**Flow Proportional Composite Sampling ProcedureS**

1. Record information from autosampler display (see attached Sample Retrieval Form), and stop the sampling program by pressing the red button to pause the program and then selecting STOP PROGRAM.
2. Record approximate sample volumes in each carboy.
3. Select the appropriate carboy(s). Carboy 1 should contain a minimum of 300 mL for sample splits to be prepared for analysis.
4. Fill out and affix labels to the appropriate containers. The correct container for each analyte is given in Table 1.

*Table 1. Sample containers, preservation, and permissible holding times*

| **Analyte** | **Container** | **Preservation** | **Hold Time (days)** |
| --- | --- | --- | --- |
| TP | 60-mL glass vial | None | 28 |
| TDP | 60-mL glass vial | Filtered (0.45 µm) in field | 28 |
| TN | 50-mL plastic centrifuge tube, blue cap | Cool (<6°C), 0.1 mL H2SO4 | 28 |

The Sample ID field is a concatenation of the Site ID (JBT01, JBT02, etc.), the collection date (mmddyy), and the carboy(s) from which sample splits are taken [1, 2, 3, 4, or 1+2, 3+4 (if the samples from multiple carboys are added together in the churn splitter)]. See step 5 regarding the sample splitting procedure. The following examples illustrate the sample IDs syntax:

* A sample collected at JBT01 on May 2, 2017 only from carboy 1: **JBT01-050217-1**
* A sample collected at JBT02 on September 27, 2017 by combining the contents of carboys 1 and 2 in the churn splitter: **JBT02-092717-1+2**

1. Put on lab gloves and pour sample from the selected carboy(s) into the churn splitter. Try to swirl the water to suspend sediment as you pour the sample into the churn splitter.

In many cases, only the first carboy will contain sample. If the second carboy also contains sample, this can be added to the churn splitter so long as the combined volume will not exceed 14 liters, the capacity of the churn splitter. For example, if carboy 1 contains 9 liters and carboy 2 contains 4 liters, these can be composited in the churn splitter; and the resulting sample ID would be in the form: SiteID-mmddyy-1+2. If the combined volume will exceed 14 L, each carboy should be split individually, resulting in two sets of sample splits for analysis.

1. Operate the churn splitter for 5-10 seconds. With sample containers in hand, open the stopcock and let spill on the ground for 1-2 seconds to clear the line. Then prepare:
   1. TP sample split: While operating the churn splitter, fill the glass vial up to the line.
   2. TN sample split: While operating the churn splitter, fill a blue capped centrifuge tube to the 50 mL line.
   3. Let the contents of the churn splitter settle for 1-5 minutes.
   4. TDP sample split: Sample splits for TDP analyses will be filtered in the field by dispensing sample from the churn splitter directly into a filtration apparatus containing a 45-mm Durapore® 0.45-µm acetate membrane filter. Use forceps to place a clean filter in the filter holder. Wet the filter with a spray of distilled water. Remove the plunger and attach the filter holder to the syringe. Fill a syringe with settled water from the churn splitter. Squirt approximately 10 mL onto the ground and then fill a glass vial to the 50-mL line. If the filter clogs prematurely, it may be replaced with a new filter and the process repeated.
2. Preservation. Put on safety glasses. Add 1 drop of concentrated sulfuric acid to preserve the TN sample. Place all samples on ice and store on ice or refrigerate until delivery to the laboratory. Clean up acid spills with acid neutralizing solution or copious amounts of water. To use acid neutralizing solution, shake bottle of acid neutralizing solution and cover affected area until bubbling stops.
3. Washing equipment. The standard washing procedure is for three rinses with distilled water. After each event, the churn splitter, filter holder, and carboys should be washed.
4. Reinstall carboys in the following clock positions: 1 at 6:00, 2 at 3:00, 3 at 12h, and 4 at 9:00.
5. Press the red button and select “run program” on the autosampler to ready the station for the next event. Confirm that the sampler program is running.
6. Complete the Chain of Custody form, including sample IDs, number of containers of each sample being sent to the lab, and the analyses to be performed. The Chain of Custody form must be kept with the samples, either by sticking it into the plastic sleeve taped to the underside of the cooler lid or in a ziplock bag with the samples.
7. Samples must be delivered to the laboratory within the holding times indicated in Table 1.

