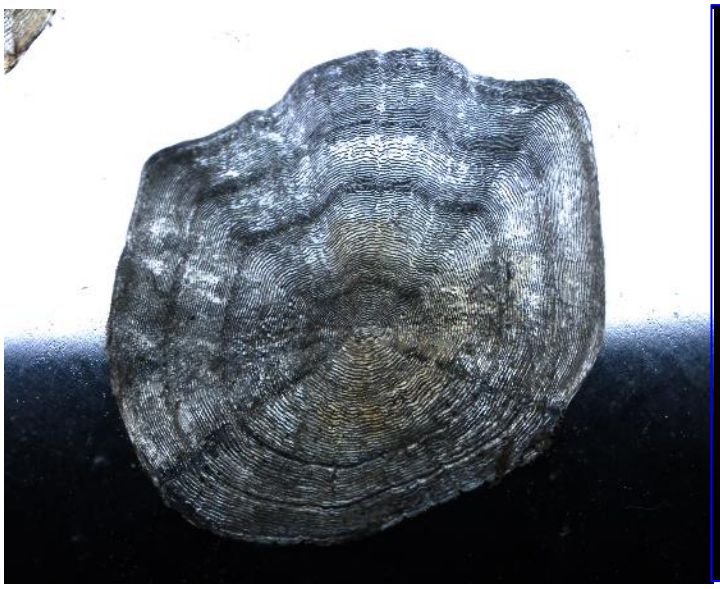
DEPARTMENT OF NATURAL RESOURCES

Division of Fish and Wildlife

Guidelines for Age and Growth Estimation of Minnesota Fishes



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Introduction

The intent of this manual is to standardize fish aging procedures for Minnesota DNR staff and to provide instruction on preferred methods. Given the time and expense of aging fish, the first question that should be asked is whether you need to age any fish at all. Is the information needed to address a specific management issue? Will you be able to collect the required sample size? Will examining size structure alone be sufficient? Details about fish aging and analyses are found within the pages of this manual. The "Recommendation" section at the beginning of the manual gives the user a quick look at statewide protocols. The later sections give more detail on the recommended procedures.

There are many ways to age fish, from which structures to use, to which procedure to age them with. This manual covers many methods; however, we also state the "preferred method".

Quality age data are important for making informed management decisions. Collecting quality age data may take more staff time than previous aging efforts. Therefore, rather than age most of your fish collected during a routine lake survey, you will want to focus your efforts on your primary species of interest. For example, if your primary species of interest is Bluegill for a particular lake, you'll want to focus most of your aging efforts on that species and not age crappie, bass, perch, etc.

Age data is routinely sampled by fisheries managers and used to estimate growth, mortality and recruitment. In a review of freshwater fish aging procedures used by state and provincial fisheries, Maceina et al. (2007) found that nearly every agency used aged fish to assess growth (100%), mortality (86%), and/or recruitment (82%). These data are commonly used to develop management strategies and evaluate successes or failures. Inaccurate data can lead to errors in these estimates and can therefore hinder the ability of fisheries managers to understand the dynamics of the fish population in question.

Preferred Methods

- Otoliths are the preferred structure for aging most fishes in Minnesota.
- The crack and burn method of otolith preparation provides the most accurate and precise estimates of age.
- Subsample 10 fish per 25-mm size-group for large bodied species (Walleye, black bass, Northern Pike) and 10 fish per 10-mm size-group for small bodied species (Bluegill, crappie, Yellow Perch).
- A minimum of 20 fish are needed for the simplest age and growth questions. For more in depth questions, 100-200 fish may need to be aged.
- Double reads should be done for all fish if using scales and for a 25% subsample if using cracked and burned otoliths.
- Back calculation should be done if you need to increase sample size, compare growth to other populations, or need information on past growth.
- Back-calculations should also be done if your sampling gear is biased for faster or slower growing fish.
- Fish should be sampled in early spring or late fall to avoid confusion with annulus formation.

Sample Collection

Time of Year

Estimating age of fishes collected during times when annuli are forming will result in greater error in age estimates, regardless of the type of aging structure analyzed. Annulus formation often requires several months for completion, and lengths of time needed for annuli formation could differ among species. Generally, annuli on structures from young fish form earlier than on structures from older fish. In Minnesota, many commonly aged fish form their annuli between May and July (Figure 1). During this time period, it can be difficult to determine whether the individual fish has laid down an annulus for the year. If it has not, then 1 year must be added to the number of annuli for the correct age. The best time to collect fish for age estimation is usually in early spring (before annulus formation) or late summer and fall (after annulus formation) so that there is no confusion about whether an annulus has been laid down that year.

References for annulus formation

- Time of year (Hales and Belk 1992) (Blackwell and Kaufman 2012) (Quinn and Ross 1982)

Figure 1. Timing of annulus formation on Bluegill otoliths taken in May, June, July, and August.

May 29, add one year, annulus not yet formed along edge. Age 4

June 29, annulus forming along outside edge, but not visible around entire otolith. Age 4

August 10, new growth beyond last annulus. Age 3

Otoliths

Removal

Otoliths can be removed using several different methods. The most common methods for removing otoliths involve accessing the otic capsule through the bottom of the fish by cutting through the isthmus (Figure 2) or by cutting through the top of the head (Figure 3). The bottom method requires either a knife or side cutters to first cut through the isthmus and then cut at the posterior end of the otic capsule. Care must be taken not to cut too deep into the capsule, otherwise you may damage the otoliths. If the cuts are made correctly, oftentimes you can apply pressure to the head and break open the otolith cavity using only these two cuts. If the capsule does not open up with these two cuts, the tissues surrounding the otic capsule and the gills may need to be removed before making a third cut at the posterior end of the capsule. Coming in from the top usually requires a hack saw to cut through the upper portion of the skull. One cut is made in line with the preopercle, being careful not to cut too deep. Care must be taken not to cut too deep otherwise you risk damaging the otoliths. When cutting through the top of the head, listen for a change in sound and feel to know when to stop. After this cut, pressure is put on the head to break the capsule open. Once you have access to the otolith capsule, the otoliths are removed with a tweezers. After some practice, you should be able to extract otoliths with minimal effort. If the cuts are in the right spot, then there is little time spent searching for the otoliths. The method you choose is personal preference; however, it is often easier to go thru the bottom on small fish (age 0). Once removed, wipe away any blood or tissue on the back of a gloved hand or paper towel. This is especially important if you are going to be reading them in whole view or are going to be toasting the otoliths prior to reading. Typically sagittal otoliths are removed and stored in scale envelopes or placed in plastic vials for added protection. Otoliths must be allowed to dry prior to reading, so plastic vials need to remain open for a few days. Otoliths stored in scale envelopes risk being broken if they are bound tightly with a rubber band. It is best if scale envelopes are stored loosely to avoid breaking the otoliths into smaller pieces. Fish can be frozen and otoliths taken at a later date. Fish should not be stored in formalin if otoliths are to be used because they become unreadable (Figure 4).

Figure 2. Otolith removal from the bottom of the head.

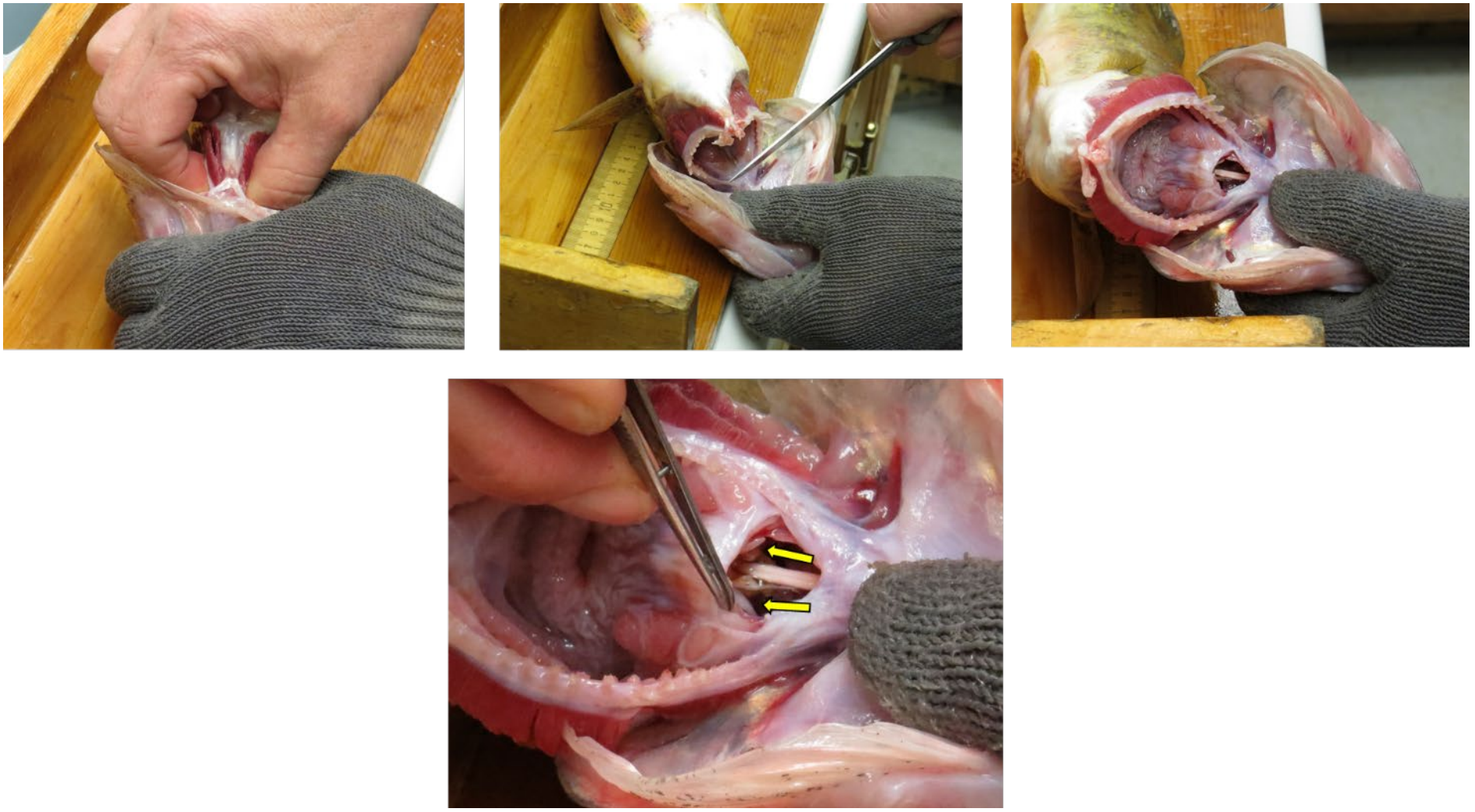
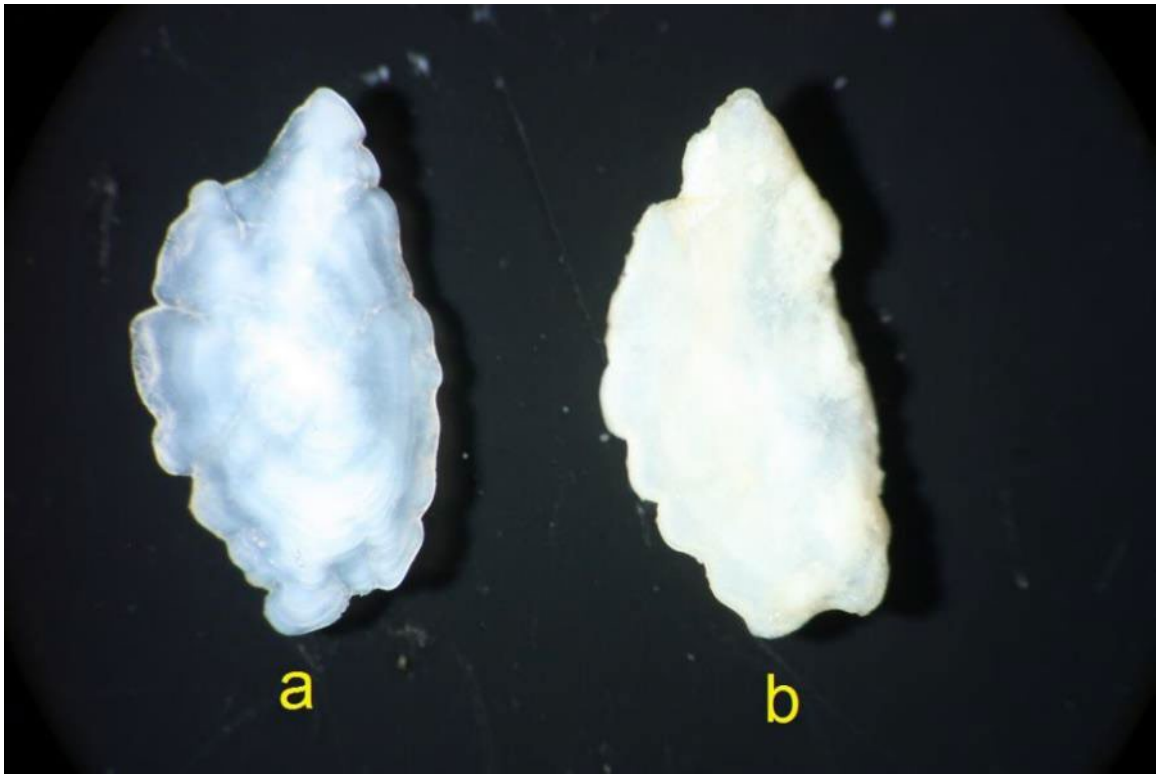


