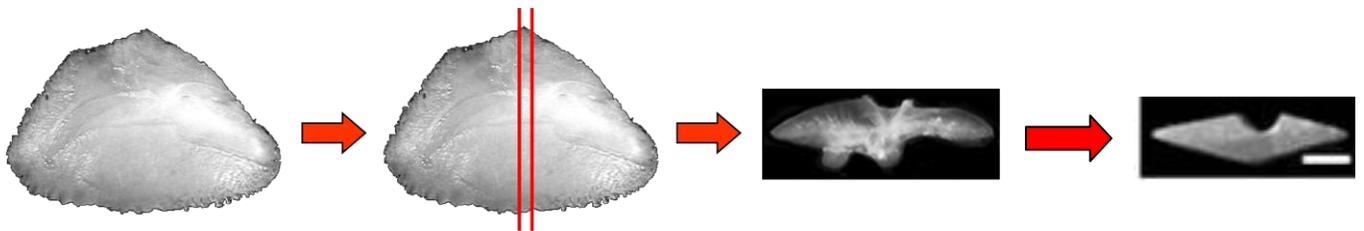




# Otolith Chemistry Procedure Manual



National Marine Fisheries Service

Southeast Fisheries Science Center

Panama City Laboratory

## Otolith Extraction Method

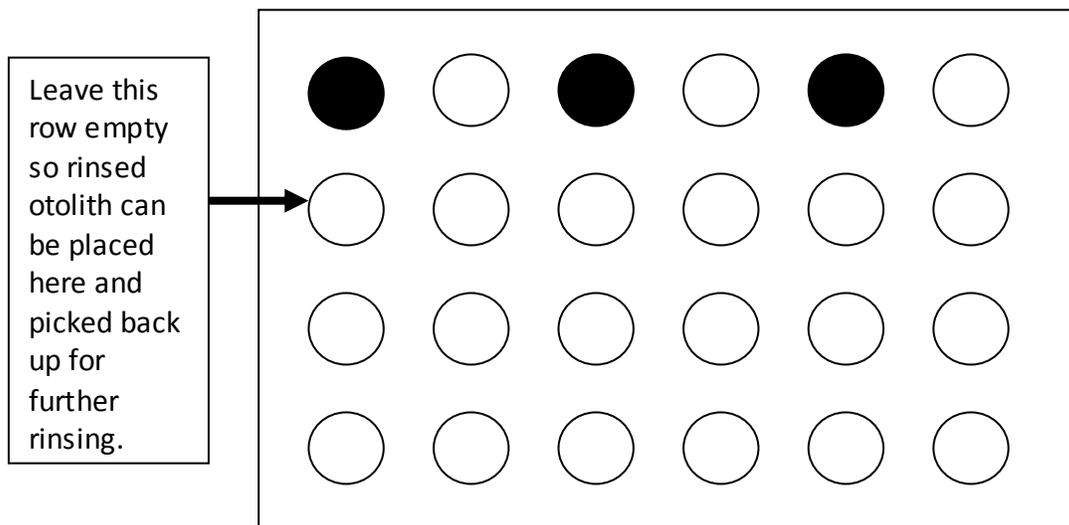
1. Wash all instruments that are used in extraction process with soap & water.
  - collection bowls (large & small)
  - glass probes (large & small)
  - plastic, clear ruler or measuring board
  - Teflon-coated forceps (large & small)
2. Rinse all instruments 3 times with regular water.
3. Rinse all instruments 3 times with de-ionized water.
4. Clean countertop where extraction process takes place with isopropyl alcohol.
5. Measure the length of the fish.
6. Weigh the fish.
7. Remove the left or right otolith by:
  - pulling the gill arch down to expose the otolith capsule
  - use the glass probe to break open the otolith capsule
  - use the Teflon-coated forceps to remove the otolith
8. Rinse the otolith for 10-15 seconds with de-ionized water while holding the otolith with the Teflon-coated forceps. Remove any of the membrane that might still be attached to the otolith.
9. Repeat step #8 two more times (for a total of 3, 10-15 second rinsings). Between each rinsing set the otolith down so that you can grab it @ different points with the forceps to ensure that the entire otolith is being rinsed thoroughly.
10. After the 3<sup>rd</sup> rinsing, place the otolith on the cell tray lid that was pre-labeled with the UWF-ID #. Be sure to place the otolith on the correct corresponding UWF-ID #.
11. After all the otoliths have been removed, place the cell tray under the “clean room” hood to help facilitate drying.
12. After 3-5 days of drying, place the otoliths in the cell tray and cover with lid. Place a couple of rubber bands around the cell tray to ensure the lid remains in tact.

