Stegen Early Career Collaboration - Protocol for Sediment Sampling

Contact Amy.Goldman@pnnl.gov with questions.

This protocol describes sediment sampling and iButton retrieval for the Perturbation Response Traits project. Please keep in mind that it is your responsibility to ensure compliance with any environmental regulations associated with the field sampling (e.g., permits to sample). Also note that your sampling location must be within ~100 m of a location that measures river discharge, height, or pressure. Data must be public and at a logging frequency of at least 2 hours; it is preferable to have 1 year of data. Sampling must be completed on Sunday, Monday, or Tuesday, and samples must be shipped overnight the day of or day after sampling. If any of these stipulations will be problematic, contact Amy.Goldman@pnnl.gov to discuss solutions.

Two people are needed to accomplish part of this protocol (see Figure 3). We recommend wearing safety glasses due to working with the liquid chemical RNAlater. The MSDS is included in the box.

MATERIALS

In addition to the sampling kit, you will need the following:	**The sampling kit should contain the following:**
1) Cooler with wet or blue ice for keeping samples cold in the field 2) Method for collecting latitude and longitude in decimal degrees in the field (smart phone is sufficient) 3) Method for taking pictures of the field site (smart phone is sufficient) 4) 1 long meter tape if the sampling sites are a long horizontal distance from the stream 5) 1 clean container (preferably glass*) for stream water (or DI water) to rinse tools, unless it's easy to rinse in the stream. 6) -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) 7) Refrigerator to store samples prior to shipping 8) Access to a FedEx office for shipping samples back overnight	1) 1 hard copy data sheet + pencil 2) 1 shipping label that is pre-filled for sending samples back via FedEx overnight 3) Freezer "blue ice" packs (place these in a -20C freezer for at least 48 hours prior to shipping) 4) 10 pairs of nitrile gloves to minimize contamination 5) 1 small tape measure 6) 10 Flags (to temporarily mark sediment sampling locations) 7) 2 foil-wrapped sterile sediment scoops 8) 2 foil-wrapped metal scoopulas to assist in collecting sediment from the sediment scoop 9) 20 50mL empty tubes 10) 10 25mL empty tubes 11) 6 50mL tubes containing RNAlater 12) String+level and extra string 13) 7 ziplocks, one for each iButton, plus more for containing sample tubes for shipment to PNNL

*Clean glass has preferably been acid washed and rinsed with DI water

OVERVIEW

This protocol describes the following activities: (1) identifying 10 sediment sampling locations along the transect previously marked with iButtons; (2) collecting metadata for the locations; (3) collecting sediment samples at each location; (4) retrieving the previously deployed iButtons; (5) reporting metadata online; and (6) shipping samples back to PNNL.

Please note that if you don't have iButtons for any reason or if there isn't enough sediment to get material from 10 locations along the transect, it is still useful to collect sediment. See the **Contingency Plan** section below.

SECTION 1: IDENTIFY SAMPLING LOCATIONS

After confirming that you have all of the needed gear, identify the five variably inundated locations used for the iButton deployments (Figure 1). They should be the locations marked as U, UM, M, DM, and D. Sediment will not be collected at the always inundated (IN) and never inundated (OUT) iButtons. 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 during low-flow conditions (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.

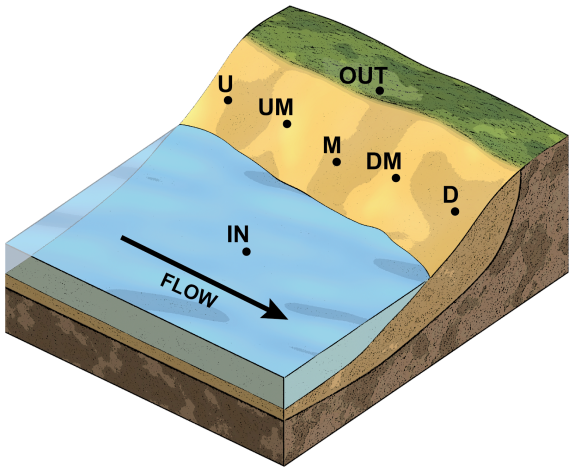

Figure 1. Layout of the idealized iButtons deployment. Sediment samples will be taken near the U, UM, M, DM, and D iButtons (see Figure 2)

