

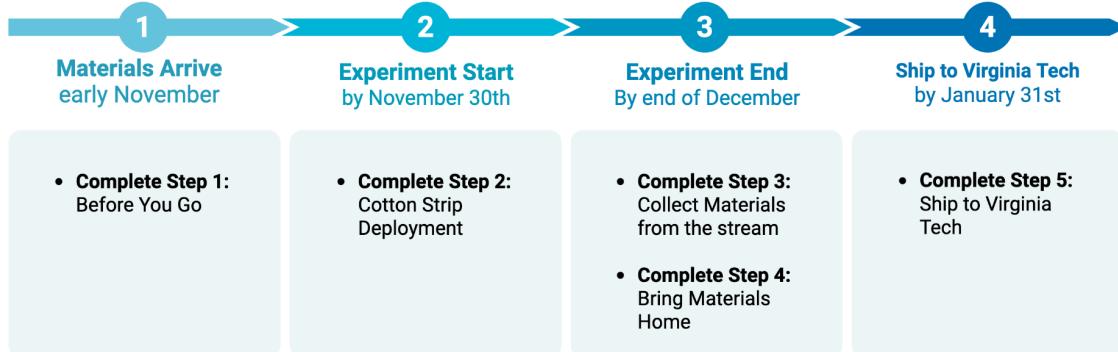
# Chasing Salt Methods Manual

A Community Science Study Understanding  
How Salt Creates Stress  
in Riparian-Stream Ecosystems

Research Project Partners:



# Quick Start Guide



## Step 1: Before You Go

- Check your materials list
- Watch the methods video

## Step 2: Cotton Strip

### Deployment

- Choose two sections of your site
  - Upstream
  - Downstream
- Install cotton strips
  - 2 strips in each section of the stream
  - 2 cotton strips in riparian zone within 5m of stream bank
- Deploy temperature loggers
  - Stream and Riparian
- Record stream characteristics on the provided datasheet
  - Width
  - Depth
  - Sediment size
  - Coordinates
- Check on your site at least weekly until the collection

## Step 3: Collect Materials from Stream

- Cotton strips
  - Rinse in alcohol
  - Wrap in foil with label
- Soil sample
  - 15cm depth
  - Double paper bag
  - Cardboard soil box
- Collect temperature loggers

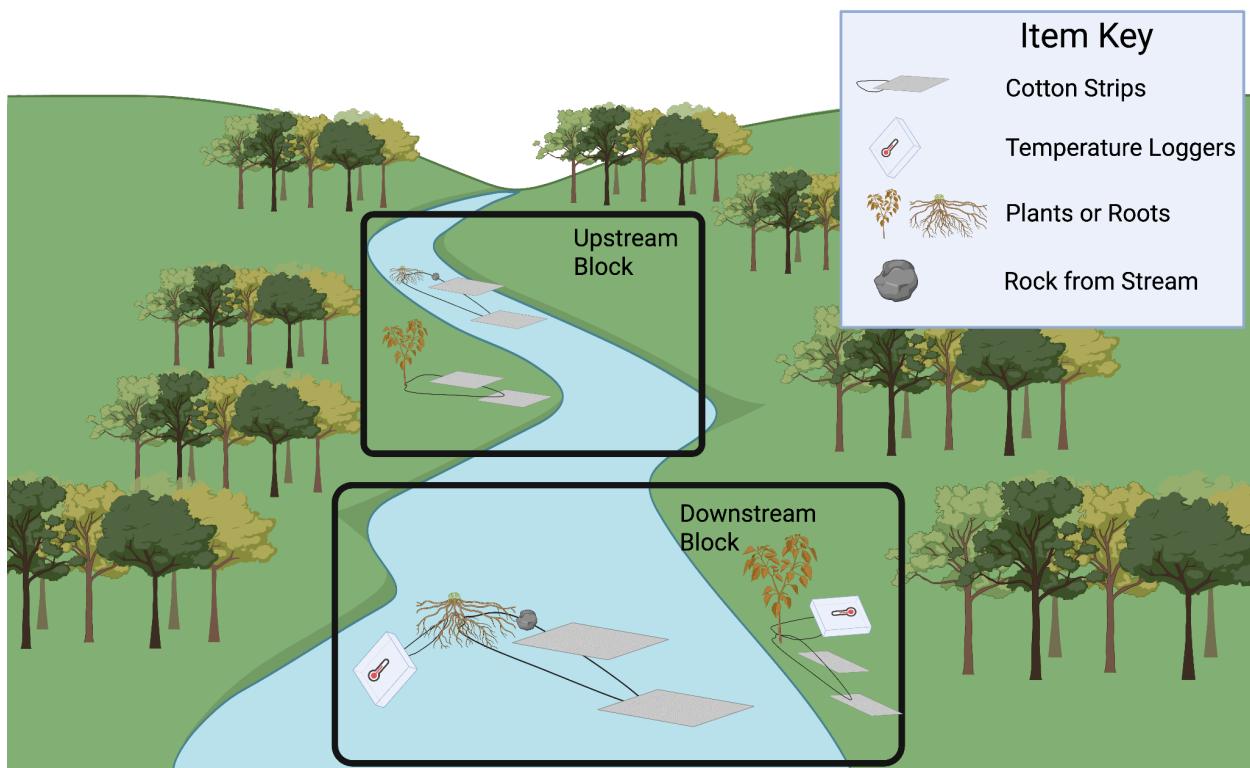
## Step 4: Bring Materials Home

- Dry cotton strips at 40 C/104 F for 1 hr
- Dry the soils at air temperature
- Keep labels with samples at all times
- Pack samples, loggers, and data sheets