Figure 3. Otolith removal from the top of the head.

Figure 4. Otolith from a frozen age 0 Smallmouth Bass (a) versus an otolith from an age 0 Smallmouth Bass stored in formalin (b).



Preparation

Whole view

Usually there is no preparation needed for whole otoliths prior to reading as long as they were cleaned and dried previously. However, they can be placed in glycerin for 2 weeks prior to reading to enhance annuli, but this is usually unnecessary.

Transverse/Crack and burn

There are several methods for preparing otoliths when reading the transverse plane. The otolith can either be thin sectioned with an Isomet saw or broken in half with slight pressure. Some sectioned surfaces require sanding/polishing to create a smooth viewing surface.

Crack-and-burn Methodology for Otoliths

- Take an otolith and crack it at the nucleus (kernel) in a transverse configuration using your thumbnail. If the break does not occur at the nucleus, you risk missing the first annulus.
- If needed, sand the cracked surface of the otolith with 600-1000 grit sandpaper to make a flat plane or to get the plane of the crack nearer the kernel of the otolith.
- Using a small flame (candle or alcohol burner), singe the transverse plane of the cracked otolith by holding it over the flame with a forceps for approximately 4 to 6 seconds, or until the otolith has a slightly browned or blackened appearance.
- Place the unsinged end of the otolith into mounting clay to view the aging plane of the otolith. Add mineral or immersion oil to the singed surface of the otolith to increase clarity.

Figure 5. A crack and burned otolith ready for aging

Burn-and crack methodology for Otoliths

This method uses a hot plate or a toaster oven to brown the otoliths before they are cracked and read. This method produces evenly toasted otoliths which cannot be overdone.

- Heat hotplate to 300 - 320°C.
- Place otolith (whole or cracked) concave surface up on the hotplate directly above the heating element.
- Toast until otolith assumes a uniform dark caramel color
  - Between 10 sec for small otoliths like age 0 Walleye and around 4 minutes for large otolith like White Bass.
- Remove with tweezers.
  - Otoliths (particularly large ones) will remain very hot and should be allowed to cool before cracking
  - Otoliths cracked prior to toasting can be transferred directly to your clay base for aging.
- If the toasting was insufficient to obtain an age, the otolith can be removed from the clay and placed back on the hotplate to darken further.
  - Note: the liquid you use to obtain better visualization under the scope makes a difference.

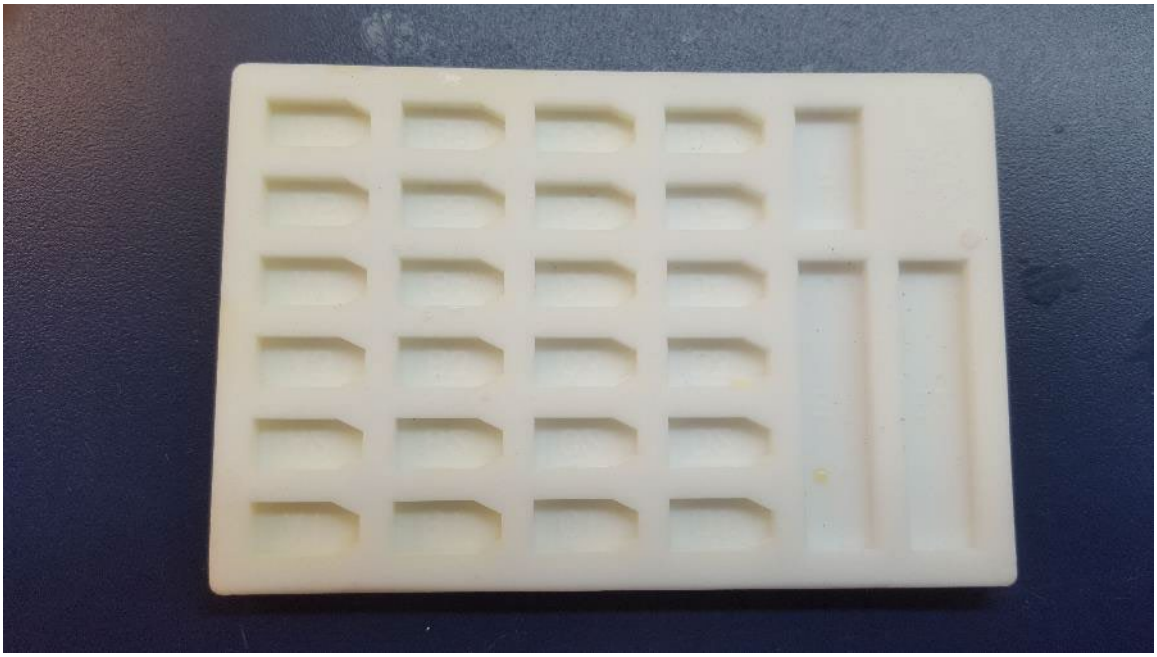
- Glycerin, Baby (Mineral) Oil, and Immersion Oil were evaluated for the re-toasting process
    - Mineral Oil (Flash point: 135C (275F) CC and Autoignition temperature: 260 – 370C (500 – 698F)) and Immersion Oil both need to be treated with care as they could potentially combust at temperatures the hotplate can reach.
    - Glycerin smokes less than the other options, but in some cases can leave a residue that may impede a second read on the otolith.
- Similar results can be obtained using a toaster over for browning otoliths. Otoliths can be placed in labeled silicon or metal trays and placed in a 500° F toaster oven. Monitor the otoliths until they reach a uniform caramel color.

Figure 6. Hot plate used to brown otoliths.

Thin sectioning methodology for otoliths

When otoliths are fragile or irregularly shaped, they may need to be embedded in epoxy and sectioned with a low-speed saw. The easiest way to do this is by placing the otolith in a silicon embedding mold (Figure 7) and covering it with a two-part epoxy. This method allows for large batches of otoliths to be embedded at one time. Mixing in small batches works well to limit wasted epoxy. Once otoliths are covered, leave overnight to harden. Consult “Appendix A. Purchasing Supplies” for where to purchase epoxy and silicon molds.

Figure 7. A silicone tray can be used to embed structures in epoxy. The structure can be placed in a hole and epoxy can be poured over the top.



Once embedded, otoliths are easily removed from the mold by bending the mold away from the epoxy block. Place otoliths in the same envelope they were initially stored in. When cutting, the epoxy block is easily held in the chuck of an Isomet saw. Otoliths can either be cut through the kernel and viewed, or they can be thin sectioned.

When viewing otoliths cut through the kernel or approximate center and stand the epoxy block up in a clay with the cut surface facing up. Place a drop of immersion oil on the cut otolith and view with a fiber optic light. This method is similar to viewing a cracked and burned otolith, without burning (burning will melt the epoxy and give off fumes that are likely toxic).

When sectioning otoliths, first make a cut near the kernel. Cut thin sections from the otolith between 0.5 and 1 mm thick. Some experimentation will be required to determine the thickness that works best with otoliths being used. Sections can be viewed by placing them directly on the stage of a dissecting microscope or by gluing the sections to a glass slide for easier handling. Sections may also be polished with 1000 grit sandpaper to improve clarity of annuli.

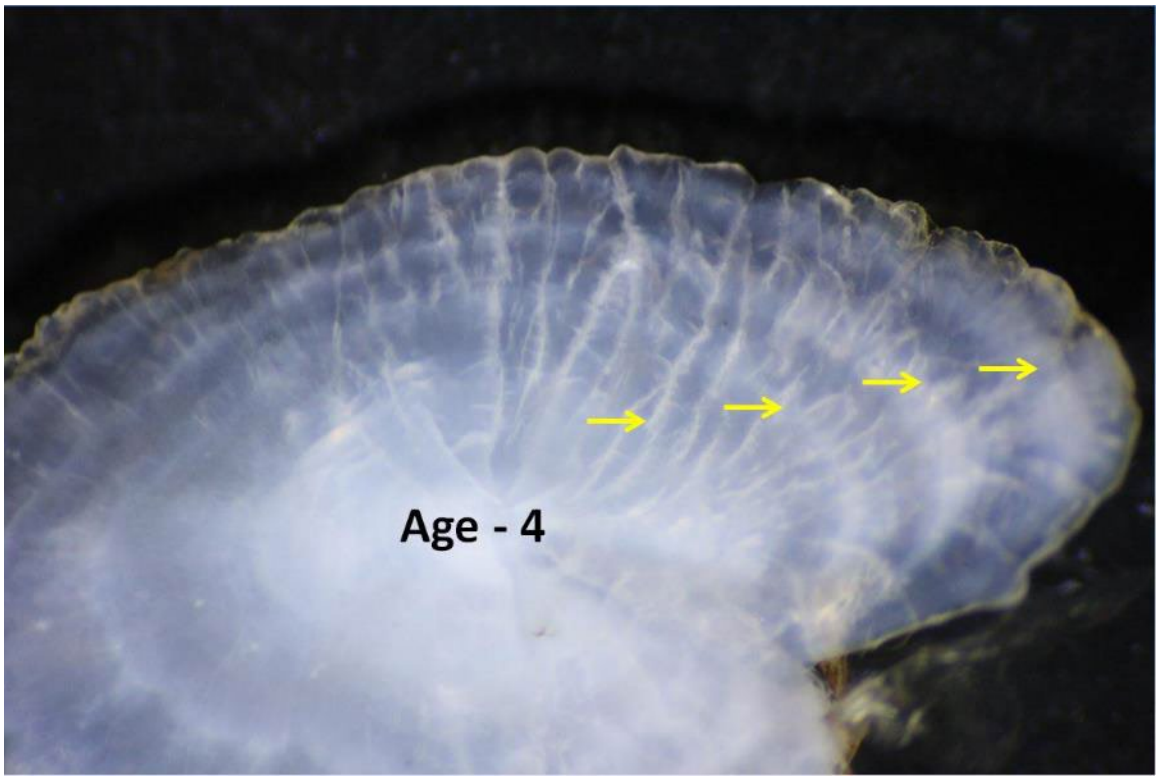
Figure 8. Thin section otolith with transmitted light

