# An Assessment of Otoliths, Spines, and Scales for Assigning Ages to Ruffe

Ian C. Woest, Kert K. Wuestenhagen

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Northland College
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# Abstract

Ruffe (*Gymnocephalus cernuus)* is a percid native to Europe and Asia that was first observed in Lake Superior in 1988. Age is an important component to understand the life histories and population dynamics of fish. The age of Ruffe has been estimated from a variety of calcified structures, but no study has determined which calcified structure or method provides the most accurate estimate of age for Ruffe. Our objective was to determine which of scales, spines, or otoliths provided the most interpretable structure for estimating the age of Ruffe. We removed scales, spines, and otoliths from Ruffe collected from various tributaries and bays in the Lake Superior watershed in 2008. For age estimation, scales were pressed into acetate, spines and otoliths were set in epoxy and sectioned, and otoliths were also cracked and burnt. Scale impressions were examined under a microfiche reader, whereas spines and otoliths were viewed under a microscope. Spines and scales generally showed the clearest and most distinct annuli. Annuli were difficult to identify on spines from young, small Ruffe and on scales from large, old Ruffe. Otoliths did not exhibit distinct annuli and included several other checks or false annuli. Our results suggest that scales and spines hold the most promise for assigning accurate ages to Ruffe due to the presence of clear and distinct annuli. Additionally, it appears that scales may be used for small, young fish and that spines may be used for large, old fish. Further research should assess the precision and accuracy of scales and spines for assigning age, especially in relation to the size of the fish.

# Contents

# Introduction

Ruffe (*Gymnocephalus cernuus*) were introduced to the Great Lakes watershed in the late 1980's (Ogle 1998). Ruffe are native to parts of Europe and Asia, but are non-native to Lake Superior. When Ruffe were first accidentally introduced, possibly through ballast water discharge from transoceanic ships, researchers questioned the effects on trophic levels of another fast growing, relatively high fecundity, spiny-rayed, small percid in Lake Superior waterways (Ogle *et al.* 1996). Ruffe are an important fish to study and manage because they can disturb Great Lakes native fisheries such as Yellow Perch (*Perca flavescens*) or other species within trophic cascades. The diet of adult Ruffe varies among locations, but Ruffe consume microcrustaceans, chironomid larvae, macrocrustaceans, and the eggs of other fish (Ogle *et al.* 1995). The diet of larval Ruffe consists of mostly zooplankton, cladocerans, rotifers, and copepods, which is similar to the diet of larval native Yellow Perch, which creates possible interspecific competition between the two species (Ogle *et al.* 2004).

Age and growth information, is important to gain insight into the ecology of invasive fishes and to guide management decisions. Various calcified structures, including scales, otoliths, fin rays, cleithra, and opercular bones can be used to estimate the age of fishes (Faust and Breeggemann. 2013). Scales are commonly used to estimate the age of centrarchids and moronids, whereas fin spines are the structure most commonly used to estimate the age of ictalurids (Quist *et al*. 2007). There are several factors that determine the use of one calcified structure over the other: the ease of collection, accuracy and precision of age estimates, and the ability to sacrifice fish. For example, scales often underestimate the age of older fish, but are easy to collect without permanently harming an individual fish (Faust and Breeggemann. 2013). Fin rays are more intrusive than scales, but are commonly used to assign ages to fishes because they provide more precise estimates of age for older fish. Otoliths are much more intrusive than both spines and scales but also provide more precise estimates of age for older fish. The choice of which structure to use for age estimation may, however, influence estimates of recruitment, growth, or mortality for a fish population (Quist *et al*. 2007).

The most accurate and precise calcified structure for assigning age to Ruffe has not been determined (Ogle 1998). Scales, spines, opercula, and otoliths have all been used in previous studies to assign age to Ruffe. One study found scales to be inadequate for assigning age to Ruffe. However, the accuracy and precision between multiple methods have not been compared (Ogle 1998).

Our primary objective was to determine the most interpretable structure for estimating the age of Ruffe. The calcified structures that we assessed were otoliths, scales, and dorsal spines. Our secondary objective was to provide a thorough lab manual for the techniques used to process and view these three calcified structures.

# Methods

In summer 2008, Ruffe were captured with bottom trawls from bays and tributaries in the Lake Superior watershed including the Flag River, St. Louis River Harbor, Amnicon River, Ontonagon River, and Thunder Bay. Scales, otoliths, and the third dorsal spine were removed from 535 fish. Scales were removed from the middle side of the fish slightly above the lateral line. Total length (nearest mm), weight (nearest g), sex, and maturity were recorded prior to the removal of the calcified structures. Each calcified structure was then processed using the methods described in the appendix. We processed 535 scales, 75 otoliths and 15 dorsal spines. Scales were done by a previous researcher and the method of selecting which fish to use was not recorded. We selected otoliths to process by selecting fish that we were able to extract two otoliths from and which putative scale ages had already been determined. We then randomly selected as many as three females, three males, and three unknown fish in each 10 mm length bin between 40 and 170 mm. Some bins had fewer fish than others as a result of a bin having a majority of one sex of fish or the bin having a low number of fish. There were less otoliths processed than scales as a result of the otoliths taking longer to process. We also used two methods to process all otoliths (thin sections and crack-and-burn). Spines were selected by randomly choosing up to two spines per length bin for fish that were also in the otolith sample. There were fewer spines in the sample due to time constraints.

