

Aging of Clams (Hard & Soft)
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Preparing Shells

1. Each shell needs to be cleaned by hand in a water and 10% bleach solution. Make sure to remove any tissue and/or fouling from the internal and external parts of the shells. Use shucking knife and/or plastic bristle brush (plastic in order to not scratch each shell) for cleaning.
2. Shells and their corresponding labels are placed in Nestier Trays and set to dry overnight in the lab (Figure 1).



Figure 1: Nestier Tray used to dry cleaned shells.

3. Shells are placed in bags with their labels and are ready to be measured, aged and/or archived. Labels are also written on outside of bag for easier identification.

Measuring/Labeling Shells

1. Measure all shells (Figure 2), shells may break during aging and/or archiving.
 - a. Length
 - b. Width
 - c. Weight
 - d. Any other essential measurements based on project needs

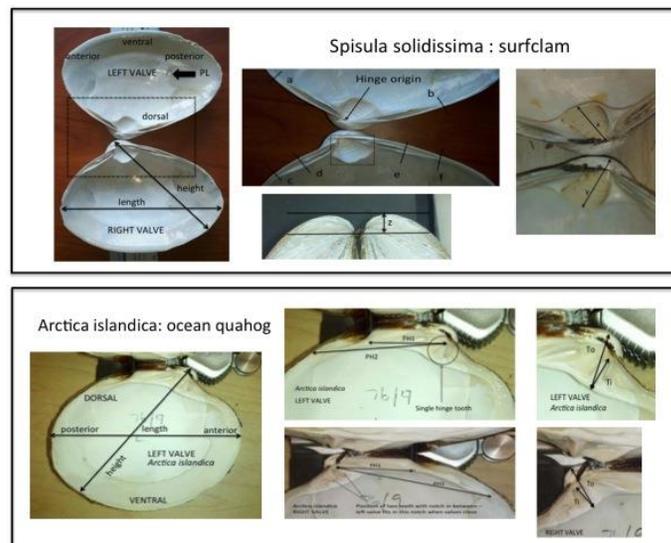


Figure 2: Examples of possible measurements for Spicula solidissima and Arctica islandica.

2. Label all shells.
 - a. Collection Date
 - b. Station
 - c. Sample Number/Shell Number
 - d. Right/Left Valve
 - e. Sex (Male/Female/Indeterminate)
 - f. Any other essential measurements based on project needs

Cutting Shells for Aging

1. A tile saw and/or slow speed saw with diamond blades are needed for cutting shells (Figure 3).



Figure 3: Examples of tile saw and slow speed saw used to cut shells.

2. Before cutting shells, stuff each shell with plasticina clay. Make sure to cram clay into hinge section first. The clay will help reduce shell breakage while cutting. Also, the clay will help hold shell in place while cutting. The clay can be reused as hot water will soften it up after each cutting session.
3. Clamp each side down onto the cutting stage using a screwdriver to loosen and tighten screw flange.
4. Be sure to wear safety goggles and ear protection when cutting (watch your fingers!). Slowly push the shell through the blade. Each shell must be cut through the hinge. Make sure to cut to the left/right of the origin to ensure grinding will not go past the aging surface of the origin (Figure 4).



Figure 4: Examples of cutting location for A. islandica.

5. Each cut shell should resemble Figure 5.



Figure 5: Example of cut valve for *A. islandica*.

Grinding/Polishing Shells for Aging

1. Once cut, the hinge surface needs to be prepared for aging. The first step is to grind the hinge to the origin. Grinding the shell includes a series of grinding stages with decreasing grit size – the higher the number, the finer the grit. For example, *A. islandica* requires for grinding stages: 240, 320, 400, and 600 grit sand paper.
 - a. Our lab uses a variable speed grinder-polisher (Figure 6), however wet/dry sanding by hand can be suitable.



Figure 6: Example of a variable speed grinder-polisher.

1. Start with the coarsest sand paper then work towards the finer grits to ensure a smooth aging surface.
2. Grinding speed is based on shell type/size. For example, *A. islandica* uses a speed of 200-250 based on shell size.
3. Grinding times is based on how long it takes to get shell level with the hinge origin. Use coarsest grit the longest and shorten grinding time as grits become finer.
4. When grinding, be sure to grind shell flat and not on an angle. Having a flat/level shell will allow for better focus when imaging the hinge.

5. Once grinding is complete, the hinge needs to be polished. Two polishing solutions, 6 μ m and 1 μ m, are used for this process working from coarse to finer solution. Polishing pads can be secured to the variable speed grinder-polisher and/or used by hand.
6. Polishing speed should be approximately 100-150. However, one can alter this based on shell type/size.
7. Polishing time should be approximately 45 – 60 seconds with each solution.
8. The resulting hinge/shell should be level and flat with a mirror-like finish.