## Washing Otoliths with 1% Nitric Acid

NOTE: Everything for this procedure will be performed in the “clean room”.  
Wipe off countertop. Wear gloves & lab coat at all times.

Only use Q-water (polished water) directly from filter in “clean room”.

1. Label cell trays on top and bottom to correspond to Analytical ID.  
Remove only 1 tray at a time while only partially removing the sealed cover back in order to remove cell tray. Label the cell trays (top & bottom). Place cell tray back in partially sealed container & place under clean hood.
2. Get 2 new cell trays – only keeping the lids (tops).  
1% Nitric Acid squirt bottle. If filling squirt bottle, shake big bottle to ensure mixing. Hold over blue water tub & make 3-4 passes over the entire cell tray tops with Nitric Acid.  
Flood with Q-water in squirt bottle @ least 5 times.  
Shake dry.  
Do not touch inside of trap top.  
Make sure there are no big droplets of water left on top before using – a few small drops is okay.  
Carefully, wipe back of top with kim wipe to remove excess water.
3. Transfer 1 row @ a time to new cell top leaving a space (cell well) between each otolith. You may want to only transfer 3 otoliths when first beginning.



Example: prepared cell tray lid.

● = spot on cell tray lid where otolith will be placed in nitric acid.

## Washing Otoliths with 1% Nitric Acid (cont'd)

4. Squirt bubble of nitric acid over each otolith and leave otolith in acid for ~ 20 – 30 seconds.
5. Use leached tweezers & remove an otolith from lid.
6. Hold over small beaker & flood with Q-water – rinsing tweezers, as well.
7. Set otolith back on prepared cell tray lid in clean, dry spot. Pick up with tweezers in different area of otolith so that entire otolith is rinsed.
8. Hold over small beaker & flood with Q-water.
9. Set otolith on correctly labeled cell tray lid. It will leave a bubble of water when you 1<sup>st</sup> set it down. Pick otolith back up with tweezers and move to a different spot on the correctly labeled cell tray lid.
10. Rinse tweezers between samples with Q-water.
11. When completed wash 3 (or 6) samples, rinse prepared cell tray lid thoroughly with Q-water.  
Shake dry.  
Place under clean hood to air dry.
12. Get the other prepared cell tray lid that has been drying under clean hood & repeat steps 3 through 11.  
Be sure there are no big droplets of water left on cell tray lid.
13. After water droplet has dried on labeled cell tray lid, use dry tweezers to flip otolith over & move to dry spot on correct cell well lid to dry.
14. Okay to leave uncovered while working on them, but COVER with cell tray bottom @ night.  
Do not leave otolith exposed overnight.

## Leaching 60-mL and 15-mL Nalgene bottles

1. Decide how many samples you will be processing for ICP-MS, then add about 5 – 10 extra bottles for leaching for “Blanks” and “Reference Material”.
2. Fill 60-mL and 15-mL Nalgene bottles approximately  $\frac{1}{2}$  -  $\frac{3}{4}$  full using 1N HCL. Place lid on tight and place bottles in double-bagged Ziploc bags (Use gallon-size bags for 60-mL bottles and quart size for 15-mL bottles).  
Note: You want to add enough HCL so that a little over  $\frac{1}{2}$  of bottle is leached when bottle is lying on its side.
3. Once bottles are filled, place Ziploc bags, with bottles lying on their side, inside box. Label box with date & time that leaching began. This will allow for leaching of a little over  $\frac{1}{2}$  of the bottle lying on its side.
4. Next day: @ 24 hours, flip box @ 180 degrees so other side of bottle will leach for 24 hours. Again, label date & time that leaching began.
5. Next day: pour HCL back into big bottle. Mark big bottle of HCL with sharpie to show that it has been used.
6. Rinsing: Each 60-mL and 15-mL bottle will be rinsed 5 times with Q-water.