# Discussion

Otoliths

We used two methods to process otoliths: crack and burn (n=74) and sectioning (n=74). These methods both produced similar results, with several accessory checks and false annuli. Figures 1A and 1B both show an otolith from a 161 mm female Ruffe caught in Thunder Bay for which a different putative age was obtained from each method (4+ from the sectioned and 3+ from the cracked-and-burnt otolith). The sectioned otolith shows possible false annuli along with a double nucleus (Figure 1A). The sectioned otolith also shows annuli in different locations compared to the cracked and burnt otolith (Figures 1A & 1B). Figure 2 shows a sectioned and crack and burnt otolith from a 101 mm male Ruffe caught in the Ontonagon River with a putative age of 3+ from both methods. Figure 3 shows a sectioned and crack and burnt otolith from a 56 mm male Ruffe also caught in the Ontonagon River with a putative age of 2+ for both methods. In general, sectioning appeared to produce more distinguishable annuli compared to the crack and burn method for Ruffe. The crack and burn method was less successful in producing distinguishable annuli, possibly due to variability of heat exposure and size of otoliths. It was also difficult to process otoliths from fish smaller than 90 mm using the crack and burn method because smaller otoliths were harder to crack and handle with tweezers. We recommend that otoliths not be used for assessing the age of Ruffe, because otoliths have several checks and false annuli which make it difficult to distinguish true annuli.

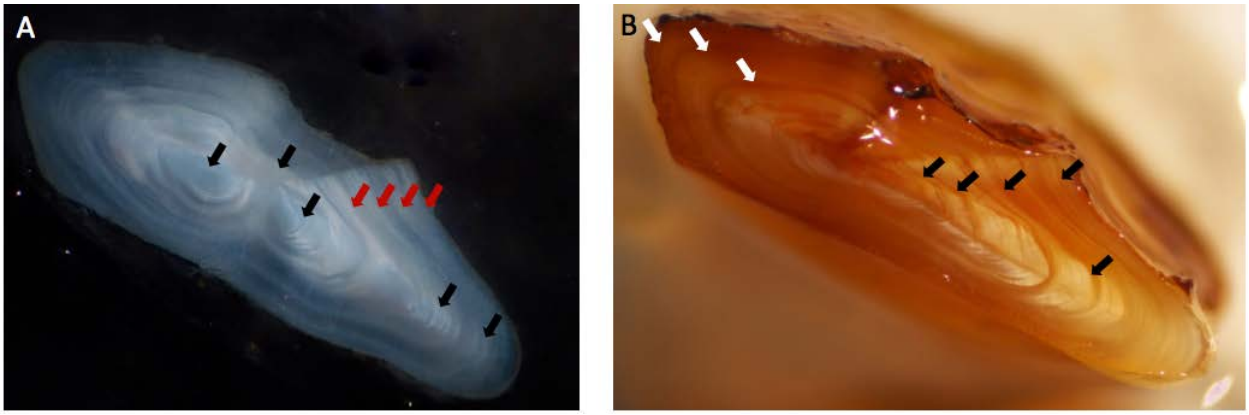



Figure 1. Sectioned (A) and crack and burnt (B) otolith from a 161 mm Ruffe. Red and white arrows depicted show putative annuli, and black arrows depict false annuli and checks.

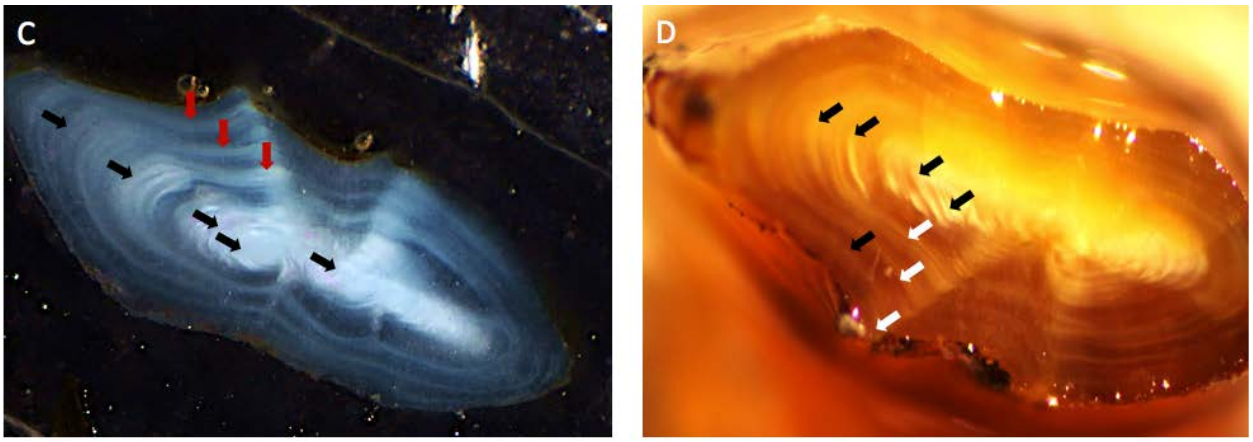



Figure 2. Sectioned (C) and crack and burnt (D) otolith from a 101 mm Ruffe. Red and white arrows depicted in Figures 1-3 show putative annuli, and black arrows depict false annuli, along with checks.

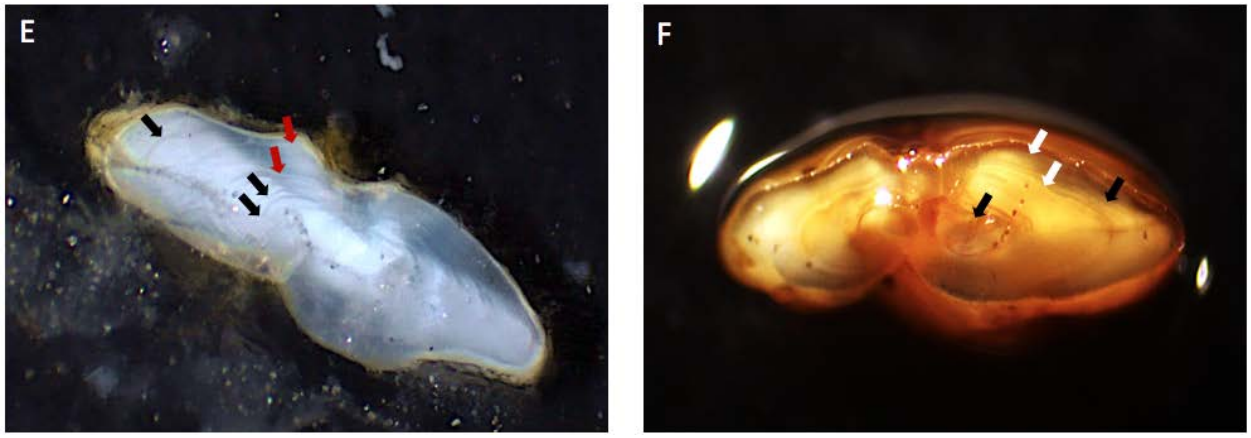



Figure 3. Sectioned (E) and crack and burnt (F) otolith from a 56 mm Ruffe. Red and white arrows depicted in Figures 1-3 show putative annuli, and black arrows depict false annuli, along with checks.

