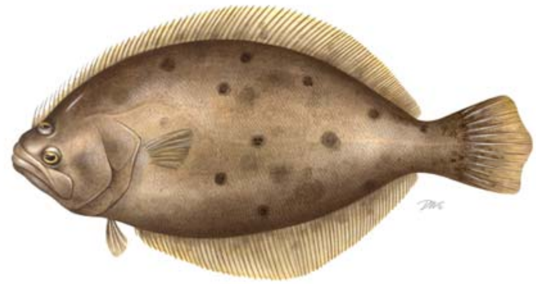
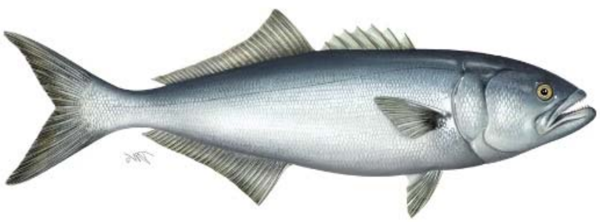
Atlantic States Marine Fisheries Commission

Report of the Quality Assurance/Quality Control Fish Ageing Workshop





April 5 & 6, 2017

Vision: Sustainably Managing Atlantic Coastal Fisheries

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Statement of Problem

Many of the fish species managed by the Atlantic States Marine Fisheries Commission (ASMFC) identify the collection of ageing hard parts, development of sample processing and reading protocols, and regular sample exchanges as research priorities in their stock assessments. Several species managed by the ASMFC have had their own ageing structure exchange and workshop to address this. However, there is a continued need for a quality assurance/quality control (QA/QC) workshop because any gradual decline in ageing accuracy could have detrimental effects on stock assessments and consistency should be monitored over time (Campana 2001). Following the Gulf States Marine Fisheries Commission (GSMFC) protocol to hold annual QA/QC workshops for its participating members, the ASMFC made a QA/QC fish ageing workshop a research priority.

The first annual ASMFC QA/QC Fish Ageing Workshop was held in 2016 (ASFMC 2016) and Atlantic croaker *Micropogonias undulatus*, black sea bass *Centropristis striata*, bluefish *Pomatomus saltatrix*, river herring (alewife *Alosa pseudoharengus* and blueback *A. aestivalis*), striped bass *Morone saxatilis*, and tautog *Tautoga onitis* were aged. Each of these species previously had their own ageing workshop and the group felt that these would be most productive to include in the QA/QC exercise. When planning for the 2017 workshop, the QA/QC fish ageing group expressed interest in rotating some species with high agreement out and others in every few years. Atlantic croaker, Atlantic menhaden *Brevoortia tyrannus*, bluefish, summer flounder *Paralichthys dentatus*, tautog, and winter flounder *Pseudopleuronectes americanus* were identified as species of interest for the 2017 workshop which took place from April 5-6th at the Florida Fish and Wildlife Research Institute (FL FWRI) in St. Petersburg, FL.

Workshop Objectives

The objectives of the workshop were to:

(1) Age samples collected and prepared from labs along the Atlantic coast for Atlantic croaker, Atlantic menhaden, bluefish, summer flounder, tautog, and winter flounder

(2) Identify areas of inconsistency that persist for processing or reading ageing structures

(3) Provide information on ageing error for each species to inform future stock assessments, including APE for group consensus ages and comparisons between individual agers that routinely age each species

(4) Develop recommendations to address any problems that emerge from this workshop so as to improve age data along the Atlantic coast

(5) Maintain samples as a reference collection for future QA/QC workshops as well as archive in a digital library

(6) Consider tautog pelvic spine samples provided by MA DMF as an ageing structure following the recommendation and protocol from Elzey and Trull (2016)

Previous Ageing Workshops

All species aged during the 2017 QA/QC Fish Ageing Workshop have previously had their own ageing workshop. Complete reports and results from those ageing workshops are available at http://www.asmfc.org/fisheries-science/research and are summarized below along with the history of how age data is used in their respective stock assessments.

I. Atlantic Croaker

Age data is used to describe the life history of Atlantic croaker in stock assessment reports, as well as in the statistical catch-at-age model in the 2010 and scheduled 2017 benchmark assessments. All ages used in these assessment reports have been from otoliths. Recommendations from the stock assessment subcommittee and the review panel during the 2005 and 2010 stock assessments identified the need to standardize ageing protocols for this species (ASMFC 2010).

The ASMFC hosted a joint ageing workshop for Atlantic croaker and red drum in 2008 to standardize methods for processing and reading otoliths (ASMFC 2008). Additionally, a goal of the workshop was to resolve the issue of identifying the first annulus from any smudges, or check marks, laid down near the core. Otolith sections were exchanged and read by participants from NJ to GA and the Southeast Fisheries Science Center (SEFSC). The workshop concluded that the smudge should not be counted but rather the first distinct ring is the first annulus.

II. Atlantic Menhaden

The most recent stock assessment for Atlantic menhaden (SEDAR 2015) used an age-structured model based on scale age data. Age data is used throughout the assessment and age is an integral part of the modeling effort and management. All age data provided for the assessment was aged at the NOAA-Beaufort Lab by Ethel Hall who retired in 2015. Hall’s duties were passed to Jennifer Potts and some state ageing labs have begun planning to age their own samples in the future.

To address future plans for states to age Atlantic menhaden scales and the research recommendation to conduct an ageing workshop, the ASMFC organized and held a workshop in 2015 (ASFMC 2015a). An exchange of scale samples took place and was followed with an in-person workshop to discuss the results. False annuli, poor storage of samples, and damaged scales were common issues identified at the workshop. The use of otoliths as an ageing structure was discussed, but more work is needed to compare ageing structures.

III. Bluefish

Both scales and otoliths have been used to age bluefish, although scale ages tend to overestimate younger fish and underestimate older fish. Scale ages were used in the stock assessment through 1997 and in 1998 the model began using otolith ages. Inaccuracies due to false annuli, regenerated scales, varying annuli counts between scales from the same fish, identifying the first annulus, and identifying annuli on scales from larger fish have all been documented (Richards 1976; NCDMF 2000; Robillard et al. 2009; NEFSC 2015). Because of these challenges, the stock assessment has used a 6+ age group in the statistical catch-at-age model to minimize the effects of ageing error for scales ages from 1985-1995.

In 2011, an ageing workshop was held for bluefish to standardize sample processing and reading procedures (ASMFC 2011). The results of this workshop established sectioned otoliths as the preferred ageing method over scales or whole otoliths and the standard protocol for processing and reading samples is that of ODU and Robillard et al. (2009). Following the workshop, Addendum I to the bluefish fishery management plan was established that required all states with substantial bluefish landings to collect and age at least 100 bluefish samples annually. Additionally, the ASMFC maintains a digital reference collection for reference and training purposes.

IV. Summer Flounder

The most recent benchmark stock assessment for summer flounder (NEFSC 2013) used a forward projecting age-structured model. Age data was used throughout the assessment including length and sex at age data, age-dependent values of natural mortality, and discards at age and therefore accurate and precise age data is critical for this assessment and its updates. There are several age-related research recommendations for summer flounder including a need for age frequency data from recreational discards, continued collection of otoliths for catch-at-age matrices, and the need for a reference collection of scales and otoliths to facilitate quality control of summer flounder production ageing.

A significant amount of summer flounder ageing work has been done by the Northeast Fisheries Science Center (see NEFSC 2013 for a more thorough description). Both scales and sectioned otoliths have been used to age summer flounder. The ASMFC sponsored an ageing workshop for scup *Stenotomus chrysops* and summer flounder in December, 2014, through a partnership with Virginia Institute of Marine Science (VIMS). While summer flounder does not have a published age validation study, increased interest in the species necessitated that labs ageing samples used the same protocol and ageing method. Samples were paired scale and otoliths from the NEFSC and VIMS. Agreement between readers for summer flounder was low and attributed to difficulties finding the first annulus and distinguishing check marks from true annuli.

V. Tautog

From 1995-2011, benchmark stock assessments for tautog and the updates used a VPA model that relied on age data. A statistical catch-at-age model was developed for the 2015 stock assessment and age data was used to develop life history parameters as well (ASMFC 2015b). Most states use opercular bones for ageing, but in 2001, Virginia began using otoliths to standardize readings of the operculum. Recognizing the importance that age data plays in the assessment of tautog and addressing concerns that were raised over the change in protocols in Virginia, it was recommended that a workshop be organized and conducted among participating states.

In 2012, the ASMFC organized a hard part exchange and ageing workshop for tautog to evaluate the age precision among states and establish best practices for consist age readings (ASMFC 2012a). The workshop aged operculum and otoliths, when available, and determined that precision was similar for both hard parts. Participants of the workshop recommended that operculum remain the standard for biological sampling but also encouraged otolith collection for paired sub-samples. Additionally, it concluded that the Virginia data is not significantly different from other states and it should be used in the assessments going forward. In 2013, a follow-up to the workshop was done and states remained consistent in their readings.

VI. Winter Flounder

Winter flounder was assessed using an age-structured model (NEFSC 2011) and ages were used throughout the assessment for size at age, fishing mortality at age, and calculations of spawning stock biomass and life history parameters. As part of the research recommendations, the assessment suggested that port samplers collect otoliths from large flounder since scales cause under-ageing in larger fish and that the amount of age samples from MRFSS/MRIP should be maintained or increased.

In 1998, the ASMFC organized a winter flounder otolith ageing comparison study between four readers that exhibited systematic differences between them and inconsistent age readings. Identifying a need to develop a protocol for processing and age reading for winter flounder, the ASMFC sponsored a workshop in 2001 (ASFMC 2012b). Participants found that whole otoliths could be used to age samples and that this method was superior to ageing scales for older fish. From this workshop, it was recommended that both scales and otoliths should be collected when possible and age samples from both retained and discarded fish in the recreational fishery should be collected.

Sample Collection, Preparation, & Ageing Methodology

I. Atlantic Croaker

New Jersey Division of Fish and Wildlife (NJ DFW)
Since 2006, Atlantic croaker have been collected during dockside sampling by NJ DFW staff. Fishery independent samples are also seasonally collected aboard the NJ DFW Ocean Trawl Survey. Samples are weighed, measured, and otoliths are removed as samples are being offloaded from commercial fishing vessels. Once otoliths are extracted, they are sectioned and aged under a microscope at NJ DFW's Nacote Creek Research lab. To date, 4,153 samples have been collected, with 170 samples collected in 2015.

Maryland Department of Natural Resources (MD DNR)
Maryland Atlantic croaker otoliths were collected from commercial pound nets in 2000 and then from 2002 through present (2016). A minimum of 20 samples were taken in 20 mm TL bins annually for all size groups available. Additional randomly collected pound net, gill net, and trawl commercial samples were obtained from fish dealers from 2009 to 2014. These were opportunistic sampling events, did not collect all gear in all year, and may or may not occur in the future. In 2012, croaker otoliths were also sampled randomly from commercial gill nets. All fish sampled for age were measured to the nearest mm TL, weighed to the nearest gram and sex was determined from internal examination of the gonads.

Prior to 2011, Atlantic croaker otoliths were processed and aged by the South Carolina Department of Natural Resources (SC DNR). Otoliths from 2011 to 2015 were aged by MD DNR biologists. The left otolith from each specimen was mounted to a glass slide for sectioning. If the left otolith was damaged, missing, or miscut the right otolith was substituted. Otoliths were mounted in Crystalbond 509 and were sectioned with a Buehler IsoMet® Low SpeedSaw using two blades separated by a 0.4 mm spacer. The Buehler 15 HC diamond wafering blades are 101.6 mm in diameter and 0.3048 mm thick. The 0.4 mm sections were then mounted on microscope slides and viewed under a microscope to determine the number of annuli. All age structures were read by two readers. If readers did not agree, both readers reviewed the structures together, and if agreement still could not be reached the sample was not assigned an age.

Virginia Institute of Marine Science (VIMS)
The Northeast Area Monitoring and Assessment Program (NEAMAP) is a cooperative state-federal program that has operated a Near Shore Trawl Survey in the mid-Atlantic Bight and southern New England since fall 2007. The Virginia Institute of Marine Science (VIMS) has been awarded the contract to carry out the survey. It continues and extends the methods of the Chesapeake Bay Multispecies Monitoring and Assessment Program (ChesMMAP) which started in 2002. Atlantic croaker is a "Priority" species for NEAMAP, meaning that length, weight, sex, maturity state, stomach, and otoliths are collected for 5 individuals from each length bin on each tow. VIMS uses sectioned otoliths for age determination. A total of 10860 Atlantic croaker

have been aged by the two surveys (CM 6958, NM 3906). VIMS has disputed that an interior 1st annulus should be counted for accurate age determination due to the time of year the species spawns and their annuli deposition. For continuity VIMS has made separate ages for including the first annulus as well as excluding it, which is recommended by the Commission for stock assessment purposes among other agencies (ASMFC 2008). Ages have ranged from age-0 to a max age of 18.

There are three readers at VIMS and the mode age for each sample is provided as the final age. If there is no mode from the initial read, the readers reread the sample and if there is still no mode, they examine the sample together and come to a consensus age. If a consensus age cannot be determined the sample is discarded. Very few samples are discarded. Precision tests are preformed within each reader (multiple reads of the same sample) and between readers. VIMS uses similar precision and symmetry tests to the NEFSC.

Old Dominion University (ODU)
The otoliths collected through the Virginia Marine Resources Commission's (VMRC) Biological Sampling Program are processed and read by the ODU's Center for Quantitative Fisheries Ecology (CQFE) laboratory. Atlantic croaker otoliths have been collected by VMRC since 1998. Otoliths are processed following the methods described in Barbieri et al. (1994) with a few modifications. The left or right sagittal otolith is randomly selected and attached to a glass slide with Aremco's clear Crystalbond™ 509 adhesive. One transverse section is cut through the core of each otolith using a Buehler Isomet low-speed saw equipped with a three inch, fine-grit Norton diamond-wafering blade. Otolith sections are placed on labeled glass slides and covered with a thin layer of Flo-Texx mounting medium.

All fish are aged in chronological order based on collection date, without knowledge of the specimen lengths. Two readers must age each otolith independently. When the readers' ages agree, that age is to be assigned to the fish. When the two readers disagree, both readers must re-age the fish together, again without any knowledge of previously estimated ages or specimen lengths and assign a final age to the fish. When the readers are unable to agree on a final age, the fish is excluded from further analysis.

Atlantic croaker are assigned a January 1st birthdate by convention. The sample date is used to assign the final age. If the sample was taken before the period of annuli formation (April to May), the age is the annulus count plus one. If the sample was taken after that, the age is the annulus count.

Historically, Virginia has counted the wide band/smudge closest to the otolith core as the first annulus, whereas most other states do not; however, since all Atlantic croaker in Virginia form that band and because Virginia uses the January 1 model birth-date, the sampled fish should be scored as the same age-class assignment as those scored in other states.

The following are links to the preparation and ageing protocols for Atlantic croaker.

- [Otolith Preparation Protocol](https://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/atlantic-croaker-otolith-preparation-protocol.pdf)

https://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/atlantic-croaker-otolith-preparation-protocol.pdf

- [Otolith Ageing Protocol](https://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/atlantic-croaker-otolith-ageing-protocol.pdf)

https://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/atlantic-croaker-otolith-ageing-protocol.pdf

North Carolina Division of Marine Fisheries (NC DMF)
Atlantic croaker sagittal otolith samples are collected monthly from the winter trawl, long haul seine, pound-net, sink-net, recreational hook-and-line fisheries, and NC DMF fisheries independent programs. Sagittal otoliths have been collected since 1996. Each month, samples (n=15) are distributed across the length range in 15-mm length classes starting at 100 mm total length. Sagittal otoliths are removed, cleaned, and stored dry. Samples are weighed to the nearest 0.01 kg and measured for total length to the nearest millimeter. Date, gear, and water location are also recorded for each sample.

A transverse section through the focus on a plane perpendicular to the horizontal axis of the left otolith is prepared using a Hillquist thin-sectioning machine as described by Cowan et al. (1995). The system is calibrated with an ocular micrometer before each reading session. Sections are viewed under reflected light at 21X magnification. Annuli, marginal increment, and otolith size are measured (mm) on an image projected on a high resolution monitor from a video camera mounted on a microscope. Ages are assigned based on the number of otolith annuli viewed. The ageing lab biologist reads the otolith section and measures the annuli. The samples are then independently read by the species lead biologist. If any differences are not resolved, the data are omitted.

The NC DMF publishes three-year reports that include species-specific age-length keys, which have been applied to expanded length-frequency data to estimate length-at-age for total commercial landings on an annual basis. The age-length keys and expansions are applied on a seasonal basis: winter (January–March and October– December); and summer (April–September).

South Carolina Department of Natural Resources (SC DNR)
Croaker samples are collected from several different methods in South Carolina including inshore trammel net survey (2014), SEAMAP nearshore trawl (2001 to present) and MRFSS/MRIP survey. SC DNR Inshore Fisheries section also processes croaker otoliths from National Marine Fisheries Service’s Northeast groundfish survey since 1996. Otoliths are embedded in resin to facilitate cutting, cut on a low speed saw to obtain a 0.4mm transverse cross-section and then mounted on microscope slide. The sections are read using a dissecting microscope with an attached camera so that the image can be viewed with a computer program like Image Pro. All samples are aged independently by two readers to insure accurate ages. Some Atlantic croaker otoliths vary with respect to diffuse, undefined marking near the

core of the otolith. These diffuse areas are not interpreted as being a ring. The first annulus is considered the first well-defined, opaque band that can be traced around the entire section.

Georgia Department of Natural Resources (GA DNR)
Atlantic croaker were collected from Georgia's coastal waters using a variety of gear types in 2010-2012 as part of a graduate thesis (Franco 2014). Transverse sections were read from 2,401 otolith samples from age 0-6. The majority (98%) of otoliths exhibited the dark, opaque area near the core that is the smudge or check mark. While the majority of age samples for GA croaker came from this project, in the fall of 1997 GA DNR initiated the Marine Sportfish Carcass Recovery Project. This project takes advantage of the fishing efforts of hundreds of anglers by turning filleted fish carcasses that anglers would normally discard into a source of much needed data on Georgia's marine sportfish. The project is a true partnership of saltwater anglers, marine businesses, conservation groups, and the Coastal Resources Division (CRD). Since 1999, a total of 43 Atlantic croaker have been donated to the project. It was decided that the largest of the croaker would be sectioned and aged for the QA/QC Fish Ageing Workshop.

II. Atlantic Menhaden

Rhode Island Division of Fish and Wildlife (RI DFW)
The RIDFW has been ageing scales from Atlantic menhaden since 2015 following the ASMFC Atlantic Menhaden Ageing Workshop. Prior to 2015, all scale samples collected by RI DFW were sent to the NOAA-Beaufort Laboratory for ageing. A target number of 100 scale samples are collected annually from the commercial bait fishery. Scales are cleaned and sandwiched between two glass microscope slides. Scales are aged by a single reader using a microfiche reader. A second read is conducted by the same reader on at least 10% of the samples for each structure to obtain precision estimates.

MD DNR
MD DNR has been collecting length and age (scales) data from Atlantic menhaden since 2005. We collect scales from a fishery independent gill net survey and a dependent pound net survey. We collect an average of 20 samples from each survey each week. This provides us with around 450 scales a year to age. From each sample we select between 4 and 8 non-regenerated scales that are cleaned in soap and water and placed between 2 slides. These slides are then read with a microfiche reader by two trained DNR biologists. These ages are compared and an agreed age is assigned. If an age cannot be agreed upon we will not assign an age.

NC DMF
Scale samples are currently collected from fishery-dependent sampling of bait fisheries. No scale samples are collected during fishery-independent sampling. Scales are processed and aged in-house with a microfiche reader. For Atlantic menhaden samples, NC DMF selects six scales from each envelope, choosing only scales that are symmetrical, uniform in size, and free of defects for mounting. The selected scales are cleaned with water. Scales are arrange the on

the bottom slide with pectinations pointed up and the smooth or concave scale side down, and covered with the second slide.

III. Bluefish

Massachusetts Division of Marine Fisheries (MA DMF)

The MA DMF has been sampling and ageing bluefish since 2009. Samples come from a combination of commercial and fishery independent sources. Otoliths are the only hard part aged for bluefish in MA. Otoliths are baked, sectioned, and aged with transmitted light on a compound microscope.

RI DFW

Bluefish otoliths have been collected by the RIDFW since 2012 on fishery-independent surveys and from the recreational and commercial fisheries. The annual target number of samples is 100 per the requirements of Addendum I to Amendment I to the Bluefish Fishery Management Plan. Whole otoliths are embedded in epoxy resin, sectioned, mounted on microscope slides, and aged by a single reader annually. A second read is conducted by the same reader on at least 10% of the samples to obtain precision estimates.

New York State Department of Environmental Conservation (NY DEC)

The NY DEC has been collecting length, sex (when available), and age (otoliths) data from bluefish since 2012. The majority of samples are collected from fishery dependent sampling of commercial markets, with additional samples of larger bluefish coming from the recreational fishery. Staff sample as many bluefish as possible, but age a maximum of 10 fish per 1 cm bin. Otoliths are embedded in West System Epoxy and sectioned using an Isomet Low-Speed Saw to a thickness of ~0.3mm. Otoliths are aged on a compound microscope using transmitted light. Samples are processed and read by one person. The NY DEC has aged 1,275 bluefish since the project began in 2012.

NJ DWF

The NJ DFW initiated a sampling program for bluefish in 2010 with the intent of filling gaps in the stock assessment age-length key. Otoliths have been collected exclusively for bluefish ageing (no scales), and samples have been derived from fishery-independent survey efforts and fishery-dependent sources. Through 2014, the average number of bluefish sampled by the NJ DFW is 90 in the spring (SD = 16 ages) and 101 in the fall (SD = 27). Ageing is complete through 2015, though a summary is not yet available for 2015.

All otolith samples are sent to the NEFSC annually for processing and age determination and protocols follow those specified in the 2011 ASMFC bluefish ageing workshop. The age distribution of samples collected by the NJ DFW is available through 2014. As recommended by the bluefish Technical Committee, NJ DFW will report ages through 8+ (including retrospectively) as ageing techniques have been validated through age 8 (Robillard et al. 2009).

VIMS
Bluefish is a "Priority" species for NEAMAP, meaning that length, weight, sex, maturity state, stomach, and otoliths are collected for 5 individuals from each length bin on each tow. VIMS uses sectioned otoliths to age bluefish. Otoliths are sectioned using a method similar to ODU's. However, VIMS wet-sands the sections to a thinner width than ODU and does not bake the sections. Annulus counts are adjusted to reflect the timing of sample collection relative to ring formation. Age is assigned as the mode of three independent readings. VIMS has aged 5679 total bluefish between ChesMMAP and NEAMAP from 2002-2016 (CM 528, NM 5151). Bluefish have been aged from age-0 to a max age of 10. The majority of the specimens sampled were ages 0-2. There are three readers at VIMS and the mode age for each sample is provided as the final age. If there is no mode from the initial read, the readers reread the sample and if there is still no mode, they examine the sample together and come to a consensus age. If a consensus age cannot be determined the sample is discarded. Very few samples are discarded. Precision tests are preformed within each reader (multiple reads of the same sample) and between readers. VIMS uses similar precision and symmetry tests to the NEFSC.

ODU
VMRC obtains bluefish otoliths from the commercial catch and fishery independent sampling programs. Bluefish otoliths have been collected by VMRC since 1998. These otoliths are processed and read by ODU CQFE. ODU CQFE chooses a random subsample of otoliths collected in each length bin to age. In 2015, VMRC collected 682 bluefish otoliths and ODU CQFE aged 442 of them.

ODU CQFE uses sectioned otoliths to age bluefish. Each section is read under transmitted light using a polarizing filter. The characteristics described in Robillard et al. (2009) are used to identify the first ring and false annuli. Bluefish are assigned a January 1st birthdate by convention. The sample date is used to assign the final age. If the sample was taken before the period of annuli formation (March to June), the age is the annulus count plus one. If the sample was taken after that, the age is the annulus count.

Each section is aged by two readers. If the first readings disagree, the readers re-age the fish together. If a consensus cannot be reached, the sample is excluded from further analysis and, if available, another sample from the same length bin replaces it. Each year, readers revisit a reference collection of samples from 2000 to increase consistency across years.

The following are links to the preparation and ageing protocols for bluefish.

- Otolith Preparation Protocol

https://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/bluefish-otolith-preparation-protocol.pdf

- Otolith Ageing Protocol

https://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/cqfe-bluefish-otolith-ageing-protocol-black-white-2011.pdf

NC DMF

NC DMF has collected and aged bluefish scales from 1983 – 1998, and collected and aged otoliths from 1996 – 2000 and from 2006 to the present. From 1996 – 1998, NC DMF collected paired samples of scales and otoliths for a comparison of the two structures (NC DMF 2000). NC DMF did not collect any hard parts for bluefish from 2001 – 2005, when the Bluefish TC switched to a surplus production model for assessment purposes. The SAW/SARC review of that assessment (NEFSC 2004) found a lumped biomass model inappropriate for bluefish and recommended the use of an age-structured model instead. Thus, NC DMF began collecting otoliths for bluefish again in 2006. Despite training at ODU's lab, NC DMF could not replicate ODU's process to produce readable otolith sections and discontinued processing of annual samples in favor of archiving whole otoliths.

SC DNR

The Southeast Area Monitoring and Assessment Program (SEAMAP) is cooperative state-federal program that has operated a fishery independent Shallow Water Trawl Survey in the nearshore waters from Cape Hatteras, NC to Cape Canaveral, FL since 1986. The survey is conducted by South Carolina Department of Natural Resources (SC DNR).

In 2011, bluefish was added to the list of species that received a full work-up including the collection of otoliths for ageing. As with the NEAMAP samples, the majority of bluefish samples are small, young fish; this is not surprising in a trawl survey, as older bluefish can easily out-swim a trawl. From 2000 to 2010 before SEAMAP took over sample processing, SC DNR Inshore Fisheries section was using SEAMAP caught bluefish for otolith ageing.

Florida Fish and Wildlife Research Institute (FL FWRI)

Bluefish otoliths are collected on fishery-independent monitoring surveys. Most bluefish otoliths are incidental collections and are not targeted or regularly encountered. Otoliths are embedded in a plastic resin and sectioned on an Isomet ® low speed saw and aged under transmitted light on a stereo microscope.

IV. Summer Flounder

RI DFW

Summer flounder are sampled by the RI DFW on fishery-independent surveys and from the recreational and commercial fisheries. Each year a target number of 100 samples are collected with scales as the primary ageing structure. Paired scale/otolith samples are collected when either the rack or whole fish is available. Scales are cleaned and pressed onto acetate and aged on a microfiche reader. Otoliths are embedded in epoxy resin, sectioned, mounted on microscope slides, and aged with a microscope. Both structures are aged by a single reader annually. A second read is conducted by the same reader on at least 10% of the samples for each structure to obtain precision estimates.

VIMS

Summer Flounder is a "Priority" species for NEAMAP, meaning that length, weight, sex, maturity state, stomach, and otoliths are collected for 5 individuals from each length bin on each tow. VIMS uses sectioned otoliths to age summer flounder. Otoliths are sectioned using a method similar to ODU's. However, VIMS wet-sands the sections to a thinner width than ODU and does not bake the sections. Annulus counts are adjusted to reflect the timing of sample collection relative to ring formation. Age is assigned as the mode of three independent readings. VIMS has aged 15,097 total summer flounder between ChesMMAP and NEAMAP from 2002-2016 (CM 6437, NM 8660). Summer flounder have been aged from age-0 to a max age of 13. The majority of the specimens sampled were ages 0-7. There are three readers at VIMS and the mode age for each sample is provided as the final age. If there is no mode from the initial read, the readers reread the sample and if there is still no mode, they examine the sample together and come to a consensus age. If a consensus age cannot be determined the sample is discarded. Very few samples are discarded. Precision tests are preformed within each reader (multiple reads of the same sample) and between readers. VIMS uses similar precision and symmetry tests to the NEFSC.

ODU

VMRC has obtained summer flounder otoliths and scales from the commercial catch and fishery independent sampling programs since 1999. Ageing hard parts are processed and read by ODU CQFE. ODU CQFE chooses a random subsample collected in each length bin to age. In 2015, ODU CQFE aged 884 summer flounder samples, 293 were paired scale and otolith readings.

Otoliths samples are cleaned and baked in a Thermolyne 1400 furnace. After baking, otoliths are embedded in epoxy resin and sectioned. Each section is read under transmitted light using a polarizing filter. Summer flounder are assigned a January 1st birth date by convention. The sample date is used to assign the final age. If the sample was taken before the period of annuli formation (January to June), the age is the annulus count plus one. If the sample was taken after that, the age is the annulus count.

Each section is aged by two readers. If the first readings disagree, the readers re-age the fish together. If a consensus cannot be reached, the sample is excluded from further analysis and, if available, another sample from the same length bin replaces it. Each year, readers revisit a reference collection of samples from 2000 to increase consistency across years.

The following are links to the preparation and ageing protocols for summer flounder.

- Scale Preparation Protocol

http://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/summer-flounder-scale-prep.pdf

- Otolith Preparation Protocol

http://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/summer-flounder-otolith-preparation-latex-main-document.pdf

- Otolith Ageing Protocol

http://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/summer-flounder-otolith-ageing-protocol.pdf

NC DMF
For all otoliths that need to be sectioned, such as summer flounder, a standard methodology is used regardless of species. Sectioned otoliths are processed using a Hillquist High-Speed Thin Sectioning Saw (Cowan et al. 1995). Two otolith sections are set onto each slide, as long as they are from the same collection. The Hillquist saw is again used to grind off the remaining half of the otolith attached to the slide, so that a section about 0.75mm thick remains on the slide. The sectioned otolith can then be hand polished, if necessary for that species or size. The sectioned otolith is then ready to be aged.

V. Tautog

MA DMF
Tautog otoliths and operculum are collected from several sources; cooperation from commercial fisherman, within division fish potting, and cooperation with several recreational anglers. Opercula have been collected since 1995 and otoliths have been collected since 2012. Opercula are boiled and brushed clean before being dried and aged without magnification. Otoliths are baked, sectioned and aged with transmitted light under a compound microscope.

Tautog pelvic fin spines have been collected from primarily recreational sources since 2014. Spines are boiled for 1-2 minutes, brushed clean with a small brush then allowed to air dry for at least 48 hours. The spines are embedded in epoxy and 0.75 mm sections are cut. Three successive sections are removed starting just above the condyle.

RI DFW
Opercula have been collected by RIDFW since 1987, primarily from donated recreational carcasses. The annual target number of samples is 200 per the requirements of Addendum III to the Fishery Management Plan for Tautog. Sample collection primarily includes operculum; however, a subsample of otoliths has also been collected since 2012 following the recommendations of the 2012 Tautog Ageing Workshop. Operculum are removed from fish racks and subsequently boiled to remove all flesh and tissue. Opercula are aged by a single reader annually by holding the structure up to fluorescent lighting. A second read is conducted by the same reader on at least 10% of the samples for each structure to obtain precision estimates.

NY DEC
Fishery dependent tautog samples are primarily collected from commercial markets and headboat fish racks. While the current goal is to satisfy the requirements of the FMP, availability of samples has fluctuated over time. The total length of each fish is measured, and the opercula bone is removed and frozen until further processing. Otoliths from a subset of these fish are also collected. Previously frozen samples are thawed and boiled for 2 minutes and the flesh is gently scraped off the opercula. The bones are allowed to air dry overnight and

are then read without magnification using overhead lighting. Aged samples are available from 1993 to the present.

NJ DFW

Sampling for tautog was initiated in 2007, collecting samples primarily from Commercial and Party/ Charter vessels. Fishery Independent samples are also occasionally collected aboard the NJDFW Ocean Trawl Survey when caught. Racks are collected from fishery dependent vessels, where lengths and sex are recorded, and opercula are removed. The opercula are processed and aged at the Nacote Creek Research lab, where they are viewed under a magnisight machine. Since initiation, 7,013 samples have been collected, with 387 samples collected in 2015.

MD DNR

Maryland has collected tautog opercula for ageing since 1996. The current FMP requires that each state to collect 200 opercula and 50 otolith samples per year. Tautog have been collected by hook and line, commercial fish pots and on rare occasion spearfishing. Juvenile tautog have also been collected by seining eel grass beds in 2015 which provided samples of the smallest length groups in the population. The most productive method is hook and line with a partnering professional charter boat.

The goal is to randomly sample and fill each 10mm length group with five samples. Each fish is measured (mm total length) and weighed (kg) using the digital scale. The gonads are observed to determine the sex of the fish. These data are recorded on scale envelope. Both opercula are removed and placed in the envelope(s). The fish heads are tagged with a tuna or yellow perch tag and that tag number is recorded on the opercula envelope(s). All heads are frozen until the otolith bins are calculated to ensure all 10 mm length groups have ample representation; all large fish (>600mm) have otoliths removed. Starting in 2013, DNA was collected for scientists at VIMS.

Each operculum is boiled in water, cleaned, and placed in a new envelope for reading. All readers must re-read the reference collection that contains 20 opercula samples for each year since 1996, (except for 1997 and 1998 which has less than 20) prior to reading the current year samples. The reader uses no magnification. The first year annular line is typically 7-8 mm from the articular apex and the second year around 12-15 mm. The spacing between year's decreases as the fish gets older. The outer edge (new growth) is counted to promote (X+1) if the operculum was collected between 1 Jan to 30 June, otherwise it is not counted. A representative sample of 20 aged opercula is added to the reference collection for the following year.

VIMS

Tautog are collected for both NEAMAP and ChesMMAP surveys and additionally is considered a "Priority" species for NEAMAP, meaning that length, weight, sex, maturity state, stomach, and otoliths are collected for 5 individuals from each length bin on each tow. VIMS uses sectioned otoliths and opercula for age determination. Both opercula and otoliths have been collected since 2010 as per comparison purposes due to the low number of encounters by each survey over their time series. Prior to 2010 only opercula were collected. A total of 280 Tautog have been aged by the two surveys (CM 50, NM 230). To date VIMS tautog data has not been requested due to the low number of samples across the surveys time series.

There are three readers at VIMS and the mode age for each sample is provided as the final age. If there is no mode from the initial read, the readers reread the sample and if there is still no mode, they examine the sample together and come to a consensus age. If a consensus age cannot be determined the sample is discarded. Very few samples are discarded. Precision tests are preformed within each reader (multiple reads of the same sample) and between readers. VIMS uses similar precision and symmetry tests to the NEFSC.

ODU

Tautog have been collected as part of VMRC's Biological Sampling Program since 1998. Both otoliths and operculum are collected. Operculum are removed and frozen until prepared for age reading. Thawed samples are boiled 5-6 minutes to loosen attached tissue. When sample is removed from the water, skin and tissue are removed. Clean opercula are read using transmitted light, usually from a window or overhead light. Otoliths samples are cleaned and baked in a Thermolyne ™ 1400 furnace. After baking, otoliths are embedded in epoxy resin and sectioned.

All tautog samples are aged by two different readers. When readers disagree, they re-age the fish together without knowledge of lengths or previously estimated ages. Fish that do not result in agreement are excluded from analysis.

Tautog are assigned a January 1st birthdate by convention. The sample date is used to assign the final age. If the sample is taken before the period of annuli formation (May to July), the age is the annulus count plus one. If the sample is taken after that, the age is the annulus count.

The following are links to the preparation and ageing protocols for tautog.

- Otolith Preparation Protocol

https://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/tautog-otolith-preparation-protocol.pdf

- Otolith Ageing Protocol

https://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/tautog-otolith-ageing-protocol.pdf

- Operculum Preparation Protocol

https://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/docs/tautog-operculum-prep.pdf

VI. Winter Flounder

RI DFW

RI DFW began sampling winter flounder on fishery-independent surveys in 2014. Additionally, a small number of samples were donated by the commercial fishery in 2016. Each year a target number of 100 paired scale and otolith samples are collected. Although scales are collected, the primary ageing structure for winter flounder is otoliths. Scales are cleaned and pressed onto acetate and aged on a microfiche reader. Otoliths are embedded in epoxy resin, sectioned, mounted on microscope slides, and aged with a microscope. Both structures are aged by a single reader annually. A second read is conducted by the same reader on at least 10% of the samples for each structure to obtain precision estimates.

MA DMF

Winter flounder otoliths are collected from our resource assessment trawl survey. Collected otoliths have been aged in the MADMF age and growth lab since 2012. Samples collected from 1982-2011 were aged by the NMFS NEFSC. Otoliths are typically read whole with reflected light. Samples assigned an age of 5 or older are then thin sectioned and read either with a stereoscope using reflected light or with a compound microscope with transmitted light.

NY DEC

NY DEC has not processed or aged winter flounder since the late 1990s, although archived samples were provided for this workshop. Winter flounder otoliths were embedded in Buehler Epoxy, sectioned to a thickness of ~.4mm on an Isomet low-speed saw and read on a compound microscope with transmitted light.

VIMS

Winter Flounder is a “Priority” species for NEAMAP, meaning that length, weight, sex, maturity state, stomach, and otoliths are collected for 5 individuals from each length bin on each tow. VIMS uses sectioned otoliths to age winter flounder. Otoliths are sectioned using a method similar to ODU’s. However, VIMS wet-sands the sections to a thinner width. Annulus counts are adjusted to reflect the timing of sample collection relative to ring formation. Age is assigned as the mode of three independent readings. VIMS has aged 5,248 total winter flounder from the NEAMAP survey from 2007-2016. Winter flounder have been aged from age-1 to a max age of 19. Young of the year fish have not been recruited by the NEAMAP survey gear. The majority of the specimens sampled were ages 2-6. There are three readers at VIMS and the mode age for each sample is provided as the final age. If there is no mode from the initial read, the readers reread the sample and if there is still no mode, they examine the sample together and come to a consensus age. If a consensus age cannot be determined the sample is discarded. Very few samples are discarded. Precision tests are preformed within each reader (multiple reads of the same sample) and between readers. VIMS uses similar precision and symmetry tests to the NEFSC.

Workshop Proceedings & Methods

Participants in the workshop met on Wednesday, April 5th, in a conference room at the FL FWRI building in St. Petersburg to go over the goals of the workshop, agenda, and to make introductions. Jessica Carroll and the staff at Florida's Fish and Wildlife Conservation (FL FWC) Commission including Kristin Cook, Kristen Rynerson, David Westmark, Chris Swanson, Brittany Barbara, and Alison Brett set up stations ahead of the workshop for the hard part reading exercise. Participants broke into five groups, each led by one of the FL FWC employees, and began ageing the structures at each station. Not all states or labs routinely age all the species at the workshop, so the groups were developed to mitigate the effects of readers unfamiliar with a species.

For each of the six species, every member of the group aged the samples (n=19-20 per species) and the group came to a consensus for annulus count, margin code, and final age. In addition to group ages, the participants also recorded their individual age readings for additional analysis. Each structure was assigned a margin code from 1-4. A code 1 represented a structure with an annulus just forming or having just finished forming at the edge of the structure. Code 2 was assigned when the growth outside the last visible annulus was less than 1/3 the growth between the two previous annuli. Code 3 represented 1/3 to 2/3 growth and code 4 was for more than 2/3 growth. A catch date was provided for each sample to make final age determinations, but no other information was provided during reading.

Ageing precision between groups for consensus ages were evaluated using average percent error (APE). Following a recommendation from the 2016 QA/QC Fish Ageing Workshop, participants also reviewed individual age comparisons for agers who routinely age each of the species. Exact agreement was tested using Bowker's test of symmetry around the diagonal 1:1 line (Evans and Hoenig 1998) where a significant p-value (<0.05) indicates systematic bias between the age readings. Without knowing the true age of the fish, this test does not identify which reader is more accurate, but rather identifies whether there are differences or not. Mean coefficient of variation (CV), percent of exact agreement between readers, and percent agreement within 1 year was also calculated for each lab and reader to provide a measure of precision. While this does not serve as a proxy for accuracy, it does indicate the level of ease for assigning an age to that ageing structure, the reproducibility of the age, or the skill level of the readers. Generally, CVs of 5% serve as a reference point for determining precision, where greater values indicate ageing imprecision (Campana 2001).

Workshop Results

On April 6th, the attendees of the workshop met to go over the APE for each species, results from individual age readers, and to revisit samples with high disagreement. For all the species that were aged the previous year, APE increased (Table 1). Discussion and results for each species follows and sample images can be found in Appendix B.

I. Atlantic croaker

The APE for Atlantic croaker increased from 7.76% in 2016 to 10.57% in 2017. It was noted that identifying the annuli is not an issue for Atlantic croaker rather the disagreement can likely be attributed to margin or scope issues this year, not the "smudge" issue that was discussed in 2016 (ASMFC 2008, 2016). While there is still disagreement among the group about whether or not to count the smudge or check mark found between the core and first annulus, all participants are following the agreed upon protocol to not count the smudge when providing ages to ASMFC. The participants continued to discuss this issue, expressing concerns about some fish not getting grouped in the appropriate year-class for modeling approaches and affecting calculations such as mean length at age and the length and age at maturity. For example, Atlantic croaker otolith samples #2 and #15 are both spawned in the fall and then caught the following fall so they should be in the age-1 age class but since they have the smudge and no annulus, they are being grouped with the age-0s.

The group revisited some of the samples with high disagreement, such as #12, 13, and 14 (Table 2). Sample #12 was provided by NC DMF as a 6 year old although participants at the workshop aged it as a 5 or a 6. Most agreed that this is not a good sample. Katherine Messer from MD DNR said she counted 5 annuli and rounded to 6, but that MD would not use this sample because it was unlikely two readers would have agreed on the age. Scott Elzey from MA DMF expressed concern that that practice would result in all the fish that grew the most in the first year would be thrown out and the results would be biased. For sample #13, participants attribute disagreements to issues reading the edge. Nicole Lengyel from RI DFW said that she counted an annuli on the edge. Eric Robillard from the NEFSC stated that if it laid an annuli in May, it should show more growth on the edge and one would not be looking for an annuli on the edge for a fish caught in October like this sample. Sample #14 also was an edge issue.

When comparing individual readers for Atlantic croaker, readers from NJ DFW, MD DNR, VIMS, ODU, NC DNR, and SC DNR all reported that they routinely age this species and provide ages to ASMFC for the stock assessment. Jameson Gregg from VIMS indicated that he aged the samples the way he thinks is correct for this exercise by counting the smudge, although he adjusts his ages when providing data to ASMFC. There were no significant p-values from Bowker's test of symmetry, indicating no systematic bias between the readers, and 12 out of the 15 comparisons had CVs greater than 5%, indicating imprecision (Table 3). Exact agreement varied from 0-100% and increased to 95-100% for agreement within one year (Table 4). Most of the disagreement can be attributed to counting the smudge. Again, the participants confirm that they are all ageing the same way when providing ages to ASMFC, but concerns persist that the current protocol is not accurately assigning Atlantic croaker to their age or year-classes.

II. Atlantic Menhaden

The APE for Atlantic menhaden was 15.42% and differences in ageing were attributed to lack of experience ageing this species and issues using the microfiche at the workshop that resulted in some samples appearing blurry. Messer routinely ages Atlantic menhaden scales and led the group through the samples with high disagreement (Table 5). She advised that it is important to look at many scales on the slide, not just the “best” one. For sample #1, Messer reminded participants that the core is not counted as “1” and that an annulus should be visible all the way around the scale including the top and both sides. For sample #2, one of the groups aged it as a 7 year old, which is unlikely, but that group did not have any members who routinely age Atlantic menhaden. Most thought this was a 4 or a 5 year old, but because it was a June-caught fish, it likely just laid an annulus down so it should not be bumped and the age should be 4. Only two groups had people who age Atlantic menhaden which accounted for the high disagreement overall. Participants inquired about the use of whole otoliths as an ageing structure and recommended pursuing this protocol if possible due to trouble reading Atlantic menhaden scales.

All age samples in the most recent stock assessment (SEDAR 2015) were aged at the Beaufort Laboratory although there is increased interest in moving the ageing responsibilities to the states. For individual reader comparisons, readers from RI DFW, CT DEEP, DE DFW, MD DNR, and VIMS reported that they now routinely age Atlantic menhaden. When comparing the experienced Atlantic menhaden readers, there were no significant p-values from Bowker’s test of symmetry indicating no systematic bias between the readers and nine out of the ten comparisons had CVs greater than 5%, indicating imprecision (Table 6). Exact agreement varied from 26-89% and increased to 79-100% for agreement within one year (Table 7).

III. Bluefish

The highest APE at the workshop was for bluefish at 25.30%, which was also the species with the highest APE in the 2016 workshop at 23.06%. Workshop participants revisited some samples with high disagreement (Table 8). Similar to 2016, problems distinguishing between age-0 and age-1 bluefish dominated the discussion. For sample #11, Elzey suggested that this sample should be tilted to see the cut. Due to its length of 278 cm, many argued that this should be an age-1. Robillard noted that this sample was caught in August and that it is likely this is an April spawned fish with fast growth and that the first annulus would be much farther out. Samples #3, #7, and #18 are other examples of difficulty differentiating age-0 from age-1 or 2 bluefish that the group revisited and debated. Robillard reminded the agers that one wants to see clear separation from the smudge and if a fish is an age-2, it would have the “fish tail” or protrusion on the edge. For age-0s caught in August or September, Robillard warned the group to not jump to conclusions that it is an age-1. Therefore, samples #7, #11, #18 are likely age-0 fish. The APE was inflated by the four samples discussed and the persisting issue of determining an age for age-0 and age-1 bluefish. When these samples are removed from the analysis, APE dropped to 7.00%.