Sediment will be collected from 10 sites that should each be ~1 meter away from one of the 5 iButton sensors. That is, sediments will be collected ~1m upstream of each iButton and ~1m downstream of each iButton, for two sediment locations per iButton site (Figure 2). More important than the exact distance from the iButton is sampling in depositional locations that are undisturbed, have fine sediment, **and that will have experienced the same inundation dynamics as the iButton**. This is vital for linking the iButton data to the sediment samples. For each sampling location, you will also estimate the vertical offset between the location and the water's edge using the string, level, and measuring tape provided in your kit (Figure 3).

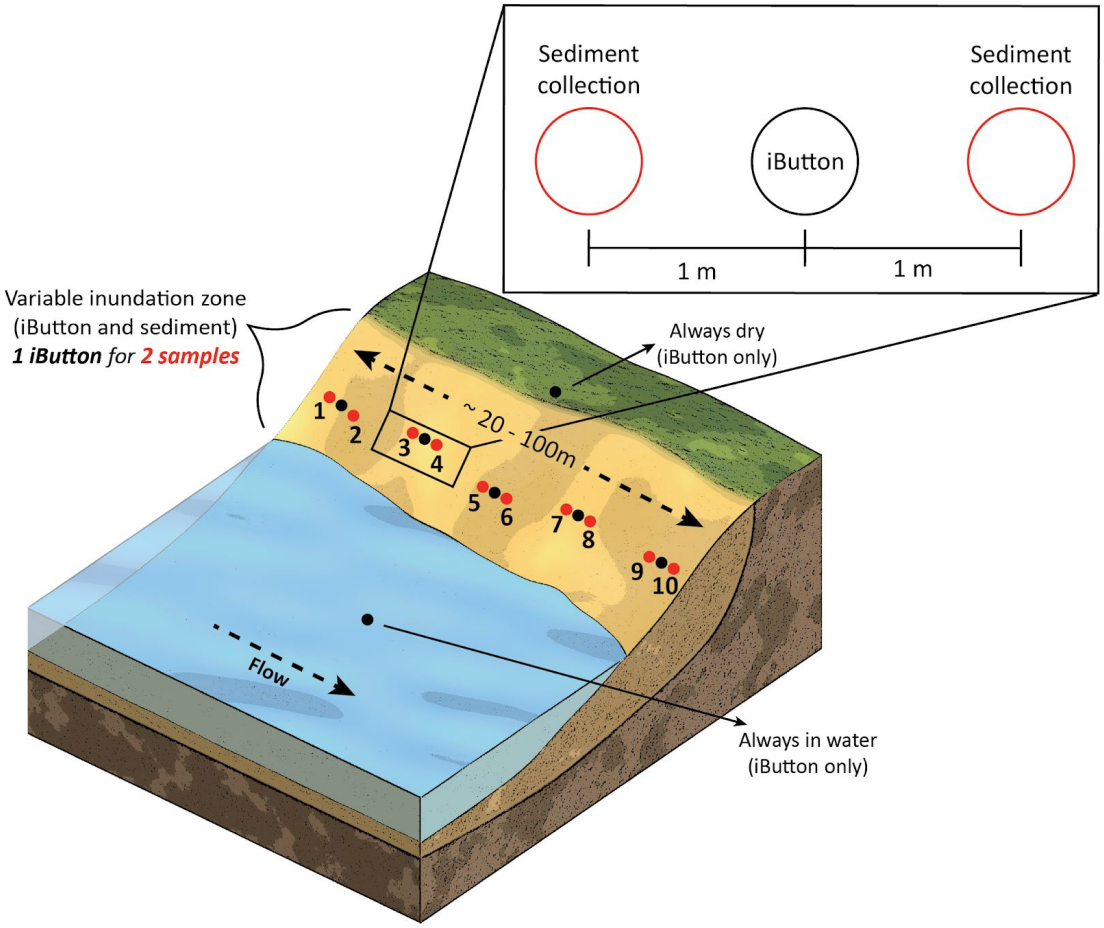

Figure 2: Overview of the sediment sampling locations. For each iButton there should be one sediment sampling location ~1m upstream and another sediment sampling location ~1m downstream of the iButton. More important than the exact distance from the iButton is sampling in depositional locations that have **fine** sediment **and that will have experienced the same inundation dynamics as the iButton**. This means that the location needs to be close to the iButton and at approximately the same elevation. If the sediment samples are taken further into the channel (relative to the iButton) or further up the bank (relative to the iButton), the data from the iButton will not be relevant to the collected sediment. If there is not a good sampling location near an iButton, please select the closest useful location and describe its spatial relationship to the iButton.

CONTINGENCY PLAN: WHAT TO DO IF YOU DON'T HAVE iBUTTONS OR THERE ISN'T ENOUGH SEDIMENT NEAR THE iBUTTONS

The #1 most important goal for the project is getting multiple sediment samples from each field site. Even if there is no iButton data, the project will make good use of the sediment samples. If you were unable to deploy iButtons or if there isn't enough sediment near your iButtons, it is still useful to collect sediment. In this case, please collect 10 sediment samples from depositional zones within 20-100m of your site's stream gauge/pressure sensor. Importantly, if you do not have any iButtons, please sample near the water line, but high enough above the water line so