Spines

Sectioning the third dorsal spine (n=15) from Ruffe generally showed the clearest and most distinct annuli, especially for large, old fish. Figure 4 shows a sectioned spine from a 161 mm female Ruffe captured in Thunder Bay with a putative age of 3+ (4A), a sectioned spine from a 134 mm female Ruffe captured in the St. Louis River Harbor with a putative age of 4+ (4B), a sectioned spine from a 61 mm female Ruffe captured in the Ontonagon River with no distinct annuli and a putative age of 0+ (4C), and a sectioned spine from a 56 mm male Ruffe captured in the Ontonagon River with no distinct annuli and putative age of 0+ (4D). For small, young fish (Figures 4C & 4D) it was difficult to assign an age because there was a lack of distinct annular marks. For Ruffe, annuli were more distinguishable in spines as compared to otoliths and scales because there were no false annuli or indistinguishable marks. We recommend that spines from large, old fish (Figures 4A & 4B) be used to assess the age of Ruffe because they show distinct and clear annuli with no false marks.

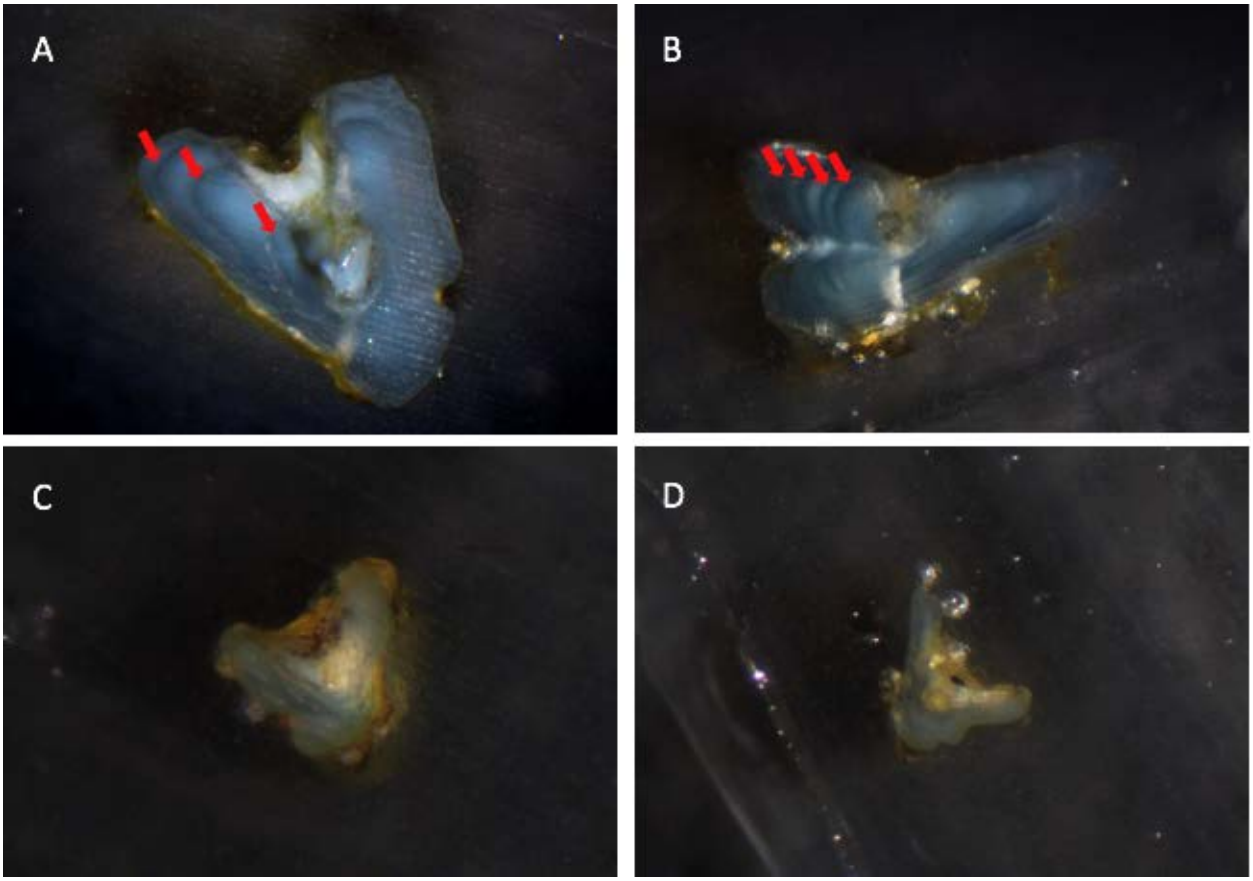


Figure 4. Sectioned spine from a 134 mm fish (A), 161 mm fish (B), 61 mm fish (C), and 56 mm fish (D).

Scales

Pressed scales from small, young fish generally showed noticeable annuli. Small, young fish (Figure 5A) showed easily distinguishable wide-spaced circuli during summer and tightly-spaced circuli during winter periods. Annuli were difficult to identify on the margins of scales from large, old fish (Figure 5B) because later annuli nearer the scale margin became too compact. Figure 5 shows a pressed scale from a 61mm female Ruffe captured in the Ontonagon River with a putative age of 0+ (5A) and a pressed scale from a 161 mm female Ruffe captured in Thunder Bay with a putative age of 2+ (5B). We recommend that scales from small, young fish (Figure 5A) be used to assess the age of Ruffe because they show easily distinguishable annuli.