Reading

Whole view

Otoliths are removed from their vessel, submersed in a clear liquid (water, alcohol, immersion oil) concave side up within a small container and viewed under a dissecting microscope with reflected light. When using water, some smaller otoliths tend to float on the surface and have to be sunk to the bottom of the dish. Using alcohol eliminates this and provides a clearer medium to view otoliths (less air bubbles to interfere with viewing). Whole view otoliths are often accurate and precise up to around age 6 for most fish species. Identifying the first annulus is often the most difficult part when using whole otoliths. Use a dissecting scope at the highest magnification and best lighting possible to view the entire section when aging the otolith. If back calculation estimates are needed, take a picture of the otolith using digital camera software (see Appendix.).

Figure 9. Whole otolith from an age 4 Bluegill.



Crack and Burn

Whether you used a hot plate, toaster oven, or a flame source, the reading process is the same. Place the cracked otolith in clay under a dissecting microscope. Place oil on top of the burned surface and use reflected light. Use either a gooseneck or a handheld fiber optic lightsource. Annuli will generally appear as thin, dark bands and should be visible on both sides of the sulcus groove (Figure 10 and Figure 11).

Figure 10. Crack and burn otolith from an age 5 Largemouth Bass.

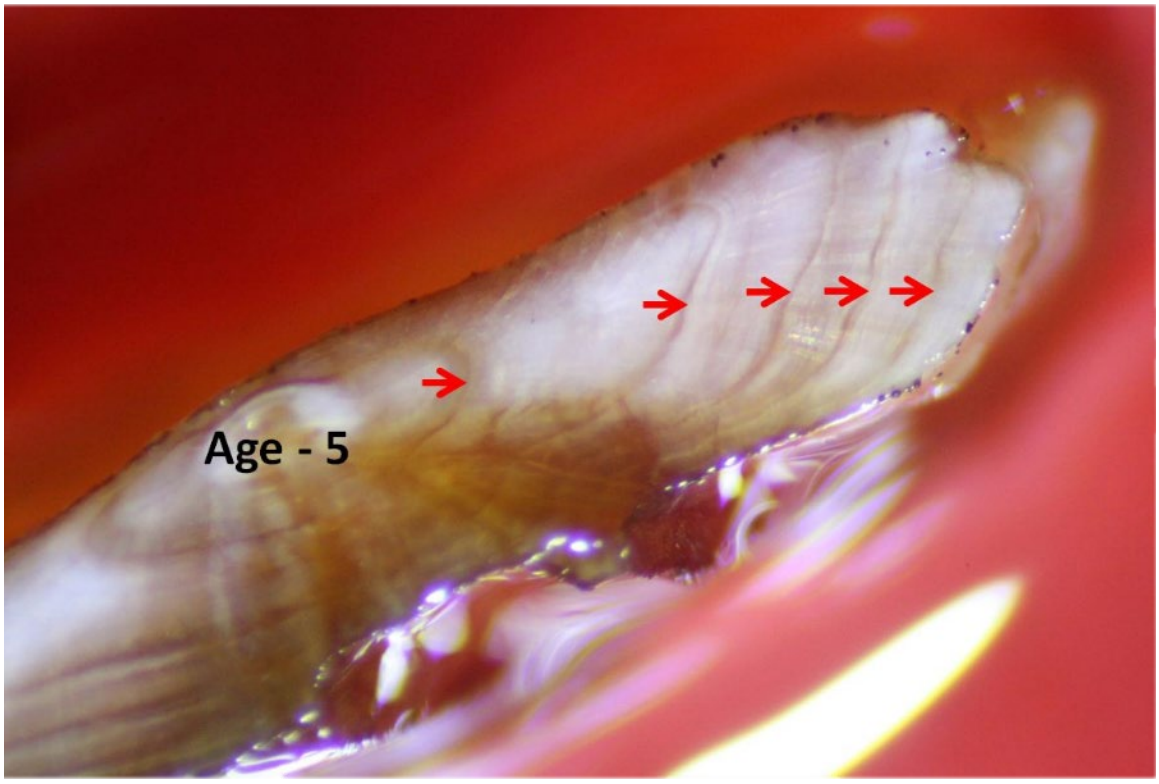
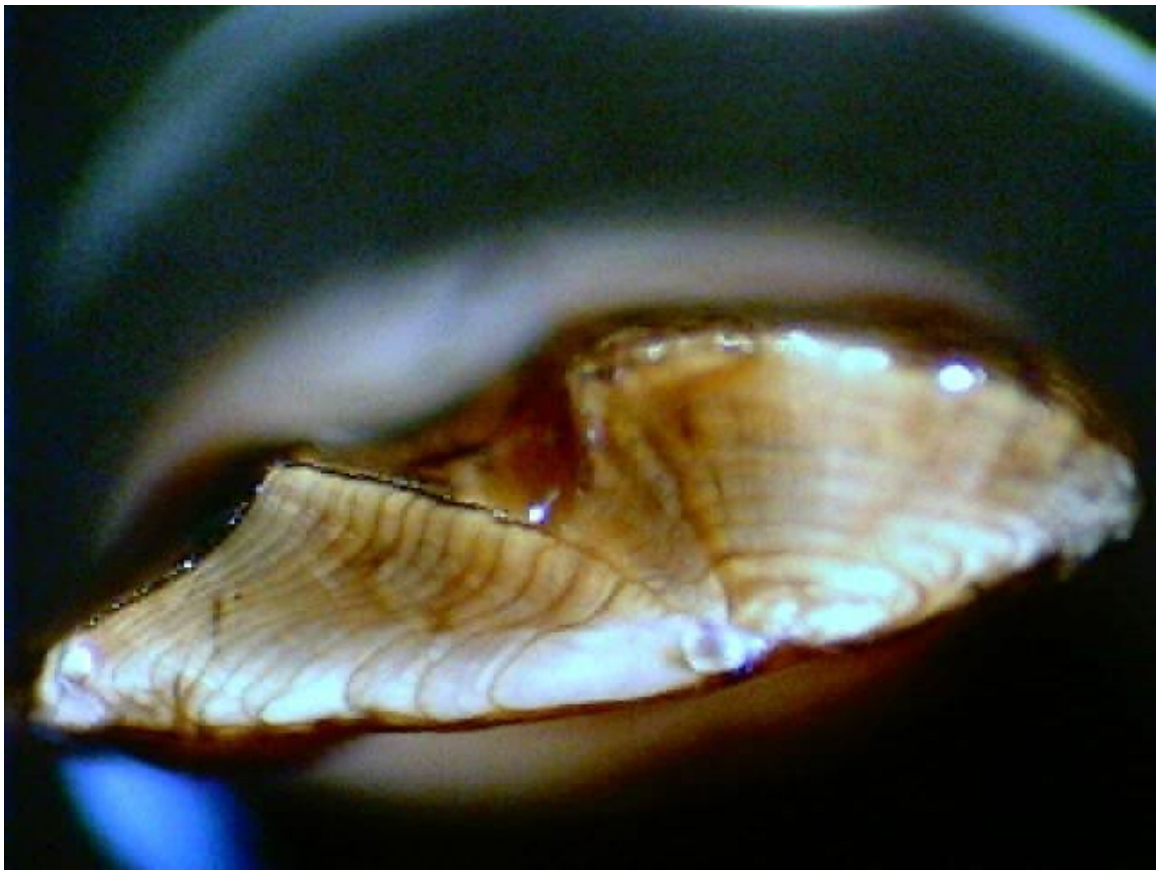


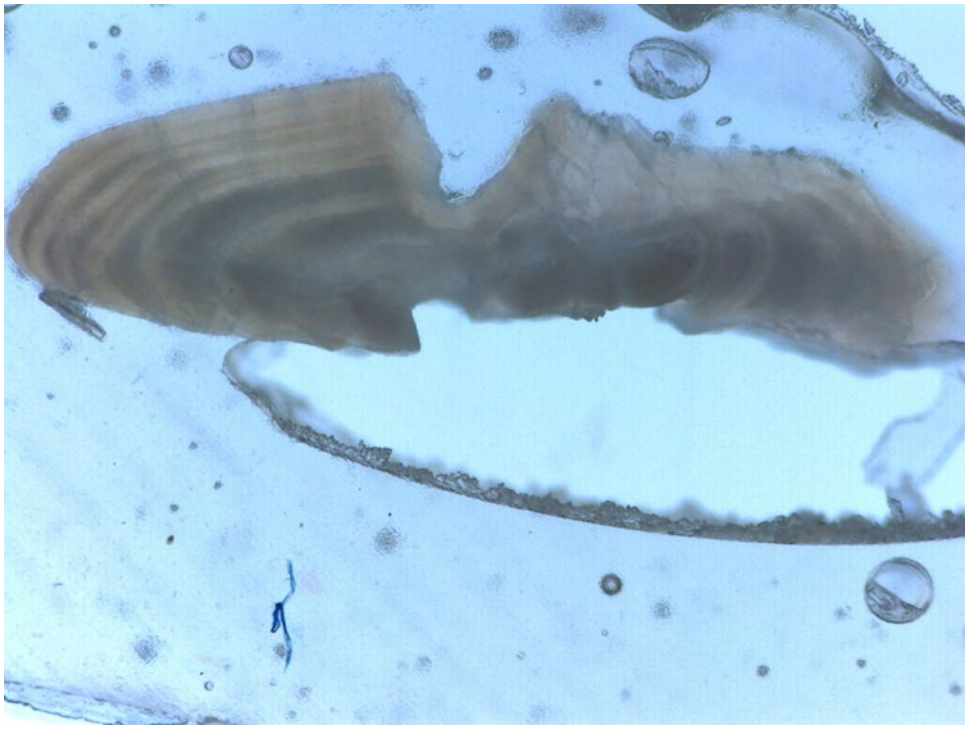
Figure 11. Crack and burn otolith from a 15 year old Walleye from Lake Pepin.



Sectioned Otoliths

Sectioned otoliths are read under a dissecting microscope using transmitted light. The annuli appear more diffuse compared to annuli found using the crack and burn method.

