

Sassafras Samplers Monitoring Program

Standard Operating Procedures

1.0 Introduction

The Standard Operating Procedures (SOP) outlined in this manual are designed to help guide and sustain a high quality water monitoring program. The procedures are utilized by the Science Committee, Riverkeeper and volunteers to assure that each individual or sampling crew follow the same protocols. Quality assurance in a monitoring program is essential for providing representative and accurate data for a water body, which is vital for the continuation of a monitoring program.

The SOP's are listed by section, with added instructions for safety and tips on keeping up proper maintenance of testing equipment. All volunteers are to follow these guidelines and procedures to ensure reliable, high quality data. The Science Committee is to use the SOP as a tool for training new volunteers during an initial training class and during coordinated periodic training sessions.

2.0 Safety

Safety to staff and volunteers is of the utmost importance to the Sassafras River Association. There are hazards in both the field and the lab, so please use caution. For instance, during inclement weather conditions, the sampling site may become a hazard due to wet and/or slippery conditions, high winds, or torrential water that can be dangerous to the individuals sampling. It is also recommended that more than one person be on site in case of an emergency. If at any time a volunteer feels that unsafe conditions exist, they are advised to terminate that activity immediately.

The tests that are performed on water samples require the use of reagents that can become hazardous if handled incorrectly. Follow basic handling procedures such as washing hands before and after use, using appropriate cleaning materials for spills, disposing of wastes properly and supervising young children that may come in close contact with reagents or their containers.

3.0 Testing Time Frame

The time frame for collecting water samples and performing tests is important for maintaining a quality monitoring program. When samples are collected from all sites during the same time period, it allows for the comparability of data across the watershed. Schedules are also crucial in maintaining coordination between the Science Committee and volunteers.

3.1 Non-Tidal Sampling

In non-tidal monitoring sites, it is preferable to sample between 10:00 AM and 2:00 PM. This is the time period of greatest activity of aquatic species and will also provide coordination to allow for comparisons to be made from site to site.

3.2 Sample Period

SRA understands that volunteers are not paid employees and may have prior or more urgent commitments. As a result, SRA has established a testing window. The testing window is from Friday through Monday on the second full weekend of every month.

Site Number	Site Description
NT1	Loyd's Creek Bloomfield Rd.
NT4	Dyer Creek West Starkey Farm Rd.
NT5	Island Creek East Starkey Farm
NT6	Mill Creek Rt. 290
NT7	Swantown Creek Rt. 290
NT10	Jacob's Creek Massey Rd.
NT11	Jacob's Creek Rt. 290
NT12	Mill Pond Rt. 299
NT13	Sassafras River Rt. 299
NT18	Hall Creek Sandy Bottom Rd.
NT19	Dowdel Creek Entrance Indian Acres
NT21	Hen Island Creek Shumaker's
NT22	Duffy Creek Wards Hill Rd.
NT23	Duffy Creek Wards Hill Rd.
NT24	Unnamed Branch Rt. 301
NT25	Unnamed Branch Edgar Price Rd.

4.0 Field Sampling

The Sassafras Samplers Monitoring program is divided into two types of activities: field collection and lab testing. This section will cover procedures that are necessary to provide accurate, high quality data that represents the body of water being sampled.

There are some basic techniques for collecting water samples that are outlined in the following sections of this manual. Training will be provided prior to the April sampling weekend with the Science Committee or qualified trainers, and then periodically thereafter. Volunteers may request further training at any time.

4.1 Prior to First Sampling

1. Label all necessary dissolved oxygen and plastic sample bottles. Three dissolved oxygen and 1 plastic sample bottle will be needed per sampling site.

4.2 On-Site

The water samples collected will be used to quantify the level of contamination or loading of pollutants (e.g., nutrients), to the Sassafras River and its tributaries. Therefore, it is imperative that samples are collected following protocols to accurately represent the body of water being sampled. Prior thought has already been used in locating each monitoring site. Sites have been located at bridge crossings or areas that have access to the stream. At each stream crossing, the water flowing past the sampling point is confined, allowing a representative sample from upstream areas to be collected. When locating the proper position or placement of the sampling container and during sample collection, follow these general rules:

- Safety comes first - if it looks unsafe, do not attempt collecting a sample.
- If possible, sample upstream of road crossing.
- If possible, look for a part of the stream that is flowing.
- If the entire width of the stream is flowing at the same rate, then sample anywhere within that span of the stream.
- Avoid sampling behind an obstruction in the water column.
- Avoid disturbance of the stream bed, which could lead to inaccurate readings.
- Avoid sampling in non-flowing pooled or stagnant areas of the stream.
- Avoid disturbance of the area that you are standing on and depositing any materials that may influence results.