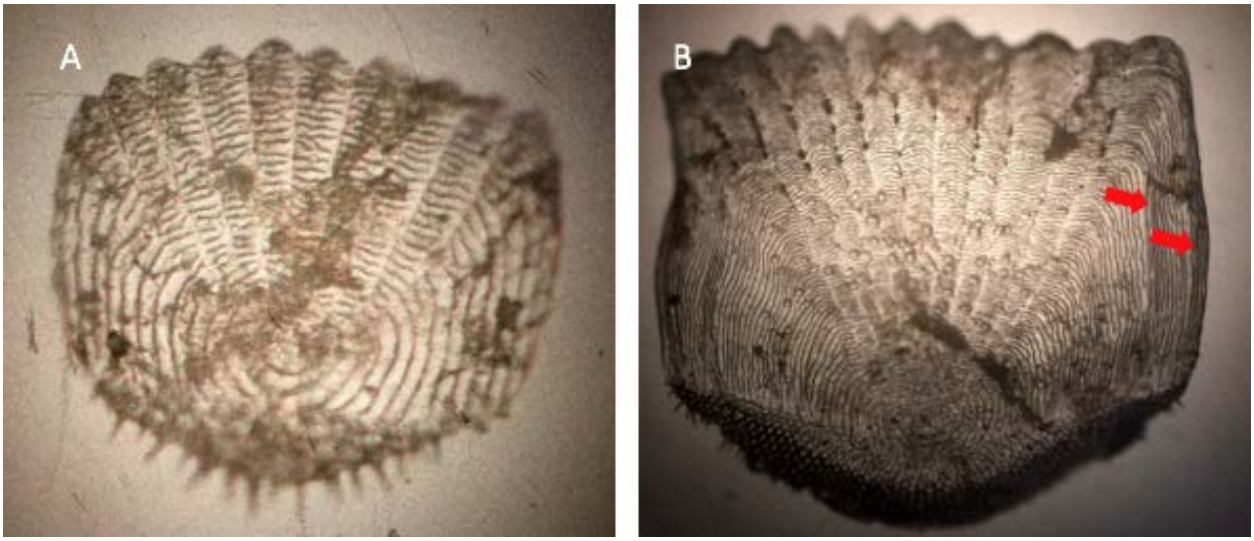



Figure 5. Scales pressed in acetate from 61 mm (A) and 161 mm (B) Ruffe.

## Future Work

We have determined that spines for larger Ruffe and scales for smaller Ruffe are most interpretable and hold the most promise for assigning age to Ruffe, but we did not determine the accuracy and precision of spines and scales for assigning ages to Ruffe. Continued research should assess the precision of scales and spines for assigning age, especially in relation to the size of the fish. Accuracy for scales of small, young fish and dorsal spines of large, old fish could be determined by comparing putative age to the true age of each fish, but knowing the true age of each fish is expensive and time consuming. We recommend that further research focuses on determining the precision, not accuracy, of spines and scales for assigning ages to Ruffe. This should be conducted by collecting Ruffe from pre-established collection sites with known populations of Ruffe. A sample size of at least 90 fish with each length category well represented (at least six fish per 10 mm increment) between 45 mm and 195 mm should be used. Each sex should also be well represented (at least three males and three females per 10 mm increment). 10 mm increments would then be grouped into three length categories: small fish (45 mm to 95 mm), medium fish (105 mm to 145), and large fish (155 mm to 195 mm). These categories were selected because we found that scales for small, younger fish and spines for large, older fish hold the most promise for assigning age to Ruffe. By separating fish into small, medium, and large categories it will be easier to determine whether or not our finding was correct. All fish in the sample would then be assigned ages using both spines and scales. Multiple technicians should individually assign these ages and compare ages among each other to determine precision. An average percent agreement, mean coefficient of variation, and average percent error will be determined for the three length categories. The length categories should be statistically compared using a two-way anova to determine which length categories are the most precise for both spine and scales. Three two-way ANOVAs, one for percent agreement, one for mean coefficient of variation, and one for average percent error, would provide any significant differences among the three length categories, along with differences between spines and scales. We would hypothesize that scales from the small length category and spines from the medium and large categories would be most precise. Percent agreement is the percent of age estimates for a fish that agree or agree to a certain extent (Ogle 2016). Coefficient of variation is the ratio of the standard deviation of age and the mean age. The mean coefficient of variation is the mean of many CVs. Average percent error is a measure of how imprecise a measurement is, and is the mean of many percent errors (Campana 1995).

# Appendix

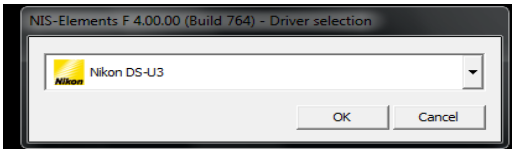
## Microscope and Computer Display

**Step 1**
Turn on the DS-U3 Digital Sight.

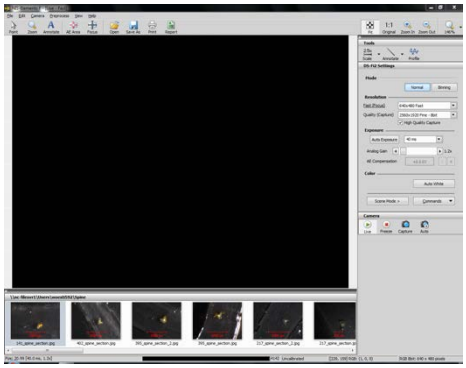
**Step 2**
Assure that the Nikon DS-F12 attachment (A) is on the Nikon SMZ 74ST microscope (B) and the cord (C) is plugged into the DS-U3 Digital Sight.