## Step 5: Ship to Virginia Tech

- Ship everything in cooler
- Use pre-printed label

*Diagram of the cotton-strip assay deployment configuration. In the stream, the cotton strips will be tied to roots or plants in the stream. Then a rock can be used to weigh down the nylon string and keep the cotton strips (enlarged for the image) in place. In the riparian zone, the nylon string will be tied to shrubs or roots to keep the cotton strips in place. The temperature loggers will be added in the downstream reach.*



## Resources:

- [Project Website](#)
- [Method Videos](#)
- [Data sheets](#)
- [Upload site photos and selfies](#)
- [Instagram](#)
- [Facebook](#)

Share online with #CommunitiesChasingSalt

# Get started with the Chasing Salt Quick Guide in English and Spanish

To get started:

1. Read the instructions in your packet
2. Watch the methods video
3. Choose a safe and accessible stream

Once you do these three things, you are ready to start your field adventure!

\*It may be possible for a project partner to involve students, postdocs, etc. in publications and other products stemming from the project by developing the assay in more than 1 stream. Please email us ([tmuenz@stroudcenter.org](mailto:tmuenz@stroudcenter.org)) if this is something you would like to do.

## **Project Introduction, Methods, and Links to More Information**

|                                                                                  |          |
|----------------------------------------------------------------------------------|----------|
| <b>Introduction</b>                                                              | <b>5</b> |
| Vocabulary Terms                                                                 | 5        |
| Kit Materials                                                                    | 6        |
| Materials Not Provided                                                           | 6        |
| <b>Monitoring with Cotton Strips</b>                                             | <b>7</b> |
| Overview: Research Methods for Deploying the Assay in the Field                  | 7        |
| Overview: Field Procedure                                                        | 7        |
| Stream Selection                                                                 | 7        |
| Experimental Design                                                              | 8        |
| Cotton Strip Assay Deployment Timing                                             | 8        |
| Riparian and Stream Incubation Period                                            | 9        |
| Sample Labelling                                                                 | 9        |
| COTTON STRIPS:                                                                   | 10       |
| SOIL SAMPLES:                                                                    | 10       |
| WATER SAMPLES:                                                                   | 11       |
| Part I: Methods for Deployment of the Cotton Strip Assay and Temperature Loggers | 12       |
| Construct the Cotton Strip Assay                                                 | 12       |
| Specific Methods: Deploying the Cotton Strip Assay in the Field                  | 12       |
| Materials Needed:                                                                | 12       |
| Installing the Cotton Strips in the Stream (2 locations)                         | 13       |
| Installing the Cotton Strips in the Riparian Zone (2 locations)                  | 14       |
| Temperature                                                                      | 14       |
| Sediment Size in the Stream                                                      | 14       |

|                                                                                                                                                                                                                                    |    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Wetted Channel Width and Depth                                                                                                                                                                                                     | 15 |
| Site Location Recording                                                                                                                                                                                                            | 15 |
| Monitoring Your Cotton Strips Weekly                                                                                                                                                                                               | 15 |
| Part 2: Methods for Retrieving the Samples from the Field,<br>Processing, and Shipping                                                                                                                                             | 16 |
| Overview                                                                                                                                                                                                                           | 16 |
| Post-Incubation Cotton Strip Processing                                                                                                                                                                                            | 16 |
| Materials Needed:                                                                                                                                                                                                                  | 16 |
| Soil Sample Collection                                                                                                                                                                                                             | 17 |
| All materials needed are in your packet except a small<br>trowel:                                                                                                                                                                  | 17 |
| Water Sample Collection                                                                                                                                                                                                            | 18 |
| Materials Needed:                                                                                                                                                                                                                  | 18 |
| Collecting Temperature Loggers                                                                                                                                                                                                     | 18 |
| Drying the Samples After Returning from the Field                                                                                                                                                                                  | 19 |
| Cotton Strips                                                                                                                                                                                                                      | 19 |
| Soil Samples                                                                                                                                                                                                                       | 19 |
| Shipping Samples                                                                                                                                                                                                                   | 19 |
| Shipping Address:<br>Thank you! We appreciate your participation in our<br>community science study! If you have any questions or<br>would like to hear about the status of the project, please<br>contact tmuenz@stroudcenter.org. | 20 |

## **Resources:**

- **Link to methods videos**
- **Field data sheet**
- **Pictorial checklist of materials in the sample packet**
- **Safety in the field for riparian and stream sampling**
- **Media waiver**
- **Google drive link to folder for site photos, selfies, and site coordinates**
- **#CommunitiesChasingSalt**

# Introduction

The overall goal of this study is to look at the influence of salt on decomposition. We have two main goals:

1. Compare organic-matter decomposition in streams and riparian areas that are influenced by salt using standard assays to trace element content of soil and water.
2. Establish U.S. baseline trace element-decomposition relationships to track changes to this fundamental ecosystem process in riverine habitats in the context of human-caused and natural salinization.