Figure 12. Sectioned otolith from a 7 year old Cisco.



Scales

Removal

Standard locations are to be used for scale removal so that consistent estimates of back-calculated lengths at age can be made. Ctenoid scales (typically found on spiny-rayed fishes) are to be removed from the area near the posterior tip of the pectoral fin and below the lateral line (Figure 13). Cycloid scales (typically found on soft-rayed fishes) are removed from the region above the lateral line and below the dorsal fin (Figure 13). A knife or forceps are usually used to extract scales, but any tool that can grab or loosen scales from the fish's skin will work. First, use the scale-extraction tool to scrape away any mucus or other debris from the area where the scales will be removed. In a posterior direction, either pull with forceps or poke/push with a knife the targeted area and remove scales (Figure 14). Collect about 6-10 scales from each fish and then place into a labeled scale envelope these collected scales. At a minimum, write on the scale envelope the serial number that links the fish with other data collected with the fish. The other pertinent details can be added to scale envelopes either before or after the fish are processed for scale removal.

Figure 13. Location of scale removal for ctenoid (e.g. Bluegill and Walleye) and cycloid (e.g. Northern Pike) scaled fishes.

Figure 14. Scale removal process on a Bluegill.

Preparation

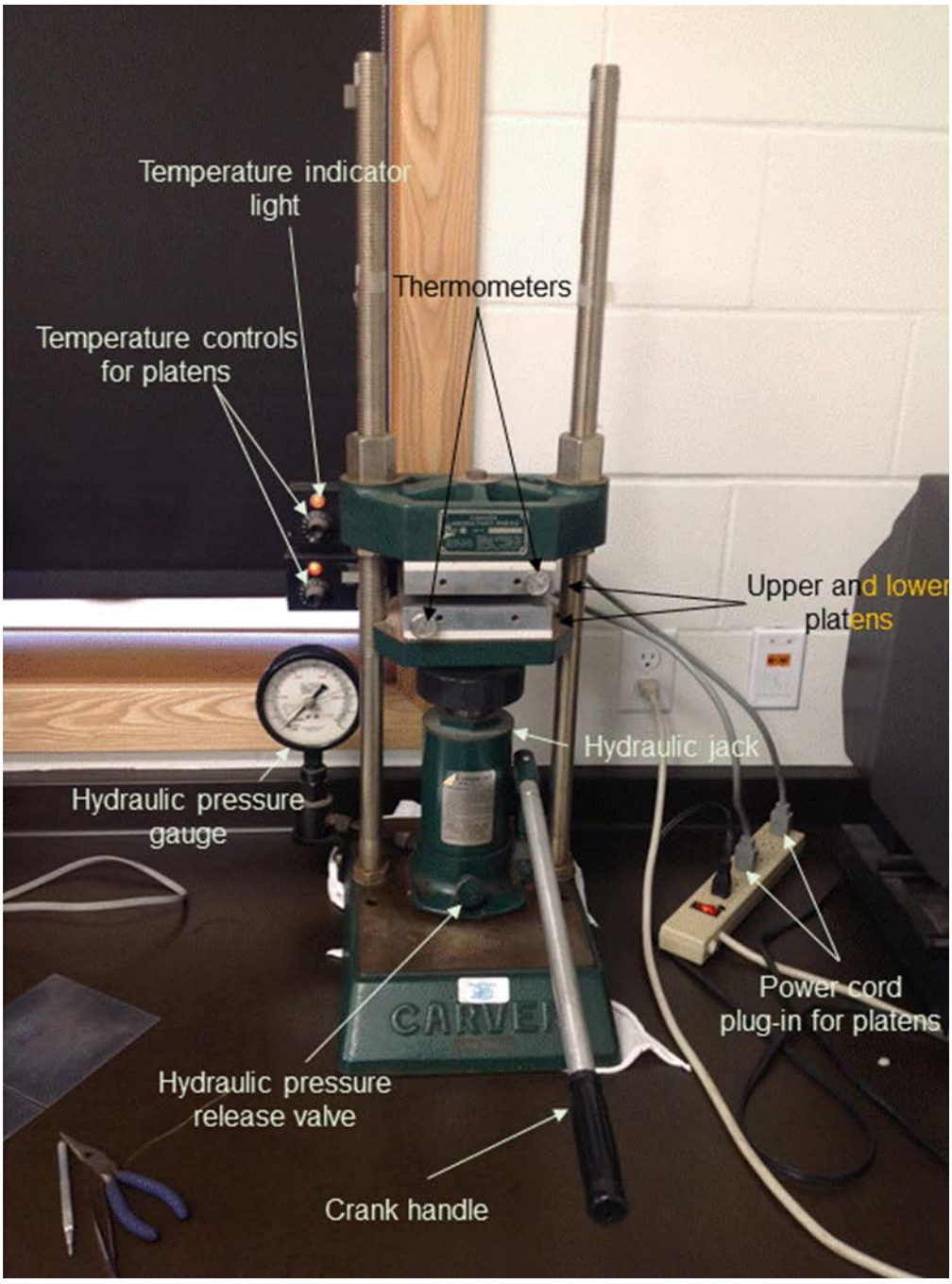
Scales can be viewed via mounting between two glass plates or slides and magnified with either a microscope or microfiche reader. Also, scale impressions made on acetate may be magnified with a microfiche reader, scale projector, or wall projector. Alternatively, digital photographs of scales can be taken and examined with the aid of computer software.

Glass mounting on a microfiche reader is discouraged for estimating age of fish of most species older than age 2 or 3. However, glass mounting of scales of age 0 and age 1 Walleye from fall electrofishing is encouraged because age estimates are reliable and small scales are more difficult to press. Scales thicken with age and increases in thickness is greater in the vicinity of the focus. Annuli near the focus on older fish are often not visible because of excess scale thickness. Studies have demonstrated lower between-reader precision or less accurate age estimates are made with annuli counts from glass-mounted scales than with scale impressions.

Making scale impressions on acetate

Cut acetate to desired dimensions. Using a guillotine-type paper cutter is an efficient way to cut a number of acetate pieces; however, a moderately heavy-duty scissors will also accomplish this task.

Figure 15. Heated hydraulic shop press typically used by MN DNR fisheries staff.

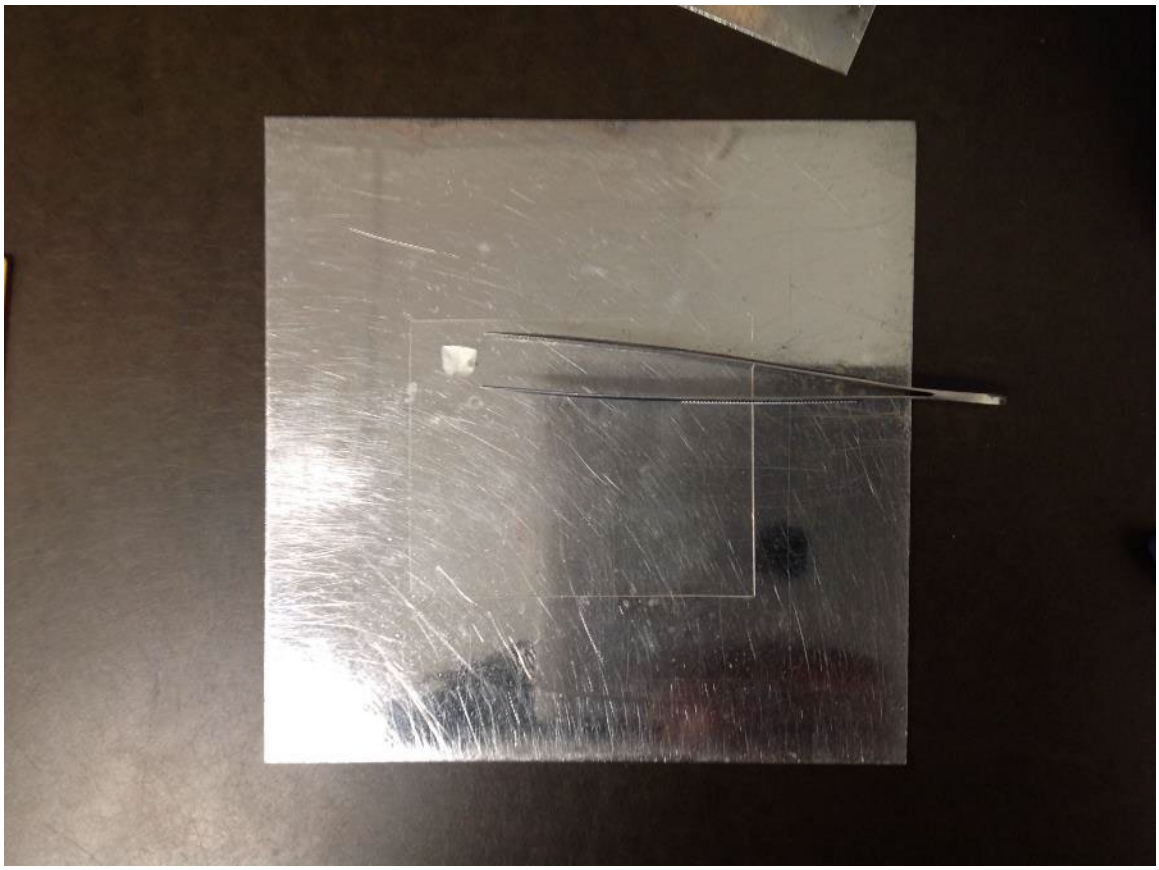


A hydraulic bench top lab press with heated platens is generally used to make scale impressions on acetate. Platens need to be completely parallel with each other so that they are flush when pressed together so that scale impressions are even and consistent on the acetate. The nuts above and below the upper platen can be loosened so that the platen can be adjusted. When adjustments are made, tighten nuts. This one-time adjustment usually is enough.

Both platens should be heated at similar temperatures (around 240 to 250 ºF) otherwise acetate will warp. However, thermometers on these presses are crude; thus, some tinkering with temperatures should be expected. Adjusting the temperature controls will increase or decrease platen temperatures. When satisfactory impressions become consistently made, leave the temperature settings as is or record the setting for use at a later date. The orange light above the temperature control turns off when the platen temperature reaches its maximum point for the particular setting of the temperature control.

Transfer scales from envelope to acetate. Hold scales up to the light to potentially see if they are regenerated. Do not use particular scales if they are obviously regenerated. With a forceps, pick a scale and then lightly rub the rough side onto the skin of your palm; the residual oil on the skin presumably acts as a mild adhesive so that static electricity cannot repel the scale from the acetate. Place acetate with the appropriate dimensions onto one of the metal plates. Make sure the dull, rougher side of the scale rests on the acetate and the smooth shiny side is away. Larger scales also curl during drying but the degree of curling declines with decreasing scale size. The convex surface of the scale is also the dull side; therefore, scales with the concave side away from the acetate are correctly positioned. The smallest scales are usually flat and the dull side and shiny side are also more difficult to distinguish.

Figure 16. Acetate on metal plate.



Arrange scales so that readers can easily determine which scales belong to the same fish, and try to press at least 3 scales per fish. Doing so increases odds that readers will find at least one useful scale per fish.