**Step 3**
Open the NIS-Elements program on the desktop.


**Step 4**
Select the Nikon DS-U3 driver.

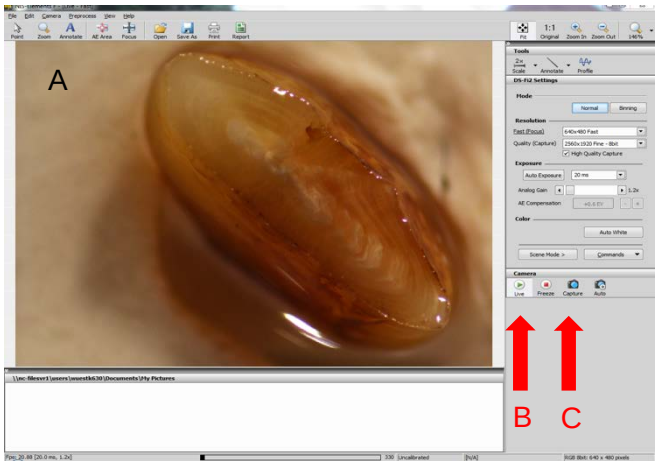



**Step 5**
Once this screen appears, the computer and microscope are ready to capture images.

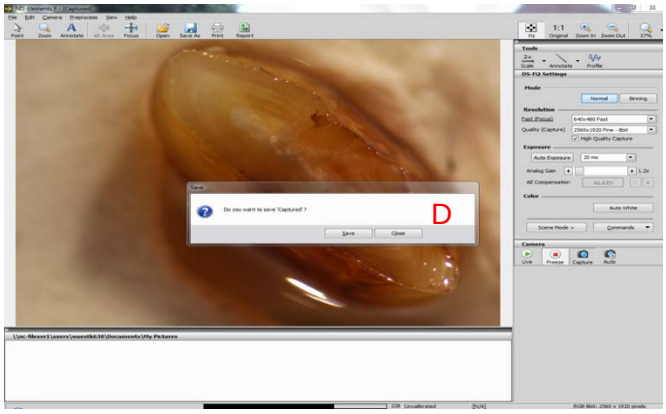


# Capturing and Saving Images

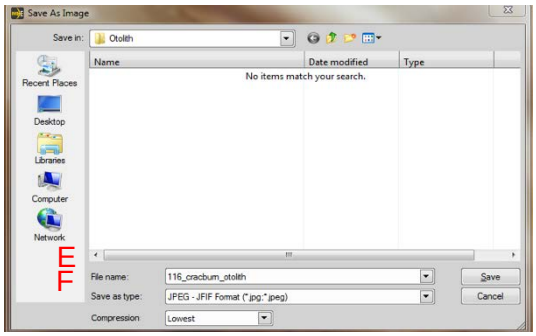
**Step 1**
Bring the image into focus and, by adjusting the microscope and lighting, zoom in until the structure fills the screen (A). Make sure the camera setting is on Live while adjusting the zoom and focus (B). When the structure becomes the most clear and shows the most distinguishable annuli, click capture (C).



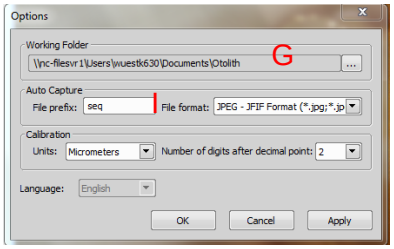

**Step 2**
A message will appear (D) and will ask if you would like to save the captured image. Click save.

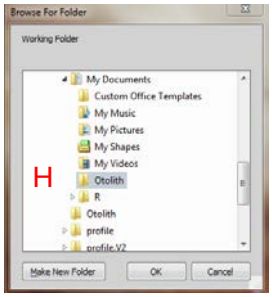



**Step 3**
Create a folder specific to the structure you are working with. Save the captured image in this folder with a file name (E) that consists of the fish's unique identification number, structure type (e.g., otolith), and, possibly, the method type (e.g., section) and image number (if multiple images taken). Change the type of file (F) to a JPEG-JFIF format and click save.



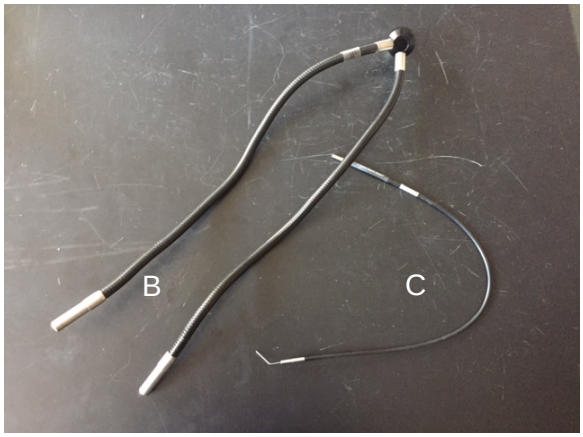

**Step 4**
Once you have created a folder you will need to set it as your working folder so that you can view your images on the bottom of the viewing screen. This also sets this folder as the default when you save other images. Select File: Options: Working Folder (G) and select the new folder you created (H). Also select the File Format as a JPEG-JFIF Format (I).



# Light Source

You will need to use the NI-150 High Intensity Illuminator (A) to view the structures under the microscope. The intensity of light used will vary, but higher intensity light generally works better. This illuminator also works better if the room is as dark as possible; therefore, turn off the room lights and close the window blinds. There are two attachments for the illuminator -- the dual broad light attachment (B) and the focused single light attachment (C). In general, the dual broad light attachment works better, but in some situations the focused light will produce a better quality image. One of these attachments will likely be plugged into the illuminator and the other will be on the shelf directly above the illuminator. To insert attachment B, align the indent on the attachment with the screw on the illuminator and tighten the screw until it is snug. To use attachment C, you will need to first put attachment D into the illuminator by aligning the indent on the attachment with the screw on the illuminator. Then tighten the screw until it is snug. You can then insert attachment C into attachment D.