**Grab Sampling ProcedureS**

The autosampler programs will be stopped during the winter months when temperatures are expected to remain below freezing. During this period, field technicians will visit each station approximately weekly to collect grab samples if tile lines are flowing.

1. Fill out and affix labels to the appropriate containers. The correct container for each analyte is given in Table 1.
2. For grab samples, the Sample ID field is a concatenation of the Site ID (JBT01, JBT02, etc.), the collection date (mmddyy), and the word “GRAB”. The following example illustrate the sample IDs syntax:

* A grab sample collected at JBT01 on February 2, 2017: **JBT01-020217-GRAB**

1. Grab sample collection.
   1. Put on lab gloves
   2. If the air temperature is above freezing:
      1. Collect samples for TP and TN analysis directly into the sample container. The preferred method is to use the autosampler to pump a sample directly into the sample container, using the manual sample mode. The autosampler pump tubing should be detached from the autosampler housing and a stream of water directed into the sample container. Set the sample volume to 200 mL and dispense the first approximately 5 pump cycles (50 mL) onto the ground, then collect sample up to the fill line on the sample container.
      2. Samples for TDP analysis may be dispensed directly into the filtration apparatus containing a 45-mm Durapore® 0.45-µm acetate membrane filter. Use forceps to place a clean filter in the filter holder. Wet the filter with a spray of distilled water. Remove the plunger and attach the filter holder to the syringe. Use the autosampler to pump sample into the syringe, using the manual sample mode. The autosampler pump tubing should be detached from the autosampler housing and a stream of water directed into the syringe. Set the sample volume to 200 mL and dispense the first approximately 5 pump cycles (50 mL) onto the ground, then collect appromately 60 mL of sample in the syringe. Squirt approximately 10 mL onto the ground and then fill a glass vial to the 50-mL. If the filter clogs prematurely, it may be replaced with a new filter and the process repeated.
   3. If the air temperature is below freezing:
      1. The autosampler may be damaged by ice accumulation. If the tile line continues flowing under freezing conditions, grab samples may be withdrawn using a portable centrifugal pump inserted into the flow metering chamber via a sampling port. Using this pump, sample should be dispensed directly into the sample containers, dispensing the first approximately 50 mL onto the ground, then collecting sample up to the fill line on the sample containers.
      2. Because field filtration is not generally successful under freezing conditions, grab samples collected for TDP analysis will be filtered at VAEL. In this case, TDP samples must be brought to VAEL for processing on the day of collection.
2. Preservation. Put on safety glasses. Add 1 drop of concentrated sulfuric acid to preserve the TN sample. Place all samples on ice and store on ice or refrigerate until delivery to the laboratory. Clean up acid spills with acid neutralizing solution or copious amounts of water. To use acid neutralizing solution, shake bottle of acid neutralizing solution and cover affected area until bubbling stops.
3. The filter holder and syringe should be washed by rinsing three times with distilled water after sampling at each station.
4. Complete the Chain of Custody form, including sample IDs, number of containers of each sample being sent to the lab, and the analyses to be performed. The Chain of Custody form must be kept with the samples, either by sticking it into the plastic sleeve taped to the underside of the cooler lid or in a ziplock bag with the samples.
5. Samples must be delivered to the laboratory within the holding times indicated in Table 1.

**Routine Maintenance**

**Tasks to be performed by sampler after each sampling event**

1. On the Sample Retrieval Form, record the amount of rainfall collected in any manual gauges and the date and time. Record the amount of rainfall collected in the graduated cylinder to the nearest 0.01 inch then empty it. If water is present in the outer (overflow) cylinder, carefully decant this into the graduated cylinder and add this amount to the first reading. Repeat if necessary until the overflow cylinder is empty.
2. Confirm that the sampler program is running.
3. Confirm that the sampling line and pump tubing are attached.
4. Confirm that the sample carboys are installed properly.
5. Describe field/crop condition.
6. Verify that sufficient sampling supplies (bottles, filters, gloves) remain for at least two sampling events. Notify the Stone project manager if any supplies are low.