In plain language, we want to know how salts are influencing the breakdown of materials in streams and on nearby stream banks. Using data collected from a large portion of the US, we will create a baseline of how the breakdown of these materials changes due to human-caused salinization and naturally occurring salinization.

## Vocabulary Terms

The methods use a few technical terms, so we put together a list of terms that would be helpful.

Cotton Strip Assay = 8 cotton strips per site used to measure decomposition

Decomposition rate = How fast organic material (like leaves) breaks down

Deploy = Place item in its location

Riffle = Shallow part of a stream with fast-moving water over rocks

Riparian zone = Stream Bank

Stream Order = Way to describe stream size (1 = smallest, headwater stream)

Trace Elements = Naturally occurring minerals and salts in water and soil

## Kit Materials

Honorarium

Cotton Strips (8 total, already attached to the nylon)

Plastic Sheet for Cotton Strips

Aluminum Foil Sheets (10)

Pre-printed Waterproof Labels

Temperature Loggers

I-dot (Riparian Zone)

Hobo (Stream Reach)

Nitrile gloves

- Medium (2 pairs)

- Large (2 pairs)

Plastic Tube

Brown Paper Bag (2)

Compostable Container (2)

Lids (2)

Spoon (1)

Flagging Tape

Ice Packs (2)

Desiccant Packs (2)

Tape

Waterproof Data Sheets

Shipping Label

## Materials Not Provided

These items can be picked up at your local retail or hardware store:

- Meter Stick or Measuring Tape
- Small Trowel or Spoon
- 600 mL of Isopropyl Alcohol
- Scissors

# Monitoring with Cotton Strips

## Overview: Research Methods for Deploying the Assay in the Field

The overall goal is to deploy the cotton-strip assay in a 1st, 2nd, or 3rd order stream and adjacent riparian zones (vegetated areas along stream banks). Stream order is a general way of discussing the size of a stream. First order streams are the headwater streams. A second order stream occurs when two first order streams join together, and a third order stream is when two second order streams join, and so on. You can determine whether your stream is a 1st, 2nd or 3rd order stream here: [link](#). Select a stream near your home, work, park or school.

We will mail each partner a kit with the materials to conduct the data collection. The kit will have 8 cotton strips that will be used for measuring decomposition rates along the riparian zone and in the water, two paper bags for soil, one plastic sterile tubes for collecting stream water, and two temperature loggers for the riparian area and the stream water. See table 1 below.

Table 1. Kit components and location for use.

| Item/location          | Soil | Stream | Riparian Zone |
|------------------------|------|--------|---------------|
| 4 cotton strips        |      |        | X             |
| 4 cotton strips        |      | X      |               |
| 2 paper bags           | X    |        |               |
| 1 plastic sterile tube |      | X      |               |
| 1 temperature logger   |      | X      | X             |

After collecting the data, you will mail all the samples back to us in the provided box (that also functions as a cooler) with the frozen ice pack. We will then measure the cotton strips for tensile strength (a measure of decomposition), and trace elements (like Na, Cl, Mg, K) and their concentration in the soil and water.

## Overview: Field Procedure

### Stream Selection

The stream and riparian zones of your site should have vegetated and stable banks, and be representative of streams in your region.

Determine the type of stream that you are sampling. Forested streams will have at least 60% forest in the watershed, urban streams will have at least 10% impervious surface, and agricultural streams will have at least 25% agriculture (cropland, pasture, or a combination). To figure out the land cover of your watershed, use this online tool <https://modelmywatershed.org/draw>. Note that you will need to know where you are in the watershed. If you need assistance in determining what type of stream you have, please contact [tmuenz@stroudcenter.org](mailto:tmuenz@stroudcenter.org).

In the stream, you will establish a “reach”, a section of stream to work within for collecting the data. The reach should have riffle habitat in the upper and lower portions. The reach will be approximately 50 meters long (160 feet) that is safe and accessible to work in. To establish this, you can walk off the reach with a step being approximately 1 foot.

## Experimental Design

A total of 8 cotton-strips will be deployed at each field site, 4 cotton strips in the riparian zone, and 4 cotton strips in the stream. These strips will be distributed in pairs in an upstream area and in a downstream area so that there are 4 strips upstream (2 in water and 2 on land) and 4 strips downstream (2 in water and 2 on land). We will pre-assemble the cotton strips for you with nylon string and zip ties, so all you need to do is find a place to put them in the riparian area and the stream.

Partners (you!) will also deploy two temperature loggers, one in the stream (Hobo Pendant) and one in the riparian zone (I-dot). Partners will also measure a few characteristics of the streams (water depth, stream channel width, and stream bottom sediment size). Additionally, partners will sample soil and water for chemical analysis.

