Lake Superior Fish Age Assessment Workshop
Great Lakes Fishery Commission

Ramada, Marquette, MI, January 9 – 10, 2023

WORKSHOP REPORT

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DISCLAIMER

The research summaries presented in Appendices 1-5 are simply the notes of the compilers of this report; therefore, the information herein should be considered preliminary and should not be cited (See Appendix 1 for a list of participants). In addition, this report represents a compilation of the material presented and discussed at the Lake Superior Fish Age Measurement Workshop; therefore, it does not reflect the views or policies of the writers, the Great Lakes Fishery Commission, or any other agency.

WORKSHOP OVERVIEW

A review of age assignment procedures used by various management agencies in Lake Superior was held in a workshop on January 9th and 10th, Marquette, MI. Specifically, the goals of this meeting were to update the Lake Superior Technical Committee (LSTC) fish aging manual (Schreiner and Schram, 2000), and to review and standardize age measurement methods for key Lake Superior species (i.e., lake trout *Salvelinus namaycush* and Coregonines), including the organization of a cloud-based species-specific LSTC age structure reference collection(s). The push to adopt rigorous species-specific standardized age assessment protocols arises from the desire to reduce bias in age assignment and to improve the consistency of age interpretations used in multi-agency stock assessments (e.g., Lake Trout Statistical Catch at Age models). Thirty attendees representing 12 state, provincial, federal, and tribal governments participated, Appendix 1. Dan Isermann, University of Wisconsin Stevens Point, was invited to present on the foundations of the *Age Measurement Process*, with the remaining eight presentations, focused on various aspects of fish age measurement, presented by workshop organizers.

The first presentation (*Importance of Age in Fisheries Assessment Programs*, Mike Seider) addressed the importance of high-quality age estimation data in fisheries assessment programs. Specifically, how the introduction of error from the processing and/or interpretation of age structures can have significant impacts on the results of analyses. Biased age estimation data have sometimes led managers to implement faulty decisions which have had negative ramifications on Great Lakes fish species. Well-developed age measurement protocols combined with analytical tools can help alleviate sources of error and improve the quality of analyses that incorporate age data. Additionally, a strong understanding of the calcified structures used to measure age in fish and the limitations of various techniques can help in the development of a well-designed age assessment program. For this reason, the next three presentations in the workshop focused on preparation, processing, and use of calcified structures in fish age estimation, highlighting the pros and cons of the most used structures. Discussion surrounding these presentations focused on identifying the age structure(s) and procedure(s) best suited to measure the age of key Lake Superior fish species. For example, thin sectioned otoliths read with transmitted light microscopy was suggested as the best method for Coregonines. It is important that when changing the age structure and/or preparation method for a species, a transitional evaluation be conducted with age assignments of paired-structures (from the same fish) that covers the size and age range of the species. This allows a measure of the bias or error of the older method or structure(s).

The morning of January 10th included presentations focused on sample size determination. Stratifying smaller sampling events to achieve greater spatiotemporal coverage better represents fish populations than large samples collected from a limited number of locations and events. Furthermore, study design should take into consideration a species' life history to account for seasonal variations in fish distribution to ensure samples are representative of the population. Documented and updated age measurement protocols help to ensure the quality of data produced during production remains constant across time. A well-developed protocol will cover topics ranging from structure preparation to age assignment decision rules. Of the subjects covered in an age measurement protocol the detailed description of quality assurance and quality control (QA/QC) procedures may be arguably the most important. Pre-production QA/QC, in particular the annual review of reference collections to recalibrate readers, helps to both eliminate individual bias and improve precision of estimates. Just as with sampling design, reference collection development needs careful thought to ensure it represents the sampled population. The development of lake-wide, species-specific reference collections may help reduce agency-level biases. Cloud-based reference collections would facilitate access, improving the ease with which agencies could train new employees and annually test existing readers. The workshop ended with an hour and a half discussion of cloud-based reference collection(s) as well as standardizing the structures used to measure age of various key Lake Superior fish species. The standardization of age measurement protocols for various important species should ultimately improve the quality of data used to guide fisheries management decisions in Lake Superior.

AGENDA - FISH AGE ASSESSMENT WORKSHOP II

Lake Superior Technical Committee
Ramada, Marquette, Michigan January 09-10, 2023

Time	Presentation	Speaker
Monday, January 9, 2023		
1:00 PM	Introductions and summary of previous workshops	Shawn Sitar
1:20 PM	1.Importance of Age in Fisheries Assessment Programs	Mike Seider
1:40 PM	2.Age Measurement Process Overview	Dan Isermann
2:20 PM	3.Otolith Extraction, Cleaning, Sectioning, and Reading	Dan Traynor and Lydia Doerr
3:00 PM	**Break**	
3:20 PM	4.Maxilla Extraction, Cleaning, Sectioning, and Reading	Dan Traynor and Lydia Doerr
4:50 PM	Closing discussion	
Tuesday, January 10, 2023		
8:00 AM	Quality Assurance and Control in Age Assessment Programs	Shawn Sitar
8:30 AM	Production Age Measurement Process	Shawn Sitar
8:50 AM	Automation and Efficiencies for Age	Ian Harding
9:15 AM	Age structure Collection in Survey Programs	Mike Seider
9:35 AM	**Break**	
9:55 AM	Discussion on Standardization: LSTC Reference Collection Archive (i.e., Cloud Based)	
10:30 AM	Workshop Summary and Future Research and Development	

WORKSHOP PROCEEDINGS

1. *Importance of Age in Fisheries Assessment Programs* - Mike Seider, US Fish and Wildlife Service

The closeness of estimates to the true age (i.e., accuracy) and the reproducibility of repeated measurements for a single structure (i.e., precision) represent two types of error found in fish age estimation. These two error types need to be considered in tandem, as it is possible to maintain a high degree of precision but low accuracy and vice versa. When developing age measurement protocols for a specific species of fish it is important to use validated structures that capture the complete growth sequence. The failure to do this introduces processing error which often can lead to over or underestimation of ages. Conversely, interpretation error is created by the subjectivity of readers, originating with the preparation and interpretation of periodic features in calcified structures. Interpretation error can be systematic or random and vary markedly among readers and laboratories (Campana, 2001). The standardization of the processing and use of validated age structures coupled with the adherence to a well-developed quality assurance and control training procedure can help to monitor and mitigate sources of error. Age assignment errors can have significant impact on the outcome of analyses used to assess population dynamics. The systematic underestimation of fish ages can affect projections of recruitment, maturity, growth, and survival. For example, Yule et al. (2008) demonstrated that the historical perspective of cisco *Coregonus artedi* survival was likely incorrect due to systematic under estimation of scale-based ages, leading to a false understanding of stock resiliency. Age error matrices can help account for biased data, such as providing a correction for scale-based estimates. For stock assessment models to be informative, age data (e.g., length at age, weight at age, mortality at age, etc.) needs to be accurate and consistent across agencies. Inconsistencies in age data can bias spatial and temporal comparisons of population metrics (e.g., growth and maturity) leading to potential overharvest or unnecessary limitations on fishing opportunities. The goal of this workshop was to identify and discuss methods/techniques that can be adopted or refined to diminish age estimation error. The adoption of a well-documented standardized approach, founded in evidence-based science, by agencies across Lake Superior will diminish systematic biases, improving the quality of the data guiding important fisheries management decisions.

Discussion

The post-presentation discussion focused on the use and creation of age error correction matrices which are species, age structure, and preparation method dependent. Correction matrices are very important but need careful development because they can add more errors if not properly constructed. Age error correction matrices are commonly used to modify historical data, allowing its incorporation into updated and/or new population analyses. For example, the original lake trout correction matrix was created to address error associated with scale-based age estimates. Although it was created using known age fish it has become difficult and/or inappropriate to use for two main reasons. First, since the creation of the matrix, environmental conditions in Lake Superior have changed leading to shifts in lake trout growth patterns. Since lake trout stocking has largely ceased throughout Lake Superior there is a lack of data from known age fish that could be used to update the age correction matrix. Second, the matrices were created from hatchery reared individuals, and the growth of captive bred lake trout differs from wild individuals introducing biases. One possibility would be to update the correction matrix using consensus-based age assignments for wild caught lake trout from reference collection(s).

This would require a careful systematic approach using a validated age assignment method. For instance, readers would be required to pass a test before production age measurements, as this calibrates the reader by increasing the precision of age assignments. Scales are not appropriate to measure age of lake trout in Lake Superior and those agencies still using scale-based age estimates are urged to use maxillae and/or otoliths. There is abundant evidence that the latter two methods are not only easier to interpret but provide more accurate age estimates for lake trout. Furthermore, sectioned otoliths and maxilla are validated techniques (Campana et al., 2008; Wellenkamp et al., 2015).

2. *Age Measurement Process* - Dan Isermann, University of Wisconsin Stevens Point

The development of a standardized operating procedure (SOP) for age measurement is important, as it both records the method used to age a species as well as allows others to replicate and interpret results. The structures used to age fish and their interpretation can vary among species, meaning SOPs need to be species specific. An excellent illustration of this is the handling of edge and plus growth during age assignment, as it can vary from agency to agency and if not recorded it can create uncertainty when using shared data. At the USGS Wisconsin Cooperative Fishery Research Lab it is handled by recording both the number of annuli counted and the age assigned. For example, the age assignment may be one higher than the number of annuli present in spring captured fish where the outer edge is counted. Commonly, SOPs will include capturing images of the fish aging structure measured, and when this occurs a scale bar needs to be present, as this helps to: 1) create a baseline that facilitates switching between imaging software; 2) allows recalibration in case of equipment drift or accidental manipulation of settings; and 3) permits the use of images in future analysis (e.g., back calculation) (Isermann and Quist, 2017).

Each of the five calcified structures (i.e., scale, otolith, spine/ray, cleithra and operculum) commonly used in age estimation have their own pros and cons. While scales, maxillae, spines, and rays all have the benefit of being non-lethal, they tend to underrepresent the true age of fish for two reasons. First, the outer edge of these structures can become compressed obscuring annuli, due to the slower somatic growth of older fish. Second, it can be difficult to identify the first annulus particularly in spines and rays. In some species of fish, the first anulus can be obscured by a central lumen (i.e., a hole that develops in the center of the structure). For each species the location of the first annulus needs to be validated. Misinterpretation of the first annulus is a common issue when structures are read at high magnification, which can be avoided by taking images at a consistent level of magnification. Although the identification of the first annulus in otoliths can be challenging, overall this structure best captures the complete growth sequence. The main limitation to the use of otoliths is that their collection is lethal to the fish. In addition to reading polished otoliths whole to produce age estimates, they can be cracked or sectioned through the nucleus. While thin sectioned otoliths are read with a transmitted light microscope, cracked otoliths can be read with either a fiberoptic light or can be burned to contrast the annuli.

Prior to production level age measurement, it is essential to validate that the age structure utilized accurately captures the complete growth sequence of that species. Although known-age fish are extremely beneficial to age validation, variability and/or error can be introduced if individuals are hatchery reared. For instance, hatchery reared salmonids can have different growth and annuli formation than wild individuals. Once a structure has been validated the development of a SOP and reference collection can improve the quality of age estimates. Specifically, it can help to avoid age measurement error associated with personnel changes, such as the retirement of a long-term reader(s). Additionally, ensuring structures are blind read (i.e., randomized and no information provided to reader) and accuracy checks, by including known/consensus aged structures, can both help to ensure the quality of age estimates.

Discussion

Methods to mitigate both process (intrinsic variation in age structure) and interpretation (measurement) error were the first topics addressed during the discussion of Dan Isermann's presentation. Not all populations and/or species of fish, such as brook trout *Salvelinus fontinalis*, produce interpretable or complete growth sequences. This means process error needs to be carefully considered when establishing a protocol for production age assessment. When establishing an age measurement protocol, it is important to validate the structures used at a population level, especially for species that are known to be difficult to age, because environmental conditions are known to alter the development and clarity of annuli. Often, for species with difficult to read age structures, it is necessary to evaluate multiple structures to identify those that provide the most reliable estimate. For some species that have high longevity, such as lake trout, it is possible to use a dual structure approach. Maxilla have been reported to be reliable for smaller younger fish (lean ≤ 649 mm; siscowet ≤ 576 mm) while otoliths provide a more accurate estimate for larger older individuals (lean ≥ 650 mm; siscowet ≥ 575 mm). Even if an age structure is validated, it is necessary to periodically reevaluate the reliability of that structure as environmental fluctuations may have altered growth patterns.

