VERMONT VERNAL POOL MONITORING PROJECT



VPMON 2021 MANUAL



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For more information, please see:

https://vtecostudies.org/projects/forests/vernal-pool-conservation/vermont-vernal-pool-monitoring-project/



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CHAPTER I INTRODUCTION TO VPMON

What is a Vernal Pool?

Vernal pools are small seasonal wetlands that generally share four characteristics:

1. Seasonal wet-dry cycle

Vernal pools typically fill during the spring or fall, and then dry, at least partially, in the summer (and fully in drought years).

2. No permanent inlet or outlets

They are hydrologically isolated from permanent water. Although some may have groundwater connections, most are fed by surface runoff.

3. No resident fish populations

This is key to vernal pool indicator species, which are all highly vulnerable to fish predation.



4. Provide breeding habitat for vernal pool indicator species

In Vermont, "mole" salamanders, Wood Frogs, & fairy shrimp are all adapted to the unique conditions of vernal pools and use them as their primary breeding habitat (see page 25 for details).

In northeastern North America, vernal pools are generally associated with forest ecosystems (though they *can* be found elsewhere). As islands of water in a sea of forest, these ephemeral wetlands are considered "keystone ecosystems," meaning that they play a greater role in forest ecology than one may guess from their size alone (Calhoun and deMaynardier, 2008). An average sized vernal pool, for instance, can have thousands of Wood Frogs visiting it to breed every single spring (Kenney and Burne, 2000); one biologist estimated the biomass of pool-breeding amphibians in the 50 acres around a vernal pool to be greater than 150 pounds (Windmiller, 1996); and yet one hardly sees the thousands upon thousands of amphibians hiding in the leaf litter, within a few hundred meters of their breeding pool.

What is the Vermont Vernal Pool Monitoring Project?

Considering the importance of vernal pools, both for forest health and as critical breeding habitat for several animal species, relatively little is known about them. On top of that, numerous threats—including climate change, airborne pollution, and development—make the future viability of vernal pools uncertain. The Vermont Vernal Pool Monitoring Project (VPMon for short) uses community science to confront this uncertainty.

Building upon the work of the Vermont Vernal Pool Mapping Project, VPMon volunteers annually collect a set of vital data on vernal pools across Vermont, providing an annual snapshot of the health of these pools each year. Over the long-term this dataset will provide a baseline with which we can compare against future changes, whatever they may be.

The overall goals of the Vernal Pool Monitoring Project are to:

- Build a base of knowledge regarding the state of vernal pools in Vermont
- Raise awareness about the value of vernal pools by involving the public in vernal pool monitoring
- Advance vernal pool conservation planning at the state and local levels

The project is managed by the Vermont Center for Ecostudies, with support from the ECO AmeriCorps program. However, all the data is gathered by a cadre of passionate community scientists. Recognizing the significance of these ephemeral wetlands, these Vernal Pool Monitors donate their time and energy to accomplish essential work that would not otherwise be accomplished so cost-effectively. As such, they deserve our thanks.

The Vermont Vernal Pool Mapping Project (VPMap)

From 2009 to 2012, VCE partnered with Arrowwood Environmental and Vermont Fish & Wildlife to use color-infrared aerial imagery to remotely map the locations of almost 5,000 potential vernal pools in Vermont.

More than 1,000 of these potential pools have been visited in the field to verify their existence, but many more remain unvisited or just unmapped in the first place. This is where community science can come in! If you find a vernal pool that hasn't yet been verified, you can benefit vernal pool conservation by filling out a VPAtlas data sheet.

An interactive map of all these pools and a printable VPAtlas data sheet can be found on VPAtlas.org.



The Purpose of the VPMon Manual & Trainings

The goal of this manual is to assist trained VPMon community scientists in monitoring vernal pools. It provides both detailed instructions of the monitoring protocols and information to help identify vernal pool species and their egg masses. While all monitors must attend a VPMon Training Session before they can begin monitoring, this manual should allow you to quickly and easily refresh that information. As such, it's written specifically for VPMon Monitors and this is who is being addressed whenever the word "you" is used.

Though you are not required to attend a VPMon training session every year, appropriate review of this Manual is required! In the future, other training materials (e.g. how-to videos) may be posted online to make review even easier. Understanding the monitoring protocols—as well as how to accurately identify vernal pool indicator species and their egg masses—is absolutely essential to the credibility and utility of VPMon data.

CHAPTER II THE ESSENTIAL INFORMATION

The Who, When, and Why of Each Monitoring Visit

Table 1 — **The Basic Information of Each Vernal Pool Visit**: you can use this table to quickly understand the essential information for each of the four visits to your adopted vernal pool. The last two columns explain what to do in each visit, but more details on each protocol can be found in Chapter 3.

	How Many Monitors Required?	When?	Purpose	What data is collected?*
Visit 1: Setup	1	About 1-2 weeks prior to the pool thawing in the spring	 Set up the HOBO Logger (unless the ice is dangerous**) Set up the Acoustic Monitor <i>If There's Open Water:</i> Collect amphibian / macroinvertebrate data 	 Physical Data: % ice-covered & water level <i>If There's Open Water:</i> Egg Mass Survey Macroinvertebrate Survey <i>If you have a smartphone:</i> HOBO Water Temperature data
Visit 2: Early Survey	2	~1-2 weeks after the peak of the Wood Frog breeding period See Timing Guide, page 7	- Collect data during the peak for Wood Frog & Jefferson/Blue- Spotted Salamander egg masses	 Physical Data: % ice-covered, water level, & % vegetation-covered Egg Mass Survey Macroinvertebrate Survey
Visit 3: Late Survey	2 ^{~2-3} weeks after the peak of the Spotted Salamander breeding period (should also be ~2-3 weeks after Visit 2) See Timing Guide, page 7		 Collect data during the peak for Spotted Salamander egg masses Take the Audio Monitor out of the field download its data. 	 Physical Data: % ice-covered, water level, & % vegetation-covered Egg Mass Survey Macroinvertebrate Survey Audio Data If accessible & you have smartphone: HOBO Water Temperature data
Visit 4: Fall Visit	1	Anytime between late- August and October. Best when pool is likely to be dry.	- Collect HOBO Logger (or just download its data and reconfigure it in the field) <i>(See page 8)</i>	- Physical Data: water level - HOBO Water Temperature data

*Completely fill out the "Pool & Observer Info" & "Weather" sections of the data sheet every time you visit.

** If the ice is too dangerous during Visit 1, set up the HOBO Logger & PVC pipe during Visit 2. See page 11.

Egg Mass Survey Timing Guide

Two of the four required visits to your adopted vernal pool involve egg mass surveys, but the ideal day on which to conduct these surveys will vary from year to year and from pool to pool. The map and figure below can help you roughly estimate when to time your visit, **however if the microclimate at your adopted pool is affected by any of the geographic or meteorological factors listed below, amphibian breeding—and thus egg mass counts—will be delayed.** As well, posting the conditions of your adopted pool on the VPMon Google Group is highly encouraged, as it will give other monitors a sense of when to time their own visits.

Delaying Geographic Factors

- Elevation (>1000 ft)
- North-facing slopes
- Extensive conifer shading

Delaying Meteorological Factors

- Late snowmelt / deep snowpack
- Dry conditions / low rainfall
- Cold temperatures (below 40°F)

Generally, the first egg mass survey (Visit 2) should occur 1-2 weeks after the peak of the Wood Frog breeding period. The second egg mass survey (Visit 3) should happen about 2-3 weeks later, which is also



about 2-3 weeks after the peak of Spotted Salamander breeding.

The breeding patterns of the amphibian species monitored by VPMon differ in several ways, which influences the timing of egg mass surveys.

Wood Frog migration is triggered by warmth and humidity. They tend to migrate to pools on the first night with warm rains (>40° F), but some may migrate on warm, sunny days. There may still be some snow on the ground. The breeding season lasts only a few days to a week, and the eggs hatch after 2-4 weeks, depending on water temperature.

Jefferson / Blue-Spotted Salamanders generally the first to arrive at vernal pools, often when just the edges of the pool are thawed.

Spotted Salamanders begin migrating at the same time as Wood Frogs, but they are more dependent on warm nighttime rains. Their breeding season is prolonged, and their eggs take about 3-6 weeks to hatch, depending on water temperature.



Egg Mass Survey Timing Guide: Monitors can use this diagram and its accompanying map to estimate the ideal time to visit an adopted vernal pool. Each bar represents the potential range of ideal surveying dates for each species in a region of Vermont. The darker section within each bar shows the typical peak (e.g. the best time to survey if conditions are average in that spring and at that pool). Each region is signified by a color, which correspond with the colors on the map. Remember that the presence and severity of the listed geographic and meteorological factors may call for a visit to be delayed.

Field Equipment

Table 2 — What to Bring for Each Pool Visit: use this table to know exactly what equipment you need for each vernal pool visit. The basic details of each visit are laid out in Table 1 on page 6. Checklists for what's included in each Equipment Category can be found on the next page.