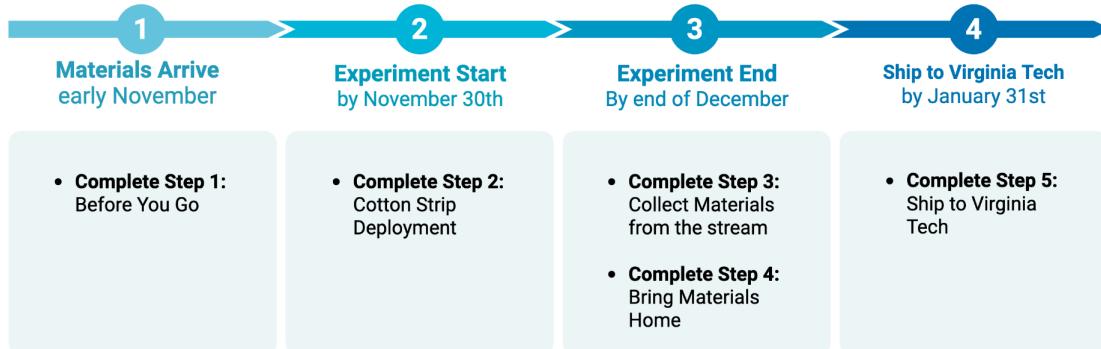
### Cotton Strip Assay Deployment Timing

Each partner will deploy the assay (8 cotton strip design) in their respective streams during the period of peak when leaves are falling in deciduous forests.

Our ideal timeline is as follows:

1. Kits Arrive to Partners by early November
2. By November 30th:
  - a. Deploy (place) riparian zone and stream cotton strips and loggers
  - b. Measure stream depth and width
  - c. Determine sediment size
  - d. Check on strips at least once per week
3. 30 days later +/- 7 days (By end of December)
  - a. Collect cotton strips from both the riparian zone and stream
  - b. Collect loggers
  - c. Collect water sample from the stream

- d. Collect soil sample from the riparian zone
- 4. By January 31st
  - a. Send us the cotton strips and temperature loggers along with the water and soil samples. You can keep everything else!



### Riparian and Stream Incubation Period

The cotton strips will stay in the field (both stream and the riparian zone) for approximately 30 days. This has proven to be the time that results in approximately 50% tensile-strength loss, an amount of decomposition thought to give the assay the greatest sensitivity.

Following the incubation period, the strips will be removed from the field, processed, and shipped back to us.

### Sample Labelling

The project will receive thousands and thousands of cotton strip samples and a clear labeling system is needed to link each strip with its particular stream and habitat.

**The soil, water, and cotton strip labels will use the following format and will be labeled upon shipment to you.**

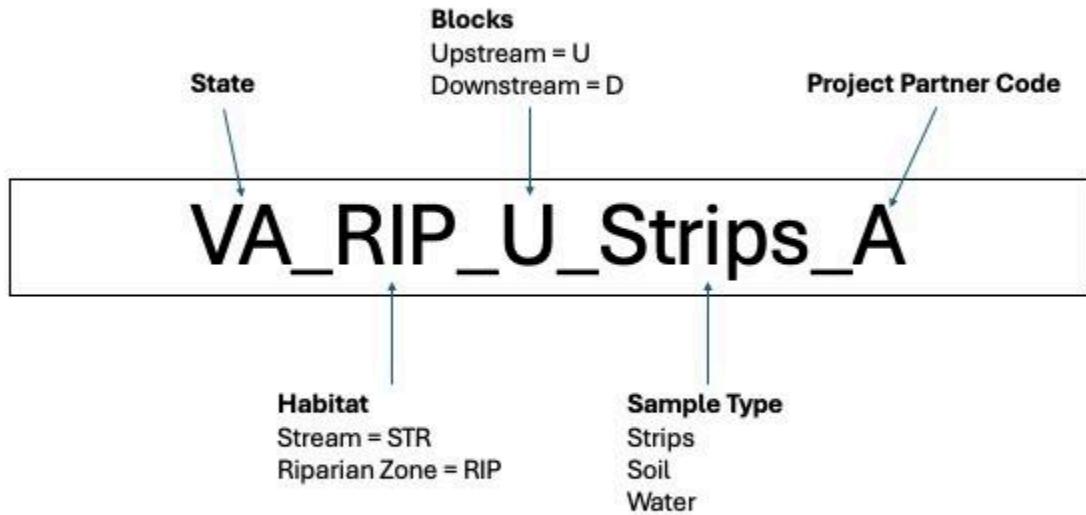
**For example:** State – Stream – Habitat – Block - Initials of project leader

- State: e.g. Ohio is 'OH'
- Stream: stream name
- Habitat: Cotton strips will be deployed in 2 habitats. STR = Stream, RIP = Riparian
- Block: Cotton strips were dispersed between two experimental blocks. "U" being upstream, "D" being downstream.
- Project Partner ID: We will assign each partner a unique identification.
- Sample Type: Soil will be soil, water will be water, and cotton strips will be strips

We created unique labels for each partner based on your State, Latitude and Longitude of your sample sites. So, for example, our field labels for Sally Entrekin at Virginia Tech, Department of Entomology in Price Hall will be:

COTTON STRIPS:

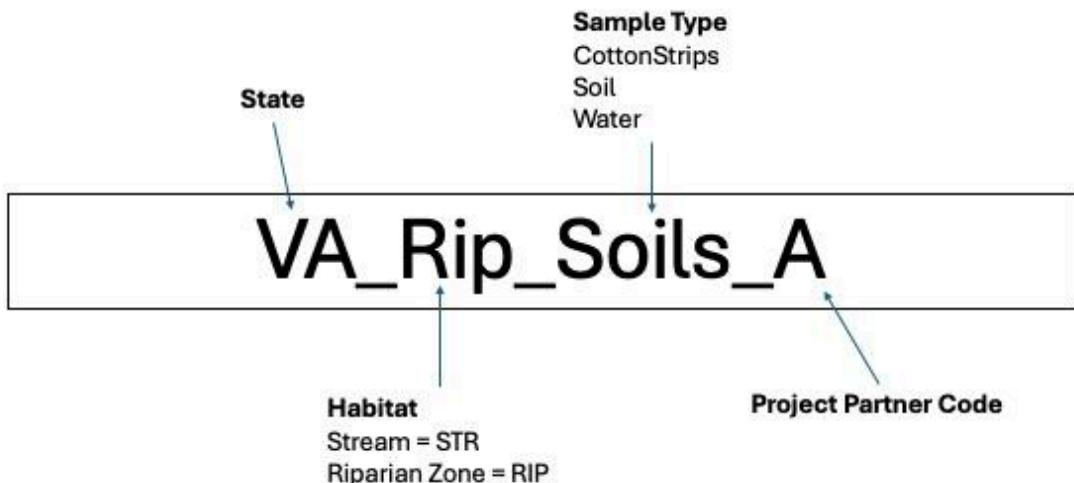
State\_Habitat\_Blocks\_Strips\_PartnerID



SOIL SAMPLES:

The soil composition samples will be labeled to match the corresponding cotton strips with the additional text “-soil” at the end.

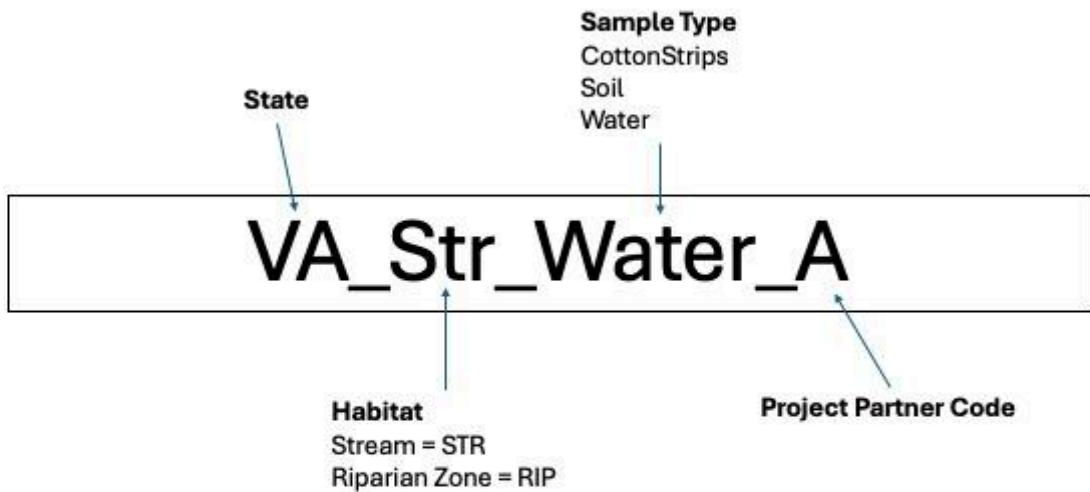
State\_Habitat\_Soils\_PartnerID



WATER SAMPLES:

The water samples will be labeled similarly, but with the additional text “-water” at the end.

State\_Habitat\_Water\_PartnerID



## Part I: Methods for Deployment of the Cotton Strip Assay and Temperature Loggers

### Construct the Cotton Strip Assay

The cotton strips will arrive to partners already tied together, or constructed.

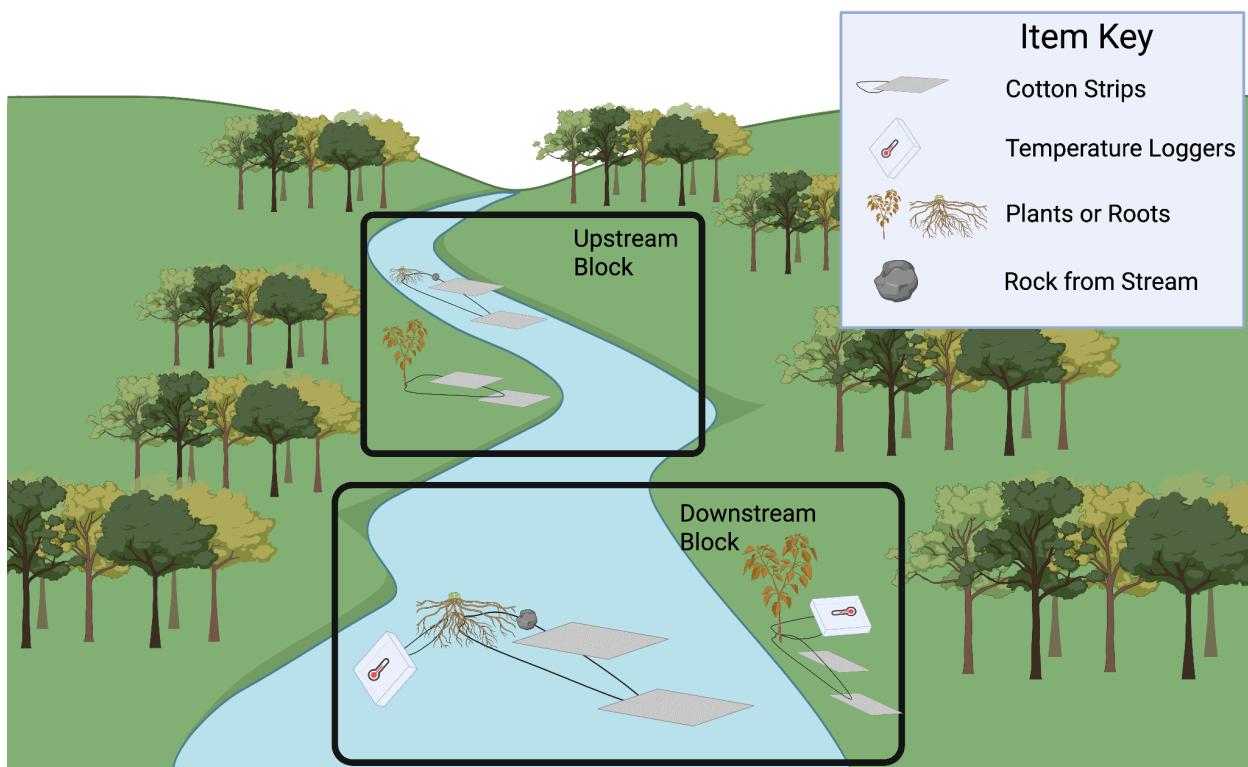
### Specific Methods: Deploying the Cotton Strip Assay in the Field