Figure 17. Scales from multiple fish on acetate before being pressed.

Arrange coin envelopes in the same order as sets of scales placed on acetate so that serial numbers written later on acetate correctly match those on envelopes. Place the second metal plate over the scales and then place this set of plates in between the two platens on the hydraulic shop press. Make sure hydraulic pressure release valve is closed; do so by turning knob clockwise until snug. Do not tighten too much because operators will need to quickly open the valve when the designated press time has been reached. Too tight of valve closure will lead to over-pressing of scales. Insert crank handle on the crank stem. Handle is usually off because it can stick out too far and become a safety hazard. In an up and down motion with crank handle, crank up the hydraulic jack so that the lower platen meets the upper platen and then continue cranking until reaching the desired hydraulic pressure.

When desired press time has been reached, open hydraulic pressure release valve by turning knob counterclockwise. The lower platen should drop immediately. Remove crank handle. With a pliers or heat-resistant glove, grab both metal plates (pinching them together) and place onto a table or cooling rack and let cool.

With a permanent marker or etching tool, write on acetate next to the appropriate set of scale impressions the serial numbers for the same individual fish. The date, lake/DOW, and species/species code should also be written somewhere on the acetate.

If making second reads, a scale impression can be identified by circling with either an etching tool or permanent marker, and a glass mounted scale can be marked with a permanent marker.

Reading

Scales are typically read through the use of a microfiche reader (Figure 18). Annuli are conspicuous marks that can be distinguished from circuli via the following criteria:

- Crossing-over pattern in the lateral and posterior-lateral region
- Crowding of circuli, a conspicuous space between two circuli, intermittent breaks or stippling in a circulus, or intermeshed circuli appearing in a chain-like pattern
- In the posterior region, continued crossing over, crowding or thinning of annuli, conspicuous space between circuli that continues around the focus, and boundaries between bands of ctenii
- Occurs in the same region in scales from the same fish
- Should be able to visualize a small scale within the scale after connecting the mark with the above criteria from all regions of the scale

Figure 18. Microfiche reader used to magnify scale images.

Fin Rays and Spines

Removal

Fin rays and spines should be removed by clipping the first two or three rays/spines, on the leading edge of the fin, as close to the body as possible, using a side cutters. The first dorsal spine is usually small and not useful for aging, so two or three spines should be taken to ensure a quality structure is collected. Typically the first pectoral/anal fin ray is the largest and the best for aging, but subsequent rays may also be adequate. Dorsal spines are typically used for Walleye, Largemouth Bass, Smallmouth Bass, Yellow Perch and common carp. Pectoral fin rays are used for brown trout, brook trout, lake sturgeon, and shovelnose sturgeon. Anal fin rays are used for Northern Pike and muskellunge.

Removal of pectoral spines from catfish follows a different process, because the entire pectoral spine is needed. Catfish pectoral spines are removed by disarticulating the spine from the body. First, you must unlock the catfish spine by pulling outward on the spine and then pushing the spine flat against the body. Once unlocked, rotate the spine towards the head until it disarticulates from the joint. On larger fish, a knife may be helpful to cut the skin and connective tissue holding the spine to the body. The articulating process should be intact after the spine is removed (Figure 19). The spines can be stored in scale envelopes and allowed to dry before cutting and reading.

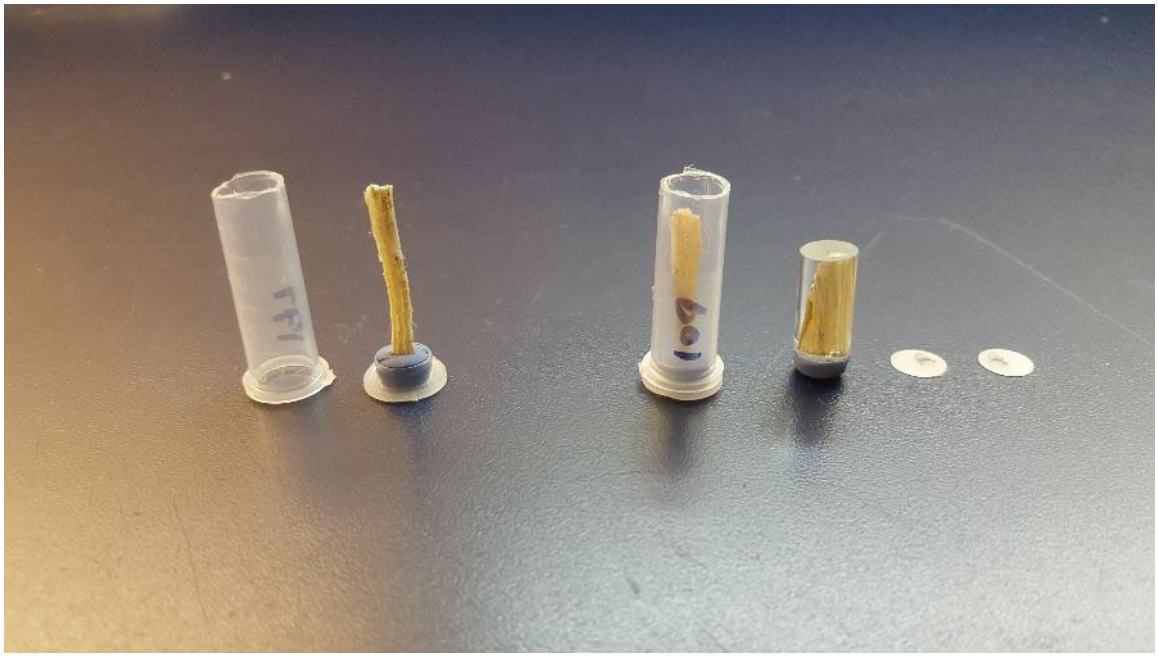
Figure 19. Photo sequence of channel catfish pectoral spine removal

Preparation

Fin rays and spines (excluding catfish) can be sectioned and viewed under a microscope with transmitted light, or left whole so the proximal end can be viewed under a microscope with a fiber optic light.

When sectioning, thin sections are cut from the proximal end of fin rays or spines, polished with fine grit sandpaper and viewed under a dissecting microscope using transmitted light. Fin rays and spines from large fish may be sectioned as is, but small fin rays and spines may need to be mounted in two-part epoxy prior to sectioning. Fin rays and spines can be mounted by standing them up in clay inside of a microcentrifuge tube which is filled with epoxy (Figure 20). With this method, the tip of the tube is cut off, the structure is stood up in the tube cap using a small piece of clay, making sure the proximal end is up, and the tube is placed cap side down. The structure may need to be broken in half to fit inside the tube. The tube is then filled with epoxy. When hardened, the embedded structure can be pushed out of the tube and sectioned with a low speed saw. A block of wood with a nail partially pounded in works well for pushing the epoxy out of the tube. Place the nail head inside one end of the tube and lightly tap the edge of the tube with a hammer and the embedded structure will slide out. Embedding can also be done by placing the structures in mounting trays and covering with epoxy as described in the otolith mounting section.

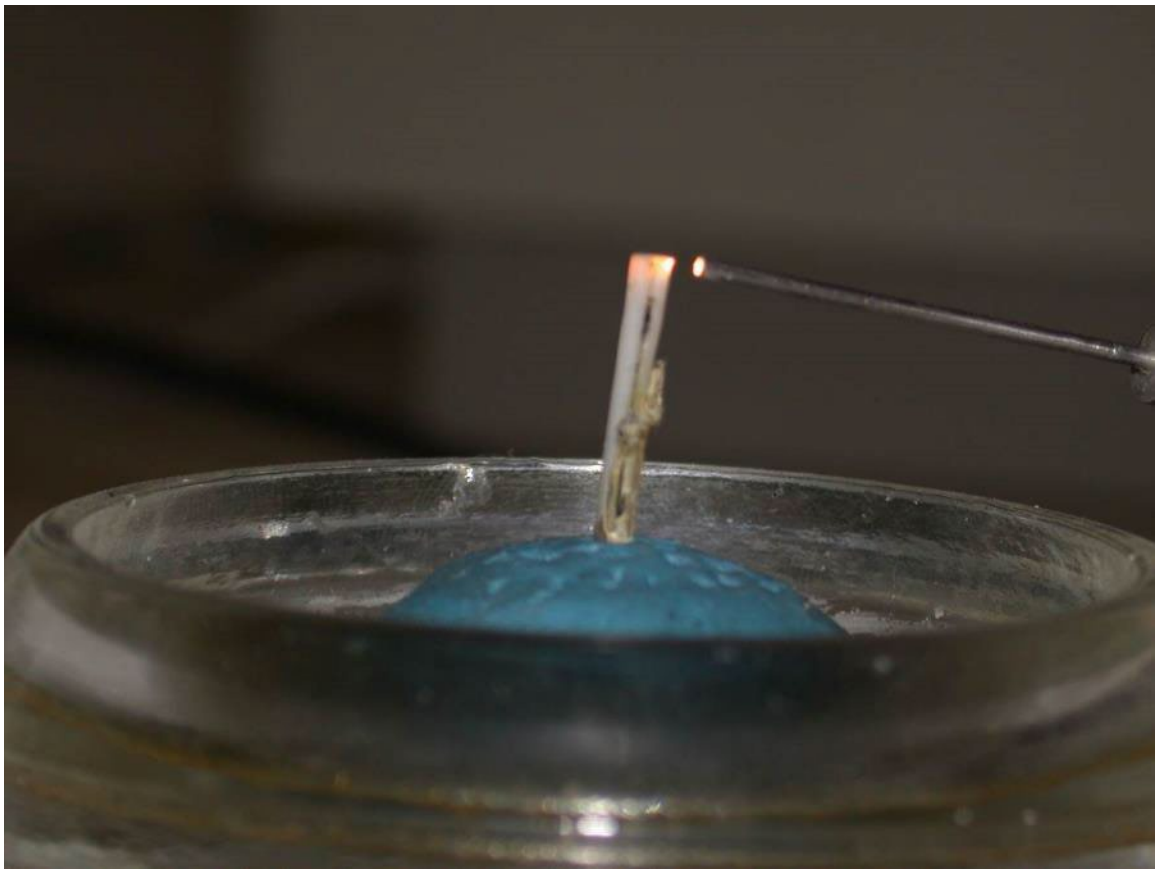
Figure 20. Microcentrifuge tube used for mounting small fin rays and spines in epoxy.



Sections should be cut using a low-speed saw at a thickness of 0.5-1.0 mm. Some trial and error may be required to determine the best thickness for the fish being aged. Two or three sections should be cut from each structure to ensure that all annuli are visible on at least one section. After sectioning all pieces of the structure should be placed back into the scale envelope.

When viewing fin rays or spines whole, the proximal end should be cut or sanded to produce a flat viewing surface. The structure can then be polished with 1000-grit sandpaper, if necessary, covered with a drop of immersion oil (or baby oil) and viewed with a fiber optic light source (Figure 21).

Figure 21. Fiber optic light placement for reading the basal end of a Walleye dorsal spine.