1. Rinse water sampling body with sample water prior to collecting the dissolved oxygen samples.
2. Collect and fix the three dissolved oxygen samples.
3. Collect the sample to be used for all subsequent testing in the plastic squeeze bottle.
4. Record the air temperature at the sampling site and water temperature from the sample. Measure the water sample temperature quickly upon collection as this temperature can change rapidly in the bottle.

4.2.1 First Sampling

1. During the first sampling, evaluate the sampling site and determine the sampling depth. Collect samples from 8 inches below the water surface, if possible, by lowering the water sampler to a depth with the water just above the highest knot.
2. If visiting a new site that has not been sampled in subsequent years, record GPS coordinates in latitude and longitude using hand-held GPS unit provided by SRA.

4.3 Sample Preparation for Transport

To acquire accurate readings for each water quality parameter, proper handling preparation of the water sample is critical. The chemistry of water is primarily influenced by temperature. Temperature increases or decreases the reactions of chemical compounds that are commonly found in natural waters. Dissolved oxygen is a priority parameter and directly relates to changes in temperature. If temperature increases, then the ability of the water to hold oxygen is reduced and therefore invalid results will occur. That is why this sample must be fixed on site. Other parameters that are being tested, such as Ammonia-Nitrogen, require temperatures to remain cool and testing within six hours of collection. In order to maintain the quality of the sample, keep the sample container on ice.

4.4 Sampling Kit Contents

4.4.1 Field Kit (black tool box)

- Water Sampler Body, orange cap, and weight
- Rope with clip
- Plastic sample bottle (for reagent tests)
- Three glass sample bottles (for D.O. test)
- Armored thermometer

4.3.2 Smart or Smart II colorimeter lab box

- SMART Colorimeter and power plug
- 6 test vials (10ml mark)

4.4.3 White Bucket

- D.O. fixing chemicals
 - Manganous Sulfate (white cap, pink fluid)
 - Alk Pot Iodide Azide (white cap, blue wrapper, clear fluid)
 - Sulfuric Acid (red cap)
- D.O. Titration
 - Sodium Thiosulfate
 - Starch Ind
 - Titrator ((0377) looks like a syringe)
 - Titration Tube (25 ml – 0608)
- Phenol Red pH bottle with 0.5 ml dropper
- NO₃ test cube (3649-SC)
- PO₄ test cube (3653-SC)
- AM test cube (3642-SC)

4.4.4. Other

- D.O. titrating instructions
- Laminated Field/lab instructions
- Owners manual and test procedures
- Data Sheets/Log Sheets
- Kitchen Timer
- Four additional plastic squeeze bottles
- Six additional Dissolved Oxygen glass bottles
- Handheld GPS

4.5 General Field Sampling Method

1. Record basic site information on the data sheet prior to sampling (e.g., time and date).
2. Collect water sample from eight inches below the surface. Be careful not to disturb the bottom if in shallow water.
3. The Rule of Three: The rule of three is simply a process that one should use in the field, in the lab, during clean-up, and when preparing a sample for testing. In the field, always rinse out the water sampler body as well as the DO bottles and plastic squeeze bottle with water from the site. Using the sampler, collect water from the site, shake and dump to be sure there are no remnants from the previous site. Take another sample, and using this water add a small amount to each DO bottle and the plastic bottle, shake and discard water downstream of the site. Add some more water from the sampler to the DO bottles and plastic bottle and repeat twice. There is no need to dip the sampler two more times, just make sure each bottle has been rinsed out three times using the water from the sampler's second dip. This process will ensure that carry over from the previous sample will have no effect on the new sample.
4. Suspend a dry thermometer in shade to record air temperature.
5. Now collect a sample using the sampler and fix 3 distinct dissolved oxygen samples according to specific kit instructions.
6. Pour excess sample water from dissolved oxygen sampler into plastic sample bottle.
7. Collect another sample if insufficient to fill plastic sample bottle.
8. Record air temperature; then place thermometer in plastic sample bottle or dissolved oxygen sampler with sample water; record water temperature.

9. Make any general observations: (Note any unusual things such as: moon phase, water level, animal activity, surface phenomena, recent rain activity, weather condition).
10. Look for life and signs of changes: e.g. ducks, geese, sea nettles, crabs, minnows. Give numbers or estimates when possible.

