



Worm Species Richness

AIM

To monitor soil health through the presence/absence and abundance worm species based on the protocols developed by Agriculture and Agri-Food Canada.

RATIONALE

The biological, chemical and physical properties of soil are essential for plant growth, regulating and partitioning of surface to ground water, and buffering, detoxifying and scrubbing of hazardous chemicals. The quality of our soil depends heavily on earthworms as they are considered very important in soil organic matter cycling.

BACKGROUND

Earthworms are invertebrates, they have no back bone. As the earthworm tunnels through the soil, it excretes mucus from its body. This mucus reacts with the soil of the tunnel walls and forms a type of cement which makes the tunnel walls stable. These tunnels allow rain water to enter into the soil, increasing the infiltration rate of soils, preventing erosion and allowing water to flow to the rooting zones for plant uptake.

Although earthworms can be an indicator of soil changes, not much is currently known about earthworm diversity or biogeography. Increasing our knowledge about earthworms can lead to better decision-making on soil improvement and conservation. Certain species are responsible for burying surface residue, while other species are actively involved in the decomposition process, making available important nutrients for other living organisms in the soil - like plants.

This protocol is based on the national WormWatch programme administered as part of NatureWatch. NatureWatch provides suites of monitoring protocols which encourage researchers, education centres, naturalists and other organisations or individuals to engage in monitoring indicators for environmental quality.

CHECKLIST OF MONITORING ACTIVITIES

- ✓ Select location of a monitoring station;
- ✓ Complete the site description sheet and select location of monitoring point;
- ✓ Become familiar with earthworm identification;
- ✓ Select monitoring method and collect data;
- ✓ Record data and manage data sets;
- ✓ Submit observations online at <http://www.wormwatch.ca/>.

EQUIPMENT

Site Description datasheet
WormWatch Data Sheet
Pen/Pencil
3 Well rinsed plastic containers (e.g. yoghurt tubs, margarine containers)
Taxonomic Key
General earthworm diagram

Sampling frame – 175cm of string, 4 large nails/stakes and metre stick OR 65x40 piece of cardboard (for Flip & Strip Protocol)
Spade or shovel (for Quantitative Hand-sort Protocol)
2 Plastic sheets or large garbage bags (for Quantitative Hand-sort Protocol)
Soil thermometer (optional – do not use glass)

LOCATION

Establishing a site for monitoring earthworm diversity can be done in a forested area, open field or a backyard.

This protocol can also be done in association with **forest biodiversity plots**. Four 25x50cm plots should be located 4 metres from the each corner of the 20x20m quadrat. (see Figure 1 for placement of earthworm stations).

Sites need to be easily accessible and free of hazards. In all cases once a site is chosen, *fill out the site description datasheet*. Site location and characteristics need to be recorded and earthworm stations should be plotted on a site map. Photographs of the site may also be useful in describing the sample area.

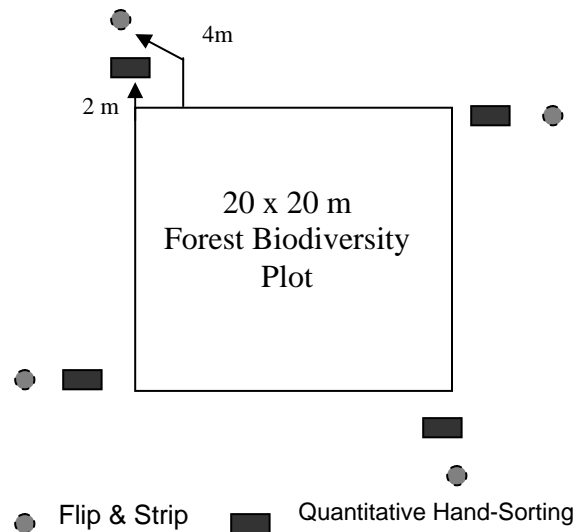


Figure 1. Example of earthworm monitoring with forest plots

SAMPLING METHODS

Timing

Earthworm monitoring can be done when undertaking data collection in forest biodiversity plots or anytime throughout the summer/fall season. For the purposes of standardisation it is important that earthworm diversity protocols are carried out at the same time each year.