**Example:** Pour HCL into big bottle.  
Fill bottle with Q-water.  
Invert bottle numerous times.  
Pour Q-water into blue tub.  
Refill with Q-water.  
Continue rinsing for a total of 5 times.

## How to Make 1% Nitric Acid

1. Fill 500-mL and 1000-mL bottles with ultrapure water.
2. Wipe off counter top
3. Place kim wipes down on counter top
4. Place bag with bottles on kim wipes

### Under Hood

1. Clean off bench under hood
2. Place paper towels down under fume hood
3. Cover paper towels with kim wipes
4. Remove nitric acid from box & bag.
5. Place nitric acid under fume hood on kim wipe
6. Place bag back into box & store under hood
7. Bring graduated cylinder (with parafilm) from clean room & lay bag on side under fume hood.
8. Bring bottle from counter top & place on kim wipe under fume hood
9. Remove graduated cylinder from bag
10. Remove parafilm
11. Remove cap from nitric acid bottle
12. Remove cap from water bottle
13. Pour acid into graduated cylinder
  - 1000 mL (1 L) bottle = 10 mL of nitric acid
  - 500 mL bottle = 5 mL of nitric acid
14. Pour acid from graduated cylinder into water-filled bottle.
15. Place graduated cylinder in bag
16. Place cap on 1% nitric acid bottle
17. Place cap on nitric acid bottle.
18. Place parafilm on graduated cylinder & return to bag.

## Dissolving Otoliths with 1% Nitric Acid

NOTE: Everything done in clean room:

wipe off countertop. Wear gloves & lab coat @ all times.

1. Turn Clean Hood off next to scale and close plexiglass shield to hood. You may have to turn off both hoods.
2. Place large kim wipe on area next to scale. Tape kim wipe down to countertop if necessary.
3. Place bottles 1 through 12 on kim wipe (leached & rinsed bottles only).
4. Place bottle on scale.
5. Tare scale.
6. Add otolith to bottle with leached Teflon tweezers & record weight.
7. Tare scale.
8. Add 1% nitric acid via squeeze bottle to bottle on scale.

**For every 1 milligram (mg) of otolith weight, add 1 gram (g) of 1% nitric acid.**

Example: Otolith weight = 0.03182 g (= 31.82 mg)

Tare scale.

Add 1% nitric acid = 31.82000 g

9. Repeat steps 3 through 8 for all subsequent samples (example: place bottles 13 through 24 on kim wipe –leached & rinsed bottles only).

## How to Pipette Dissolved Otoliths from 60mL Bottles to 15mL Bottles

### **Setup**

1. Make 2% nitric acid for leaching pipette tips.
2. Leach ~5 (50 mL) disposable beakers with HCL in bath
3. Turn off hoods so it doesn't interfere with weighing process.
4. Set up kim-wipes on tabletop next to balance (tape down if necessary).
5. Put 15 mL bottle in balance.
6. Tare balance.

### **Leaching Pipette tips**

7. Place 5 mL pipette tips in small Ziploc bag to avoid being in big bag frequently.
8. Put parafilm across disposable beaker leaving a small space to get pipette tip into beaker.
9. Do not touch pipette tips.
10. One beaker will contain 2% nitric acid (draw a circle on the beaker to indicate nitric acid)
11. One beaker will contain 18 Q-water (only take directly from filter – do not use carbuoys).
12. Pipette 5 times with acid & squirt it out each time into waste beaker.  
Pipette 5 times with water & squirt it out each time into waste beaker.
13. Shake off any residual water.
14. Lay pipette down under hood with tip hanging over the side of table.
  
15. Invert bag containing samples a couple of times to mix well.
16. Place cap under hood upright to avoid dust from settling.
  
17. Pipette 1 time using 5 mL pipette tips to 15 mL bottle in balance.
18. Record weight to 4 decimal places.
19. If weight is a little off (ex: 4.875 g), don't worry about it.
  