4.6 Specific Sampling Procedures

4.6.1 Dissolved Oxygen Sampler

This device is designed for use in the field and is a simplified water sampler. The sample is collected in a removable inner bottle which is overflowed 5 times to insure a representative sample. Samples may be taken at a controlled depth by using a calibrated line. Attaching a weight to the bottom of the sampling device insures rapid descent and minimizes the amount of drift due to currents. More weight should be attached to the sampling device in strong currents.

It is necessary to maintain a position directly over the water sampling body when lowering it so that it remains in an upright position. This permits the displacement of all of the air in the sampler so that it will fill completely. As it fills, bubbles of air displaced from the sampler will be observed downstream.

1. Remove the plastic center plug with inlet tubing attached.
2. Insert the collecting bottle with the cap removed, into the inner chamber of the cylinder.
3. Replace the plastic center plug and make sure the inlet tubing is in the collecting bottle.
4. Attach a weight to the bottom bridle of the sampler.
5. Attach the snap clamp on the calibrated line to the bridle on top of the sampler.
6. Quickly lower the water sampler to the desired depth and leave until full. This can be determined when the bubbles from the displaced air in the sampler cease to appear. This usually takes from 3-5 minutes.
7. Carefully retrieve the water sampler.
8. Remove the plastic center plug to expose the collecting bottle in the inner chamber.
9. Remove DO bottle and proceed to fixing instructions.
10. Pour excess water from the sampler into the plastic squeeze bottle provided. Make sure that there is no air space in the sample bottle.

4.6.2 "Fixing" Dissolved Oxygen Sample

1. Tap the sides of the submerged bottle to dislodge any air bubbles clinging to the inside.
2. Once a satisfactory sample has been collected, proceed immediately with the next steps, to "fix" the sample.
3. Be careful not to introduce air into the sample while adding the reagents. Simply drop the reagents into sample, holding the reagent bottles vertically.
4. Add 8 drops of Manganous Sulfate Solution (4167) and 8 drops of Alkaline Potassium Iodide Azide (7166).
5. Cap and mix by inverting several times. A precipitate will form. Allow the precipitate to settle below the shoulder of the bottle before proceeding.
6. Add 8 drops of Sulfuric Acid.
7. Cap and gently shake until the reagent and the precipitate have dissolved. A clear-yellow to brown-orange color will develop, depending on the oxygen content of the sample.

Following the completion of Step 7, contact between the water sample and the atmosphere will not affect the test result. Once the sample has been "fixed" in this manner, it is not necessary to perform the actual

test procedure immediately. Thus, several samples can be collected and "fixed" in the field, and then carried back to a testing station or laboratory where the titration procedure is to be performed within 6 hours.

4.6.3 Temperature Readings

1. Measure air temperature in an area that is out of direct sunlight or in the vicinity of any external heat.
2. Allow 2 minutes before reading thermometer.
3. Measure water temperature either in the plastic sample bottle, the dissolved oxygen sampler or in the water source it self immediately following sample collection.
4. Record data on site data sheet.

5.0 Lab Testing Procedures

The lab testing procedures that are outlined in this document and in Appendix A are to be followed with no deviations. Collecting quality data requires careful practice of good lab techniques and following all specific directions when mixing reagents. The directions for each test have been developed by LaMotte and guarantee accurate results. A laminated Water Testing Procedures card book has been developed along with a sequence on each Colorimeter. Sequence 1 is used for Sassafras Samplers and is pre-set for all tests that will be analyzed. Please use caution and double check that the test number on the sequence list is the same on the laminated cards.

The use of the Smart Colorimeter is an upgrade from the program's past experience with color cards. The Smart Colorimeter utilizes a light wavelength technology that detects minute color changes in a sample after specific reagents are combined and allowed to react. Using glass test tubes, the colorimeter selects a filter that will analyze color development and targets a beam of light through the water sample matrix. Because of this process, it is extremely important that all glassware is free of water drops, finger smudges and solid residue on the surface of the glass. If at anytime the glassware becomes worn and excessive scratches show, contact the Riverkeeper and have the equipment replaced. Keep track of this on your log sheet. For a description of how the Smart Colorimeter works, see the manual that is provided for each kit.