Data Collection

Select protocol to be used;

- Flip and Strip:** This protocol is the easiest sampling method to use and recommended if you're sampling in a small area or don't want to disturb the vegetation in the sample area. It is for use in casual surveys.
- Quantitative Modified Hand-Sorting:** This is the protocol recommended if you are concentrating on monitoring earthworm populations for research purposes and can remove soil and disturb vegetation.

Flip and Strip Method

- Laying out the sample frame: This can be done in two ways, using stakes and string or cardboard.
 1. Layout the stakes into a rectangle 25cm by 50cm, using the metre stick for exact measurements. Wrap the string around the stakes to show the sample frame (see figure 2) OR
 2. Take a flat piece of cardboard and cut out a rectangle in the centre that measures 25cm by 50cm. Lay on the area for sampling.



Figure 2. Sample frame for the flip and strip methods

- Place your sampling grid on the ground in the designated area. Keep in mind that rocks, pieces of wood and logs often have earthworms living under them in the cool moist soil. If you are looking under rotting wood, strip back the bark in moist areas and check for bark worms. Bark worms are usually small and very red.
- Gently flip the rocks and strip the pieces of wood that are within your sampling frame. Even if you don't find any earthworms, be sure to carefully replace the rocks, wood and logs that you moved. **juveniles:** Those earthworms without genital markings such as the clitellum, tubercula pubertatis, or genital tumescence. This stage of the life cycle is located between the hatchling phase and the appearance of genital markings (adult stage).
- Adult worms, juveniles, cocoons and aestivating worms (dormant worms found curled in knots with a pinkish colour) will be recorded. Sort the worms found into groupings, colour, and appearance (refer to key). Count the total number of juveniles, cocoons and aestivating earthworms then put them back in the soil. Identify and count the numbers of adults

(clitellum) per species using the Earthworm Key (see appendix 1). Record the numbers on the data sheet (enter 0's for species not found).

- After you have identified your adults, return earthworms to the soil or the place you found them and put all the rocks and litter back the way you found it.
- Record the species, number of adults, number of juveniles (if any) and number of cocoons (if any) on your Observation Form. If you do not find any earthworms, that's important information too.
- If you have found an earthworm that you can not identify, please see the *earthworm verification* section below.

Quantitative Modified Hand-Sorting

- Dig a hole of known volume and repeat for each designated earthworm sampling station at a site.
- Measure the width of the blade of your spade. If your spade is 15 cm across then note that you will dig a 15 cm wide by 15 cm long by 20 cm deep hole. Place the plastic sheet on the ground in a convenient place near where you are digging and get your containers out. If there is a lot of litter (dead leaves, old plant parts, or twigs) on the surface then trace an outline of the square hole with the spade on the soil surface and then remove and sort through the surface material (see figure 3). Carefully look under the bark on dead twigs, and through the leaves because you might find surface or litter dwelling earthworms. Often these earthworms deposit their egg capsules or cocoons in the plant litter, so look for them too. Put the earthworms and cocoons you find in a container and record how many earthworms and cocoons you found in the *litter layer* on the Observation Form and that you found them in the litter layer.