Equipment Item / Category	Visit 1: Setup	Visit 2: Early Survey	Visit 3: Late Survey	Visit 4: Fall Visit					
HOBO Logger / Water Temperature Equipment	Set up at Pool <i>See below*</i>		Download data if accessible & you have a smartphone	Download data! <i>See below</i> **					
Egg Mass & Macroinvertebrate Survey Equipment	Bring (hopefully won't need)	Bring	Bring	Don't bring					
Audio Monitor & Case	Set up at pool	Don't collect equipment/data	Collect equipment/data	Don't bring					
VPMon Data Sheet & Writing Utensil	Bring	Bring	Bring	Bring					
ThermoPro Indoor Humidity & Temperature Monitor	Bring	Bring	Bring	Bring					
Camera or Smartphone	Bring	Bring	Bring	Bring					
= Bring with you = Don't Bring = Already in Field = Collect equipment									

*Ice Thickness and the HOBO Logger during Visit 1: Setup — If the thickness of the ice at the vernal pool makes it dangerous to reach the deepest point, wait until Visit 2 to set up the HOBO Logger. See page 11 for more information on when to set up the HOBO Logger.

****Reconfiguring the HOBO Logger during Visit 4: Fall Visit** — It is absolutely essential to collect the data from the HOBO logger during Visit 4, whether that means downloading it with a smartphone or taking the HOBO logger out of the pool and then downloading the data at home.

• For instructions on how to collect and submit Water Temperature / HOBO logger data (with or without a smartphone) see page 12.

The HOBO logger can be left out over the winter if: 1) you have already downloaded its data for the current year, 2) its battery is more than 50% full at the time of the fall visit, and 3) it has been reconfigured to start monitoring March 1st of the following year.

• For instructions on how to reconfigure the HOBO logger see page 13.

Monitoring Equipment List

Water Depth & Temperature / HOBO Logger

Measures water temperature, hydroperiod (how long the pool has water in it), & water level.

- HOBO Logger (Onset HOBO[®] Bluetooth Pendant Temperature Data Logger)
 - Automatically measures water temperature.
 - Requires a CR2032 3V lithium battery.
- 5' PVC Pipe with 5 cm markings
 - Depth Gauge
 - HOBO Logger is attached to it.
- Meter stick / Tape Measure
 - To know how to number the 5 cm markings. on the PVC pipe after it's put into the pool.
- Sharpie
 - To number PVC pipe markings.
- Zip Ties (at least 2)
- To attach the HOBO Logger to the PVC pipe
- Smartphone with "HOBO Mobile" app*
 - * Necessary for configuring the HOBO logger, though it doesn't have to be configured in the field
- Waterproof boots / Waders (If the vernal pool will be filled with water)
- A tool to break through ice (If there will be thick ice when you set up the HOBO Logger)

Egg Mass & Macroinvertebrate Survey

Conducted by Vernal Pool Monitors to assess the breeding population sizes / presence of pool-breeding amphibians, fairy shrimp & caddisflies.

• Meter stick / tape measure / etc.

- To identify survey areas for the egg mass survey (within 1 m of the pool's edge) and the macroinvertebrate survey (1 m² area).
- Polarized Glasses (Recommended, but optional)
- To counter glare on the water.
- **Binoculars** (*Recommended, but optional*)
 - $-\,$ To survey egg masses far from the pool's edge.
- Camera / Smartphone

- To take photos of vernal pool indicator species or their egg masses.
- 1 m² PVC Pipe Square (Optional)
 - To delineate 1 m² during surveys.

Frog Phenology / Audio Recorder

Records frog calls, from which we can determine phenology and species composition.

- Audio Recorder (AudioMoth)
 - To record the timing and species composition of chorusing frogs.
 - Requires three AA batteries.
 - Should contain a 16 GB microSD card.
- Waterproof case
 - Protects the audio recorder.
- Zip tie / strap / etc.
 - To strap the audio recorder to a tree.
- Flagging tape (if the pool is on public land)
 - Write your or the VPMon Project Coordinator's phone number on it.
 - Tie it to the tree the acoustic monitor is strapped to.
- Smartphone with "AudioMoth" app* * Necessary for configuring the AudioMoth internal clock once batteries are inserted

<u>Other</u>

- VPMon data sheet & writing utensil
 - Use a sheet for every visit, even if you don't need to fill out the entire form.
- ThermoPro Indoor Humidity & Temperature Monitor
 - Measures the air temperature and humidity around the pool.
- GPS / Smartphone with an app that can show GPS coordinates
 - To obtain the GPS coordinates of the pool and ensure that you're in the right place.

For Home Use

- Boot/Wader Decontamination Equipment
 - To clean waders and thus prevent the spread of amphibian diseases.
 - See page 22.

Data Submission

Data collected during each visit to the vernal pool should be submitted as soon as possible after returning from the field. This is to make sure that no data is lost or forgotten. All data from Visits 1 through 3 should absolutely be submitted by July 15th. Below you'll find exactly what should be submitted within a few days after each visit to an "adopted" vernal pool.

• Visit 1: Setup

- <u>Data Sheet</u>: Pool & Observer Info, Physical Parameters (except for % vegetation-covered), and Weather.
 - Egg Mass and Macroinvertebrate Survey sections should also be completed if the pool has open water; <u>Photos</u> of egg masses and macroinvertebrates should also be submitted.

• Visit 2: Early Survey

- <u>Data Sheet</u>: all sections.
- <u>Photos</u> of egg masses and macroinvertebrates.

• Visit 3: Late Survey

- <u>Data Sheet</u>: all sections.
- <u>Photos</u> of egg masses and macroinvertebrates.
- <u>HOBO Water Temperature Data</u> (If HOBO logger is accessible and you have a smartphone to download the data with).
- o <u>Audio Recordings</u>
- Visit 4: Fall Visit
 - <u>Data Sheet</u>: Pool & Observer Info, Physical Parameters (except for % vegetation-covered), and Weather.
 - HOBO Water Temperature Data

How to Submit Data

Data Sheet: Scan the datasheet to a computer and email it to vpmondata@vtecostudies.org

- The subject line of this email and the name of the file should be "Pool ID_Your Last Name & First Initial Year Visit Number."
 - Example for Visit 1: MLS123_DarwinC_2021_1
- If you can't scan or email the datasheet, please mail a copy to:

Vermont Center for Ecostudies Attn: Vernal Pool Monitoring Project PO Box 420 | Norwich, VT 05055

<u>Photos</u>: See page 33 for instructions for submitting photos to the VPMon iNaturalist project.

HOBO Water Temperature Data: See page 12 for a guide on how to submit this (requires a smartphone).

- If you simply cannot obtain a smartphone, please contact the VPMon Project Coordinator.

<u>Audio Recordings</u>: Download & save the recorded data to a computer using the USB that extends from the bottom of the recorder. Then mail the microSD card from the audio recorder to VCE's P.O. Box (see above).

 The microSD card is very fragile, so when mailing it please place it between two small pieces of cardboard (tape these together). If possible, send it in a padded envelope.

NOTE: VCE is currently developing a public, online database for VPMon. Once it is available, volunteers will be able to submit all data online, and this section will need to be updated.

CHAPTER III VPMON PROTOCOLS

HOBO Logger Protocols

Strapped to a PVC pipe that is inserted into the deepest point in the pool, the compact and durable HOBO Logger automatically collects water temperature data. Once the pool dries up and the HOBO is out of the water, it begins measuring the air temperature; this transition is very obvious and it tells us when a vernal pool dries. As well, the PVC pipe, which is marked in 5 cm increments, is used as a depth gauge to determine the water level at the time of each vernal pool visit.

Go to page 9 to see what equipment is needed to carry out these protocols.

When to set it up

Generally, HOBO Loggers and PVC pipes will be set up when Monitors first visit their adopted vernal pool in the early spring (Visit 1). If possible, it is best to set up this equipment in the summer or fall prior to your first monitoring season, when the pool is at its driest.

Monitors should take care to only set up the HOBO Logger during Visit 1 if the ice is thin enough to easily break through while walking with waders along the pool bottom or thick enough that it can support their weight comfortably. If the ice is at an intermediate thickness, (where it may break with a Monitor standing on top of it) it is best to wait until Visit 2 to set up the HOBO Logger.

Installing the PVC pipe & HOBO Logger in water

- 1. Use 2 zip ties to attach the HOBO logger to one end of the 5' PVC Pipe.
 - $-\,$ The HOBO Logger should be configured before you attach it to the PVC Pipe.
 - For a guide on how to configure the HOBO Logger see page 31.
- 2. Locate the deepest point in the pool.
 - This will likely require breaking through ice and wading into potentially deep water, so be sure to bring waders and a tool to break through ice (i.e. an ice spud or auger).
 - Don't worry if you can't locate the exact deepest point in the pool. The PVC pipe can be moved during a later visit when there is less water and ice.
 - Remember to wash your boots / waders afterwards to prevent the spread of amphibian diseases. See page 23 for instructions on this.
- 3. Push the end of the PVC pipe into the pool bottom, so that it is both vertical and feels very secure.
- 4. Slide the HOBO Logger from the top of the PVC pipe to the bottom so that it sits just above the pool floor, but isn't buried in leaf litter or muck.
 - You will likely have to stick your arm into the cold water, so dress preparedly.
- 5. Use a measuring tool to determine the depth of the water at the PVC pipe. Write this number on the 5cm marking at the water level and number every marking above it in 5 cm increments.
 - As the water level drops you can add in lower increments.