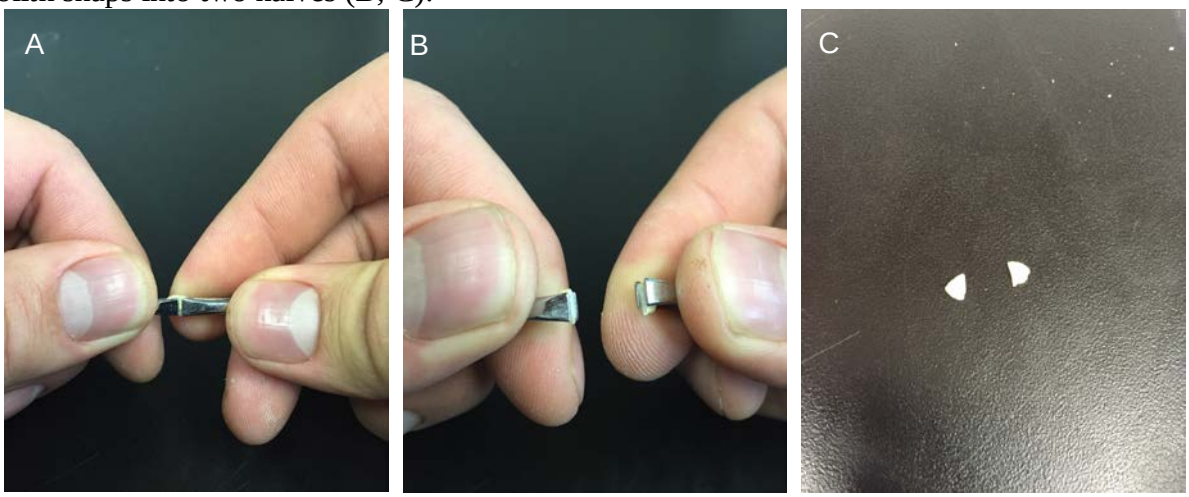

# Crack and Burn Otoliths


**Materials**

- 2 Pairs of Straight Tip Tweezers
- 1 Tea Candle
- 1 Container of Putty
- 1 Clear Dish
- Bamboo Oil

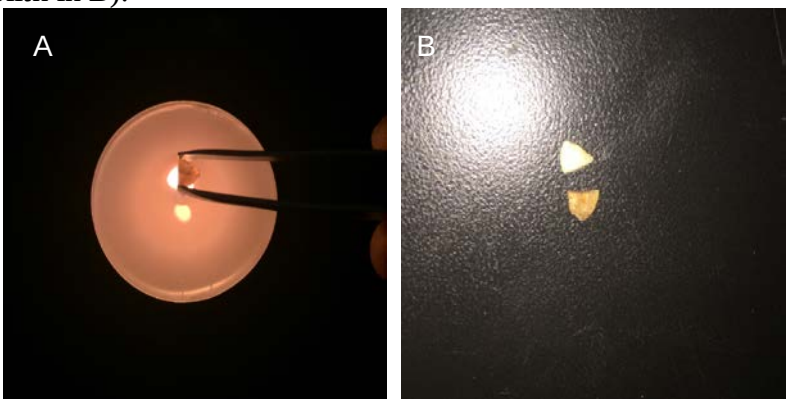
**Step 1**

Pick up the the otolith with one pair of tweezers. Use the second pair of tweezers to position both on the center of the otolith length wise, sulcus (a groove along the medial surface of the sagittal otolith) side up (A). Once the tweezers are positioned, force downward on either side until the otolith snaps into two halves (B, C).

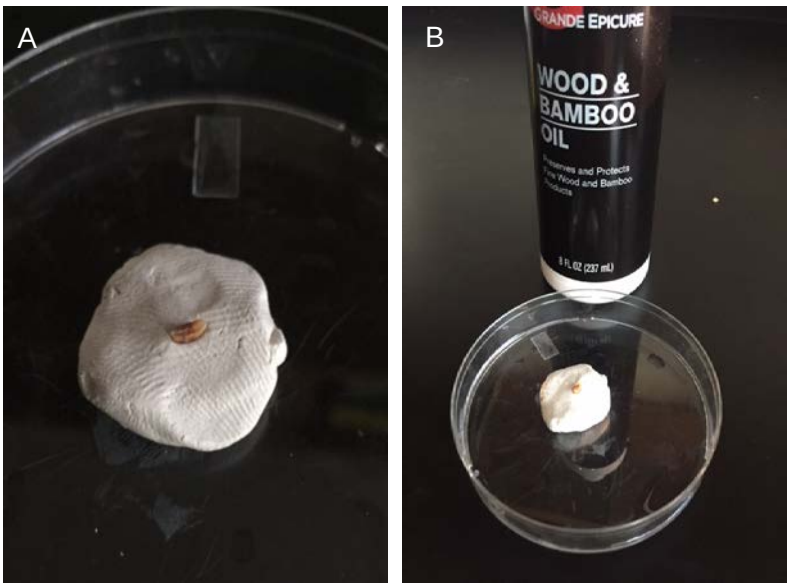



**Step 2**

Burn the halves separately over a tea candle by holding the otolith (A) approximately 8 cm above the flame for approximately 45 seconds or until the halved otolith appears golden brown in color (bottom half of otolith in B).