that the sediments that are collected (at ~1-3cm depth) are above the water table (within the sediments) at time of collection. This will help to infer previous inundation dynamics using the stream gauge data. Even if you don't have any gauge/pressure data, it is still useful to collect the sediments. If you can't get all 10 samples, getting at least 3 would still be helpful. When collecting the sediments, keep the vertical offset between the sampling location and the water's edge as consistent as possible across sampling locations. And please make sure to measure the vertical offset for each sampling location (Figure 3).

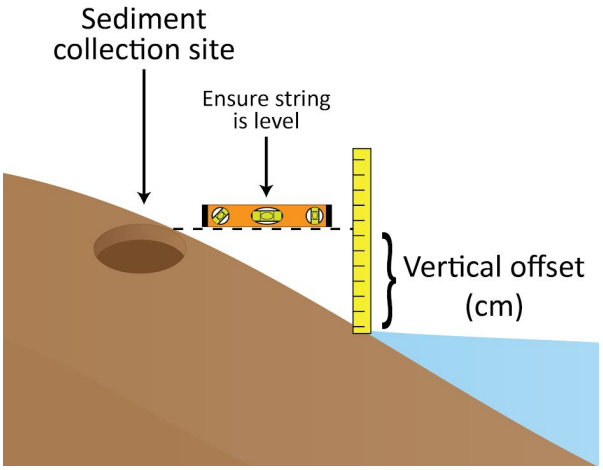

Figure 3: Idealized layout for measuring the vertical offset between the water's edge and the sediment sampling location. This should be done for each of the 10 sampling locations. It is critical to make sure the string (dashed line) is level. Please use the string, level, and measuring tape provided in the sampling kit. See Section 2 step 4 for instructions.

SECTION 2: COLLECT METADATA ON THE PROVIDED FORM: We strongly recommend doing all the metadata collection prior to collecting the sediments. This will generate better samples and be more efficient for your time. Your kit comes with two metadata forms. **You only need to fill out one of them**. One is for people who have deployed iButtons, and one is for people who were not able to deploy iButtons (for any reason).

1) When collecting metadata and sampling be sure to keep track of sampling locations, which are numbered 1-10 (Fig. 2). Site #1 should be the most upstream. Site #10 should be the most downstream. Mark your chosen sediment locations with the provided flags.

2) You will need to identify your site ID, which is the four digit number included on all of your sample tubes.