The HOBO Logger, strapped securely to the PVC pipe.

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Installing the PVC pipe & HOBO Logger in a dry vernal pool

- 1. Find the deepest point in the pool basin and stick the PVC pipe into the ground there, such that it is both vertical and feels very secure.
 - One of the 5 cm markings should line up with the ground.
 - If necessary, use a rock or hammer to pound the PVC pipe into the ground.
- 2. Attach the HOBO Logger to the bottom of the PVC pipe with 2 zip ties, so that it sits about 1 inch off the ground.
- 3. Label the 5 cm marking at ground level as 0 and label all the markings above it in 5 cm increments.

Downloading Data without a Smartphone

A Bluetooth enabled smartphone is necessary for downloading data from the HOBO Logger. If you do not have a smartphone in the field for Visit 3 then do not worry about collecting data from the HOBO Logger. If you do not have a smartphone in the field for Visit 4 then you will have to take the HOBO Logger out of the field and reattach it to the PVC pipe during a future visit.

If there is absolutely no way for you to obtain a smartphone at home with which to download HOBO Logger data, contact the VPMon Project Coordinator. You may need to mail the logger to VCE so that the data can be downloaded.

Downloading & Submitting Data with a Smartphone (Bluetooth cannot transmit data through water, so the pool must either be dry to download the data, or you will need to slide the Logger up the PVC pipe.)

- 1. Open the <u>HOBO Mobile</u> app (^{H000}) and tap the name of your HOBO Logger in the light blue rectangle under IN RANGE to connect with it. (Your logger name is "Pool ID Your Last Name & First Initial.")
 - Make sure that your phone's Bluetooth is turned on
 - Press the white target icon in the center of the HOBO Logger's blue face to open a connection.
 - If you're trying to connect to the HOBO it doesn't appear in the app, power your phone totally off and then turn it (and the Bluetooth) back on.
 - If that fails, the Logger will need to be retrieved from the pool to have its data downloaded.
- 2. Tap <u>Readout</u> (Readout) to download the data to the smartphone.
- 3. When you have phone service or WiFi, tap <u>Data Files</u> (Data Files) at the bottom of the screen.
 - If you're asked to close the connection, click yes.
- 4. Tap <u>Select</u> in the top right corner and then tap the graph or graphs that you want to submit (the graph you just downloaded will be the first one).
- 5. On the bottom left corner of the screen, tap Share and then tap .XLSX (
- 6. Finally, tap in the upper right corner of the screen and email your data to <u>vpmondata@vtecostudies.org</u>.
 - Title the email "Pool ID_Your Last Name & First Initial_Year_HOBO"
 - Example: MLS699_DarwinC_2019_HOBO



Step 1



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Step 4

Reconfiguring the HOBO Logger

After your first spring of monitoring, the HOBO logger can be reconfigured when you visit the pool in latesummer or fall (Visit 4). It is much easier to access and reconfigure the HOBO Logger at this time since pools are typically dry or quite shallow. If you reconfigure the HOBO Logger in the field every time you visit the vernal pool in late-summer/early-fall, you'll never have to take it out of the field. Of course, the HOBO Logger can also be reconfigured when you first visit the pool in the spring (Visit 1), but the water will be at its deepest (and frigid!).

To reconfigure the HOBO Logger in the field and prepare it for another season of temperature monitoring, you'll need **a smartphone with the HOBOmobile app** and (just in case) **a new CR2032 3V lithium battery**.

- 1. Open the <u>HOBO Mobile app</u> (^{HOBD}) and tap the name of your HOBO Logger in the light blue rectangle under IN RANGE to connect with it.
 - Make sure that the Bluetooth on your smartphone is turned on.
 - Your phone should be within 5-10 feet of the HOBO Logger.
 - If the logger is submerged in water you will have to slide it up the PVC pole above the water in order to make a connection (water inhibits the Bluetooth signal).
 - Manually press down on the white target icon in the center of the HOBO's blue face, the HOBO should show up as IN RANGE. Sometimes the button requires a solid push.
 - If the logger doesn't appear, shut down your phone and then turn it back on. This resets the Bluetooth signal.
- Download the temperature data from the HOBO Logger

 See the previous page for instructions on how to do this
- 3. Tap on <u>Full Status Details</u> () and then check the <u>Battery</u> <u>Level</u> of the HOBO Logger.
 - If the HOBO Logger has less than 50% battery when you check it during the fall then you will need to replace its old battery with a new CR2032 lithium battery.
 - See the next page for instructions on how to change out the battery.
- 4. Tap on <u>Configure</u> (\checkmark) and enter in the same information that you would for configuring the HOBO Logger in the springtime (see the previous page).

Tap on Start in the upper right hand corner of the screen. Your HOBO Logger is now reconfigured and ready to be hunkered down for the winter in the vernal pool!



After configuring or reconfiguring the HOBO Logger, the configuration menu should look like this.

Acoustic Monitor Protocols

What sound more evokes the thawing of winter than a chorus of spring peepers? By setting out an audio recorder at a springtime vernal pool, VPMon can investigate both which species of frog are calling at a vernal pool and when they are calling. The latter is particularly important in the face of climate change; as winter grows shorter and shorter, how will the breeding phenology of vernal pool amphibians respond?

Go to page 9 to see what equipment is needed to carry out these protocols.

When you receive your AudioMoth, it will already be programmed with the correct settings. Once you insert the microSD, followed by three AA batteries, you'll need to use the AudioMoth smartphone app to synchronize its internal clock. Lithium batteries are ideal, as they are longer-lasting, but Duracell Coppertop Alkaline batteries work as well. New batteries are needed for each new monitoring season.

When to Set Up & Collect the Acoustic Monitor

The audio recording equipment should be placed in the field when you first visit the vernal pool in the spring (Visit 1). It is essential that it is placed in the field prior to the first date of frog chorusing, so that the timing of this event can be recorded. Frog chorusing occurs on "big nights," which are typically triggered by an evening rain event with temperatures above 40° F and some bare, snowless ground. To be safe, it is best to conduct Visit 1 before or when the pool begins to thaw.

The audio recorder should be removed from the field on Visit 3 and its data should be promptly downloaded to your computer and submitted to VCE.

Deploying the Acoustic Monitor in the Field

Prior to going into the field

- 1. Ensure the AudioMoth's switch is set to "USB/OFF".
- 2. Insert the microSD received from the VPMon coordinator into the AudioMoth.
 - The microSD slot is marked with a white icon.
 - The microSD must be inserted with the metal contacts facing upwards.
- 3. Insert three AA batteries in the battery compartment.
- 4. Switch the AudioMoth's switch from "USB/OFF" to "Custom".a. The red light should remain constant while the green light blinks.
- 5. Using the AudioMoth smartphone app*, synchronize the AudioMoth's internal clock by hitting "play chime".
 - a. If the system is programmed correctly, once the chime is registered the red light will shut off while the green light stays lit for 2s.
 - b. It is imperative that the internal clock is synchronized every time the batteries are removed and replaced. When not synchronized, the red light will stay constant while the green light flashes.

*Contact the VPMon coordinator for instructions on how to do this using a computer instead of smartphone.



How to insert a MicroSD to an AudioMoth.





<u>In the field</u>

While "sleeping" between recording periods, the red light will remain off while the green light blinks.

- 1. Place the AudioMoth into the waterproof case.
 - Ensure that the AudioMoth's microphone is lined up with the black acoustic vent.
- 2. Using a zip tie, strap, or cord, mount the audio recording equipment on a sturdy, living tree nearest to the northernmost edge of the pool.
 - If there is no tree within 3 m of the northernmost point of the pool, attach it to the closest tree and note the distance between the audio recorder and the pool on the datasheet.

Downloading & Submitting Data from the Audio Recorder*

- Remove the microSD from the AudioMoth by pressing it in gently and letting it eject.
 Never pull on the green circuit board, it may separate from the battery compartment.
- 2. If possible, backup the microSD card files to your computer before returning to VCE.
- 3. Mail the microSD card to the following address:
 - It should be mailed inside of a padded envelope after being placed between two pieces of cardboard for safekeeping.

Vermont Center for Ecostudies Attn: Vernal Pool Monitoring Project PO Box 420 Norwich, VT 05055

- 4. Please remove the batteries between seasons to reduce potential leakage & corrosion.
- * You will soon be able to submit these data online. If you are monitoring in the 2022 season or beyond, then check the VPMon page of the VCE website to see if you should submit frog call data online.

Egg Mass Survey Protocols

Likely the most time-intensive task for each Vernal Pool Monitor will be conducting egg mass surveys of pool-breeding amphibians. By counting egg masses and accurately identifying the species, we can estimate the amphibian breeding population size at a given vernal pool. A key part of this protocol is knowing how to correctly identify the egg masses of the vernal pool indicator species in Vermont, which is covered in Chapter 4 (page 24).