20. At end of day, place beaker into bath.
21. Next morning, rinse beaker with Q-water.

## Pulverizing Otoliths for IR-MS Analysis

**NOTE: Everything is done in Clean Room.**

**Wear gloves & lab coat at all times.**

1. Be consistent as to which otolith is used for IR-MS (always use left; or always use right).
2. Make sure that glass mortar & pestles have been leached for at least 24 hours in 1N HCL and rinsed 5-times with Q-water.
3. Place otolith in glass mortar (distal side facing upwards).
4. Cup your hand over the mortar so the otolith cannot bounce out of the mortar.
5. Press down, while turning, with the pestle. This should break the otolith into multiple pieces.
6. Continue pulverizing the otolith until there is a smooth powder.
7. If processing Age-0 fish, use a piece of wax paper to transfer ½ of pulverized material to labeled microcentrifuge tube. Keep the other ½ of the pulverized material for archive purposes.
8. If processing Age-1 cores, use a leached and rinsed, L-shaped, cutout from a Ziploc storage container to transfer the pulverized material. In this process, you will be processing both the elemental and stable isotopes at the same time.

### **Elemental (ICP-MS)**

Place 60-mL bottle on scale.

Tare scale.

Transfer at least 2 mg (preferably more, if possible) of pulverized material to the bottle using the L-shaped, Ziploc cutout.

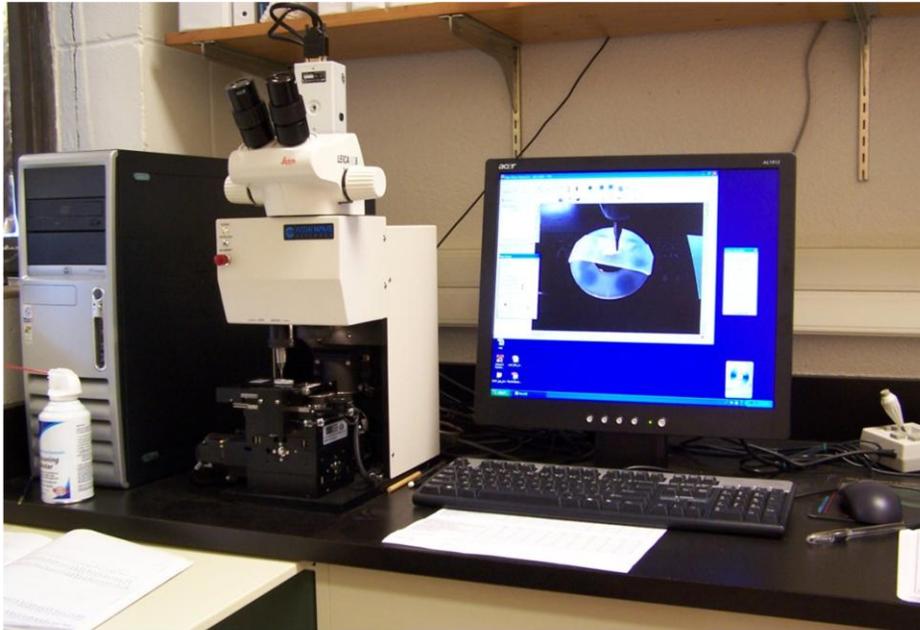
### **Stable Isotopes (IR-MS)**

Transfer the remaining material to a labeled, microcentrifuge tube using the same L-shaped, Ziploc cutout.

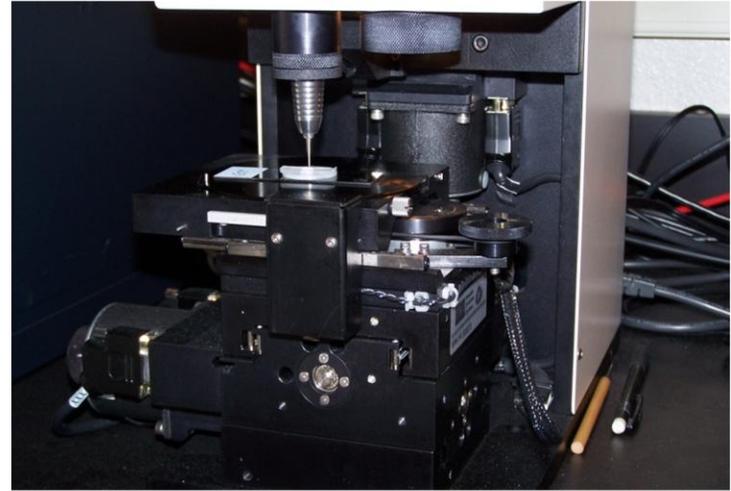
NOTE: After you have used all the leached mortar & pestles, as well as the L-shaped Ziploc cutouts:

1. Wash both the mortar & pestle with 1% Nitric Acid @ least 3 times, swirling acid around in mortar.
2. Rinse with Q-water @ least 3 times, swirling water around in mortar.
3. Hold the L-shaped, Ziploc cutout with leached Teflon tweezers while washing with 1% Nitric Acid @ least 3 times.
4. Continue holding with Teflon tweezers and rinse with Q-water @ least 3 times, placing the cutout down on a kim wipe between each rinse and picking it back up in a different location.
5. Air-dry under Clean Hood.

## Merchantek Micromill

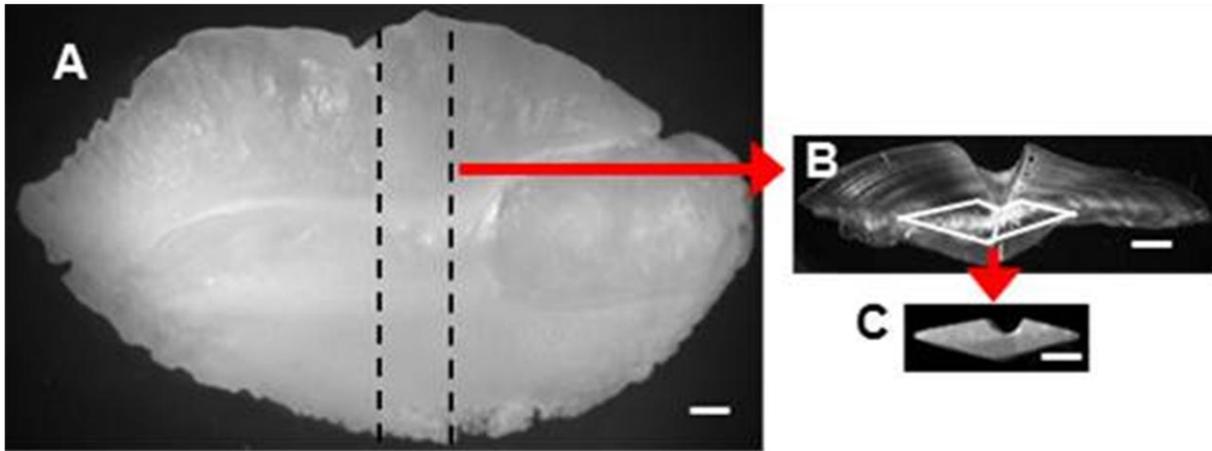


The Merchantek Micromill is a computer-controlled microsampling device used to remove otolith cores from sub-adult and adult fish.



← Milling Bit

## Coring Otoliths from Sub-Adult and Adult Fish



Digital images of A) a whole red snapper otoliths from a 563 mm TL 5-year old female, B) a thin section cut from the otolith, and C) the core of the otolith extracted with a Micromill precision drilling instrument. Scale shown on each is 1 mm.

## Sectioning and Mounting Otoliths to Slides for Micromilling

1. Leach glass slides in 1N HCL for at least 24 hours.  
Rinse 5 times with Q-water.  
Air-dry.  
Double-bag.
2. Leach nylon washer in 1N HCL for at least 24 hours.  
Rinse 5 times with Q-water.  
Air-dry.  
Double-bag.

### **Mounting Otolith in Epoxy Prior to Sectioning**

1. Use West Epoxy System with their measuring dispensers.
2. Pour a small amount of epoxy into Peel-a-way mold. This will give the otolith the necessary support it needs to be milled.  
Let air-dry for 24 hours.
3. Label Peel-a-way mold with Analytical ID #.
4. Place otolith on top of dried-epoxy surface.
5. Cover with epoxy.  
Let air-dry for 24 hours.

### **Sectioning Otolith Using Isomet Saw**

1. Use only Distilled Water for lubrication of the blades.
2. Only use blades, spacers, and water tray that have not been exposed to oil or other contaminants.
3. Use 2 blades that are separated by 1.4 mm using plastic spacers.
4. Remove the otolith mounted in epoxy from Peel-a-way mold and mark core.
5. Mount in the isomet arm so that the mark is centered between both blades.
6. Section the otolith.
7. Remove the section from saw.  
Rinse with Distilled water.  
Air-dry.