Materials Needed:

- 8 cotton strips
- Measuring Tape or Meter Stick
- Field Data Sheet
- Flagging Tape
- 1 Hobo temperature logger for the stream
- 1 i-dot temperature logger for the riparian zone

You will repeat all of the steps for “Installing Cotton Strips in Stream” and “Installing Cotton Strips in the Riparian Zone” in the upstream area and downstream area of the stream section.

*Figure 1. Diagram of the cotton-strip assay deployment configuration. In the stream, the cotton strips will be tied to roots or plants in the stream. Then a rock can be used to weigh down the nylon string and keep the cotton strips (enlarged for the image) in place. In the riparian zone, the nylon string will be tied to shrubs or roots to keep the cotton strips in place. The temperature loggers will be placed in the downstream reach.*



#### Installing the Cotton Strips in the Stream (2 locations)

1. Select a riffle-type habitat in the stream.
2. Tie either end of the string that does not have a cotton strip, to the base of the tree root to hold the strip in place. If you are unable to find tree roots, you can use place a piece of rebar and attach the cotton strips to that. Ensure cotton strips are fully submerged under water. To prevent the cotton strips from flapping in the current, a small flat rock can be placed on the string immediately upstream from the cotton strip (BE CAREFUL not to place the rock on the strip itself).
3. Find a location in the riparian area (a tree or shrub) to mark where you have placed the cotton strips in the stream by tying off brightly colored flagging tape. This will help you find the cotton strips if they get buried during a storm or from natural movement of rocks or sediment in the stream.
4. Repeat this procedure in the next riffle habitat downstream of the other location.

## Installing the Cotton Strips in the Riparian Zone (2 locations)

1. Deploy cotton strips in an area adjacent to the location of the in-stream cotton strips. The side of the stream can be chosen at random using a coin. The exact distance of the riparian site from the stream site will vary but for most streams will be within 5 meters from the in-stream cotton strips.
2. Tie the end of the nylon string to a small tree or tree root. Make an effort to place the strips such that they come into direct contact with the soil or decomposing organic matter, mimicking a recently fallen leaf. This can be achieved by covering the cotton strips with leaves.
3. Use flagging tape to provide a marker for yourself to indicate where these cotton strips are located.
4. Repeat this procedure in the downstream riparian zone location.

## Temperature

Water and air temperature will be measured with data loggers sent to partners. These will take data at a certain time frequency and will be useful in our research. Water temperature will be measured with the hobo logger. This will be tied to the same tree or root as one of the cotton strips in the stream. Air temperature will be measured with the i-dot logger. This will be tied to the same small tree or tree root as the cotton strip in the riparian area. All loggers were initiated before they were sent and will be ready to place at the stream and riparian locations when they arrive in the kit.

## Sediment Size in the Stream

Partners will visually estimate the predominant size class of the sediment in the 50 meter reach of the stream where the cotton strip assay is deployed. The sizes classes are on the field data sheet and are summarized here:

- Coarse-sand and smaller (>2 mm diameter)
- Fine-Pebble (2-8 mm diameter)
- Pebbles (8-32 mm diameter)
- Coarse Pebbles (32-64 mm diameter)
- Cobbles (64-256 mm diameter)
- Boulders (>256 mm diameter)

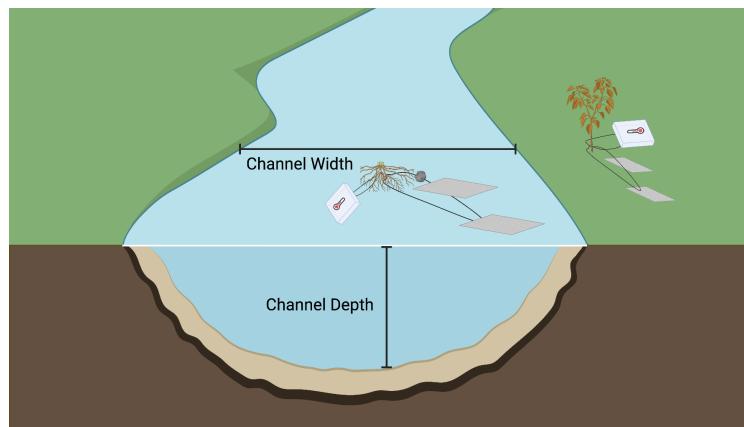
To estimate the predominant size class of sediment within the stream reach, partners will choose 4 cross-sections within their 50 meter reach to assess.