For individual reader comparisons, readers from MA DMF, NEFSC, RI DFW, CT DEEP, NY DEC, VIMS, ODU, NC DMF, and SC DNR reported that they routinely age bluefish. The 2015 stock assessment has ages from all Atlantic states from MA to FL (NEFSC 2015, Table B5.1), although the reader at the workshop may not be the one responsible for ageing this species. When comparing the experienced bluefish readers, there were nine significant p-values from Bowker’s test of symmetry, indicating some systematic bias between the readers (Table 9). CVs ranged from 1-45% and 32 out of the 36 comparisons had CVs greater than 5%, indicating imprecision. Exact agreement varied from 45-95% and increased to 90-100% for agreement within one year (Table 10).

IV. Summer Flounder

Summer flounder had the lowest APE at 3.63% which was inflated by including the scale samples in the analysis. When split into otoliths (n=14) and scales (n=6), APEs were 1.33% and 9.00% respectively (Table 11). Agers suggested that disagreement on the scale samples was because participants were using a microscope to read the scales rather than a microfiche like they do in their labs. FL FWC does not use microfiches, so only one was available for this workshop and it was used for reading Atlantic menhaden scales. Paired otolith and scale summer flounder samples included #4 with #19, #7 with #16, and #11 with #17. The group revisited the first set of paired samples. Jessica Gilmore from ODU pointed out where she saw the annuli on scale sample #19 and described how the annuli are getting compressed at the edge where she saw some growth so she counted 9 annuli and bumped it to an age-10. Several people agree that the paired otolith, #4, is a bad cut and it is difficult to determine if it is an age-10 or age-11. For sample #20, participants disagreed between age-2 and age-3 and attributed this to an edge issue.

For individual reader comparisons, readers from NEFSC, RI DFW, CT DEEP, DE DFW, VIMS, ODU, and NC DMF reported that they routinely age summer flounder. When comparing the experienced summer flounder readers, there were no significant p-values from Bowker's test of symmetry, indicating no systematic bias between the readers (Table 12). CVs ranged from 0-9% and 4 out of the 21 comparisons had CVs greater than 5%, indicating some imprecision. Exact agreement varied from 50-100% and increased to 95-100% for agreement within one year (Table 13). When the six scale samples were removed from the analysis, all CVs were less than 5% (Table 14) and agreement was increased between readers with all otolith samples having 100% agreement within one year (Table 15).

V. Tautog

The APE for tautog went up from 6.09% in 2016 to 10.89% in 2017 (Table 16). It was suggested that sample #1 be removed from future workshops since it is now broken and pieces are missing. Otherwise, the disagreement between age determinations was mainly attributed to older age samples and issues identifying the first annulus. The group reviewed sample #6 which was either an age-1 or an age-2. David Molnar from CT DEEP suspected that some readers missed the first annulus and incorrectly counted it as an age-1. A representative from FL FWC said that the sample was so small compared to the others, their group did not count the ring Molnar was referring to. Sample #6 was from a tautog with a total length of 163 cm. Both Messer and Elzey agree that all visible annuli should be counted and that with tautog opercula one does not consider the spacing of the annuli like in some other species. Sample #7 had high disagreement, but participants attribute that to the sample coming from an older fish and described how the annuli get packed together on the edge making age determinations more difficult. Messer described a method used in MD for making age determinations based on distance in mm. Sample #16 had a lot of disagreement that was attributed to 2-3 bands that appeared close to each other that people interpreted and counted differently.

For individual reader comparisons, readers from MA DMF, RI DFW, CT DEEP, NY DEC, DE DFW, VIMS, and ODU reported that they routinely age tautog. The 2015 stock assessment has age data from MA to VA (ASMFC 2015b), although the reader at the workshop may not be the one responsible for ageing this species. When comparing the experienced tautog readers, there were no significant p-values from Bowker's test of symmetry, indicating no systematic bias between the readers (Table 17). CVs ranged from 4-18% and 18 out of the 21 comparisons had CVs greater than 5%, indicating imprecision. Exact agreement varied from 10-75% and increased to 35-95% for agreement within one year (Table 18).

The workshop participants reviewed the pelvic spine samples provided by Elzey and discussed the methods and possibility of using spines as a regular ageing hard part for this species. Collecting the samples is non-lethal and the annuli appear very clearly on the samples the group reviewed. Processing the samples is more time consuming than other methods, but the actual ageing is faster since annuli are clearer than on opercula. Identifying the first annulus is a challenge regardless of the hard part used. Overall, participants were interested in this method and recommended that if possible paired spines, opercula, and otoliths should be collected from tautog so that some training could take place in the future to formally evaluate this as a method for routine ageing.

VI. Winter Flounder

The APE for winter flounder was 10.13%, although when broken up by ageing hard part APE was higher for the scale samples at 32.33% (n=5) than the otolith samples at 2.72% (n=15; Table 19). Robillard described how the NEFSC, who provides many ages for the stock assessment, was using scales to age up to age-4 and then would use otoliths for older fish, although now they only use otoliths for all ages. Overall the group recommended using otoliths, not scales, as an ageing hard part for this species. The only sample the group reviewed was #4 which was aged as both an age-2 and an age-3. This sample was from a winter flounder 355 cm in length and participants discussed how location matters for this species since some grow faster than others. Changing the light on this sample to reflective light convinced the participants that this is in fact an age-3 fish.

For individual reader comparisons, readers from MA DMF, NEFSC, CT DEEP, and VIMS reported that they routinely age winter flounder. When comparing the experienced winter flounder readers, there were no significant p-values from Bowker's test of symmetry, indicating no systematic bias between the readers (Table 20). All six CVs were greater than 5%, indicating imprecision, and ranged from 6-15%. Exact agreement varied from 50-80% and increased to 85-95% for agreement within one year (Table 21). When the five scale samples were removed from the analysis, only one CV was greater than 5% (Table 22) and agreement was increased between readers (Table 23).

Workshop Recommendations

Overall, the participants of the workshop felt that there were no major sample processing issues along the coast that needed to be addressed. They were satisfied with the ageing agreement among species, noting the persisting disagreement among agers regarding the protocol for Atlantic croaker ageing and the high APE for bluefish due to difficulties assigning age for young fish. The group made the following recommendations:

- River herring, striped bass, black sea bass, bluefish, red drum, scup, tautog, and Atlantic menhaden should be aged at the 2018 QA/QC Fish Ageing Workshop. The addition of American eel otoliths, which is undergoing an ageing exchange currently, should be considered for the 2019 workshop.

- Summer and winter flounder should be aged using otoliths rather than scales when possible.

- The use of whole otoliths instead of scales for ageing Atlantic menhaden should be explored.

- For the 2018 QA/QC Fish Ageing Workshop, individual ages and group ages should still be collected but assignments to groups should be strategic rather than random. For example, a representative who routinely ages each species should be in each group when possible.

- Agers did not identify an ASMFC species that needs its own ageing workshop, but rather recommended that the ASMFC hold a "New Methods" workshop (i.e., Tautog spine processing and ageing, Atlantic menhaden whole otolith-scale comparison).

- A coast-wide age validation study for Atlantic croaker should be conducted to fully evaluate the presence/absence of the smudge in different regions and identify if it in fact represents the first annulus. The Atlantic croaker stock assessment subcommittee should discuss the concern some agers have that Atlantic croaker may not be assigned to their correct year and age classes based on current ageing protocols and should consider a sensitivity run in future stock assessment models with adjusting the ages by 1.

References

Atlantic States Marine Fisheries Commission (ASMFC). 2008. Proceedings of the Atlantic Croaker and Red Drum Ageing Workshop. ASMFC, Arlington, VA. 9p.

Atlantic States Marine Fisheries Commission (ASMFC). 2010. Atlantic Croaker 2010 benchmark stock assessment. ASMFC, Arlington, Virginia.

Atlantic States Marine Fisheries Commission (ASMFC). 2011. Proceedings of the Atlantic States Marine Fisheries Commission Bluefish Ageing Workshop. ASMFC, Arlington, VA. 26p.

Atlantic States Marine Fisheries Commission (ASMFC). 2012a. Proceedings of the Tautog Ageing Workshop. ASMFC, Arlington, VA. 88p.

Atlantic States Marine Fisheries Commission (ASMFC). 2012b. Proceedings from the Winter Flounder Ageing Workshop. ASMFC, Arlington, VA. 60p.

Atlantic States Marine Fisheries Commission (ASMFC). 2015a. 2015 Atlantic Menhaden Ageing Workshop Report. ASMFC, Arlington, VA.

Atlantic States Marine Fisheries Commission (ASMFC). 2015b. Tautog 2015 Benchmark Stock Assessment. ASMFC, Arlington, VA.

Atlantic States Marine Fisheries Commission (ASMFC). 2016. Report of the Quality Assurance/Quality Control Fish Ageing Workshop. ASMFC, Arlington, VA.

Barbieri, L. R., M. E. Chittenden Jr, and C. M. Jones. 1994. Age, growth, and mortality of Atlantic croaker, Micropogonias undulatus, in the Chesapeake Bay region, with a discussion of apparent geographic changes in population dynamics. *Fishery Bulletin*, 92: l-12.

Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of fish biology*, 59(2): 197-242.

Cowan Jr, J. H., R. L. Shipp, H. K. Bailey IV, and D. W. Haywick. 1995. Notes: Procedure for Rapid Processing of Large Otoliths. *Transactions of the American Fisheries Society*, 124(2): 280-282.

Elzey, S. P., and K. J. Trull. 2016. Identification of a nonlethal method for aging tautog (*Tautoga onitis*). *Fishery Bulletin, 114(4*).

Evans, G. T., and J. M. Hoenig. 1998. Testing and viewing symmetry in contingency tables, with application to readers of fish ages. Biometrics: 620-629.

Franco, D. M. 2014. Determining life history characteristics of Atlantic croaker, *Micropogonias undulatus*, within coastal Georgia waters (Doctoral dissertation, Savannah State University).

North Carolina Department of Marine Fisheries (NCDMF). 2000. Comparison of age assignment and reader agreement for bluefish (*Pomatomus saltatrix*) based on scales, whole otoliths, and sectioned otoliths. NC Dept. of Environment and Natural Resources, Division of Marine Fisheries Annual Progress Report 1999.

Northeast Fisheries Science Center (NEFSC). 2004. 39th Northeast Regional Stock Assessment Workshop (39th SAW) assessment summary report. US Dept Commerce, Northeast Fish Sci Cent Ref Doc. 04-10a; 16 p.

Northeast Fisheries Science Center (NEFSC). 2011. Northeast Regional Stock Assessment Workshop (52nd SAW) Assessment Report. US Dept Commerce, Northeast Fish Sci Cent Ref Doc. Technical Report 11–17; 962 pp.

Northeast Fisheries Science Center (NEFSC). 2013. 57th Northeast Regional Stock Assessment Workshop (57th SAW) Assessment Report. US Dept Commerce, Northeast Fish Sci Cent Ref Doc. 13-16, 967 p.

Northeast Fisheries Science Center (NEFSC). 2015. 60th Northeast Regional Stock Assessment Workshop (60th SAW) Assessment Summary Report. US Dept Commerce, Northeast Fish Sci Cent Ref Doc. 15-07.

Richards, S.W. 1976. Age, growth, and food of bluefish (*Pomatomus saltatrix*) from East-Central Long Island Sound from July through November 1975. *Transactions of the American Fisheries Society*, 105: 523-525.

Robillard, E., Reiss, C.S., Jones, C.M. 2009. Age-validation and growth of bluefish (Pomatomus saltatrix) along the East Coast of the United States. *Fisheries Research,* 95: 65-75.

SEDAR (Southeast Data Assessment and Review). 2015. SEDAR 40 – Atlantic menhaden stock assessment report. SEDAR, North Charleston, South Carolina.

Tables

Table 1. The ageing structure with sample size in parentheses and average percent error (APE) between the four ageing groups for each species aged at the 2016 and 2017 QA/QC Fish Ageing Workshops.

Species	Ageing structure (sample size)	2016	2017
Alewife herring	scales (5), otoliths (5)	13.23%	------
Blueback herring	scales (5), otoliths (5)	13.23%	------
Black sea bass	scales (4), otoliths (16)	3.67%	------
Striped bass	scales (10), otolith (10)	4.96%	------
Tautog	opercula (20)	6.09%	10.89%
Atlantic croaker	otoliths (20)	7.76%	10.57%
Bluefish	otoliths (20)	23.06%	25.60%
Summer flounder	scales (6), otoliths (14)	-------	3.63%
Winter flounder	scales (5), otoliths (15)	-------	10.13%
Atlantic menhaden	scales (19)	-------	15.42%

Table 2. Ageing worksheet for Atlantic croaker at the workshop with the sample number, lab providing the sample and their assigned age, catch date of the sample, workshop group annulus counts, margin codes (scored from 1 to 4), and final age as well as average percent error (APE) values between groups. All samples were otoliths.

Sample #	Lab	Age	Catch date	Group 1			Group 2			Group 3			Group 4			Group 5			Average Age	APE
				Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age		
1	ODU	10	9/23/2014	10	4	10	11	2	11	10	4	10	10	4	10	10	4	10	10.2	3.1%
2	NJ	0	10/1/2012	0	3	0	0	4	0	0	4	0	0	4	0	0	4	0	0	0.0%
3	GA	6	7/1/2014	5	2	5	5	2	5	5	2	5	5	2	5	5	1	5	5	0.0%
4	GA	1	5/15/2012	0	4	1	0	4	1	0	4	1	0	4	1	0	4	1	1	0.0%
5	SCDNR	2	5/14/2014	2	1	2	2	1	2	2	2	2	2	1	2	1	4	2	2	0.0%
6	NJ	12	9/16/2010	12	2	12	12	3	12	12	3	12	12	3	12	12	2	12	12	0.0%
7	GA	5	6/29/2011	4	2	4	4	1	4	4	2	4	4	2	4	4	1	4	4	0.0%
8	VIMS	9	5/10/2014	8	1	8	8	1	8	8	2	8	8	1	8	8	3	9	8.2	3.9%
9	ODU	3	4/21/2014	3	1	3	3	1	3	3	1	3	3	1	3	3	1	3	3	0.0%
10	SCDNR	3	5/14/2014	3	1	3	3	1	3	3	1	3	3	1	3	3	1	3	3	0.0%
11	NJ	4	10/3/2006	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0.0%
12	NCDMF	6	3/26/2013	4	4	5	6	1	6	4	4	5	5	1	5	4	4	5	5.2	6.2%
13	MD	2	10/1/2019	2	4	2	3	2	3	2	4	2	2	4	2	2	4	2	2.2	14.5%
14	ODU	6	8/18/2014	6	3	6	7	2	7	6	3	6	6	3	6	6	3	6	6.2	5.2%
15	VIMS	1	11/5/2014	0	4	0	1	2	1	0	4	0	0	4	0	0	4	0	0.2	160.0%
16	NCDMF	7	3/26/2013	5	4	6	5	4	6	5	4	6	6	1	6	5	4	6	6	0.0%
17	MD	3	10/1/2018	3	3	3	4	1	4	3	4	3	3	4	3	3	3	3	3.2	10.0%
18	MD	7	10/1/2017	7	2	7	8	1	8	7	3	7	7	3	7	7	2	7	7.2	4.4%
19	NCDMF	3	6/13/2013	3	2	3	3	2	3	3	2	3	3	2	3	3	1	3	3	0.0%
20	VIMS	9	4/26/2014	7	2	7	8	1	8	7	4	8	8	1	8	7	3	8	7.8	4.1%
																			Average APE	10.57%

Table 3. **Symmetry test p-values for inter-lab age comparisons using Bowker's test and CVs (%) for Atlantic croaker otoliths. P-values appear under the shaded diagonal line and CVs are above.**

	NJ	MD	VIMS	ODU	NC	SC
NJ	N/A	19	27	0	0	7
MD	0.213	N/A	8	19	19	26
VIMS	0.074	0.416	N/A	27	27	34
ODU	0.317	0.163	0.055	N/A	0	8
NC	0.317	0.163	0.055	1	N/A	8
SC	0.055	0.306	0.317	0.082	0.082	N/A

Table 4. **Percent exact agreement (below the shaded diagonal line) and agreement within one year (above the shaded diagonal line) between readers for Atlantic croaker otoliths.**

	NJ	MD	VIMS	ODU	NC	SC
NJ	100	100	95	100	100	100
MD	55	100	100	100	100	100
VIMS	5	45	100	100	100	95
ODU	95	60	5	100	100	100
NC	95	60	5	100	100	100
SC	95	50	0	90	90	100

Table 5. Ageing worksheet for Atlantic menhaden at the workshop with the sample number, lab providing the sample and their assigned age, catch date of the sample, workshop group annulus counts, margin codes (scored from 1 to 4), and final age as well as average percent error (APE) values between groups. All samples were scales.

Sample #	Lab	Age	Catch date	Group 1			Group 2			Group 3			Group 4			Group 5			Average Age	APE
				Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age		
1	RI	3	5/27/2014	3	2	3	3	2	3	2	2	2	4	4	5	5	4	6	3.8	35.8%
2	MD	4	6/1/2016	3	4	3	4	2	4	3		3	6	4	6	5	1	5	4.2	24.8%
3	MD	1	6/1/2016	1	4	1	1	2	1			1	1	4	1	1	1	1	1	0.0%
4	NC	5	3/20/2014	3	4	4	4	2	4			3	5	4	6	4	3	5	4.4	20.0%
5	MD	3	9/7/2016	3	4	3	4	1	4			3	4	2	4	3	3	3	3.4	14.1%
6	NC	4	1/6/2014	2	4	3	3	2	3			3	3	4	4	2	4	3	3.2	10.0%
7	MD	2	6/1/2016	2	4	2	2	2	2			2	2	4	2	1	4	1	1.8	17.8%
8	RI	3	10/7/2014	3	2	3	4	1	4			2	3	2	3	3	2	3	3	13.3%
9	NC	1	1/6/2014	1	4	2	1	4	2			1	1	4	2	1	4	2	1.8	17.8%
10	RI	2	5/14/2014	3	4	4	4	1	4			2	3	1	3	3	3	4	3.4	21.2%
11	MD	5	7/5/2016	3	2	3	5	2	5			3	4	2	4	5	2	5	4	20.0%
12	MD	1	9/7/2016	4	2	4	3	2	3			3	3	2	3	4	1	4	3.4	14.1%
13	RI	2	10/7/2014	3	2	3	3	1	3			2	3	2	3	3	2	3	2.8	11.4%
14	MD	3	7/6/2016	4	2	4	4	2	4			3	4	2	4	3	4	4	3.8	8.4%
15	NC	5	3/20/2014	3	4	4	4	1	4			4	3	4	4	3	4	4	4	0.0%
16	MD	5	8/23/2016	4	2	4	5	2	5			4	4	2	4	4	1	4	4.2	7.6%
17	MD	1	9/8/2016	1	4	1	1	4	1			1	2	1	2	1	3	1	1.2	26.7%
18	NC	2	10/26/2016	2	2	2	2	3	2			1	2	2	2	2	2	2	1.8	17.8%
19	RI	4	7/8/2014	5	2	5	5	4	5			4	6	1	6	6	1	6	5.2	12.3%
																			Average APE	15.42%

Table 6. Symmetry test p-values for inter-lab age comparisons using Bowker's test and CVs (%) for Atlantic menhaden scales. P-values appear under the shaded diagonal line and CVs are above.

	RI	CT	DE	MD	VIMS
RI	N/A	18	14	3	20
CT	0.619	N/A	17	20	15
DE	0.502	0.174	N/A	16	17
MD	0.368	0.54	0.377	N/A	20
VIMS	0.395	0.174	0.174	0.501	N/A

Table 7. Percent exact agreement (below the shaded diagonal line) and agreement within one year (above the shaded diagonal line) between readers for Atlantic menhaden scales.

	RI	CT	DE	MD	VIMS
RI	100	84	95	100	84
CT	37	100	89	84	95
DE	53	42	100	95	79
MD	89	26	42	100	79
VIMS	32	32	37	37	100

Table 8. Ageing worksheet for bluefish at the workshop with the sample number, lab providing the sample and their assigned age, catch date of the sample, workshop group annulus counts, margin codes (scored from 1 to 4), and final age as well as average percent error (APE) values between groups. All samples were otoliths.

Sample #	Lab	Age	Catch date	Group 1			Group 2			Group 3			Group 4			Group 5			Average Age	APE
				Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age		
1	NJ	4	6/4/2014	3	4	4	4	1	4	3	4	4	4	1	4	3	4	4	4	0.0%
2	NCDMF	5	3/29/2014	3	4	4	4	1	4	3	4	4	4	4	5	3	3	4	4.2	7.6%
3	VIMS	1	9/25/2009	1	2	1	1	2	1	1	2	1	1	2	1	0	4	0	0.8	40.0%
4	ODU	12	3/10/2015	10	4	11	11	1	11	10	4	11	10	4	11	11	3	12	11.2	2.9%
5	SCDNR	1	7/12/2014	1	4	2	1	1	1	1	2	1	1	2	1	0	3	0	1	40.0%
6	MA	6	9/16/2015	6	2	6	6	2	6	6	2	6	6	2	6	6	2	6	6	0.0%
7	SCDNR	1	9/22/2014	2	3	2	1	3	1	1	3	1	1	3	1	0	3	0	1	40.0%
8	RI	2	11/2/2012	2	3	2	2	3	2	2	4	2	2	3	2	2	2	2	2	0.0%
9	FL	7	5/23/2012	6	4	7	6	4	7	6	4	7	7	1	7	6	3	7	7	0.0%
10	NJ	3	6/14/2014	2	4	3	2	4	3	2	4	3	3	1	3	2	4	3	3	0.0%
11	ODU	0	8/12/2015	0	3	0	1	2	1	0	2	0	0	3	0	0	2	0	0.2	160.0%
12	NY	4	5/3/2012	3	4	4	3	4	4	3	4	4	3	4	4	3	3	4	4	0.0%
13	RI	6	6/10/2012	6	4	7	6	4	7	6	4	7	6	4	7	6	4	7	7	0.0%
14	VIMS	1	10/9/2009	1	3	1	1	3	1	1	3	1	1	2	1	0	3	0	0.8	40.0%
15	NY	5	10/23/2013	3	4	3	4	2	4	4	2	4	4	3	4	3	3	3	3.6	13.3%
16	NCDMF	7	2/20/2014	6	4	7	6	4	7	6	4	7	6	4	7	5	3	6	6.8	4.7%
17	NCDMF	10	2/20/2014	8	4	9	8	4	9	8	4	9	8	4	9	8	3	9	9	0.0%
18	MA	0	8/28/2015	0	3	0	0	2	0	0	4	0	1	3	1	0	3	0	0.2	160.0%
19	VIMS	9	5/11/2014	8	4	9	8	4	9	8	4	9	8	4	9	9	4	10	9.2	3.5%
20	NY	2	5/31/2013	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	2	0.0%
																			Average APE	25.60%

Table 9. Symmetry test p-values for inter-lab age comparisons using Bowker's test and CVs (%) for bluefish otoliths. P-values appear under the shaded diagonal line and CVs are above. Asterisks indicate significant p-values.

	MA	NEFSC	RI	CT	NY	VIMS	ODU	NC	SC
MA	N/A	31	45	37	7	45	30	30	31
NEFSC	0.067	N/A	14	8	23	15	1	1	7
RI	0.406	0.021 *	N/A	8	38	1	15	15	21
CT	0.03 *	0.368	0.021 *	N/A	30	9	9	7	13
NY	0.091	0.221	0.163	0.136	N/A	38	23	22	23
VIMS	0.321	0.04 *	0.156	0.04 *	0.253	N/A	15	16	22
ODU	0.042 *	0.317	0.03 *	0.392	0.199	0.051	N/A	2	7
NC	0.042 *	0.317	0.03 *	0.317	0.199	0.061	0.368	N/A	6
SC	0.091	0.135	0.067	0.261	0.321	0.197	0.172	0.223	N/A

Table 10. Percent exact agreement (below the shaded diagonal line) and agreement within one year (above the shaded diagonal line) between readers for bluefish otoliths.

	MA	NEFSC	RI	CT	NY	VIMS	ODU	NC	SC
MA	100	100	100	100	100	100	100	100	90
NEFSC	60	100	100	100	100	100	100	100	100
RI	50	90	100	100	100	100	100	100	100
CT	60	90	90	100	100	100	100	100	100
NY	95	65	55	65	100	100	100	100	90
VIMS	45	85	95	85	50	100	100	100	95
ODU	65	95	85	85	70	80	100	100	100
NC	65	95	85	95	70	80	90	100	100
SC	60	80	70	80	65	70	75	85	100

Table 11. **Ageing worksheet for summer flounder at the workshop with the sample number, lab providing the sample and their assigned age, catch date of the sample, workshop group annulus counts, margin codes, and final age as well as average percent error (APE) values between groups. Samples 1-14 were otoliths and samples 15-20 were scales. APEs are provided for all samples, only otoliths, and only scales.**

Sample #	Lab	Age	Catch date	Group 1			Group 2			Group 3			Group 4			Group 5			Average Age	APE
				Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age		
1	ODU	5	3/17/2015	4	4	5	4	4	5	4	1	4	4	4	5	4	3	5	4.8	6.7%
2	NCDMF	3	2/3/2014	3	1	3	2	4	3	2	4	3	2	4	3	2	4	3	3	0.0%
3	VIMS	0	10/12/2015	0	4	0	0	4	0	0	4	0	0	3	0	0	4	0	0	0.0%
4	ODU	11	3/21/2015	9	4	10	10	4	11	10	4	11	10	4	11	10	2	10	10.6	4.5%
5	NCDMF	7	2/26/2014	6	4	7	6	4	7	6	4	7	6	4	7	6	3	7	7	0.0%
6	VIMS	2	5/22/2015	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	2	0.0%
7	ODU	3	3/17/2015	2	4	3	2	4	3	2	4	3	2	4	3	2	3	3	3	0.0%
8	VIMS	4	10/10/2015	4	3	4	4	3	4	4	3	4	4	2	4	4	2	4	4	0.0%
9	NCDMF	2	12/5/2013	2	4	2	2	3	2	2	4	2	2	3	2	2	4	2	2	0.0%
10	ODU	7	7/21/2015	6	3	6	7	2	7	7	1	7	7	2	7	7	1	7	6.8	4.7%
11	ODU	1	11/20/2015	1	4	1	1	3	1	1	4	1	1	4	1	1	2	1	1	0.0%
12	VIMS	6	10/24/2015	7	3	7	7	2	7	7	3	7	7	3	7	7	1	7	7	0.0%
13	NCDMF	11	2/3/2014	10	4	11	11	4	12	11	4	12	11	4	12	11	2	12	11.8	2.7%
14	VIMS	9	5/16/2015	8	4	9	9	1	9	8	4	9	9	1	9	8	4	9	9	0.0%
15	RI	5	7/3/2015	4	2	4	5	1	5	4	4	5	4	4	5	4	4	5	4.8	6.7%
16	ODU	3	3/17/2015	3	4	4	2	4	3	2	4	3	3	4	4	2	4	3	3.4	14.1%
17	ODU	1	11/20/2015	1	4	1	1	4	1	1	4	1	1	4	1	1	4	1	1	0.0%
18	RI	2	9/18/2015	1	4	1	1	4	1	1	4	1	1	3	1	1	3	1	1	0.0%
19	ODU	11	3/17/2015	6	4	7	9	4	10	9	4	10	7	4	8	10	2	11	9.2	14.8%
20	RI	4	7/3/2015	2	3	2	3	1	3	3	2	3	2	2	2	2	4	3	2.6	18.5%

Average APE	3.63%
Average APE (otoliths)	1.33%
Average APE (scales)	9.00%

Table 12. **Symmetry test p-values for inter-lab age comparisons using Bowker's test and CVs (%) for summer flounder scales and otoliths. P-values appear under the shaded diagonal line and CVs are above.**

	NEFSC	RI	CT	DE	VIMS	ODU	NC
NEFSC	N/A	4	4	9	8	3	3
RI	0.321	N/A	0	5	3	2	2
CT	0.321	1	N/A	5	3	2	2
DE	0.647	0.287	0.287	N/A	2	6	6
VIMS	0.433	0.392	0.392	0.157	N/A	5	5
ODU	0.321	0.607	0.607	0.549	0.736	N/A	0
NC	0.321	0.607	0.607	0.549	0.736	1	N/A

Table 13. **Percent exact agreement (below the shaded diagonal line) and agreement within one year (above the shaded diagonal line) between readers for summer flounder otoliths and scales.**

	NEFSC	RI	CT	DE	VIMS	ODU	NC
NEFSC	100	95	95	95	95	100	100
RI	80	100	100	100	100	95	95
CT	80	100	100	100	100	95	95
DE	50	65	65	100	100	95	95
VIMS	65	85	85	80	100	95	95
ODU	90	90	90	60	75	100	100
NC	90	90	90	60	75	100	100

Table 14. Symmetry test p-values for inter-lab age comparisons using Bowker's test and CVs (%) for summer flounder otoliths. P-values appear under the shaded diagonal line and CVs are above.

	NEFSC	RI	CT	DE	VIMS	ODU	NC
NEFSC	N/A	2	2	4	2	1	1
RI	0.368	N/A	0	2	0	1	1
CT	0.368	1	N/A	2	0	1	1
DE	0.423	0.287	0.287	N/A	2	3	3
VIMS	0.368	1	1	0.287	N/A	1	1
ODU	0.287	0.221	0.221	0.321	0.221	N/A	0
NC	0.287	0.221	0.221	0.321	0.221	1	N/A

Table 15. Percent exact agreement (below the shaded diagonal line) and agreement within one year (above the shaded diagonal line) between readers for summer flounder otoliths.

	NEFSC	RI	CT	DE	VIMS	ODU	NC
NEFSC	100	100	100	100	100	100	100
RI	86	100	100	100	100	100	100
CT	86	100	100	100	100	100	100
DE	64	79	79	100	100	100	100
VIMS	86	100	100	79	100	100	100
ODU	93	93	93	71	93	100	100
NC	93	93	93	71	93	100	100

Table 16. Ageing worksheet for tautog at the workshop with the sample number, lab providing the sample and their assigned age, catch date of the sample, workshop group annulus counts, margin codes, and final age as well as average percent error (APE) values between groups. All samples were opercula.

Sample #	Lab	Age	Catch date	Group 1			Group 2			Group 3			Group 4			Group 5			Average Age	APE
				Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age		
1	VIMS	2	10/6/2011	1	3	1	2	4	2	1	3	1	1	3	1	2	3	2	1.4	34.3%
2	MD	28	2/20/2014	24	4	25	28	2	28	27	4	28	27	4	28	28	4	29	27.6	3.8%
3	RI	3	9/8/2015	2	4	2	2	4	2	2	3	2	3	2	3	2	3	2	2.2	14.5%
4	VIMS	4	10/6/2011	4	4	4	5	2	5	4		4	5	3	5	5	3	5	4.6	10.4%
5	MA	12	11/6/2015	9	4	9	12	4	12	9		10	10	3	10	12	3	12	10.6	10.6%
6	RI	2	9/8/2015	1	3	1	2	4	2	1		1	2	3	2	1	4	1	1.4	34.3%
7	VIMS	20	10/6/2011	16	3	16	20	2	20	17		17	17	3	17	21	2	21	18.2	10.1%
8	MD	19	2/20/2014	16	2	16	18	4	19	18		19	18	2	18	19	3	20	18.4	6.1%
9	NY	7	5/19/2015	6	4	7	7	4	8	6		7	7	4	8	7	4	8	7.6	6.3%
10	NY	8	6/14/2015	6	2	6	7	2	7	7		7	7	2	7	8	1	8	7	5.7%
11	NY	10	11/19/2015	7	4	7	8	1	8	8		8	9	2	9	8	4	8	8	5.0%
12	MD	6	12/6/2014	6	4	6	6	4	6	6		6	6	4	6	6	4	6	6	0.0%
13	ODU	6	4/25/2014	5	4	6	7	3	8	5		6	5	4	6	7	4	8	6.8	14.1%
14	ODU	17	4/27/2014	14	4	15	16	4	17	18		19	15	2	15	18	4	19	17	9.4%
15	MD	3	12/16/2014	2	3	2	3	3	3	3		3	3	3	3	3	2	3	2.8	11.4%
16	ODU	3	11/22/2014	3	4	3	5	4	5	4		4	5	3	5	5	3	5	4.4	16.4%
17	MA	6	10/31/2015	5	4	5	5	4	5	5		5	5	2	5	5	3	5	5	0.0%
18	MA	9	11/6/2015	8	4	8	8	4	8	9		9	8	4	8	9	3	9	8.4	5.7%
19	NJ	9	1/11/2012	8	4	9	10	4	11	9		10	9	4	10	10	4	11	10.2	6.3%
20	NJ	5	1/10/2012	3	4	4	4	4	5	4		5	3	4	4	5	4	6	4.8	13.3%
																			Average APE	10.89%

Table 17. Symmetry test p-values for inter-lab age comparisons using Bowker's test and CVs (%) for tautog opercula. P-values appear under the shaded diagonal line and CVs are above.

	MA	RI	CT	NY	DE	VIMS	ODU
MA	N/A	8	8	6	18	14	11
RI	0.199	N/A	4	4	17	7	12
CT	0.202	0.437	N/A	4	16	10	10
NY	0.214	0.261	0.358	N/A	16	9	9
DE	0.199	0.433	0.446	0.276	N/A	11	7
VIMS	0.529	0.207	0.386	0.269	0.165	N/A	8
ODU	0.532	0.307	0.45	0.199	0.256	0.721	N/A

Table 18. Percent exact agreement (below the shaded diagonal line) and agreement within one year (above the shaded diagonal line) between readers for tautog opercula.

	MA	RI	CT	NY	DE	VIMS	ODU
MA	100	95	90	95	35	70	75
RI	40	100	95	90	65	80	80
CT	45	75	100	85	65	80	80
NY	60	60	70	100	60	80	90
DE	15	10	15	20	100	85	80
VIMS	20	50	40	45	30	100	95
ODU	30	35	50	50	45	50	100

Table 19. **Ageing worksheet for winter flounder at the workshop with the sample number, lab providing the sample and their assigned age, catch date of the sample, workshop group annulus counts, margin codes (scored from 1 to 4), and final age as well as average percent error (APE) values between groups. Samples 1-15 were otoliths and samples 16-20 were scales. APEs are provided for all samples, only otoliths, and only scales.**

Sample #	Lab	Age	Catch date	Group 1			Group 2			Group 3			Group 4			Group 5			Average Age	APE
				Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age	Annulus count	Margin code	Final age		
1	VIMS	3	10/9/2015	3	3	3	3	2	3	3	2	3	3	2	3	3	2	3	3	0.0%
2	NY	7	3/21/2002	6	4	7	6	4	7	7	1	7	7	1	7	7	4	8	7.2	4.4%
3	MA	10	5/14/2013	9	4	10	9	4	10	4	4	10	9	4	10	9	4	10	10	0.0%
4	VIMS	3	10/8/2015	2	4	2	3	2	3	3	3	3	3	2	3	2	4	2	2.6	18.5%
5	NY	6	4/30/2002	5	4	6	5	4	6	5	4	6	5	4	6	5	4	6	6	0.0%
6	VIMS	5	10/8/2015	5	2	5	5	2	5	5	3	5	5	2	5	5	1	5	5	0.0%
7	NY	6	3/24/2003	5	4	6	5	4	6	5	4	6	5	4	6	5	3	6	6	0.0%
8	VIMS	7	10/8/2015	7	3	7	7	2	7	7	2	7	7	2	7	7	2	7	7	0.0%
9	VIMS	11	5/21/2015	10	4	11	10	4	11	10	3	11	10	4	11	10	4	11	11	0.0%
10	MA	2	5/9/2013	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	2	0.0%
11	NY	4	4/3/2003	3	4	4	3	4	4	3	4	4	3	4	4	2	4	3	3.8	8.4%
12	MA	8	5/8/2013	7	4	8	7	4	8	7	4	8	8	4	9	8	4	9	8.4	5.7%
13	MA	5	5/7/2013	4	4	5	4	4	5	4	4	5	4	4	5	4	4	5	5	0.0%
14	VIMS	12	5/17/2015	12	1	12	12	4	13	12	1	12	12	4	13	12	3	13	12.6	3.8%
15	MA	7	5/6/2013	6	4	7	6	4	7	6	4	7	6	4	7	6	4	7	7	0.0%
16	RI	3	8/20/2015	2	3	2	3	2	3	3	2	3	2	4	2	1	3	1	2.2	29.1%
17	RI	3	5/19/2015	2	2	2	3	1	3	3	4	4	2	4	3	1	4	2	2.8	22.9%
18	RI	2	5/21/2015	1	4	2	2	4	3	3	1	3	3	4	4	1	4	2	2.8	22.9%
19	RI	2	5/21/2015	0	4	1	2	1	2	2	1	2	2	1	2	1	4	2	1.8	17.8%
20	RI	3	5/20/2015	0	4	1	5	4	6	1	1	1	1	1	1	1	4	2	2.2	69.1%
																			Average APE	10.13%
																			Average APE (otoliths)	2.72%
																			Average APE (scales)	32.33%

Table 20. **Symmetry test p-values for inter-lab age comparisons using Bowker's test and CVs (%) for winter flounder otoliths and scales. P-values appear under the shaded diagonal line and CVs are above.**

	MA	**NEFSC**	**CT**	**VIMS**
MA	N/A	15	13	9
NEFSC	0.213	N/A	8	8
CT	0.501	0.095	N/A	6
VIMS	0.333	0.158	0.392	N/A

Table 21. **Percent exact agreement (below the shaded diagonal line) and agreement within one year (above the shaded diagonal line) between readers for winter flounder otoliths and scales.**

	MA	**NEFSC**	**CT**	**VIMS**
MA	100	85	90	95
NEFSC	50	100	90	95
CT	60	80	100	95
VIMS	70	65	80	100

Table 22. Symmetry test p-values for inter-lab age comparisons using Bowker's test and CVs (%) for winter flounder otoliths. P-values appear under the shaded diagonal line and CVs are above.

	MA	NEFSC	CT	VIMS
MA	N/A	7	4	4
NEFSC	0.238	N/A	3	3
CT	0.156	0.317	N/A	1
VIMS	0.156	0.317	1	N/A

Table 23. Percent exact agreement (below the shaded diagonal line) and agreement within one year (above the shaded diagonal line) between readers for winter flounder otoliths.

	MA	NEFSC	CT	VIMS
MA	100	93	100	100
NEFSC	60	100	93	93
CT	73	87	100	100
VIMS	80	80	93	100

Appendix A: Agenda

Atlantic States Marine Fisheries Commission's
QA/QC Fish Ageing Workshop

Wednesday, April 5th, 2017 – 9:00 a.m. to 5:00 p.m.
Thursday, April 6th, 2017 – 9:00 a.m. to ~3:00 p.m.