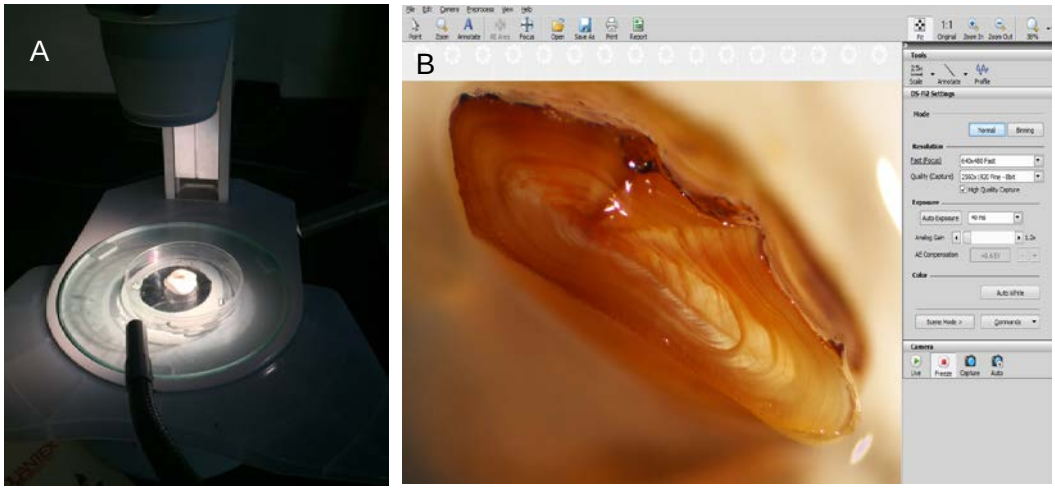


**Step 3**
Place a 1-2 cm diameter ball of putty in the clear dish (A). Place the burnt otolith in the putty, cracked side up. Apply 1-2 drops of bamboo oil as needed to the face of the otolith (B).




**Step 4**
Set up the microscope, computer, and light source as described previously.

**Step 5**
Once the NIS-Elements program is open, place the dish you prepared in Step 3 under the microscope (A) and bring the otolith into focus under the microscope. The light will need to be positioned specifically for each halved otolith in order to display the best image. Once you have focused the otolith and have the best lighting possible take a picture by pressing capture (B; see capture and saving section above for more detail).



# Spine and Otolith Sectioning

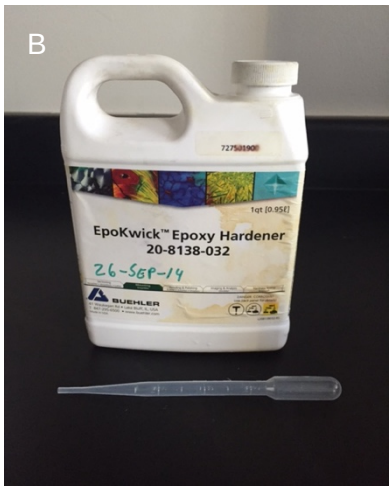
Materials

- EpoKwick Epoxy Resin
- EpoKwick Epoxy Hardener
- 1 Disposable Dish
- 3 Disposable Pipettes
- 1 Scale (grams)
- 1 Disposable Stirring Rod
- 1 Red Rubber Mold
- 1 Space Heater
- Buehler Slow Speed Saw



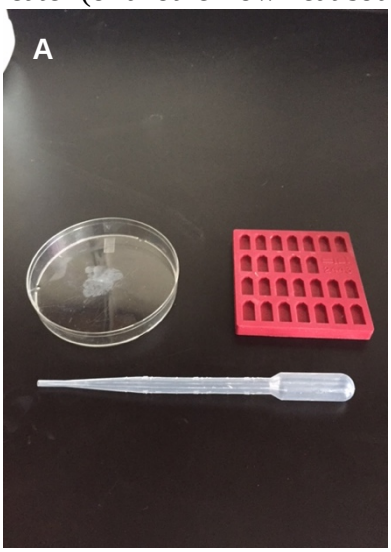
**Step 1**

Mix the epoxy with the hardener in the clear dish. You need five parts resin for every one part hardener (5:1 resin:hardener). It is crucial to be very precise with your measurements, otherwise the epoxy will not set properly. Begin by placing the clear dish on the scale and zeroing the scale. Using a new, clean pipet, draw Epoxy resin from the container and put precisely 5.0 g into the dish on the scale (A). Once you have measured out the resin, use a different new, clean pipet and draw Epoxy Hardener from the container (B). Put precisely 1.0 g of hardener (to reach the 5:1 ratio) into the same dish that you put the resin in. Discard the unused hardener in the pipette; do not return unused hardener to the container! Mix the resin and hardener together for at least 3 minutes using the stirring rod. It is important to mix the epoxy and hardener slowly but thoroughly. The mixture will become cloudy after approximately one minute, but will then clear with continued stirring. This amount (i.e., 5.0 g of Epoxy resin and 1.0 g of epoxy hardener) should be enough to fill all spaces in one mold half-full with epoxy. If a larger mold is used, then more epoxy can be mixed but in the same precise 5:1 ratio.

**Step 2**

Using a new, clean pipet, draw the epoxy mixture from the dish (A) and use it to fill the desired amount of cells in the molds half-full with the epoxy mixture. Place the epoxy-filled mold in front of the space heater (or another low heat source) to dry/harden overnight (B).