- The label structure for the white-capped 50ml tubes is "ECA2_####_##" in which #### indicates your unique site ID and ## indicates the locations (01-10) that you will be sampling at your site. The metadata form asks for the #### portion of the label, which will be the same on all of your tubes. The tubes also have -a or -b on them. These should be filled at the same location (PNNL will homogenize them later).
- The label structure for the 25ml tubes is "ECA2_####_##_SM" and for the green-capped RNAlater tubes is "ECA2_####_##_R." In both cases, #### is your same unique site ID and ## indicates the sampling locations (01-10). The green-capped tubes also have -1 or -2 on them. These should be filled at the same location (PNNL will homogenize them later).

3) For each of the 10 sampling locations record latitude and longitude in decimal degrees. You can use a GPS or a smartphone app such as ‘My GPS Coordinates’. If you have poor coverage for GPS measurements, you can instead provide a known set of coordinates for the location and then measure the distance between the sampling locations and the reference point. Cross out and write new text as needed on the metadata sheet if you do this. For those who never deployed iButtons, you will also record the latitude and longitude of the stream gauge.

4) Record the distance from each sampling location to the nearest iButton. The metadata sheet is set up for the ideal layout. If your layout deviates, cross out needed components on the form’s diagram and make sure to indicate which iButton is closest to the sampling location whether the sampling location is upstream or downstream from the iButton, and make sure to record the sample number along with the distance and iButton information. This is vital to link each sediment sample to temperature data from the nearest iButton. If you do not have iButtons, record the distance between the sampling locations instead.

5a) At each sediment sampling location, **if the location is not inundated (i.e., not under water)**: use the provided string, level, and measuring tape to estimate the vertical offset (in cm) between the water’s edge and the riverbed surface at the sampling location (see Figure 3). Also measure the horizontal distance from the water’s edge to the sampling location. If the locations are far from the water’s edge, measure the horizontal distance and vertical offset as best you can, but please provide a written comment about the level of uncertainty. If needed, tie the multiple lengths of string together. To measure the vertical offset (Figure 3),

1. One person holds one end of the string without the level at the sampling location against the sediment.
2. A second person extends the measuring tape and places the end of it at the water’s edge so that it extends vertically to the sky.
3. The person holding the measuring tape takes the other end of the string with the level and places the end of the string against the measuring tape. **It is critical to pull the string tight so it doesn’t droop/sag**.
4. The second person adjusts the string up and down along the measuring tape until the level’s bubble is exactly between the two lines.
5. The person at the measuring tape records the point on the measuring tape (in centimeters) at which the string is level. This distance is the vertical offset between the sampling location/string and the water’s edge.
6. After recording the value, extend the measuring tape from the water’s edge to the sampling location, ensure it is level, and record the horizontal distance.

5b) At each sediment sampling location, **if the location is inundated**, ignore step 5a and measure the water depth where sediments will be collected and record it as a **negative value** on the datasheet in cm. Then measure the horizontal distance from the water’s edge to the sampling location and record it as a **negative value**.

6) Repeat step 5a/5b for the 5 variably inundated iButtons (U, UM, M, D, DM) to capture vertical and horizontal distance from the water line.

7) With a smartphone or camera, take a photo looking down on the **undisturbed** sediment at each sampling location. Ensure a measuring tape extended to 30 cm is visible in each of the photos. It is not always possible to read the measuring tape in photos, so please ensure it is extended to exactly 30cm. Note that these will be uploaded along with the metadata. Record which picture goes with which site so that you can label them appropriately when you upload them.

8) At the midstream sampling location **only**, take the following photos (note these will be uploaded along with metadata). For the last two pictures (b, c), 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.

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

9) 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.