## Sectioning and Mounting Otoliths to Slides for Micromilling (cont'd)

### **Mounting to Glass Slide and Micromill Sample Plate**

1. Mount nylon washer to glass slide using Loctite glue. Be sure that no glue is in the washer center.  
Let dry for at least 15 minutes.
2. Label glass slide with Analytical ID #.  
Once sectioned otolith is dry, mount to top of washer using Loctite glue. Be sure to leave a little space between the section and the washer to be able to remove the cored piece.
3. Mount slide to micromill sample plate using paraffin wax.  
Place sample plate on top of slightly heated hot plate.  
Use tweezers to dissolve a minimal amount of paraffin wax on sample plate.  
Place slide on top of wax and immediately remove from hot plate.  
Place sample plate on cool surface.

NOTE: Once core has been milled, keep slide in slide boxes for archive purposes.

## Micromill Drilling Procedures

1. Raise Drill up from sample plate.
2. Spray canned air to remove dust particles on drill bit.
3. Mount sample (ensure that sample plate thumbscrew is tightly fastened)
4. Click on "Drill Bit Position" and follow message prompts.
5. Center drill bit over empty space on sample plate (visually check that it will not come down on sample).
6. Click "next".
7. Record drill bit position in notebook.
8. Click "OK".
9. Click on "Sample Thickness" and follow message prompts.
10. Click on Drill (the Drill button is the 1<sup>st</sup> button to the left of the Scope button). The sample plate will slide over to the drill position.
11. Center drill bit over sample.
12. Click "next".
13. Record sample thickness in notebook.
14. Click on Scope (the Scope button is the 1<sup>st</sup> button to the right of the Drill button). The sample plate will slide over to the microscope view.
15. Center sample in middle of screen. You may have to raise the Drill up a little bit so that the sample comes into better view.
16. (Only used when making new pattern) Mark 6 reference marks for pattern.
17. (Only used when making new pattern) Click on Line pattern and connect the 6 reference marks. At each reference mark, left click on the mouse to draw the next line segment.
18. SAVE SCAN as the # shown on the sample slide.
19. TAKE A PICTURE of OTOLITH SHOWING PATTERN ON TOP.
20. SAVE PICTURE of OTOLITH SHOWING PATTERN ON TOP in PHOTOSHOP.
21. Make sure properties is set to 24 passes.
22. Click on Surface Profile. Sample plate will move over under drill position.

### Micromill Drilling Procedures (Cont'd)

23. Click on "Profile" and record time in both notebooks.
24. When Profiling is complete, record Profile End time in red notebook.
25. Click on "Scan".
26. When next message box appears, click on little box next to "Enable Drill".
27. Then click on "Scan".
28. Record Scan Start time in red notebook.
29. When sample falls through, Abort scan.
30. Record End time in both notebooks.
31. Raise Drill up from sample plate.
32. Click on Scope (the Scope button is the 1<sup>st</sup> button to the right of the Drill button).  
The sample plate will slide over to the microscope view.
33. Remove sample plate and dump material onto back of Ziploc bag.
34. Remove biggest pieces and place in the corresponding sample microcentrifuge tube #.
35. (Only used when keeping excess milling powder) Pour powdered material into small microcentrifuge tube.

Checklist for Drilling Samples with Micromill

Analytical ID										
Spray Air on Drill bit to clean										
Mount Sample (ensure that thumbscrew is tight)										
Record Drill Bit Position										
Record Sample Thickness										
Recall Scan Pattern from File										
Take a picture with camera										
Save image in photoshop & label w/ Analytical ID#										
Save image as Analytical ID #										
Highlight pattern under "Desired Patterns"										
Run Surface Profile										
Click on Scan										
Click on Enable Drill										
Run Scan										

Analytical ID										
Spray Air on Drill bit to clean										
Mount Sample (ensure that thumbscrew is tight)										
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Save image as Analytical ID #										
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Run Surface Profile										
Click on Scan										
Click on Enable Drill										
Run Scan										

Analytical ID #'s \_\_\_\_\_ cored on \_\_\_\_\_.

Analytical ID #'s \_\_\_\_\_ cored on \_\_\_\_\_.