the flip top cap of the 60mL amber vial (if there is one) to uncover the septum. **Do not unscrew the vial lids**. Please **don't touch the septum** that is exposed after removing the flip top cap. This is important to minimize contamination. Only remove the white disc that rests on top of the cap and conceals the septum
14) Pierce the septum of the vial with the needle and expel water using the syringe plunger. Fill one of the acidified **amber** glass vials **to the premarked line**.
15) Shake the vial gently to incorporate the acid into the sample. Store vial on ice. There is no need to replace the flip top cap that previously covered the septum.
16) Prior to collecting the second replicate, remove the filter from the syringe, and push out all the water, then refill the syringe and push at least 5ml of water through the filter (this is to push new sample into the filter housing).
17) Collect the second replicate as described above into the second vial, then refill the syringe, flush the filter, and collect the third replicate into the third vial. Store vials on ice. *See the note above this section if your filter clogs. One of the extra,* unlabeled *filters has a luer-lok connection to attach a needle. Find that extra filter if needed. The others do not have the luer-lok connection on the outlet. If your filter clogs, skip down to the filter preservation section before continuing on with a new filter here.*
18) **Make sure you remove the needle before moving on to non-acidified glass vials and/or plastic tubes. This is to avoid any acid cross-contamination.**

SAMPLE PHASE 2: FILTERED WATER SAMPLES - Three 40mL AMBER GLASS vials *These vials are for DOC and total N. The label suffix after the kit ID is "OCN" and a replicate number (-1, -2, -3)*

19) From the non-sediment materials, locate the 40mL amber vials labeled as "OCN".
20) Collect water into the syringe as described above and flush 5 ml through the filter.
21) Open the vial and fill one of the 40ml glass vials up to the pre-marked line (~ ¾ full)) by holding the filter outlet above the vial opening. Then re-cap the vial (don't let anything touch the inside of the vial or the inside of the cap, to avoid contamination).
22) Refill the syringe as described above, flush 5 ml through the filter and collect the second replicate in the second vial, and repeat for the third replicate in the third vial. Store vials on ice.

SAMPLE PHASE 3: COLLECT FILTERED WATER SAMPLES - Three 15mL WHITE CAP PLASTIC TUBES *These tubes are for ions. The label suffix after the kit ID is "WIN" and a replicate number (-1, -2, -3)*

23) From the non-sediment materials, locate the plastic 15mL tubes labeled with "WIN."
24) Collect water into the syringe as described above and flush 5mL through the filter.
25) Without attaching a needle, fill one of the white cap tubes to 10mL by holding the filter outlet above the tube opening. Don't let anything touch the inside of the vial or the inside of the cap, to avoid contamination.
26) Refill the syringe as described above, flush 5mL through the filter and collect the second replicate, and repeat for the third replicate. Store tubes on ice.

SAMPLE PHASE 4: COLLECT FILTERED WATER SAMPLES - One 500mL OPAQUE BROWN PLASTIC BOTTLE
This bottle is for bulk filtered water and is labeled with "BWT" and a replicate number (-1), a piece of tape over the top, and a square label that says "filtered."

27) From the non-sediment materials, locate the two opaque brown plastic bottles labeled with "BWT" and "filtered." **Do not use** the bottle labeled "BSD" or the bottle labeled "BWT" without a label that says "filtered."
28) Collect water into the 60ml syringe as described above, but there is no need to flush 5mL through the filter. (Ensuring you do not touch the outlet of the syringe or the inlet of the filter).
29) Fill the 500ml bottle to the neck by filling the syringe, filtering, and refilling the syringe multiple times as described above. (Do not let anything touch the inside of the bottle or the inside of the cap, to avoid contamination).
30) It is likely that filters will clog during this step. Once flow has slowed down **for the filter that is labeled**, close the bottle you are filling, then preserve the filter as described below in the next section titled "SAMPLE PHASE 5: PRESERVE THE USED, LABELED FILTER"
31) Once you have preserved the labeled filter, replace it with a new unlabeled filter and continue filtering. Replace filters as needed to continue good flow while filling the 500mL bottle.
32) While in the field, if you cannot fill the bottle with filtered water due to the filters clogging, then fill it with unfiltered water. For this, face upstream and simply fill the bottle from ~50% of the water column depth and cap it. In this case, clearly mark the bottle as 'unfiltered.' It is vital that the bottle be filled with water, even if it is unfiltered. All of the small vials/tubes must have filtered water, however.
33) Store on ice.