FWC Fish and Wildlife Research Institute
100 8th Ave SE
St. Petersburg, Florida

Agenda

Wednesday, April 5th

1. Call to Order/Introductions
2. Conduct Hard Part Readings Exercise for Atlantic menhaden, Winter flounder, Atlantic Croaker, Bluefish, Tautog, and Summer flounder

Thursday, April 6th

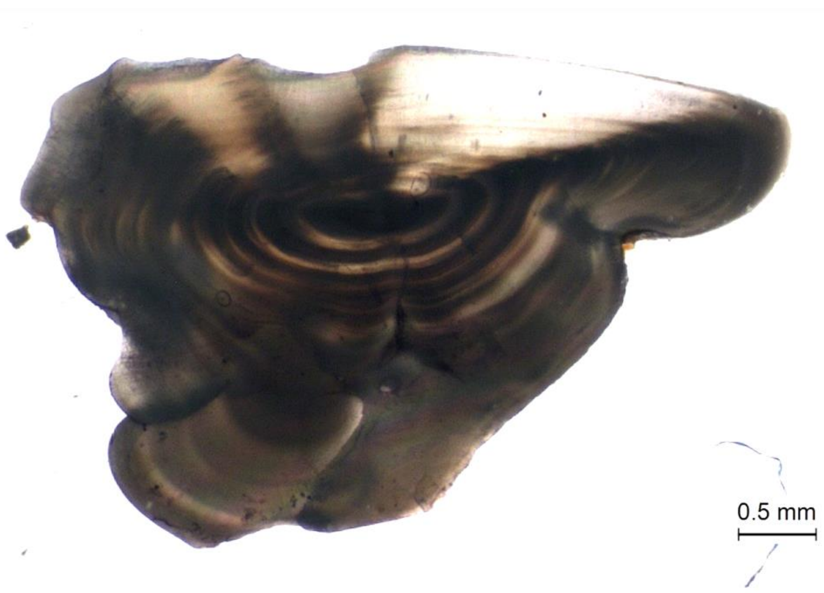
3. Review and Comparison of Otolith Reading Exercise by Groups and by States
4. Discussion and Review of Issues and Differences Encountered during Reading Exercise
5. Make Recommendations
6. Other Business

Adjourn

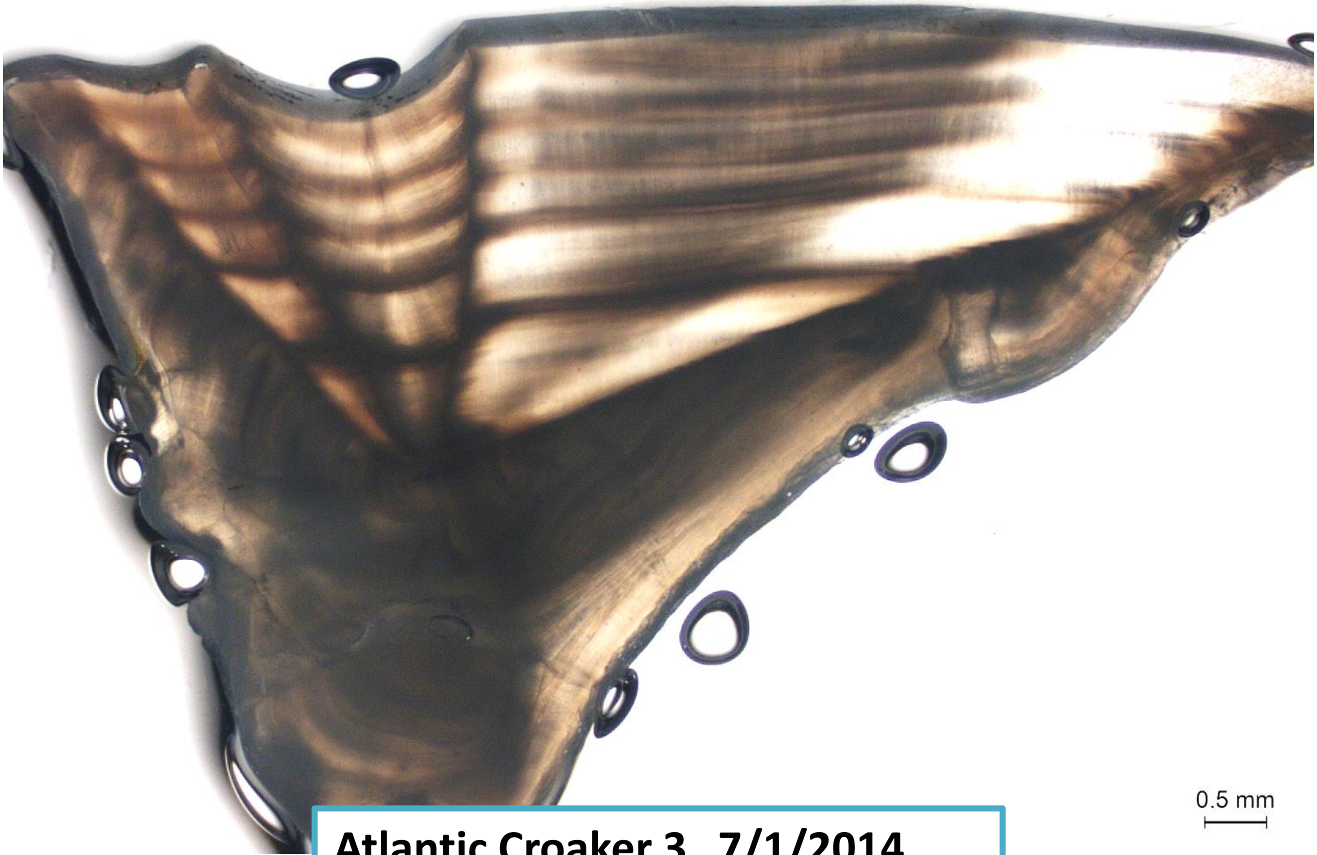
Name	State/Lab	Provided 2017 Samples of:
Scott Elzey	MA	Bluefish, Tautog, Winter Flounder
Eric Robillard	NEFSC	
Nicole Lengyel	RI	Bluefish, Tautog, Atlantic menhaden, Summer flounder, Winter flounder
David Molnar	CT	
Paul Nunnenkamp	NY	Bluefish, Tautog, Winter flounder
Heather Corbett	NJ	Atlantic croaker, Bluefish, Tautog
Michael Greco	DE	
Katherine Messer	MD	Atlantic croaker, Bluefish, Tautog, Atlantic menhaden
Jessica Gilmore	ODU	Atlantic croaker, Bluefish, Tautog, Summer flounder
Jameson Gregg	VIMS	Atlantic croaker, Bluefish, Tautog, Summer flounder, Winter flounder
Kelly McDonald	NC	Atlantic croaker, Bluefish, Summer flounder, Atlantic menhaden
Jonathan Tucker	SC	Atlantic croaker, Bluefish
Donna McDowell	GA	Atlantic croaker
Jessica Carroll	FL	Bluefish
Kristen Anstead	ASMFC	

Appendix B: Sample Images

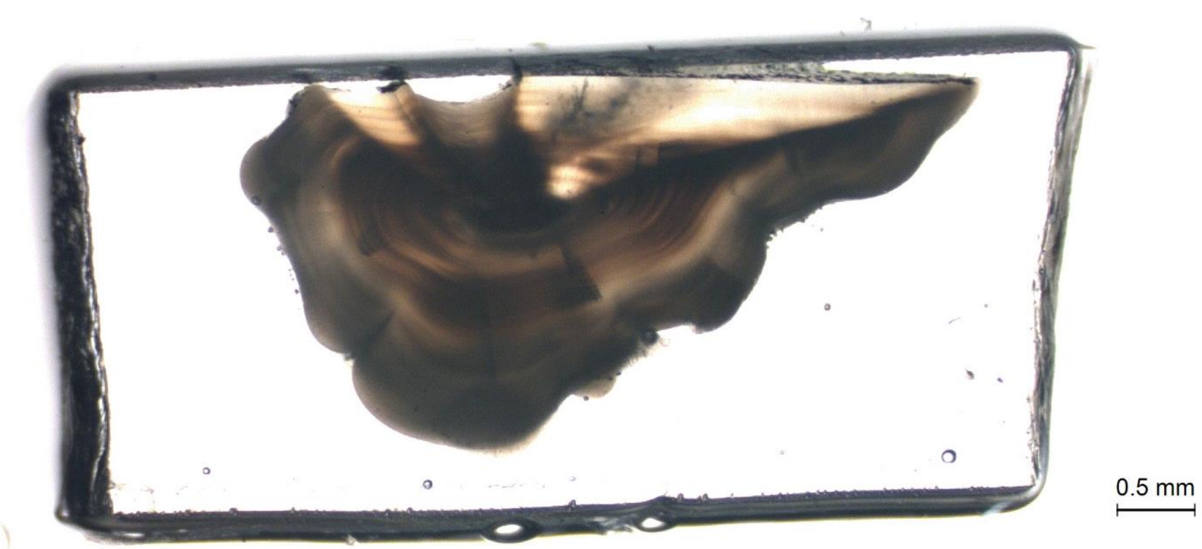
Atlantic Croaker 1   9/23/2014



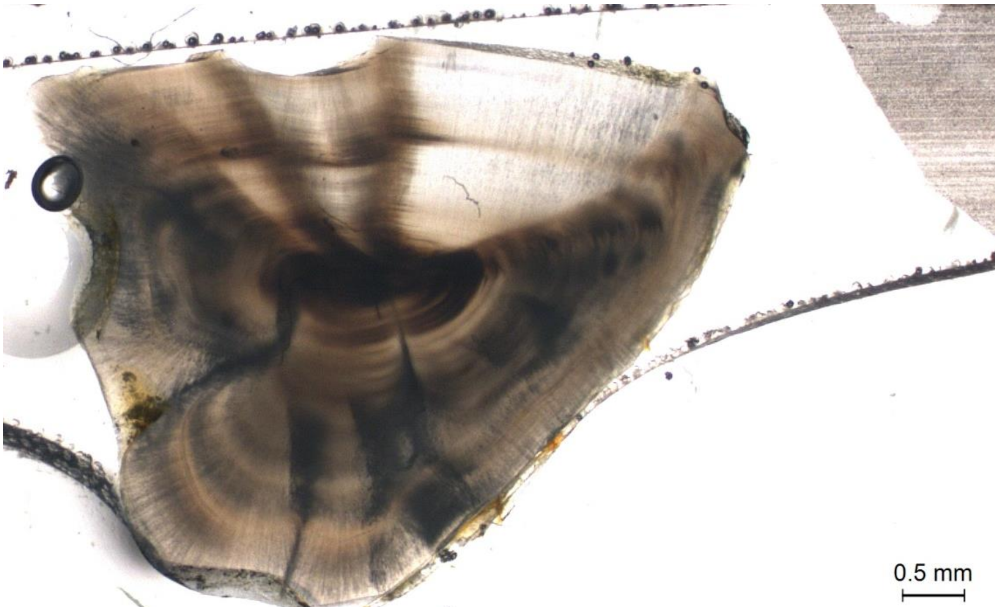
Atlantic Croaker 2   10/1/2012



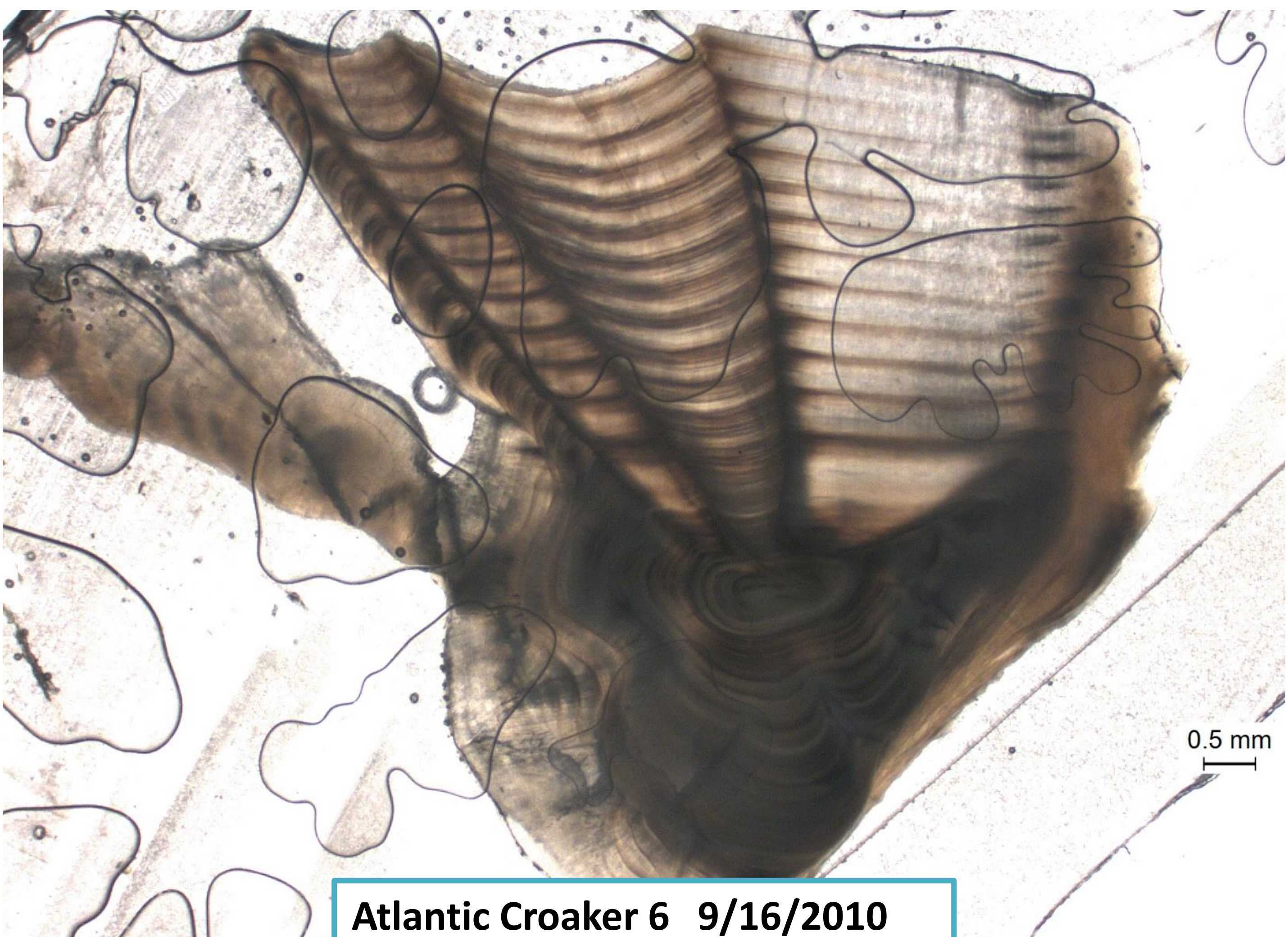
Atlantic Croaker 3   7/1/2014



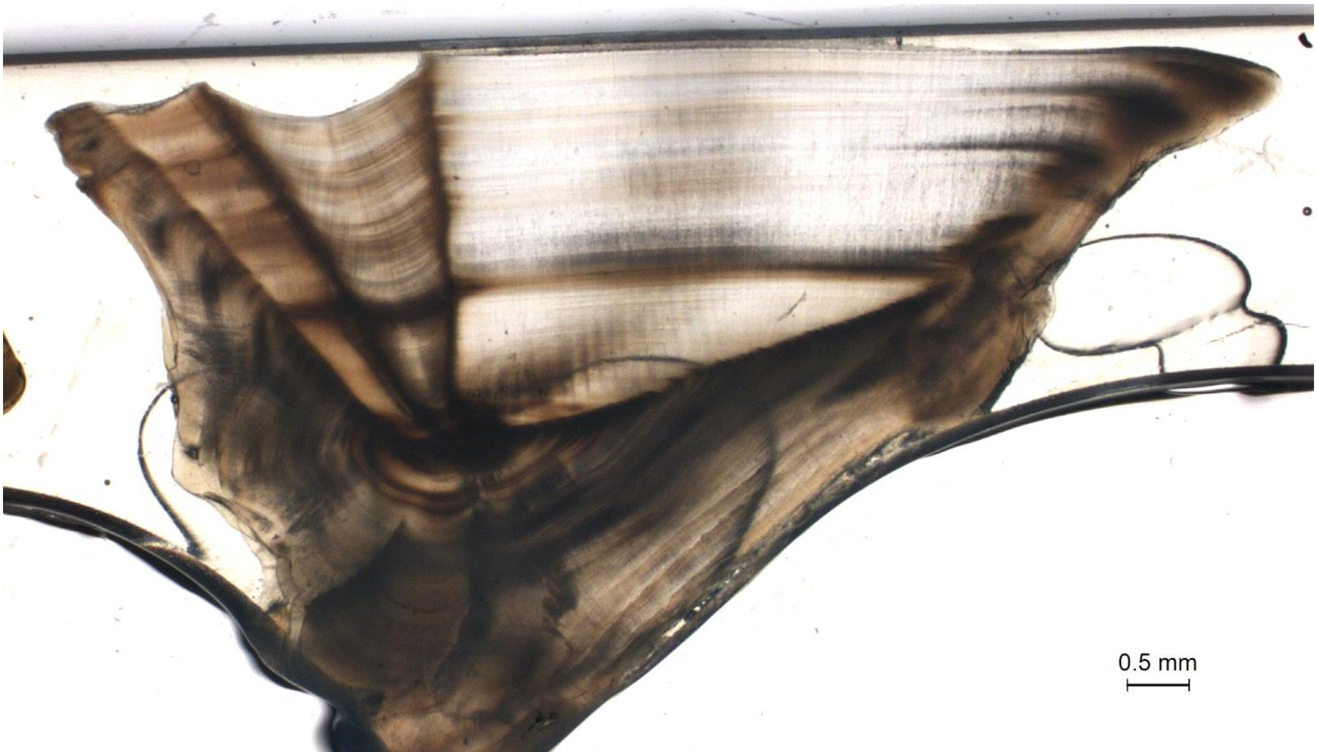
Atlantic Croaker 4   5/15/2012



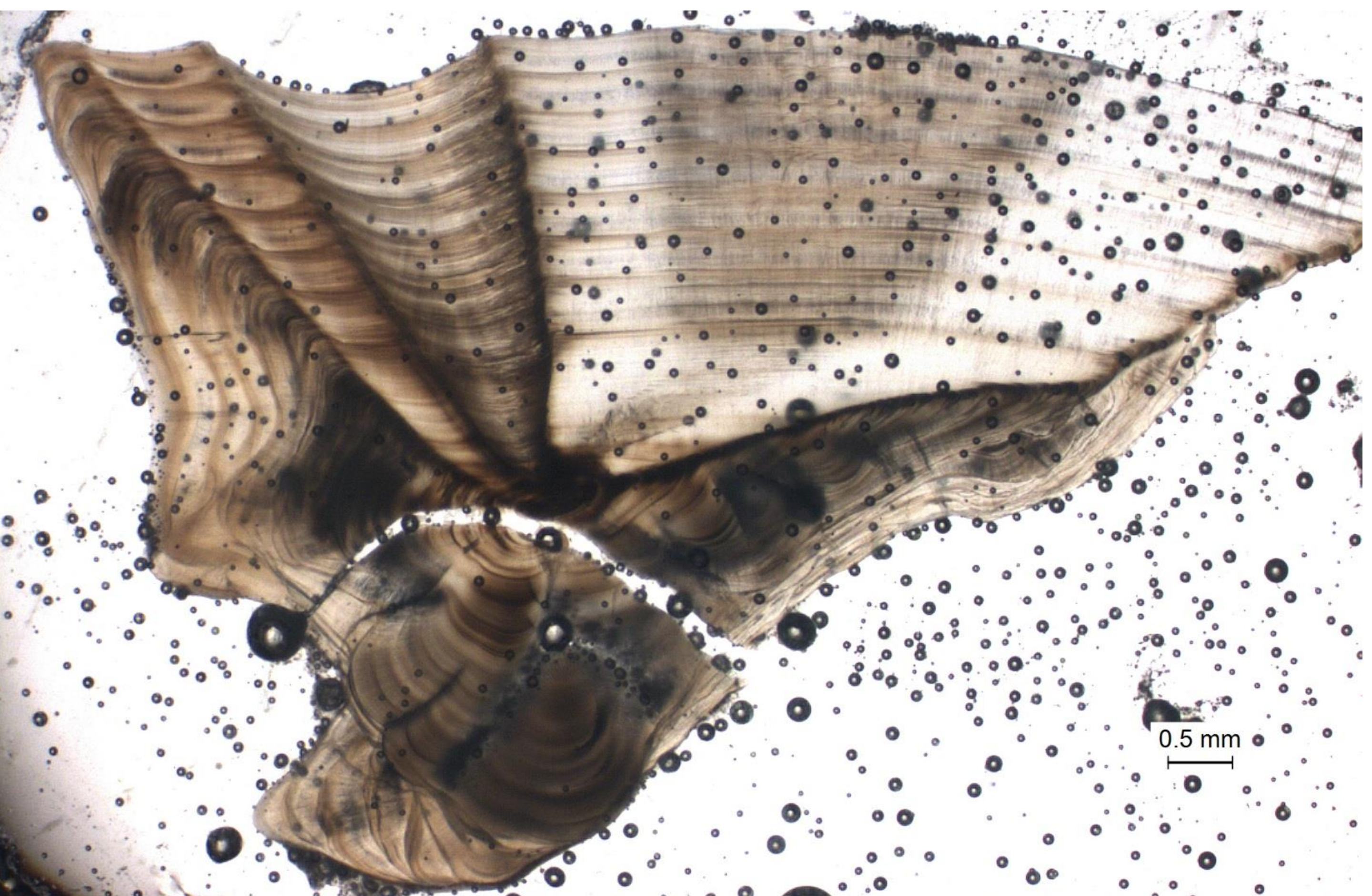
Atlantic Croaker 5   5/14/2014



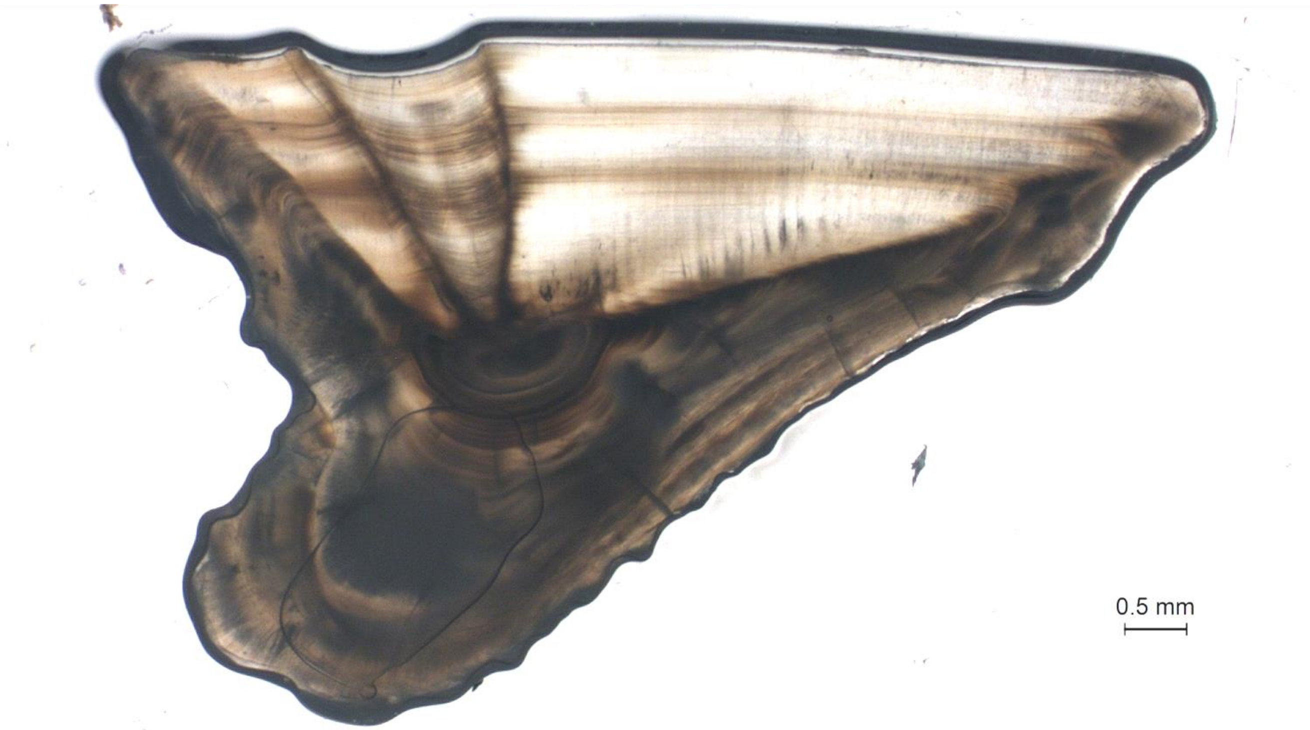
Atlantic Croaker 6   9/16/2010



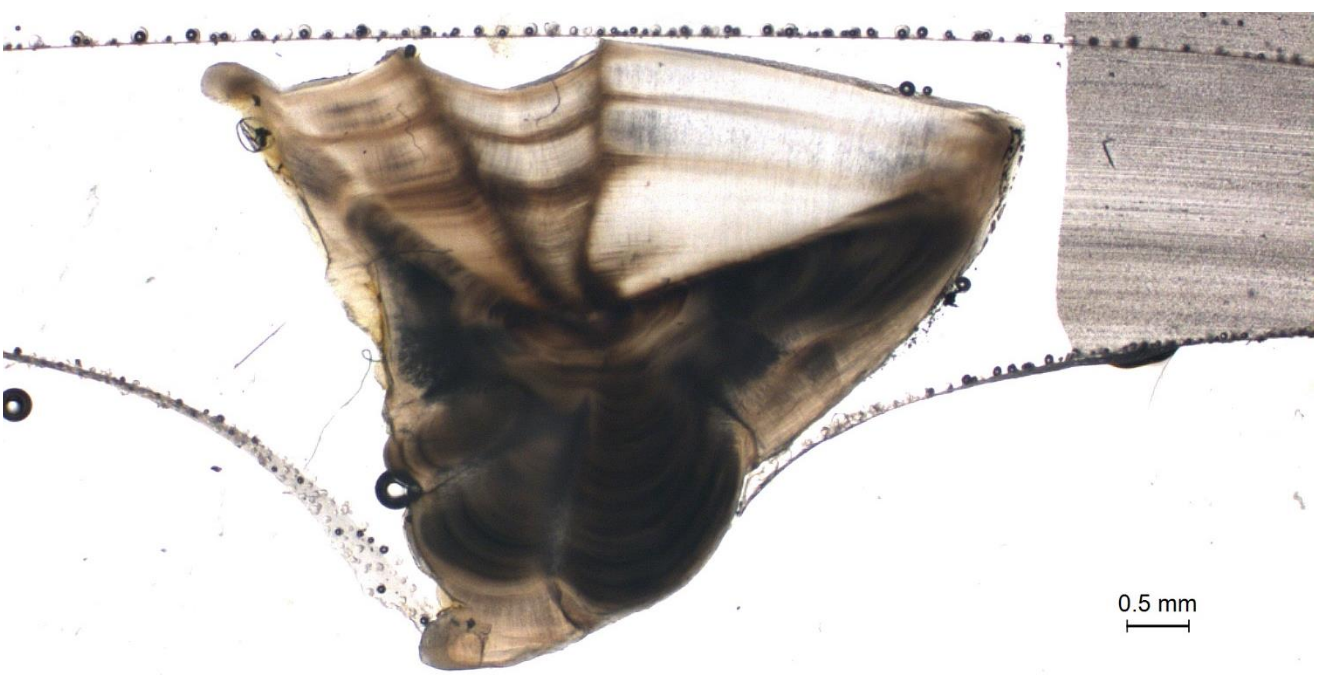
Atlantic Croaker 7   6/29/2011



Atlantic Croaker 8   5/10/2014



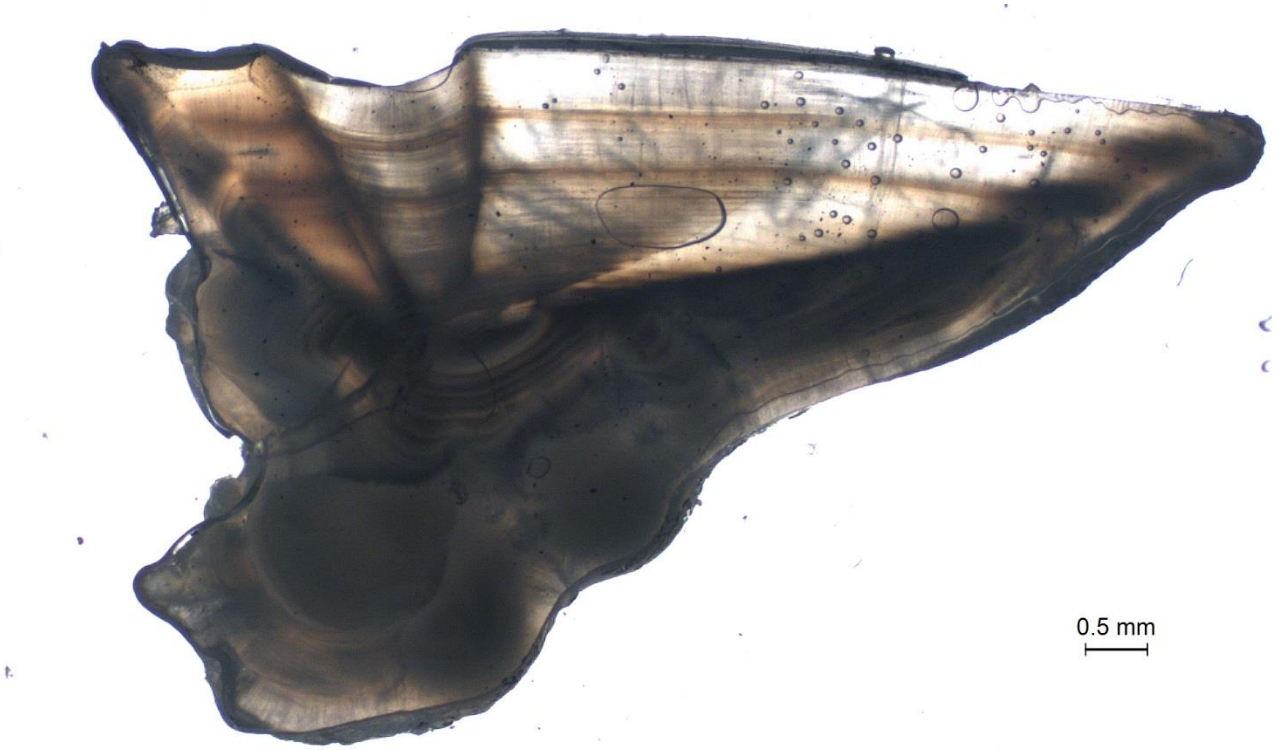
Atlantic Croaker 9   4/21/2014



Atlantic Croaker 10   5/14/2014

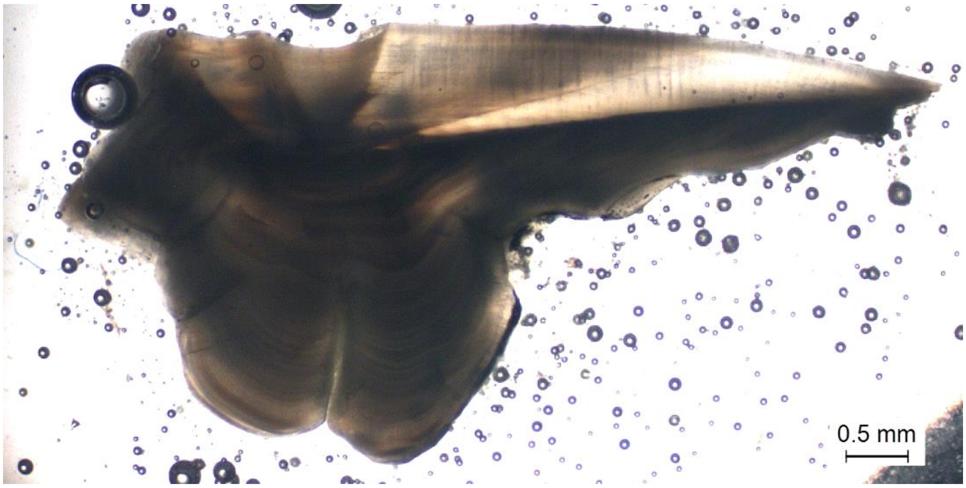
Atlantic Croaker 11 10/3/2006

Atlantic Croaker 12 3/26/2013

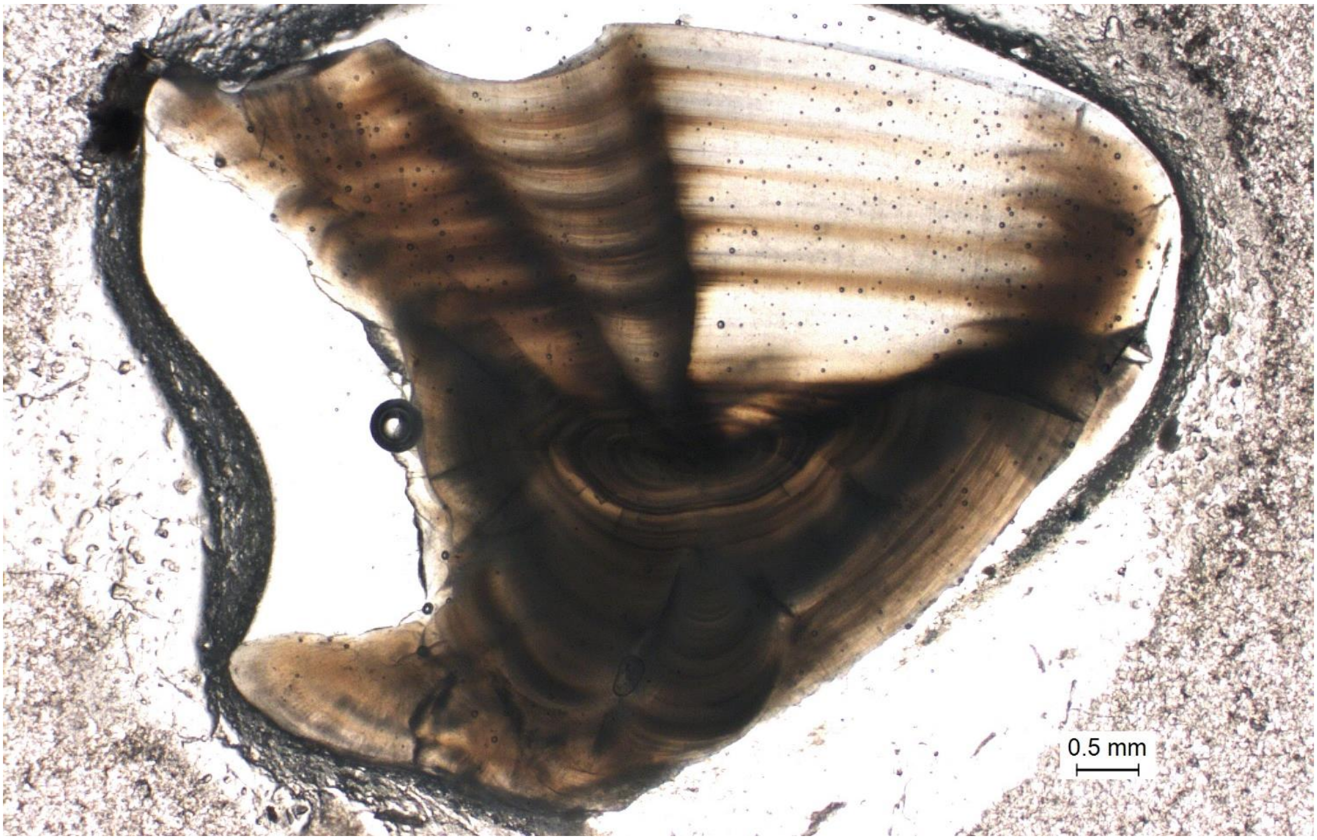


Atlantic Croaker 13 9/15/2015

Atlantic Croaker 14 8/18/2014

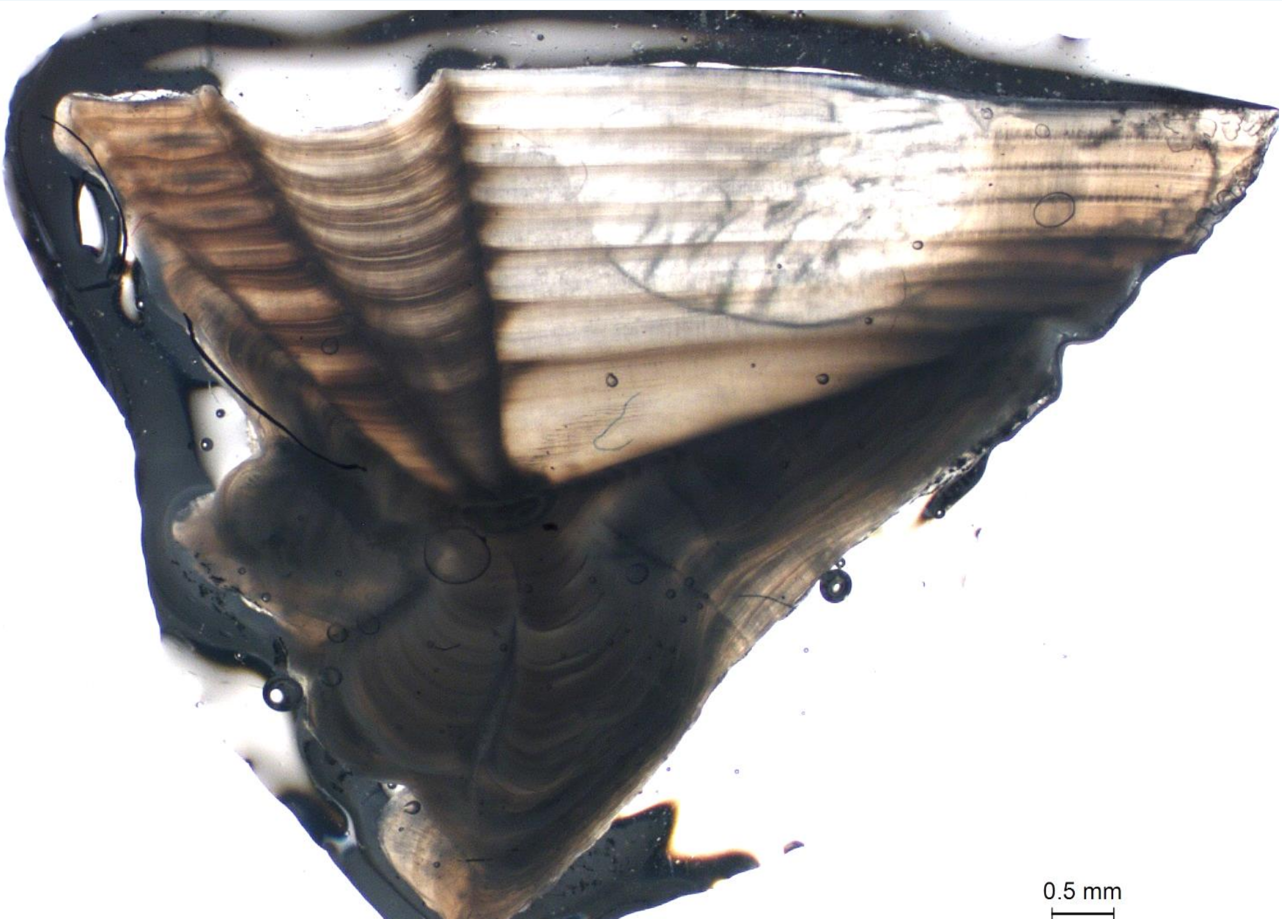


Atlantic Croaker 15 11/5/2014



Atlantic Croaker 16 3/26/2013

Atlantic Croaker 17 9/15/2015



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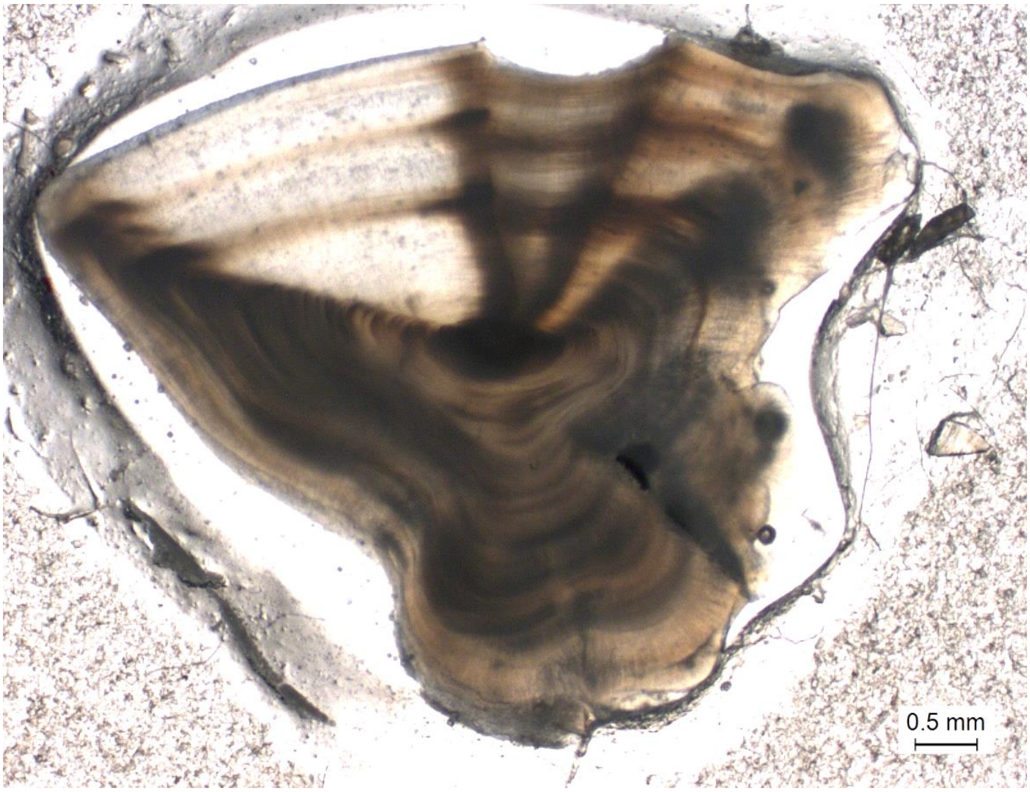
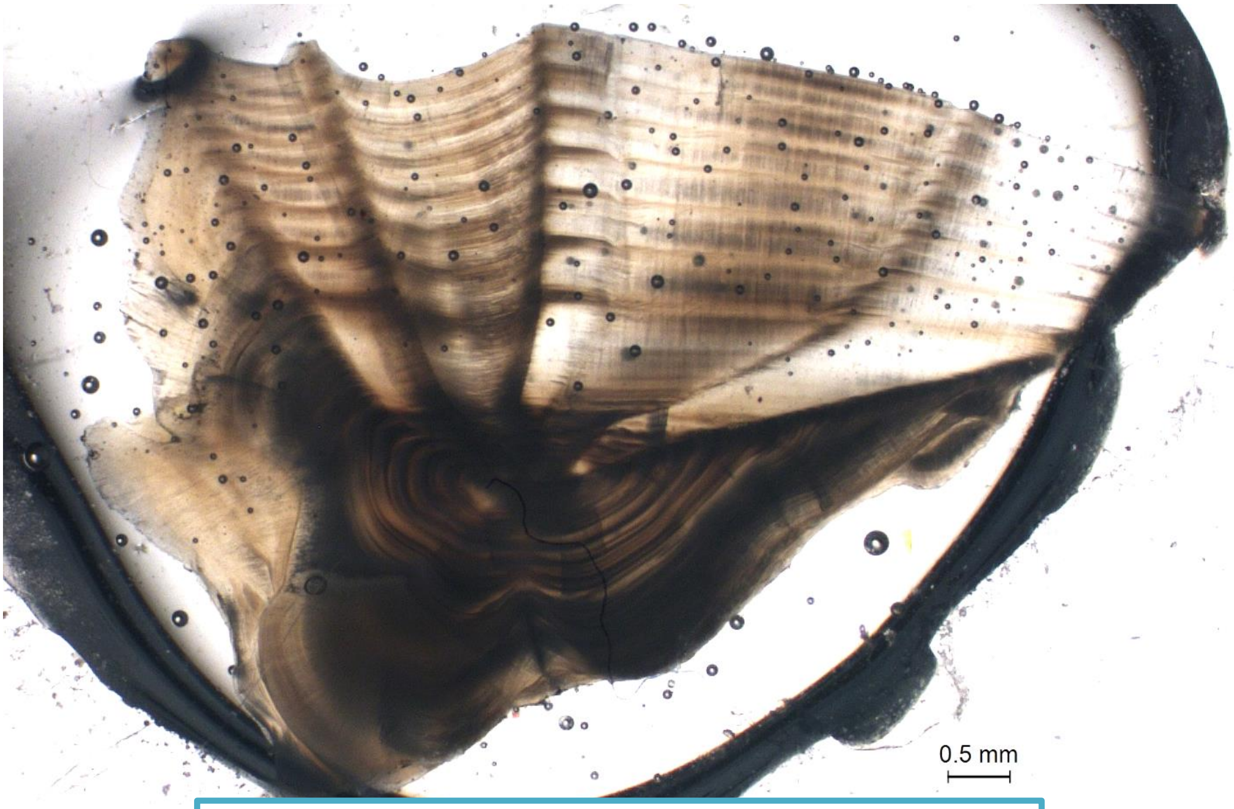
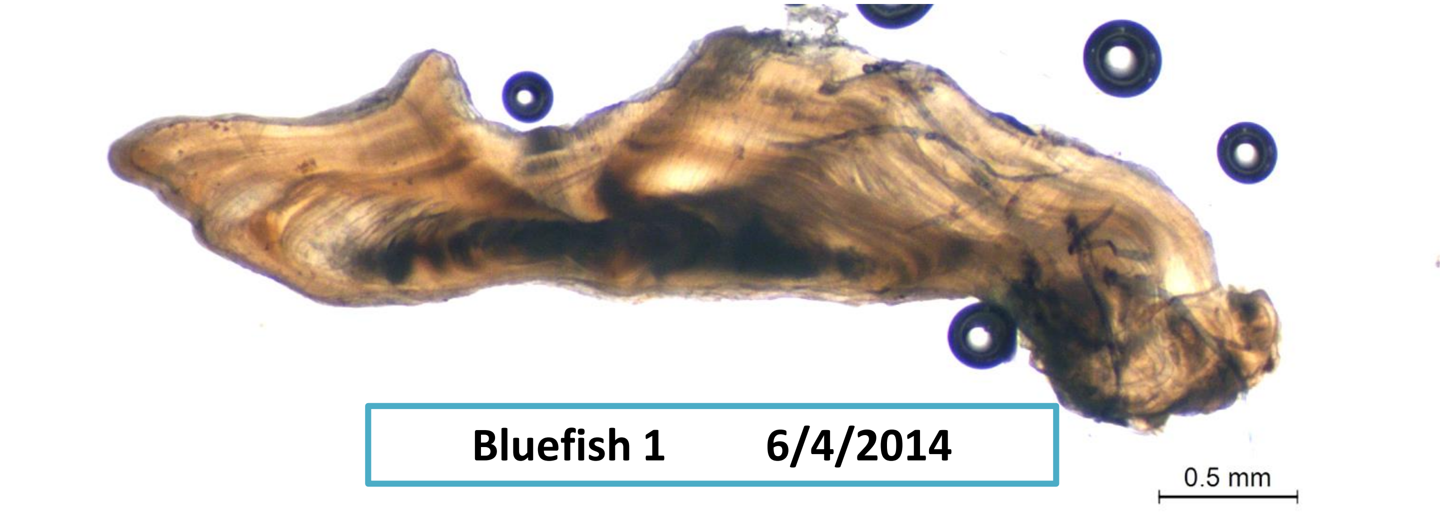