Egg mass survey results are submitted as part of the VPMon data sheet. Instructions for completing and submitting the data sheet can be found on pages 19 to 21. Any photos taken as part of the egg mass survey should be submitted to the VPMon iNaturalist Project (see page 33).

Go to page 9 to see what equipment is needed to conduct egg mass surveys.

When to Survey for Egg Masses

Ideally, you will only survey for amphibian egg masses during Visit 2 (1-2 weeks after the peak of the Wood Frog breeding period) and





Location of AM's microphone.

Visit 3 (2-3 weeks after the peak of the Spotted Salamander breeding period).

However, if there is at least 1 m² of open water in the pool during Visit 1, then you should conduct an egg mass survey during this visit as well.

How to Conduct an Egg Mass Survey

Because the egg mass surveys are based on a **double-observer independent protocol** (meaning that both Monitors conduct surveys, but they do so independent from one another), it is not necessary for the two Monitors to visit their adopted vernal pool together. However,

both Monitors must conduct egg mass surveys on the same day.

If you do visit your adopted vernal pool with your monitoring partner, then while you conduct your egg mass survey, your partner should not be paying attention to you. This is to avoid biasing their own independent survey.

- Mark your starting location and walk the perimeter of the pool while counting the number of egg masses of each species that you see within 1 meter of the pool's edge. Tally these in the "Egg Mass Edge Survey" section of the data sheet.*
 - A meter stick or walking stick marked at 1 meter in length is helpful for identifying what is within the survey area.
- 2. After returning to your starting point, scan all areas beyond the 1 meter survey area for egg masses. Tally these in the "Egg Mass Interior Survey" section of the data sheet.*



<u>Red</u>: survey area for the Egg Mass Edge Survey at a given vernal pool. <u>Blue</u>: survey area for the Egg Mass Interior Survey at a given vernal pool.

— Binoculars are highly recommended for this. If you use them mark so on the data sheet.

* Keep the following points in mind when conducting Steps 1. and 2.

- Consult Chapter 4 for help on identifying amphibian egg masses (page 25).
- Polarized glasses are highly recommended for the Egg Mass Surveys, as they help to counter glare on the water surface. If you do use polarized glasses, mark this on the data sheet. If your pool is well-shaded by conifers or it is an overcast day, polarized glasses may be too dark and actually hinder observations, so use your best judgement.
- If wood frog egg masses have formed into a "raft" and are uncountable, then estimate the dimensions of this "raft" in the "Amphibian/Macroinvertebrate Notes" section.
- For <u>each</u> species of amphibian egg mass that you observe, take a photo of one egg mass and submit it to the VPMon iNaturalist project (See the iNaturalist Submission Guide on page 33).
- If you observe signs of **amphibian presence** (tadpoles, adult amphibians, etc.) note this in the "Amphibian/Macroinvertebrate Notes" section of the data sheet.
- If you observe spermatophores (see page 28), mark this on the data sheet and submit a photo to the VPMon iNaturalist project.

- If you observe any sign of **amphibian disease** (See Table 4, next page) write all details (including the affected species) in the "Amphibian/Macroinvertebrate Notes" section.
 - As well, be sure to take photos and submit them to iNaturalist.
 - Contact the VPMon Project Coordinator if you see any sign of amphibian disease.
- 3. Estimate the proportion of the water within 1 meter of the pool's edge that is visually impaired. Then estimate visual impairment for all areas of the vernal pool beyond this 1 meter area.
 - Use Table 3 Visual Impairment (also on the data sheet) to quantify the impairment level.
 - Visual impairment may be due to depth, surface reflection, particulate matter, algae, etc.
 - Do not consider areas covered with ice within this estimate.
 - If you used polarized glasses to counter pool reflectance, you should wear them while estimating visual impairment.
- Table 3 Visual Impairment

 Level
 Description
 Impaired

 0
 Clear Survey area not visually impaired

 1
 Slightly impaired Less than 25% of the survey area is visually impaired

 2
 Impaired 25 to 50% of the survey area is visually impaired

 3
 Severely Impaired More than 50% of the survey area is visually impaired
- 4. If there are any non-contiguous vernal pools within 20 meters of the survey area is visually impaire your adopted vernal pool, check them qualitatively for egg masses, but do not conduct another full Egg Mass Survey.
 - If you observe egg masses in these nearby vernal pools note this in the "Amphibian/ Macroinvertebrate Notes" section with a rough estimate of their quantity.

Table 4 - Amphibian Diseases: Be alert to any sign of these diseases while at your adopted pool. If you see any sign of an amphibian disease note it in the "Amphibian/Macroinvertebrate Notes" section of the data sheet and **contact the VPMon Project Coordinator as soon as possible.** Take plenty of photos and submit them to the VPMon iNaturalist Project (See the iNaturalist Submission Guide on page 33).

Disease	Background	Signs / Symptoms
Chytridiomycosis / Bsal (Batrachochytrium salamandrivorans)	 Not documented in North America, but high global concern due to risk of import through pet trade Susceptibility of species in U.S. unknown 	 Skin lesions and deformities on salamanders.
Ranaviruses / Rv	 Can infect amphibians, reptiles, & fish Usually occurs in larvae and metamorphs Hundreds of sick/dead individuals may be observed at affected sites over a few days. Mass mortality events have been documented in New England 	 Dead and/or decomposing individuals found in / along pool. Extensive reddening (hemorrhaging) on underside, especially at base of legs and vent. Swelling (edema) in body and legs
Chytridiomycosis / Bd (Batrachochytrium dendrobatidis)	 Kills frogs and toads within 2-3 weeks of metamorphosis Occasionally lethal in adult newts Die-offs gradual: rarely more than two dead frogs or toads at a site. Detected in New England, but there have been no documented cases of mass mortality. 	 Adults: skin abnormalities, extended back legs, lethargy, loss of righting reflex. Larvae: jaw sheaths and tooth rows lack pigment or appear deformed (non-lethal).

Perkinsus-like Organism	 Kills tadpoles in the family <i>Ranidae</i> (rarely other species). Die-offs may proceed for weeks at a pool Often causes greater than 90% mortality Has been detected in New England 	 Dead, floating tadpoles Enlarged light yellow liver/spleen Abdominal distention 			
Ichthyophoniasis	 Infects newts and larval / adult frogs in the family <i>Ranidae</i> Slight to severe infection of skeletal muscle 	 — "Swollen rumps" in recently metamorphosed frogs. 			

Macroinvertebrate Survey Protocols

Invertebrates are key to the functioning of all ecosystems, and vernal pools are no exception. As both predator and prey, macroinvertebrates fill key roles across the vernal pool food web, and they make up the majority of the species and biomass in each one (Calhoun and DeMaynadier 2008). Vernal Pool Monitors conduct surveys for two kinds of macroinvertebrate: fairy shrimp and caddisfly larvae. In Vermont, fairy shrimp are only found in a subset of vernal pools, where they hatch, develop, and breed shortly after ice-out. Caddisfly larvae and their cases, on the other hand, are readily found in a variety of water bodies.

Macroinvertebrate survey results are submitted as part of the VPMon data sheet (see pages 19 to 21). Any photos taken as part of the survey should be submitted to the VPMon iNaturalist Project (see page 33).

Go to page 9 to see what equipment is needed to conduct macroinvertebrate surveys.

When to Survey for Macroinvertebrates

Ideally, you will only survey for macroinvertebrates during Visit 2 and Visit 3.

However, if there is at least 1 m² of open water in the pool during Visit 1, then you should conduct a macroinvertebrate survey during this visit as well.

How to Conduct a Macroinvertebrate Survey (Visits 2 and 3)

Unlike the egg mass survey, the macroinvertebrate survey does not require a double-observer independent protocol and it can be completed by just one Monitor. As well, this protocol assumes that you have already estimated the Visual Impairment Level of the vernal pool as part of the egg mass survey.

- 1. Survey a $1m^2$ area at the northern end of the vernal pool.
 - Macroinvertebrate surveys occur within four 1 m² survey plots distributed evenly around the pool (one plot at each cardinal direction: North, East, South, & West).
 - Use a tape measure or a walking stick marked at 1 meter to demarcate each survey plot.
 - Constructing a 1m x 1m frame out of PVC pipe is a good method of delineating sample plots.
 - If you aren't sure which end of the pool is the north end, just make your best guess and be sure to survey for macroinvertebrates at 90°, 180°, and 270° away from this first plot.
- 2. Within this area, record the number of fairy shrimp & caddisfly larvae (including caddisfly cases) that you observe. Write this number in the "N. Square" box on the data sheet.
 - Look, but don't touch while observing. Don't turn over any leaf litter or sticks in the survey area.
 - Polarized glasses are highly recommended for this, as they help to counter the glare on the water. If you use polarized glasses, mark this on the data sheet.
 - Fairy shrimp in particular can be difficult to count, but try to do so to the best of your ability.
 - If you observe fairy shrimp or caddisfly larvae, be sure to take a photo and submit it to the VPMon iNaturalist group (See the iNaturalist Submission Guide on page 33).