SAMPLE PHASE 5: PRESERVE THE USED, LABELED FILTER (Wear safety glasses) ***for microbial sequencing***

34) For the **labeled filter only**, use the following procedure to preserve the microbes. Be careful to keep the inlet and outlet of used filters clean so no microbial contamination is introduced.
35) Detach the 60mL syringe from the **labeled** filter, expel remaining water from the syringe, and **fill the syringe with air.**
36) 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. There will be a small amount of water remaining; just do your best.
37) Take the used labeled filter off the 60mL syringe, taking care not to touch the inlet of the filter.
38) Take the "female" luer lock cap, dip it into the stream to rinse it and then **attach it to the open (discharge) end of the filter**. Note that the filter has a "male" side and a "female" side and therefore there are two types of caps provided.
39) From the provided supplies, take out a small plastic epi tube filled with RNALater. This is the preservative for the filter. Also take out a 3mL syringe and a new needle.

40) Connect the new needle to the 3mL syringe, carefully open the small epi tube, and fill the syringe with all the RNALater by simply putting the needle down into the liquid.
41) After filling the syringe with RNALater, invert the syringe so that the needle is facing up. Safely discard the needle.
42) Attach the 3mL syringe to 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.
43) Detach the syringe and place it back into the syringe wrapper. You can ship this used syringe back with your samples. Locate the remaining luer lock cap, dip it into the stream to rinse it, and then attach to the filter. Gently shake the sealed filter to distribute the RNALater.
44) Put the capped filter into a small whirlpak bag, tie up to seal, and place in the cooler. Please ensure no water enters the whirlpak bag.

SAMPLE PHASE 6: COLLECT UNFILTERED WATER SAMPLES - One 500mL OPAQUE BROWN PLASTIC BOTTLE

*This bottles is for bulk **un**filtered water and is labeled with "BWT" and a replicate number (-2). It **does not** have a small square label that says "filtered," unlike the 500ml BWT bottle used for filtered water.*

45) From the non-sediment materials, locate the opaque brown plastic bottle labeled with "BWT." **Do not use** the bottle labeled "BSD" or the bottle labeled "BWT" and "filtered."
46) Face upstream and simply fill the bottle from ~50% of the water column depth and cap it.
47) Store on ice.

SAMPLE PHASE 7: COLLECT BULK SEDIMENT

1) Locate the sediment materials set aside earlier. These should be:
    a) One opaque brown plastic bottle marked as "BSD"
    b) Two 50ml white-capped conical tubes containing RNALater marked as "MIC".
2) Locate the foil-wrapped scoop and scoopula. You can use the scoopula to remove plant material and larger (>4mm) debris and help get sediment off the scoop and into the container
3) Put on a new pair of nitrile gloves to be used for sediment collection.
4) **Never scrape the sides of the plastic bottles/vials with the sediments or tools as this could introduce plastic contamination. If needed, use the scoop and scoopula in tandem to drop sediment down from the opening.**
5) Remove the 30mL metal scoop from packaging; ensure you only touch the scoop handle and not the spoon. Unwrap the scoopula and ensure you only touch the scoopula handle. Wash the scoop and scoopula by dipping them in the stream. If you need to set down the scoop or scoopula, place them on the clean side of the foil to minimize contamination.
6) Use the scoop to collect **underwater** surface sediments (**1-3 cm depth**, ideally, but can go a little deeper if needed) from the streambed. The goal is to fill the brown plastic bottle with sediment with as little water as possible. To do this, decant any bulk water (e.g., hold the scoopula against the scoop to retain sediments as you tip the scoop to drain water). Sampling should avoid plant material and avoid sediments > 4 mm so there is enough < 2mm sediment for analysis.
    NOTE: If you receive a sieve with or without a catch pan in your sampling kit, carefully unwrap the foil wrapped sieve and use the metal tools to work/push sediments through the sieve for a couple minutes and then rinse the sediments out of the sieve in the stream. This is to clean the sieve and avoid contamination. **Do not use your hands (even if gloved) to push material through the sieve.** (there's a lot of microbes and organics on fingers and even the nitrile gloves). If you have a catch pan, rinse it in the stream after unwrapping it from the foil. After cleaning the sieve, collect the sediment sample by holding the sieve over or setting it on the

opening of the brown plastic bottle if you do not have a catch pan. Drop your scoops of sediment onto the sieve, and use the metal tools to push the sediment through the sieve into the bottle. If you have a catch pan, sieve sediment into the catch pan and then use the metal tools to scoop the sieved sediment in the catch pan into the bottle. **Do not use your hands (even if gloved) to push material through the sieve or scoop the sediment.** Also, once your bottle is nearly full, scoop sieved sediment out of the bottle or catch pan and into the 50ml tubes for microbial analysis (see below), and then continue to fill the bottle with sieved sediment.

