Protocol for Tree Core Collection and Preparation for Ring Width and Nitrogen Isotope Analysis

Updated June 2014

Written by Laci Gerhart and Kendra McLauchlan
Kansas State University Paleoenvironmental Lab

Part 1: Collecting Tree Cores

Selecting a borer: Häglof increment borers are the most commonly used borers, though other brands also exist. They are offered in a variety of lengths (4-28"), diameters (4.3-12 mm), and threads (2 or 3). Wider diameter borers provide more tissue for analysis, but can be more difficult to drill into the tree, particularly for hardwoods. Longer borers allow you to sample larger trees, but can be unwieldy on smaller trees. Three-thread borers 'bite' into the tree more easily and produce less friction than 2-thread borers. The KSU Paleoenvironmental Lab uses 5.15 mm borers ranging from 12" to 26" in length.

Borers consist of three parts: a handle (blue in color for Häglof, or orange for other brands), a bit (black), and an extractor (silver). When not in use, the extractor and bit fit inside the handle for easy storage.

Assembling the borer

1. Untwist and remove the extractor from the handle.
2. Remove the bit from inside the handle, and anchor it in the handle. Anchoring requires lifting the small latch on the hole in the handle, inserting the square end of the bit into the opposite side of the handle through the hole, and latching the handle into the notch on the bit.

Coring the Tree: Check out the Novus RCN You Tube video of tree coring

1. Choose large, straight, naturally-occurring (ie. Not irrigated or fertilized) trees, not immediately next to roadways, buildings, man-made clearings, or other disruptive features.

2. Choose a location on the trunk near breast height and free of limbs, knobs, or other growths. If the tree has a branching trunk, choose a section lower than breast height below the branching.
   Note: if you are interested in the date of establishment, you may wish to core near to the base to obtain the youngest years (ie when the tree was not yet as tall as breast height)
3. Core the tree by twisting the bit clockwise into the trunk of the tree. We have found it is easiest to hold the bit with your non-dominant hand to keep it steady while slowly twisting the handle with your dominant hand. Push as hard as you can while keeping the borer stable and lean into the borer with your bodyweight to apply more pressure. Make sure to hold the borer very steady until it 'bites' into the tree. If there is much 'wiggling' of the borer, the end portion containing the most recent years will break off and make processing in the lab more difficult (though not impossible).
   Note: You may require an extra person to hold the bit steady as you twist the handle. This person should hold onto the bit as you turn it and push or pull (depending on their angle to the borer) towards the center of the tree.
4. Continue twisting the borer into the tree until at least half of the tree trunk has been breached. Distance into the tree can be estimated with the extractor, which is equal to the length of the bit.
   Note: while coring, make sure to push INTO the tree and not pull down on the borer. Pushing into the tree increases the effectiveness of each turn of the handle, and pulling down can also warp, bend, or even break the bit. If you find yourself pulling down on the handle, try coring lower on the trunk of the tree.

Removing the Core

1. Insert the extractor into the bit, curve down (to make a little tray for the core), and push it all the way flush with the end of the bit. This sometimes takes a bit of strength if the core is 'sticky'.
   Note: be careful not to push too hard on the extractor, as it may warp, bend, or even break. Warped extractors may still be used if the warping is minor, but ones that are

bent or broken must be replaced. It is better to not get the extractor fully inserted into the bit than to risk breaking the extractor. If you do break an extractor, it is possible to use an extractor from a longer borer so long as you are careful to not jam it too far into the bit, but an extractor from a shorter borer will be virtually useless.