3. Repeat steps 1. and 2. as you progress clockwise around the vernal pool, making observations at the eastern, southern, and western ends of the pool.

How to Conduct a Macroinvertebrate Survey (Visit 1)

If there is at least 1 m² of open water when you first visit the vernal pool in the spring (Visit 1), you should survey for macroinvertebrates within up to four 1 m² survey plots of open water. Spread out these 1 m² survey plots as much as possible within the area of open water. Count the number of fairy shrimp and caddisfly larvae/cases, and write these numbers on the data sheet.

VPMon Data Sheet Protocols

Most of the information collected each time you visit your adopted vernal pool is recorded on the VPMon Data Sheet. An example of a VPMon data sheet can be found on the very last page of this manual.

Filling out the Data Sheet

POOL & OBSERVER INFO

Date: When is this field visit taking place?

Time: At what time did you begin data collection?

Observer Names: Who is/are collecting the data on this data sheet?

- Be sure observer names are listed in accordance with "Obs. 1" & "Obs. 2" for the egg mass survey.

Pool ID: A code of 3+ letters followed by numbers, which identifies a given vernal pool (e.g. MLS699).

- If the pool was mapped as part of the Vernal Pool Mapping Project, it will already have a Pool ID.
- If this was a previously unmapped pool you will be given an ID by the VPMon Project Coordinator.

Town: In which town is the vernal pool located?

GPS Coordinates: What is the latitude and longitude of the vernal pool?

- Only fill out this section on Visit 1: Setup, to ensure that you're at the right location.
- During future visits the presence of the PVC pipe will indicate that you're at the correct location.
- For instructions on finding your GPS location with a smartphone, see page 24.

Field Visit: Check whichever of the four VPMon field visits this data sheet describes.

- For an explanation of the four VPMon field visits, see page 6.
- If this is Visit 1: Setup, be sure to deploy the audio recorder and note this date in the Amphibian/Macroinvertebrate Surveys section.
- If this is Visit 3: Late Survey, be sure to collect the audio recorder, and note this date in the Amphibian/Macroinvertebrate Surveys section.

Is Pool Mapped?: Has a Vernal Pool Atlas (VPAtlas) data sheet been filled out for this pool?

- <u>Yes, through VPAtlas</u>: If the pool was mapped through VPAtlas.
- Yes, by VPMon volunteer: If you or another VPAtlas volunteer have filled out a VPAtlas data sheet.
- Not mapped: If the pool hasn't been mapped, you'll need to fill out a VPAtlas data sheet on Visit 3.
 - See page 22 for instructions on filling out the Vernal Pool Mapping data sheet

Acoustic Monitor: Was the acoustic monitor deployed or collected on this visit?

- <u>Deployed (Visit 1)</u>: If you set up the acoustic monitor on this visit.
 - If you're deploying the acoustic monitor it is essential to turn all presets ON (see page 13).
- <u>Already deployed (Visit 2)</u>: If the acoustic monitor was set up in the previous visit.
- <u>Collected (Visit 3)</u>: If you have collected the acoustic monitor on this visit.

• If you're collecting the acoustic monitor remember to turn all presets OFF (see page 13).

HOBO Logger: Have the HOBO Logger and PVC Pipe been set up on this visit?

- <u>Set up on this visit</u>: If you set up the configured HOBO Logger on this visit (likely Visit 1).
- <u>Already in the pool</u>: If the HOBO logger is already collecting data in the pool (likely Visits 2 or 3).
- <u>Reconfigured on this visit</u>: If the HOBO Logger was already in the field, but you reconfigured it on this visit (likely Visit 4).

PHYSICAL PARAMETERS

% Ice-Covered: What percentage of the pool is covered in ice?

- Use the Veg / Ice Percent Cover table at the bottom of the data sheet to estimate this.

Water Level: How high is the water in the vernal pool, according to the PVC pipe?

- If you set up the PVC pipe on this visit, measure water level after having done this.
- If the vernal pool is dry, record 0 cm.

HOBO Water Temperature: Did you download water temperature data from the HOBO logger?

- Except for on Visit 4, downloading HOBO data is not required if you do not have a smartphone.
- For instructions on how to download data from the HOBO Logger see page 12.
- If you have trouble downloading the HOBO logger data, note this in Physical Parameter Notes.

% Vegetation-Covered: What percentage of the pool bottom is covered/shaded by each category?

- Only fill out this section on Visit 2: Early Survey and Visit 3: Late Survey
- Use the Veg / Ice Percent Cover Table at the bottom of the data sheet to estimate this.
- The vegetation categories are:
 - <u>Submerged Aquatic</u> <u>Floating Aquatic</u> <u>Emergent</u> <u>Shrubs</u> <u>Trees</u>
 Emergent is defined as vegetation that emerges out of the vernal pool.
- Percent cover should account for any vegetation that covers or shades the pool (including tree canopy), regardless of whether the vegetation emerges from the pool itself.
- If trees and other vegetation aren't fully leafed out, don't attempt to predict percent cover under full leaf out. Instead, just report the actual, current percent cover.

Physical Parameter Notes: Any additional notes on the physical parameters of the vernal pool.

This may also include any trouble you had connecting to the submerged HOBO Logger or any
physical changes that have occurred at the vernal pool since the VPAtlas data sheet was filled out.

WEATHER

Air Temperature: What is the air temperature at the vernal pool?

 Place the ThermoPro Indoor Humidity & Temperature Monitor in a shaded location within 1 m of the edge of the vernal pool and leave it for at least one minute before recording the humidity.

Relative Humidity: What is the humidity at the vernal pool?

 Place the ThermoPro Indoor Humidity & Temperature Monitor in a shaded location within 1 m of the edge of the vernal pool and leave it for at least one minute before recording the temperature.

Wind (Beaufort Force): What is the wind speed at the vernal pool?

- Use the Wind: Beaufort Scale table at the bottom of the data sheet to estimate this.

Conditions: Which option best describes the weather conditions at the time of data collection?

Weather Notes: Any additional notes on the weather conditions at the vernal pool.

AMPHIBIAN & MACROINVERTEBRATE SURVEYS

Egg Mass Survey: Survey the vernal pool for egg masses using the protocols described on page 16.

- Only fill out this section on Visit 2: Early Survey and Visit 3: Late Survey
 - And on Visit 1: Setup (if there is at least 1 m² of open water)
- The Egg Mass Survey is broken up into two sections:
 - The Edge Survey, in which all water within 1 m of the pool's edge is surveyed.
 - The Interior Survey, in which all water more than 1 m from the pool's edge is surveyed.
- This survey follows a double-observer independent protocol, so while "Obs. 1" conducts their survey, "Obs. 2" should not be paying attention.
- Each acronym refers to a vernal pool-indicator amphibian:
 - <u>WOFR</u>: Wood Frog <u>JESA</u>: Jefferson Salamander
 - <u>SPSA</u>: Spotted Salamander <u>BLSP</u>: Blue-Spotted Salamander
- Remember to take photos and submit them to the VPMon iNaturalist Project (see page 33).

Macroinvertebrate Survey: For instructions on how to conduct this survey, see page 18.

- Only fill out this section on Visit 2: Early Survey and Visit 3: Late Survey
 - And on Visit 1: Setup (if there is at least 1 m² of open water)
- Remember to take photos and submit them to the VPMon iNaturalist Project (see page 33).

Spermatophores Found?: Did you notice any spermatophores in the vernal pool?

- Remember to take photos and submit them to the VPMon iNaturalist Project (see page 33).

Amphibian/Macroinvertebrate Notes: Record any additional notes or observations from the surveys.

- Did you notice any sign of amphibian disease? See page 17 for the Amphibian Disease table.
 - If you did see signs of amphibian disease, contact the VPMon Project Coordinator immediately and submit photos to the VPMon iNaturalist Project (see page 33).
- Are there other, non-contiguous vernal pools within 20 meters of your adopted pool, and if so did you notice any egg masses in them? Roughly how many?

Visual Impairment: What proportions of the search areas are visually impaired (See page 17)?

- Use the Visual Impairment table at the bottom of the data sheet to estimate this.
- Make a separate estimate for the Egg Mass Edge Survey search area (all water within 1 m of the pool's edge) and the Interior Survey search area (all water more than 1 m from the pool's edge).
- Impairment may be due to water depth, surface reflection, particulate matter, algae, etc.
 - But do not consider areas of the pool obstructed by ice.
- Use polarized glasses to estimate the Visual Impairment Level if you will also be using them for the Egg Mass Survey.
 - If so, be sure to check the "Yes" box for "Did you use polarized lenses?"

Submitting your Data Sheet

- 1. Scan the data sheet to your computer or make a copy of it soon after returning from the field.
 - The file should be saved as "Pool ID_Your Last Name & First Initial_Year_Visit Number."
 Example for Visit 1: MLS699_DarwinC_2019_1
- 2a. Email this file to <u>vpmondata@vtecostudies.org</u>
 - The subject line of the email should be the same as the file name.