Figure 2. Example of field work for hand-sort method

- Now dig down to 10 cm, you can make a mark on your spade or measure down with a measuring stick beside your spade. Cut the hole to 10 cm on all sides with the spade then scoop the soil out with the spade from one side onto one of the plastic sheets or bags (away from the plant litter). Try as best you can to make a square hole i.e. if you are digging a 15 cm wide hole then it should measure 15 cm wide at the bottom after you have removed 10 cm of soil. Cut along the edges of the hole down another 10 cm to 20 cm and scoop the soil out onto the other plastic bag. Measure the hole you made and record the size on your data sheet. *Check to make sure it is the same size at the bottom as it is at the top and record any differences.*
- Note: If you are only able to dig to 10cm, make sure that you consistently dig to 10cm at all stations and sites and that you indicate the depth of the hole on the data sheet.
- Hand-sort the soil from the bottom half of the hole first. As you go through the soil, put any earthworms or cocoons into the container.
- To hand-sort, grab a handful of soil and gently but firmly break-up any clumps. As you are breaking the clumps look for earthworms and cocoons. Sometimes inside clumps of soil, you will find earthworms that are coiled into what looks like a ball of wool. These earthworms are in a resting state until the soil conditions are more favourable. Aestivating earthworms are smaller and pinker than regular active earthworms. Put the aestivating earthworms into a container to count - they cannot be identified.
- Count the earthworms and cocoons in each container and record the numbers on the Observation form. Count and record the earthworms and cocoons from each layer, one layer at a time starting with the deepest layer. (You should have already counted everything from the litter layer and recorded it on your data sheet). Put the juveniles, cocoons and aestivating earthworms back in the soil. Keep your adults to identify by using the key. Try to make sure you do not mix the soil layers you have carefully dug.
- Identify and record the number of adult earthworms along with each species (using appendix 1) for each soil layer on your Observation Form. If you don't find any earthworms, that's important information too.
- After you have identified your adults from each soil layer and the litter layer, return earthworms to the soil layer in which they were found and put the 20 cm soil layer back into the hole first, then the 10cm layer, pat the soil down lightly with your foot and then replace the litter layer.
- If you have trouble identifying an adult earthworm species please see the *earthworm verification* section below.

EARTHWORM VERIFICATION

WormWatch scientists are available to verify your earthworm identifications or try and identify earthworm species that are not listed on the taxonomic key.

Sending Live Specimens

Select one representative adult of each earthworm species that you would like verified. For example, if you found four different species, you will be sending four different adult earthworms. Make sure it is an adult earthworm with a fully developed clitellum and place the specimen a separate container. Add some wet bunched-up paper towels to a



Figure 3. Packing worm in container.

plastic container which will serve as bedding and protect the earthworms during transport (see figure 3).



Figure 4. Package to send to WormWatch for verification

Place the adult worm in the container and poke a few air holes in the lid, then label the container and secure the lid with packing tape. In a box, place the container(s) surrounded by newspaper for protection and include a copy of your datasheet with the specimens (see figure 4). Send the container(s) to Worm Watch at the address below.

Dr. John W. Reynolds
Oligochaetology Laboratory
18 Broadview Court
Kitchener, Ontario
Canada N2A 2X8

Please use Priority Post, Express Post or courier to make sure the earthworms get to Lethbridge alive.

NOTE: Please send earthworms at the beginning of a week and not on a Friday to minimise the possibility that the earthworms will be stored for a long period of time in too hot or too cold conditions. Earthworms can survive for approximately one week if kept in a container with wet paper towels in a cool dark or shady place.

Sending Preserved Specimens

Select specimen for species verification. Place the earthworm into a clean small screw top plastic container. For example, clean sample-size shampoo bottles work well. Do not use any flip top containers as they will leak. Fill the vial with about 20 ml of colourless drinking alcohol (vodka, white rum, and tequila are preferred), 70% ethanol, or methylated spirits. **Children and students should be supervised by an adult or teacher while handling these liquids.**

NOTE: Beer, wine, and rubbing alcohol are not acceptable for preservation.

Gently drop the earthworm into a vial and close the lid firmly. **Clearly indicate on the label what kind of liquid is in the bottle.** Unmarked vials will be discarded.

Once the earthworm stops moving (about 1 minute), open the vial, and tightly fill the vial or container with cotton balls to the top. Add enough liquid so that the cotton is completely saturated and there is a very small amount of excess liquid on top of the cotton balls (see figure 5). It is important that there is



Figure 5. Preserved earthworm packaging.



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a sufficient amount of liquid preservative in the vial so that the earthworm remains in contact with the liquid at all times during shipping.

Firmly secure the lid and turn to make sure nothing leaks out. Label the screw-top container with the name of the earthworm species or unknown (unknown #1, #2, etc.), where you found it, and your name and email address or telephone number. Place your vial inside a Ziploc bag. We recommend the freezer Ziploc bags because they are made out of a thicker plastic. Remove the air and seal the bag.

If including more than one container, make sure that all containers are labelled with your name, what is inside the container, and where you collected the worm.

Tightly package your containers in newsprint, or other packing material like Styrofoam popcorn or popcorn inside a small box or sturdy envelope. Then the containers will not shift around during transport to the lab. **Please include a copy of the datasheet** (see figure 5).