Catfish

Basic preparation of catfish pectoral spines includes removing dried skin and tissue with a scalpel and tweezers (this is easier when most tissue is removed in the field), and then cutting thin sections (0.5–1.0 mm) out of the articulating process with a low speed isomet saw (using a diamond wafer blade with a medium–high concentration of medium grit). Although not necessary, various chemicals (e.g., BIZ detergent and ammonia, ethanol) and processing steps (e.g., dermestid beetles, baking) can soften and clean spines. Spines can also be mounted in epoxy prior to sectioning, but this is unnecessary and greatly increases processing and sectioning time.

Rather than sectioning the thin distal section of the spine, catfish pectoral spines are aged by sectioning the articulating process (proximal end of the spine). Annuli are extremely visible on these sections, but the central lumen of the articulating process can erode the first couple annuli in older fish and the outer most annuli may be absent on the most proximal sections. For this reason, two–four sections should be used to estimate ages on larger fish (> 400 mm) to increase the liklihood of detecting the first and last annuli. The first section of the articulating process should begin at the base of the ventral process (Figure 22) and subsequent cuts progressing distally 0.5–1.0 mm apart (Figure 23).

Figure 22. Channel catfish pectoral spine secured in an Isomet saw and ready for sectioning. The first cut is made at the base of the ventral process.

Figure 23. Cutting locations for sectioning catfish pectoral spines. The first cut is at the base of the ventral process with subsequent cuts made distally, 0.5-1.0 mm apart.

Reading

Sections can be placed directly on the stage of the dissecting microscope. When using transmitted light to view fin ray or spine sections, annuli are identified as light bands against a dark background (Figure 24). Whole fin rays or spines should be stood up vertically in a block of clay so the flat surface can be viewed under the microscope. When viewing whole fin rays or spines with a fiber optic light, annuli appear as dark, hyaline bands against a lighter opaque background (Figure 25).

Figure 24. Sectioned white sucker fin rays viewed with transmitted light. Annuli appear as light bands against a dark background. These fin rays were embedded in epoxy prior to sectioning.

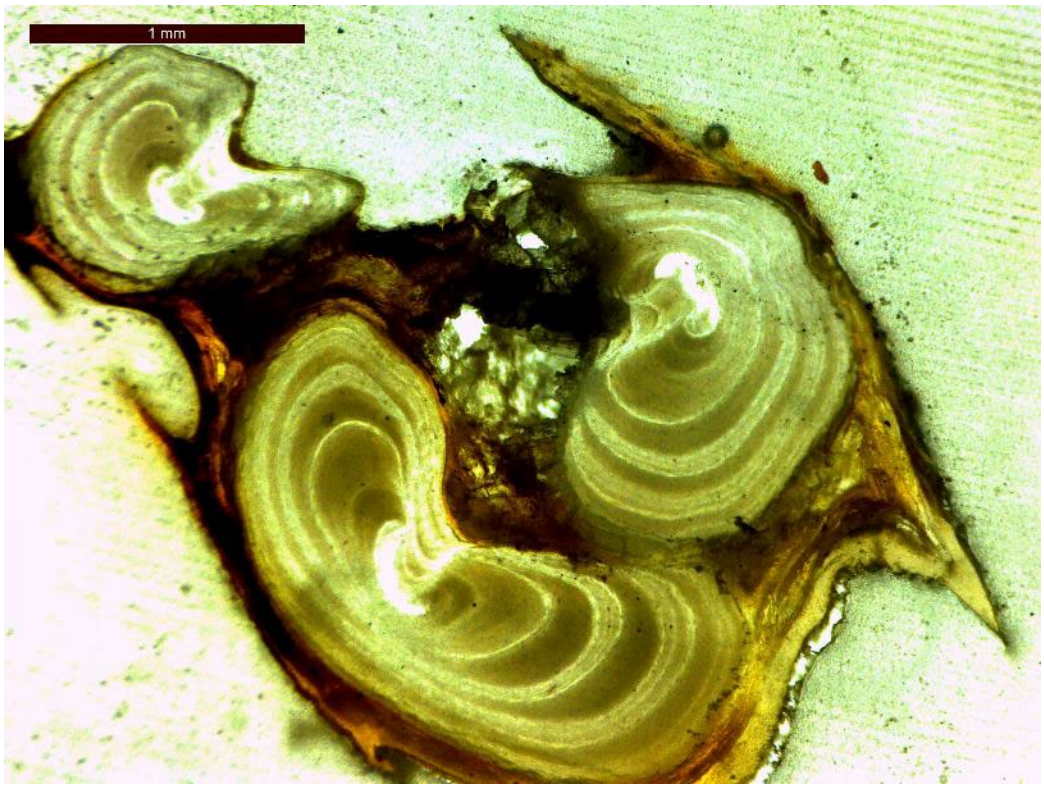
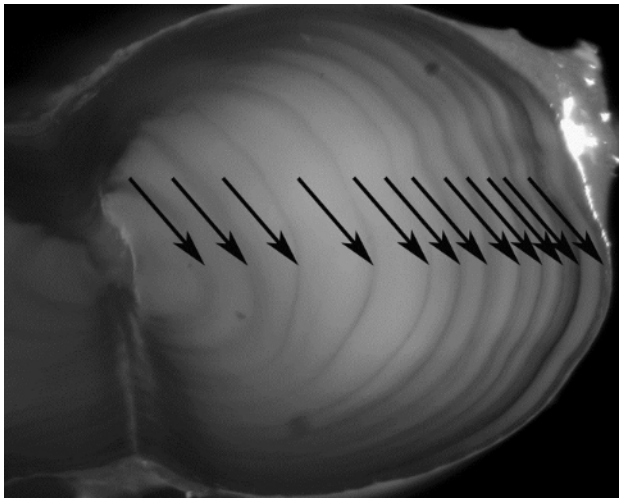
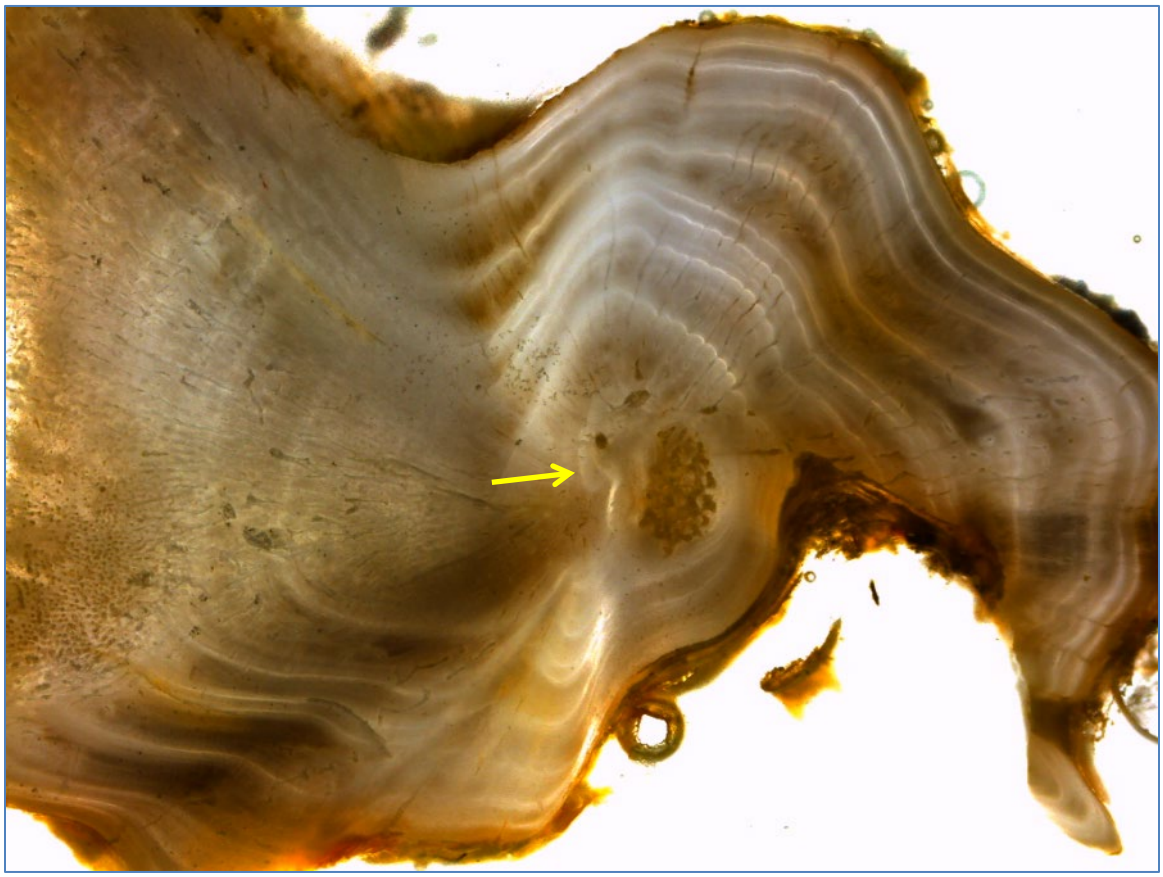


Figure 25. Whole Walleye dorsal spine viewed with a fiber optic light source. Annuli appear as dark bands against a lighter background.



Methods for reading sectioned catfish spines are the same as for reading other sectioned fin rays and spines. When using transmitted light, annuli appear as white or light colored bands against a darker, opaque background (Figure 26). The first annulus is often difficult to see but can usually be seen on at least one section. This highlights the need to have multiple sections, as it is often useful to look at more than one section simultaneously.

Figure 26. Sectioned Channel Catfish pectoral spine viewed under a dissecting microscope with transmitted light. Annuli appear as light bands against a darker background. The first annulus is often the most difficult to see (indicated with yellow arrow).



Cleithra, Opercles, Maxillae, Dentary

Bones are removed and as much tissue is removed as possible before drying.

Cleithra

Cleithra are submersed in water in a black dish and viewed with the naked eye and ambient light or using a fiber optic light source (Faust et al 2013).

Opercles

Opercles are sometimes used to age Walleye and Yellow Perch. They are typically less precise than otoliths. They can be read with the naked eye or with the aid of a dissecting scope. For instructions on their use, refer to (Baker and McComish 1998).

Maxillae

Maxillae have been used to age lake trout. For instructions on their use, refer to Wellenkamp et al (2015).

Dentary

Dentary bones are primarily used to age Paddlefish. Thin sections are made through the mesial arm of the dentary bone and read under a dissecting microscope with transmitted light. For instructions on their use, refer to (Adams 1942).

References for structure preparation

- Removal and storage (Isermann et al. 2003) (Vandergoot et al. 2008) (LaBay and Lauer 2006) (Lucchesi and Johnson 2006) (Muir et al. 2008)
- Preparation (Maceina 1988; Logsdon 2007; Williamson and Dirnberger 2010)

Sample Size

Requirements

How many fish to sample will be dependent on what growth metrics you want to report. Sample size is also dependent on species, variation in growth within your population, and how big of a growth difference you want to be able to detect (see Figure 27 as an example). Lake prioritization categories (Long Term Monitoring, Management Evaluation Lakes, and Maintenance Lakes) may also influence your sample size requirements. For example, if you want to give a rough idea of Bluegill growth at a lake association meeting, 20 fish maybe sufficient. However, if you want to propose a new angling regulation for Black Crappie, you may need over 100 fish. Unfortunately, standard lake survey sampling was not designed for growth estimates and oftentimes results in inadequate sample sizes. If previous lake surveys indicate low catch rates for a particular species, you may decide beforehand not to collect any age structures because it is unlikely that you will reach the recommended sample size. Based on lake survey data from 2006-2013, only 53% of surveys in lakes where walleyes were present had more than 20 walleyes aged. In lakes where Bluegill are present, 63% of surveys aged more than 20 Bluegill. Less than 1% of our lake surveys aged enough Bluegill (N > 100) to make informed management decisions. Targeted sampling will likely be required to obtain sufficient sample sizes.