SECTION 3: COLLECT SEDIMENT

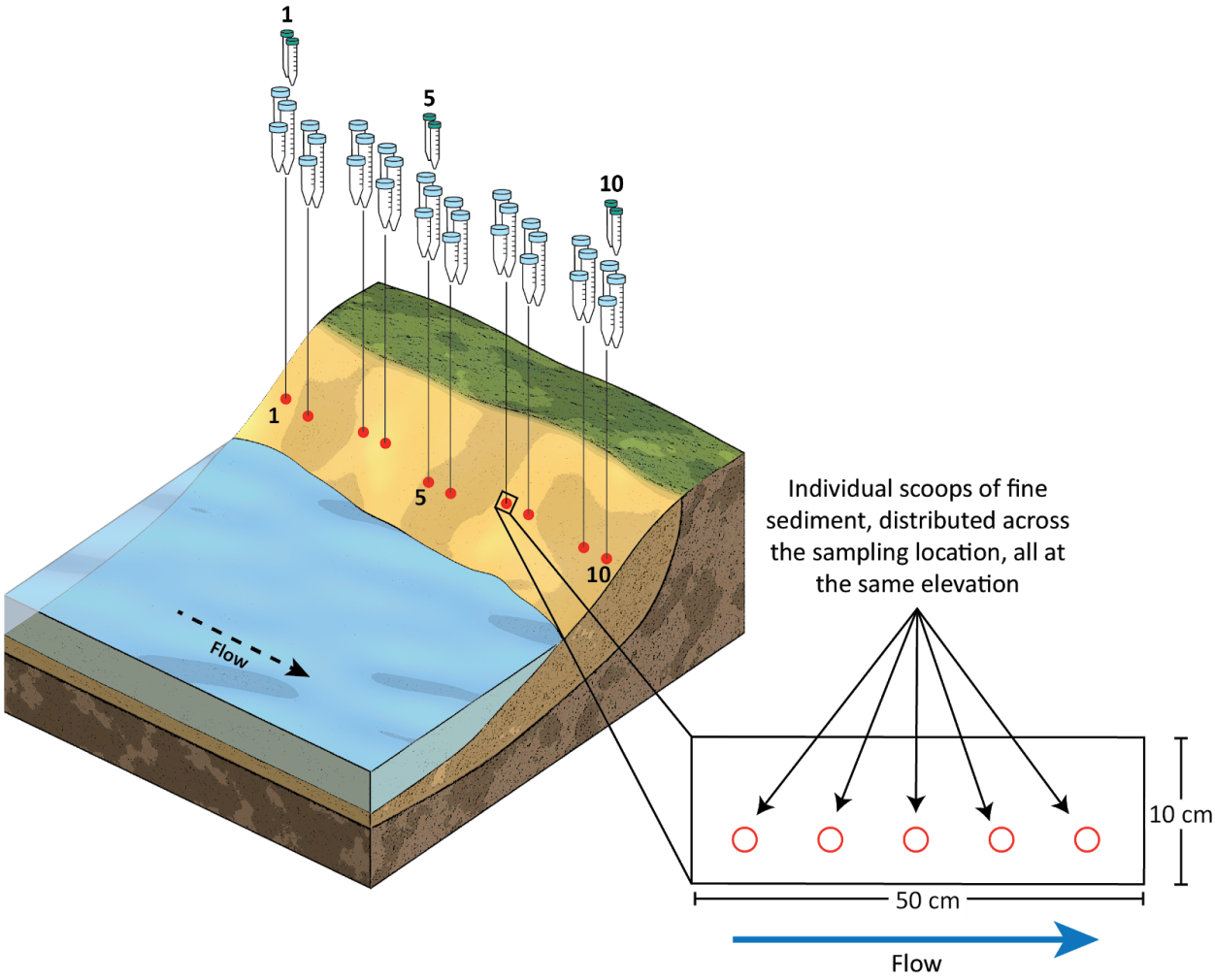

Figure 4. There are two components to the sediment sampling. At all 10 locations, undisturbed sediment will be collected to fill two empty 50ml tubes and one 25ml tube. In addition, at the most upstream (#1), most downstream (#10), and midpoint locations(#5), two additional tubes will be used to collect sediment into RNAlater (clear liquid) to preserve microbial communities. It is vital that only 7 ml of sediment be placed in each tube that has RNAlater to keep a high RNAlater-to-sediment ratio. In all cases, please try to combine sediment from 3-5 different spots within each sampling location (i.e., scoop sediment multiple times at each location). **In all cases it is critical that you do not touch the sampling parts of the scoop/scoopula or the inside of the tubes with anything but the scoop/scoopula (i.e., no gloved fingers, etc.). Touching surfaces that samples contact will contaminate the sample.**