Mitigating interpretation error (e.g., reader drift that causes loss of precision and over magnification of structures during age estimation) can be just as challenging as addressing process error. A well-developed QA/QC program can help to control much of the error introduced by the reader during production age measurements. Among other topics, QA/QC protocols could include guidelines for capturing and cataloging images of age structures. For example, structures could be photographed at a consistent level of magnification and this information along with a scale bar should be included on the saved image. Where possible during production age measurement, ages should be generated from live read structures, as some information required for accurate estimates can be lost in images. To provide clarity to readers, QA/QC protocols can also include decision rules, such as how to handle edge assignment. The development of decision rules regarding marginal increments should be species and/or ecotype specific as the formation of annuli are life history dependent. For species where full-year monitoring is possible (i.e., commercial lake whitefish *Coregonus clupeaformis*) samples can be used to determine when the marginal increment appears, which then could be used to develop the edge assignment decision rule. Additionally, when possible, it is best to avoid sampling during the transition period when the last annulus is becoming visible.

In addition to guidelines for annuli interpretation, QA/QC protocols can include a species-specific reference collection of known and/or consensus-age fish. Due to the influence that environmental conditions have on annuli formation, reference collections should be system specific. For example, a Lake Superior lake trout reference collection should include multiple regions, ages, and ecotypes, unless ecotype specific collections are developed. Then, if desired, individual labs can develop specific collections to train readers on issues seen in age measurement structures collected from their surveys. If an experienced reader generates the same age estimate from an image then associated age structure photographs can be included in reference collections. Besides training new readers these collections should be regularly reviewed by all readers to help prevent drift. Drift can further be prevented by preproduction age measurement tests, with the estimates for readers being required to fall within an acceptable coefficient of variation (CV), error range for a particular structure, or the collection as a whole. As best as possible, reference collections should include the full spectrum of ages and interpretability that readers can expect to encounter while measuring the age of wild caught samples. During testing older and/or less legible structures can have a different level of acceptable error than easily aged fish. For instance, you may expect estimates for easily aged individuals to be exact, whereas as for difficult structures estimates maybe acceptable if they fall within 2-3 years of the accepted age. This allows readers to train or refresh themselves on challenging structures without compromising the results of their test due to a high level of error.

3. ***Otolith Extraction, Cleaning, Sectioning, and Reading*** - Dan Traynor and Lydia Doerr Michigan Department of Natural Resources

Interpretation error can be significantly impacted by otolith preparation and thus the process requires careful consideration. Michigan Department of Natural Resources employees based at the Marquette Fisheries Research Station (MFRS) have put considerable time and resources into identifying the otolith preparation method that consistently provides the most accurate age estimation for lake trout and Coregonines. Specifically, thin sectioned otoliths embedded in clear epoxy has been identified as the method that provides the most interpretable age measurement plane. A summary of the method is provided here but for a complete description see the videos posted on the MFRS YouTube channel (https://youtube.com/@marquettefisheriesresearch2992?si=TunNIDATniBz4zap; Traynor 2021a, 2021b).

Initially, a thin layer of West System® epoxy (105 resin, 207 special clear hardener) was added to the Cells of SPI® 2443 silicon mold(s)and allowed to cure for 20 - 24 hours. Otoliths were then placed sulcus side up, with the ventral end extending upward into the point of the bullet shaped cell. The cells were then filled with epoxy ensuring the surface was level and bubbles were removed with a fine tip probe. Again, epoxy was allowed to harden fully before blocks were removed for sectioning with a Buehler low speed IsoMet® saw. Although otoliths can be sectioned using a single blade, staff at the MFRS prefer a duel blade setup, as this process reduces the number of adjustments, and cuts required. Dado blade spacers are used to create a section approximately 0.64mm in width. The pointed end of the epoxy block was secured in the chuck perpendicular to the blades and the micrometer was used to position the nucleus of the otolith over the center of the blades. Additionally, the block should form a 90-degree angle with the chuck. The chuck was then lowered onto the slowly running saw blade. The saw speed was then increased as the block was pressed into the blades by the weighted arm. Once the block was cut approximately three quarters of the way through the blade speed was slowed, as this prevented the section from being lost once the cut was complete (Traynor 2021b).

Both sides of the section were sanded using 400 grit sandpaper, which removed blade marks, better exposed the nucleus, and increased the amount of light that passed through the otolith. Thin sections were placed in mineral oil and initially viewed at 30x magnification on a Nikon SMZ1000 dissecting microscope. Magnification was adjusted as needed to ensure the most accurate age estimate. Otoliths were read along four axes. Two axes run from the nucleus to the outer edge along either side of the sulcus, while the others extend from the nucleus to the outer edge along the ventral and dorsal plains of the otolith, Figure 1. With transmitted light microscopy, annuli are thick translucent bands that occur at consistent intervals across the surface of the otolith. Translucent bands that split or are incomplete are considered checks and/or false anulus and therefore should not be included in the age estimate, Figure 2. Finalized age estimates were generated by counting annuli along the clearest axes on both sides of the structure. In lake trout, the two axes on the dorsal side of the sulcus typically provide the clearest planes to measure age.

Discussion

Age estimation error can be significantly impacted by otolith preparation. Each technique used to prepare structures for age measurement introduces its own set of issues, such as the risk of sanding off marginal increment(s) during whole otolith polishing. Even thin sectioned otoliths, viewed by many as the best preparation method, can introduce error if care is not taken during processing. A species/structure specific SOP and/or age measurement protocol can help mitigate error by establishing comprehensive guidelines. In addition to reducing error associated with using varying methods of otolith preparation, an interagency age measurement protocol could provide guidance on the materials and techniques that provide the most reliable results. A consistent technique may help to reduce issues that some agencies have with incorrectly cured epoxy and otoliths shattering because of exceedingly fast sectioning speeds.

4. *Maxilla Extraction, Cleaning, Sectioning, and Reading* - Dan Traynor and Lydia Doerr Michigan Department of Natural Resources

Maxilla are used to age smaller lean (≤650 mm) and siscowet (≤575 mm) lake trout at the MFRS, as this method has proven reliable for smaller faster growing lake trout when compared to otoliths. Although maxilla processing is less involved than that for otoliths, it still requires careful consideration as it can have a significant impact on interpretation error. The method summarized here was adapted from Wellenkamp et al. (2015) and full video descriptions are available on the MFRS YouTube channel (Traynor, 2021c).

Prior to sectioning, maxilla were boiled to remove desiccated skin, ensuring the outermost annulus was visible during age measurement. A securely mounted Dremel® tool, equipped with a Damascus silicon carbide separating disc (Cas-Ker Company) was used to section maxilla. Maxilla were clamped to a multidirectional adjustable sliding jig used to control section width and steady the structure during cuts, Figure 3. Once the maxilla was secured perpendicular to the blade, four cuts were made. The first cut removed the knuckle, which is the upturned portion of the maxilla that fits into the joint on the premaxilla. For the next three cuts the turnbuckle was adjusted laterally to create sections that ranged from 0.01 to 0.4 mm in width, Figure 4.

Maxilla thin sections usually do not require sanding, so are placed directly in mineral oil, and initially viewed at 30x magnification on a Nikon SMZ1000 dissecting microscope. For some fish, age interpretation was enhanced through manipulating the magnification and/or structure (i.e., tilting the structure using forceps). Similar to otoliths, with transmitted light microscopy, annuli in maxilla are light translucent bands on a dark background, distinguishable from checks and split annuli due to their consistent sizing. Annuli counts were conducted along the medial and lateral radii. The medial radius extends roughly 40 degrees from the nucleus to the medial edge, whereas the lateral radius extends approximately 150 degrees from the nucleus to the lateral edge, Figure 5. Age estimates were based on counts from the clearest radius that had the most annuli.

5. ***Quality Assurance and Control in Age Assessment Programs*** - Shawn Sitar, Michigan Department of Natural Resources

The age assessment program employed at the MFRS was based on recommendations reported by Campana (2001), as this work provides a reliable framework for systematic age estimation of Lake Superior fishes. For an age assessment program to be considered successful it must: validate that the periodic increments observed in calcified structures; accurately reflect fish age; identify the method(s) of structure preparation that provides the most interpretable outcome; include a reference collection of known or consensus aged fish to train and test readers; and conduct regular quality control assessments to ensure quality age estimates. Although all four of these components are required for an age assessment program to be considered successful, quality control may be arguably the most important aspect. A well-developed quality control program can help maintain high levels of precision and accuracy by preventing long-term age estimation drift through recalibrating existing staff and training new readers. Quality control protocols are founded on statistical evaluation, such as age-bias graphs, average percent error (APE), and CV, of age estimates by readers from an archived reference collection of known age or consensus high precision age structures. Readers undergo a calibration trial (test) by reading a sub-sample of structures from the reference collection and results are assessed in terms of the statistical benchmarks (e.g., CV, APE) to acceptable precision levels before moving on to production age assessment. Long-term tracking of quality control metrics can help identify both age estimation biases and drift in age estimates.

In addition to being a key component of quality control programs, reference collections can decrease cost by eliminating the need for a second reader. By calibrating readers to a shared standard, an interagency cloud-based reference collection would help prevent agency specific biases, thus improving the quality of age data. Structures included in a reference collection need to be representative of the fish populations being sampled, which means they should include individuals of all sizes collected across the possible geographic range during multiple seasons and years. Development of a reference collection consists of four steps. First, a pool of structures from known or consensus-age fish is gathered. For example, candidate fish could be known-age hatchery reared fish with more than one read in agreement with hatchery records of year-class or wild caught fish with two or more identical age estimates that are judged to be reliable. Secondly, candidate age structures are read by experienced readers. Third, candidate structures are retained if results of two or more age assignments from experienced readers is consistent. Fourth, continued monitoring of accumulated estimates for each structure and modification of the collection as needed. Regular review of reference collection(s) can help to ensure the samples have high precision and are useful to calibrate readers to conduct production age measurements. Reference collections are also important teaching aids during the training of new readers. Specifically, these collections can be used by experienced staff for instruction and to test the new reader to ensure they are ready to participate in production age measurements. Regardless of the readers experience the criteria for passing a reference collection review remains the same. For example, the criteria used at the MFRS requires that 50% or more of age estimates are correct, while an additional 25% must be within one year of the known/consensus age. Reader's age estimates also must be normally distributed with a mean of zero and a standard deviation of less than or equal to 0.8.

Although reference collection(s) and regular monitoring of quality control are key components of a successful age assessment program, the development and adherence to an age measurement protocol is also critical. These protocols should explicitly detail the procedures for both quality control and production age measurements. Document(s) should also include definitions of artifacts that are encountered during age measurement such as annuli, checks, and marginal growth increments. As an example, Appendix 3 provides a copy of the MFRS lake trout otolith age measurement protocol. Once an age measurement protocol has been established and readers have been tested, production level age measurements can begin. At the MFRS, sub-sampled structures (i.e., 20 samples per 25 mm length group per Michigan management unit) are divided among four readers and read once. During production age estimation, age measurement estimates from each reader are randomly checked to monitor for drift. Unique age measurement protocols, which include distinct reference collections, should be developed for each structure(s) used to age a species. It is the responsibility of the individuals in charge of an agency's age measurement protocol to monitor advancements in fish age assessment, testing new techniques and adopting them when they show to be an improvement over current method(s).

Discussion

Reference collections and inclusion of poor-quality structures or old fish was the center of the post presentation discussion. Specifically, individuals were concerned that excluding structures that were difficult to age causes reference collections to be non-representative of possible structures a reader may expect to experience during production age measurements. Currently, it is common to exclude poor quality age structures from reference collections because it decreases the probability of a reader passing a quality control test. This is because the difficulty of assigning ages in these structures makes it problematic to produce a precise age estimate, which would cause higher error. One possible solution is rather than expecting readers to accurately estimate the age of difficult structures, an acceptable range of age estimates could be developed for each structure. This method provides readers with a more realistic review of the age structures they will encounter in production samples without compromising their ability to pass quality control tests.