Imaging Shell for Aging

1. Taking a picture of the hinge/shell is the best way to age each shell (Figure 7). There is no correct way to image a hinge. One can magnify the hinge with a magnifying glass and images can be captured with any type of camera.
 - a. We use an Olympus Microscope with CellSens Software to capture images. The software also allows several images to be stitched together for analysis (Figure 8). See Manual 2019-2.

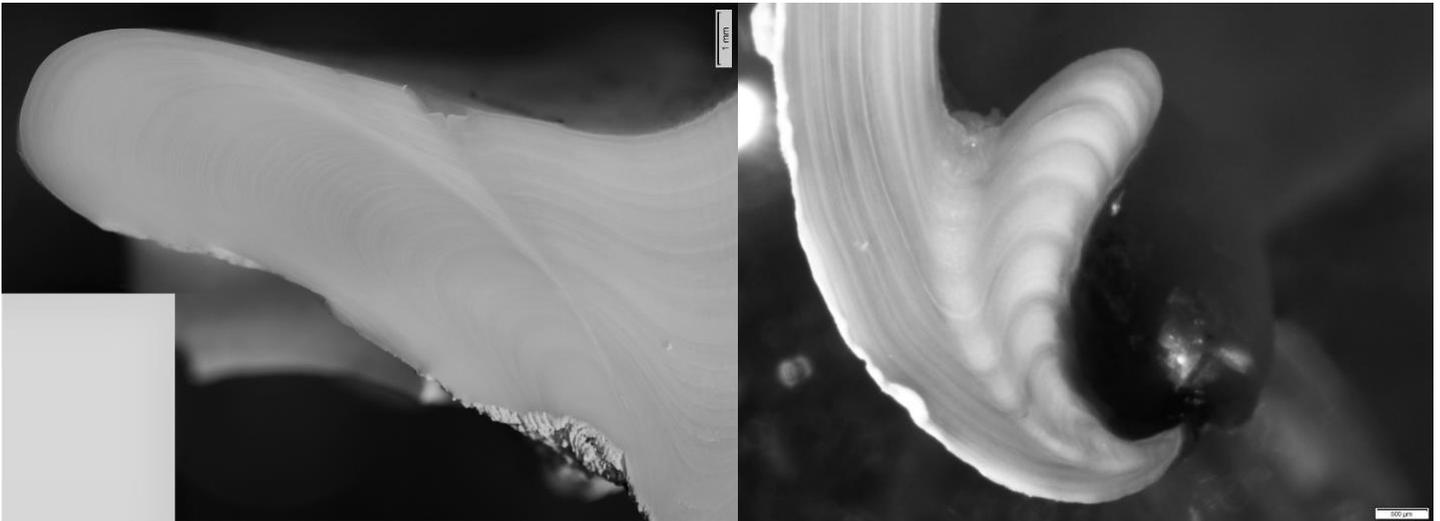


Figure 7: Example images of *A. islandica* and *Mya truncata*.



Figure 8: Example of a stitched image of *A. islandica*.

- b. For the best results, we use a finger bowl filled with glass beads to secure the shell under the microscope. Orient the specimen with the curved edge of the valve pointing down so that it is secured by the beads. Orient the polished surface of the valve so that it is as perpendicular to the microscope lens as possible, with the hinge region located as close to the center of the finger bowl as possible to provide for the most range of movement on the microscope stage.
 - c. Once the specimen is aligned, we use a fiber optic light source to illuminate the hinge region as evenly and brightly as possible from a steep angle so that reflections and glare are minimized.

d. We use CellSens Software by Olympus to capture image of hinge.

Aging Shells

1. Shell aging can be as easy as counting annual visible lines (Figure 9) on the hinge/shell to counting annual age signatures and analyzing age increments via Fiji imaging (ImageJ) software (Figure 10). See Manual 2019-3.
2. The annual lines that appear on the shell hinge should follow all the way through the cut shell valve. Beware of any sub annual signatures as they can be easily confused with annual signatures.
3. Images can be manipulated by adjusting the lighting, contrast and/or color saturation. This may help age signatures stand out in a monotone image.