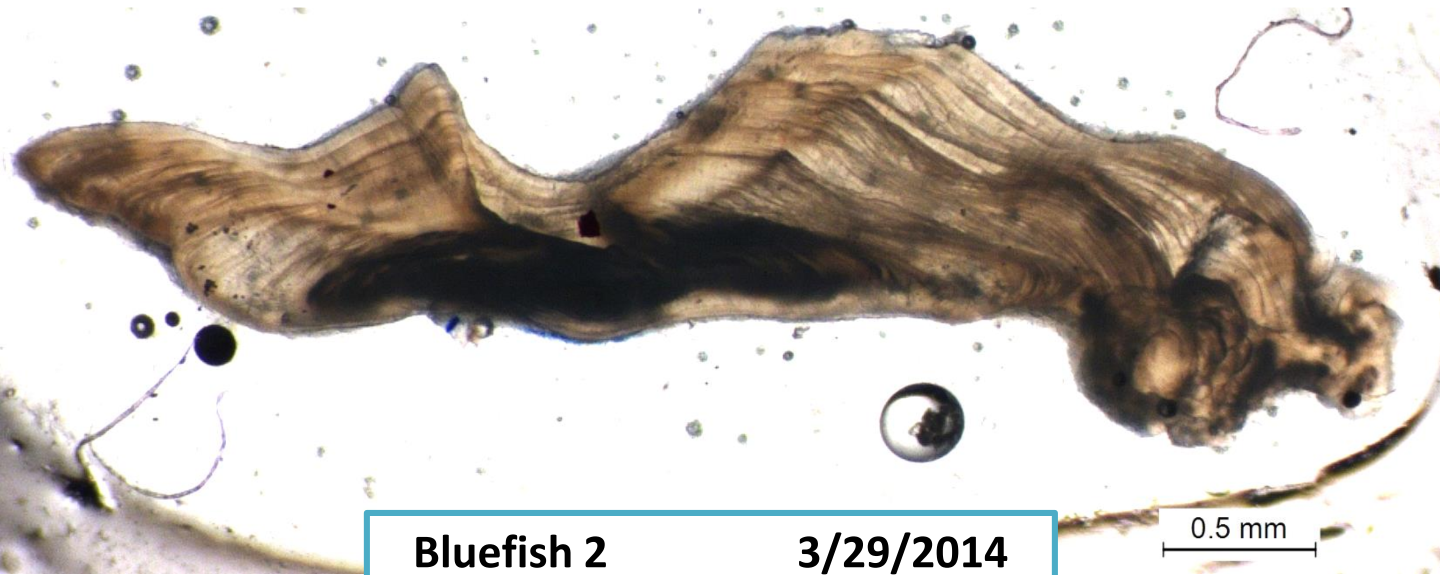
Atlantic Croaker 19 6/13/2013



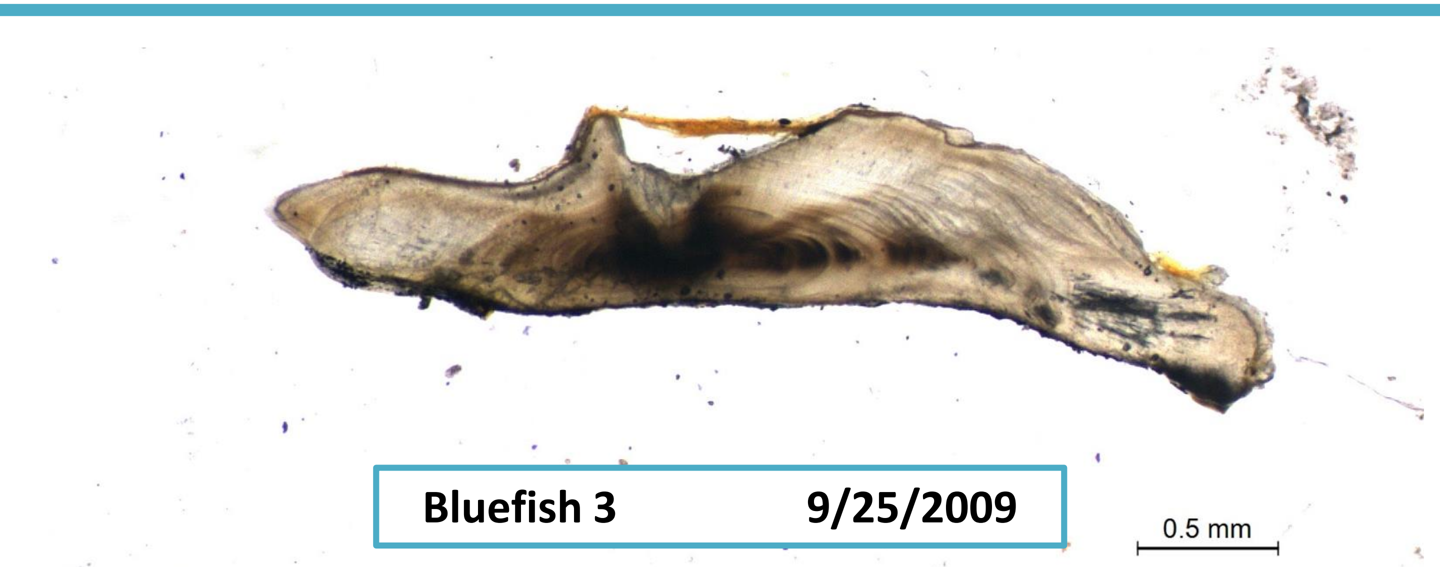
Atlantic Croaker 20 4/26/2014



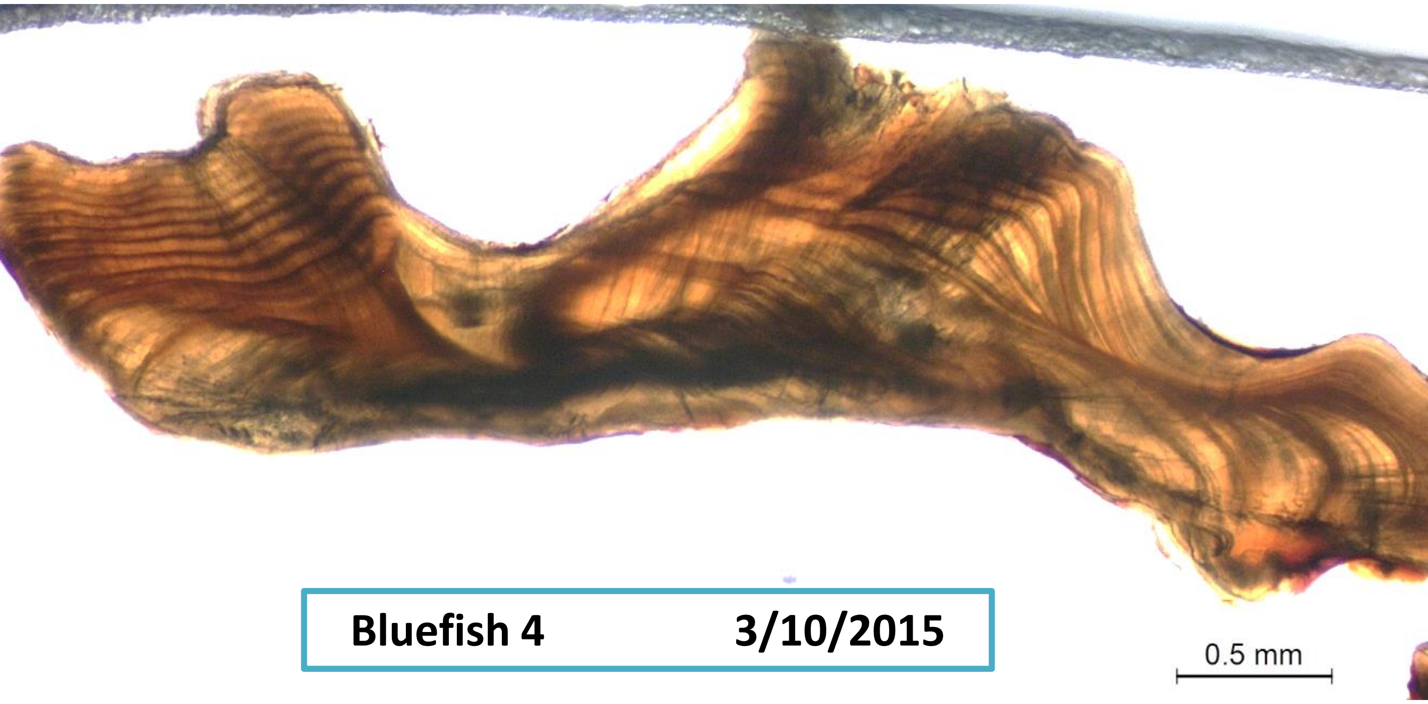
Bluefish 1 6/4/2014



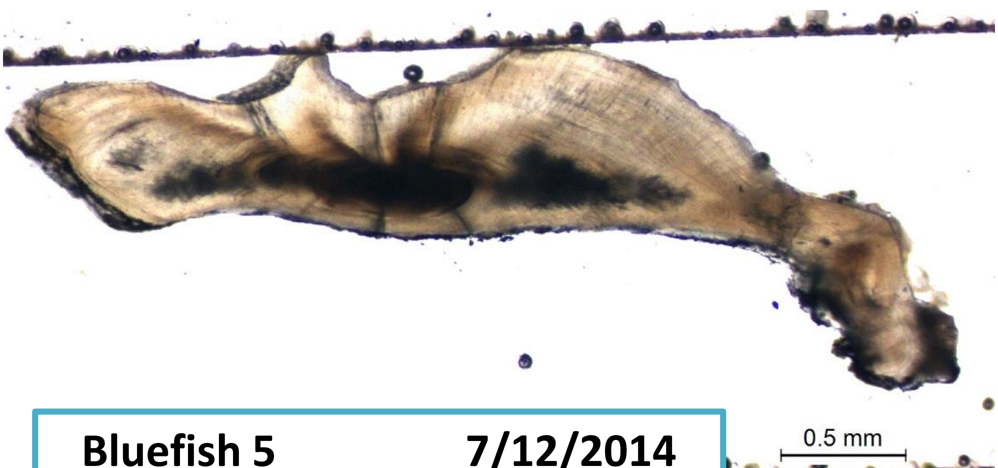
Bluefish 2 3/29/2014



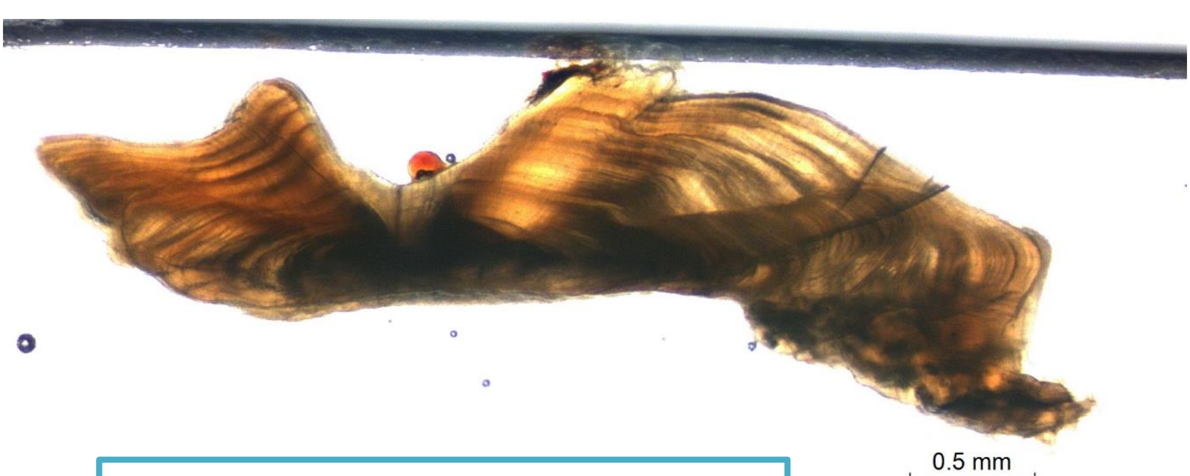
Bluefish 3 9/25/2009



Bluefish 4 3/10/2015



Bluefish 5 7/12/2014

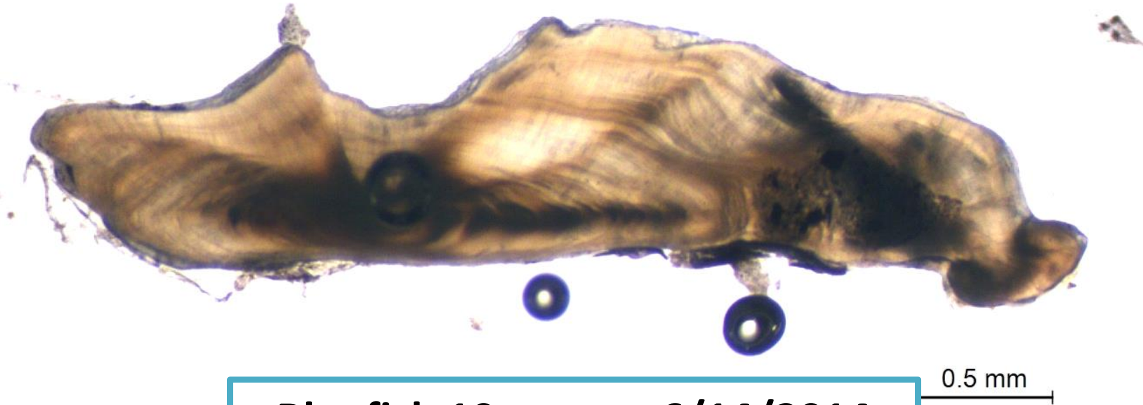


Bluefish 6 9/16/2015

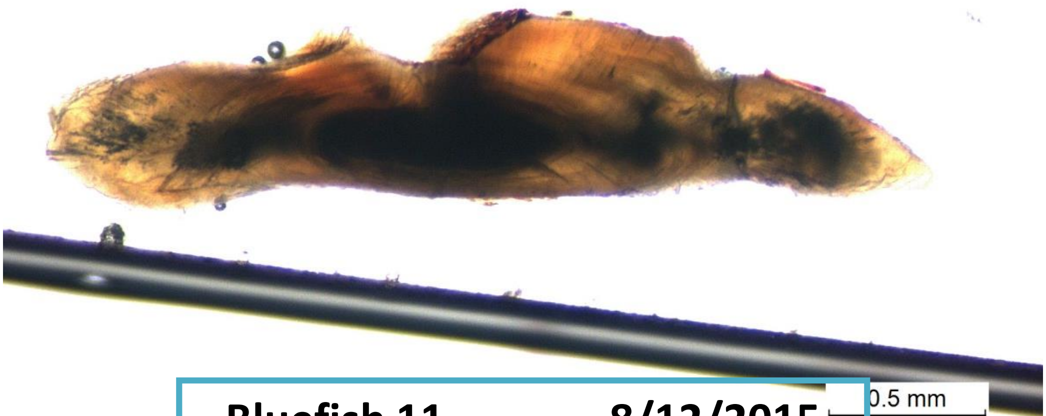
Bluefish 7 9/22/2014

Bluefish 8 11/2/2012

Bluefish 9 5/23/2012



Bluefish 10 6/14/2014



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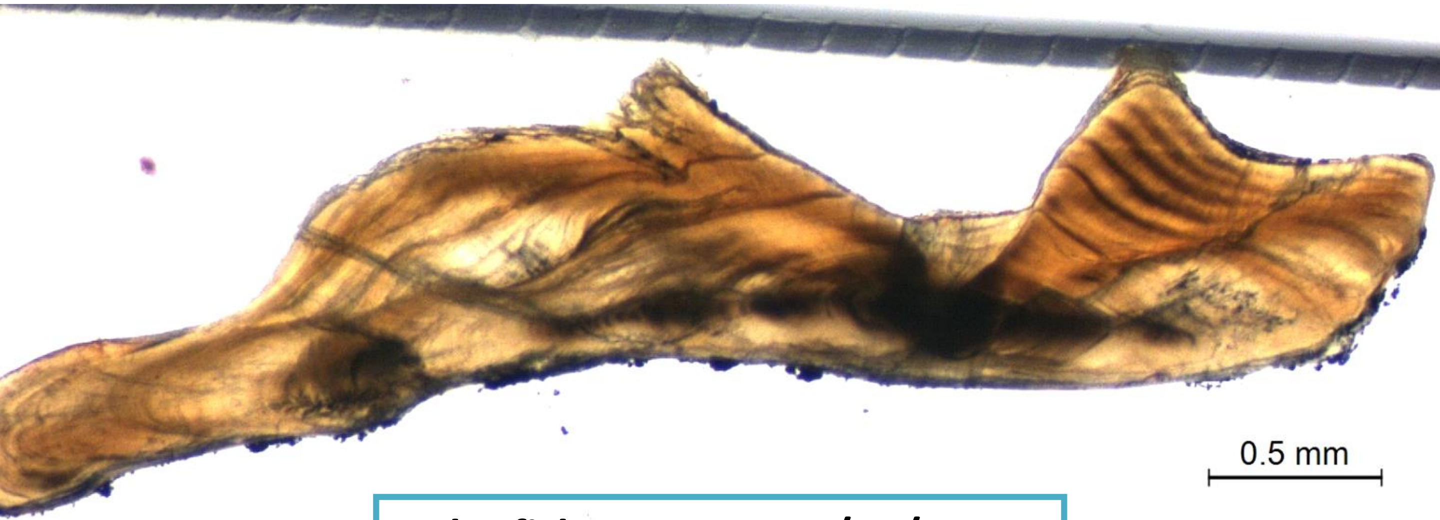
Bluefish 12 5/3/2012



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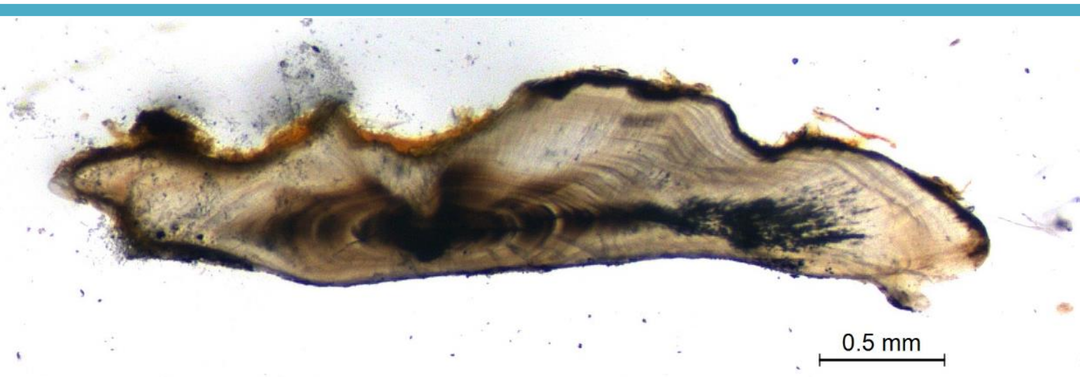
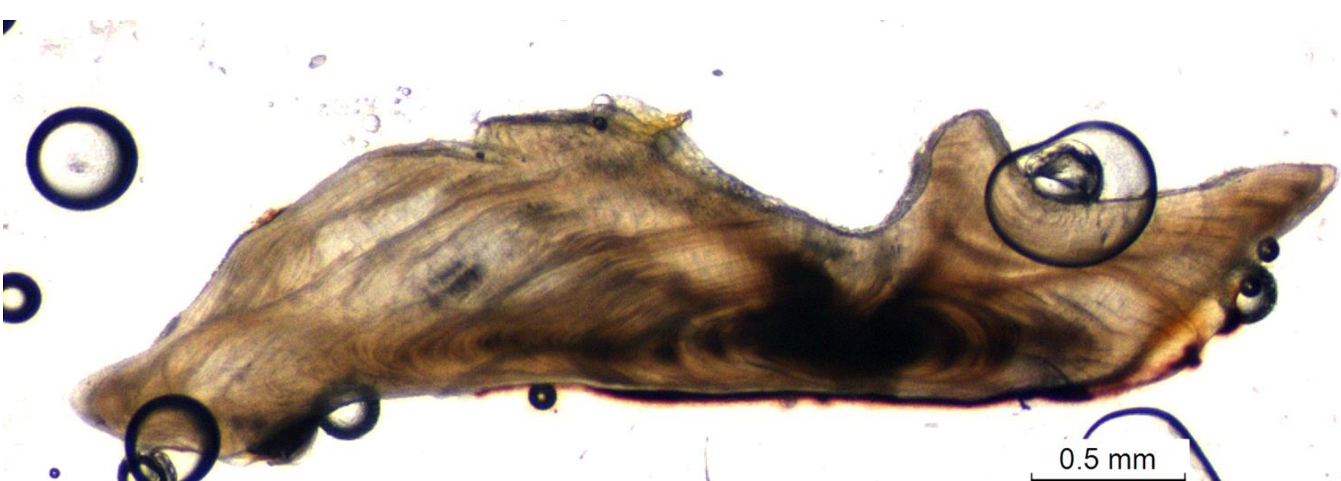
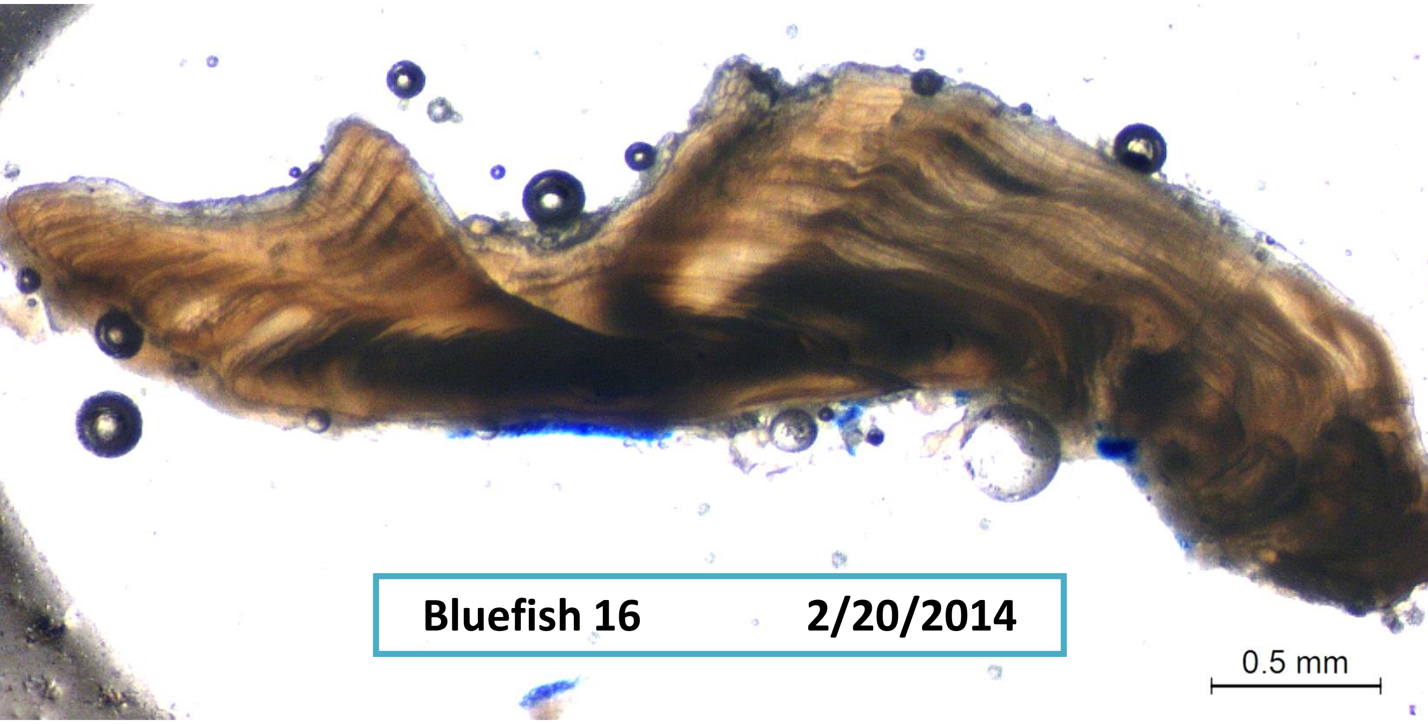


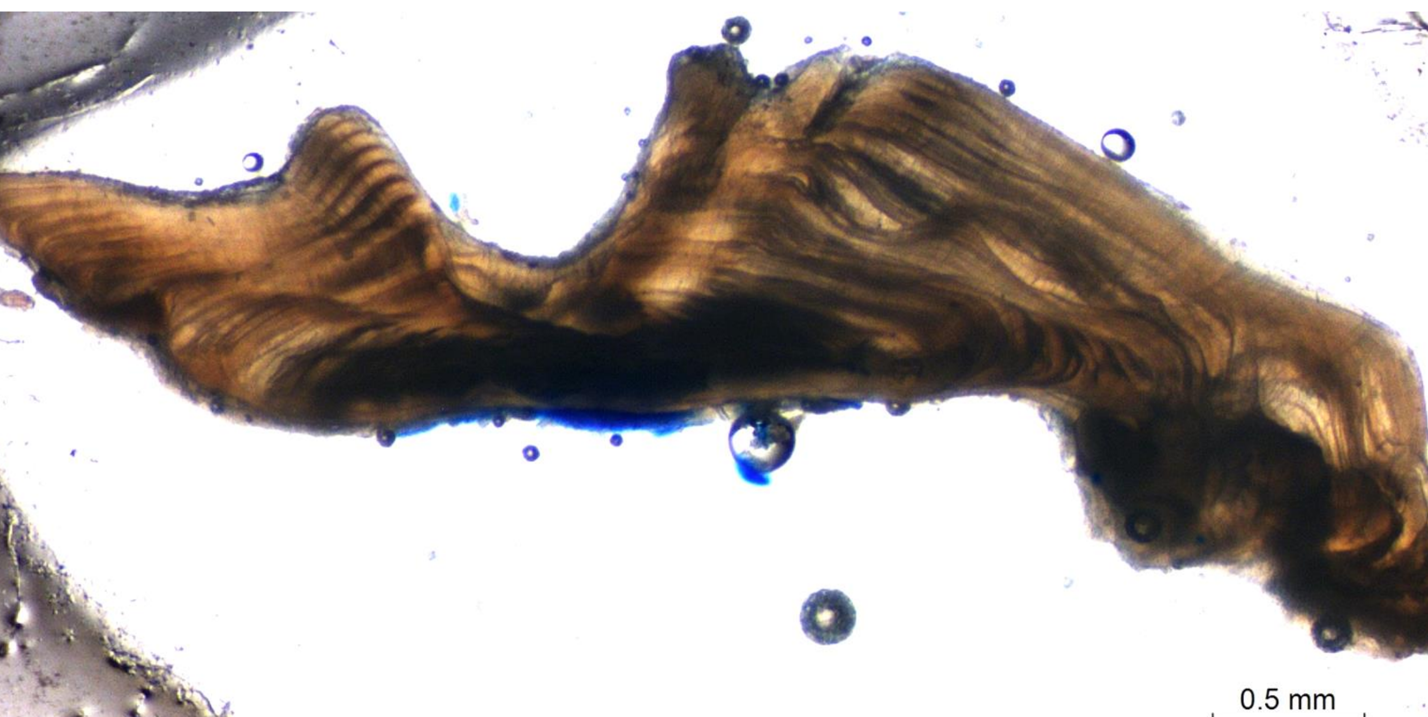
Bluefish 14 10/9/2009



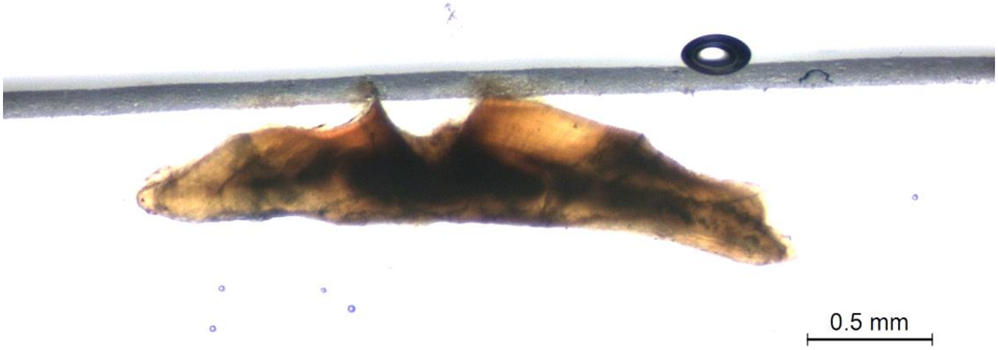
Bluefish 15 10/23/2013



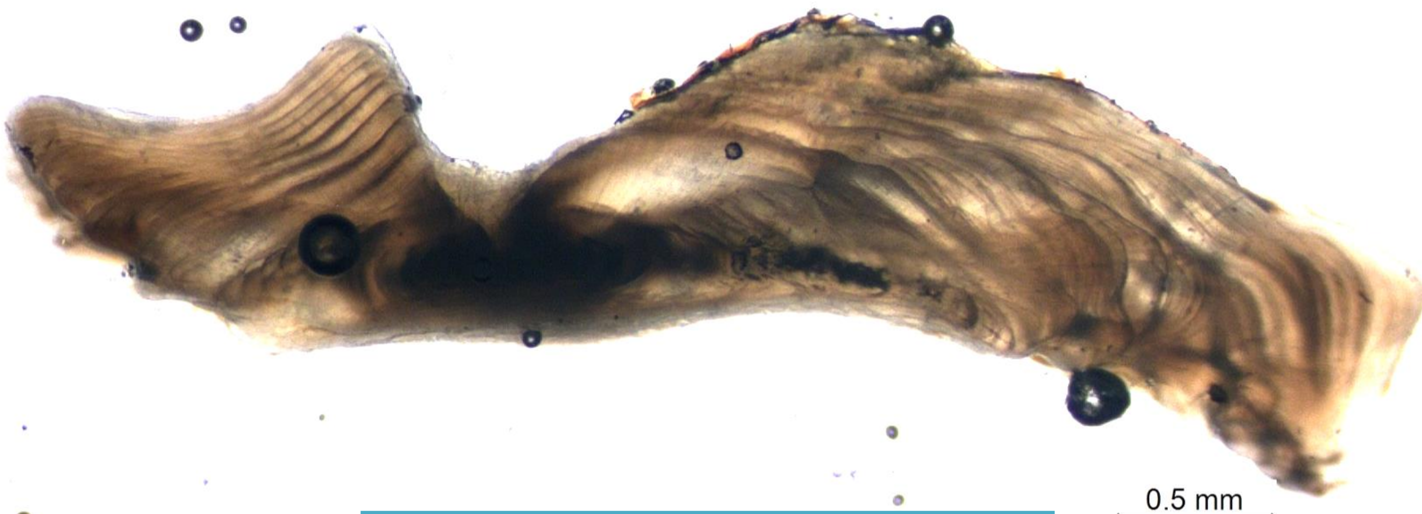
Bluefish 16 **2/20/2014**



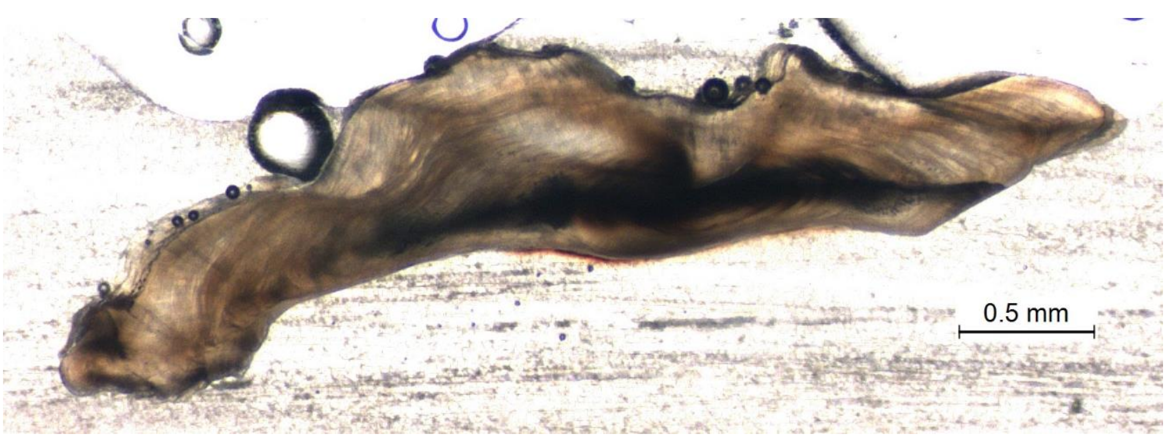
Bluefish 17 **2/20/2014**



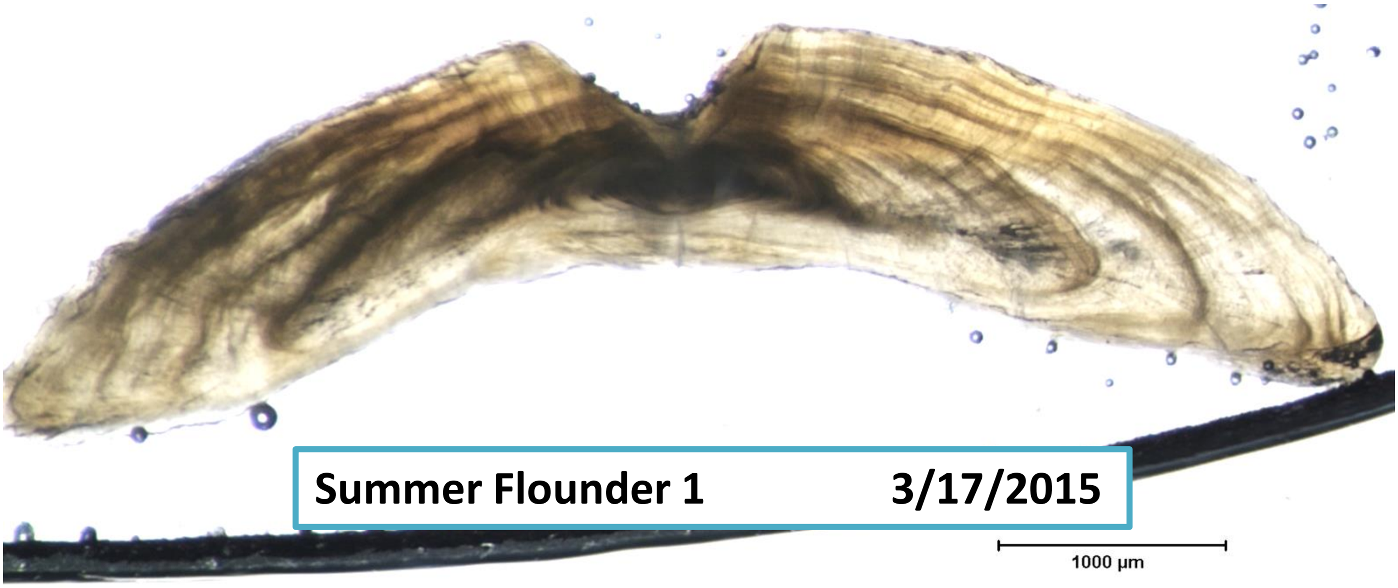
Bluefish 18 8/28/2015



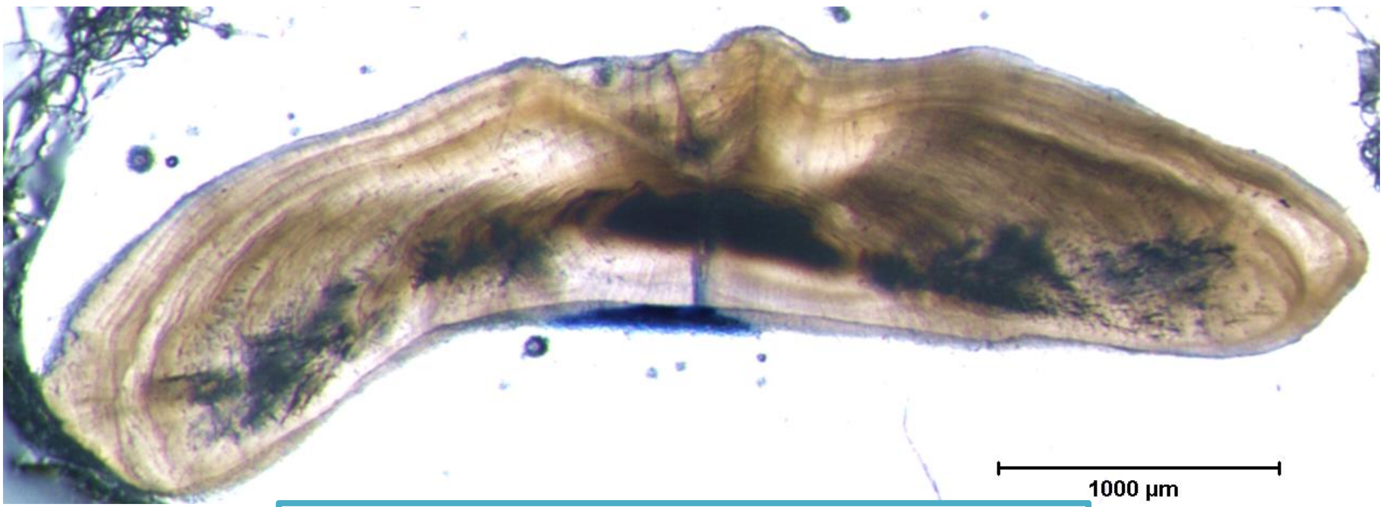
Bluefish 19 5/11/2014



Bluefish 20 5/31/2013



Summer Flounder 1 3/17/2015



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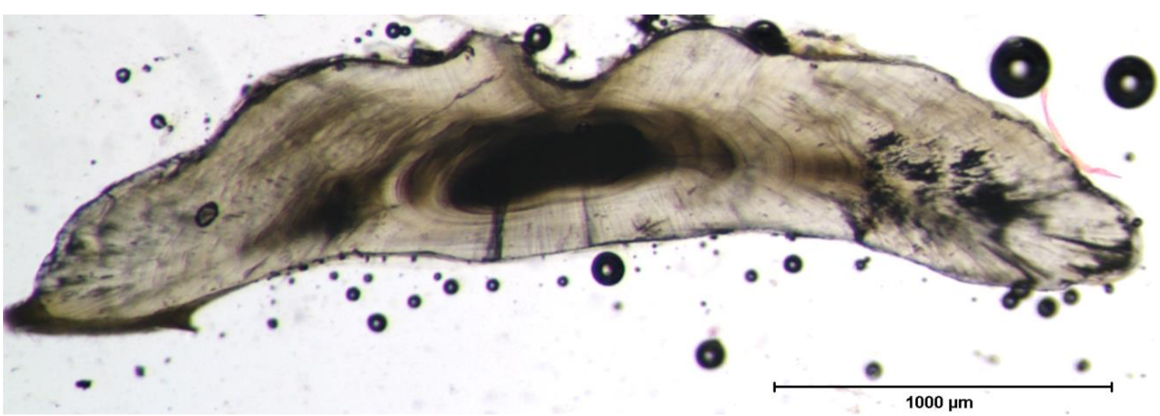
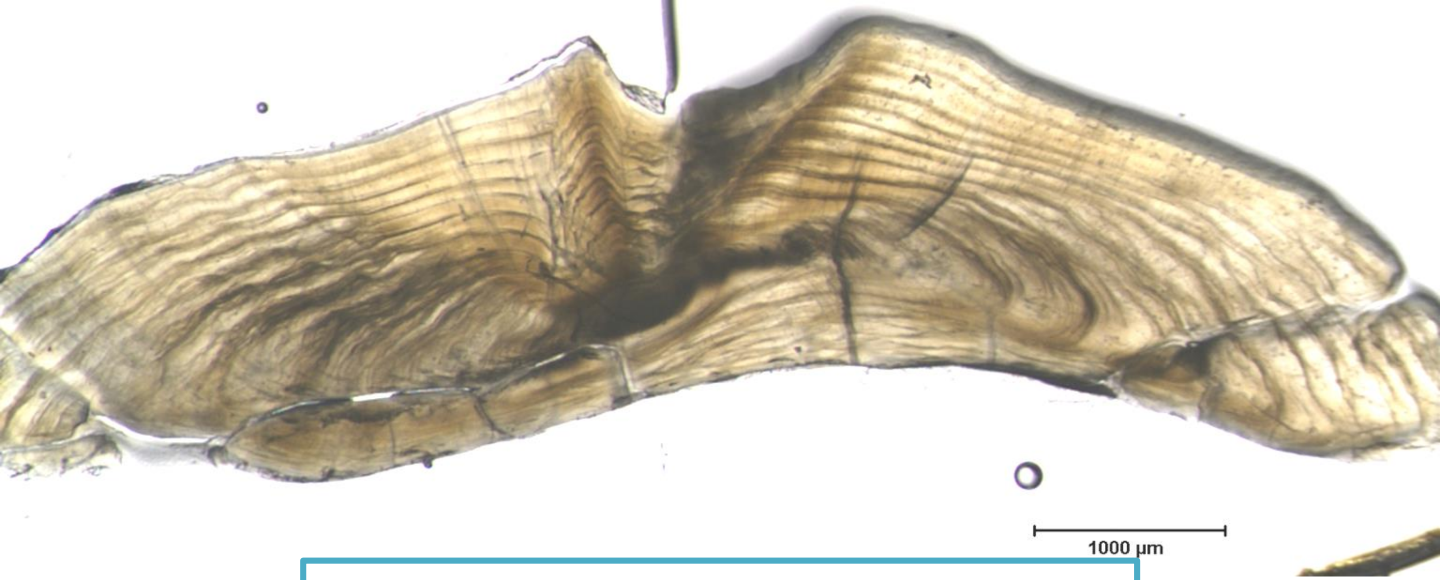
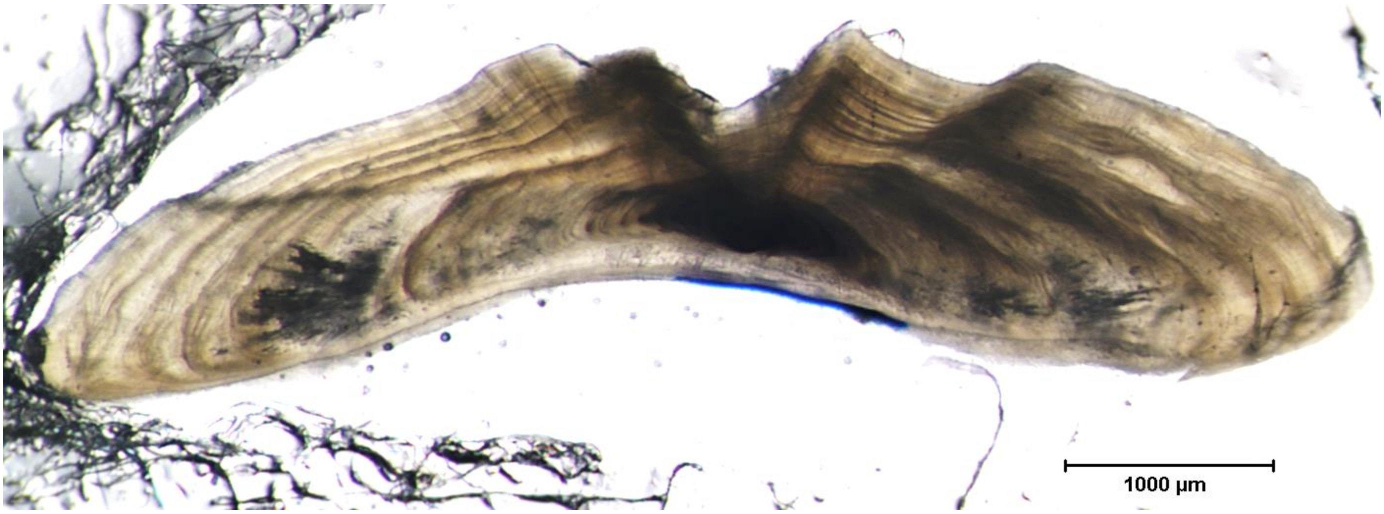