6. ***Automation and efficiencies for Age assessment programs*** - Ian Harding, Red Cliff Band of Lake Superior Chippewa

Ian Harding demonstrated software tools he developed to streamline data collection and analysis of production aging data and quality control evaluation. Relational database software (e.g., Microsoft Access) was used to construct relational databases that use unique identifiers to relate fields from multiple data tables (e.g., site data, bio data, age data, etc.). Also, the same software was used to create user interfaces to simplify quality control data entry. For example, aging structures collected by Red Cliff are uniquely and serially labeled. When an aging structure is processed for age interpretation by Red Cliff staff, the staff enters the serial ID in the user interface and information on the capture date and species is shown to help the reader make a decision on edge determination. Data regarding the age interpretation (e.g., reader name, date of the interpretation, age interpretation, edge determination, confidence, etc.) is entered in clearly defined fields that have specified data types (e.g., numeric, text, etc.), and includes drop down fields (e.g., structure: otolith, maxillae), yes or no check boxes (e.g., was a picture taken), and auto fill (e.g., date of the interpretation). Images are saved to a consistent directory and with a check box can be hyperlinked to the database and displayed on the user interface. Hyperlinking pictures to the database allows Red Cliff staff to view pictures and age interpretation data on the same interface making it easier to quality check staff's age interpretations. Because the data is entered in a relational database, data can be efficiently queried for analyses. Another advantage of relational database software (e.g., Microsoft Access) is that data can be entered by multiple users simultaneously.

Similarly, Ian demonstrated a database software tool for quality assurance. Staff to be trained/tested on age interpretation open a user interface, select a species and structure to train on, and a random subset of the reference collection is queried. The species, structure, capture date, and image are displayed. The image can be opened in a separate window to enlarge/magnify the image. The staff enters their age interpretation which is logged in the database. Ian then uses a separate interface for evaluating the staff's age interpretations. The reader and date are selected from drop-down menus and analyses (e.g., APE, observed and expected interpretations by structure, etc.) specific to that reader and date are displayed. Alternatively, the database can be linked to statistical software (e.g., program R) to automatically generate plots, tables, etc. that are useful for assessing accuracy, precision, and bias.

Discussion

Discussion focused on how an automated system may help streamline the development of a cloud-based species-specific interagency reference collection(s). By dramatically increasing the number of times a structure has been read, this shared collection could help to evaluate trends in age estimation observed over time and across Lake Superior management agencies. As mentioned previously a shared reference collection will allow consistent calibration across Lake Superior readers, eliminating agency specific biases, thus improving the quality of age data. A common reference collection among agencies could improve how transitions are made when readers join or retire from various agencies. Specifically, a cloud-based collection could be used to both assess the precision of new reader(s), as well as that of existing experienced staff. For each species, a structure-specific collection would need to be developed that would be consistent for each method conducted. To evaluate possible alternative methods of age measurement, a paired structure analysis could be employed. By comparing the results of the alternative method to those from the established technique it would be possible to identify limitations of the alternate technique, such as only producing accurate estimates for fish under a specific size.

7. ***Age structure Collection in Survey Programs*** - Mike Seider, US Fish and Wildlife Service

The primary goal of age assessment programs is to produce accurate age estimates with minimal error and bias. Although age assessment programs primarily focus on reducing error associated with artifacts in age structures and their interpretation, project leaders also need to consider the impact from sampling error and bias. Addressing sampling error and bias requires a wholistic approach, as these two issues are often linked in sampling designs. Sampling bias can be introduced by gear selectivity, sampling procedures, time, and design of surveys. It is common to want to maximize the number of samples collected during a single event but due to the correlated nature of this data this approach can introduce bias. Specifically, the schooling nature of many fish species means samples collected together tend to have similar age and size composition (i.e., highly correlated). So rather than collecting most samples on one or two occasions it is recommended to distribute samples across multiple locations and events, collecting a limited number of individuals from each site. For example, when sampling commercially harvested species, collect fewer fish from more boxes and spread sampling events across as many fishers as feasible.

Sampling bias is more related to survey design, whereas sampling error is associated with sample size and selection. Optimal sample size is impacted by life history, as longer-lived species with a higher number of cohorts will require a larger sample size than shorter lived species. It is suggested to collect many samples from multiple locations and surveys, then select a subset of structures for production age measurement. Results can then be used to create an age-length key (ALK), which can be used to assign ages to fish based on length. ALKs should be specific to not only the species but to the survey and year of collection, as environmental changes can impact the relationship between fish length and age.

Discussion

In addition to addressing questions regarding ALK development and sampling design, the discussion covered the potential adoption and use of new technologies. Specifically, due to recent advancements in forestry it may be possible to adopt dendrochronology software to assist with back calculations of growth from historic samples or increment counting. This method could be potentially useful to examine how changes in environmental condition are reflected in the growth patterns of long-lived species (i.e., lake trout and lake sturgeon *Acipenser fulvescens*). Although this method would require the collection of fewer samples, it is model intensive, which may limit its wide-scale adoption. As technology improves across various disciplines it may offer an unexpected boon to fisheries management, which is why managers and researchers alike need to accept and trial new approaches.

Even with well-accepted methods, advancements in technology can improve the ease in which calculations are made. For instance, software such as the statistical program R allows the construction of code that facilitates the easy calculation of annual ALKs from multiple years of data. The results of such an analysis could highlight the time scale at which the relationship between age and length changes and how it may vary depending on a species life history. To prevent the introduction of error, ALK must be representative of the entire population it is applied to. Due to the large population of many Lake Superior fishes, concerns about oversampling are unwarranted, rather sampling design should focus on maximizing spatiotemporal coverage. A species' life history also needs to be considered when designing a sampling regime. For example, the goal of the sampling design for commercial lake whitefish monitoring should be to collect specimens in proportion to monthly harvest levels. This method will help ensure proper representation of harvest, thus ensuring the reliability of ALKs.

8. Overall Workshop Discussion

The overall objective of this LSTC workshop was to standardize age assessment among all fisheries agencies sampling in Lake Superior. The key benefit of standardizing age assessments is to ensure quality of shared data by minimizing agency-specific errors and biases in age measurements. The introduction of bias, be it from age structure processing or interpretation, can have cascading implications. For example, by affecting the reliability of analyses, biased age data could impact the regions socioeconomics by misdirecting fish management decisions. Concerns were expressed that the current Great Lakes Fishery Commission Lake Superior Aging Manual (Schreiner and Schram, 2000) has become obsolete, as it no longer reflects the best methods(s) for age estimation for many species. The manual recommends crack-and-burn for interpreting ages for Coregonines, but some agencies have adopted thin sectioned otoliths because they have found this method to be more interpretable. This highlights the need for more regular workshops, where advancements in methodologies can be shared and discussed. If a hands-on component were included in these workshops, they could become important training events for new employees.

The results of a survey sent out prior to this workshop emphasized the lack of standardization in structure preparation and age measurement technique(s) used for various species (Appendix 2). For example, there was a lack of consensus on the method(s) and/or structure(s) used to age each of the eleven species identified in the survey. The adoption of a species-specific protocol must be done methodically. Agencies considering an alternative age measurement method should conduct paired structure analysis, such as comparing the estimates from thin-sectioned and crack-and-burn otoliths. The results of such analyses can be used to update historical data through the creation of an age correction matrix. This type of work is likely best achieved by a small task group which can share results with agencies across Lake Superior.

The task group could be responsible for compiling a dedicated age measurement protocol for species of interest, that would outline everything from structure processing to marginal increment decision rules. The development of QA/QC procedures for each structure aged for a species should be included in this process. Specifically, the QA/QC documents would detail the statistics and methods used for pre-production reader assessments. To address issues associated with interagency age measurement bias the QA/QC procedures should include a common reference collection. Shared collections would ensure all agencies are participating in QA/QC procedures helping to alleviate previously discussed issues with age bias. Furthermore, the cost of developing reference collections, as well as age measurement protocols as a whole, would be shared if the task group consists of individuals from multiple agencies. This may be particularly useful for smaller, understaffed and/or underfunded agencies, which lack the resources to develop their own reference collection(s) and age measurement protocol(s). During discussions surrounding this topic it was mentioned that some offices may be limited to the use of certain age structures because they lack the funding to purchase new equipment that would allow them to use an alternative method(s). Such limitations and solutions to address them need to be considered during the development of the standardized Lake Superior age measurement protocols.

The most convenient format for a shared reference collection would be a catalog of high-resolution cloud-based images, as this format would facilitate wide-scale use by Lake Superior fish management agencies. To address concerns about bias associated with image-based age estimates a paired structure analysis could be conducted. Age estimates should be conducted by experienced readers from multiple agencies for this analysis, as well as during development of reference collections. A possible starting point for a standardized Lake Superior reference collection and common age measurement protocol, would be the use of maxilla to measure age for smaller lake trout (lean < 650 mm; siscowet < 575 mm). Previous work at the MFRS has shown that an image-based maxilla reference collection provides reliable age estimates. Prior to wide-scale application, region-specific criteria may need to be developed for each ecotype because spatial variation in growth may prevent the use of a single length cut off across Lake Superior. Additionally, changes in environmental conditions, especially in the light of climate change, requires the periodic reevaluation of length cut off requirements to determine if an adjustment is necessary.

Similar to lake trout, maxilla may prove to be a reliable structure for brook trout age estimation. Brook trout scales are notoriously difficult to read as the reliability of periodic features in structures are significantly impacted by environmental conditions and individual growth trajectories. For this reason, it would be worthwhile to develop a research project designed to identify the structure(s) that provide the most reliable age estimates for Lake Superior brook trout. Another avenue of research worth exploring is the identification of the first annulus in otoliths. Staff at the MFRS have evaluated first annulus formation in lean lake trout to help guide readers in identifying where to expect the age-1 increment. Currently, staff with the Red Cliff Band of Lake Superior Chippewa are evaluating the first annulus in age-1 lake whitefish otoliths. To improve on this work, it is recommended to include additional year classes. The ideal manner to address this topic would be to develop funding for a graduate level project. In addition to collecting their own samples, fish could be provided by other agencies, such as the U.S. Geological Survey Great Lakes bottom trawl survey. It is likely that during the development of standardized Lake Superior age measurement protocols, additional topics, best addressed by research, will be identified. Conducting more frequent age estimation workshops would improve the quality of data used to guide fisheries management decisions in Lake Superior.

SUMMARY OF WORKSHOP RECOMMENDATIONS TO LSTC

Based on discussions following the workshop, the following set of recommendations were established for the LSTC:

- Standardize and document age structure preparation and measurement techniques (Table 1),
- Create LSTC agency protocol which includes best practices for structure preparation, training, QA/QC, and age assignment,
- Implement QA/QC process in the age assessment program,
- Establish a standard benchmark for precision of readers (e.g., CV, APE),
- Develop a reference collection for each age structure/preparation technique,
- Conduct research to define the size of the first annulus for each structure/species,
- Crack-and-burn was the preferred age measurement technique in the old (current) manual. Based on conversations during the workshop it may be that some, but not all agencies have switched to thin sectioning. This process needs further vetting and discussion as to whether it is appropriate.
- Adoption of Quist and Isermann (2017) *Age and Growth of Fishes Principles and Techniques* as the current standard reference for age estimation work.

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Wellenkamp, W., He, J.X., Vercnocke, D., 2015. Using Maxillae to Estimate Ages of Lake Trout. North Am. J. Fish. Manag. 35, 296–301. https://doi.org/10.1080/02755947.2014.1001045

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TABLES

Table 1. Lake Superior Technical Committee standard methods of age measurement for Lake Superior fishes.