2. Untwist the bit counter-clockwise two full turns. The curved bottom of the extractor should be down again, making a tray for the core.
3. Slowly and gently pull out the extractor. If the extractor is not oriented to make a little tray holding the core, continue untwisting the borer until the extractor is appropriately oriented.
   Note: it is often difficult to gently pull out the extractor, particularly with hardwoods. If you are worried about breaking the core (or if it is already broken and you are worried about losing pieces), you may gently cup your hand around the exposed portion of the core as you pull the rest of it out.
4. If the extractor comes out empty, or containing only part of the core, insert it again, twist the bit counter clockwise another turn or two and try again. Sometimes it takes a few tries.
5. Fully remove the borer from the tree by continuing to turn it counter-clockwise. It's ok if some of the core is still inside the bit.
   Note: Do not lollygag in removing the borer from the tree. As soon as the core is started, the tree will begin sealing up the wound. The process is quick enough that even a borer left in a tree for only a few minutes may be sealed into the tree and impossible to remove. Numerous researchers have lost bits this way.

<u>Storing the Core</u>: The Paleoenvironmental Lab uses paper art straws for storage. These are ideal for 4.3 or 5.15mm cores, but not large enough for the 12mm cores. Other options include plastic drinking straws (though these are too short for the longer borers, and cannot be used with the drying oven), or aluminum foil (though this is sometimes unwieldy in the field and less protective against core bending or breakage).

1. VERY GENTLY insert the core into one of the paper straws, or other storage medium, label it with the Tree ID (ex. KS1), and mark the end of the core which was

nearest the center of the tree as "In" and the end of the core nearest the outside of the tree (the bark) as "Out." Secure each end with masking tape

2. Artstraws are not sturdy enough to prevent breakage of the core by themselves, so cores must then be housed in a mailing tube or long Tupperware box to prevent breakage.
3. Clean the inside and outside of the bit, and the extractor with ethanol so as not to spread disease between trees.
4. When finished, reassemble the borer by unlatching the bit, inserting the bit threads-first into the handle, then inserting the extractor and twisting it to the handle.

Frequently Asked Questions

The core broke as I was removing it with the extractor. Is it still usable?

Yes, usually. Cores often break a long a ring boundary, making it easier to match up the ends. Make sure to note the original orientation of the pieces and insert them into the storage straw in the correct order. It is rather common for a core to have at least one break, and unless there are many breakages, the core is still probably usable. You will just need to be more careful when processing it in the lab.

The core is stuck inside the bit, and the extractor can't get it out. What do I do?

This is a common occurrence (especially with juniper, in my experience). In the field, you can try repeatedly pushing the extractor in the bit, being careful to not bend or break the extractor. Sometimes it just takes a few tries. If this is unsuccessful, once you are back in the lab, you can dry out the core by placing the entire bit in the oven for a day or two. If it is particularly resinous, you may also need to squirt some WD-40 down the inside of the bit. Though this makes the core useless for research, it will clear out your bit.

The Paleoenvironmental Lab also has a tool designed for just this purpose. The Siegwarth (named after the man who made it for us) consists of a flat metal base, attached to a long narrow metal rod. You can hold the rod steady by setting the base on the floor and standing on it. You can then force the narrow rod through the thread-side of the bit, forcing the core out the larger back-end of the bit. This usually removes the stuck portion intact, making it still possible to process it for analysis.

The core is too long for the paper artstraws. What do I do?

This is a great problem to have, as it means you have cored a really large, old tree! The easiest method is to tape two straws together around the core. You can also wrap the exposed end in aluminum foil, or some other paper (like from your field notebook). Anything to protect it in transit will suffice.

When I was coring, the bit lost all traction and just started 'spinning' in the tree. What does that mean?

That likely means the inside of the tree has rotted out, which is not unusual in large old trees, particularly oaks. The core will still be usable, but will not show the center of the rings, and the inner portion of the core may be crumbly. Remove and store the core as you normally would. Removing the bit may prove more difficult if it has lost traction. Try pulling back on the borer as you twist it counter-clockwise to remove it. It may also require bracing a foot against the trunk of the tree to pull back as hard as you can. If you aren't sure if you are getting the borer out, try placing a small piece of tape on the bit, flush with the tree bark. You can then see if the tape moves as you pull on the borer.

The core I removed has a bluish-black tinge to it. What does that mean?
It likely means the tree was infected with a kind of sap stain fungus. Sap stain is spread to pines via the antennae of bark beetles, and can also infect oaks and other species. The core can still be used for some purposes (like age of the tree, and potentially ring width), but it is unclear if sap stain infection affects isotope signatures of ring wood, so sap stain infected cores should not be used for isotope analysis.