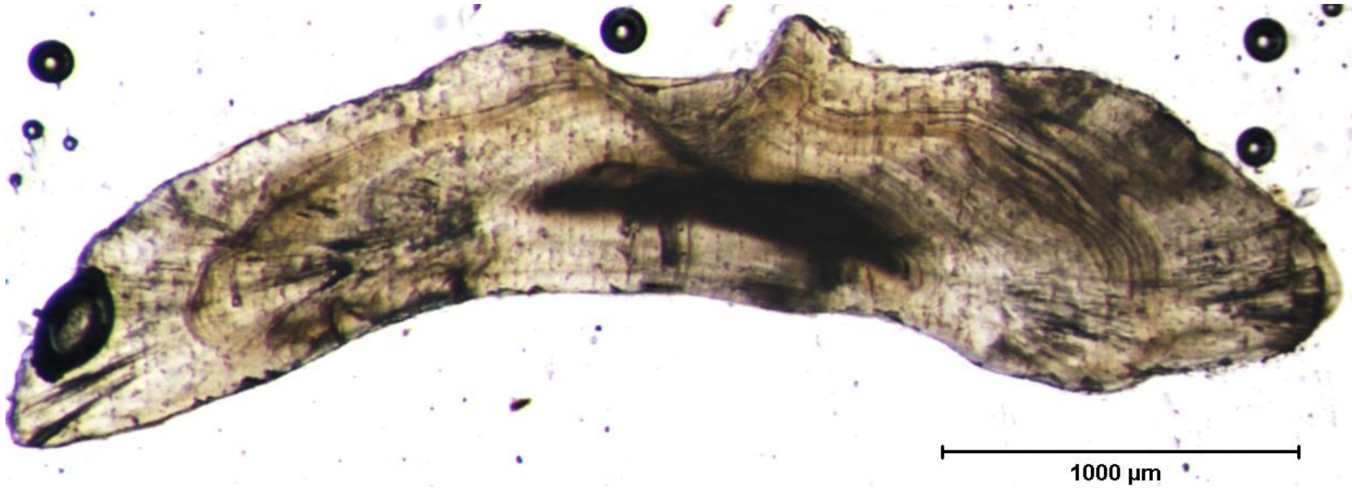
Summer Flounder 3 10/12/2015



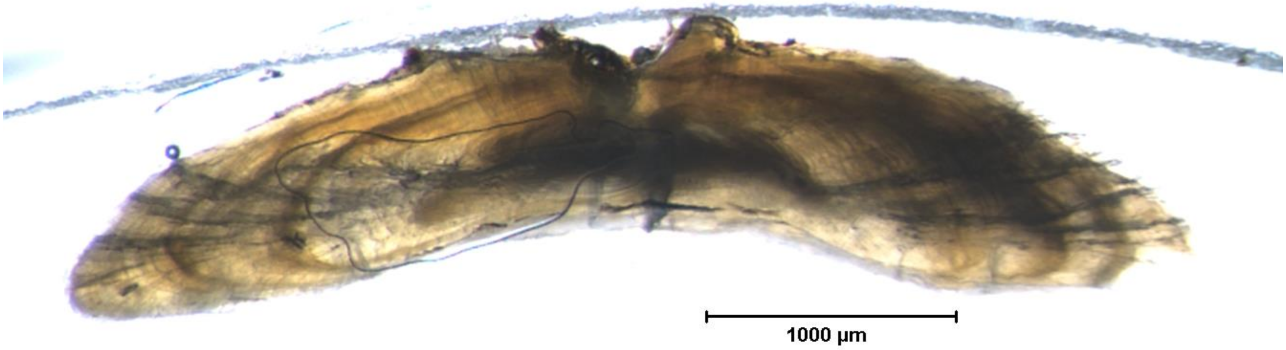
Summer Flounder 4 3/21/2015



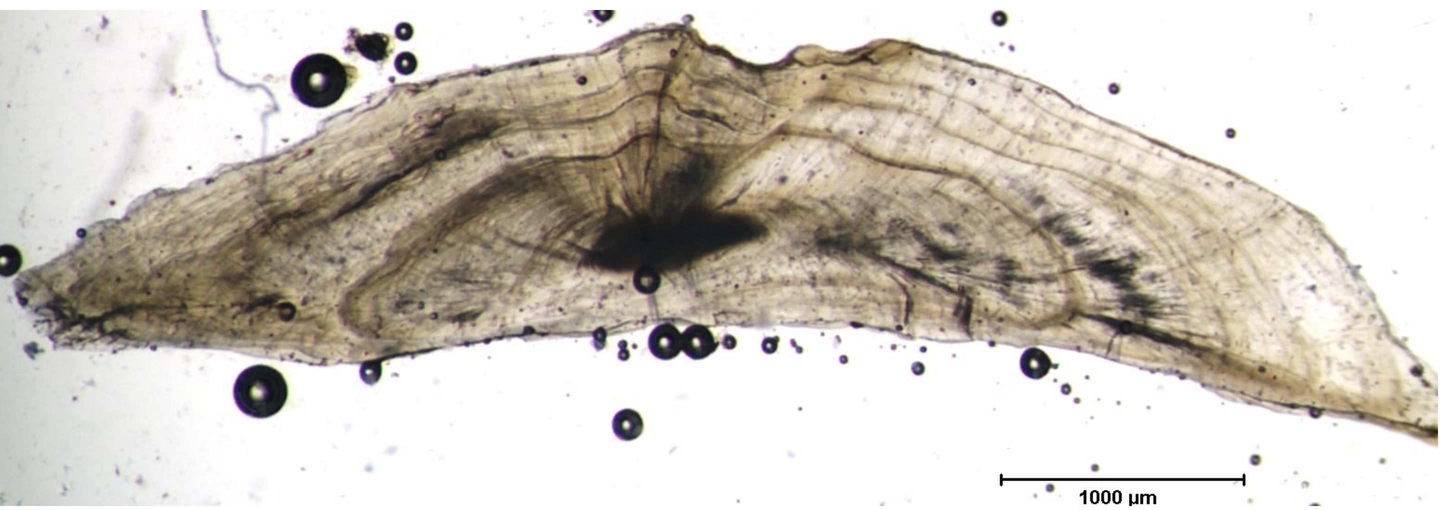
Summer Flounder 5 2/26/2014



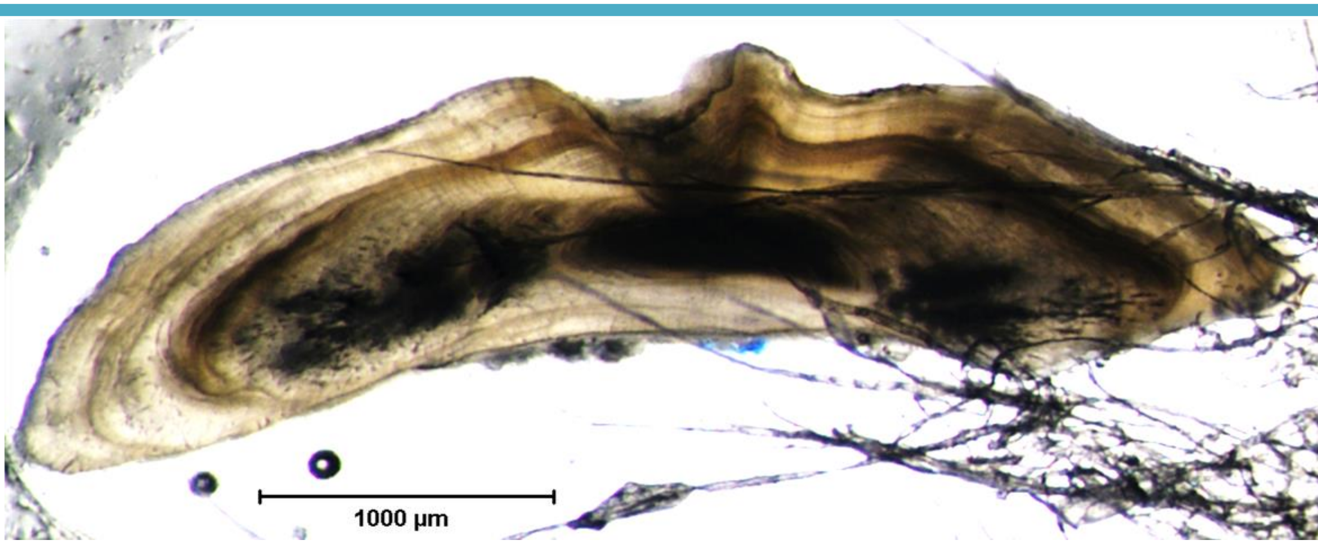
Summer Flounder 6 5/22/2015



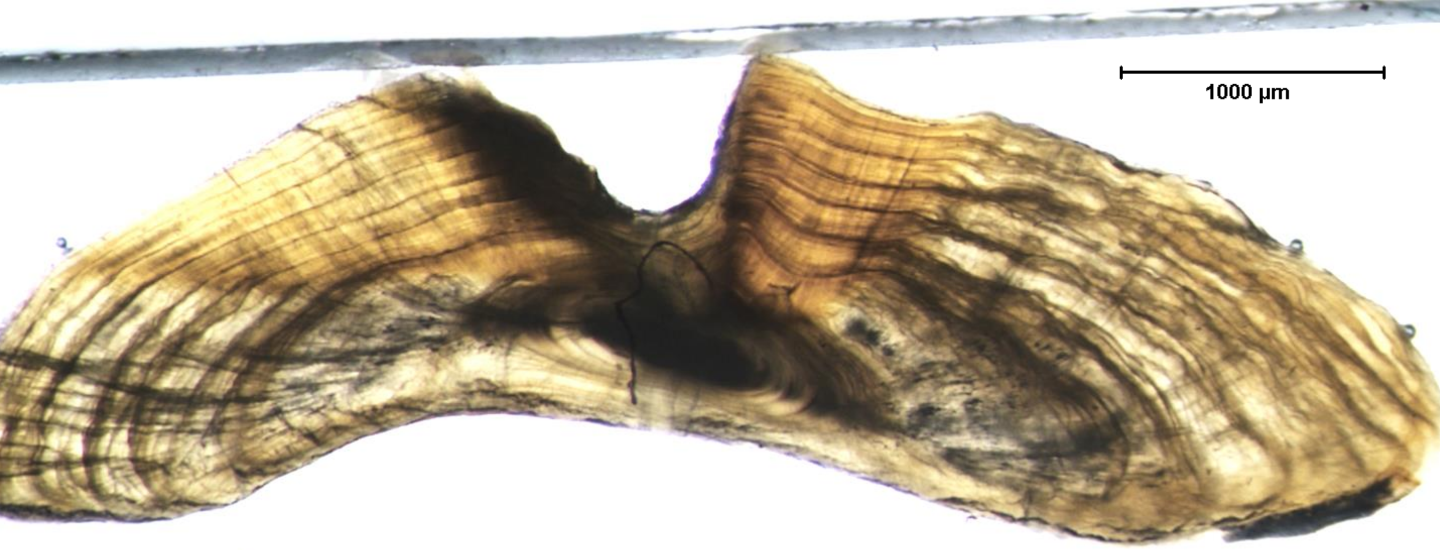
Summer Flounder 7 3/17/2015



Summer Flounder 8 10/10/2015

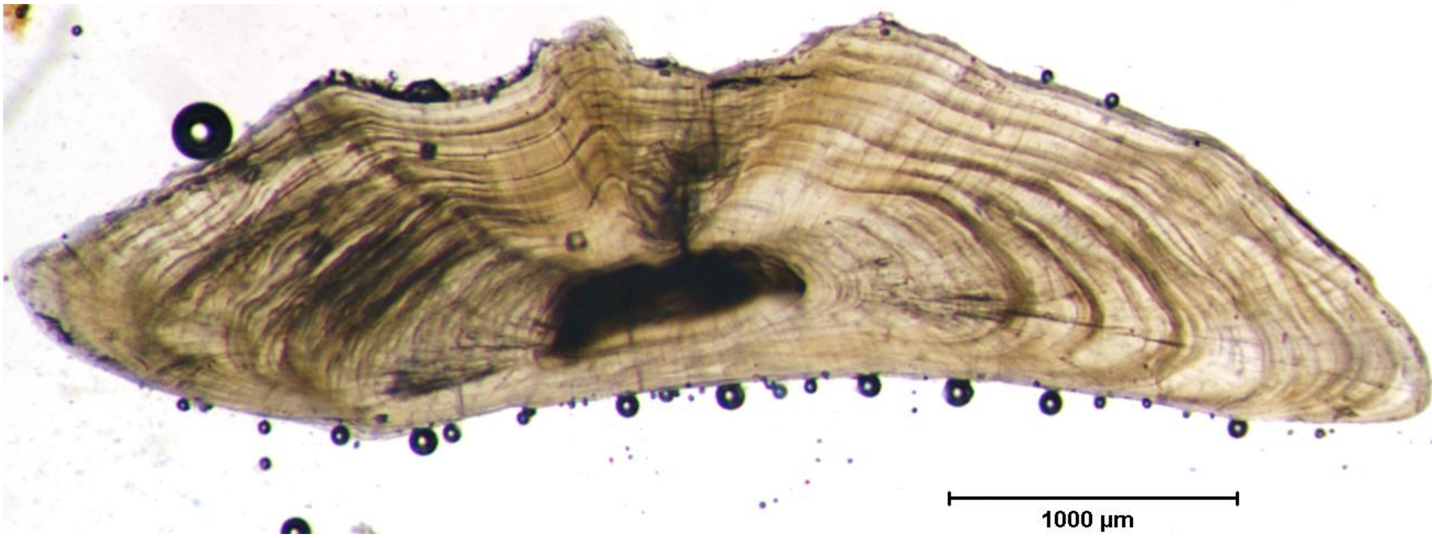


Summer Flounder 9 12/5/2013

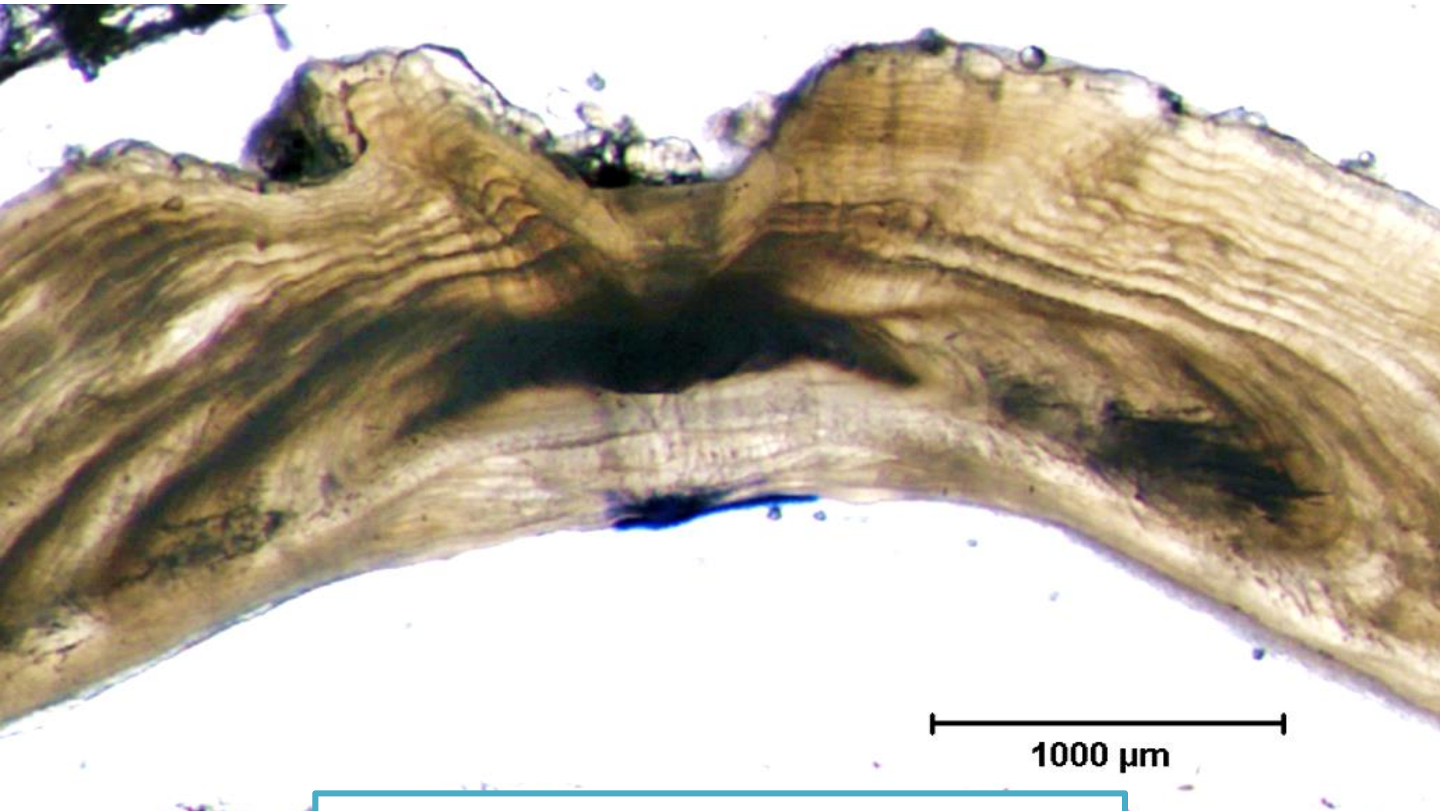


Summer Flounder 10 7/21/2015

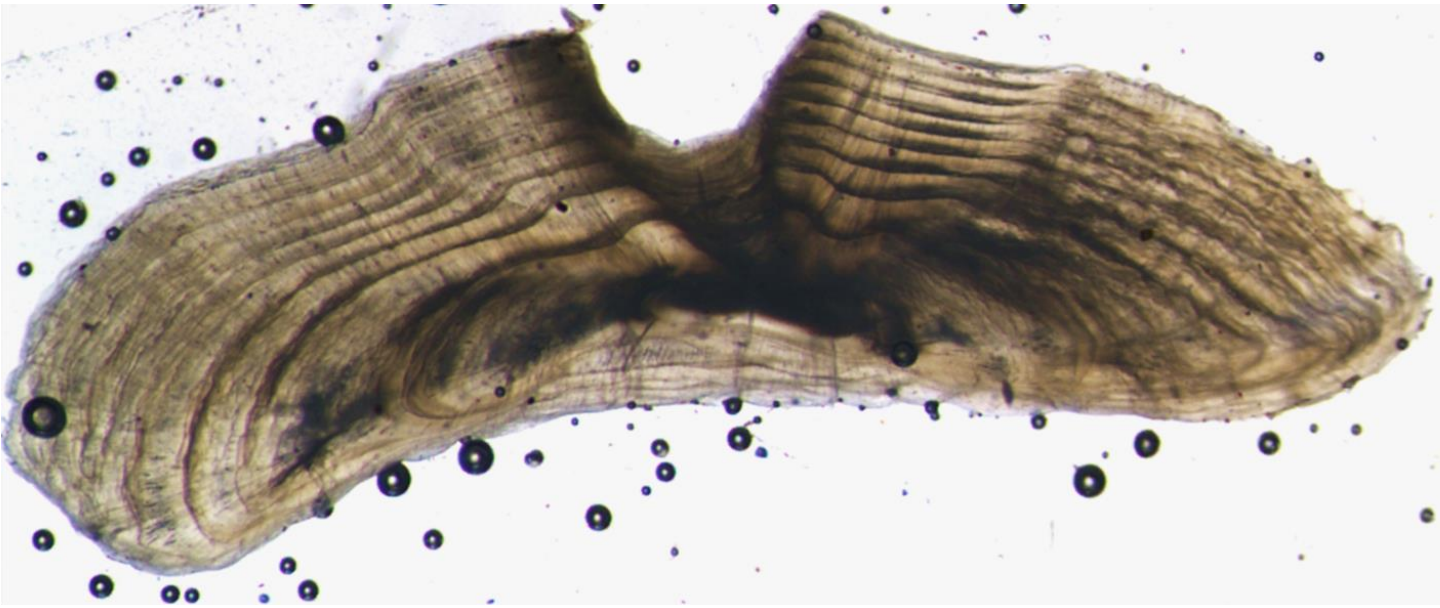
Summer Flounder 11 11/20/2015



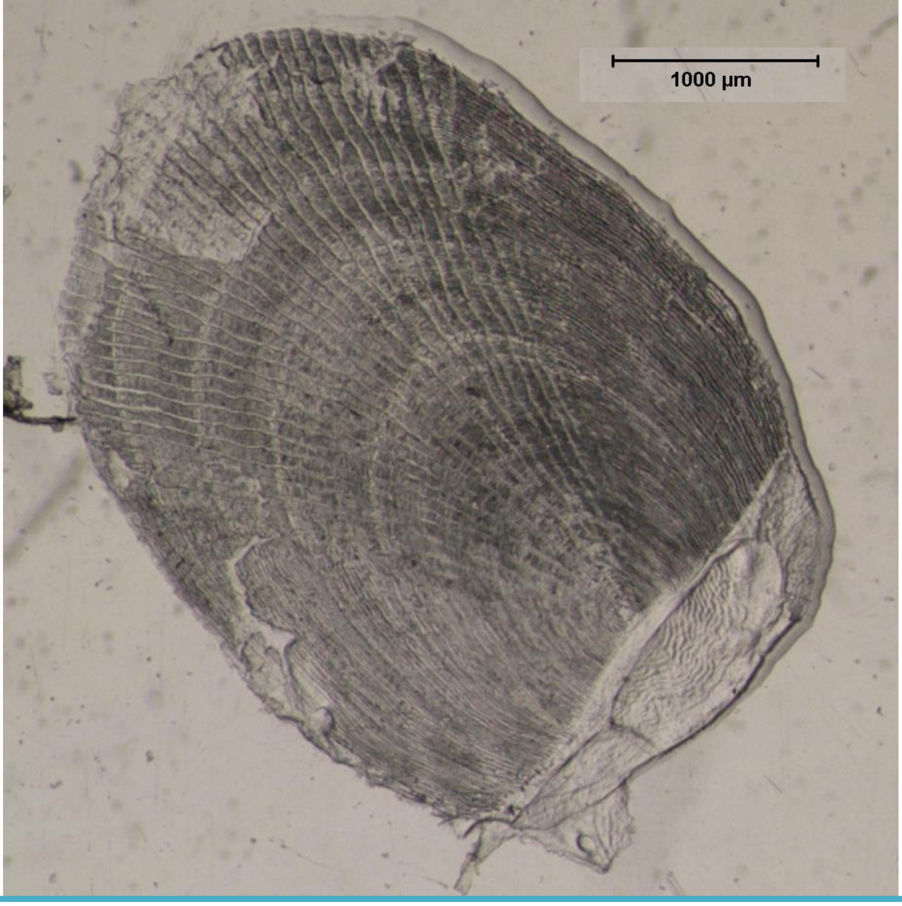
Summer Flounder 12 10/24/2015



Summer Flounder 13 2/3/2014

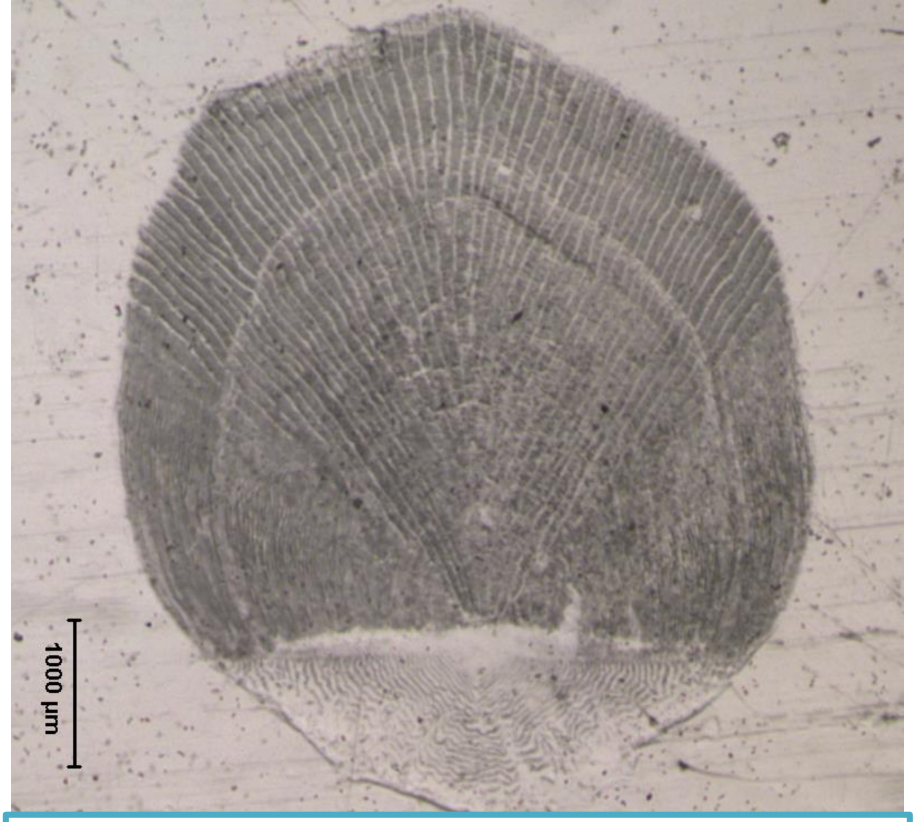


Summer Flounder 14 5/16/2015

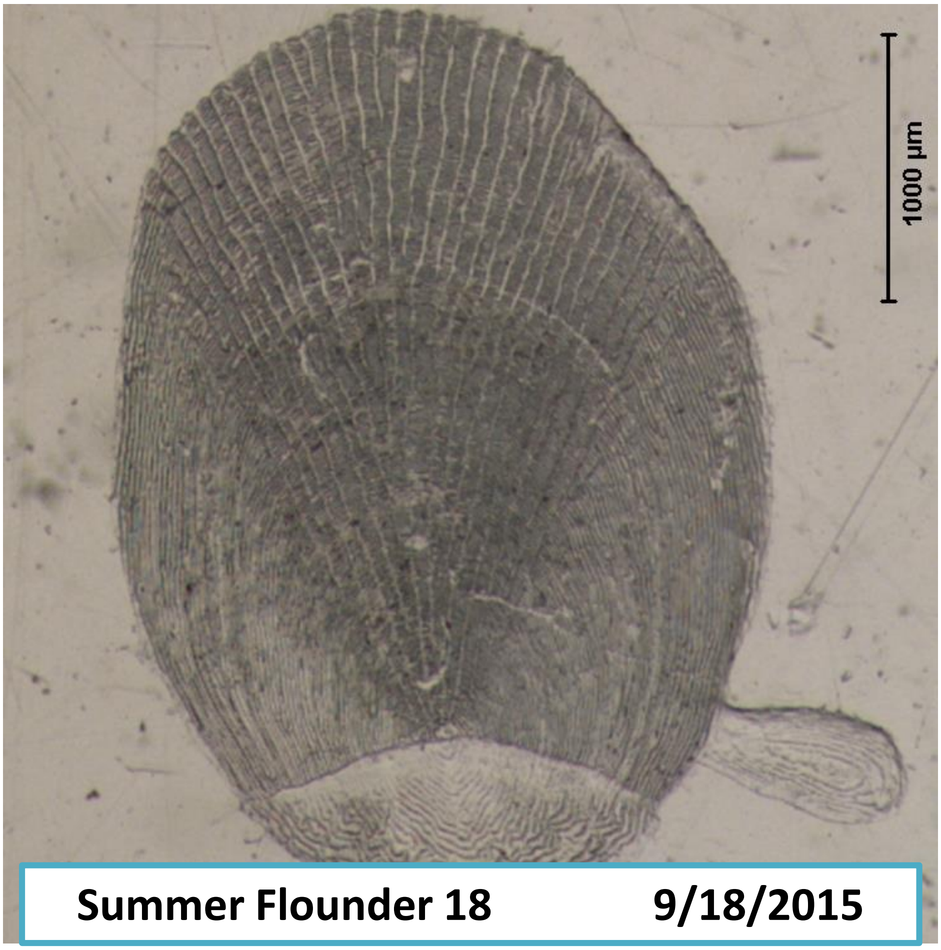


Summer Flounder 15 7/3/2015

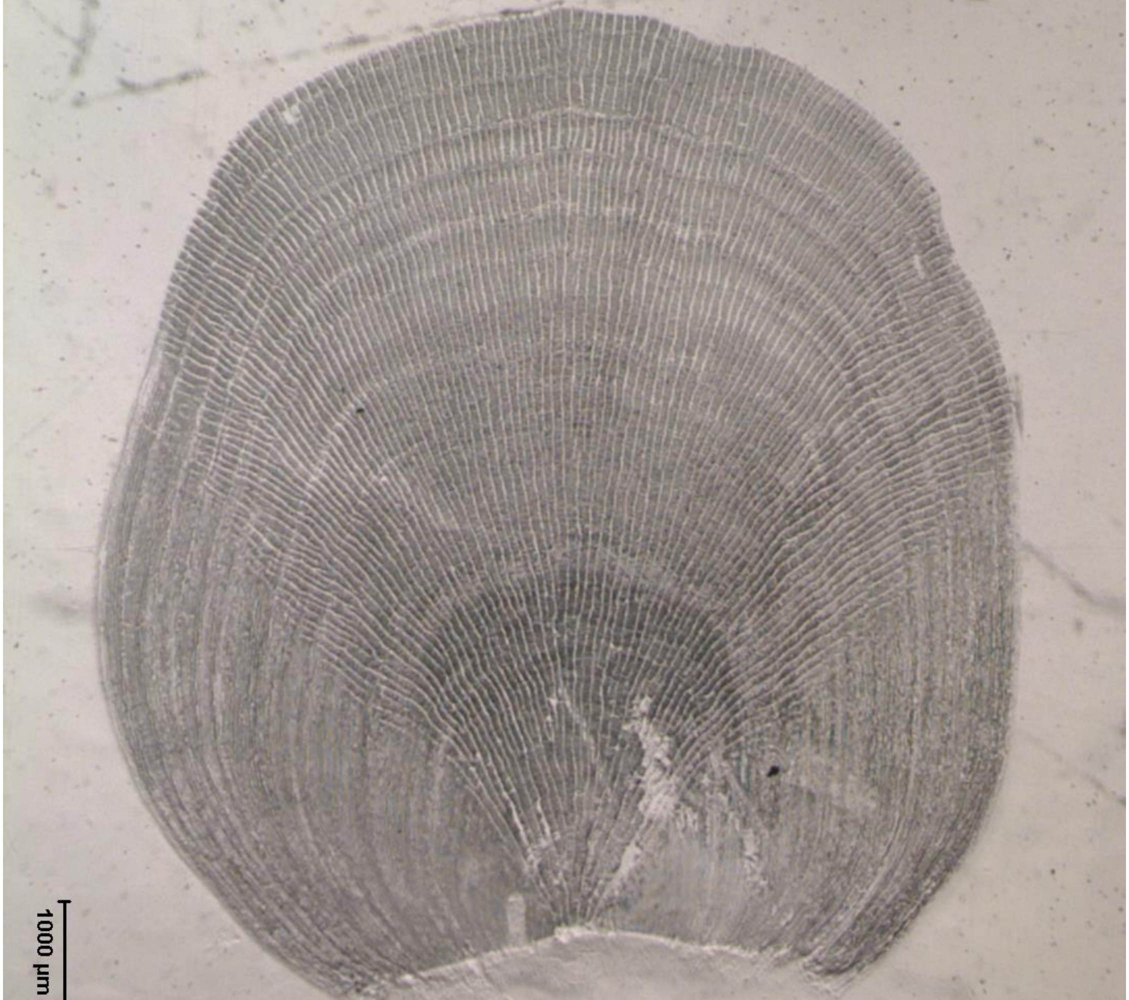
Summer Flounder 16 3/17/2015



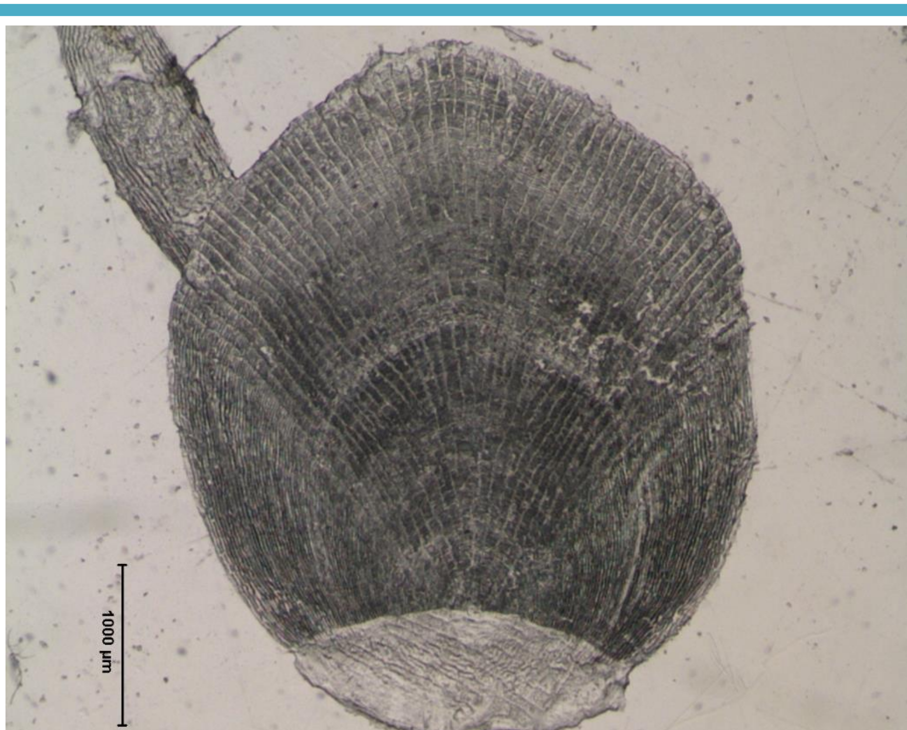
Summer Flounder 17 11/20/2015



Summer Flounder 18 9/18/2015



Summer Flounder 19 3/17/2015



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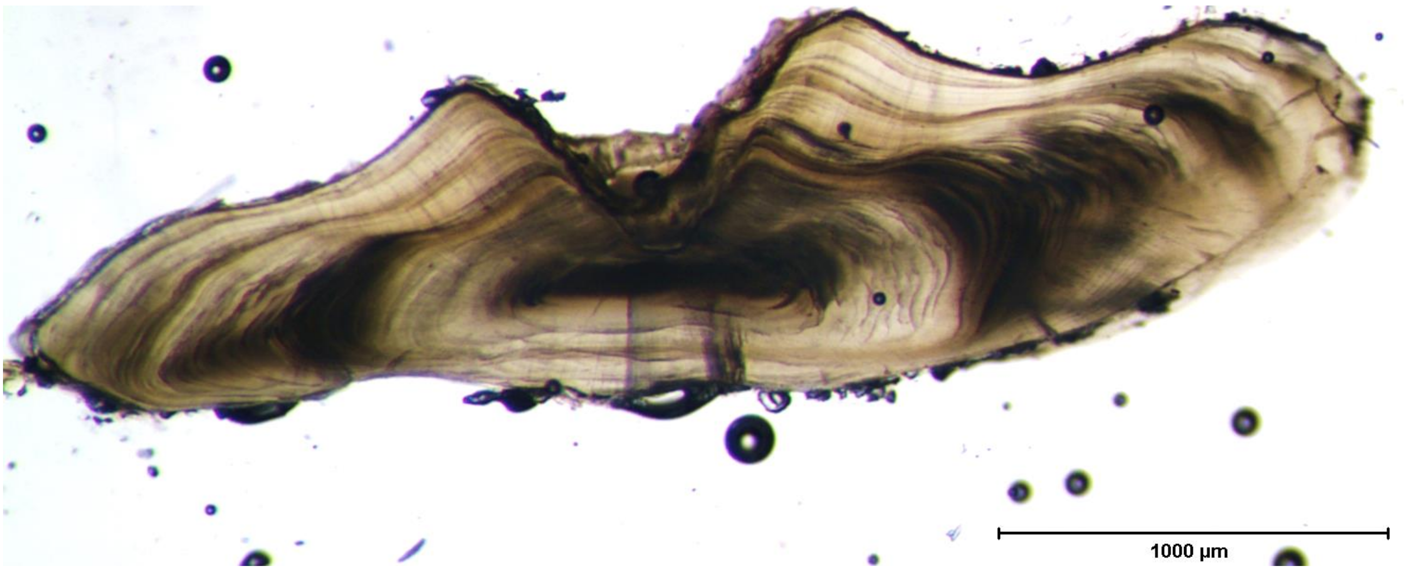
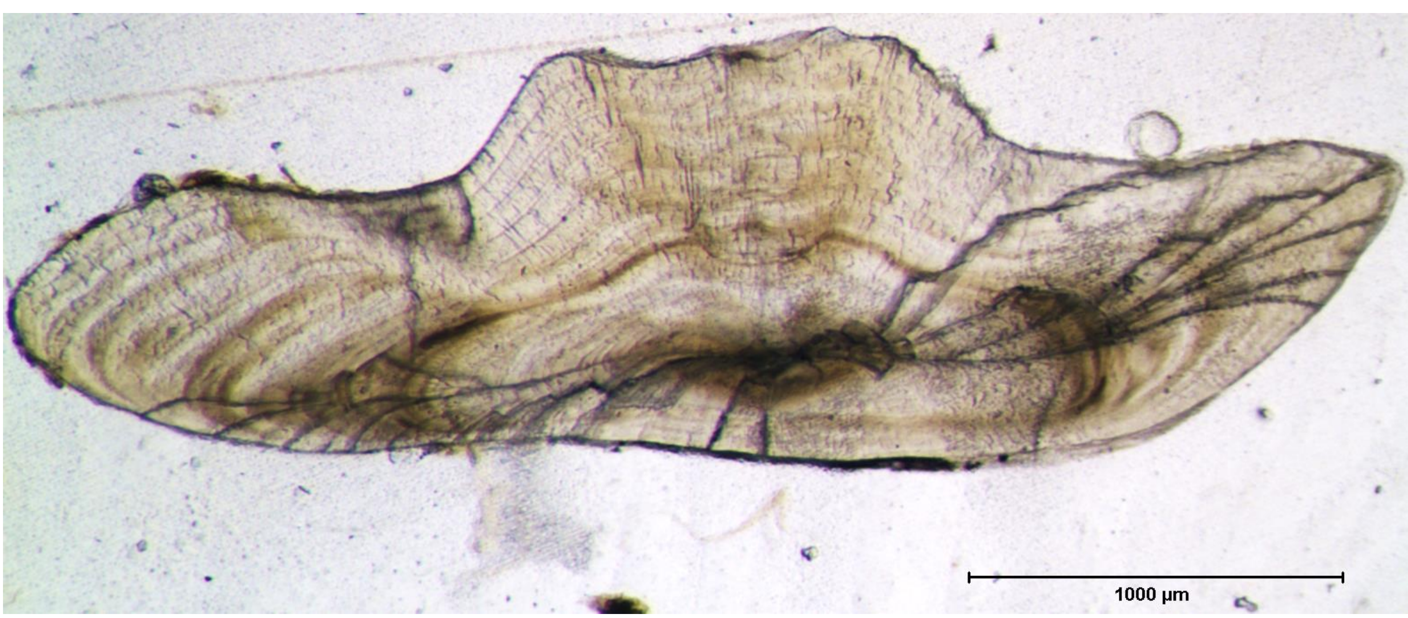


Winter Flounder 1 **10/9/2015**



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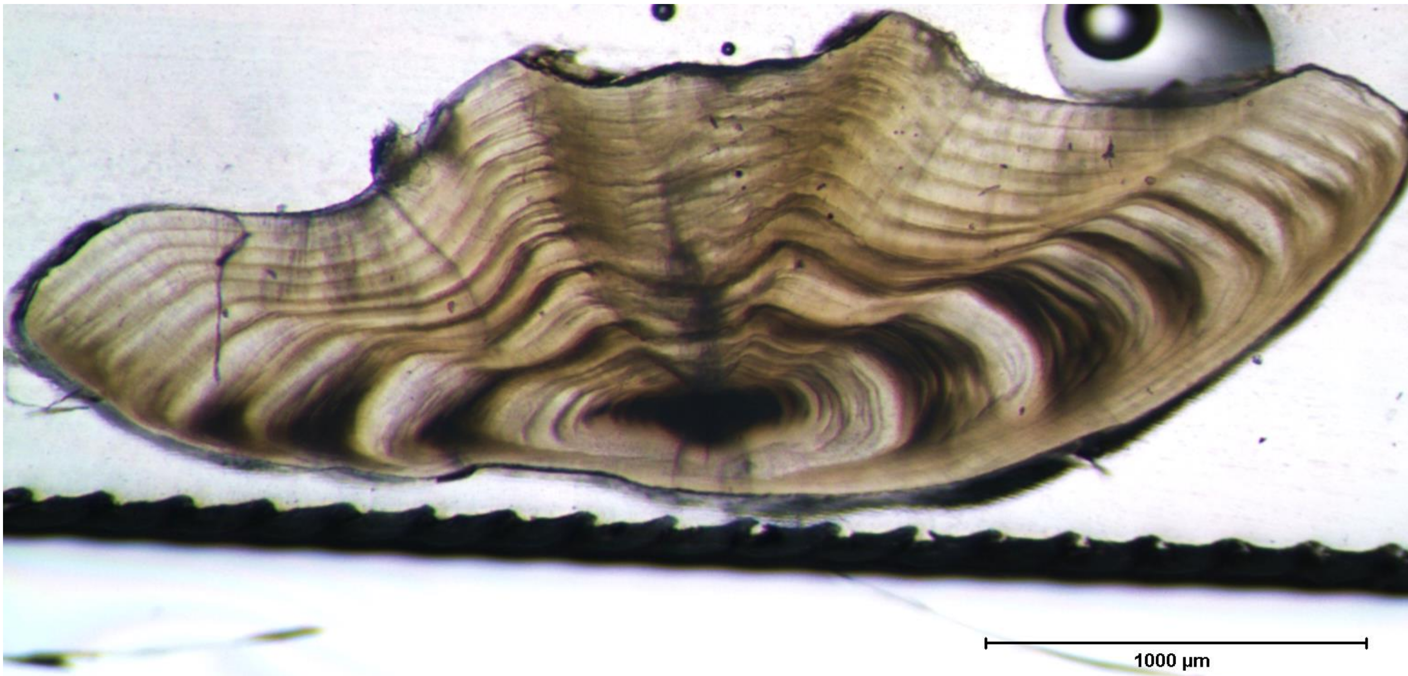
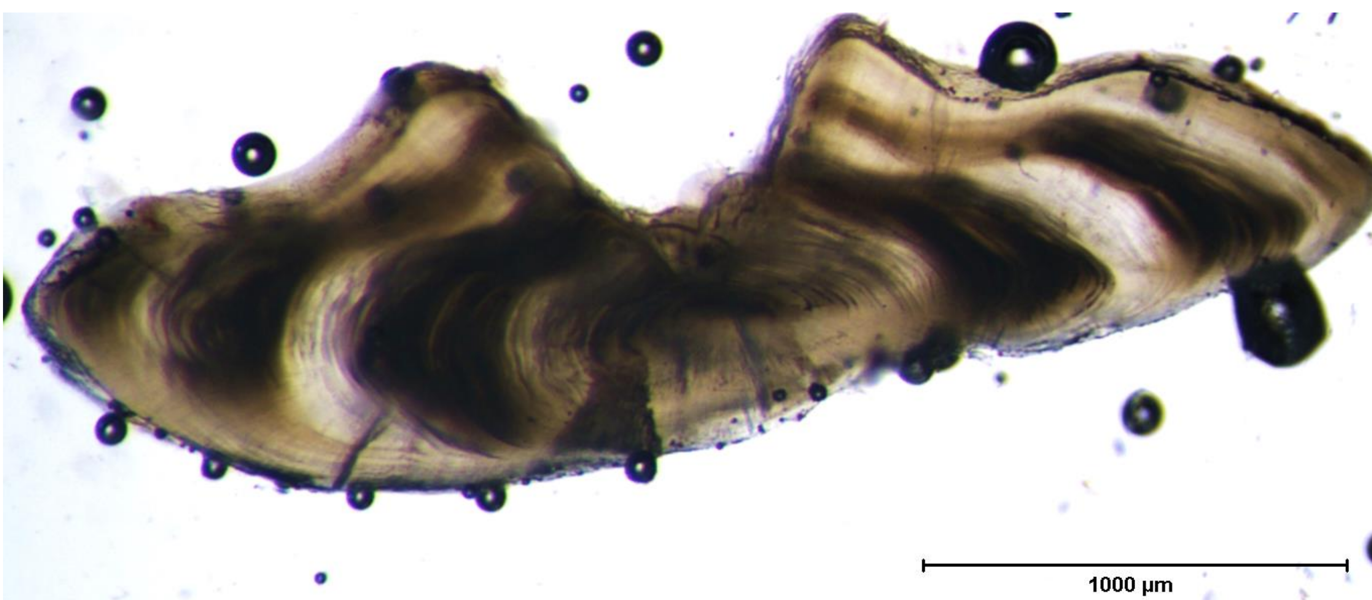
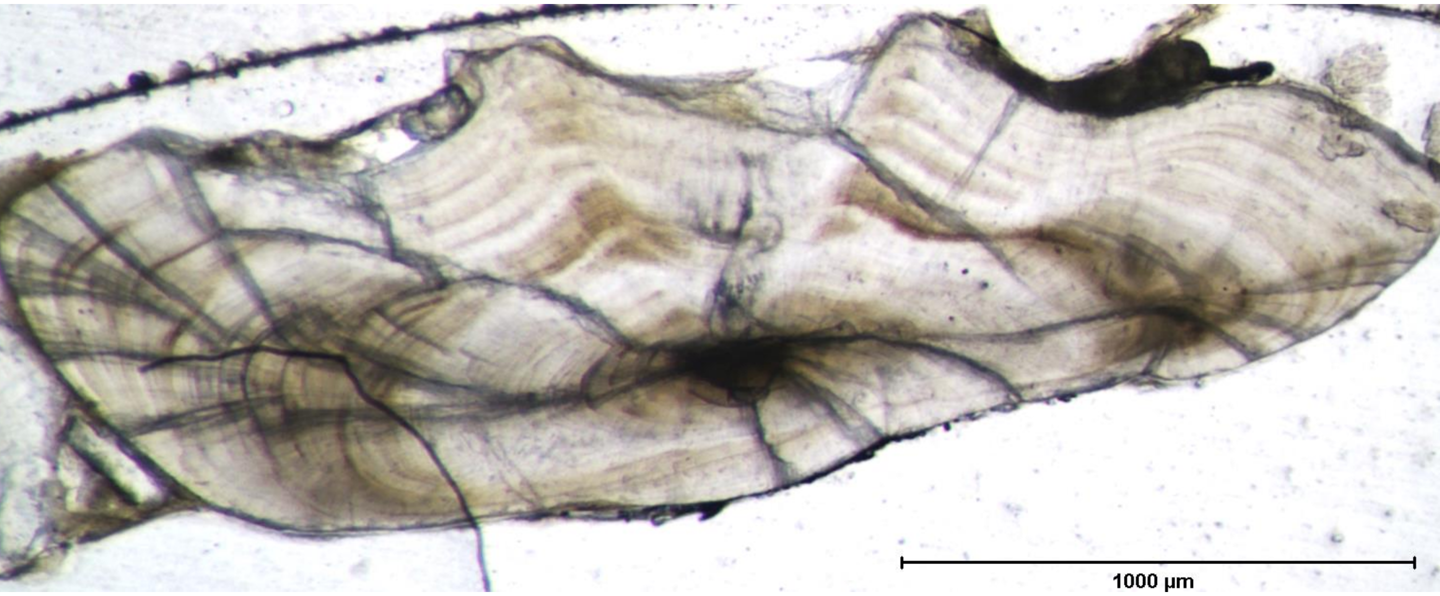


Winter Flounder 3 5/14/2013



Winter Flounder 4 10/8/2015



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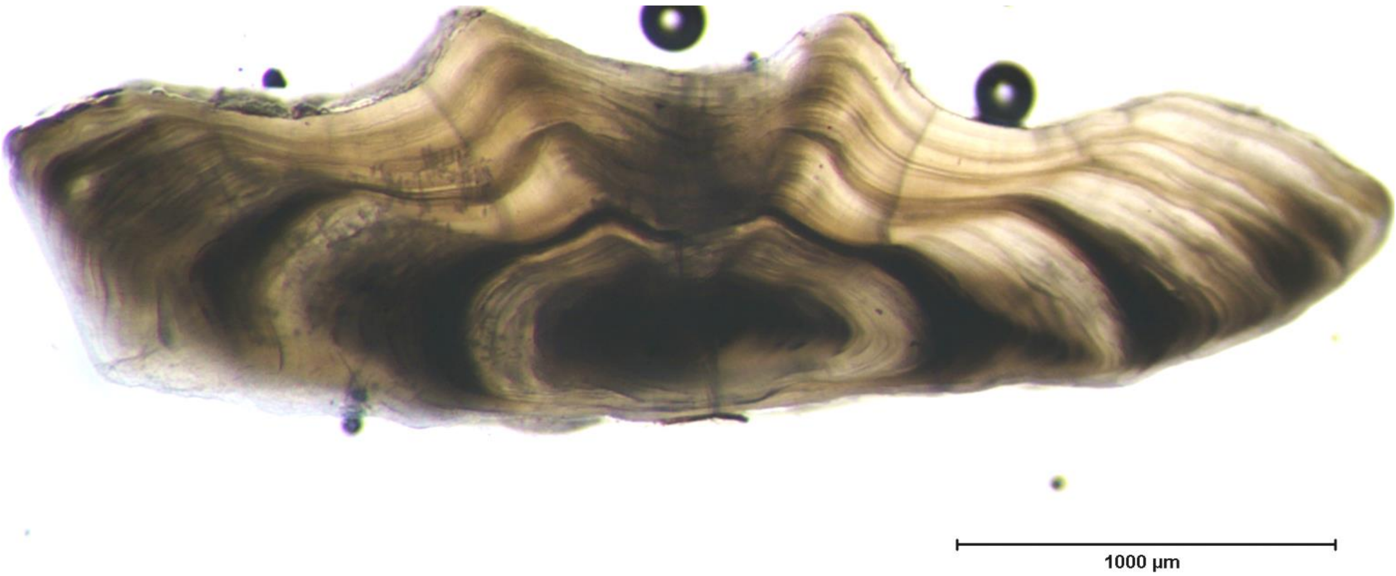