7) To get enough material, it is okay to move around and collect sediments from across the depositional zone and even to move to additional nearby depositional zones with a similar physical setting and similar sediments.
8) The most important thing is getting enough fine-grained sediment to fill the bottle.
9) Store the sealed bottle of sediment in the cooler.
10) Fill out the metadata sheet field that asks about sediment collection depth.

SAMPLE PHASE 8: COLLECT SEDIMENT FOR MICROBIAL ANALYSIS (Wear safety glasses)

11) Locate the two 50mL white-capped tubes pre-filled with RNALater and labeled with "MIC."
12) Following the same sediment collection procedures detailed above, collect ~7.5ml of <2mm sediment into each white-capped tube (if needed, scoop sieved sediment from the brown bottle as indicated above). There is a marked line on the tube (Fig. 3). Fill until the **RNAlater reaches that line**. Do not go past that line. If you accidentally go past the line, don't try to remove material as it will likely contaminate the sample and/or lose RNALater. Be careful to not splash RNAlater out of the tube. Take care not to spill the RNAlater at the sampling site.

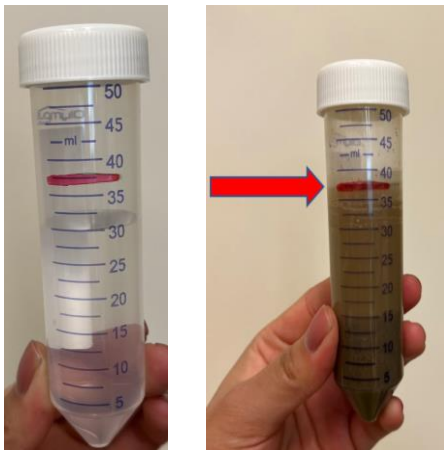

Figure 3. 50mL tube before sediment has been added (left) and after sediment has been added (right). Note that after sediment has been added, the total volume in the tube is at the pre-marked line.

13) After adding sediment, gently mix with the RNAlater by inverting the closed tube 5-10 times.
14) Store the sealed sediment/RNALater tubes on ice in the cooler.

RETRIEVE OXYGEN SENSOR

1) On the metadata sheet, record the sensor deployment end time before you remove the sensor from the water.
2) Photograph the metadata sheet.
3) Retrieve the oxygen sensor from the water and replace the soft black cap (Figure 2). You will ship it back with your samples.

TRANSPORT AND STORE SAMPLES AFTER COLLECTION

4) After all samples are collected, keep them on wet or blue ice during transit. Confirm the metadata sheet is filled out before leaving the field.
5) Place all samples (all vials/tubes/bottles and the filters preserved in RNAlater) in a **refrigerator until you are ready to ship on a Tuesday**. Please don't use a freezer as this will render samples unusable.

SHIPPING

1) Samples should be stored in the refrigerator until they are **shipped on the day after sampling (i.e., sample on Monday, ship on Tuesday) within 24 hours following sampling**. It is critical that overnight shipments be made on Tuesday in case there are shipping delays. Please **don't ship later in the week**.
2) It can be useful to remove materials from the refrigerator and pack the cooler as close as is reasonable to the time FedEx will ship the package. This maximizes the time samples stay cold.
3) When you are ready to ship, place the glass vials in the provided vial holders and then into ziplock bags. Place the other sample tubes/bottles into ziplock bags. Package the vials so they are upright in the cooler box; the brown plastic bottles can lay on their side. Surround the samples with the frozen ice packs.
4) **Also inside the cooler, include the scoops, scoopulas, sieve (if you have one), unused filters and needles, and absorbent padding that came with the supplies**. Pack those materials into the cooler and then place a frozen ice pack on top.
5) Put the lid of the cooler on, note on the paper metadata sheet the time and date the box is packed, and then place the paper metadata sheet on top of the lid. This will keep it dry.
6) Please **don't return** gloves or used needles. Dispose of the used needles in an appropriate sharps container.
7) Tape the outer box closed. Use enough packing tape to assure it will not open during shipping.
8) Adhere the provided shipping label to the outside of the box by removing the backing from the plastic sleeve that contains the shipping label.
9) Drop the package off at FedEx, or have it picked up by FedEx.
10) **On the same day you ship the package**, **notify WHONDRS@pnnl.gov that you shipped the package, and include the FedEx tracking number in your ema**il. This is critical to ensure sample integrity and timely delivery. The subject line of the email should be "SHIPPED SAMPLES – [Sample kit ID # (e.g., CM_###*)]". **If you wait until the day after you have shipped the samples, the samples may arrive prior to our notification, which will increase the likelihood of delivery issues and loss of sample integrity.**