Vermont Center for Ecostudies Vernal Pool Monitoring Project PO Box 420 Norwich, VT 05055

2b. If you cannot email the data sheet, mail a copy to this address:

Other Protocols

Vernal Pool Mapping: a Prerequisite for Monitoring

More than 5,000 potential and known pools have been mapped by the Vermont Vernal Pool Atlas (VPAtlas), some of which are now being monitored through VPMon. More information on VPAtlas can be found on the <u>VPAtlas Project page of the VCE website</u>.

One key aspect of VPAtlas is that community scientists fill out VPAtlas data sheets to confirm that remotely mapped vernal pools are actually vernal pools. These data sheets provide an idea of the important physical characteristics of a vernal pool and it is required that each monitored pool have a completed VPAtlas sheet. **If your adopted vernal pool doesn't have a completed VPAtlas data sheet, then you will need to fill one out when you visit the vernal pool for Visit 3: Late Survey.** If you don't know if your pool has a completed VPAtlas data sheet, ask the VPAtlas Project Coordinator.

A new VPAtlas data sheet doesn't need to be filled out each year, but if there have been any physical changes to the vernal pool since the data sheet was filled out, you should note this in the Physical Parameter Notes section of the VPMon data sheet. For instance, if agriculture was not noted near the pool when the pool was first mapped, but now you find that a farmer is growing crops nearby, you should note this on the VPMon data sheet.



More than 5,000 potential pools were mapped with remote imagery through the Vermont Vernal Pool Mapping Project, many of which were verified in the field with a VPAtlas data sheet.

Printable VPAtlas data sheets can be found by <u>clicking here</u>, which takes you to the VPAtlas Volunteer Materials page of the Vermont Center for Ecostudies website.

Equipment Decontamination

The VPMon protocol is designed to minimize wading into the vernal pool, but it's never a bad idea to decontaminate one's gear (boots, waders, buckets, nets, etc) after field monitoring. **It is imperative however, that you decontaminate your gear between visits to different vernal pools.** This limits the potential spread of amphibian diseases and pathogens, particularly Chytridiomycosis and Ranavirus.

How to Decontaminate your Gear

1. Mix together a 3% beach solution

- Most household bleaches are a 6% solution. To make a 3% solution add 1 part 6% bleach for 32 parts water.
- Store in an opaque container. If exposed to sunlight or air this mixed solution will only last for five days.
- 2. Thoroughly scrub and rinse all equipment
 - Be careful not to decontaminate your gear near a drainage, vernal pool, or any other aquatic ecosystem.
- 3. Liberally spray bleach solution on all equipment, and then wait 5 minutes.
- 4. Thoroughly rinse and dry the equipment.

Suggested Decontamination Equipment

- 3% bleach solution
- Clean water for rinsing
- Brush for scrubbing and/or removing mud & vegetation from equipment
- Hand sanitizers
- Handheld bottles and/or pump sprayers for applying bleach and water.

Leaf Litter Composition Survey Protocols

The composition of leaf litter in vernal pools plays a large role in both a pool's water chemistry and its detritus- based food web. It's important to develop baseline data on the species of leaves that line the basin of a vernal pool to get a better understanding of future changes that may result. This survey will be conducted once every 5 years to track major changes in leaf litter composition.

A good supplemental guide, such as The Forest Trees of Maine, is a useful resource that's available online for free from The State of Maine's website. Additionally, iNaturalist can be used to crowdsource unknown leaf species (See iNaturalist Submission Guide on page 33).

How to Conduct a Leaf Litter Composition Survey (Visit 3)

This survey is similar to the macroinvertebrate survey in that you will be surveying four 1m² plots spaced around the pool. However, rather than sampling within pool itself you will be sampling along the outside edge of the pool.

1. Mark a $1m^2$ survey area along the northern outside edge of the pool.

- You will survey four $1m^2$ plots spaced around the outside edge of the pond in the four cardinal directions (north, east, south, and west).
- Use a tape measure or walking stick marked at 1 meter to demarcate each survey plot.
 - Constructing a 1m x 1m frame out of PVC pipe is a good method of delineating sample plots.
- Within each plot the surface should be entirely leaf litter, no bare dirt or rocks.
- 2. Estimate the percent cover of each species of leaf and needle present.
 - Only the top layer of leaf litter should be sampled.
 - Leaves should be identified to as close to species as possible.
 - If in doubt about an ID, post photos (the leaf's top and underside) to the VPMon iNaturalist group to crowdsource a conclusive ID (see iNaturalist Submission Guide on page 33)
- 3. Repeat steps 1. and 2. as you progress clockwise around the vernal pool, making observations at the eastern, southern, and western ends of the pool.
 - After sampling the four plots, add up each column's percentages to ensure that each plot's total leaf litter cover comes out to 100%.

If you have access to a spreadsheet application (Excel, Numbers, etc) or Google Docs please transfer the data sheet to a spreadsheet to send to us. If you don't, please submit your data sheet as you do for regular vernal pool monitoring visits. Please name the file "Pool ID_Your Last Name & First Initial_Year_LLSurvey".

- Example: "MLS699 DarwinC 2019 LLSurvey"

Using your Smartphone as a GPS

When you fill out the data sheet for Visit 1 (or if you fill out a Vernal Pool Mapping data sheet) it is essential to note the latitude and longitude of the vernal pool. This is both to make sure that you're collecting data at the correct pool and to know whether or not this pool is already in the database of vernal pools created by VPAtlas.

If you have your own GPS unit then you should feel free to use that. However if you do not, then GPS coordinates can be easily obtained with the help of a smartphone, even if there's no phone service at your adopted vernal pool. There are several smartphone apps that can be used to find latitude and longitude, but the below instructions show how to do so with the My GPS Coordinates app.

With an Apple iPhone

- 1. On your smartphone, download the free <u>My GPS Coordinates app</u> (²⁶) from the App Store.
- 2. Allow your phone and the app to access your location:
 - a. Open the **Settings** app on your smartphone.
 - b. Tap **Privacy** and then tap **Location Services**.
 - c. Switch Location Services to on (green).
 - d. Find My GPS Coordinates in the list of apps under this on/off switch and tap on it.
 - e. Select While Using the App.
 - f. Press the Home button to exit Settings.
- 3. Open the <u>My GPS Coordinates app</u> and your latitude and longitude will be shown on the screen.
 - Make sure to write the coordinates in decimal degrees format with at least five decimal places.
 e.g. 44.76432, -72.65422

With an Android phone

- The extra options for steps b. and c. are for older versions of the Android operating system.
- 1. On your smartphone, download the <u>My GPS Coordinates app</u> (¹) from the Google Play store.

2. Allow your phone to access your location:

- a. Open the **Settings** app on your smartphone.
- b. Tap Security & Location and then tap Location.
 - b2. If you don't see Security & Location, tap **Location**.
 - b3. If you don't see either of these options, tap **Location access**.
- c. Switch Use location to on.
 - c2. If you don't see Use location, tap **Mode**, and then select **High Accuracy**. Skip to Step f.
 - c3. If you clicked on Location access in Step b., switch **Access to my location** to on, and then select both **GPS Satellites** and **Wi-Fi & mobile network location**. Skip to Step f.
- d. Tap Advanced and then tap Google Location Accuracy.
- e. Switch Improve Location Accuracy to on.
 - This allows the phone to use GPS, Wi-Fi, mobile networks, and sensors to show a more accurate location.
- f. Press the Home button to exit Settings.
- 3. Open the <u>My GPS Coordinates</u> app and your latitude and longitude will be shown on the screen.
 - Make sure to write the coordinates in decimal degrees format with at least five decimal places.
 - e.g. 44.76432, -72.65422

CHAPTER IV

IDENTIFYING SPECIES AND THEIR EGG MASSES

Amphibian Identification

There are four species of amphibian in Vermont that are specifically adapted to breeding in the ephemeral waters of vernal pools. Most populations of Wood Frogs, Spotted Salamanders, Jefferson Salamanders, and Blue-Spotted Salamanders depend on the presence of vernal pools on the landscape. This section will give you a basic understanding of these amphibians and how to identify them in the field. Distribution maps are courtesy of the Vermont Reptile and Amphibian Atlas, https://www.vtherpatlas.org.

Wood Frog — *Lithobates sylvaticus*

Description

- Has a black or dark brown patch or "robber's mask" extending back from each eve
- A dark line of the same color is usually visible from the front of the eye to the snout.
- They vary in body color from dark brown, to light tan (usually in



summer), to rust (especially in spring).

Description

spots on each side.

Two pronounced ridges are present on each side of the back.

Adults range in size from 1.5 to 3 inches and females are larger than males.

Newly metamorphosed juveniles are about

Gray brown to blue-black.

Two irregular rows of bright yellow

½ inch and resemble tiny adults

Natural History

- Courting males make a hoarse, duck-like quack.
- They are explosive breeders and may only call for 2-5 days.
- Typically migrate to pools on the first spring night with warm rains (>40° F).