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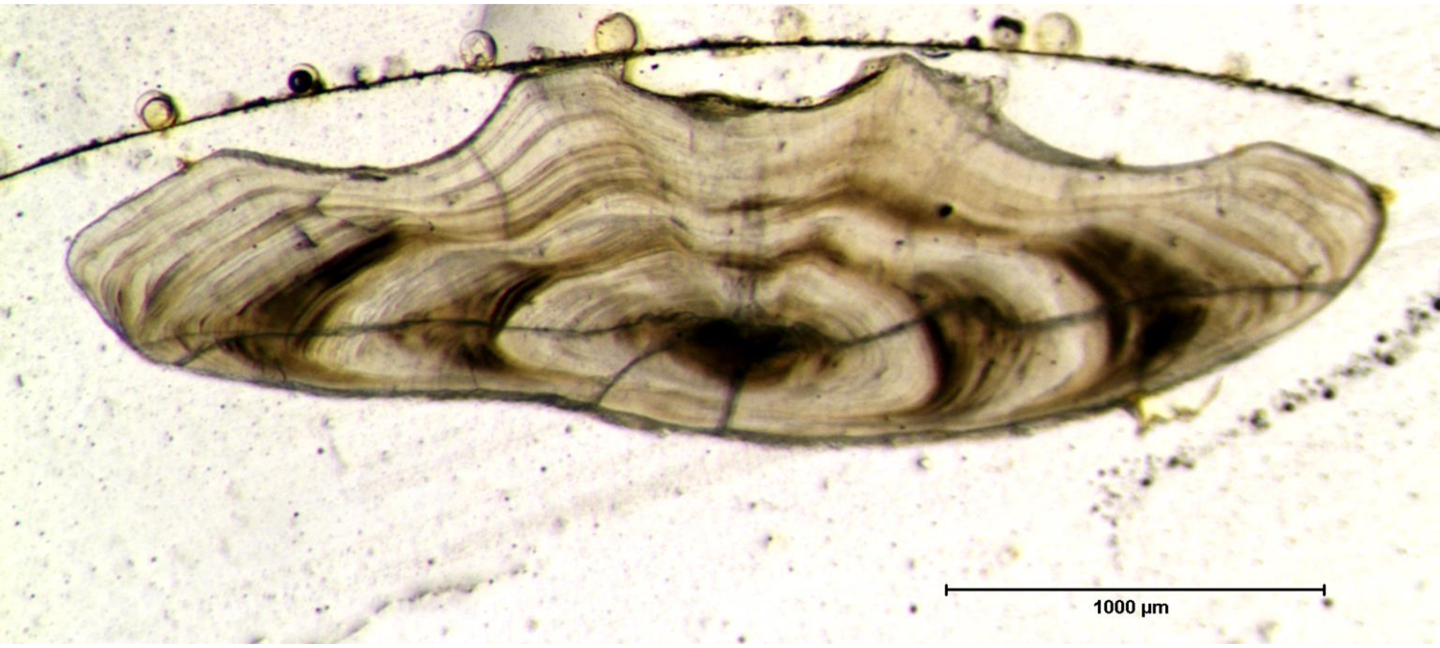
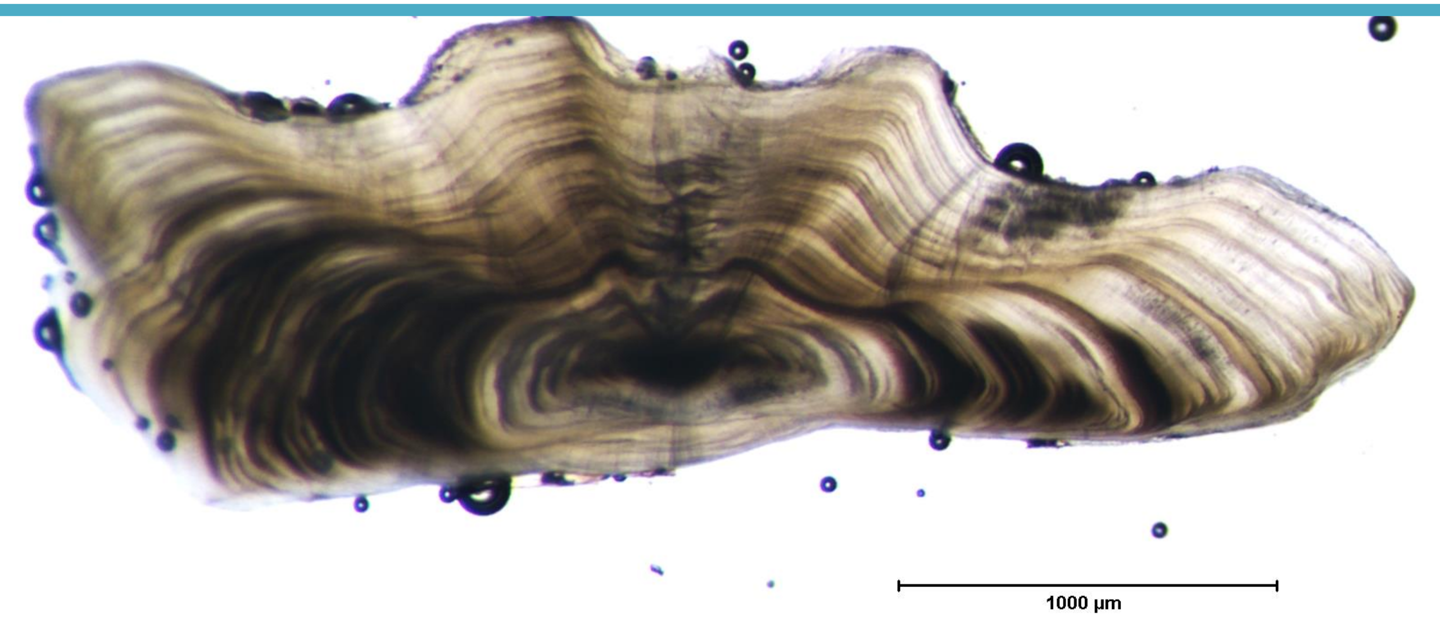


Winter Flounder 7 **3/24/2003**



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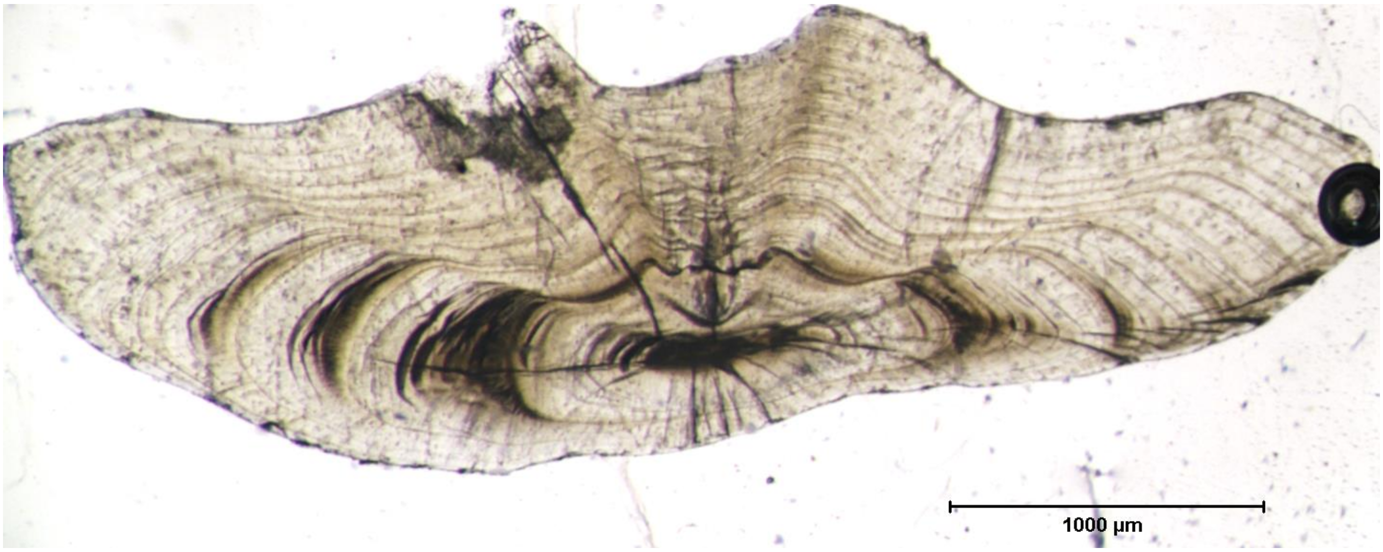
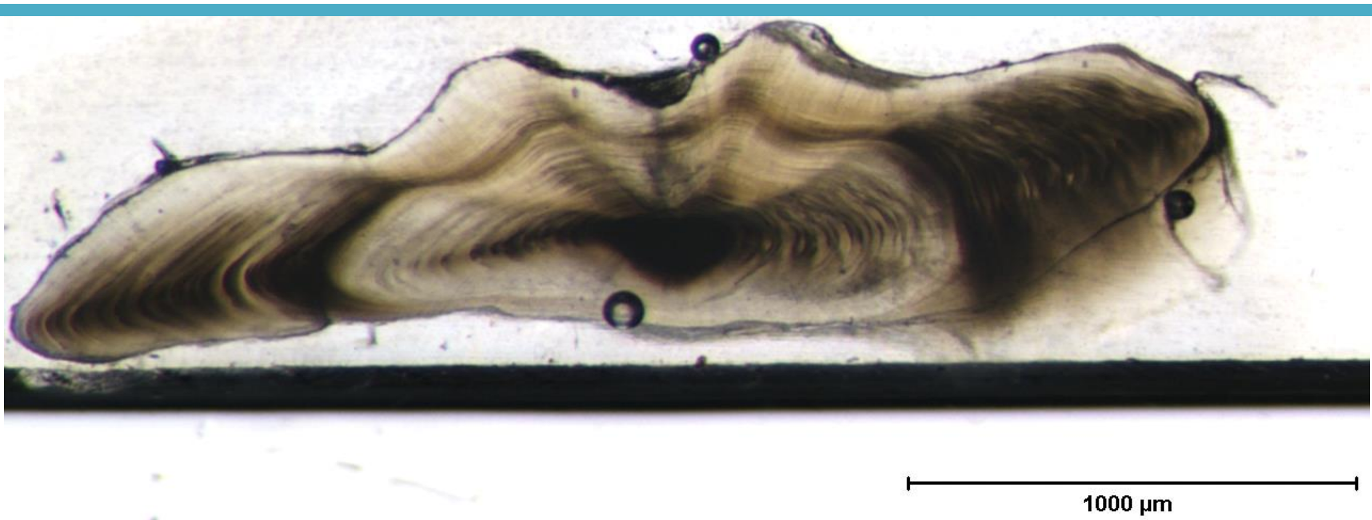
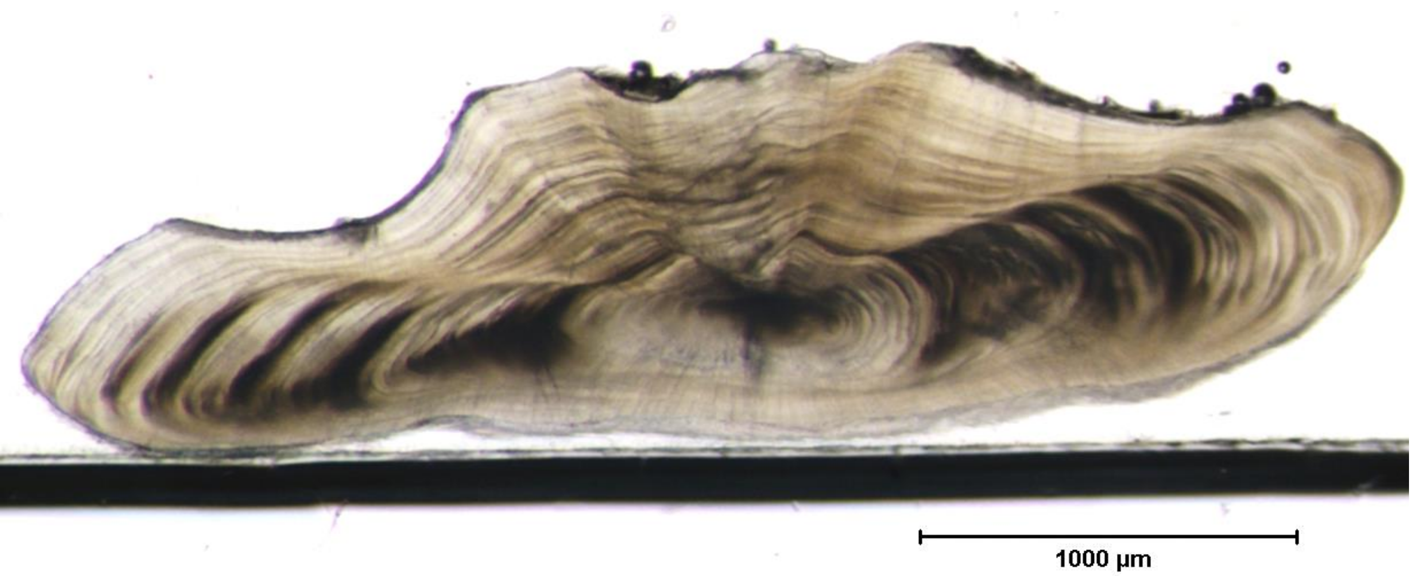


Winter Flounder 9 5/21/2015



Winter Flounder 10 5/9/2013

Winter Flounder 11 **4/3/2003**



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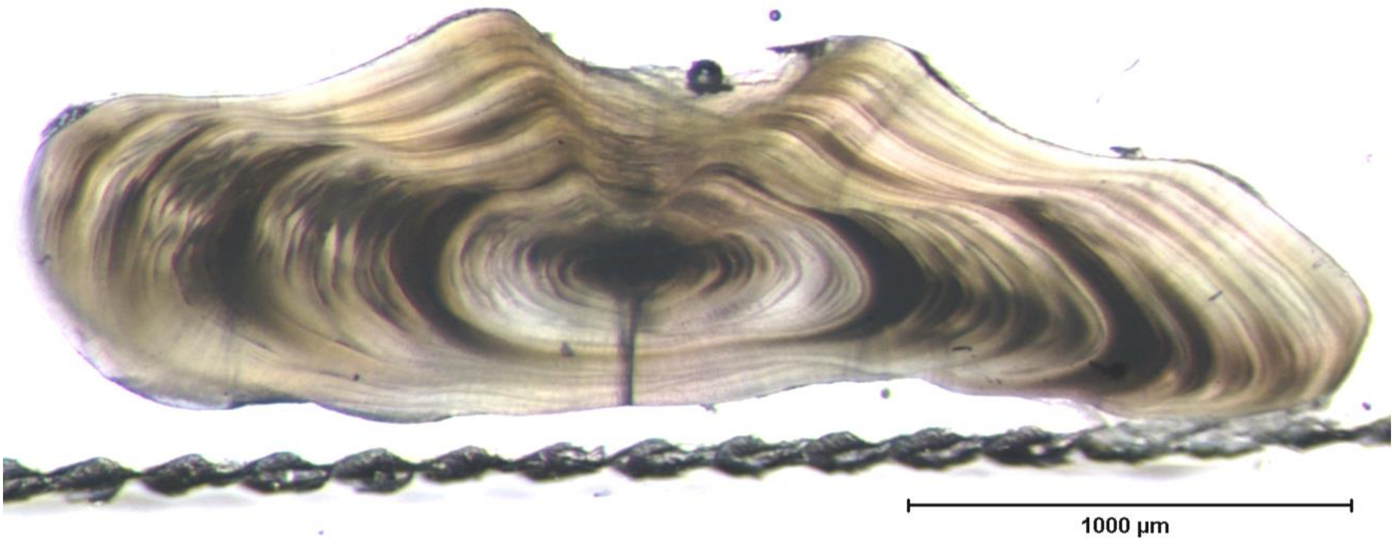
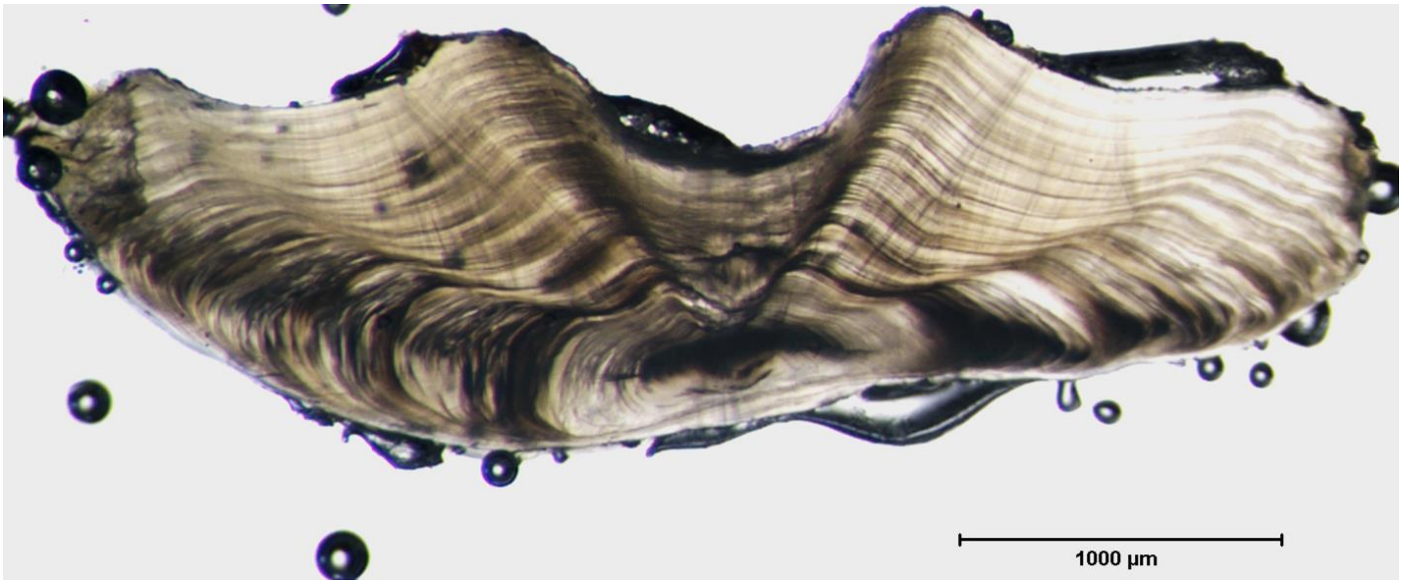


Winter Flounder 13 5/7/2013



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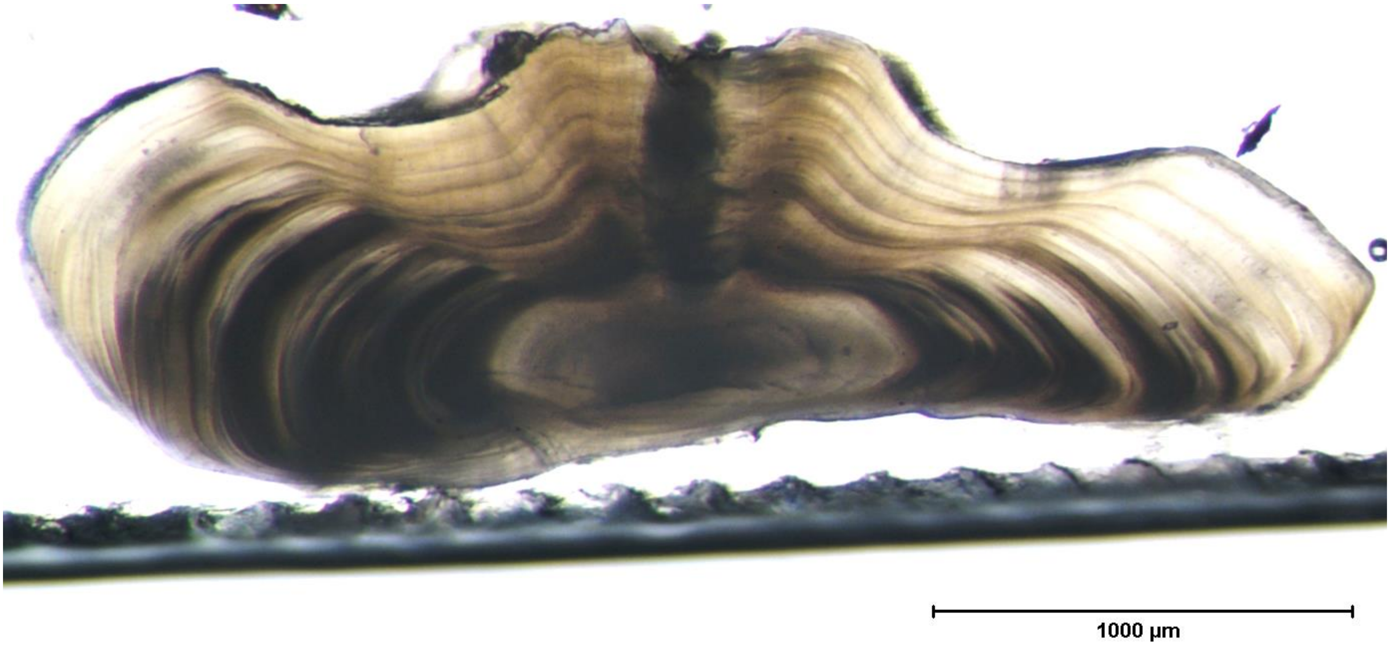
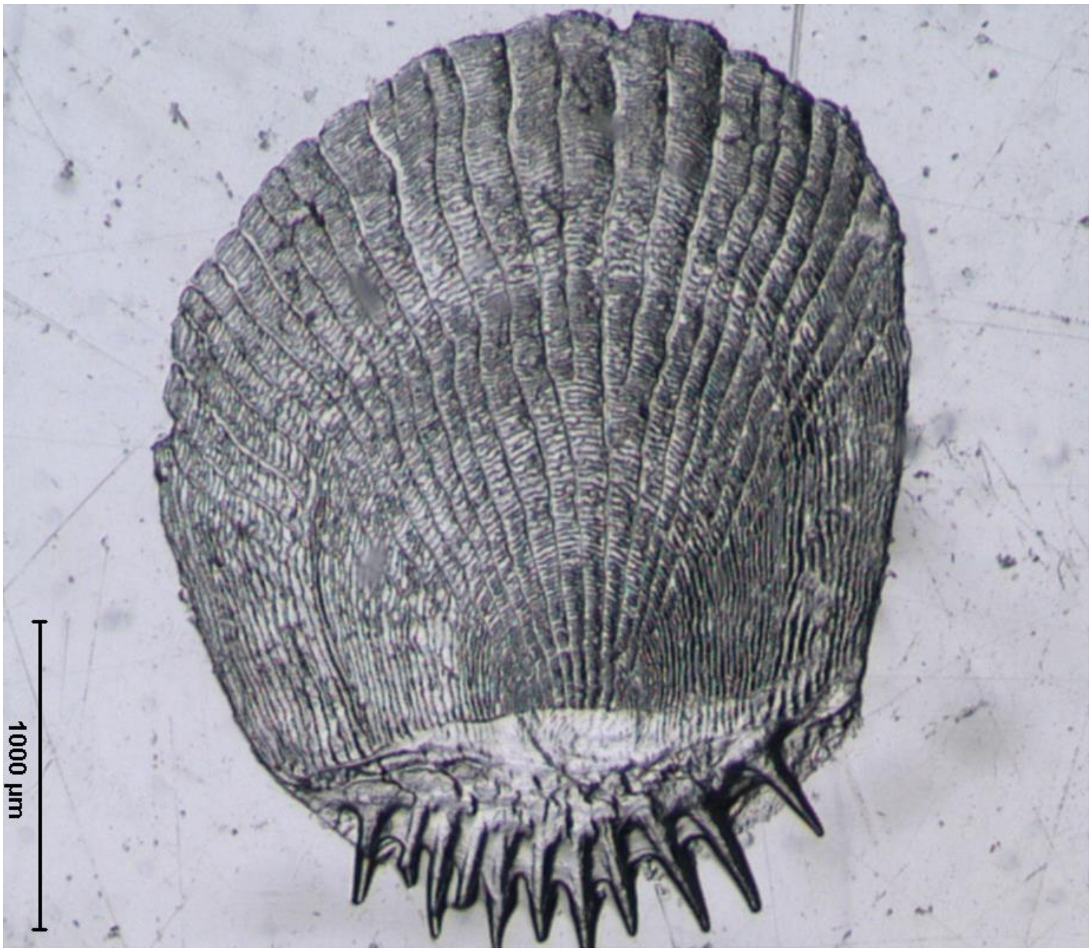
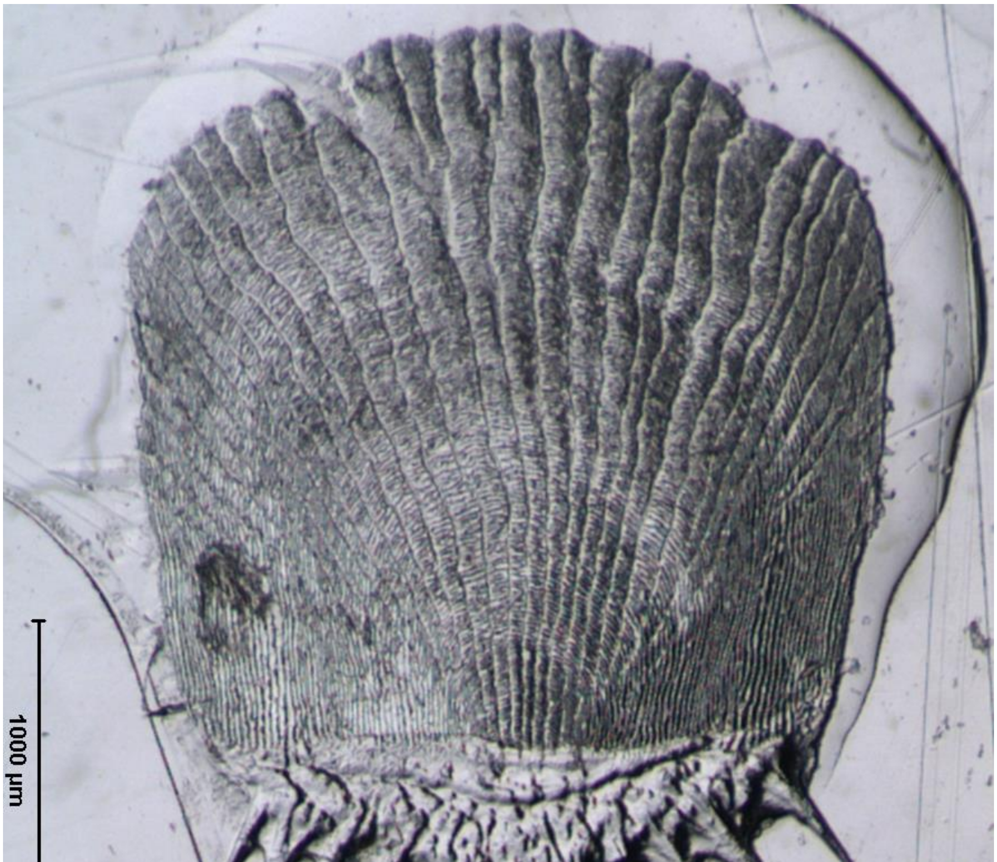


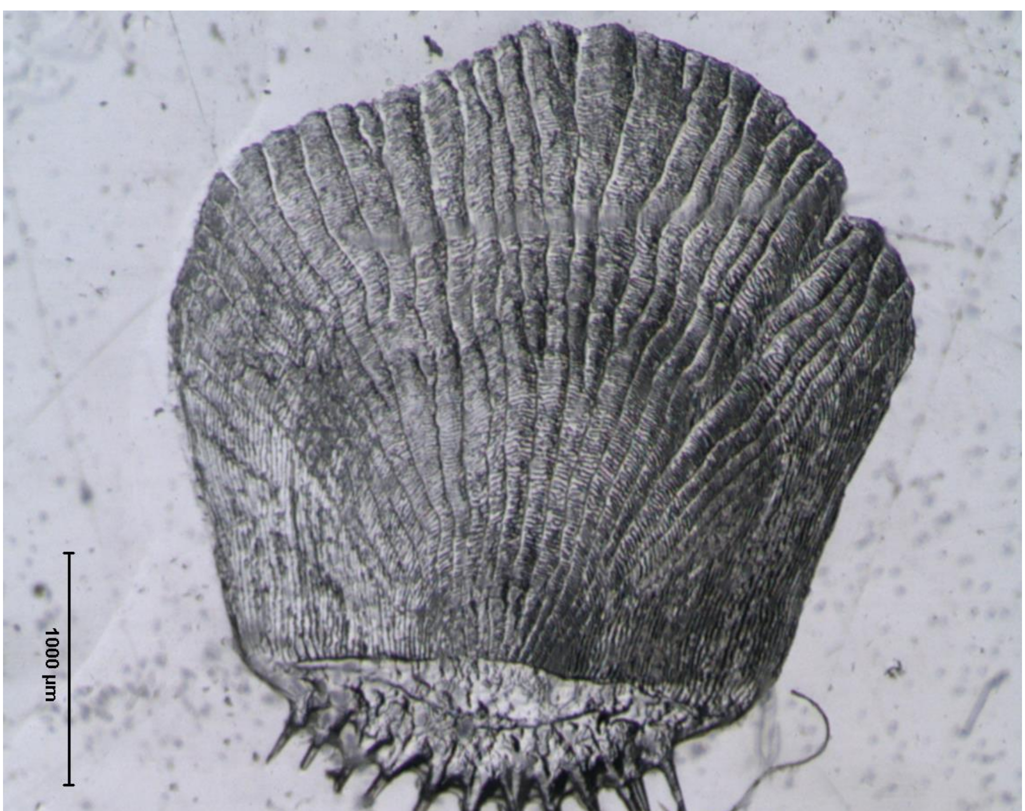
Winter Flounder 15 5/6/2013



Winter Flounder 16 8/20/2015

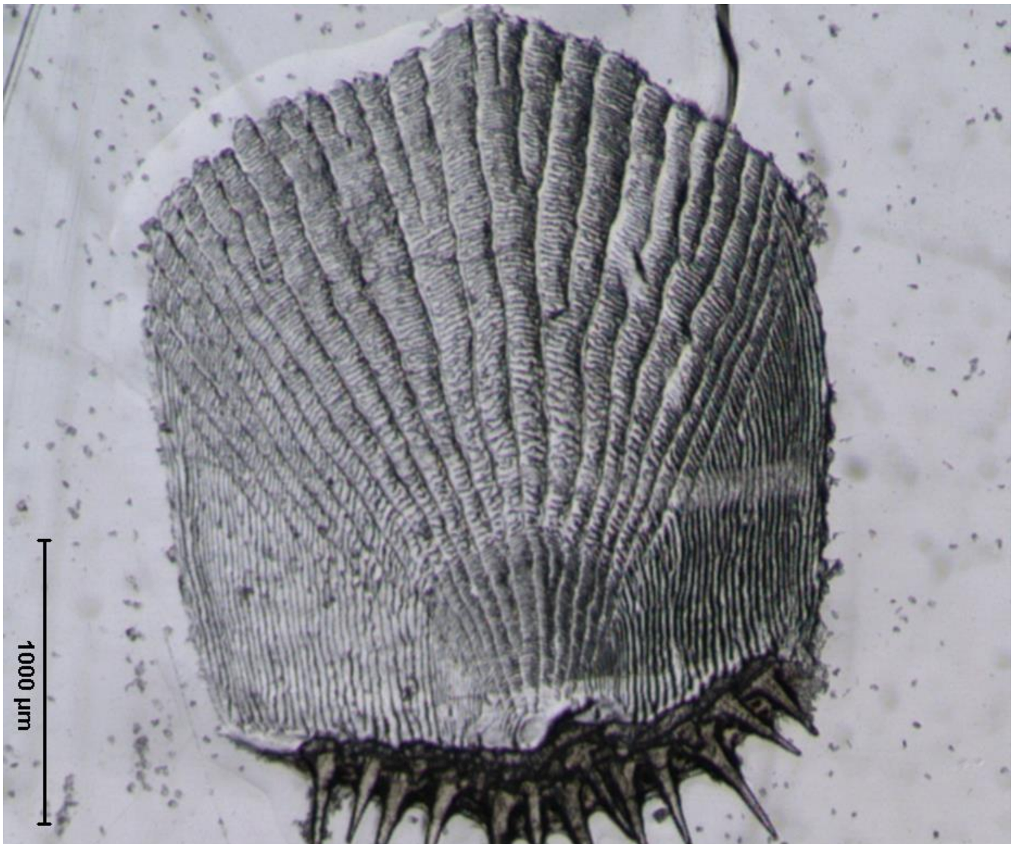


Winter Flounder 17 **5/19/2015**



Winter Flounder 18 **5/21/2015**

Winter Flounder 19 5/21/2015



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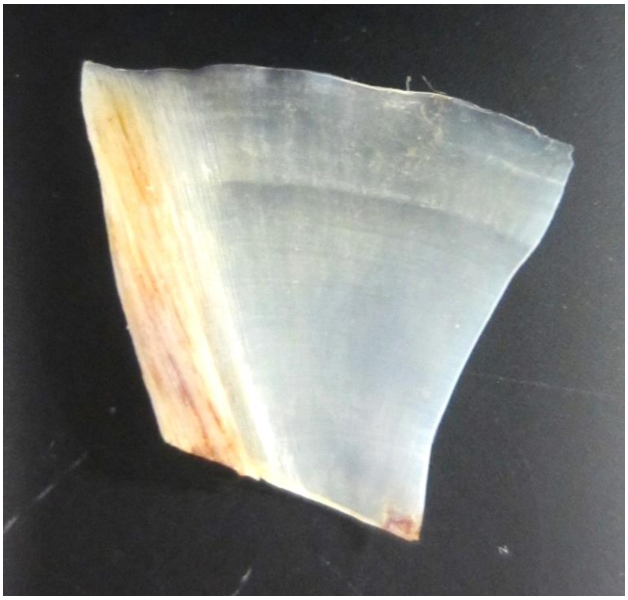

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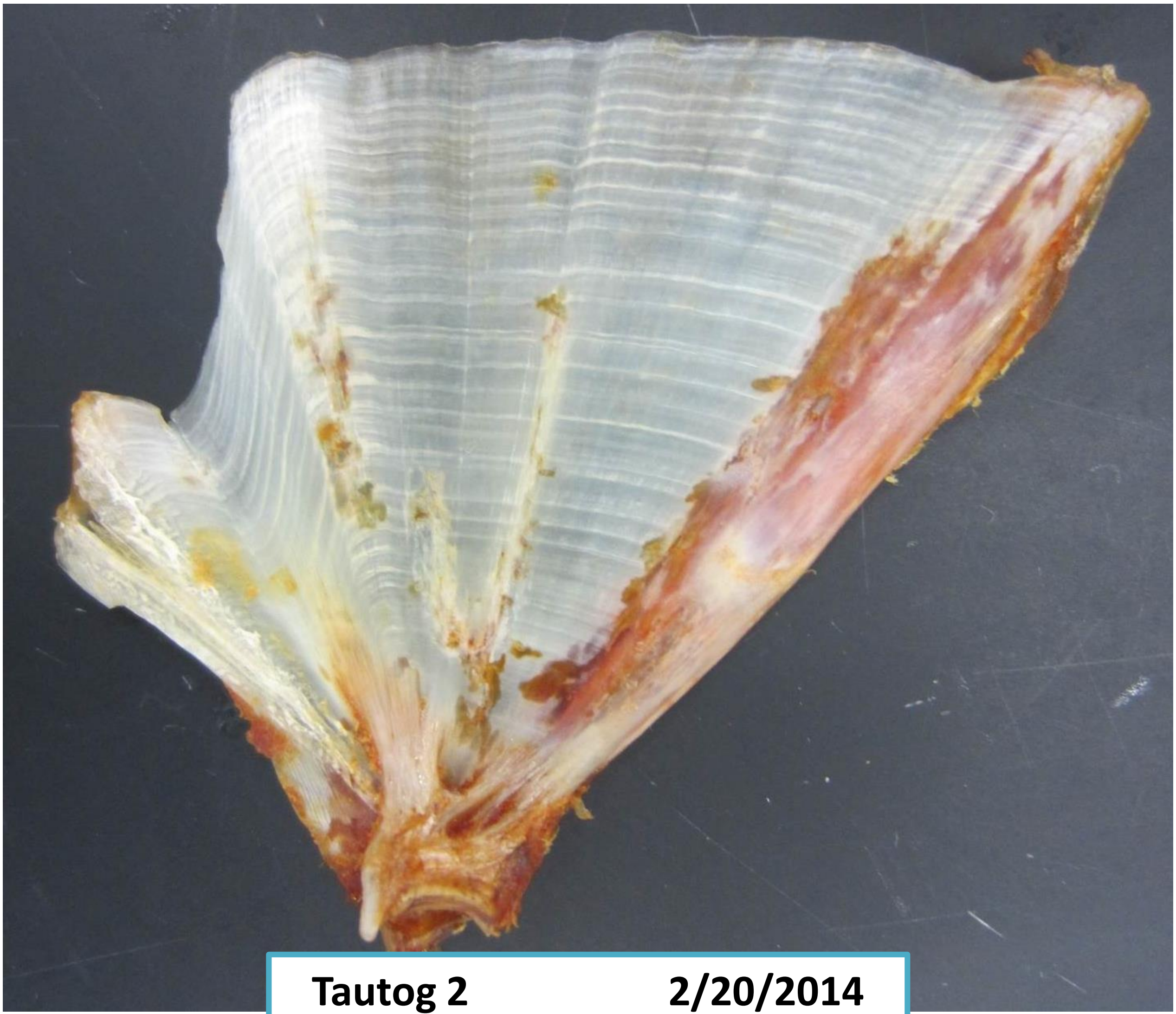

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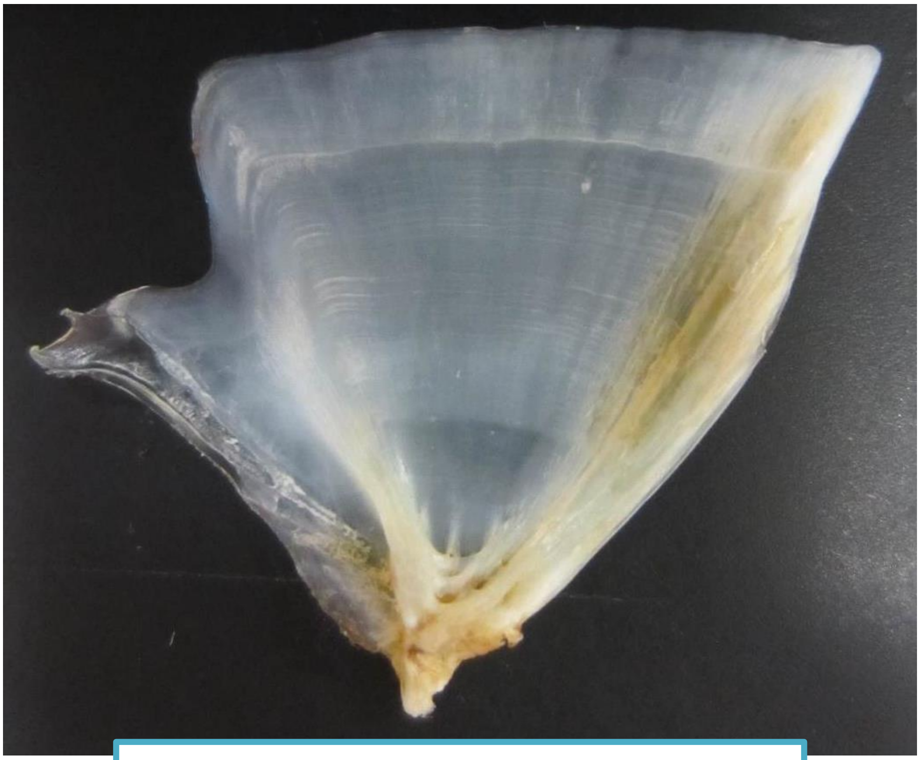

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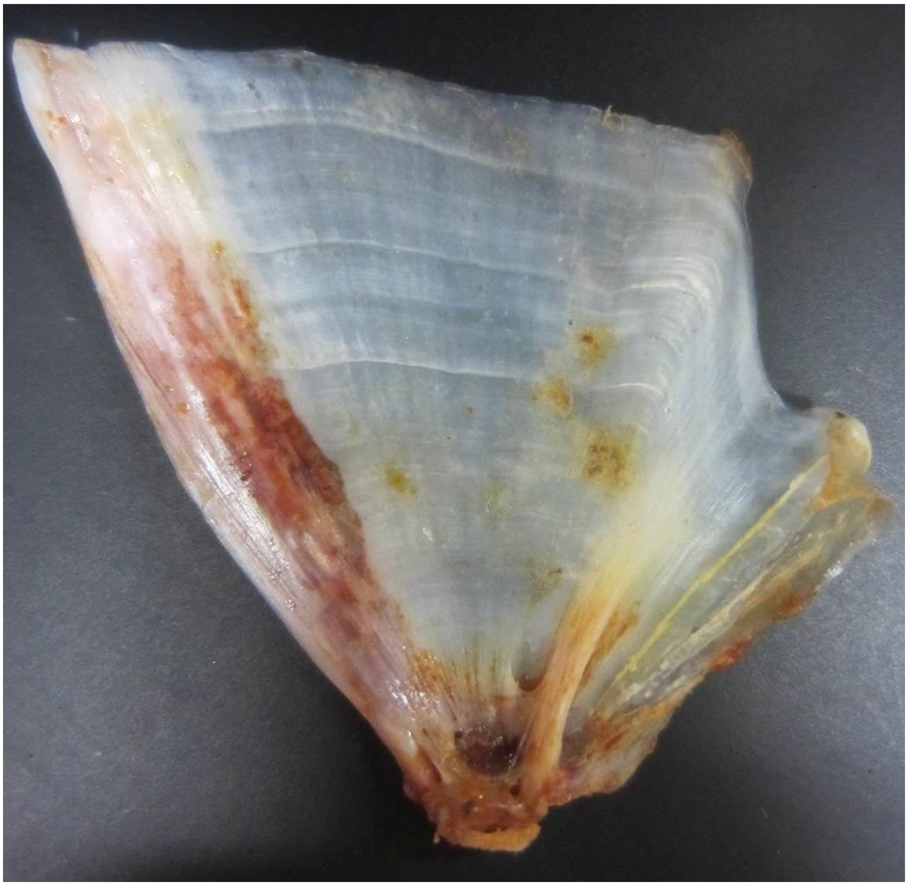

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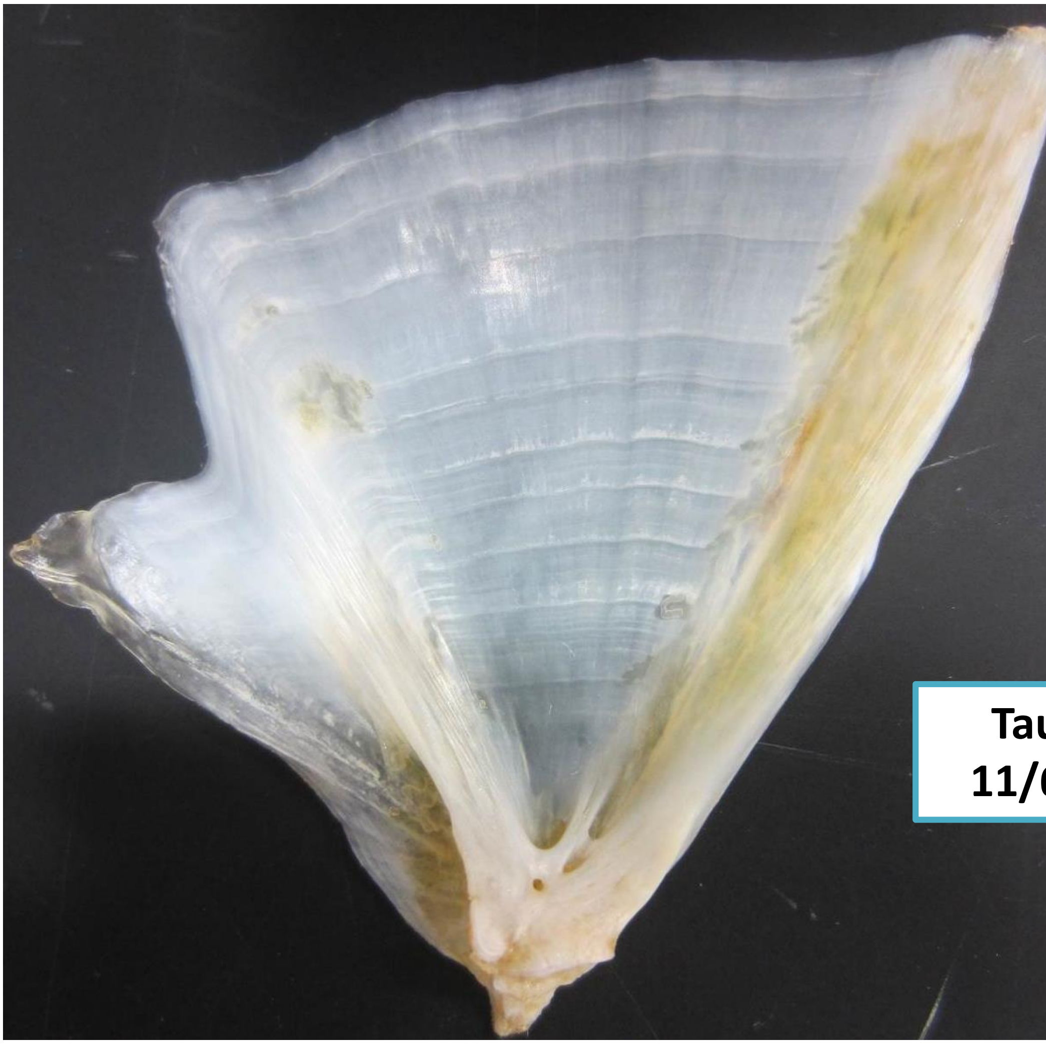