1. Walk across the stream from one side of the stream to the other.
2. Every few steps, pick up or look closely at a few pebbles and measure their general size category from the list above. Complete field data sheet on sediment size.
3. Note which size is most common in that section.
4. Repeat at all four cross-sections of the stream.

## Wetted Channel Width and Depth

We will also have you provide data on the physical stream channel. Use the measuring tape or meter stick to estimate the mean width of the wetted stream channel (water's edge) at the two locations where cotton strips are installed (Figure 2). Also measure the depth of the stream at the location where the cotton strips are installed. Enter these data on the field data sheet provided.

*Figure 2. Diagram of how to measure wetted channel width and depth. Complete this at both the Upstream and Downstream locations.*



## Site Location Recording

Please locate the stream on Google Earth or Google Maps and provide the latitude and longitude of your site. For example, Price Hall at Virginia Tech would be 37°13'32.3"N 80°25'27.2"W in Google Maps.

## Monitoring Your Cotton Strips Weekly

Be sure to check on your cotton strips at least once per week. This will be helpful especially after storms or other events that might disrupt the streams. Add any storm information (date and amount of rainfall) to the field data sheet.

## Part 2: Methods for Retrieving the Samples from the Field, Processing, and Shipping

### Overview

After cotton strips have been in the stream and riparian zone for about 30 days (+/- 7 days), they will need to be removed and processed (treated with alcohol and dried), then shipped back along with your water and soil samples to The Aquatic Entomology Lab at Virginia Tech.

Ensure that all stream characterization data is collected (Sediment Size, Wetted Channel Width and Depth, and Coordinate Reading) during Part I Deployment of the Cotton Strip Assay.

### Post-Incubation Cotton Strip Processing

Materials Needed:

- Aluminum foil
- Pre-made labels
- Scissors
- Plastic bottle with ethanol or isopropyl alcohol (approximately 600 mL)
- Small compostable tray
- Insulated shipping box
- Plastic sheet for cotton strips
- Nitrile gloves
- Tape

NOTE: Make an effort to handle the strips gently, and to hold them from only the very ends while wearing gloves.

1. Using a pair of scissors, cut the nylon string from each root that holds the cotton strips in the riparian zone and stream sites. Cut the cable binder and detach it from the strip. The ends of the strip will not influence the tensile strength measurements as much as other parts of the strip. In the field, shortly after taking the strips from where they were deployed (stream or riparian zone), place each strip in a compostable bowl with a centimeter or so (enough to cover the strips) of ethanol or isopropyl alcohol (70% or greater). These will all go through the same bowl of ethanol, since this is to stop biotic activity on the cotton strip.
2. Using a gloved finger, gently brush the strip for 10 seconds or so on each side to remove any sediment or biofilm adhering to the surface. Be gentle.

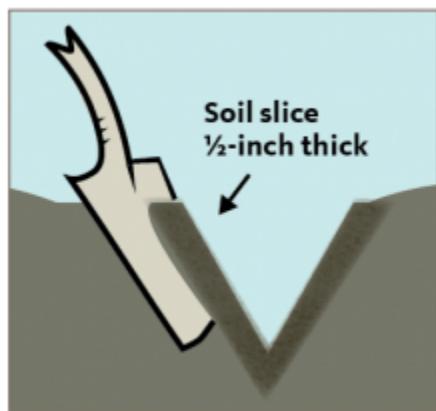
3. Place each of the eight cotton strips individually onto the center of one of the sheets of aluminum foil, and fold the sides of the foil over the strip, effectively making an envelope. Keep the strip flat (do not fold or crumple).
4. Place the corresponding pre-printed label into the foil envelope along with each strip.
5. You can place foil wrapped strips into one of the ziplocs provided for transport home. Bring the strips back from the field with you.

## Soil Sample Collection

All materials needed are in your packet except a small trowel:

- Nitrile gloves to wear during the soil and water sampling
  - Small trowel
  - Rice desiccant
  - 50 mL pre-labeled sterile plastic tube
  - Spoon and tray in packet
1. Roll over the paper bag to seal it and label it with the provided printed label. The labels on the soil samples will match their corresponding cotton strips with the word 'soil' added to the end. Ensure the trowel is cleaned and has no other soil or chemical residues.
  2. Mark the shovel/trowel with a piece of tape at 15 centimeters (or 6 inches) from the tip, to determine the depth to take the soil sample. You will collect your soil sample from right next to where the cotton strips were placed in the riparian zone.
  3. Clear away any leaf litter and vegetation until just top soil is visible.
  4. Once bare soil is exposed, use the trowel and push the blade into the soil at a slight angle to 15 centimeters (6 inches) of depth.
  5. Tilt the trowel back to remove the blade full of soil, being careful to keep the soil intact (Figure 3). This can consist of multiple scoops.
  6. Open one of the lunch-sized paper bags and place the soil sample in the bag.
  7. Place the first paper bag into the second paper bag to ensure the sample is contained.
  8. Bring the soil back from the field with you.