Spotted Salamander — Ambystoma maculatum



Natural History

- Generally live within 100 meters of the vernal pool in which they breed.
- Spend most of the year underground (hence the name "mole salamanders"), in shrew or other small mammal burrows.
- Also seek refuge under leaf-litter, rocks, logs, and other coarse woody debris.
- The timing of their migration to pools is very dependent on warm, nighttime rains.







Jefferson Salamander — *Ambystoma jeffersonianum*

Description

- Charcoal-gray to brown.
- Tiny white flecks on the belly and lower sides.
- Chunky, with a wide & long snout.
- Adults range from 5 to 7 inches long.



The Jefferson Salamander has been designated as a "High Priority Species of Greatest Conservation Need" in VT.

Blue-Spotted Salamander — *Ambystoma laterale*



Hybrids tend to be chunkier and browner.

Natural History

- Found at lower elevations, primarily in the Champlain Valley, with scattered populations elsewhere in Vermont.
- Like the Jefferson Salamander, they typically migrate to the vernal pool earlier than Spotted Salamanders.
- Unisexual hybrid populations exist with Jefferson Salamanders (See below).

Blue-Spotted / Jefferson Complex

Jefferson and Blue-Spotted Salamanders have historically interbred to create unisexual (all-female) hybrid populations, which are now widespread across Vermont. The physical appearance of these hybrids is highly variable along a gradient from pure Blue-spotted to pure Jefferson, depending on which species they're more closely related to. This can make identification difficult.



Natural History

 Usually the first amphibian to arrive at breeding pools in spring.

Typically found in low- to midelevation foothill regions of Vermont.

Breed primarily on ridgetop pools.

More sensitive to pH than other salamander species.

Unisexual hybrid populations exist with Blue-Spotted Salamanders (See below).

Dark-colored with irregular blue or

Coloration is reminiscent of old

Slender, with a narrow head that

Adults are typically 3.5 to 5 inches









Egg Mass Identification

Identification of amphibian egg masses is both one of the most difficult and most important pieces of the VPMon methodology. If you'd like to further practice your egg mass identification, check out the **Egg Mass ID Quiz** on the VPMon page of the VCE website. This section also covers spermatophores.

Wood Frog Egg Masses



Description

- Each egg mass typically contains between 800 & 1,000 eggs.
- Egg masses are **ping pong ball-sized when first laid**, but after a few hours they **swell to the size of a softball**.
- Egg masses will look lumpy, because frogs egg masses lack the outer jelly matrix found in salamander eggs.
- The embryos are dark on top (to absorb heat) and light below (to reduce predation).
- Egg masses may turn greenish from a symbiotic algae.
- They may look like bubbles at the water's surface.
- Hatch in 2 to 4 weeks, depending on water temperature.

Location in the Pool

- Generally **near the surface**, but may sometimes be at the pool bottom.
- Often attached to twigs just below the surface.
- May be laid in sunnier, warmer sections of a pool (often the north end).
- Often laid in large, multi-layered "rafts," consisting of up to 100 masses.



 After several days, the individual egg masses within the "raft" can become hard to distinguish.

Spotted Salamander Egg Masses



Description

- Each mass typically contains between 30 & 250 eggs.
- Around the size of a baseball when first laid (2-3 inches), but will roughly double in size after absorbing water
- Egg masses are **oval**, elongate, or kidney-shaped.
- Unlike other egg masses, the outer jelly matrix is very firm, like set gelatin.
- Outer jelly matrix may be clear, opaque white, or even green from a symbiotic algae.
- Vitelline membrane (cloudy halo around each embryo) is as wide as the embryo itself, unlike Blue-spotted and Jefferson Salamanders (see next pg).
- Hatch in 3-6 weeks, depending on water temperature.

Location in the Pool

- Typically within 8-10 inches of the surface, but may be much deeper.
- Egg masses are usually attached to sticks, weeds, or grasses.
- May be on whichever side of the pool melts faster.



The vitelline membrane of Spotted Salamander eggs is as wide as the embryo itself, but in Blue-Spotted and Jefferson Salamanders it is much smaller. The two images to the right show this clearly (the dark circle is the embryo of each egg, and the white ring around it is the vitelline membrane).



Spotted Salamander eggs



Jefferson Salamander eggs

Jefferson Salamander Egg Masses



Description

- Each egg mass typically contains between 5 and 30 eggs.
- Tend to be cylindrical or sausage-shaped, and about 1-3 inches long (smaller than Spotted Salamander egg masses).
- The outer jelly matrix is less firm than in Spotted Salamander egg masses, but not as drippy as Blue-Spotted egg masses.
- The outer jelly matrix is clear, making egg masses hard to see.
- Vitelline membrane very narrow, less than the diameter of embryo.
- Hatch in 3-6 weeks, depending on water temperature.

Location in the Pool

- Eggs are typically attached to submerged sticks or vegetation.
- May be on whichever side of the pool melts faster.

Blue-Spotted Salamander Egg Masses



Description

- Each egg mass typically contains **between 2 and 5 eggs**.
- Their small size and clear outer jelly matrix makes them hard to see.
- Very **drippy**; not firm whatsoever.

Location in the Pool

- May be deposited individually in loose sheets on the pool floor, or in small, drippy masses attached to sticks.
- May be on whichever end of the pool melts faster (often the north end).



Blue-Spotted / Jefferson Complex Egg Masses

These egg masses can be highly variable and typically resemble whichever species the mother is more closely related to. One distinguishing trait is that hybrid egg masses contain **many white, infertile or dead embryos.**



Spermatophores

Spermatophores are packets of sperm deposited by breeding male salamanders on the submerged sticks and leaf litter of vernal pools. They resemble small, white flecks of paint.



Macroinvertebrate Identification

Though there are hundreds of macroinvertebrate species that can be found in vernal pools across New England—many of which have specific and interesting adaptations to survive and thrive in ephemeral waters—VPMon only collects data on two groups: fairy shrimp and caddisflies.

Fairy Shrimp — Order: Anostraca; Eubranchipus species most common

Several hundred species of fairy shrimp are known worldwide; at least three of which occur in New England vernal pools. Although their distributions are not well understood, they appear to be most common in woodland vernal pools, and uncommon in roadside pools impacted by runoff. Identification to species requires examination under a dissecting microscope.

- Knob-lipped Fairy Shrimp Eubranchipus bundyi: common in Vermont and northern New England.
- Intricate Fairy Shrimp Eubranichipus intricatus: common in Maine and probably other NE states.
- Vernal Fairy Shrimp Eubranchipus vernalis: more common in southern New England.

Description

- Adults are ½ to 1 inch in length.
- All species of fairy shrimp feature stalked eyes, swim "upside-down", & are orange, red, blue, or bronze-colored.
- They swim slowly, propelled by their 11 pairs of rhythmically beating appendages.
- Females have paired egg sacs just behind their feathery legs.
- Males appear to have enlarged heads due to their "claspers," which are modified antennae used to grasp females during mating.

Natural History

- After being fertilized, the eggs (technically "cysts") may have to dry out *and* be exposed to winter temperatures before they will hatch in the late winter/early spring.
- Young fairy shrimp molt several times over a few weeks before reaching their adult stage, which lasts only 1 to 3 weeks before they die.
- Most commonly seen in spring, 2-3 weeks after ice-out at a vernal pool.

Caddisfly Larvae — Order: Trichoptera

Unlike fairy shrimp, caddisfly larvae can be commonly found in many bodies of water across Vermont. They're monitored in VPMon because they are easy to identify, widespread, and a sentinel species, meaning that changes in their abundance and/or distribution can indicate changes to the health of a pool.



Description

- They vary in size by species, but are rarely more than 1 inch in length.
- Most easily recognized by the cases that they construct with silk and pebbles, sticks, or bits of leaves.
- Larvae have 3 pairs of legs; long, cylindrical bodies, and a plate on the first thoracic segment.

Natural History

- Aquatic as larvae and pupae. Adults emerge in late spring or early summer.
- Larvae can typically be found in a vernal pool around 1 to 3 days after flooding.
- The cases allow larvae to create water currents to increase oxygen flow in non-flowing environments.









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Of course, we must also recognize everyone who has so far contributed their time and enthusiasm into monitoring the vernal pools of Vermont. They allow community science projects like this one to do effective, far-reaching work and for that we thank them.

Finally, it is essential to acknowledge and thank our funders — the Vermont Watershed Grants Program, The Lintilhac Foundation, TechFoundation, and the Canaday Family Charitable Trust — who have contributed the funds to get this project up and running.

APPENDIX

HOBO Logger Configuration

There are many advantages to using the HOBO Loggers: they are reliable, have very long battery lives, and the data they collect can be downloaded in the field (as long as the logger isn't under more than an inch or two of water!). One downside to the HOBO Logger however, is that they require a smartphone to download the data and to configure them.

The instructions in this section will show you how to both configure the HOBO Logger before it goes into the field on Visit 1 (or 2) and how to reconfigure it after it has collected its data for the year.