1) Refer to Figure 4 for the idealized sampling scheme. We strongly recommend doing all the metadata collection prior to collecting the sediments - except for the measurement of sediment collection depth, which requires that you have already collected the sediment. This will keep tools and gloves as clean as possible (to minimize contamination) and be more efficient (as opposed to switching back and forth between tasks).

2) Starting at the most downstream location (#10), put on a new pair of nitrile gloves. Only the person/people scooping sediment need(s) to wear gloves. It may be easier for two people to collect sediment. We have provided enough materials for that option. Change the gloves every couple locations to avoid cross contamination and **do not touch the sediments being sampled or the parts of the scoops and scoopula being used to collect sediments**.

3) At the most downstream location (#10), midpoint location (#5), and most upstream location (#1):

a) Remove large debris from the sediment sampling locations (>4mm). Remove large cobbles that make up an armored layer, if applicable, to reach finer sediments.

b) Locate the two empty 50ml and one 25ml empty tubes (white caps) prelabeled for the location you are sampling (e.g., #10 for the most downstream; see Fig. 2). Also locate the 2 tubes with RNAlater (clear liquid, green caps) that have the same location number (these are only for locations #10, #5, and #1). It is vital to ensure the right sample number is used for each location. See section 2 for a description of the labeling scheme. When you receive the kit, the tubes should be bundled by location, but please verify each label before use.

c) Locate the 30ml metal scoop and metal scoopula wrapped in foil. There will be two of these packages. One package per person if two people decide to collect sediment. Each contains one scoop and one scoopula. Unwrap the tools; ensure you only touch the scoop/ula handles and not the spoons (the same tools will be used for all sediment sites). Rinse both the scoop and scoopula in stream water or DI water prior to sampling. If you need to put either item down during sampling, place them on the clean side of the foil.

d) Use the scoop to collect sediments from ~1-3 cm depth. It's okay to sample deeper (down to ~10cm) to access finer sediments. The metadata sheet has a location to write collection depth after you complete sampling. Sampling should avoid large debris greater than ~4 mm to allow for the collection of enough sediment for analysis (after receiving your samples, PNNL will sieve and analyze the <2mm fraction). If possible, collect from at least 3-5 spots within the sampling location to get a representative sample (Figure 4). Keep the spots close together (within ~30cm of each other) and at a consistent elevation so they would have experienced the same inundation dynamics. As needed, use the scoopula to push large rocks out of the scoop and push sediments from the scoop into the tube. Do not use your hands for this. If there is enough sediment at each site to collect all samples according to the idealized sampling scheme, distribute sediment from the 3-5 spots into the tubes following the guidance in Table 1. If there is not enough sediment to follow the idealized sampling scheme, see step 3e.

Table 1. Description of samples and collection guidance when there is enough sediment at each location to collect all samples according to the idealized sampling scheme.

	50ml white cap	**50ml green cap with clear liquid**	**25ml white cap**
Quantity of tubes	2 (all locations)	2 (locations #1, #5, #10)	1 (all locations)
Volume to collect	Fill to sharpie line (~40ml)	Fill to sharpie line (~7ml). *Overfilling will make samples unusable*	Fill completely
Special considerations		Ensure sediment drops down into the liquid. Do not stick scoopula down inside the tube to scrape sediment down into the RNALater. Rotate or tap the tube to accomplish this if needed.	
Fill all tubes simultaneously at a given location so that each tube gets a little bit from each of the 3-5 scoops (Fig. 4).			

e) **If a given location may not have enough sediment for all samples to be collected according to the idealized sampling scheme,**