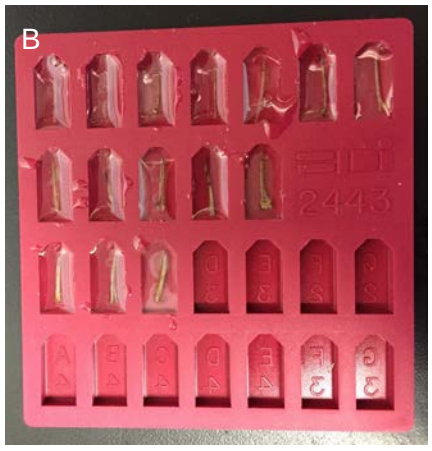




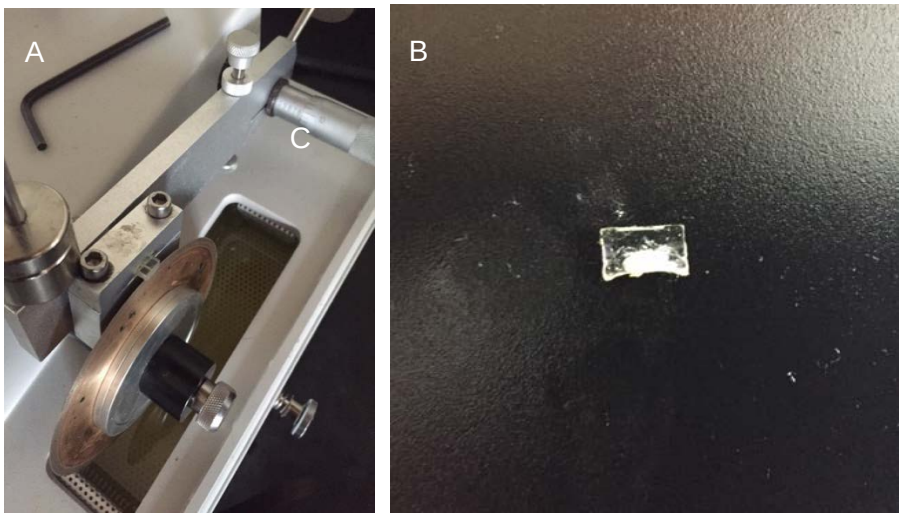
**Step 3**

Otolith. -- Once the epoxy in the mold has hardened (A), place an otolith in each of the cells that are half-filled with dry epoxy. Orient each otolith so that the rostrum (anterior and ventral projection of the sagitta) is toward the narrow, front of the cell and the sulcus side of the otolith is down. Repeat steps 1 and 2 to cover the otoliths with epoxy mixture. It is critically important to keep track of which cell you place each otolith in by recording which fish identification number corresponds to each cell number and letter on the red mold.

Spine. -- Once the epoxy in the mold is dry (A), you will place spines in each of the cells that are half-filled with dry epoxy. The spines may need to be trimmed before being placed into the molds. Hold the base of the spine with a tweezers and cut as much excess from the tip of the spine as needed to fit in the mold. Orient each spine in a parallel fashion as displayed in (B). Repeat steps 1 and 2 to cover each spine completely with epoxy mixture. It is critically important to keep track of which cell you place each otolith in by recording which fish identification number corresponds to each cell number and letter on the red mold.

**Step 4**
Once the epoxy with the otolith/spine has hardened, you can cut sections from the epoxied otoliths/spines using the low-speed Buehler saw (A). Place the epoxied otolith/spine in the clamp on the saw and tighten the screws using the Allen wrench. Otoliths should be positioned in the clamp with the rostrum facing outward, whereas spines are positioned with the base of the spine facing outward. Use the dial (C) to move the epoxied otolith until the blade is behind the rostrum for otoliths or lined up with the thickest part of the spine. Once the blade is in place, turn the saw on and set the speed to five until just before the blade reaches the otolith/spine, then slow the speed to three. After the first cut is made, turn the dial and look at the measurements on the dial so there is a thin section (approx. 1mm thick) in front of the saw and make a second cut. The second cut produces the thin section that you will use (B) -- be careful that it does not get lost!

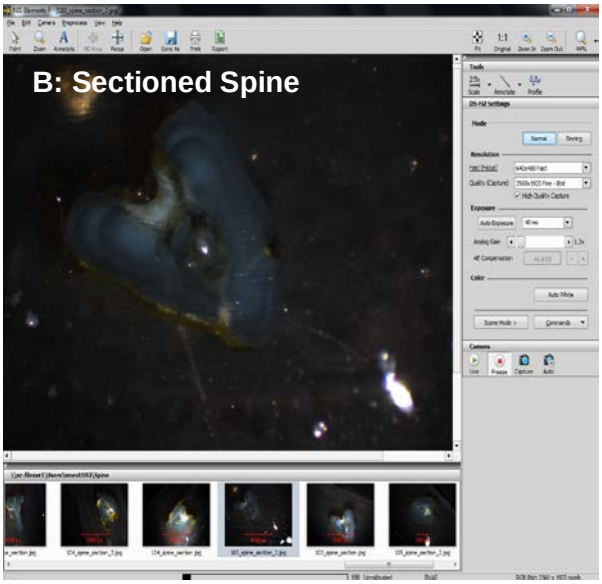


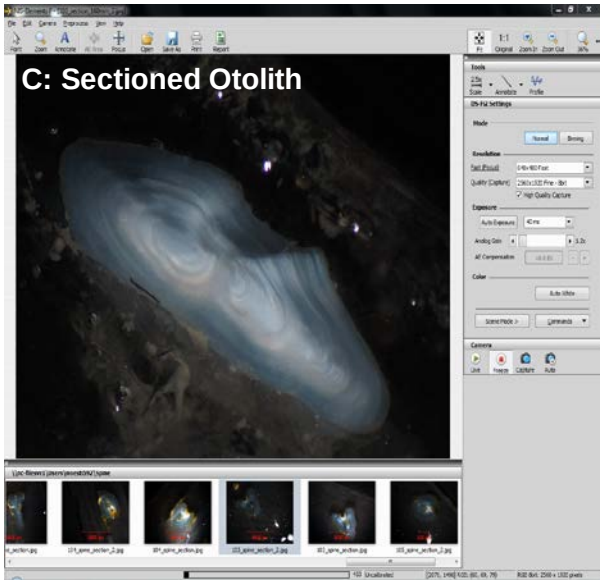

**Step 5**
Place the thin section of the structure on a black background (hard plastic) and put 1-2 drops of bamboo oil on top of the sectioned structure.

**Step 6**
Set up the microscope, computer, and light source as described previously.

**Step 7**

Once the NIS-Elements program and the microscope are ready, place the thin section on a black background that you prepared in step 5 under the microscope and bring the microscope into focus. The light will need to be positioned specifically for each section in order to display the best image. Once you have focused the structure (B and C) and have the best lighting possible (A), take a picture by pressing capture (see capture and saving section above for more detail).

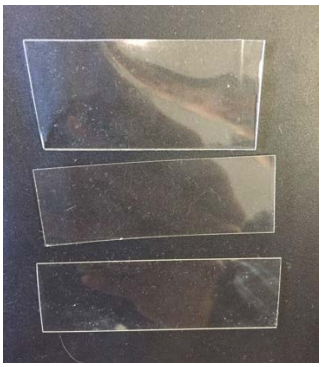



# Scale Pressing

Materials

- Microfiche
- Scale Press

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