All of these protocols require a smartphone. If you will not have a smartphone on hand when you visit your adopted vernal pool, you will have to configure (and reconfigure) your HOBO Logger at home. If there is absolutely no way for you to obtain a smartphone with which to configure the HOBO Logger at home, contact the VPMon Project Coordinator.

Configuring the HOBO Logger

- 1. Open the <u>HOBOmobile app</u> (¹⁰⁸⁰) on your smartphone.
 - Make sure that the bluetooth on your smartphone is turned on.
- 2. Press the white target icon in the center of the HOBO Logger's blue face. The HOBO Logger should appear on your phone screen in a light blue rectangle under IN RANGE. Tap this rectangle.
- 3. Tap on <u>Configure</u> (X) and enter in the following information:
 - Once you've entered the information for each option, click **Done** in the upper right hand corner to return to the main menu.
 - Only configure the following options:
 - <u>Name</u> The HOBO Logger should be named "Pool ID_Your Last Name & First Initial."

e.g. MLS699_DarwinC

Logging Interval — The HOBO Logger should take a temperature reading every hour.

Set the logging interval to "1h Om Os."

- <u>Start</u> <u>Logging</u> The HOBO Logger should start collecting data on March 1 at midnight.
 Select **On Date / Time** ...
 - Set the DATE to March 1 of the year the HOBO Logger will collect data.
 - Set the TIME to "00 00 00."
- **Stop Logging** The HOBO Logger should only stop once it has run out of memory.
 - Under STOP LOGGING (MEMORY OPTIONS), select When Memory Fills.
 - Under STOP LOGGING (TIME OPTIONS), select Never.

Bluetooth Always Off — Make sure this is switched to **on** to save battery life!

- If it is on, the button to the right of it will be totally blue, not white.

4. Tap on <u>Start</u> in the upper right hand corner of the screen. Your HOBO Logger is now configured and ready to be placed in the vernal pool!

Changing the HOBO Logger's Battery

A full battery will theoretically last a HOBO Logger for 13 years, but to be safe, VPMon requires that a new battery be put in if the battery level of the HOBO is less than 50% when you visit during the fall (Visit 4). Instructions for how to change the battery can be found below:

- 1. Twist the black cover of the HOBO Logger about 20° counter-clockwise (the directions that the arrows on the base show), so that it pops out of its socket.
- 2. Check the O-ring attached to the black cover, and make sure it isn't cracked or damaged.
 - This black, elastic band helps to keep water from entering the interior of the HOBO Logger.
 - If it appears cracked or damaged contact the VPMon Project Coordinator.
- 3. Note the orientation of the old battery and remove it. Put in a new CR2032 3V lithium battery. It is possible that the new battery will pop out of the socket on one side, but it should be pressed down by the black cover of the HOBO Logger when you put it back into place.
- 4. Set the black cover back into place and twist it about 20° clockwise so that it sits snugly in the HOBO Logger.
 - The key is to line up the groove on the side of the black base with the small, rounded tab.



Step 4. Line up the groove in the black cover with the small tab. When this occurs the cover will be offset like the middle image.

iNaturalist Submission Guide

VPMon uses iNaturalist; it inserts VPMon data into a thriving community science network, allowing the data to be looked at by the broader community and be available for any other potential research projects.

Monitors should submit a photo of one egg mass / macroinvertebrate for each unique species observed as part of the Egg Mass & Macroinvertebrate Surveys. Photos showing evidence of spermatophores or amphibian disease should also be submitted to iNaturalist.

e.g. If you observe 30 Spotted Salamander egg masses in the Egg Mass survey, several spermatophores, no evidence of amphibian disease, and 15 caddisfly larvae in the Macroinvertebrate Survey, then you should make three submissions to iNaturalist: one of a Spotted Salamander egg mass, one of a spermatophore, and one of a caddisfly larva.

You can use iNaturalist with either a computer or a smartphone. The below instructions show how to use iNaturalist with a computer. See page 34 for how to submit data to iNaturalist with your smartphone.

In 2020, we plan to stop requiring iNaturalist for photo submission, replacing it with our own online database. If you run into trouble submitting to iNaturalist, please contact the VPMon Project Coordinator.

Creating an Account

The first step to using iNaturalist is to set up an account. If you have already done so, that is fantastic. However, if you do not have an iNaturalist account, the below steps will show you how to make one:

- 1. Go to the iNaturalist website (<u>https://www.inaturalist.org</u>) and click on **Sign Up** in the upper righthand corner of the screen.
- 2. Fill out the required information (Email, Username, Password, etc.) and click on Create an Account.

Getting Access to the VPMon iNaturalist Project

- Using the same email you made an iNaturalist account with, email the VPMon Project Coordinator to request access to the VPMon Project on iNaturalist.
- 2. Once you've been granted access you should see an invitation to join the VPMon Project on the home page of the iNaturalist website. Click **Join this project**.

cel4 invit	ed you to join Vermont Vernal Pool Monitoring Project	⊙ 11:44 am						
-	Vermont Vernal Pool Monitoring Project							
	The Vermont Vernal Pool Monitoring Project (VPMon) is a citizen science-fueled initiative							
	coordinated by the Vermont Center for Ecostudies, that s	coordinated by the Vermont Center for Ecostudies, that seeks to establish a baseline of vital data						
	on vernal pools in the state of Vermont. More information	n can be found at:						
	https://vtecostudies.org/projects							

Step 2

3. You can now access the VPMon Project page by hovering your mouse over **Community** at the top of the iNaturalist homepage screen and then selecting **Vermont Vernal Pool Monitoring Project** beneath "Your Recent Projects."

Submitting Photos to iNaturalist on your Computer

- 1. When you visit the VPMon Project page on iNaturalist you should see a bright red banner that says <u>ADD OBSERVATIONS</u>. Click on this banner to submit your photos.
 - The VPMon Project page can also be found by going to the following web address: <u>https://www.inaturalist.org/projects/vermont-vernal-pool-monitoring-project</u>.
- 2. Enter your data into the provided boxes:
 - What did you see?
 - For each observation, begin typing the name of the species or taxonomic group in the provided gray box and then select the appropriate option from the dropdown menu.
 - For an egg mass observation, select the amphibian species that produced the egg mass.
 - For a spermatophore observation, select "Mole Salamanders, Genus Ambystoma."
 - For evidence of **amphibian disease**, select the species that showed signs of this disease.
 - For a **fairy shrimp** observation, select "Eubranchipus, Genus."
 - For a caddisfly observation, select "Caddisflies, Order Trichoptera."
 - When did you see it?
 - Type the date and time that you began collecting data at the pool.
 - Be sure to select your timezone in the box underneath.
 - Description
 - Type any additional notes or thoughts that you care to add.
 - If you're submitting evidence of amphibian disease or spermatophores note this here.
 - Where Were You?
 - In the box labelled "Name of the place you made the observation" type the pool ID of the vernal pool, e.g. MLS567.

• Type the latitude and longitude of the pool next to "Lat:" and "Lon:".

Add Media

- Click on the "Choose Files" button and upload your photo(s) of what you observed.
- Remember that you're only submitting a photo(s) of one specimen of each species observed. Even if you observe 50 fairy shrimp across your four sample squares, you only need to submit one photo.
- Vernal Pool ID
 - Write in the pool ID of the vernal pool that you're submitting data for; e.g. MLS567.
- 3. Click <u>Save Observation</u> to submit your data. If you have another observation to submit, click on <u>Save and add another</u> instead.

Submitting Photos to iNaturalist on your Smartphone

If you have a smartphone on-hand and are within range of cell service, you can actually submit photos to iNaturalist in the field. If you don't have service you can still submit them with your smartphone when you return home.

- 1. Download the <u>iNaturalist app</u> () from the App Store or the Google Play store. Open the iNaturalist app and log into your account on it.
- 2. In the iNaturalist app, tap on <u>Observe</u> (⁽ⁱ⁾_{(observe}) at the bottom of your screen.
- 3. Take or select a photo of your specimen and then click <u>Next</u>.
 - If you're at the vernal pool and have the egg mass, caddisfly larvae, etc.
 in front of you tap the green circle to take a photo of it.
 - If you've already taken a photo, tap the icon in the bottom right hand corner of the screen & select the photo(s) you want to submit.
- 4. Fill in the same information that you would for Step 2. of the computer submission instructions (See previous page).
 - Date / Time and Location should have been automatically filled in, but you should verify that they are correct.
 - Geoprivacy should be Open and Captive / Cultivated should be "No."
- 5. Select Projects () and switch the Vermont Vernal Pool Monitoring

Project to "On."

- You will know that it is turned on because the icon on the right hand side of the screen will be green.
- Be sure to add in the pool ID of your adopted pool in the Vernal Pool ID field.
- 6. Click the green banner labelled <u>SHARE</u> at the bottom of the screen to submit your data.





Vermont Vernal Pool Monitoring Project

Vernal Pool Monitoring Data Sheet



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Vernal Pool Monitoring Data Sheet Version 2.0

Leaf Litter Composition Survey Datasheet

Pool ID:	Date:		Time:	
Leaf Species	North	East	South	West
Sum (should equal 100%)				