Species	Structure	Preparation Method	Light Source	Applicable Lengths	Image vs Scope	Section Thickness
Brook trout	Scales	Dried	Transmitted	All	Scope	
Burbot	Otolith	Sectioned [a], Crack and burn	Transmitted, reflected	All	Scope	0.64 mm
Chinook salmon						
Cisco	Otolith	Sectioned [a], Crack and burn	Thin section -Transmitted, Crack and burn-Reflected	All	Scope	
Coho salmon						
Lake whitefish	Otolith	Sectioned [a], Crack and burn	Thin section -Transmitted, Crack and burn-Reflected	all	Scope	0.68 mm
Lake trout- humper	Otolith	Sectioned [a]	Transmitted	ALL	Scope	0.64 mm
Lake trout- lean	Maxilla	Sectioned [a]	Transmitted	≤ 625 mm (MI)	Image or scope	0.01 to 0.4 mm
Lake trout- lean	Otolith	Sectioned [a]	Transmitted	All	Scope	0.64 mm
Lake trout- lean	Otolith	Sectioned [a]	Transmitted	All	Scope	0.64 mm
Lake trout- siscowet	Otolith	Sectioned [a]	Transmitted	All	Scope	0.64 mm
Lake trout- siscowet	Maxilla	Sectioned [a]	Transmitted	≤ 575 mm (MI)	Scope	0.01 - 0.4 mm
Rainbow trout/steelhead						
Walleye	Dorsal spine	Sectioned [a]	Transmitted	All	Scope	.01 – 0.4 mm

[a] transverse plane

FIGURES

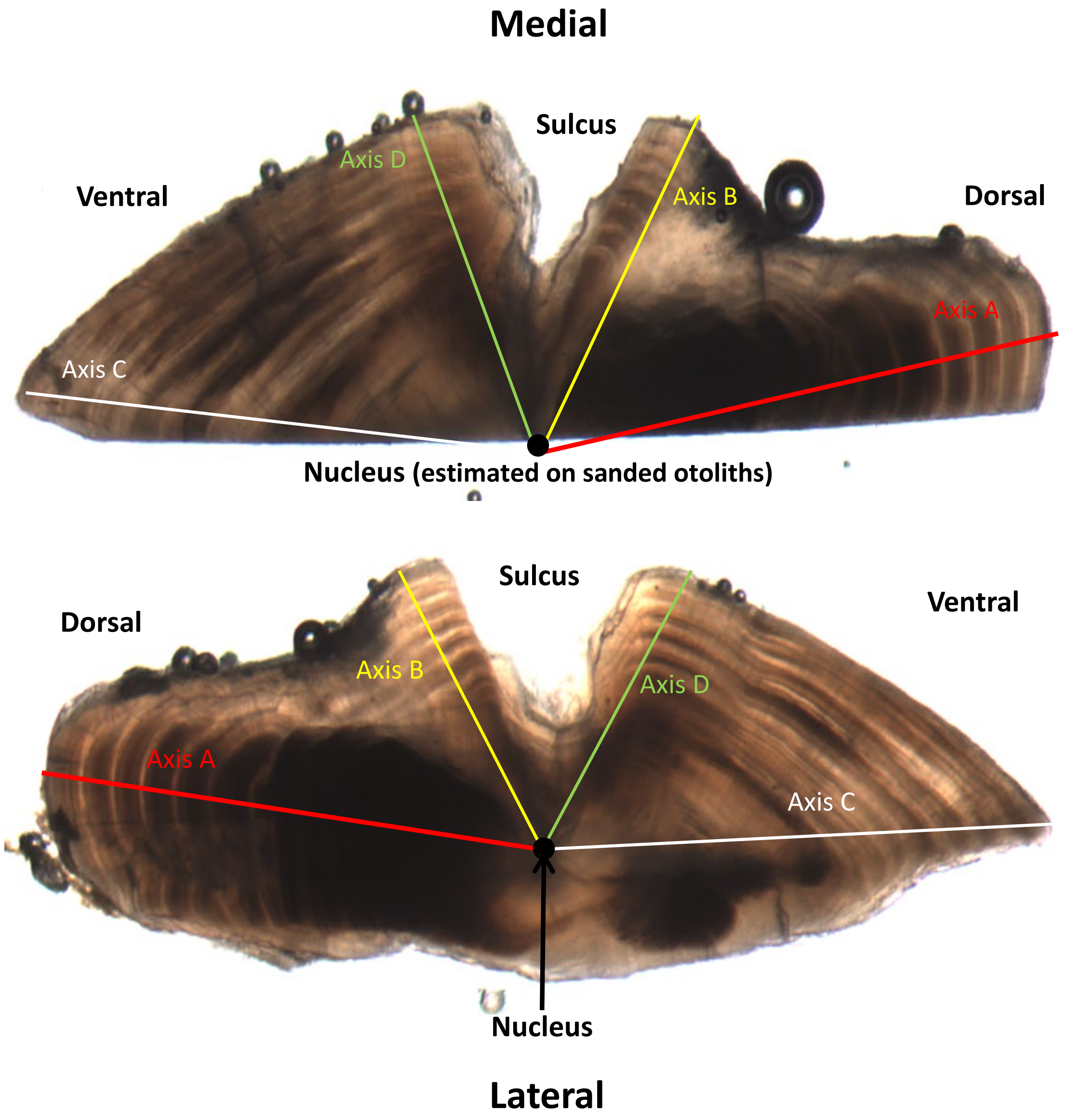


Figure 1. Lake trout sagittal otolith illustrating the four axes used during age estimation. The axes are as follows: A extends from 0 to 45 degrees on the dorsal side; B extends along the sulcus on the dorsal side; C extends from 0 to 45 degrees on the ventral side; and D extends along the sulcus on the ventral side. As the image demonstrates, the orientation of the dorsal and ventral plains will vary depending on which side of the section is examined.

Figure 2. Sagittal otolith from a 5-year-old lean lake trout collected during the summer, examined at 30x magnification using transmitted light microscopy. Annuli, indicated with white circles, appear as regular thick translucent bands, whereas checks are thinner irregular semi-translucent bands.

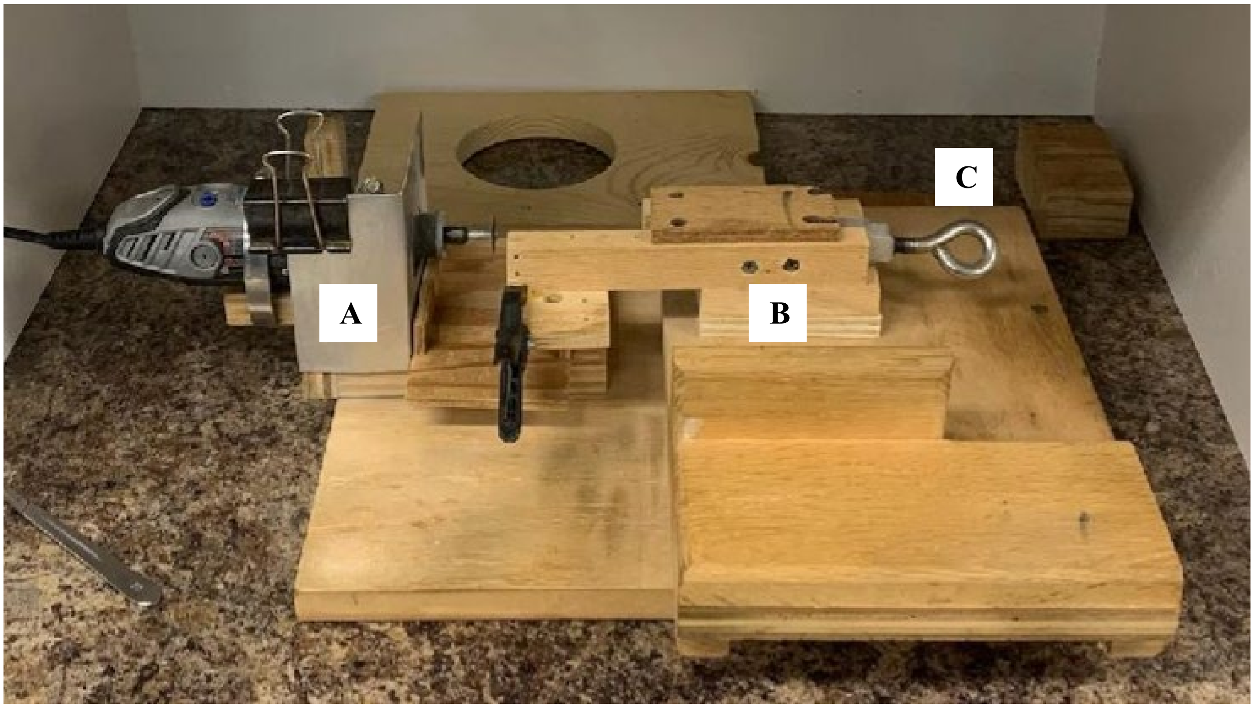


Figure 3**.** Maxilla and fin ray sectioning apparatus at the Michigan Department of Natural Resources Marquette Fisheries Research Station. The sectioning apparatus consists of two main parts, a securely mounted Dremel® tool (A) and a multidirectional adjustable sliding jig (B). In addition to controlling section width via a turnbuckle (C) the jig also steadies the structure during sectioning.

Figure 4. Lake trout maxilla demonstrating where sectioning occurs for age measurement. During sectioning the knuckle (black box) is removed first to provide a perpendicular cutting surface. This is followed by three cuts (dashed lines) that produce sections ranging from 0.1 mm to 0.4 mm in width.

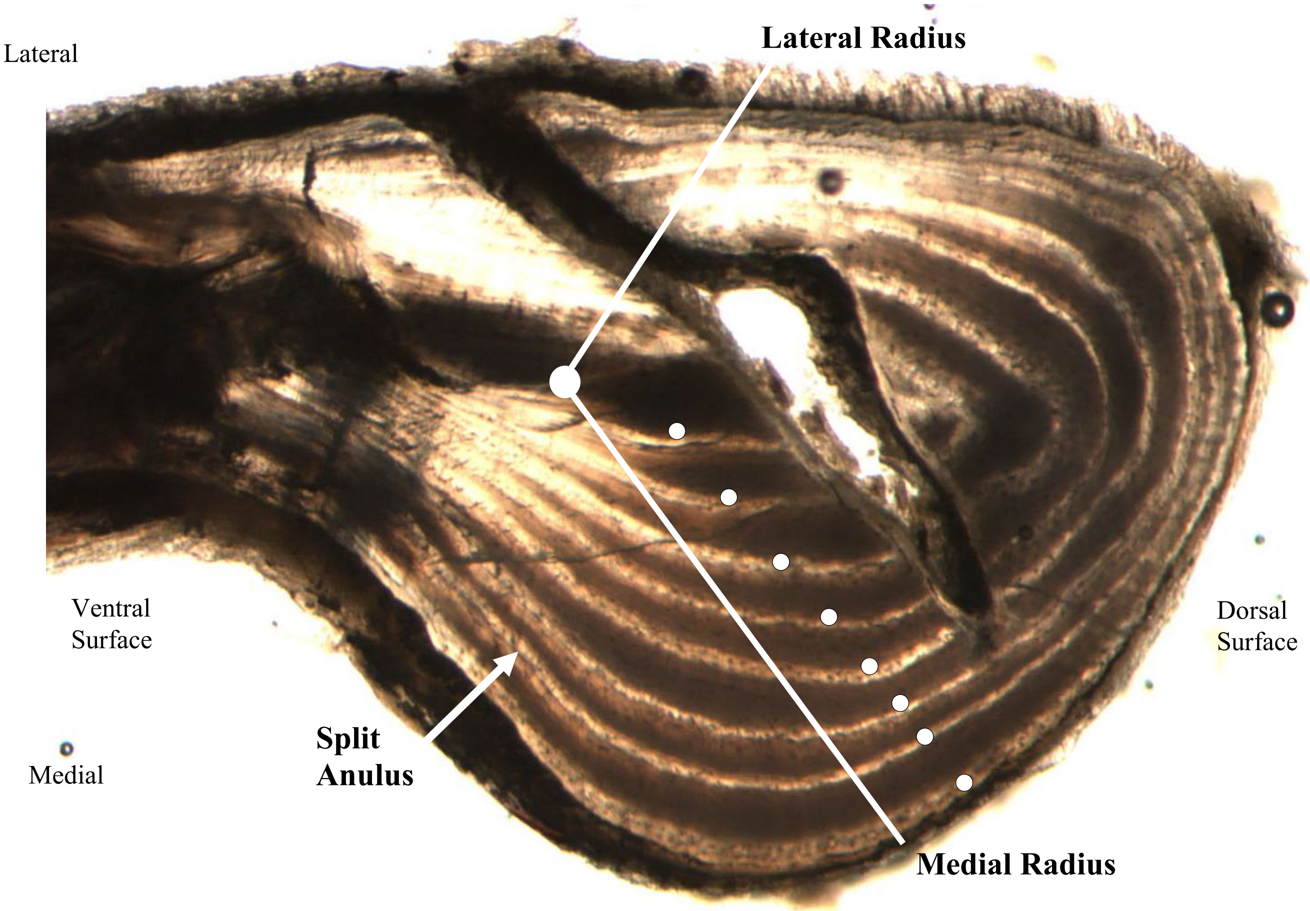


Figure 5. Maxillae thin section from an eight year old lean lake trout viewed at 30x magnification using transmitted light microscopy. On the dark surface of the maxillae annuli, indicated with white circles, appear as regular light bands, whereas split annuli are thinner irregular bands. Medial and lateral radii provide the best plains to conduct annuli counts along.