5.1 General Lab Testing Procedure and Basic Techniques

1. Make sure all glass wear is clean and dry from prior testing cycles.
2. Make sure work surface is clean and free of clutter.
3. The Rule of Three: This rule also applies in the lab prior to testing. During clean-up and in between samples, use the rule of three for washing and for rinsing the small vials, pipettes and spoons.
4. Shake sample bottle before dispensing into colorimeter test tube.
5. Each test requires a BLANK to be scanned before inserting the prepared sample for measuring. If monitoring only one site: pour sample water into one colorimeter test tube and use as the BLANK for each test. Some kits will have a test vial with a yellow cap; this is the blank with sample water. Distilled water is used for the blank in the Turbidity test. This is the vial with the blue cap and should be used for the turbidity blank only.
6. In addition to analyzing the samples collected at the sampling sites, analyze the Quality Control (QC) sample for the parameters listed in 7. If "STD" or "QC" is written by your team name in the site list, this is because you will need to run a QC sample along with your sites. This will allow calculation of the standard deviation, which will provide a statistical estimate of the

inherent variability of the test protocol. NOTE: Prior to testing, vigorously shake the QC sample.

7. The testing parameters for the colorimeter tests are:
 - Nitrate-Nitrogen
 - pH
 - Ammonium-Nitrogen
 - Phosphate
 - Turbidity
8. Follow instructions for each test. NOTE: some tests have a required development time, allowing time for other tests to be started and completed. Do not start other tests unless you are comfortable in your lab techniques and are familiar with the testing procedures.
9. Record all data on the data sheet filled out during the field sampling process.
10. Dispose of chemicals properly. Nitrate-Nitrogen test produces a cadmium waste that should be stored in a sealable container and labeled "Cadmium Waste". Once this jug is about $\frac{3}{4}$ full, drop it off at the SRA office and pick up a new waste jug. All other waste can be poured down the sink with ample amounts of water. NOTE: You should not pour any waste material down the drain if you have a nutrient reducing septic system. If this is the situation, place waste in a sealable bottle filled with inexpensive kitty litter. Once full, dispose of the bottle with household solid waste.
11. After all waste is properly contained, wash all equipment, glassware and sample bottles with tap water and with distilled or de-ionized water using the "Rule of Three" method.
12. Limit Contamination: Good lab practices limit contamination of the testing equipment and the water sample that you are analyzing. Contaminated test reagents can not be used for the next test period and will jeopardize all data and quality assurance measures. Follow these basic rules:
 - Keep chemical dispensing equipment clean (e.g., spoons, syringes, sample bottles and glass vials).
 - Make sure spoons and droppers only come into contact with the appropriate chemicals.
 - Keep track of test tubes when working with multiple sites.
 - Keep lab work area clean.
 - Make sure selected tests are measured with the appropriate chemicals in the Smart Colorimeter.

LaMotte Approved Analytical Methods

Clean glassware is a must for accurate results. Thoroughly rinse test tubes before and after each use. Caps and stoppers should also be cleaned after each use.

- When adding sample to calibrated test tubes, be sure tube is filled to the appropriate mark. The bottom of the liquid (meniscus) should be level with the desired mark (See Figure 1).
- When dispensing reagents from bottles fitted with dropper plug and cap, be sure to hold bottle vertically and gently squeeze to dispense the appropriate number of uniform drops (See Figure 2).
- For those reagents to be added with the enclosed screw cap pipette assemblies, remove polyseal cap on bottle and replace with the screw cap pipette.
- Note: We recommend placing the polyseal caps back on the reagent bottles for longer periods of storage. Be sure that both pipette assemblies and polyseal caps are thoroughly clean before placing on the bottles to avoid contamination.
- When dispensing reagents from pipettes, hold pipette vertically to assure uniform drop size (Figure 3).
- To fill pipettes, squeeze rubber bulb and immerse into reagent. Release bulb to fill (Figure 4).
- To accurately dispense powdered reagents with spoon, tap spoon on vial to remove excess reagent (Figure 5).



Figure 1



Figure 2



Figure 3



Figure 4

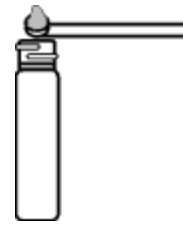


Figure 5

5.2 Specific Test Directions

5.2.1 Dissolved Oxygen Titration

The Titration method for analyzing dissolved oxygen content utilizes a chemical reaction between Sodium Thiosulfate and the prepared, or “fixed”, dissolved oxygen sample. The addition of Sodium Thiosulfate in small amounts chemically reacts with the fixed sample and results in a colorless mixture. The amount of dissolved oxygen in the water sample relates to the amount of Sodium Thiosulfate added. To determine the exact amount, a starch indicator is used to highlight the moment when all possible reactions are complete, and the mixture turns clear. Each kit is outfitted with instructions that depict the “fixing” and titration procedures.