The core I removed has regular black marks along one side. What does that mean?
A regular pattern of black marks along one or more sides of the core is likely friction from the borer itself. This is particularly true if the tree made moaning or screeching sounds as you cored it, which are indications of friction. These marks are not a problem, and can be sanded away in order to reveal the ring structure more clearly.

Part 2: Processing Cores for Ring Width Measurements

Initial Preparation: Dry the cores in a drying oven at 60-75°C for several days. Highly resinous species (like pines) will require more time than non-resinous deciduous species. Drying for long times will not harm any cores, so err on the side of more drying.

Note: If you stored your cores in paper artstraws, you can put them directly into the drying oven. If you stored them in anything plastic, they will need to be removed from the plastic before drying. If stored in aluminum foil, you will need to open up the foil to allow the core to outgas.

Artstraws are particularly good for this stage because they prevent the core from warping as it dries. Warping makes the cores more difficult, but not impossible, to process.

Measuring Ring Width:

1. Remove the dried core from its storage straw and place it in a wooden clamp.
   Note: these clamps can be bought at most home improvement stores. They are easier to use if modified to have a slight groove in the center, equal to the diameter of your core. This way, the core can settle into the groove, without falling through the center of the clamp. Non-modified clamps are still usable, but slightly more frustrating.
   If the core has warped during drying, it may break when you press it into the straight clamp. The core is still usable, just proceed with care.
2. Sand the core smooth to more clearly reveal the ring widths. This can be done with a power sander, or by hand. Be careful with cores that are broken, as pieces may fall out of the clamp, particularly when using a power sander.

Note: orientation of the core does not matter for conifers, but does matter for ring porous species. Look closely at the core – you should be able to see small dots that represent the open ends of the xylem. You want to core on a side where the dots are visible, as this will most clearly show the ring boundaries.

3. With the core still in the clamp, for stability, scan the core at high resolution (at least 1200 dpi) on a large flatbed scanner. The Paleoenvironmental Lab uses an Epson Expression 10000XL.
4. Save the image with the Tree ID name (example file name: KS1.jpg)
5. Open CooRecorder 7.7, then select File > Open image file for new coordinates. Find and open your image file. When the image file opens, a dialogue box will also open. Make sure the dpi value matches the dpi of the image, and under "Type of Data," select "Sorted data (e.g. Dendro-yearly ring widths)"
   Note: some in the Paleoenvironmental Lab prefer to measure ring widths with the image oriented horizontally, others prefer vertical. This is a matter of personal preference and makes no difference for the software or the data.
6. With the magnifier tool, zoom in until the annual rings are clearly visible.
7. Click the large D to enter data-picking mode.
   Note: if you plan to split the rings for isotope analysis, it is helpful to print off the image and note important ring boundaries on the paper. "Important" can be any easily identifiable ring, such as one that is unusually wide or narrow, or rings for which the boundary is faint, etc. This will help keep your ring splits accurate in the splitting stage.
8. Click on each ring boundary.
   Note: your first point (the zero point) should be on the outer boundary of the core, where the wood meets the bark (if the bark is present).
9. You can adjust data points by clicking the large arrow (Select a point mode) and selecting the incorrect data point. You can then delete the point by clicking the white X in a black background (Delete/Kill selected point), or move the point by clicking the double-sideways arrow (Replace selected point) and then clicking the appropriate location for the point.
   Note: If you delete the zero point, you must select a new zero point by clicking on another data point and clicking the black asterisk on a white background (Click to make the selected point the start point for writing data).
10. Once you reach the center, or the end of the core, save the file (File > Save As… OR File > Save coordinate file) using the Tree ID and the suffix COORD (ex. KS1_COORD.pos)
    Note: if you are tracking rings on a printed copy of the image, note the last ring number
11. Open CDendro 7.7 then select "Samples > Open one or more single sample files: .pos, .wid, .datw, .cat, .d, .crn" Find and open your coordinate .pos file from step 10
12. The current settings will display both the raw ring widths (in green) and the normalized data (in red). You can alter what is shown and how data are standardized using the click boxes below the curve display.
13. To save just the raw ring widths, select Samples > Save ring width data as (one single column)(.wid). Save the file with the tree ID and the suffix WIDTH (ex. KS1_WIDTH.wid)