LIST OF APPENDIXES

Appendix 1. List of workshop participants.

Name		Agency
Nick	Boggo	1854 Treaty Authority
Eric	Torvinin	Fond du Lac Resource Management Division
Dane	LaGrew	Great Lakes Indian Fish & Wildlife Commission
Jake	Parisien	Great Lakes Indian Fish & Wildlife Commission
Mike	Plucinski	Great Lakes Indian Fish & Wildlife Commission
Patrick	LaGrew	Great Lakes Indian Fish & Wildlife Commission
Shane	Cramb	Great Lakes Indian Fish & Wildlife Commission
Patrick	LaPointe	Keweenaw Bay Indian Community
Shawn	Seppanen	Keweenaw Bay Indian Community
Connor	Johnson	Little River Band of Ottawa Indians
Tina	VanDoornik	Little River Band of Ottawa Indians
Dan	Traynor	Michigan Department of Natural Resources
Lydia	Doerr	Michigan Department of Natural Resources
Shawn	Sitar	Michigan Department of Natural Resources
Chris	Palvere	Minnesota Department of Natural Resources
Cory	Goldsworthy	Minnesota Department of Natural Resources
Ian	Harding	Red Cliff Band of Lake Superior Chippewa Indians Treaty and Natural Resources Division
Paige	Sutton	Red Cliff Band of Lake Superior Chippewa Indians Treaty and Natural Resources Division
Brad	Silet	Sault Ste. Marie Tribe of Chippewa Indians
Chase	Daiek	U.S. Fish and Wildlife Service
Francesco	Guzzo	U.S. Fish and Wildlife Service
Henry	Quinlan	U.S. Fish and Wildlife Service
Josh	Schloesser	U.S. Fish and Wildlife Service
Mike	Seider	U.S. Fish and Wildlife Service
Sarah	Mansfield	U.S. Fish and Wildlife Service
Shannon	Cressman	U.S. Fish and Wildlife Service
Steve	Shrer	U.S. Fish and Wildlife Service
Zach	Kleeman	U.S. Fish and Wildlife Service
Olivia	Nyffleler	University of Minnesota -Twin Cities
Dan	Isermann	University of Wisconsin - Stevens Point

Appendix 2. Summary of responses to the *Age Assessment Programs* survey sent to Lake Superior agencies prior to the Lake Superior Fish Age Assessment Workshop.

Table 1. List of respondent agencies.

Agency Name
Bay Mills Indian Community
Fisheries and Oceans Canada-SSM, ON
Great Lakes Indian Fish and Wildlife Commission
Little River Band of Ottawa Indians
Michigan Department of Natural Resources-Marquette
Minnesota Department of Natural Resources -Duluth
Red Cliff Treaty Natural Resources
Sault Tribe
US Fish and Wildlife Service-Ashland
US Geological Survey-Ashland
Wisconsin Department of Natural Resources -Bayfield

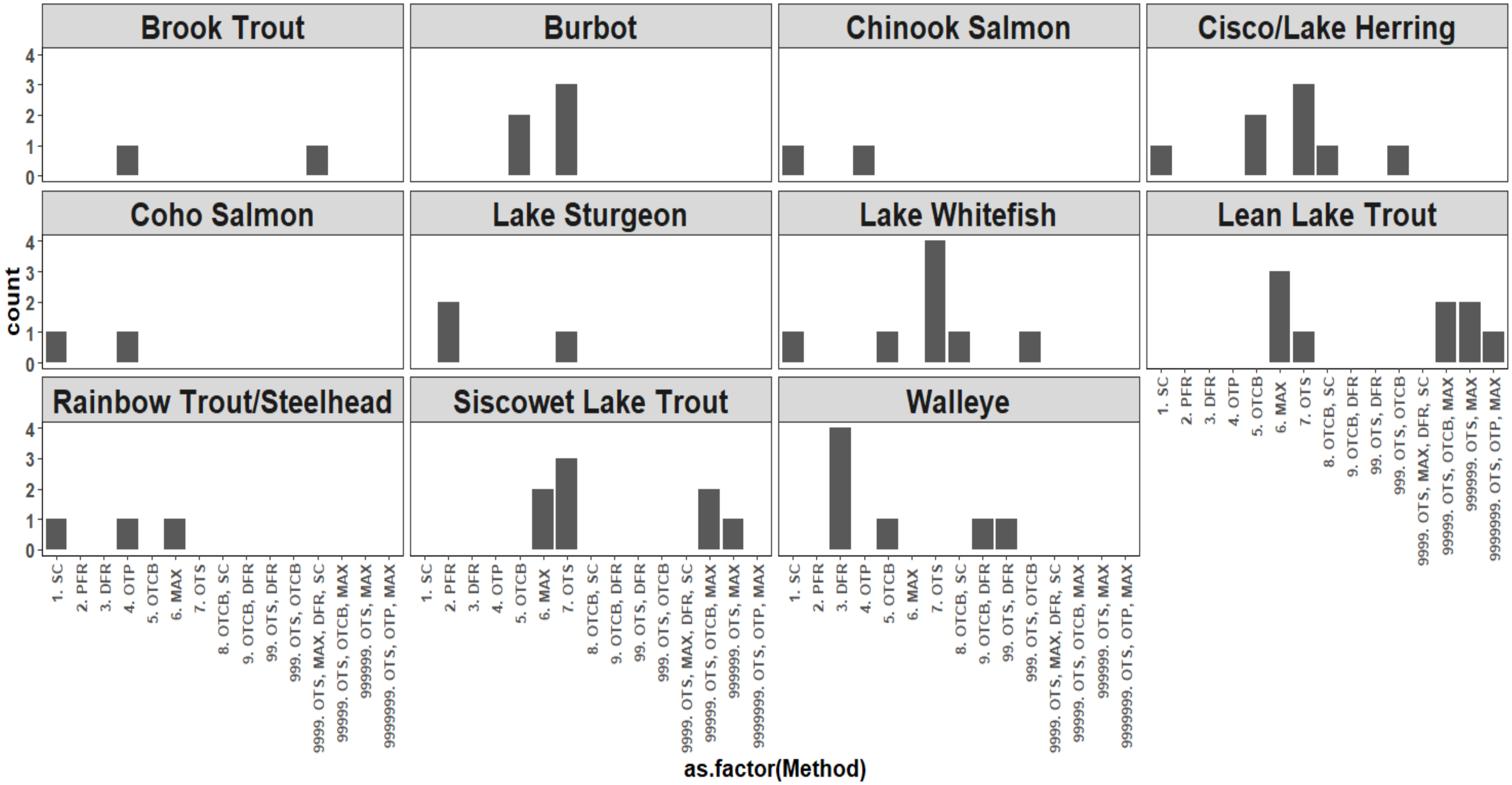


Figure 1. Summary of calcified structures used to age key Lake Superior fish species.

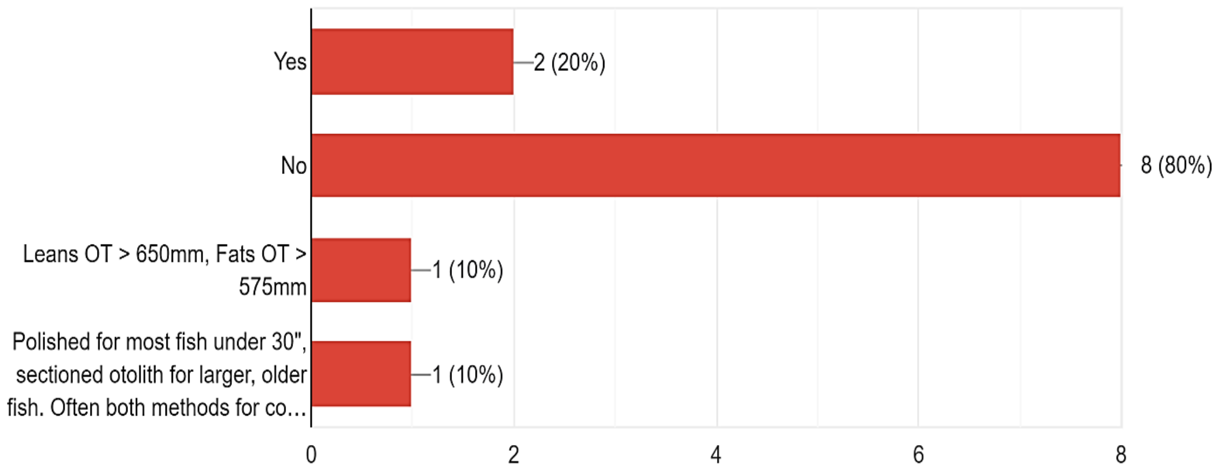


Figure 2. Results of the question does your agency use different structures to age lean lake trout dependent on length.

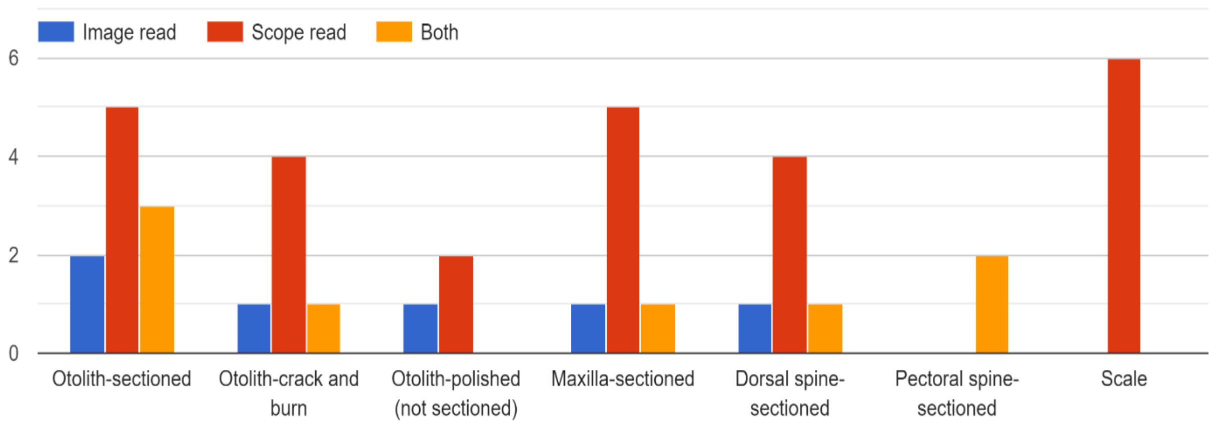


Figure 3. Bar graph illustrating the age structure preparation process and review technique used by various Lake Superior fisheries management agencies.

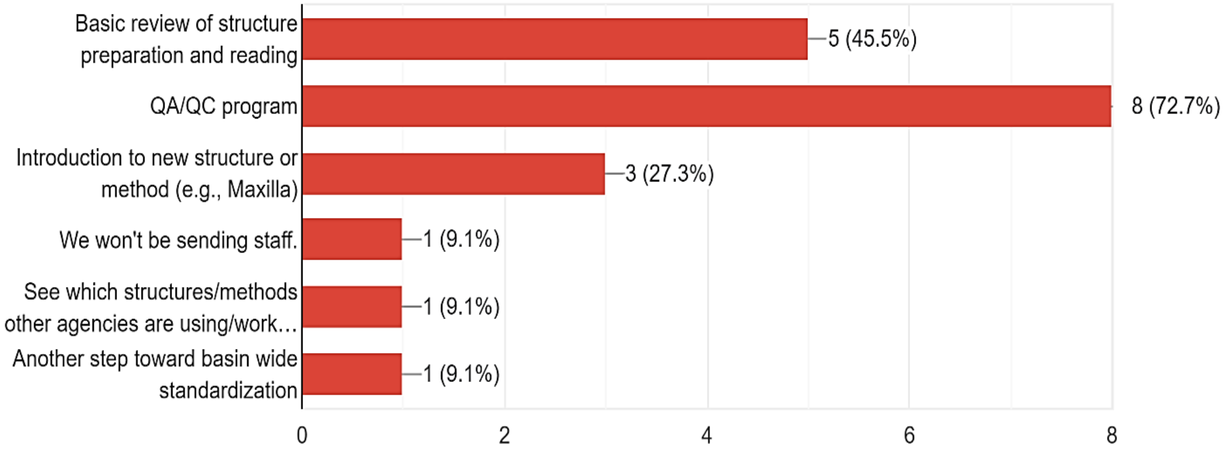


Figure 4. Results of the question inquiring what information attendees are most interested in gaining from the workshop.