METADATA

1) After the samples are packaged for shipment, **enter metadata into the digital form that can be found at https://tinyurl.com/CMX-metadata**. If you cannot access the form, email WHONDRS@pnnl.gov for a copy. Although not all fields are marked as required, please fill in as many fields as possible. Most information will be directly from your hard copy metadata sheet.
2) The google form also provides instructions on how to submit the field photos. Please name all files with the sampling kit ID as follows:
    - Photo looking down at sediments that were collected named: CM_###-collection
    - Photo looking across stream at sensor station named: CM_###-across
    - Photo looking upstream at the sensor station named: CM_###-up
    - Photo looking downstream at the sensor station named: CM_###-down
    - Photo looking down at exposed shoreline sediments named: CM_###-exposed

- Photo of the data sheet (front and back if notes are on the back) named: CM_###-data

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SDS: RNALater: https://100sb204.cims.tw/attachments/2014/7/2f52c04f27ba94d6.pdf; 85% phosphoric acid: https://www.fishersci.com/shop/products/o-phosphoric-acid-85-hplc-fisher-chemical/A260500#?keyword=A260-500

Summary

The links below redirect to the full sections of the protocol

- Before leaving for the field, freeze the blue ice packs included in your kit.
- Store samples in a cooler on wet/blue ice in the field and then transfer to a **refrigerator** until shipping.

1	**Identify sampling locations**	
2	**Deploy the oxygen sensor and record related metadata**	
3	**Record general metadata and take site photos**	
4	**Measure pH, water temperature, and water depth**	
5	**Collect filtered water samples.** *Wear nitrile gloves.*	**#1: Three pre-acidified 60mL amber glass vials** *labeled with "ICR" and a replicate number (-1, -2, -3). Inject sample into vial through septum. Do not unscrew the vial lid.* ***Wear safety glasses.*** *Shake gently before storing.*
		#2: Three 40mL amber glass vials *labeled with "OCN" and a replicate number (-1, -2, -3)*
		#3: Three 15mL white cap plastic tubes *labeled with "WIN" and a replicate number (-1, -2, -3)*
		#4: One 500mL opaque brown plastic bottle *labeled with "BWT" and a replicate number (-1) and a piece of tape that says "****filtered****."*
6	**Preserve filter** *when the labeled filter clogs. Wear nitrile gloves and safety glasses.*	**#5: One filter** *labeled with "RNA". Only preserve the labeled filter. After you have preserved it, switch to an unlabeled filter to continue filtering. Do not preserve unlabeled filters.*
7	**Collect unfiltered water sample.** *Wear nitrile gloves.*	**#6: One 500mL opaque brown plastic bottle** *labeled with "BWT" and a replicate number (-2)*
8	**Collect sediment samples.** *Wear a new pair of nitrile gloves.*	**#7: One 500mL opaque brown plastic bottle** *labeled with "BSD"*
		#8: Two 50mL white cap plastic tubes *labeled with "MIC" and a replicate number (-1, -2). Fill with sediment until pre-existing liquid (RNAlater) and sediment combined reach the pre-marked line. Do not go over the line.* ***Wear safety glasses.*** *Shake gently before storing.*
9	**Complete metadata and take metadata sheet photo**	
10	**Retrieve oxygen sensor**	
11	**Transport samples to the interim storage location. Refrigerate until they can be shipped.**	
12	**Fill out online metadata https://tinyurl.com/CMX-metadata**	

13	**Email whondrs@pnnl.gov that you plan to ship the following day**
14	**On Tuesday, ship the samples and sensor back with the frozen blue ice packs, metadata sheet, and extra unused materials.** *Sampling should occur on Mondays. Shipping should occur on Tuesdays.*