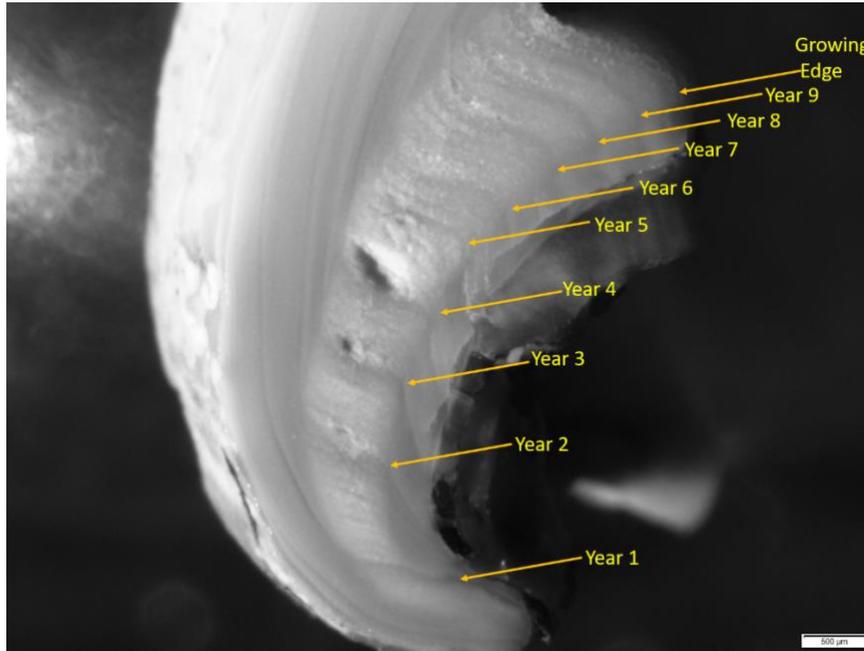


Figure 9: Example of an aged image of *M. truncate* via PowerPoint.

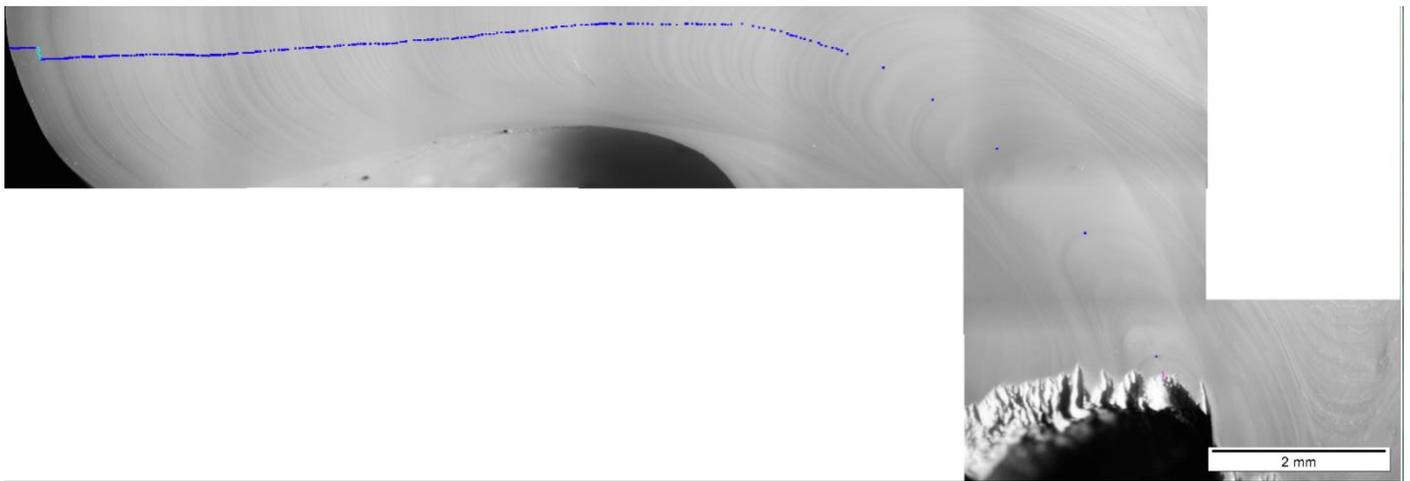


Figure 10: Example of an aged image of *A. islandica* via Fiji software.

Archiving of Aged Shells

1. Once shells are imaged/aged, they are to be archived in small plastic bins between layers of easy liner (Figure 11). Lay the shells down in a manner to protect their polished hinge. See Molluscan Collection Manager for bin and bin number.



Figure 11: Example of storage bin and easy liner for aged shell storage.

2. In a notebook, record the shell's Sample Number and whether the shell is a left (L) or right (R) valve. Also, write these identifiers on the shell with a sharpie.
3. Place the opposite valve in a sample bag that is labeled with "Matched Valves for Bin ###" based on the bin number the cut valve is archived in.
4. Once bin and bag is full, give to Molluscan Collection Manager for storage in the Molluscan Ecology Collection Room.