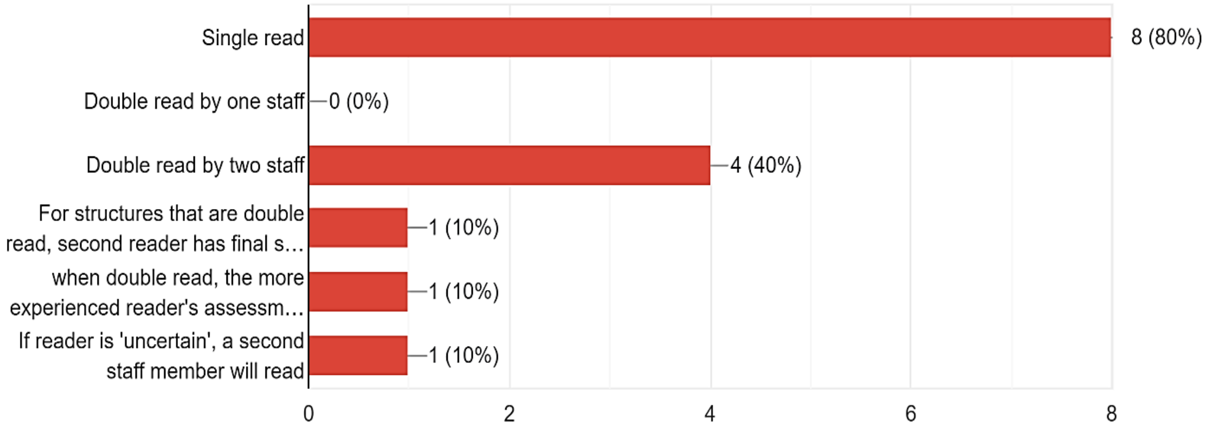


Figure 5. Summary of the responses to the question at your agency how many times is an age measured for a fish.

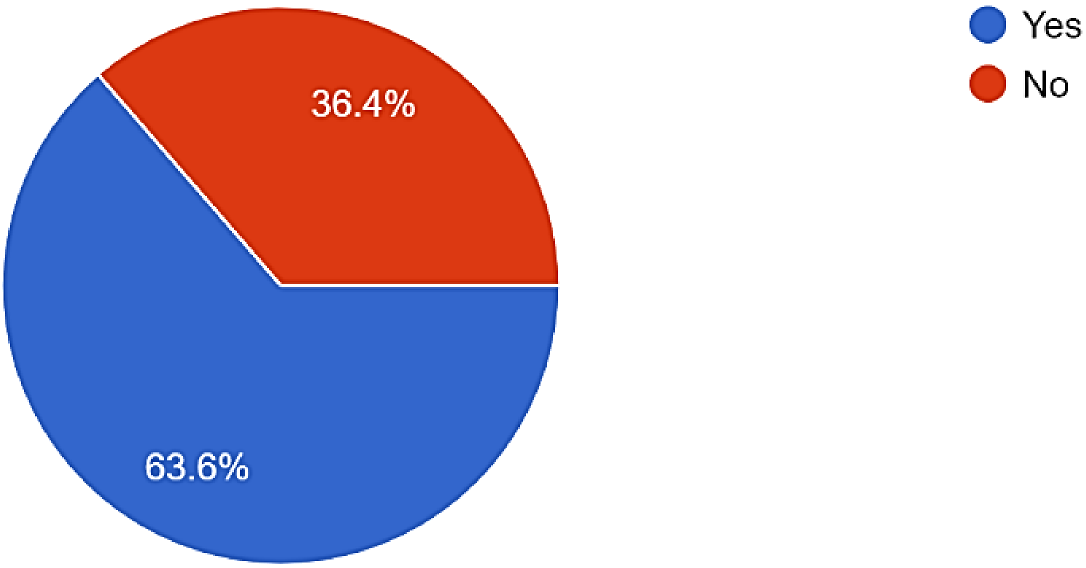


Figure 6. Pie chart representing the number of Lake Superior agencies that have a reference age collection.

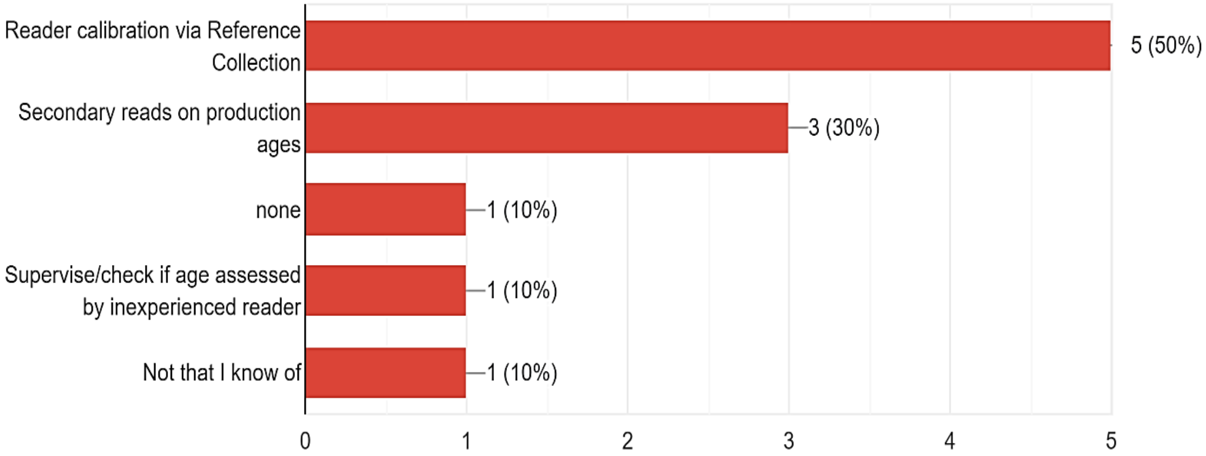


Figure 7. Responses to the question does your agency have an age assessment quality assurance and control procedure.

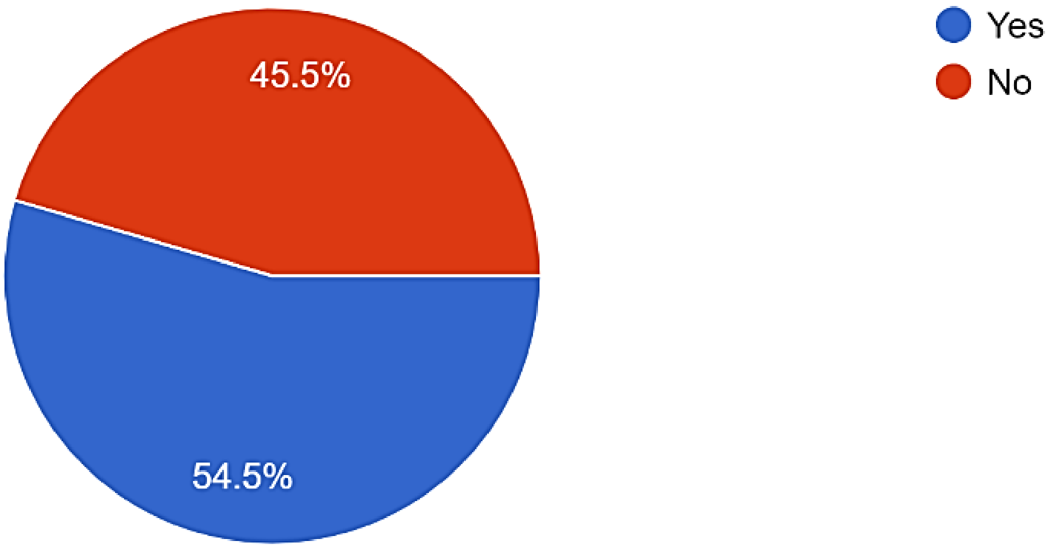


Figure 8. Summary of the proportion of Lake Superior agencies that have an age assessment protocol document.

Appendix 3. Lake trout otolith sectioning and age measurement protocol developed by the Michigan Department of Natural Resources staff at the Marquette Fisheries Research Station.

MFRS Protocol for Sectioning and Aging Lake Trout Otoliths

S. Sitar and D. Traynor, 2014

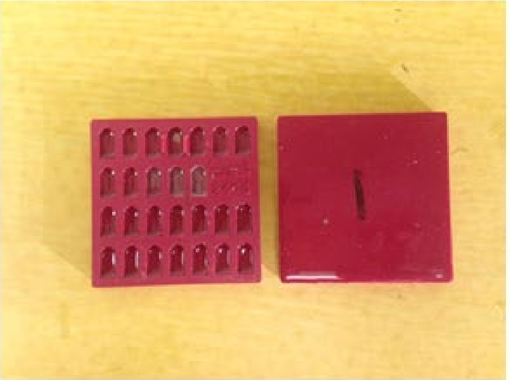
Otolith Preparation

Pre-serialized scale envelopes

Scale envelopes sorted by year, ager, survey, and species

Epoxy for embedding otoliths

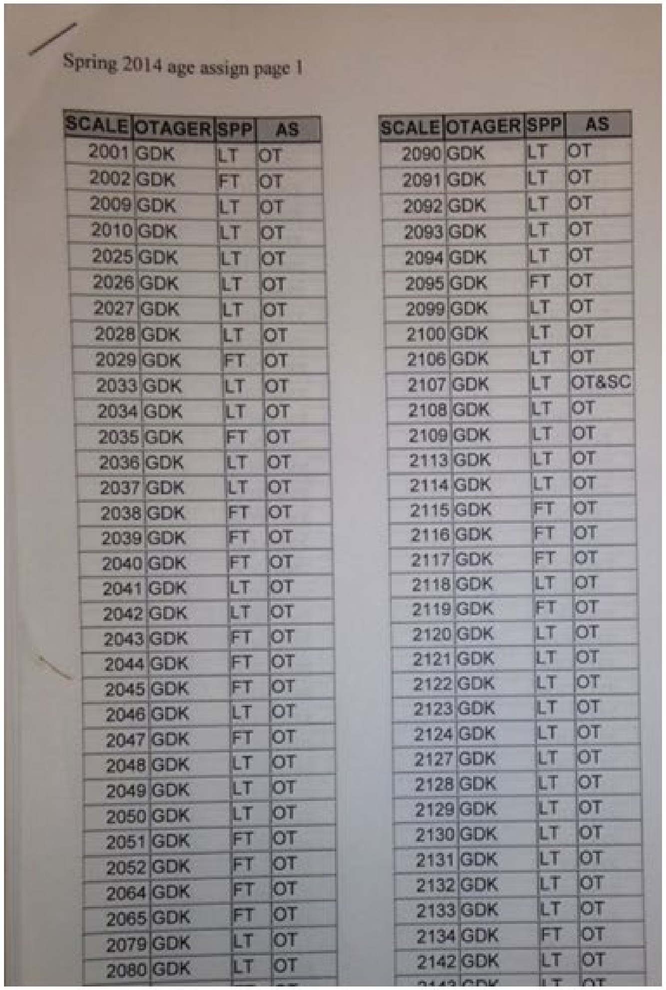
Materials for weighing/mixing/ transferring epoxy