Titration Procedure

1. Fill the titration tube to the 20 mL line with the "fixed" sample and cap.
2. Fill the Direct Reading Titrator with Sodium Thiosulfate.
3. Insert the Titrator into the center hole of the titration tube cap. While gently swirling the tube, slowly press the plunger to titrate until the yellow-brown color is reduced to a very faint yellow.
4. **NOTE:** This pale yellow endpoint is a bit vague (difficult to determine). It is suggested that you aim for a ‘post-it’ note yellow. It is better to add the starch indicator sooner (more yellow) than later.
5. Remove the Titrator and cap. Be careful not to disturb the Titrator plunger, as the titration begun in Step 12 will be continued in Step 14. Add 8 drops of Starch Indicator Solution (4170WT). Sample should turn blue.
6. Replace the cap and Titrator. Continue titrating until the blue color just disappears. Read the test directly from the scale where the large ring on the Titrator meets the Titrator barrel.
7. Record as ppm dissolved oxygen.
 - Each minor division on the Titrator scale equals 0.2 ppm.
 - If the plunger tip reaches the bottom line on the Titrator scale (10 ppm) before the endpoint color change occurs, refill the Titrator and continue the titration. Make sure to stop at exactly 10 before refilling. When recording the test result, be sure to include the value of the original amount of reagent dispensed (10 ppm).
 - In "fixing" the sample, if the precipitate does not dissolve after adding sulfamic acid, add additional drops of the acid to dissolve all of the precipitate. Precipitate should dissolve before adding Sodium Thiosulfate to the sample.
 - The sodium thiosulfate titrant will be replaced every 6 months to reduce inaccuracy due to contamination.

5.2.2 Colorimeter Test Procedures

The Colorimeter Test Procedures for each parameter that Sassafras Sampler Volunteers are testing can be found in the supplied manual with each Smart Colorimeter. The following parameters currently utilize the Smart Colorimeter:

- Ammonia-Nitrogen
- Nitrate-Nitrogen
- pH
- Phosphate
- Turbidity

A copy of the instructions for each parameter listed above can be found in Appendix A. Also, a laminated set of instructions has been provided and outlines the exact procedure using Sequence 1 on the Colorimeter.

5.3 Quality Control (QC) Samples

Each month and during training sessions, Quality Control (QC) samples will be analyzed for nitrate-nitrogen, pH, ammonium-nitrogen, phosphate, and turbidity. One bulk QC sample will be prepared monthly and for training sessions by spiking distilled water or Sassafras River water with known concentrations of nitrate, ammonium and phosphate. The bulk QC sample will be sub-sampled with the sub-samples distributed to the testing teams during the week prior to sampling or during training sessions. QC samples will be analyzed at the same time with the same protocol as the collected samples. **Prior to analysis, QC samples will be stored at cool temperatures, either refrigerated or on ice.** Analysis of the QC samples by multiple teams allows calculation of the standard deviation for each test. The standard deviation observed for QC samples prepared in distilled water provides a statistical estimate of the inherent accuracy and precision (variability) of the test protocol. The standard deviation observed for QC samples prepared in Sassafras River water provides a statistical estimate of the inherent precision (variability) of the test protocol as impacted by the river matrix.

6.0 Data Recording

1. All Sassafras Sampler Volunteers are required to record data on the provided data sheets. These must be dropped off, mailed or scanned and e-mailed to the Riverkeeper within a week after sampling.
2. Also note on the log sheet the date and your actions that day: Samples analyzed, new reagents obtained or reagents replaced.
3. If any samples are determined to be “Overrange” on the colorimeter, dilute and re-analyze the samples following the dilution procedures provided in the Test Instructions.

Thank You

Thank you for volunteering as a Sassafras Sampler. We at SRA hope you enjoy your active role in monitoring the Sassafras and helping to provide a detailed record of water quality. You are helping a great deal by providing us with the tools to identify and track sources of nutrients and pollutants to the Sassafras River.

Sassafras Sampler Monitoring Program

Standard Operating Procedures

Appendix A

Specific Test Directions

Sassafras Samplers Monitoring Program

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Appendix B

Water Quality Data Sheet