- First fill one 50ml empty tube and then the 25ml empty tube. They are the highest priority. If you have sediment available at a given location, continue filling the other empty 50ml tube. If there is enough sediment for *both* the RNAlater tubes, collect those samples. If there is enough sediment for only one RNAlater tube, move those tubes to another location. Do not collect sediment into only one RNAlater tube. Both tubes are needed from a single location.
- Assess if you have enough sediment for all five of the tubes at a different location so that you can shift the RNAlater tubes to that location. **If yes,** change the labels on the RNAlater tubes to indicate which location was sampled (e.g., location #6 instead of location #5). Also indicate this change on the hardcopy metadata form. The pair of RNAlater tubes should be kept together. Do not fill one at the originally indicated location and one at the new location.

f) Samples should go immediately into a cooler with blue or wet ice. **Do not freeze any of the samples.**

g) After sampling a location, rinse the scoop and scoopula in stream or DI water to reduce cross-site contamination. If the stream is close, just walk over and rinse the tools in the stream. **Don't wipe the tools on anything as that will likely introduce contamination.** If the stream is too far, use a container of water. The water can be from

the stream or DI. Ideally this will be an acid-washed clean glass container to avoid organic and/or microbial contamination.

4) At all other locations (i.e., #2, #3, #4, #6, #7, #8, #9):
   a) Everything is the same as described above in step 3 except that there are no tubes with RNAlater. That is, only sample sediments into the three white-capped empty tubes. The only exception is if you need to move RNAlater tubes to one of these locations due to insufficient sediment at locations #1, #5, or #10.

5) Fill out the sediment collection depth fields on the metadata sheet. Estimate the average depth of your collections (bottom of hole to riverbed surface) at each location and record this number. There isn't a need to spend time formally calculating mean depth, just quickly estimate the average as best you can.

SECTION 4: COLLECT iBUTTONS

1) After collecting sediments, collect all the iButtons.

2) Please don't remove iButtons from their housings or modify them in any way. Simply place each iButton (with its housing) in the appropriate ziplock. That is, each ziplock is labeled; please make sure the right iButton goes into the right ziplock. If you are not sure which iButton is which because some have been lost and the sharpie has worn away, flip over the iButton and read the label stamped into the metal along the underside of the rim.

3) Package the iButtons with the samples for refrigerated storage. They will be shipped separately from the samples.

TRANSPORT AND STORE SAMPLES AFTER COLLECTION

After all samples and iButtons are collected, transport both samples and iButtons to a refrigerator, keeping them (including the iButtons) on wet or blue ice during transit:

1) Once back to the lab, place both samples and iButtons in a **refrigerator**. Please don't use a freezer as this will render samples unusable. Putting the iButtons in the refrigerator, along with the samples, provides very useful data on sample temperature during transit. Ensure all sample lids are tightened.

SECTION 5: METADATA ENTRY

1) After the samples are in the refrigerator, please enter metadata into the digital metadata form. If you had iButtons, use the form at **https://tinyurl.com/ECAsed1**. If you did not have iButtons, use the form at **https://tinyurl.com/ECAsed2**. If there is difficulty in accessing the form, please email Amy.Goldman@pnnl.gov for a copy of the form.

2) Please enter as much information as possible. The form indicates what information is required and what is optional.

3) The form also provides instructions on how to submit the field photos and the stream gauge data. Use your site ID/first part of the tube labels in the first part of all file names (e.g., ECA2_0022). This was the number you put on the top portion of the metadata form. Please name all files with the Sampling kit ID as follows (using your unique 4 digit site ID in place of 0022 in this example):

- ECA2_0022-across (photo looking across stream at midstream [#5] sediment sampling location)
- ECA2_0022-up (photo looking upstream at midstream [#5] sediment sampling location)
- ECA2_0022-down (photo looking downstream at midstream [#5] sediment sampling location)
- ECA2_0022-sed-XX (photo of undisturbed sediments to be sampled at a given location, looking down at the sediments. Replace 'XX' with the location number. For example, use the value of 01 for the most upstream location as shown in Fig. 2. There should be 10 of these photos, each with the location number at the end of the name.)
- ECA2_0022-data1 and ECA2_0022-data2 (photo of the data sheet, front and back, taken after sampling is complete)

4) For the stream gauge data, if the closest stream gauge provides downloadable real-time data, provide the url in the metadata form. If the data are not available online, use the online metadata form or contact Amy.Goldman@pnnl.gov for instructions to submit data files.