Numbered silicone embedding molds, ½ filled with epoxy

Embedding mold with otoliths

Otolith Preparation



Spring 2014 age assign page 1

SCALE	OTAGER	SPP	AS
2001	GDK	LT	OT
2002	GDK	FT	OT
2009	GDK	LT	OT
2010	GDK	LT	OT
2025	GDK	LT	OT
2026	GDK	LT	OT
2027	GDK	LT	OT
2028	GDK	LT	OT
2029	GDK	FT	OT
2033	GDK	LT	OT
2034	GDK	LT	OT
2035	GDK	FT	OT
2036	GDK	LT	OT
2037	GDK	LT	OT
2038	GDK	FT	OT
2039	GDK	FT	OT
2040	GDK	FT	OT
2041	GDK	LT	OT
2042	GDK	LT	OT
2043	GDK	FT	OT
2044	GDK	FT	OT
2045	GDK	FT	OT
2046	GDK	LT	OT
2047	GDK	FT	OT
2048	GDK	LT	OT
2049	GDK	LT	OT
2050	GDK	LT	OT
2051	GDK	FT	OT
2052	GDK	FT	OT
2064	GDK	FT	OT
2065	GDK	FT	OT
2079	GDK	LT	OT
2080	GDK	LT	OT

SCALE	OTAGER	SPP	AS
2090	GDK	LT	OT
2091	GDK	LT	OT
2092	GDK	LT	OT
2093	GDK	LT	OT
2094	GDK	LT	OT
2095	GDK	FT	OT
2099	GDK	LT	OT
2100	GDK	LT	OT
2106	GDK	LT	OT
2107	GDK	LT	OT&SC
2108	GDK	LT	OT
2109	GDK	LT	OT
2113	GDK	LT	OT
2114	GDK	LT	OT
2115	GDK	FT	OT
2116	GDK	FT	OT
2117	GDK	FT	OT
2118	GDK	LT	OT
2119	GDK	FT	OT
2120	GDK	LT	OT
2121	GDK	LT	OT
2122	GDK	LT	OT
2123	GDK	LT	OT
2124	GDK	LT	OT
2127	GDK	LT	OT
2128	GDK	LT	OT
2129	GDK	LT	OT
2130	GDK	LT	OT
2131	GDK	LT	OT
2132	GDK	LT	OT
2133	GDK	LT	OT
2134	GDK	FT	OT
2142	GDK	LT	OT

Aging structure assignment sheet

Spring 2014 OT mould assign Page **1** of **7**

OTAGER	SPP	SCALE	Mould_id
GDK	LT	2001	1A1
GDK	LT	2009	1A2
GDK	LT	2010	1A3
GDK	LT	2025	1A4
GDK	LT	2026	1B1
GDK	LT	2027	1B2
GDK	LT	2028	1B3
GDK	LT	2033	1B4
GDK	LT	2034	1C1
GDK	LT	2036	1C2
GDK	LT	2037	1C3
GDK	LT	2041	1C4
GDK	LT	2042	1D1
GDK	LT	2046	1D2
GDK	LT	2048	1D3
GDK	LT	2049	1D4
GDK	LT	2050	1E1
GDK	LT	2079	1E2
GDK	LT	2080	1E3
GDK	LT	2082	1E4
GDK	LT	2084	1F1
GDK	LT	2085	1F2
GDK	LT	2086	1F3
GDK	LT	2087	1G1

OTAGER	S
GDK	LT
GDK	LT
GDK	LT
GDK	LT
GDK	LT
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GDK	LT

Embedding mold assignment sheet

Otolith Preparation

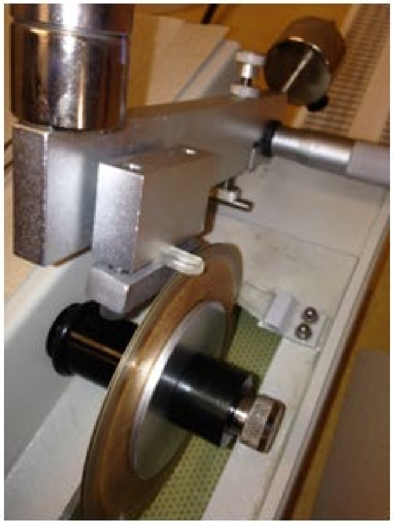

Filled embedding mold

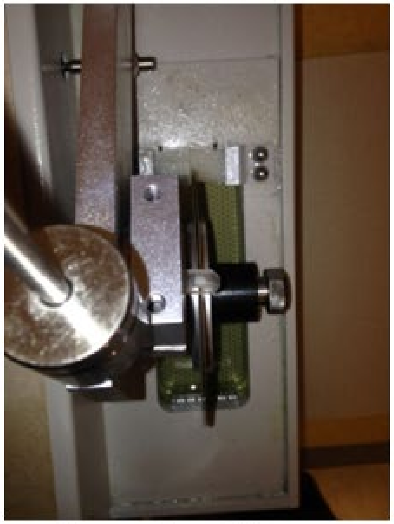

Saw blade coolant


Otoliths in epoxy block

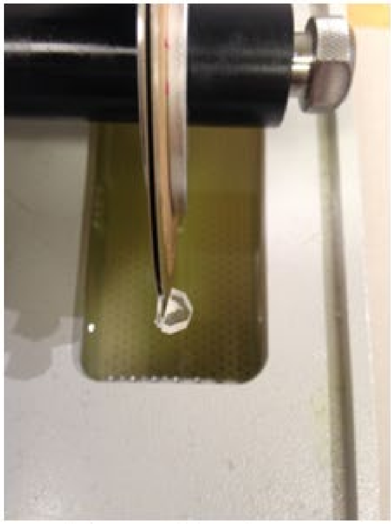

IsoMet saw

Otolith Sectioning


Otolith in saw chuck

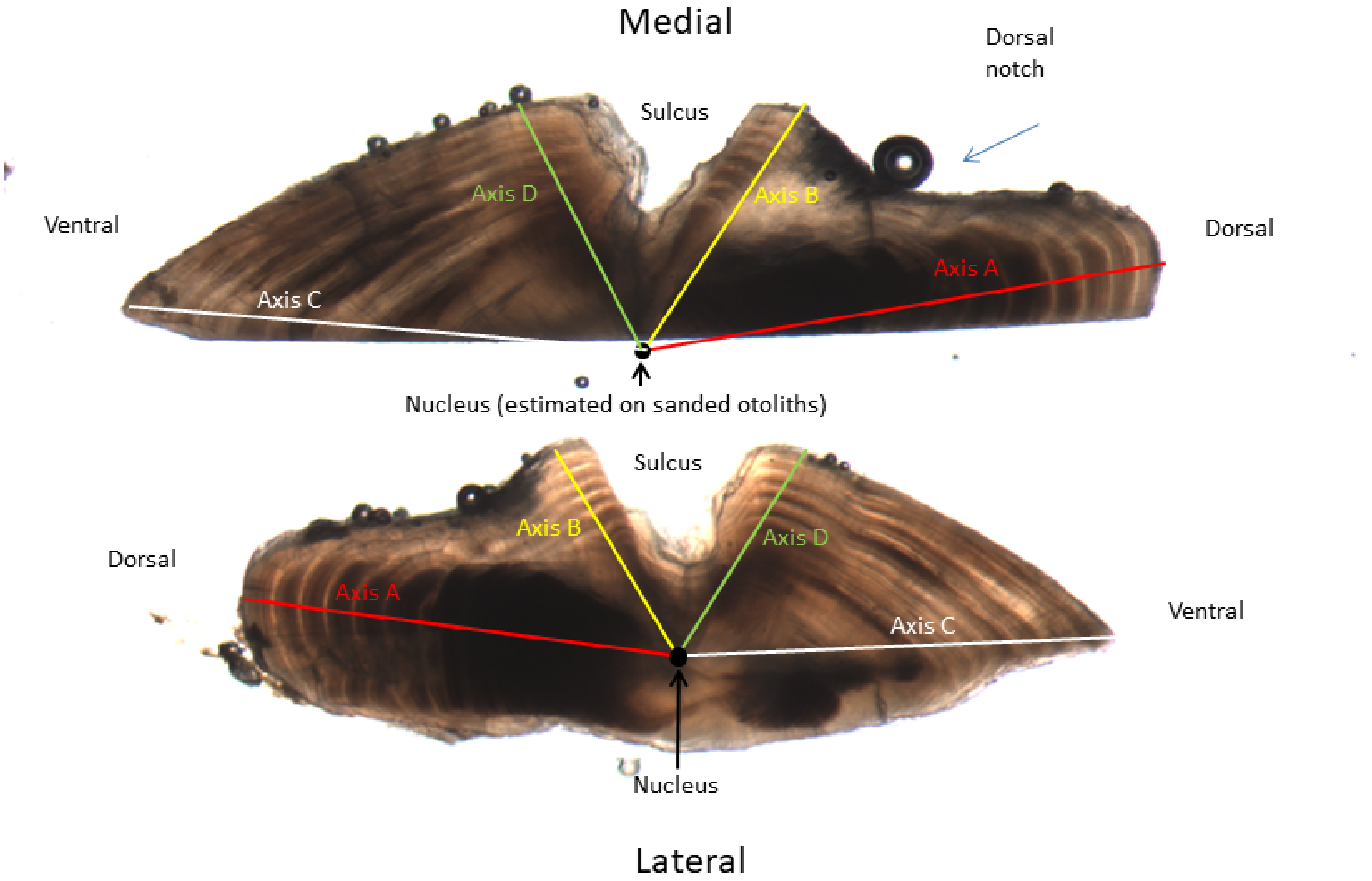

Nucleus of otolith centered between saw blades


Block of epoxy stuck to blades after cut is finished


Otolith section between blades and still attached to block of epoxy

Axis Definition

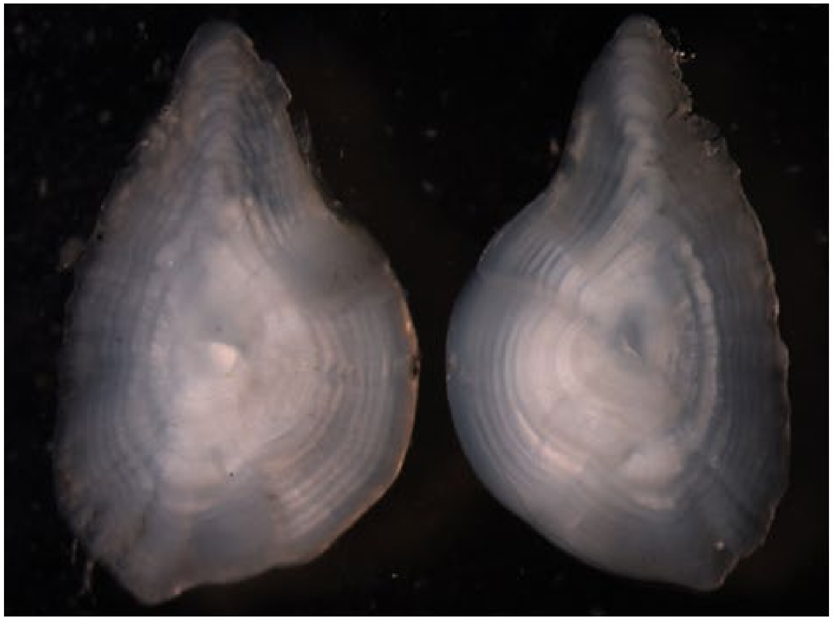
- The orientation of the otolith (dorsal or ventral/left or right side) will vary depending on which side of the section was imaged.
  1. Axis A – A line that is generally between 0 and 45 degrees on the dorsal side of the otolith.
  2. Axis B – A line that is on the same side as Axis A and is situated along the edge of the sulcus.
  3. Axis C – A line that is generally between 0 and 45 degrees on the ventral side of the otolith.
  4. Axis D – A line that is on the same side as Axis C and is situated along the edge of the sulcus.