Length based subsampling

A subsample of ten fish from each 25-mm size group for large-bodied species such as Walleye, Northern Pike, Largemouth Bass, and Smallmouth Bass is usually sufficient in terms of sample size. Collecting more than 10 per length bin has small effects on reducing your standard errors (Figure 28). For smaller bodied fishes (e.g., Bluegill, crappie, and perch), ten fish in each 10-mm size group is sufficient. Sample sizes can be increased by using back-calculated lengths at age. For estimates of mean age at length, usually 20 fish are needed within the specified length group. Sample sizes less than 20 are unlikely to provide any meaningful growth data.

Random subsampling

In random subsampling the first 100-200 fish are retained for age determination, regardless of the size of fish. This can also be done by taking the first 25-50 fish from each gear set. This method gives you an unbiased representation of age.

Figure 27. Sample size needed to detect a 25mm difference in mean length at Age-3 for Walleye.

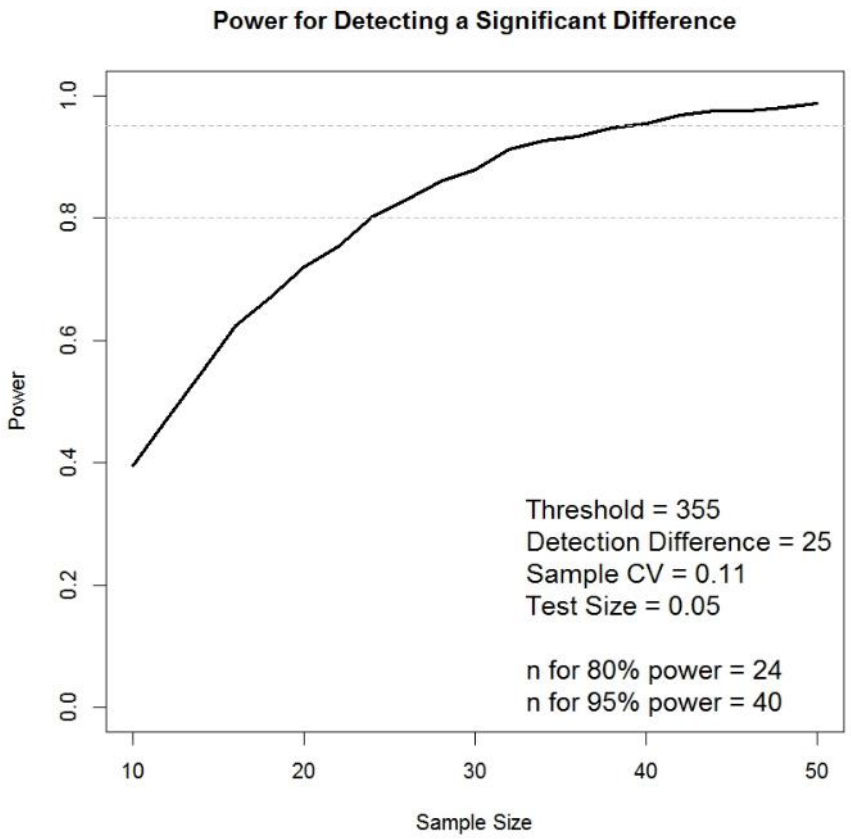
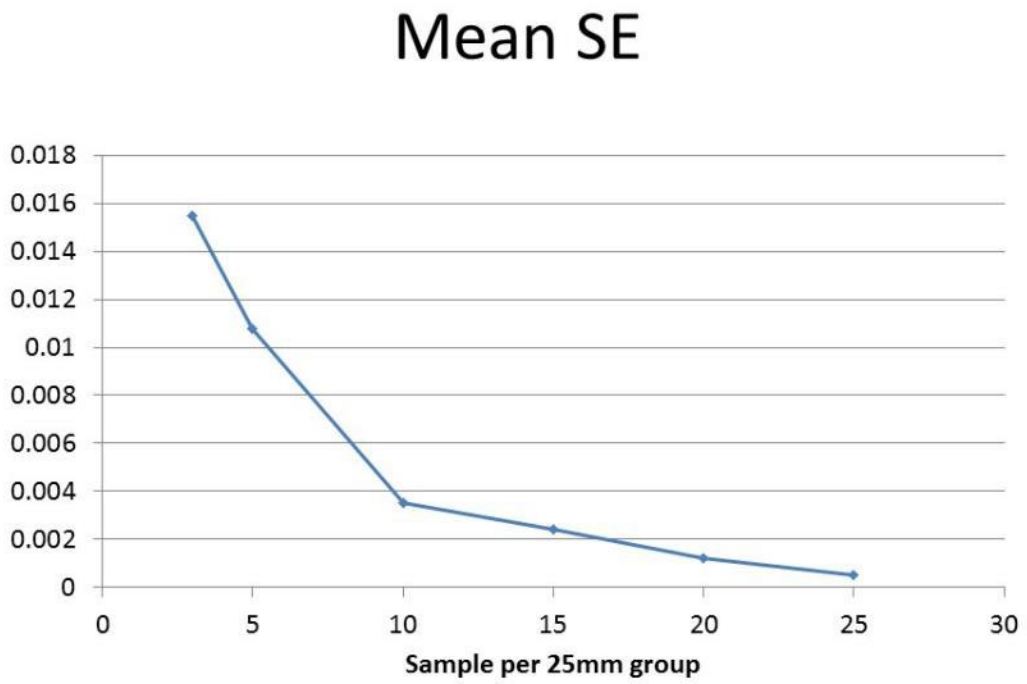


Figure 28. Mean standard error for mean length at age 3 walleyes based on how many are sampled from each 25mm length bin.



References for sample size

- Sample Size (Dumont and Schlechte 2004) (Worthington et al. 1995) (Gerow 2007) (Vokoun et al. 2001) (Miranda 2007) (Kritzer et al. 2001)
- Subsampling (Bettoli and Miranda 2001)

Structure Comparisons

Hard Structures

Scales are a common aging structure for many fish species and have been used extensively to assign age estimates to fish since they were first recognized to have age information as early as 1890. In Minnesota, scales have been the official aging structure since 1993, however, otoliths have been used more recently for aging Walleye. Other structures have also been used in age estimation, including dorsal spines, cliethra, fin rays, and opercles. However, in recent years it has been noted that scales may be neither as accurate nor as easy to process as alternative structures such as otoliths, dorsal spines, cliethra, fin rays, or pectoral spines (Ictalurids). Otolith age estimations have been found to be accurate and validation of ages has been accomplished for many species of fish. Additionally, aging otoliths instead of scales has proven to be a cost benefit and time savings compared to scales.

Whole vs sectioned otoliths

Whole view otoliths can be used to age younger fish accurately; however, transverse otolith sections are the most accurate for aging fish across many years of growth for many species.

Table 1. Maximum recommended ages for using calcified structures in Minnesota. Crack and burn otoliths represent the maximum age of that species using this method.

	Otoliths				
	CB	Whole	Scales	Rays/Spines	Other
Brook Trout			2		
Black Crappie	17		4	Do not use	
Bluegill					
Brown Trout			3		
Carpsucker spp.					
Freshwater Drum					
Green Sunfish					
Hybrid Sunfish					
Lake Trout					
Lake Whitefish	62				
Largemouth Bass	22		3	Do not use	
Muskellunge					
Northern Pike					
Pumpkinseed					
Rainbow Trout					

	Otoliths				
	CB	Whole	Scales	Rays/Spines	Other
Rock Bass					
Sauger					
Smallmouth Bass			3	Do not use	
Tullibee					
Walleye	28			7	
White Crappie			5		
White Sucker					
Yellow Perch					

Validation Techniques

Age validation is an often overlooked criteria for aging fish. Both the structure and the methodology should be validated before any age interpretation. Fortunately, many of the suggested aging techniques in this manual have been validated for commonly aged species. Although many techniques and structures have been validated, it still does not mean that their interpretation is precise or even accurate. Age interpretations can be validated in three general ways: 1) validation with known age fish, 2) validation of the periodicity of increment formation and 3) corroborating an existing set of age interpretations. Examples of methods that have been used for age validation are: Release of known-age and marked fish into the wild, bomb radiocarbon dating, date-specific markers, marginal increment analysis, length frequency analysis, and progression of strong year-classes.

The most important, and often overlooked in age validation studies, is validation across the entire longevity of the species of interest. The age-determination methods used for many species likely provide accurate age estimates for the period of rapid growth because growth increments are relatively consistent and the differential rates between rapid and slow (winter) growth are substantial enough to create distinct annular marks. However, when growth is reduced (older and occasionally younger ages) due to sexual maturity, food limitations, environmental conditions, etc., appearance of annuli may change and the method of age determination subsequently may need to be modified. Consequently, these segments of the population are often the most difficult to age accurately.

Table 2. Summary of structures that annulus formation has been validated for at least some age classes. Common species that no annulus validation studies were found are also included. Adapted from Maceina et al (2007).

Species	Otoliths	Scales	Spines	Fin rays	Vertebrae	Cleithra	Opercula
Black Crappie	x	x					
Bluegill	x						
Brook Trout	x						
Brown Trout							
Channel Catfish	x		x		x		
Cisco							
Flathead Catfish			x				
Freshwater Drum	x						
Lake Sturgeon				x			
Largemouth Bass	x	x					
Muskellunge		x		x			
Northern Pike		x		x		x	x
Rainbow Trout	x	x					
Smallmouth Bass	x						
Walleye	x						x
Yellow Perch	x						

References for structure comparisons

1.1. Hard Structures comparisons (Hoxmeier et al. 2001) (Isermann et al. 2011) (Sylvester and Berry 2006) (Kruse et al. 1993) (Isermann et al. 2003) (Maceina et al. 2007) (Erickson 1983) (Howells et al. 1995) (Long and Fisher 2001) (Ross et al. 2005) (Vandergoot et al. 2008) (Boxrucker 1986) (Hammers and Miranda 1991) (Niewinski and Ferreri 1999) (Kocovsky and Carline 2000) (Maceina and Sammons 2006) (Stolarski and Hartman 2008) (Kowalewski et al. 2012)

- Whole vs sectioned otoliths (Beamish 1979) (Skurdal et al. 1985) (Long and Fisher 2001)
- Validation
    - Bluegill (Regier 1962) (Hales and Belk 1992) (Schramm 1989)
    - Crappie (Maceina and Betsill 1987)
    - Bass (Buckmeier and Howells 2003) (Taubert and Tranquilli 1982
    - Walleye (Heidinger and Clodfelter 1987)
    - Gizzard shad (Clayton and Maceina 1999)
    - Lake Sturgeon (Bruch et al. 2009)

Species Specific Reference

The following are species specific recommendations for age and growth. More detail regarding the recommended procedures can be found in the proceeding sections.