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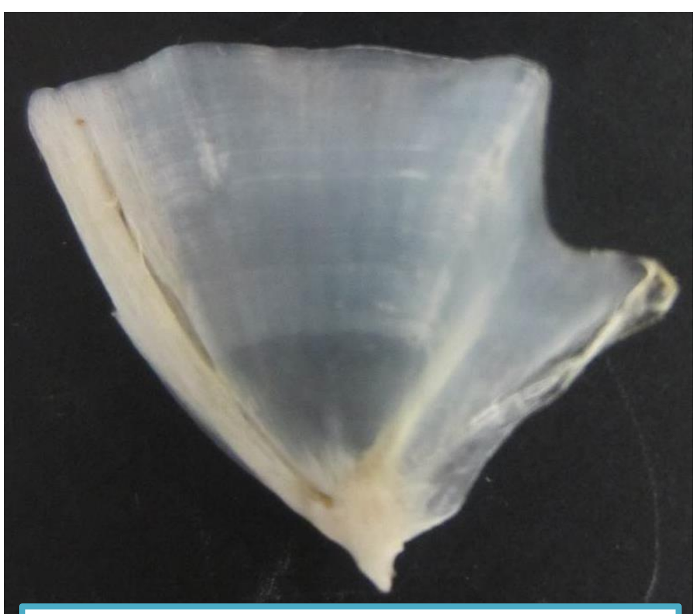

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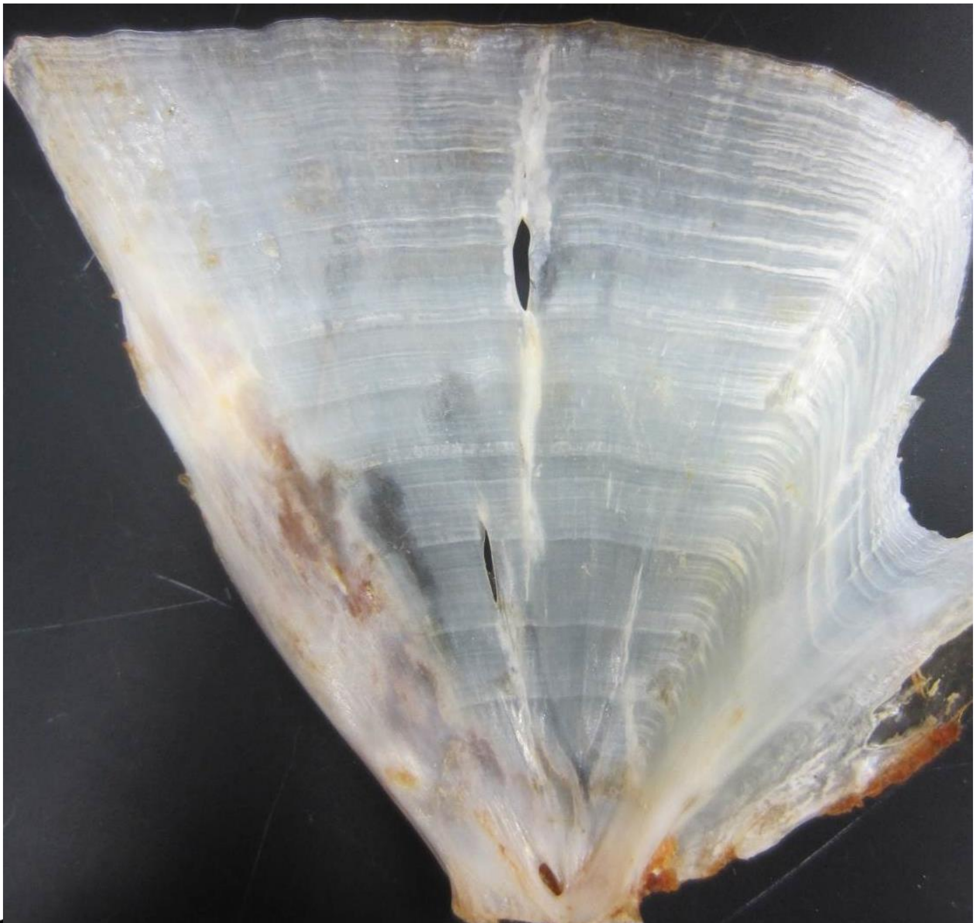

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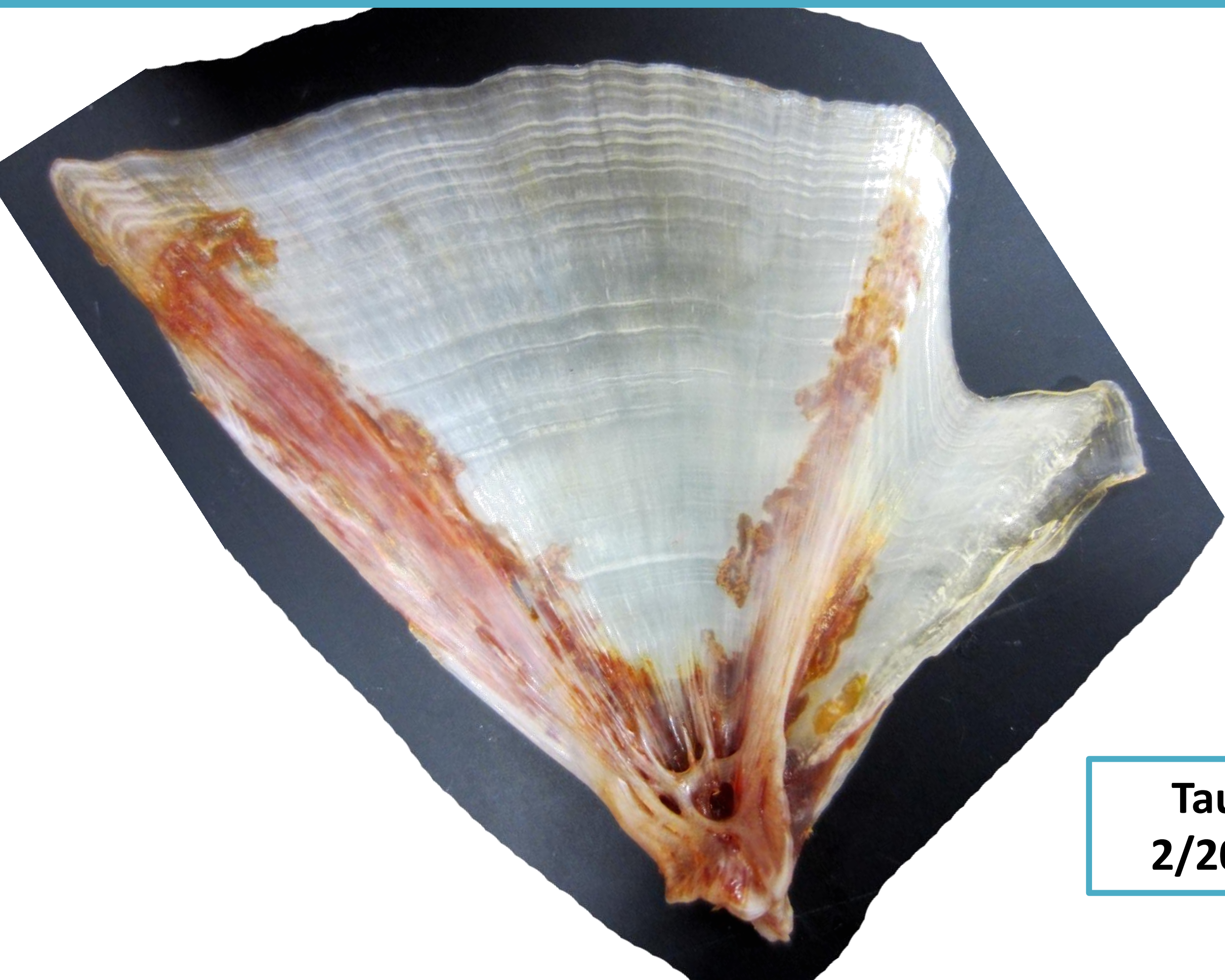

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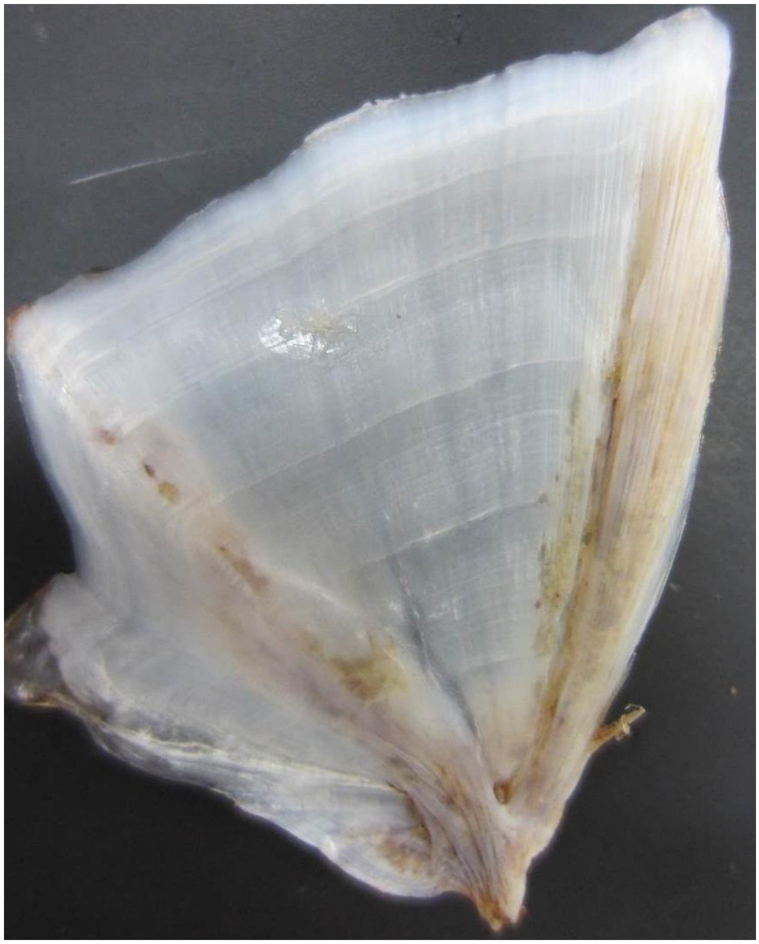

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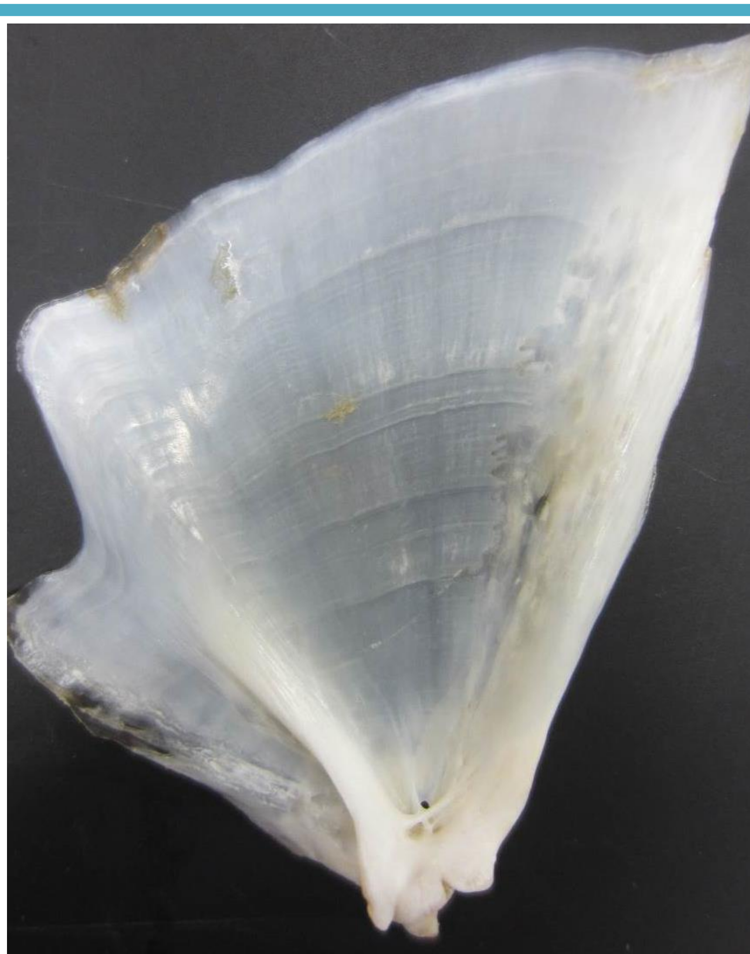

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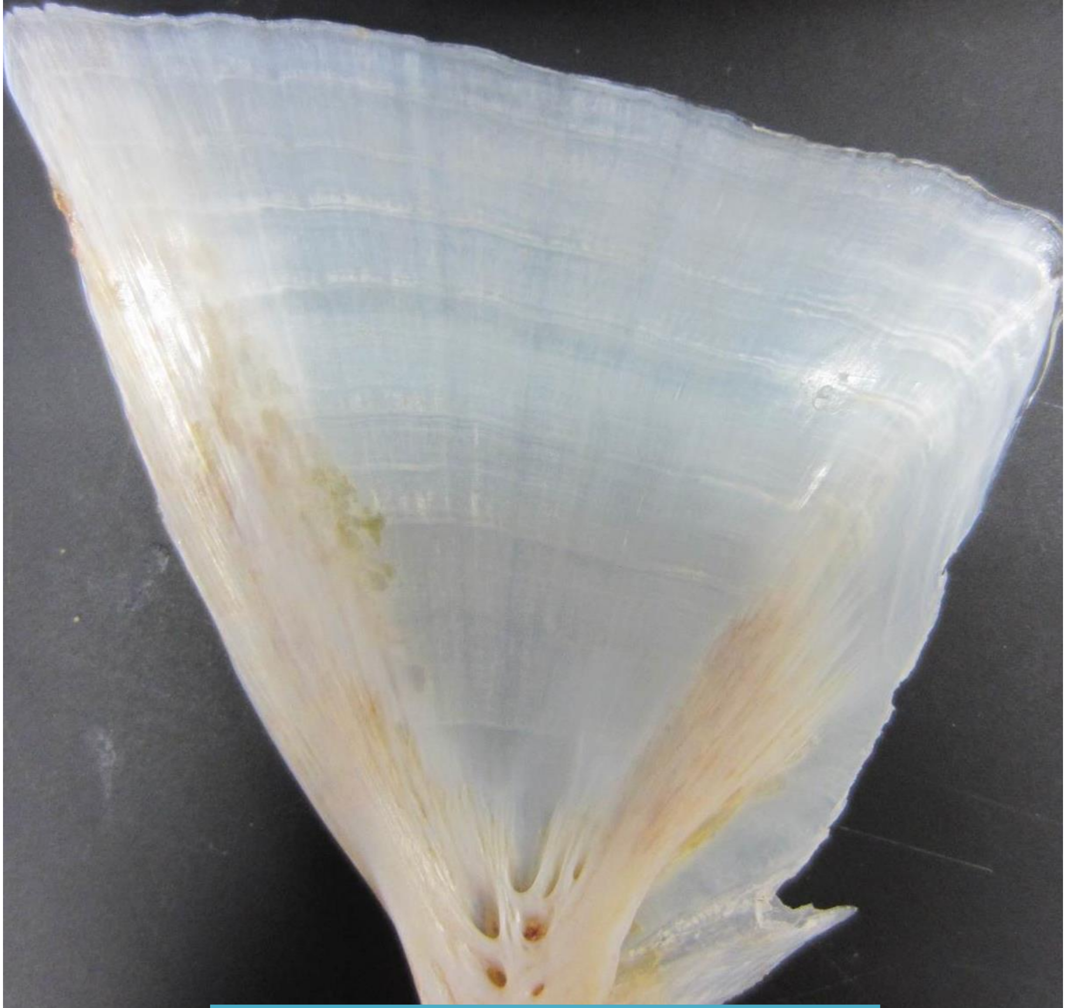

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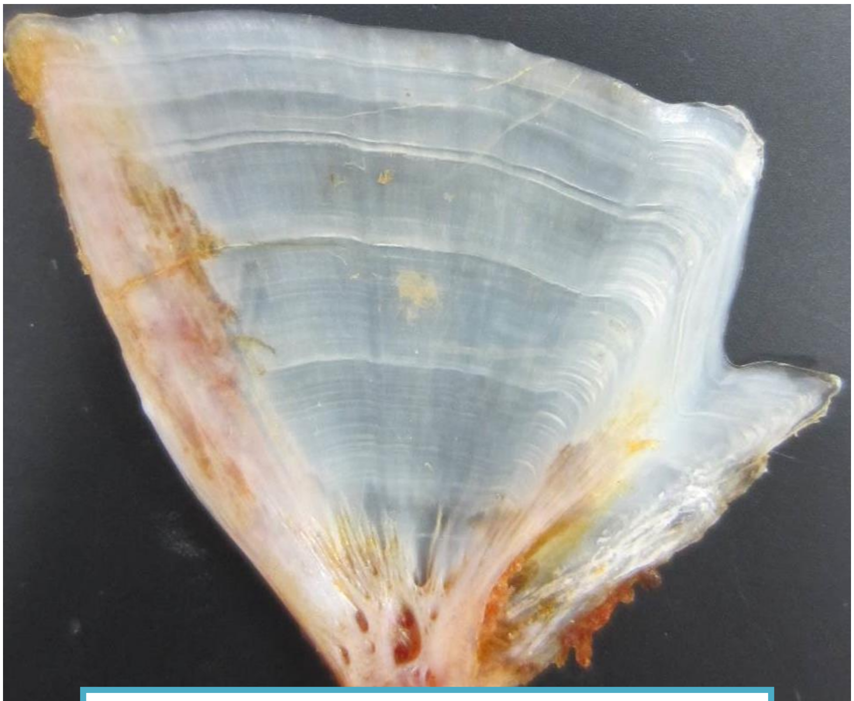

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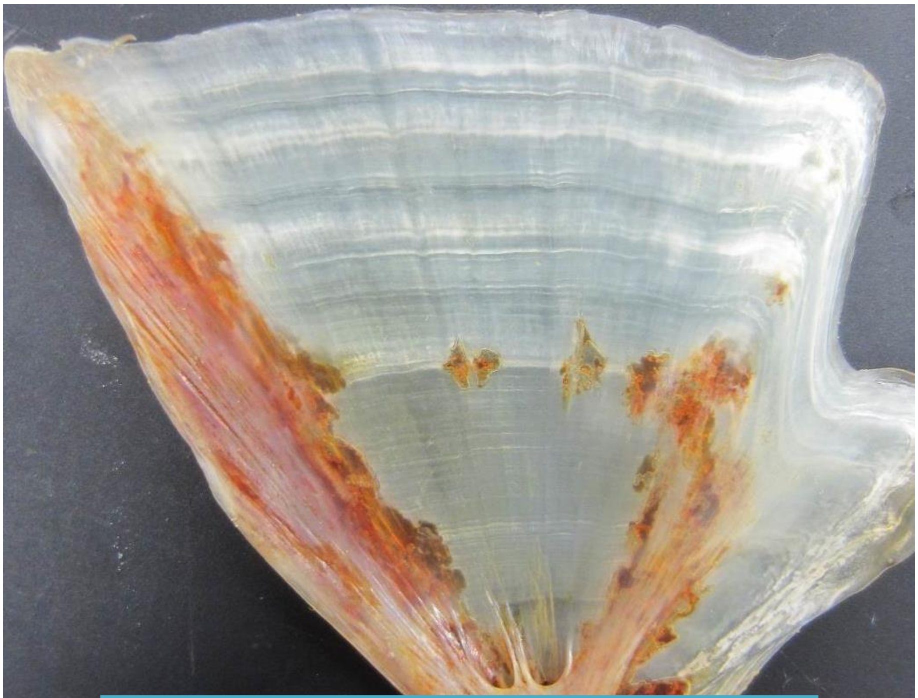

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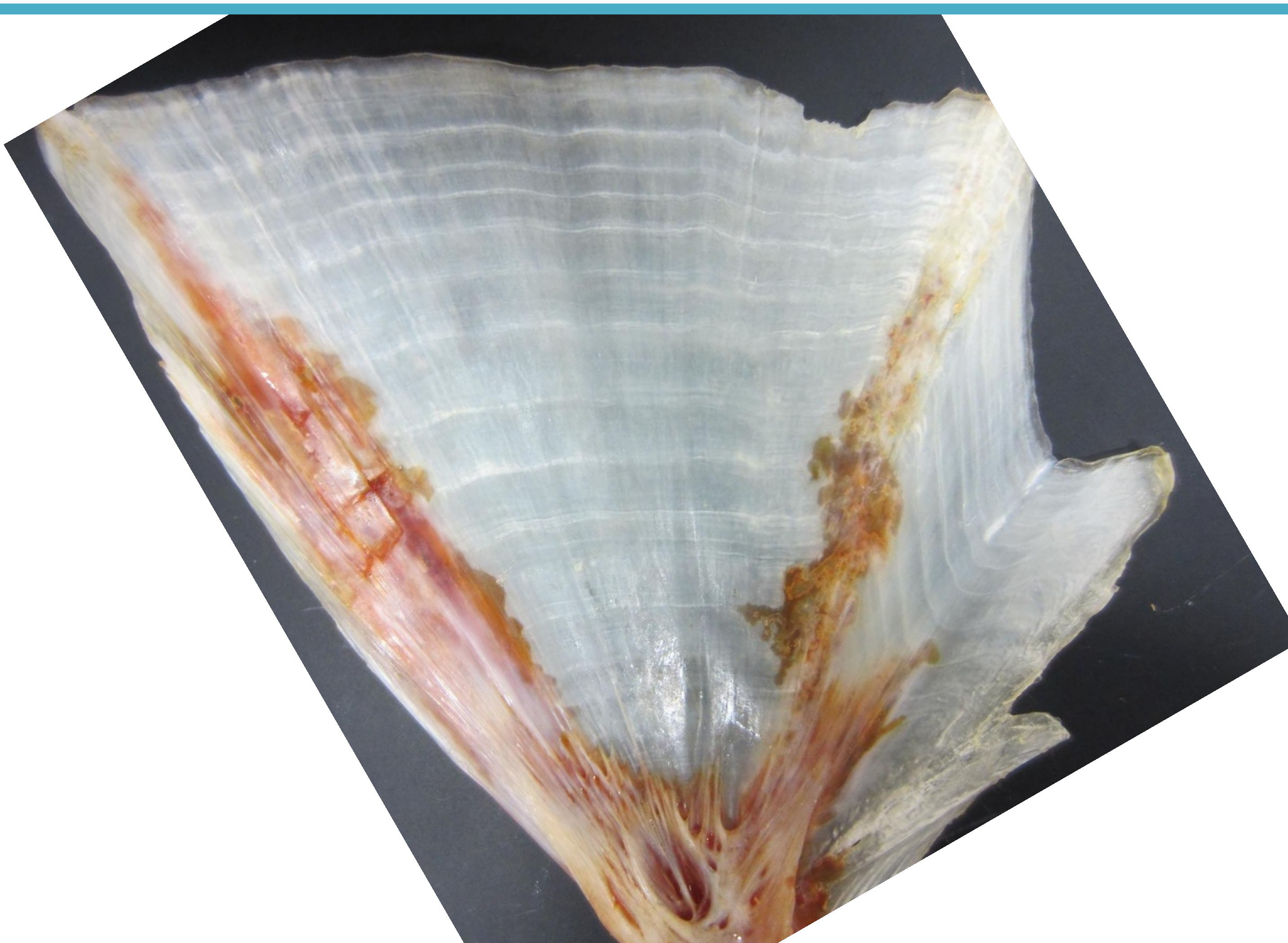

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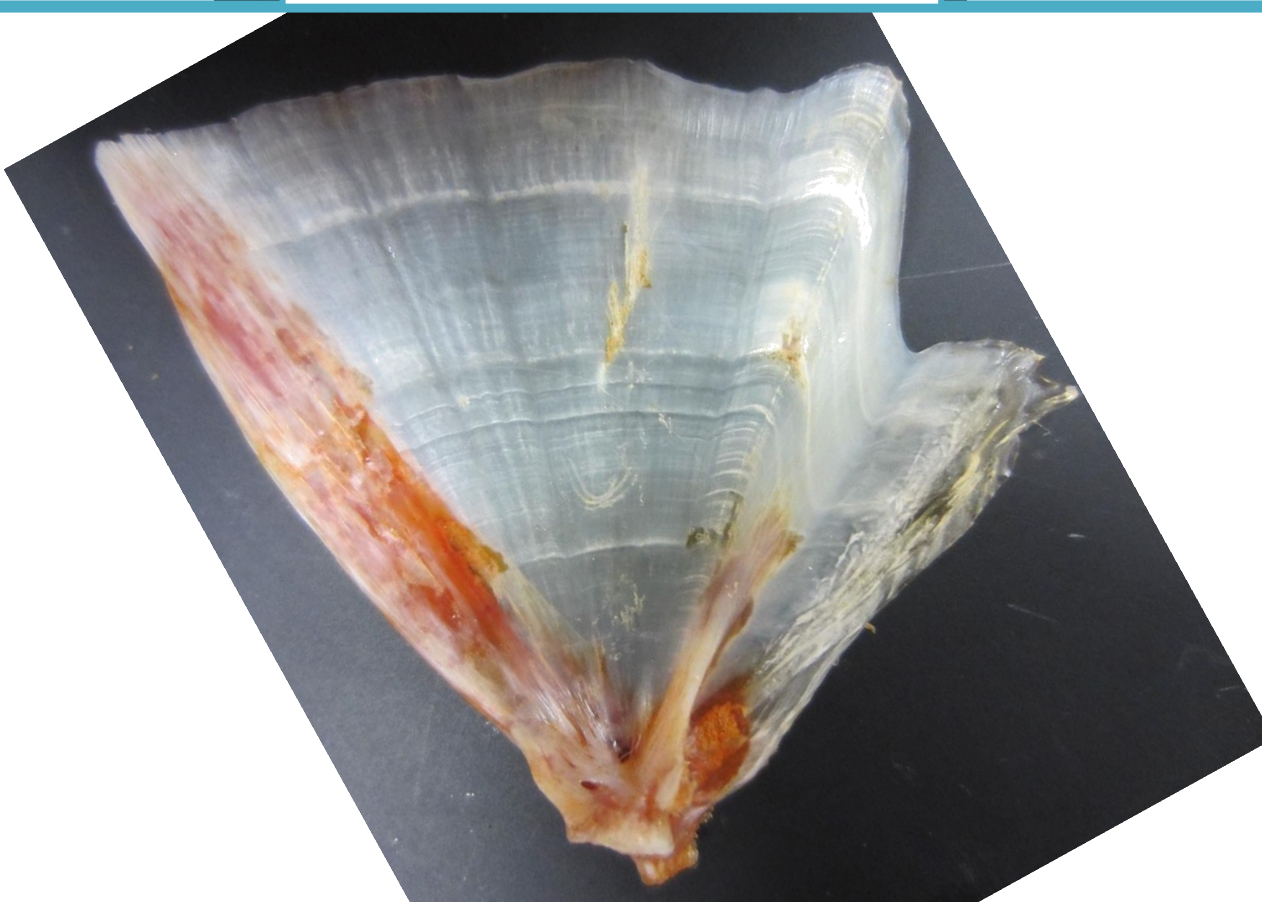

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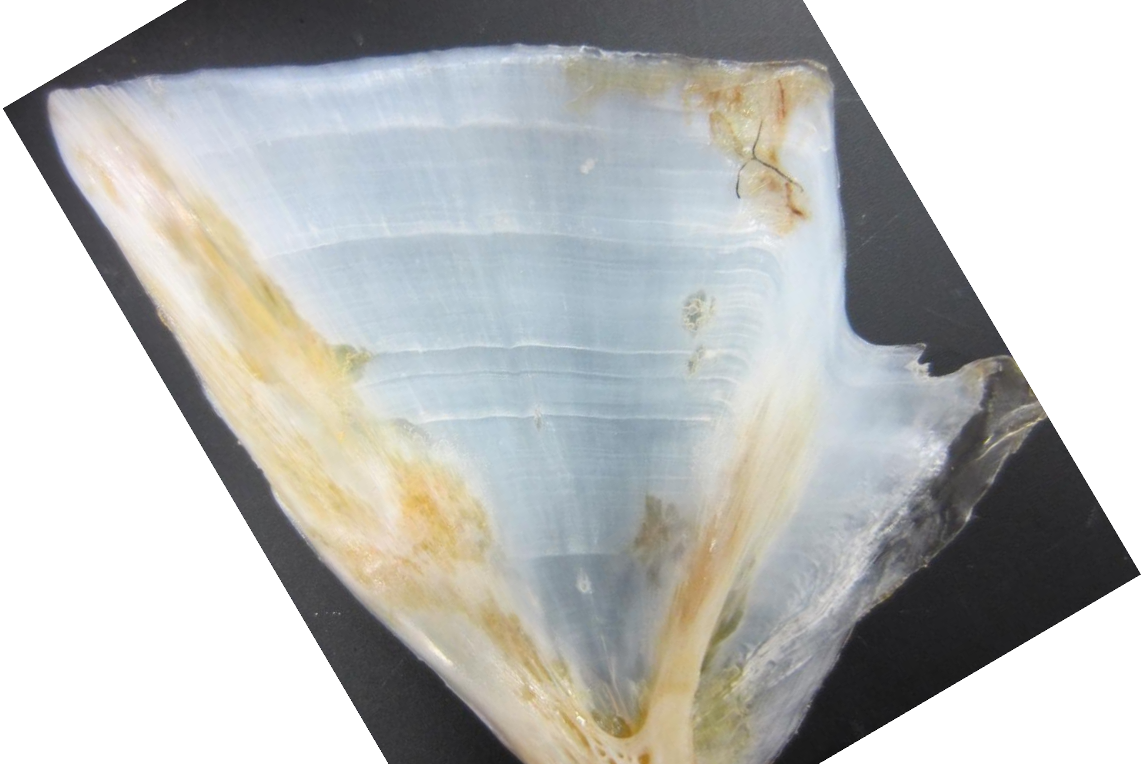

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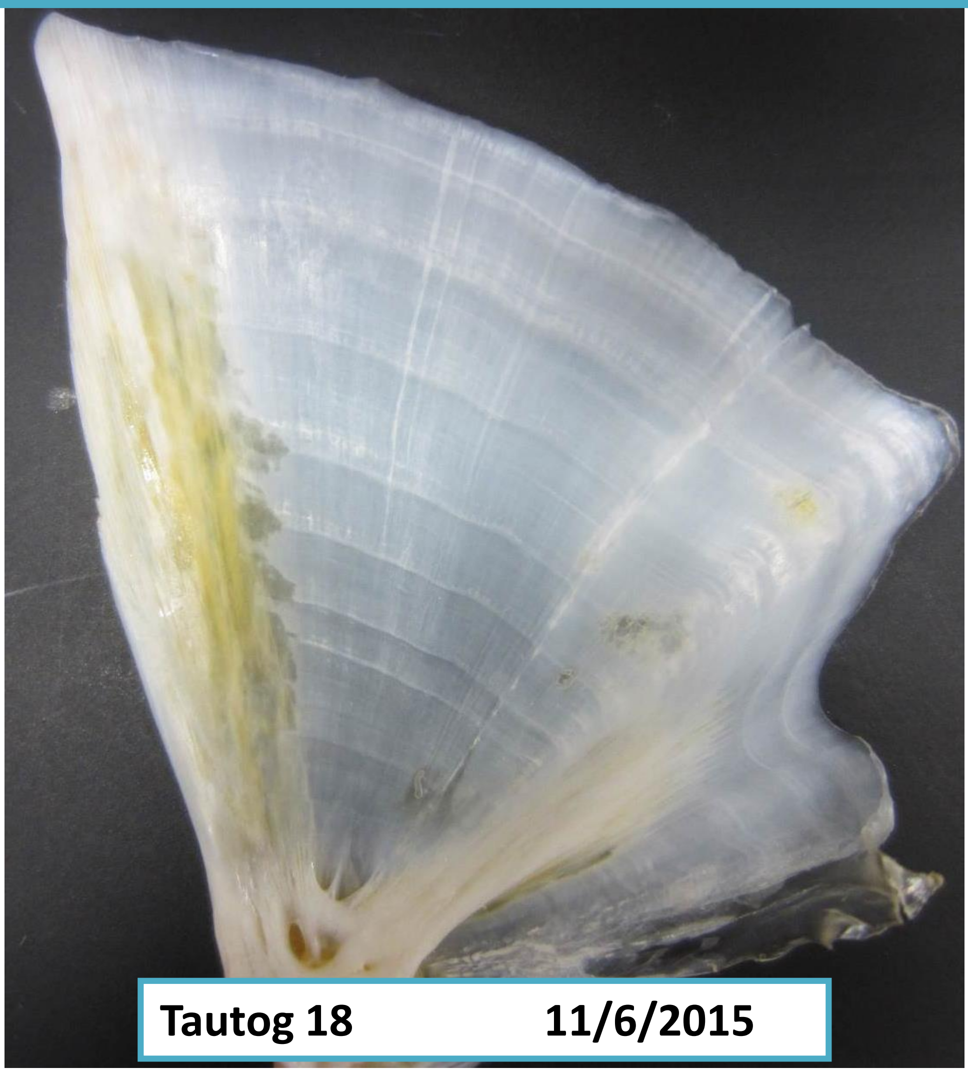

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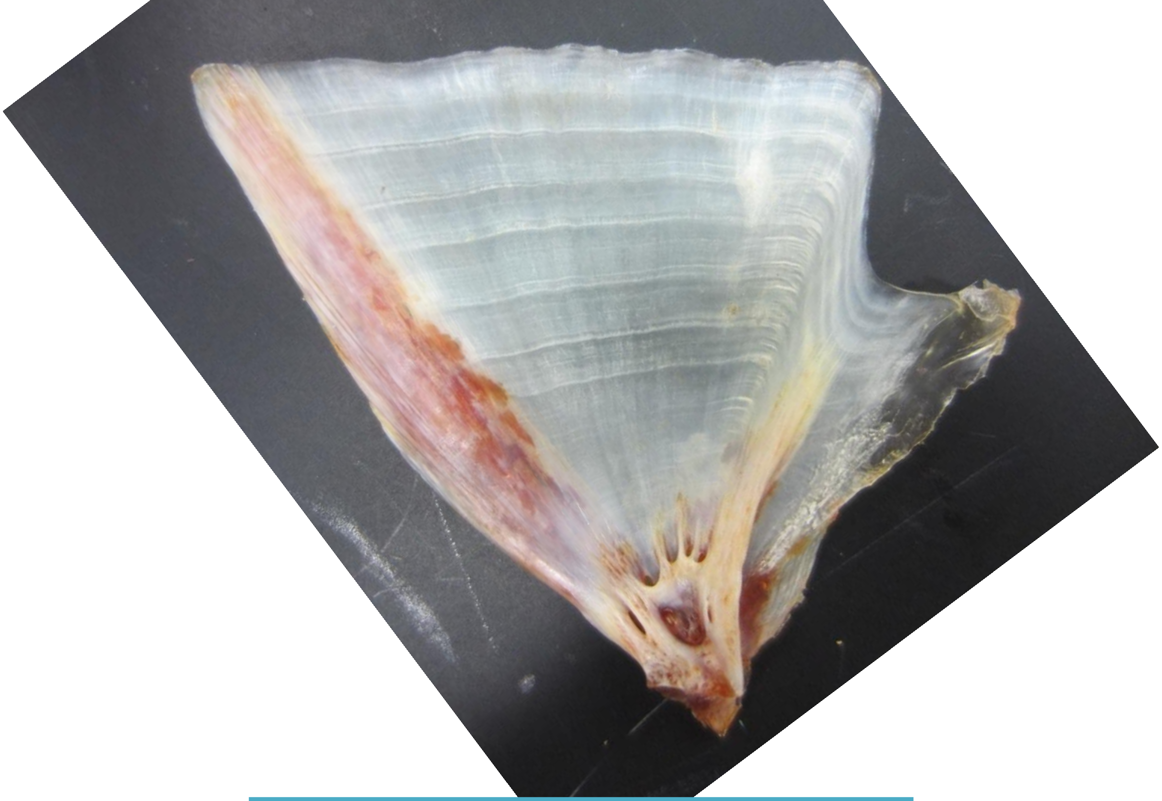

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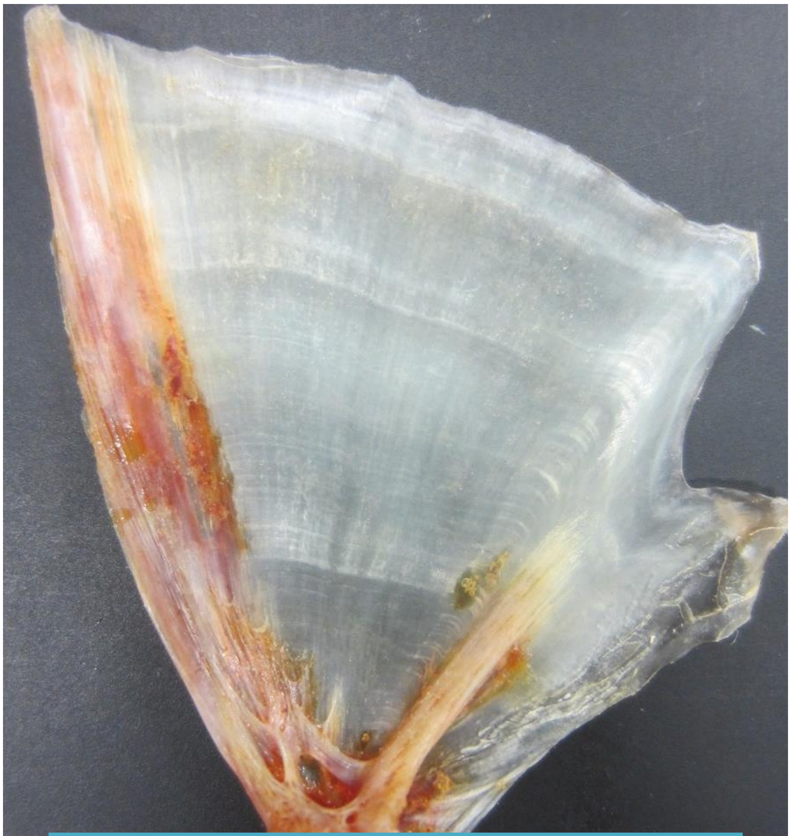

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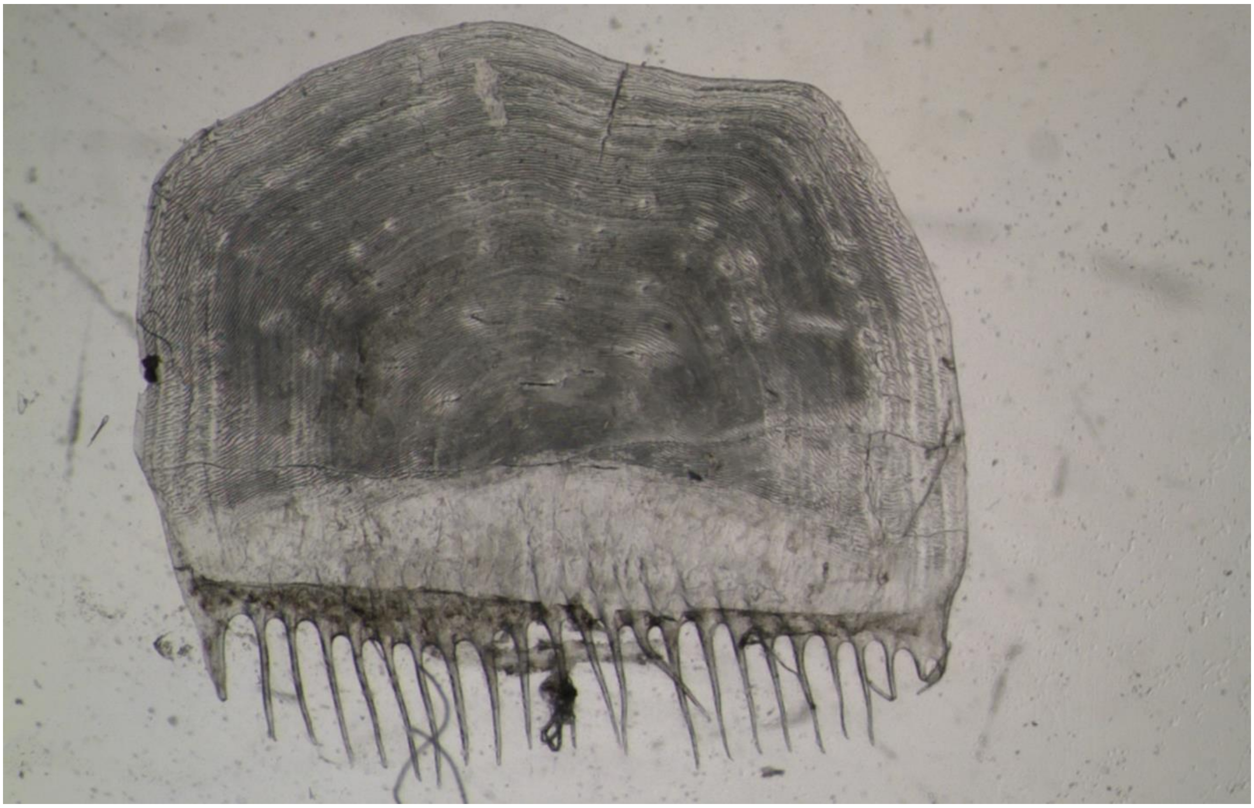

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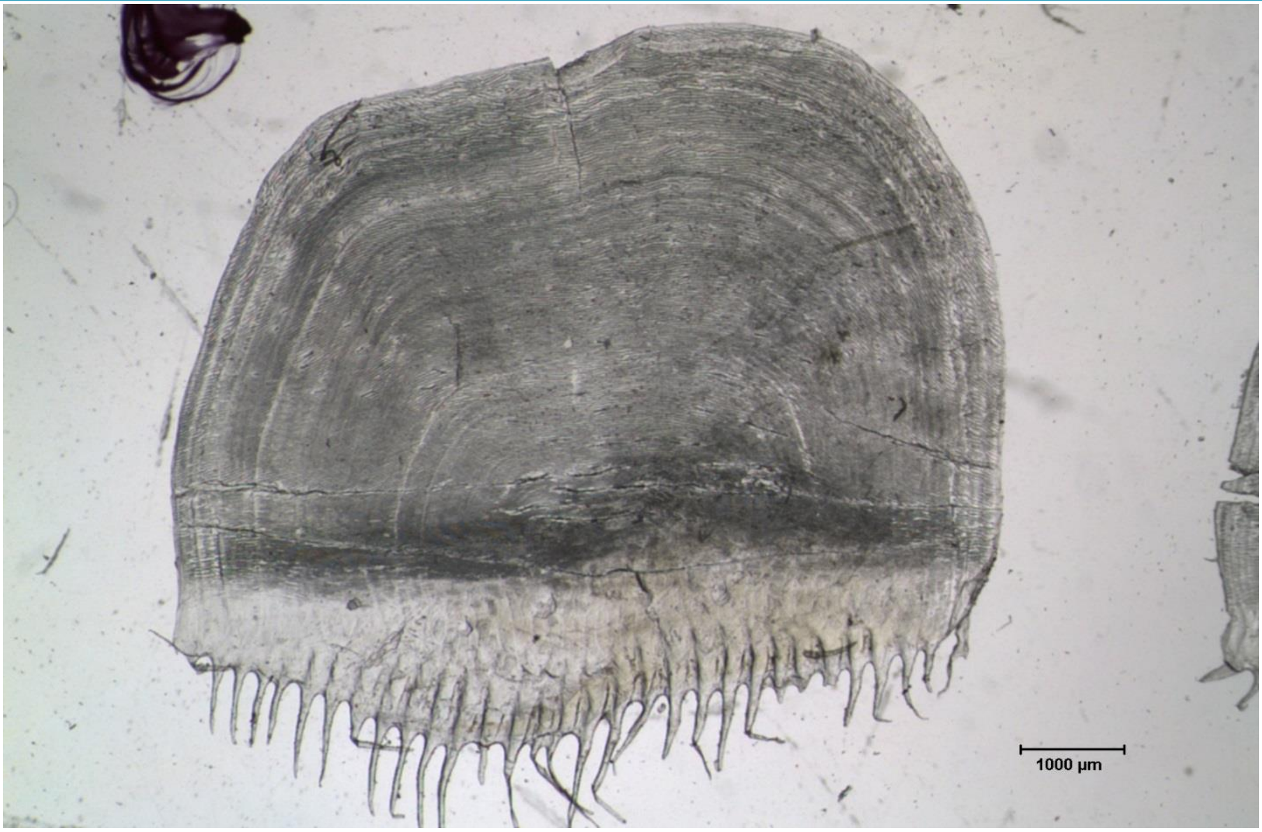