Send the container(s) to Worm Watch at the address below.

Dr. John W. Reynolds
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Apply sufficient postage to the package as **we will not accept packages C.O.D.**

Please send your live earthworms Monday - Thursday to avoid specimens being stored in either extreme cold or hot for extended periods of time. Preserved specimens are also best sent on a weekday.

DATA MANAGEMENT AND SHARING

Hard copies of the data should be kept for future use. Submit online data at

<http://www.wormwatch.ca/>.

REFERENCES

Clapperton, J. 1996. National Sampling Protocols. Agriculture and Agri-Food Canada, University of Lethbridge.

Clapperton, J. EarthWatch website. <http://www.naturewatch.ca/english/wormwatch/>



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WORMWATCH DATA SHEET

LOCATION DATA (for all methods)

SITE NAME:		NEAREST NAMED PLACE:	
OBSERVATION AREA DESCRIPTION (FOREST/WETLAND):			
LATITUDE/LONGITUDE:	COUNTY/TOWNSHIP:	PROVINCE:	
OBSERVER NAME(S):	OBSERVER ADDRESS:		
	TELEPHONE:		
	EMAIL:		

SAMPLING TECHNIQUE (FLIP & STRIP / QUANTIFIED HAND-SORT):	DATE:
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Habitat Description (check all that apply)		
<input type="checkbox"/> Hardwood Forest (deciduous)	<input type="checkbox"/> Softwood Forest (coniferous)	<input type="checkbox"/> Mixed Forest
<input type="checkbox"/> Grassland	<input type="checkbox"/> Farmland	<input type="checkbox"/> Abandoned Farmland
<input type="checkbox"/> Marsh, Bog or Wetland	<input type="checkbox"/> Residential gardens and lawns	<input type="checkbox"/> School Yard
		<input type="checkbox"/> Empty Lot

Soil Type (check all that apply)	
<input type="checkbox"/> Rich and loamy	<input type="checkbox"/> Rocky
<input type="checkbox"/> Sandy	<input type="checkbox"/> Water saturated/muddy
<input type="checkbox"/> Clay	<input type="checkbox"/> Moist
<input type="checkbox"/> Dry	<input type="checkbox"/> Other (please describe): _____

Soil Colour (check all that apply):	
<input type="checkbox"/> Light brown	<input type="checkbox"/> Dark brown
<input type="checkbox"/> Black	<input type="checkbox"/> Red
<input type="checkbox"/> Other (please describe): _____	

Soil Temperature (if available): _____

Weather Conditions:	
Air temperature: _____	Raining: _____
Dry: _____	Overcast (estimate % cloud cover): _____
Sunny: _____	Windy: _____
Other: _____	

ATTACH PHOTOGRAPHS OF OBSERVATION AREA HERE

(Digital photos can be uploaded and submitted to <http://www.wormwatch.ca/>)



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QUANTITATIVE HAND-SORT DATA SHEETS

Worm station #:

<i>Litter Layer</i>		<i>Top Layer</i>		<i>Bottom Layer</i>	
Worm Species	Number of Adults	Worm Species	Number of Adults	Worm Species	Number of Adults

All earthworms:

Type	Top layer total #	Bottom layer total #
Adults		
Cocoons		
Juveniles		
Aestivating Individuals		

Comments:

Worm station #:

<i>Litter Layer</i>		<i>Top Layer</i>		<i>Bottom Layer</i>	
Worm Species	Number of Adults	Worm Species	Number of Adults	Worm Species	Number of Adults

All earthworms:

Type	Top layer total #	Bottom layer total #
Adults		
Cocoons		
Juveniles		
Aestivating Individuals		

Comments:



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FLIP AND STRIP DATA SHEETS



Worm station #:

Worm Species	Number of Adults

Type	Total number
Adults	
Cocoons	
Juveniles	
Aestivating Individuals	

Worm station #:

Worm Species	Number of Adults

Type	Total number
Adults	
Cocoons	
Juveniles	
Aestivating Individuals	

Worm station #:

Worm Species	Number of Adults

Type	Total number
Adults	
Cocoons	
Juveniles	
Aestivating Individuals	

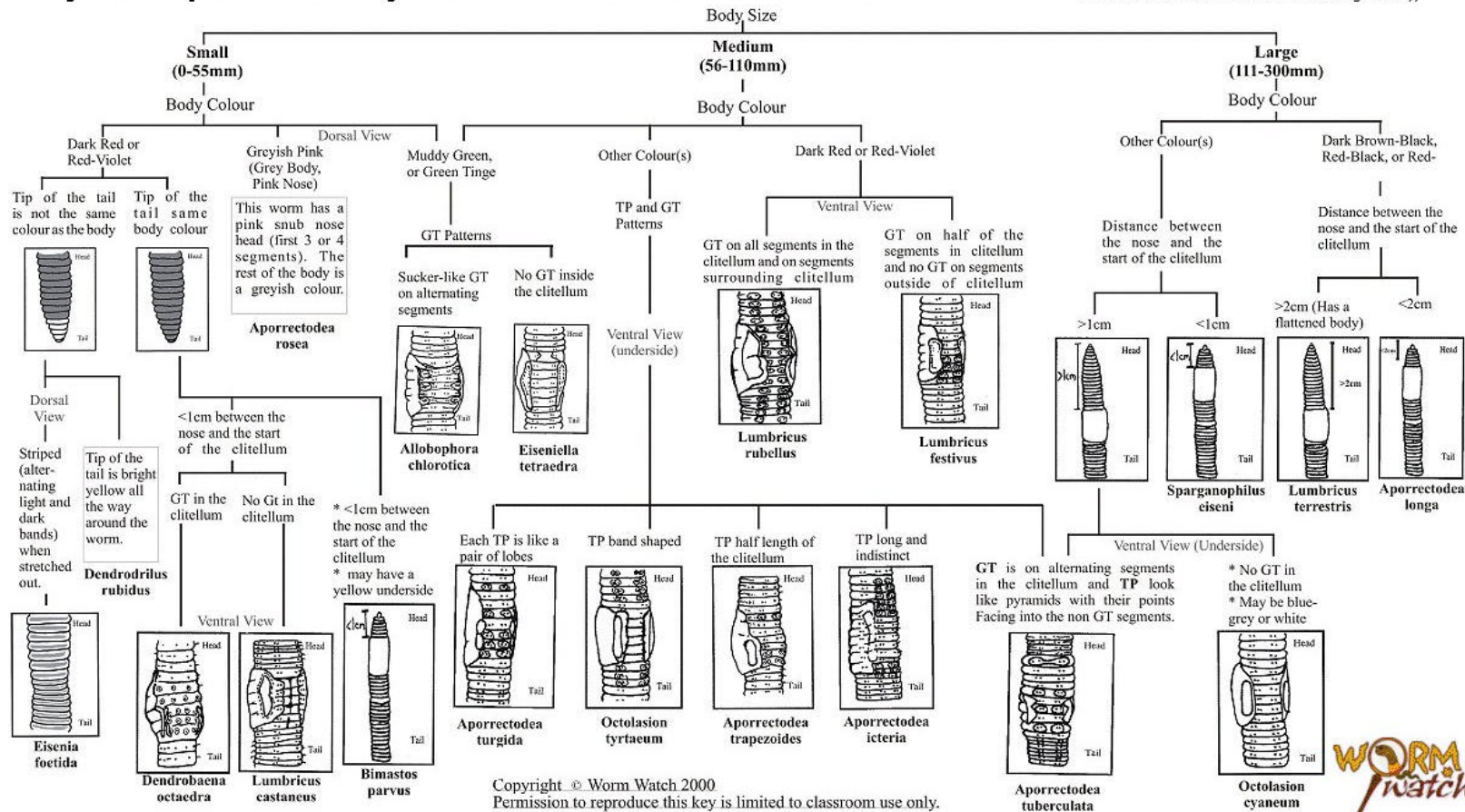
Worm station #:

Worm Species	Number of Adults

Type	Total number
Adults	
Cocoons	
Juveniles	
Aestivating Individuals	

APPENDIX A

Key to Reproductively Mature Earthworms Found in Canada (an earthworm without a clitellum is not reproductively mature and thus cannot be identified using this key)



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