*Figure 3. Soil sample collection.*



### Water Sample Collection

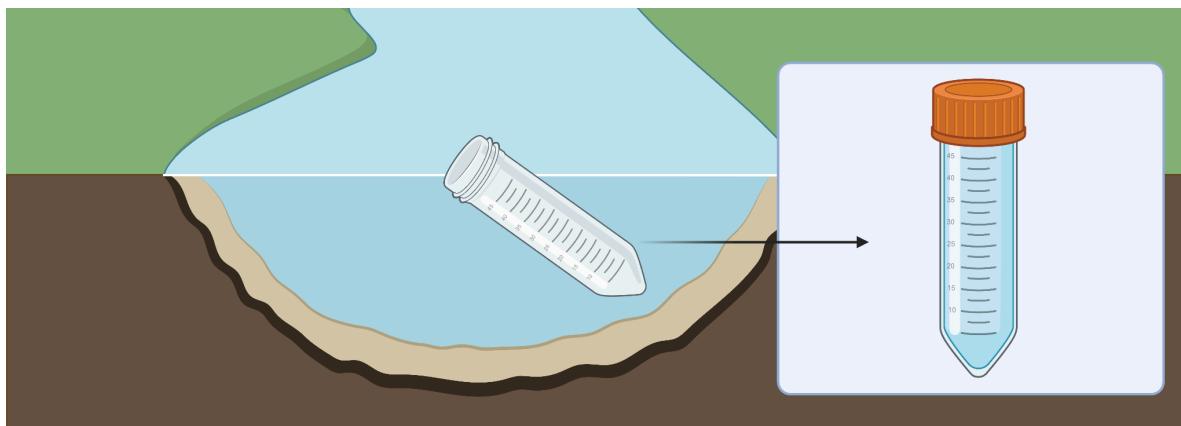
#### Materials Needed:

- 50 mL pre-labeled sterile tube

Using one plastic tube, partners will collect unfiltered water samples.

1. Be sure to collect at the downstream section of your stream.
2. Ensure that you collect water samples from the water column while avoiding disturbing the sediment (Figure 4).
3. To collect the water samples, remove the cap and rinse the tube three times and then collect the water sample, filling the tube to the 50 mL line.
4. Cap the tube.
5. Bring the water samples back from the field with you.

*Figure 4. Rinse the plastic tube with stream water three times and then collect your stream water sample, filling to the 50 mL line. Be sure not to disturb the sediment while collecting water.*



## Collecting Temperature Loggers

1. Cut the nylon string attaching the temperature sensors in the stream and the riparian zone.
2. Bring both back from the field with you.

## Drying the Samples After Returning from the Field

### Cotton Strips

1. Preheat an oven (empty) to ~105 °F (approximately 40 °C)
2. Lightly unfold each foil envelope so that the strip is exposed to the atmosphere. Be sure to keep the labels with their respective envelopes.
3. Place the strips on top of a cookie sheet to dry in the oven at ~105 °F (approximately 40 °C) for 1 hour.
4. After 1 hr, remove from the oven and reseal the foil envelopes.
5. Pack the cotton strips in a plastic envelope with pockets supplied from the kit. Be careful not to bend or crumble the strips when packing them.
6. Once foil wrapped cotton strips are placed in envelope pockets, tape the pockets closed.

### Soil Samples

1. Lay the soil out on a clean cookie sheet to dry at room temperature.
2. Put the dried soil in the provided cardboard box for soil samples.
3. Place the small soil boxes into the insulated shipping box.

## Shipping Samples

We ask that you add the following to the shipping box to return to us:

1. FROZEN ice packs
2. Cotton strips in plastic sheet with pockets taped laying flat
3. Soil samples in cardboard box provided
4. Water sample in plastic tube (make sure lid is on tight)
  - a. Please place this is the plastic bag in which extra nylon and flagging tape arrived
5. Two temperature loggers
6. Affix the UPS pre-printed lab on the shipping and ship back to The Aquatic Entomology Lab at Virginia Tech.
7. Drop off box at your local UPS drop off location

<https://www.theupsstore.com/tools/find-a-store>

Shipping Address:

ATTN: Lisa Tabor  
The Aquatic Entomology Lab  
Department of Entomology (MC0319)

Price Hall, Room 216A, Virginia Tech

170 Drillfield Drive

Blacksburg, VA 24061

Phone: 540-231-6341

Fax: 540-231-9131

Thank you! We appreciate your participation in our community science study! If you have any questions or would like to hear about the status of the project, please contact [tmuenz@stroudcenter.org](mailto:tmuenz@stroudcenter.org).

We will be analyzing the data from the cotton strips, soil samples, water samples, and temperature loggers in the spring. We will reach out to community partners as the analysis is completed and manuscripts are published so that everyone can see their hard work in action.