Whole Otolith Morphology

Lateral (distal) View, Transmitted Light

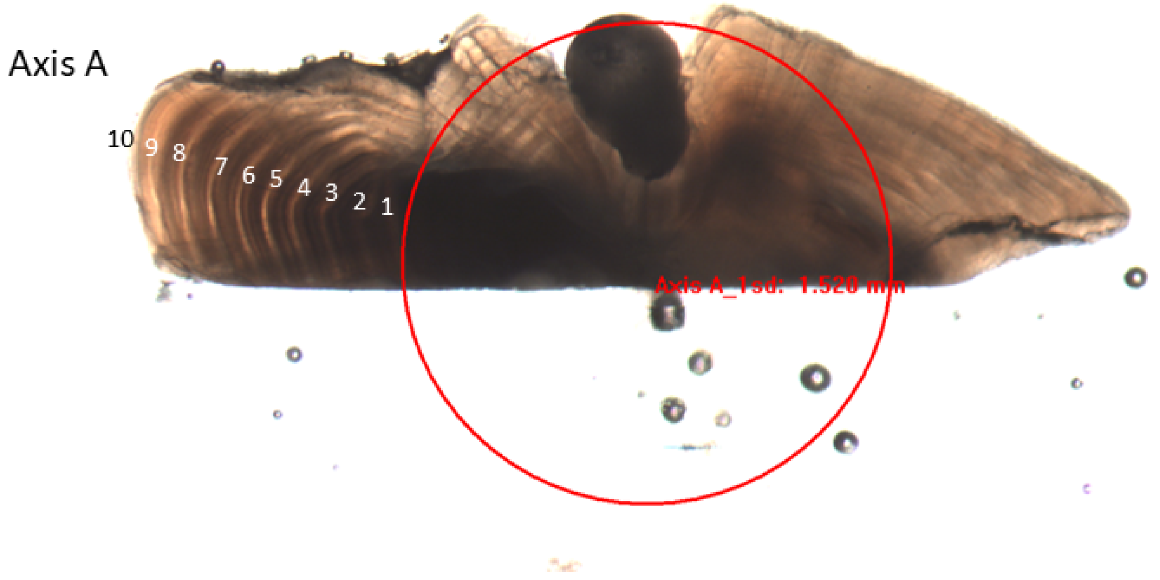
Lateral (distal) View, Reflected Light



Age Determination

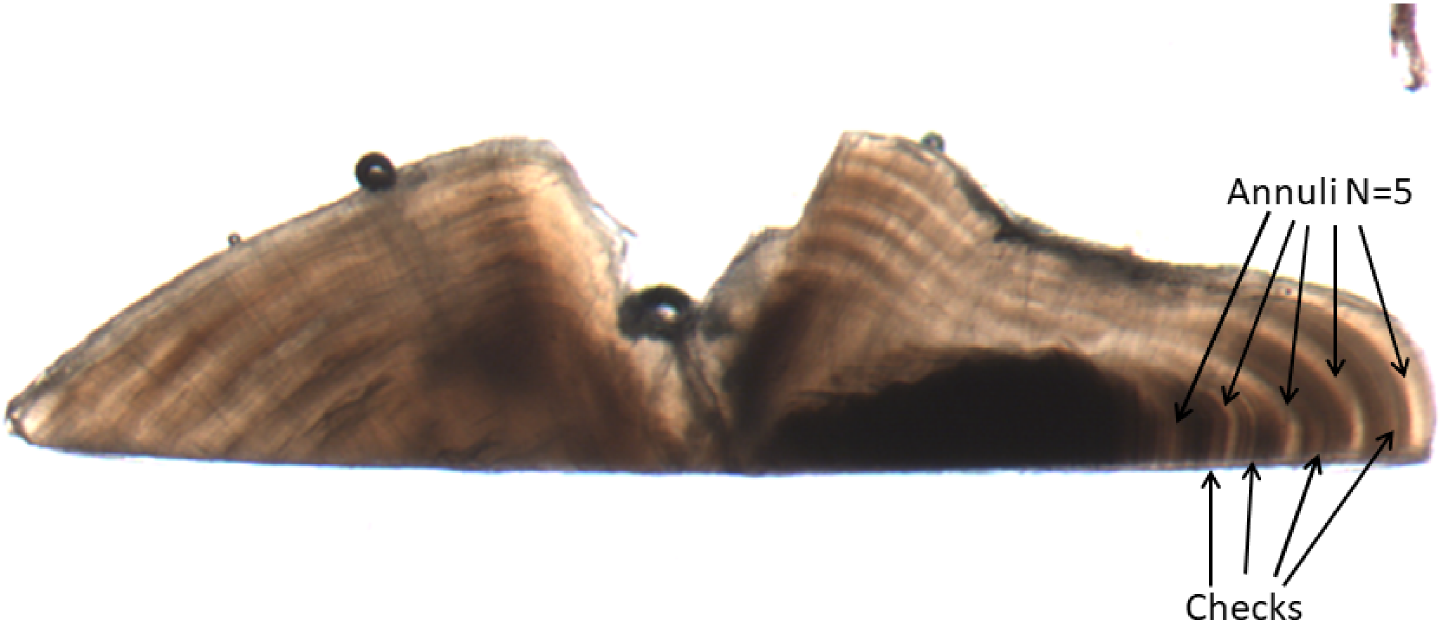
- Beginning at the nucleus annuli are counted along all aging axes on both sides of the structure. Results from the clearest aging plains will be used for the finalized age estimate.
- The treatment of the outer edge will vary depending on the season of collection.
  - When aging polished otoliths the outside edge is typically counted on spring fish although the annulus is not yet visible.
  - The outside annulus on summer fish may or may not be visible. When aging sectioned otoliths the growth on the outer edge is more visible and therefore the last annulus may be visible.

Example: Spring lake trout age 10 with the last annulus just visible on the outside edge



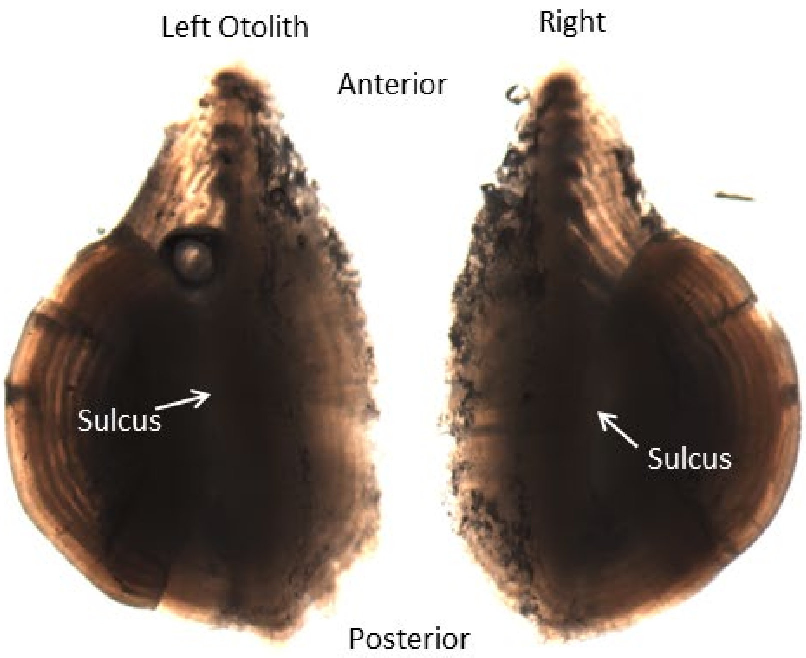
Annulus Definition

- **Band Intensity** – Annuli on images with transmitted light are the thick, translucent bands. Annuli may be split and appear as two separate annuli but should only be counted as one. Checks appear as thinner, weaker translucent bands, often at irregular growth intervals.
- **Spacing** – Otolith growth increments are more regular that growth increments on somatic structures. Spacing should be fairly uniform all the way to the outside edge. Annuli do not appear to compress on the outside edge of sectioned otoliths like they do on polished otoliths.

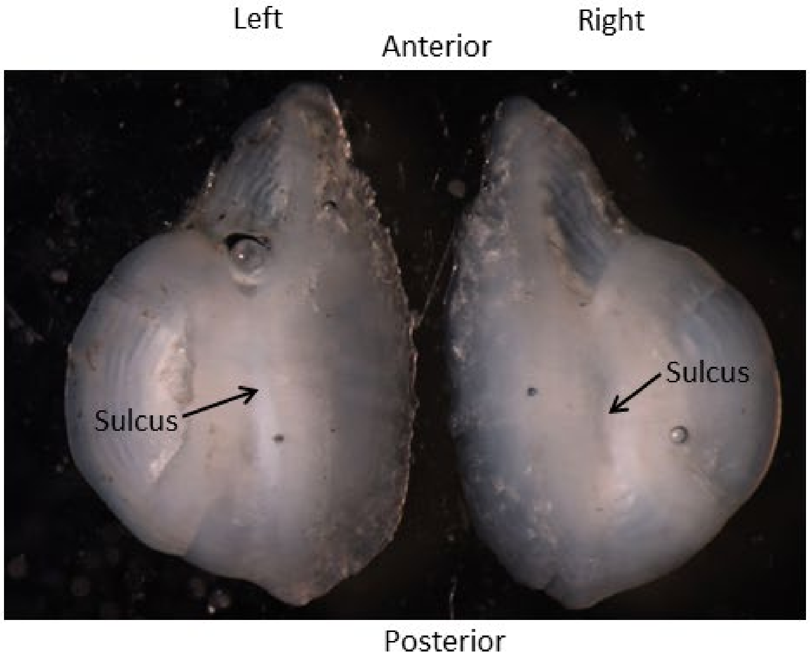


Whole Otolith Morphology

Medial View, Transmitted Light



Medial View, Reflected Light



Appendix 4. Example of procedures to develop age structure reference collection for lake trout thin-sectioned otoliths.

1. Develop a digital collection of images of age structures with known or consensus age. Campana (2001) recommends at least 200 structures but 500 is preferred. Ideally the collection includes structures that are collected from around the lake to capture regional differences in growth.
    a. Hatchery fish with confident CWT or fin clip age and > 1 otolith and/or scale readings in agreement.
    b. Fish with > 2 readings that are identical.
2. Have readers test read candidate pool.
3. Check distribution of age readings with known or consensus age.
    a. Retain structures in probationary status if readings are identical to known or consensus.
    b. Delete structures from candidate pool if readings do not agree.
4. Once probationary structures have multiple readings that are consistent with the known/consensus age, then the structure can be entered into reference collection.
5. Reference collection structures will accumulate readings and still need to be monitored for reliability.

Appendix 5. Glossary of terms used during age measurement in fish.

- **Accuracy**: the closeness of an age estimate to the true age of the fish.
- **Age assignment**: the systematic quantification of fish age through the numeration of growth increments (i.e., circuli or annuli) present in calcified structures, such as otoliths, scales, and spines.
- **Aging axes**: an imaginary straight line extending across the clearest surface of an aging structure from the core to the terminal edge, on which counts are conducted.
- **Aging plan**: the prepared surface of a calcified structure across which the growth sequence is enumerated.
- **Annulus**: in fish seasonal changes in the accretion of material on the margins of calcified structures creates an alternating pattern of incremental zones (i.e., periods of regular growth) and discontinuous (i.e., periods of slow growth) zones. If deposition results in annual formation enumeration of the discontinuous zones can be used to estimate age (Campana and Neilssra, 1985; Haglund and Mitro, 2017).
- **Check**: distinctly prominent band(s) present on the aging plane of a calcified structure, which likely signifies period(s) of stress (e.g., hatching, yolk sac absorption, metamorphosis, or spawning) (Oyadomari and Auer, 2007).
- **Edge**: the terminal surface of a calcified structure where new material is deposited continually throughout a fish's life.
- **Growth sequence**: banding pattern that portrays the period of interest (i.e., daily, annual) in the calcified structures of fish, created by seasonal changes in growth.
- **Measurement/Observation error**: the difference between the measured quantity and true value, which in age measurement arises when counts are imprecise due to misinterpretation of the growth sequence (Mcbride, 2015).
- **Nucleus**: the core of a calcified structure representing the origin where initial material was deposited by the embryotic fish. During enumeration of growth sequences, counts originate and radiate out from this point.
- **Precision**: the reproducibility of repeated measurements (i.e., circuli or annuli counts) on a specific calcified age structure (Campana, 2001).
- **Process error**: not all bony structures and/or axes represent complete growth sequences that exactly represent the period of interest (i.e., daily, annual) thus resulting in error from inaccurate age estimates (Campana, 2001; Mcbride, 2015)
- **Reader**: an observer trained to recognize and interpret growth sequences captured in calcified structures, which represents incremental growth (i.e., daily, annual) in fish.
- **Validation**: the process in which the growth sequence captured in a specific age structure is proven to accurately represent the true age for that species.