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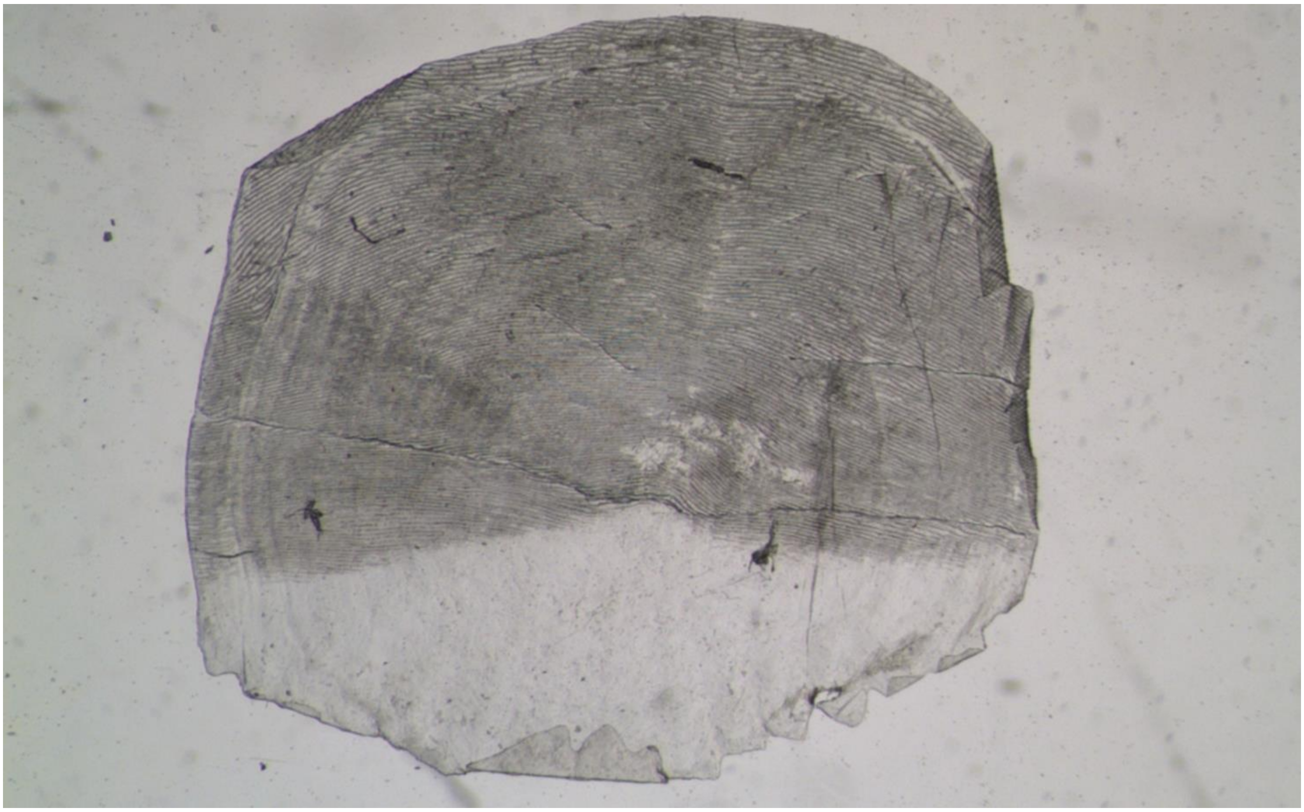

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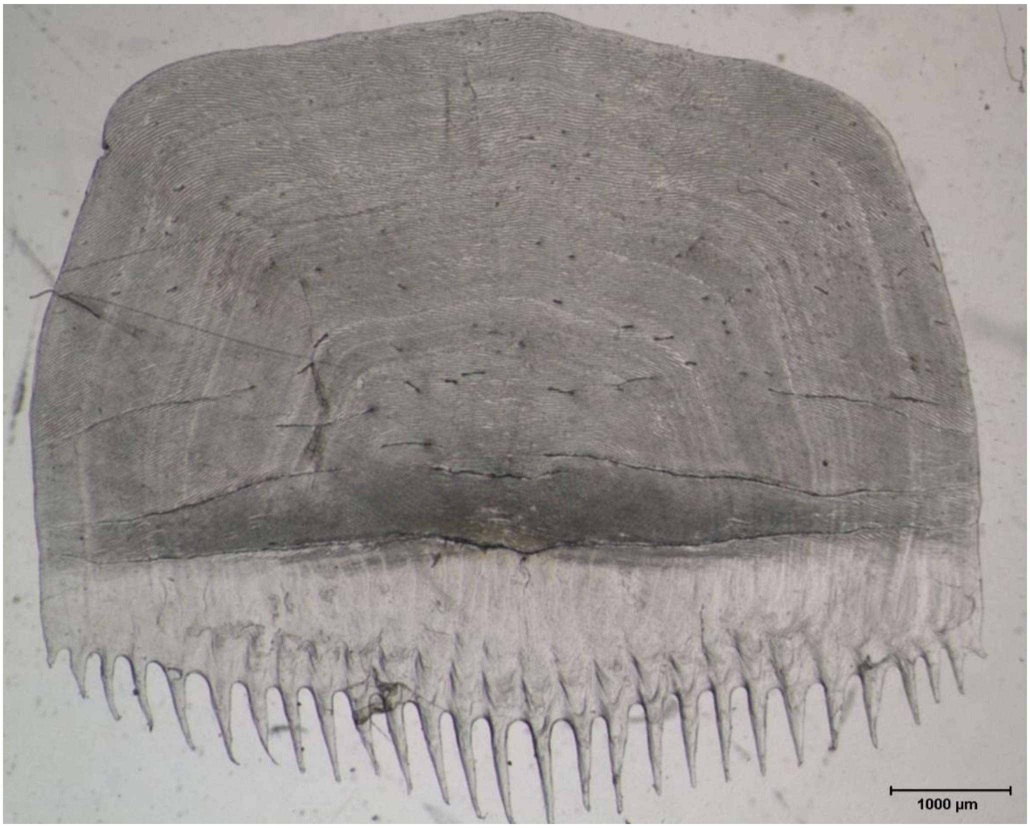

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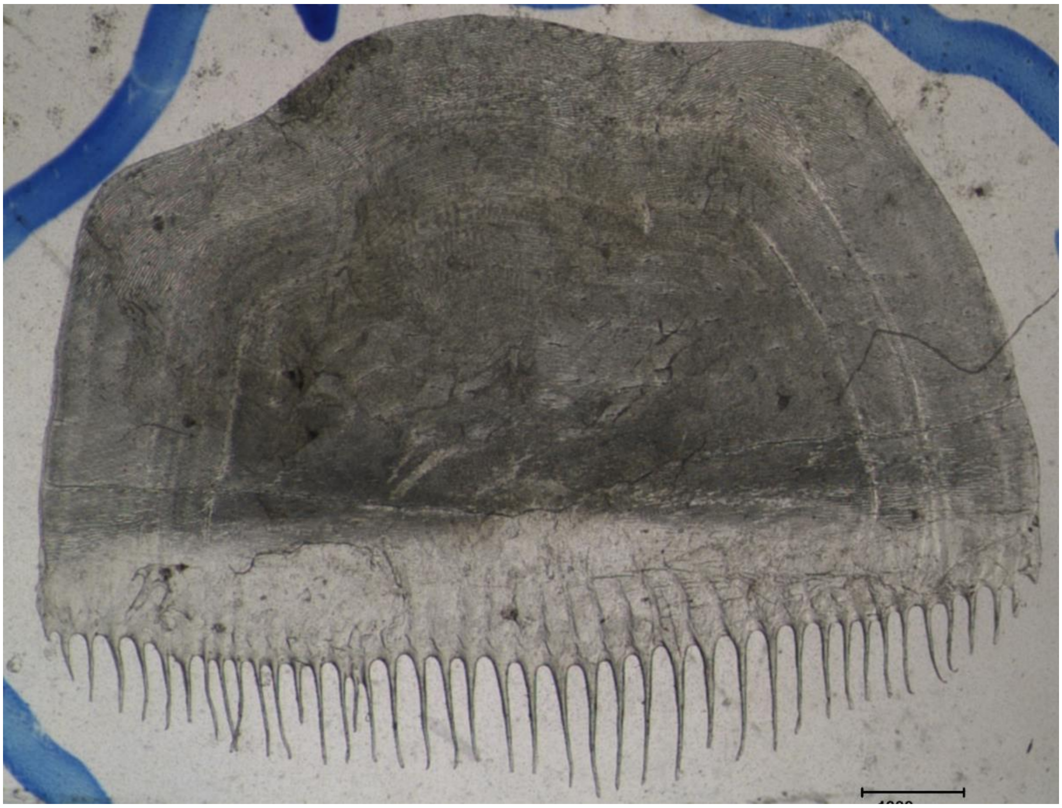

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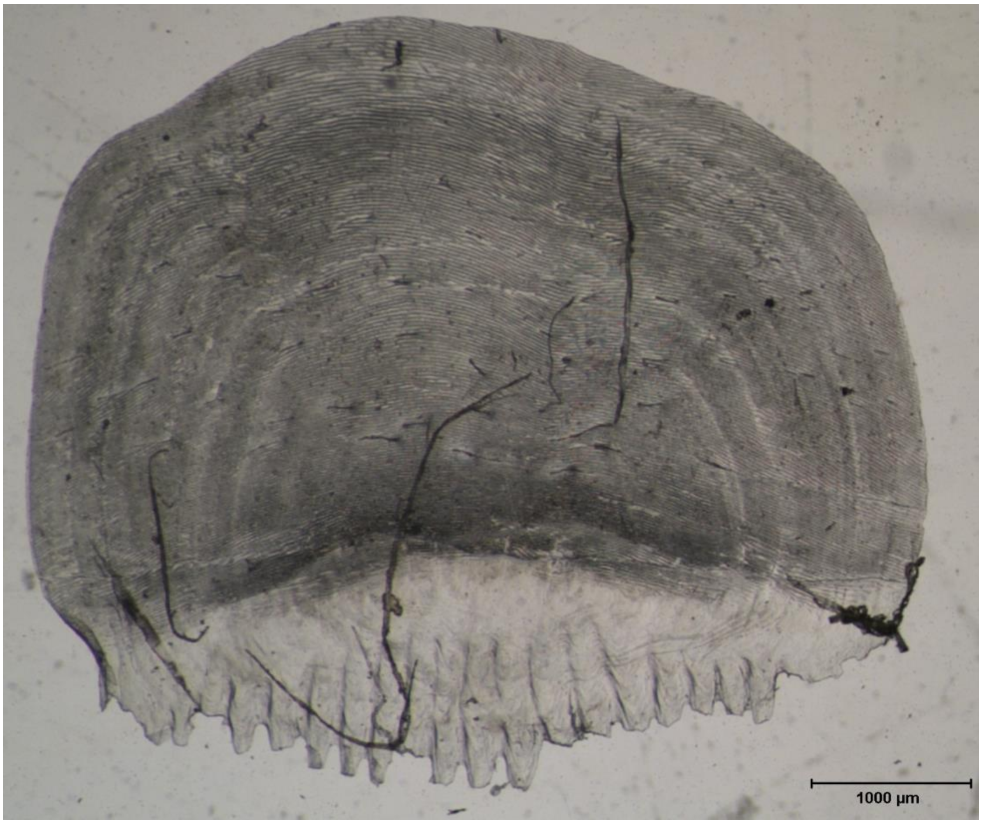

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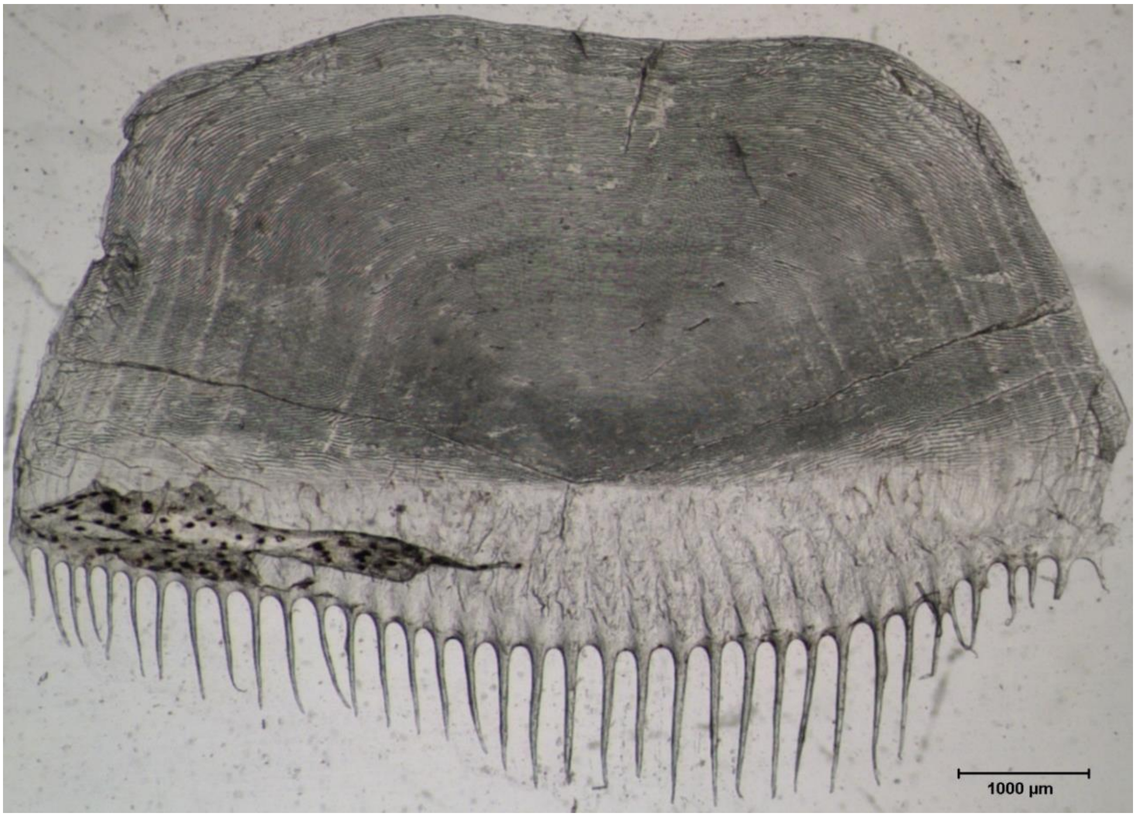

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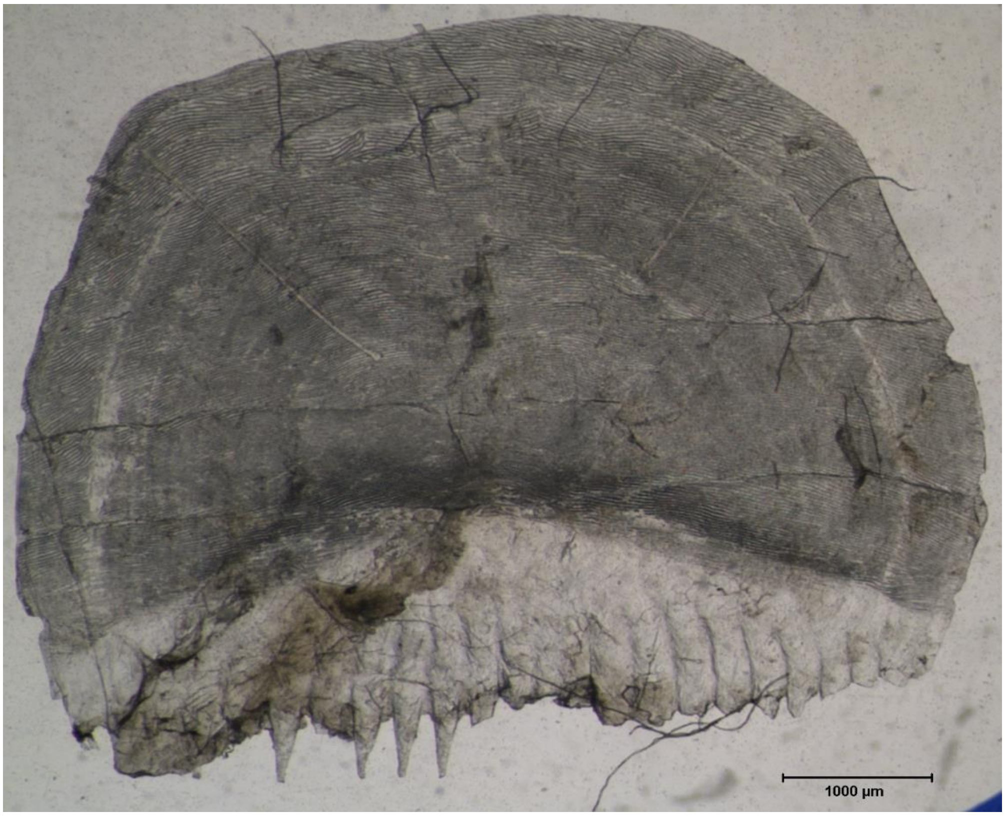

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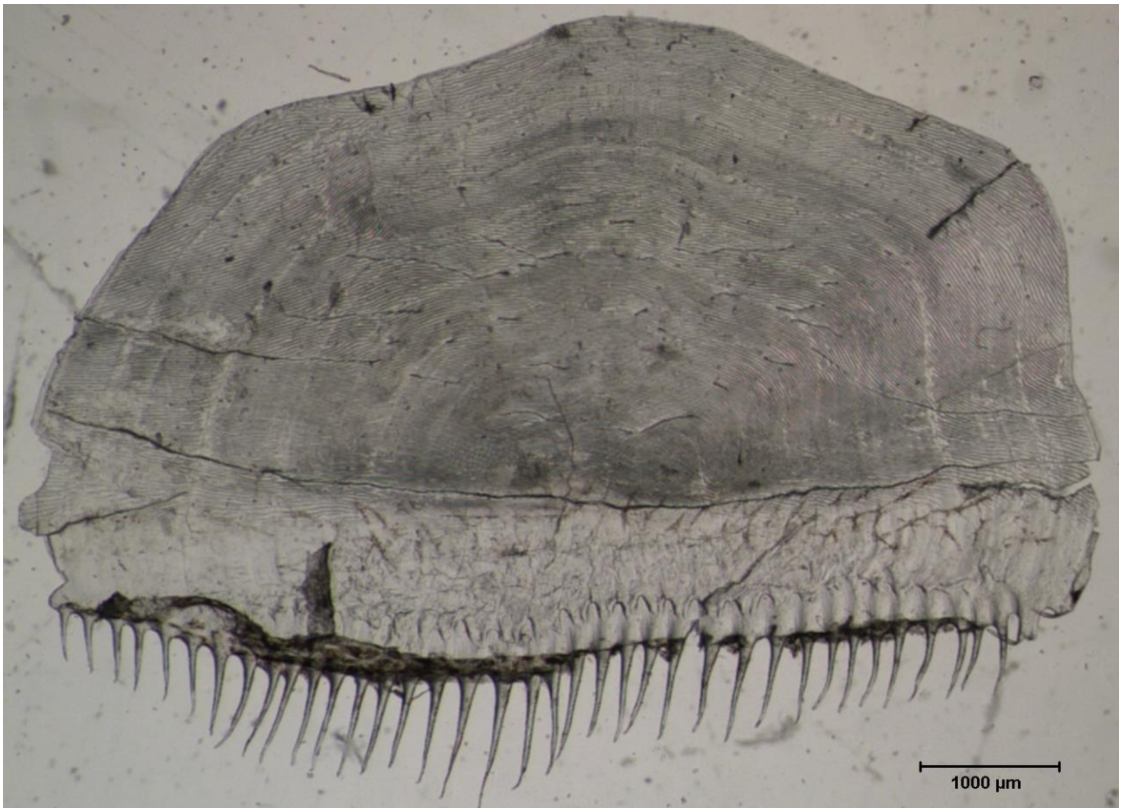

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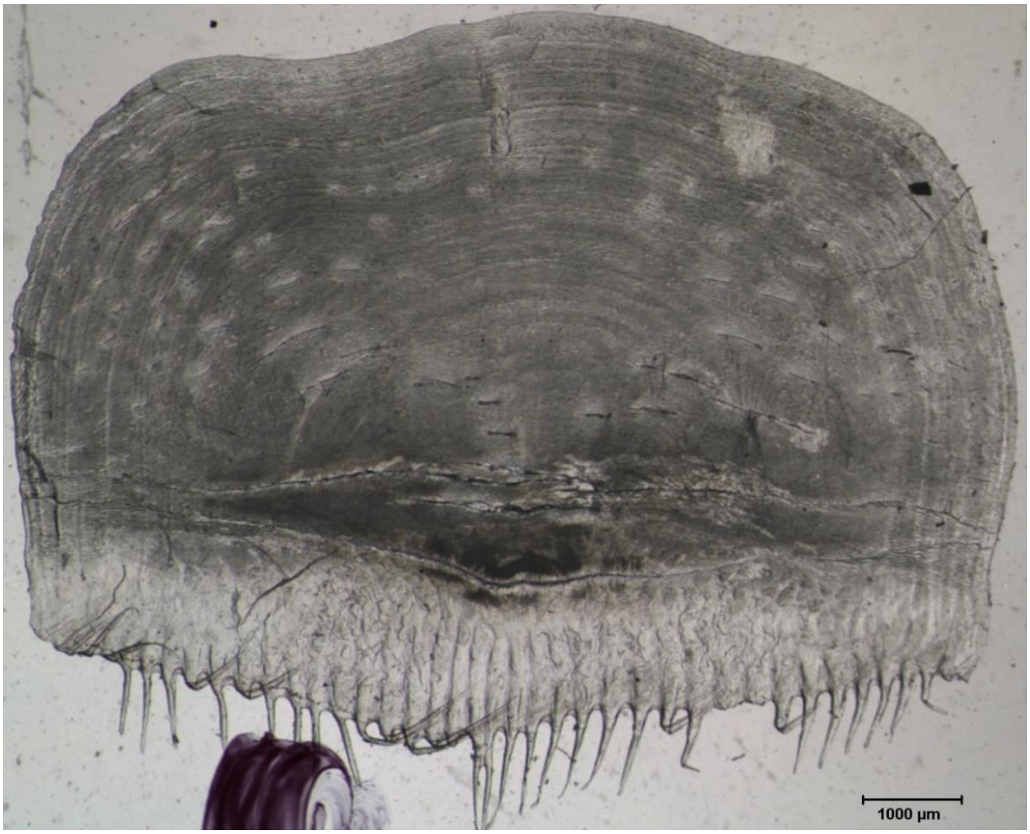

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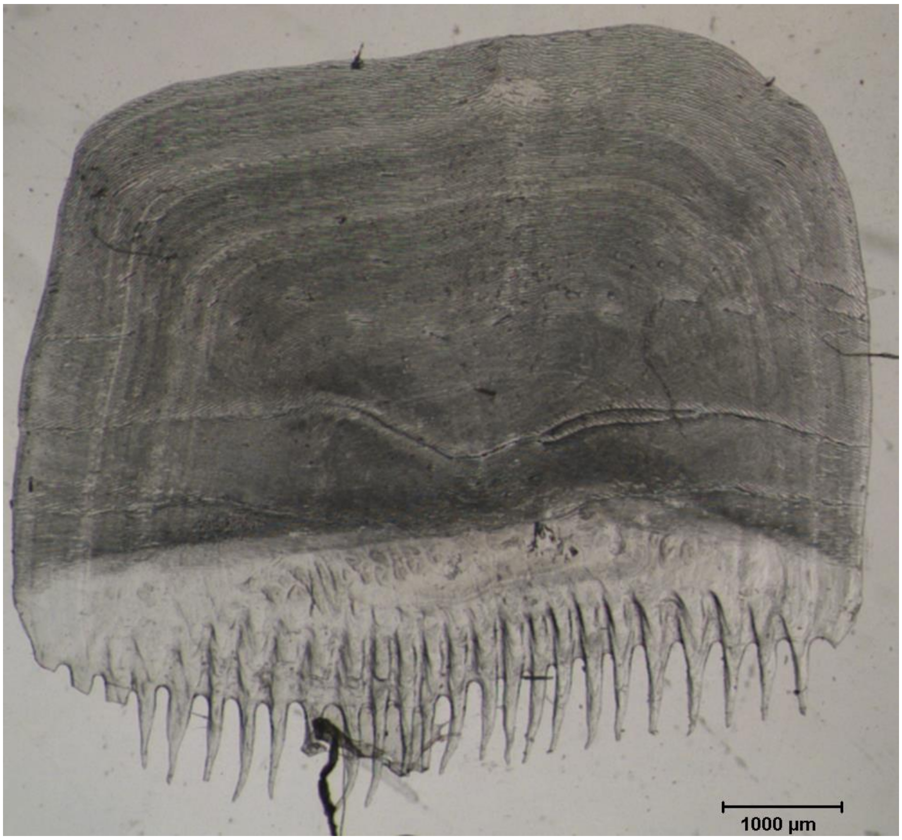

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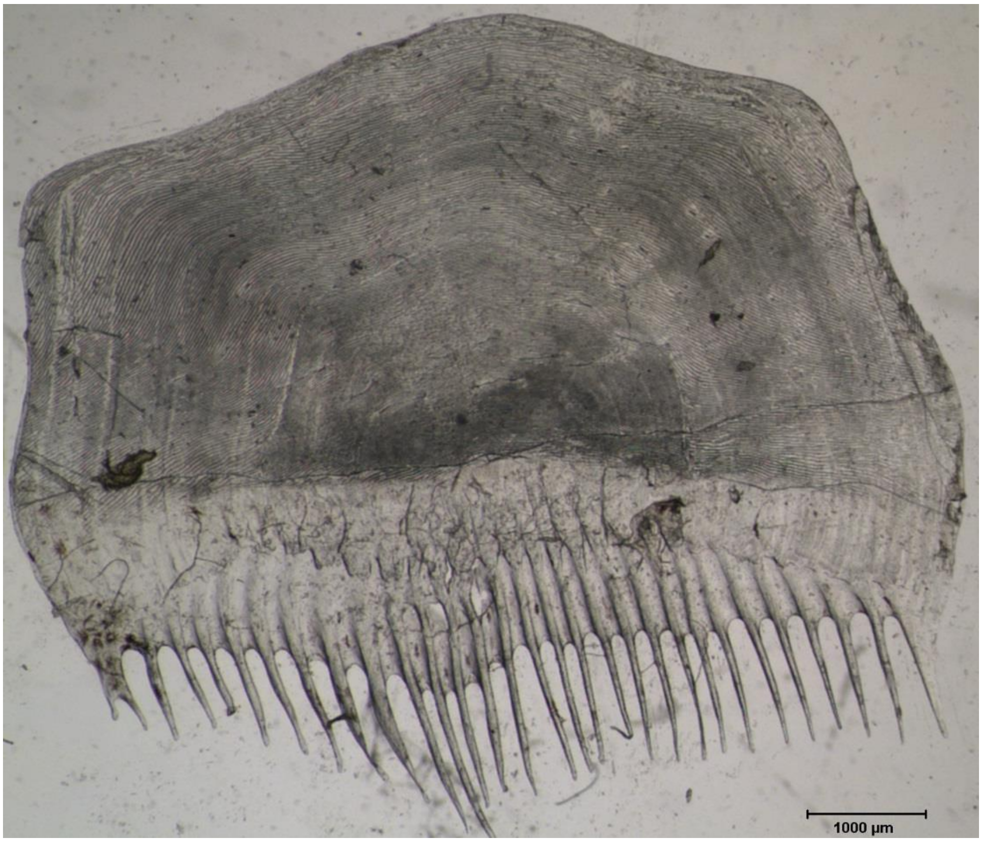

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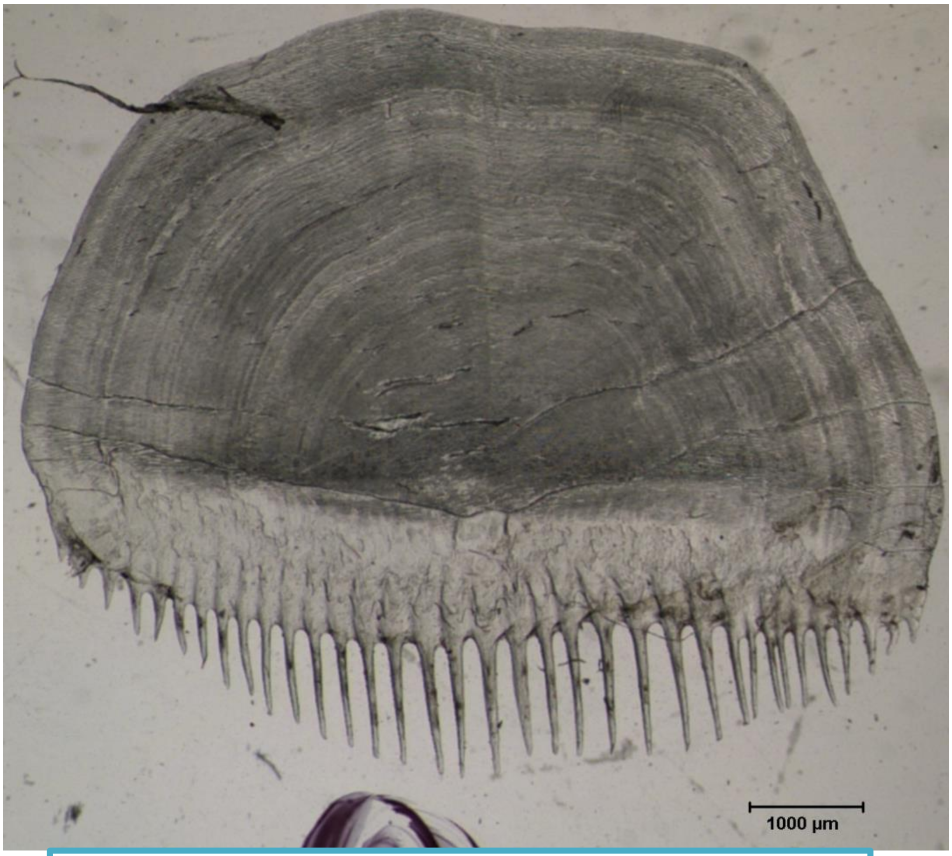

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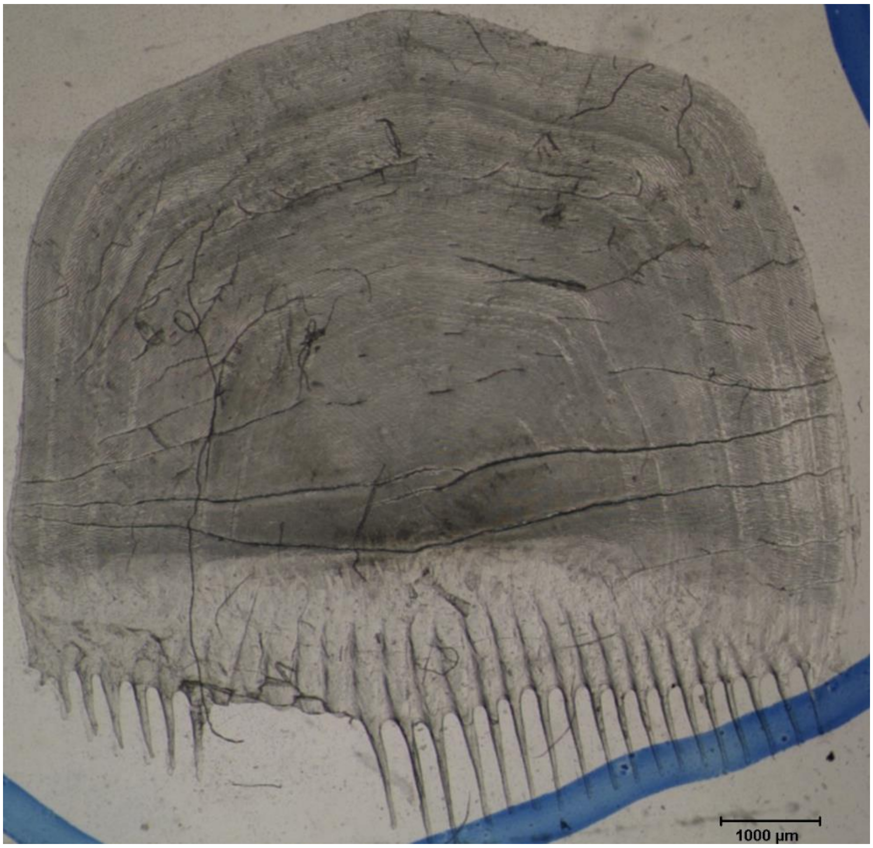

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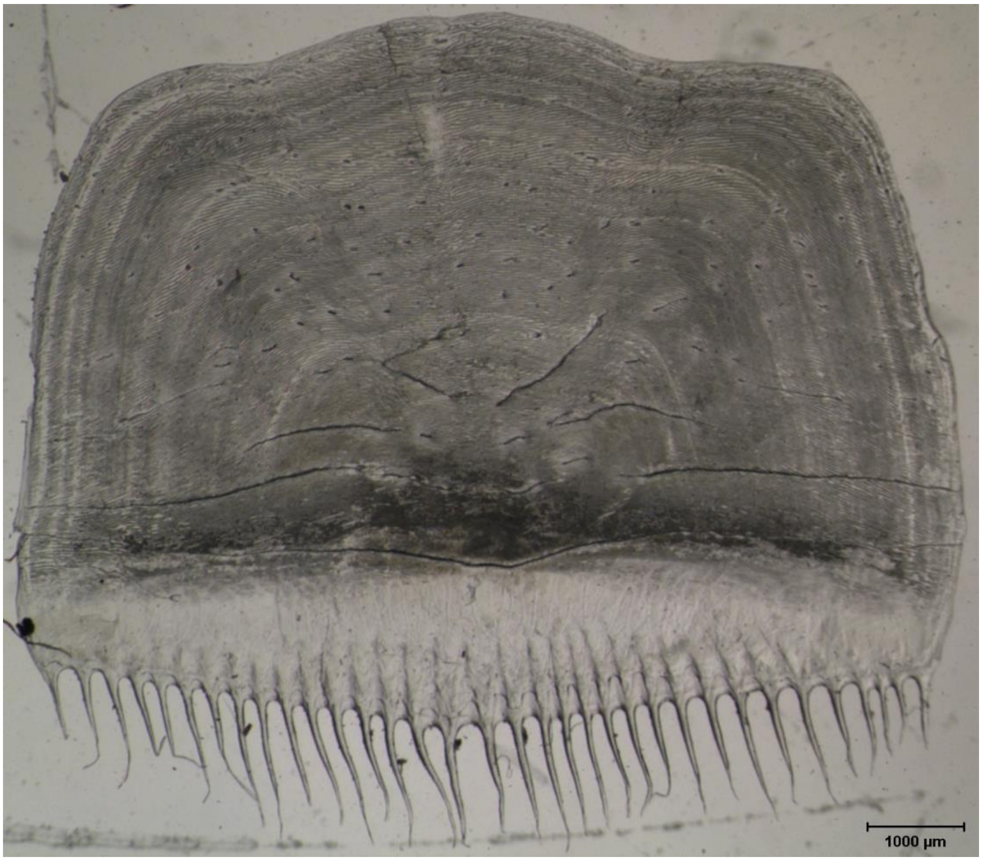
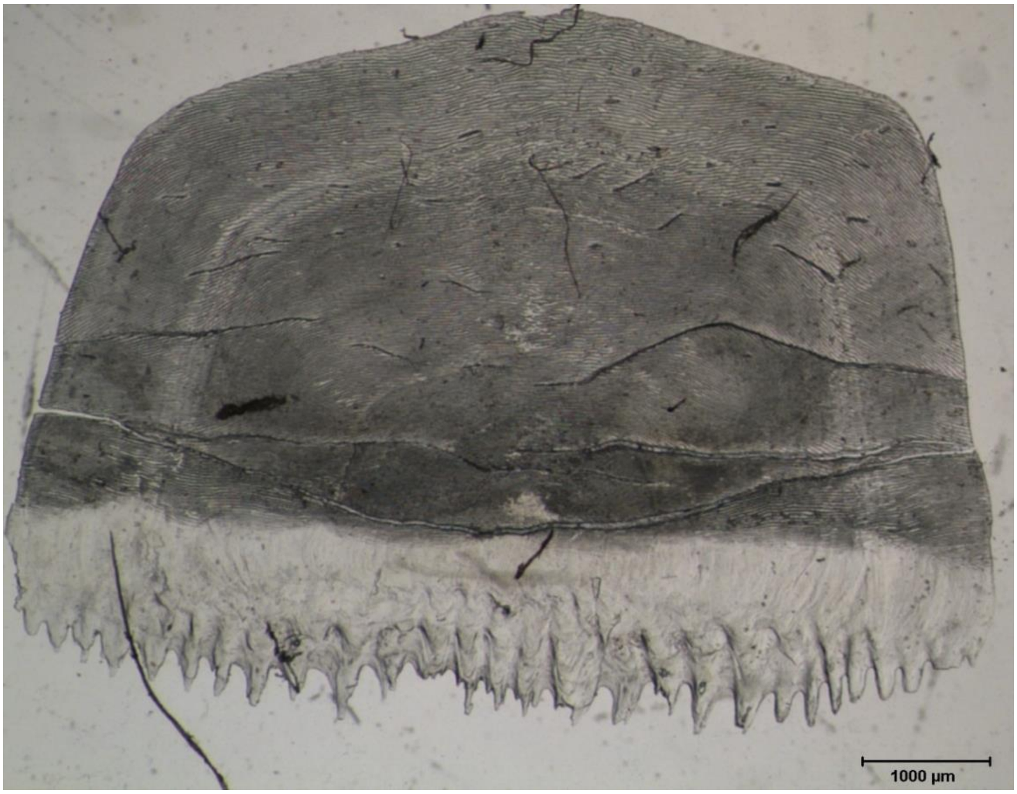
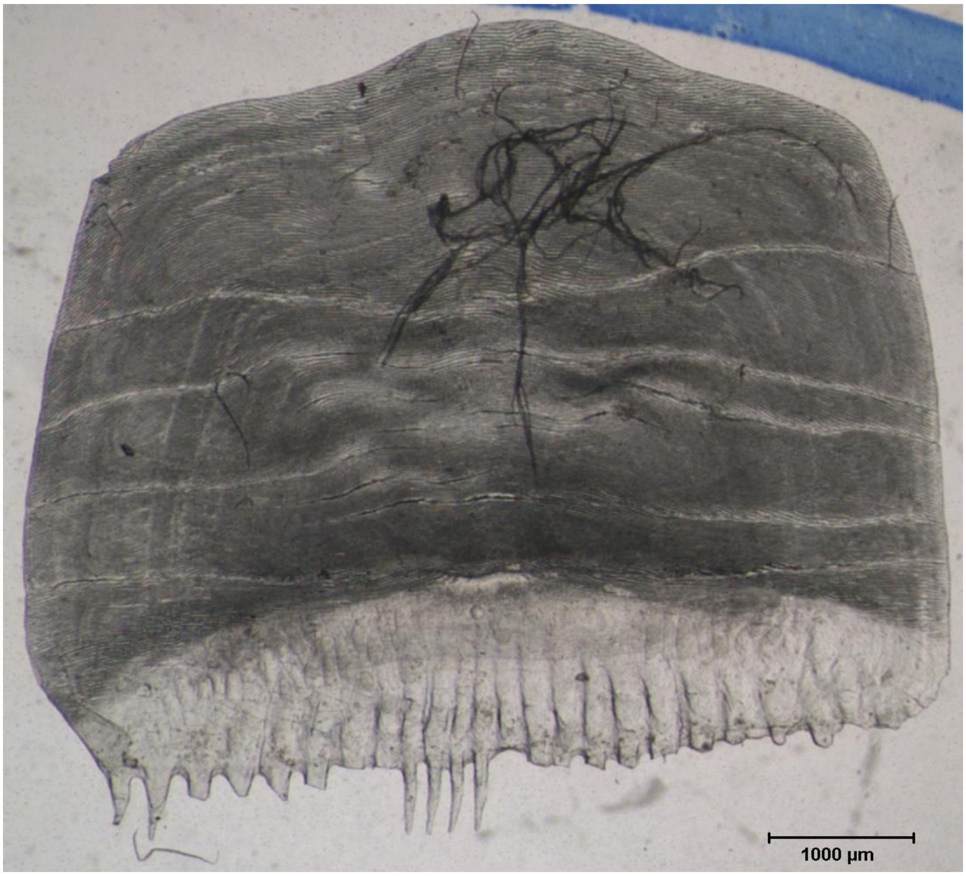


Atlantic Menhaden 16 **8/23/2016**



Atlantic Menhaden 17 9/8/2016



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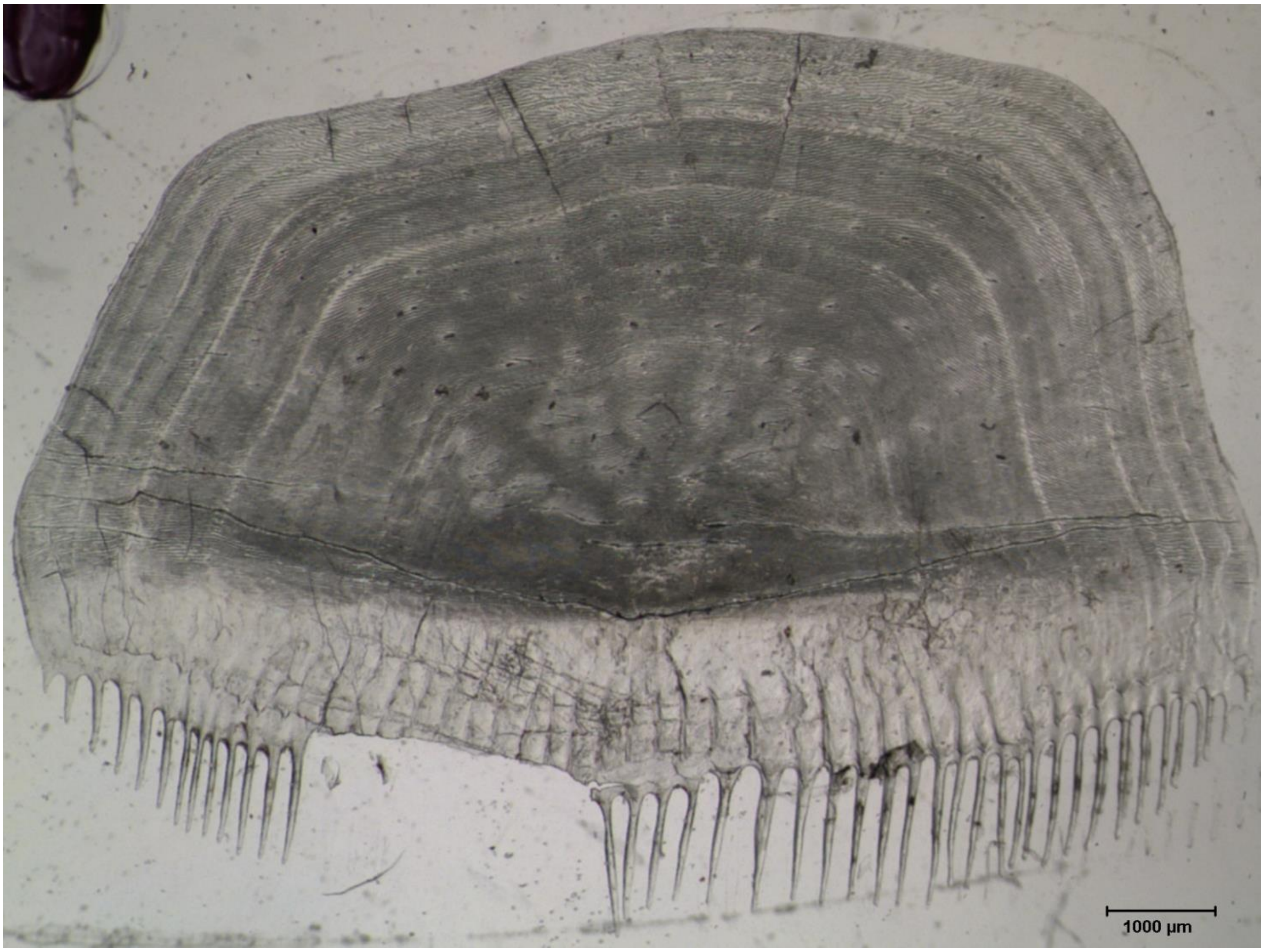

Atlantic Menhaden 19 7/8/2014