14. To save both the raw and normalized ring widths, select Samples > Save normalized data As. Save the file with the tree ID and the suffix STAND (ex. KS1_STAND.d12)
15. By this method, each tree will have its own ring width file. To combine them, open Excel, go to File > Open. To find your .wid or .d12 files, make sure to select "All Files" from the dropdown menu at the bottom right, near the Open and Cancel buttons. An import wizard will open, allowing you to select the delimitations of the file (usually semicolon from CDendro) and open the file in Excel.
16. For STAND files, the A column is standardized ring widths and the B column is raw ring widths. For both STAND and WIDTH files, the first row of data (below the header information of the file) is ring 1, second row is ring 2, etc. Ring widths can then be copied and pasted into whatever format you require for analysis.

<u>Frequently Asked Questions</u>

I don't have CooRecorder or CDendro. Can I still measure ring widths?

Yes. Raw ring width can also be measured using the ruler tool in Adobe Photoshop. Make sure to go to the preferences page and set the units to millimeters. Then use the ruler tool to draw a line across each ring. The width of the ring (ie the length of the line you drew) will be displayed at the top of the screen. This will require you to enter ring widths into Excel by hand, and detrend or standardize them on your own.

I thought cores were supposed to be glue mounted for ring width analysis. Why don't you do that?

Glue mounting makes processing for isotope analysis (Part 3) virtually impossible. When planning isotope analysis, it is easier to measure ring widths in the method described here than with glue mounting,

Part 3: Processing Cores for Nitrogen Isotope Analysis

<u>Initial Preparation:</u> follow the same guidelines described in Part 2

<u>Splitting Rings:</u> though sanding is not technically necessary for ring splitting, it does make the ring boundaries much easier to see. Also, we highly recommend scanning all cores, even if you do not intend to use ring width data.
Note: the following protocol is specific to the Paleoenvironmental Lab and may differ at different institutions. There are many suitable ways to subsample cores for isotope analysis.

1. Place the bare core (ie not in the clamp or other storage device) under a dissecting microscope. Using a razorblade, break off each ring individually. Particularly narrow rings can be 'doubled-up', but splitting each ring separately is ideal.
   Note: for hardwoods, or even large rings on softwoods, it may be necessary to keep a small hammer handy to gently tap the razorblade into the core and break off the ring.
2. Each ring should be stored separately from the others. This is done most easily using a 96-well plate in which each ring sits in a separate well.

3. Periodically check with your printed notes, and/or the CooRecorder coordinate file to make sure your ring splits agree with the ring boundaries you marked.
4. When finished, label the well plate(s) with the tree ID, and letter suffix, if there is more than one tray (ex. KS1, or KS1a and KS1b)

Loading Wood for Isotope Analysis

1. First, you must know the tissue weight requirements of the lab in which you will run your samples. Tissue weights can range from 10-30 mg.
2. Once you know the tissue weight requirements, estimate the resolution of data you could acquire with your samples. For very narrow rings and high tissue weight requirements, you may only be able to achieve a single data point per decade. For wide rings and low tissue weights, you may be able to achieve annual resolution.
3. Weigh out samples according to the lab tissue weight requirements, and your estimates of sampling resolution. For example: for decadal resolution, begin with the fifth ring, and fill in rings on either side until you reach the required tissue weights.
4. Load the rings into a tin capsule. Note the weight of the wood sample (NOT including the tin capsule) and the rings included in that sample (ex. Your notes may read something like “Well A1: KS1, 4-6: 30.2 mg” meaning in well A1, there is a tin capsule containing rings 4, 5, and 6 of core KS1, and weighing 30.2 mg)