SECTION 6: SHIPPING SAMPLES AND iBUTTONS

1) All shipments should be stored in the refrigerator until they are **shipped on** a **Monday, Tuesday, or Wednesday**. It is critical that overnight shipments be made on one of these days. Please **don't ship later in the week**. This is important in case there are shipping delays. Contact Amy.Goldman@pnnl.gov *before* you sample if this is not possible. **It is vital to ship within 24 hours following sampling.**

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're ready to ship, prepare to package the items into two boxes. If you have your original iButton box, please use it for the iButtons. If you do not, please use any box you have available. You can pick one up for free at FedEx.

4) In the sediment kit sampling box

1. Place some ice packs into the box, on top of the provided absorbent padding
2. Put the **50ml** tubes into the provided ziplocks and then place them on top of the ice packs. All sample tubes must be inside of a ziplock.
3. Place the remaining ice packs on top of and around the 50ml sample tubes. The most important component is having the 50ml tubes as cold as possible.
4. Pack the 25ml tubes in the remaining space. Their temperature is not as critical (i.e., they don't need to be surrounded by ice packs).
5. If there is extra space, please fill it with other sampling supplies just so the tubes stay in place.

5) Put the lid of the cooler on, fill out the field on the paper metadata sheet for the time and date the box is packed, and then place the paper metadata sheet on top of the lid of the cooler. This will keep it dry.

6) Tape the outer box closed. Please use enough packing tape to make sure it won't come open during shipping.

7) In the iButton box or the box you have provided,

1. Place the ziplocks with iButtons into the box
2. Also include the tools from the sediment box, including the measuring tape, scoops, scoopulas, and level. Please **don't return** gloves.

8) Tape the iButton box closed. Please use enough packing tape to make sure it won't come open during shipping.

9) Adhere the pre-filled shipping labels (provided in the kit) to the outside of the boxes by removing the backing from the plastic sleeves that contains the shipping labels. If your site is USDA regulated (certain states and Puerto Rico), you will have additional instructions required for the box containing sediment samples. **It is critical you follow the instructions for the USDA APHIS permit. If your site is USDA APHIS regulated a copy of the receiving permit will be included in your kit in a plastic sleeve ready for return shipping. The permit will need to be adhered to the outside of the box along with the pre-filled shipping label. If you have any questions at all, contact Amy.Goldman@pnnl.gov and Sarah.Fansler@pnnl.gov.**

10) Drop the packages off at FedEx or have them picked up by FedEx.

11) **On the same day you ship the packages, notify Amy.Goldman@pnnl.gov that you shipped the package, and include the FedEx tracking number in your email. This is critical to ensure sample integrity and timely delivery.** The subject line of the

email should be “SHIPPED SAMPLES – [fill here with sample site ID such as ECA2_0022]”. 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.

REFERENCES:

Jensen, B. 2019. AOS Protocol AND Procedure: Sediment Chemistry Sampling in Wadeable Streams. NEON.DOC.001193, Rev. G.

APPENDIX:

If you have contacted us to ask about sieving your samples in the field to ensure you get enough sediment, please remember the following:

- This is not a required step. This is entirely optional.
- We initially clean our sieves with hydrogen peroxide and then rinse with DI water. Between sites, we suggest rinsing with stream water or DI water.
- Never put your hands into the sieve - with or without gloves. Only use the clean metal tools we have provided to push material through the sieve. Anything else will introduce contamination.
- Do not dry the sieves with any material. It will introduce contamination. Just shake off as much water as possible.