Black Bass (Largemouth Bass and Smallmouth Bass)

Preferred structure and preparation: Crack and burn otolith

Subsample: 10 fish per 25-mm size group

Sample size:

- 20 for mean age at length
- 20 for mean length for a specific age
- 100 for age structure and growth rate

Annulus formation: Late May – Early July

Comments: Determination of first annulus and the focus can be difficult using whole otoliths.

References to black bass aging: (Long and Fisher 2001)

Bluegill

Preferred structure and preparation: Crack and burn otolith

Subsample: 10 fish per 10-mm size group

Sample size:

- 20 for mean age at length
- 20 for mean length for a specific age
- 100 for age structure and growth rate

Annulus formation: Late May – Early July

Comments: Determination of first annulus and the focus can be difficult using whole otoliths.

References to Bluegill aging: (Hoxmeier et al. 2001; Regier 1962; Hales and Belk 1992; Schramm 1989; Kowalewski et al. 2012)

Catfish

Preferred structure: Pectoral spine

Subsample: 10 fish per 25-mm size group

Sample size:

- 20 for mean age at length
- 20 for mean length for a specific age
- 100 for age structure and growth rate

Annulus formation: Late June – Early August

Comments:

References to catfish aging: (Buckmeier et al. 2002; Colombo et al. 2010; Michaletz et al. 2009)

Crappie spp. (Black and White Crappie)

Preferred structure and preparation: Crack and burn otolith

Subsample: 10 fish per 10-mm size group

Sample size:

- 20 for mean age at length
- 20 for mean length for a specific age
- 100 for age structure and growth rate

Annulus formation: Late May – Early July

Comments: Determination of first annulus and the focus can be difficult using whole otoliths.

References to crappie aging: (Isermann et al. 2011; Kruse et al. 1993; Boxrucker 1986; Hammers and Miranda 1991; Maceina and Betsill 1987; Schramm and Doerzbacher 1985)

Lake Trout

Preferred structure and preparation: Thin sectioned otolith or Maxillary bone

Subsample: 10 fish per 25-mm size group

Sample size:

- 20 for mean age at length
- 20 for mean length for a specific age
- 100 for age structure and growth rate

Annulus formation:

Comments:

References to lake trout aging: (Sharp and Bernard 1988)

Northern Pike

Preferred structure and preparation: Thin sectioned otolith

Subsample: 10 fish per 25mm size group

Sample size:

- 20 mean age at length
- 20 for mean length for a specific age
- 100 age structure, growth rate, …

Annulus formation:

Comments: Northern pike are difficult to age using any structure.

References to Northern Pike: (Faust et al. 2013; Oele et al. 2015; Blackwell et al. 2016)

Trout (stream)

Preferred structure and preparation: Thin sectioned otolith or pectoral fin ray

Subsample: 10 fish per 10-mm size group

Sample size:

- 20 for mean age at length
- 20 for mean length for a specific age
- 100 for age structure and growth rate

Annulus formation:

Comments:

References to trout aging: (Hall 1991; Hining et al. 2000; Shirvell 1981; Stolarski and Hartman 2008)

Walleye and Sauger

Preferred structure and preparation: Crack and burn otolith

Subsample: 10 fish per 25-mm size group

Sample size:

- 20 for mean age at length
- 20 for mean length for a specific age
- 100 for age structure and growth rate

Annulus formation: Late May – Early July

Comments:

References to Walleye aging: (Borkholder and Edwards 2001; Erickson 1983; Frie et al. 1989; Logsdon 2007; Isermann et al. 2003; Kocovsky and Carline 2000; Lucchesi and Johnson 2006; Olson 1980)

References to Sauger aging: (Williamson and Dirnberger 2010)

Yellow Perch

Preferred structure and preparation: Crack and burn otolith

Subsample: 10 fish per 10-mm size group

Sample size:

- 20 for mean age at length
- 20 for mean length for a specific age
- 100 for age structure and growth rate

Annulus formation: Late May – Early July

Comments:

References to Yellow Perch aging: (Blackwell and Kaufman 2012; Niewinski and Ferreri 1999; Robillard and Marsden 1996; Vandergoot et al. 2008)

Other Species

- Buffalo ;
- Gizzard shad (Clayton and Maceina 1999);
- Lake Sturgeon (Bruch et al. 2009) ;
- Paddlefish ;
- White bass ;
- White Sucker (Sylvester and Berry 2006) (Beamish and Harvey 1969) (Scidmore and Glass 1953) (Smith et al. 2008) (Ostazeski and Spangler 2001) (Thompson and Beckman 1995) (Quinn and Ross 1982)

Quality Control and Assurance

Only quality age data should be entered into the Fisheries Survey Module (FSM) (see Figure 29 for recommended procedure). There are several ways to measure accuracy and precision of age estimates. Accuracy of a structure can only be assessed using known age fish. Precision can be assessed by using multiple readers. Many methods have been developed to compare the precision of age estimates. The most common are percent agreement, coefficient of variation (CV; Chang 1982), and average percent error (Beamish and Fournier 1981). Campana et al. (1995) pointed out that all measures of precision will be artificially inflated by any bias which exists between agers and that systematic differences (bias) are more serious and should be addressed before precision. Comparisons based on matched pairs (i.e., two agers estimate age from the same structure) always provides the most statistical power and age-bias plots and CV can be used for multiple comparisons (Campana et al.1995). A goal obtaining a CV less than 5% should serve as a reference point for many fishes of moderate longevity and reading complexity (Campana 2001). A percent agreement above 80% is usually considered acceptable for an aging technique (Maceina et al. 2007).

A quality control program monitors short- and long-term aging consistency, both within and among age readers, by insuring that the age interpretation method does not drift through time. The following is a quality control protocol that is recommended by many aging laboratories:

1.) development of a reference collection consisting of known-age or consensus-aged structures.

2.) periodic aging of a randomly-drawn, blind-labelled subsample of the reference collection, intermixed with a subsample of structures recently aged as part of routine aging.

3.) use of age-bias graphs and CV as tools to evaluate the results of the monitoring (Campana et al., 1995).

4.) Procedures and equipment should be standardized.

5.) Acceptable levels of accuracy and bias be determined.

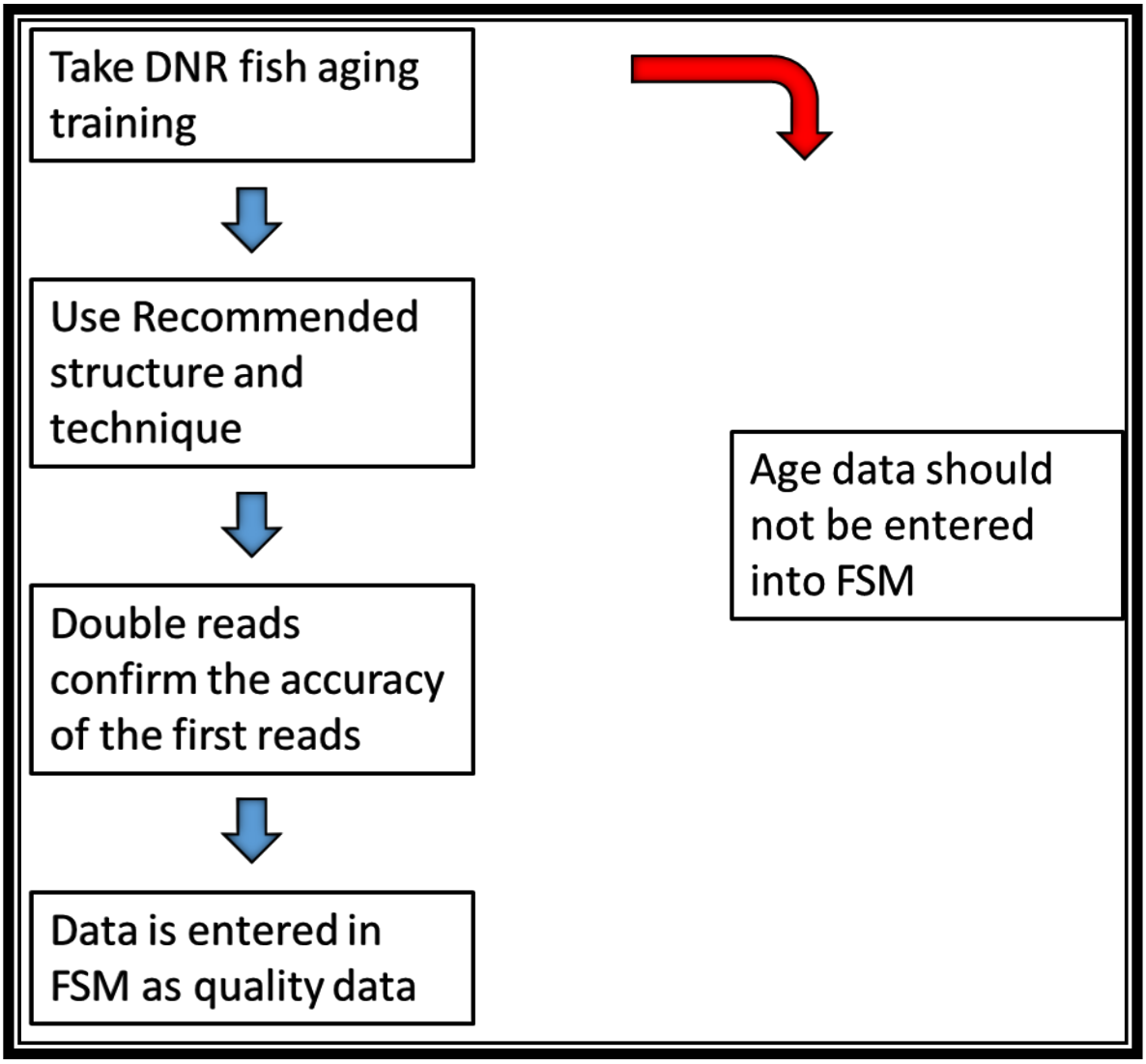
6.) Readers be tested with double reads.

Reference collections of otoliths or other aging structures are important elements of any ongoing aging program. The primary role of a reference collection is to monitor individual aging consistency over time, as well as among readers. A secondary role is for training purposes. Reference collections can be a collection of known or consensus derived ages, representative of all factors which might be reasonably expected to influence the appearance or relative size of the growth increments. Factors including: age, sex, season, source of collection, geographical range, and several years of collections. “Average” preparations are likely of more valuable than are ideal preparations (Campana 2001).

Protocol for assigning age

What information should you use to help you correctly assign an age to an individual fish? Although not necessary, it is advisable to sort age samples from a given lake by species, and then sort each species by total length (smallest to largest). Each species has different patterns in annulus formation. The smallest fish are usually the youngest, and structures from the youngest individuals of a species are often the easiest to interpret. Examination of the youngest fish first also gives readers a better idea where the first and second annuli are located on the structures of older individuals of the same species and lake. Use of length-frequency distributions to help identify annuli is also encouraged, especially if gears sample a wide range of lengths of the species being examined.

Figure 29. Recommended quality control procedure for entering age data into the Fisheries Survey Module.



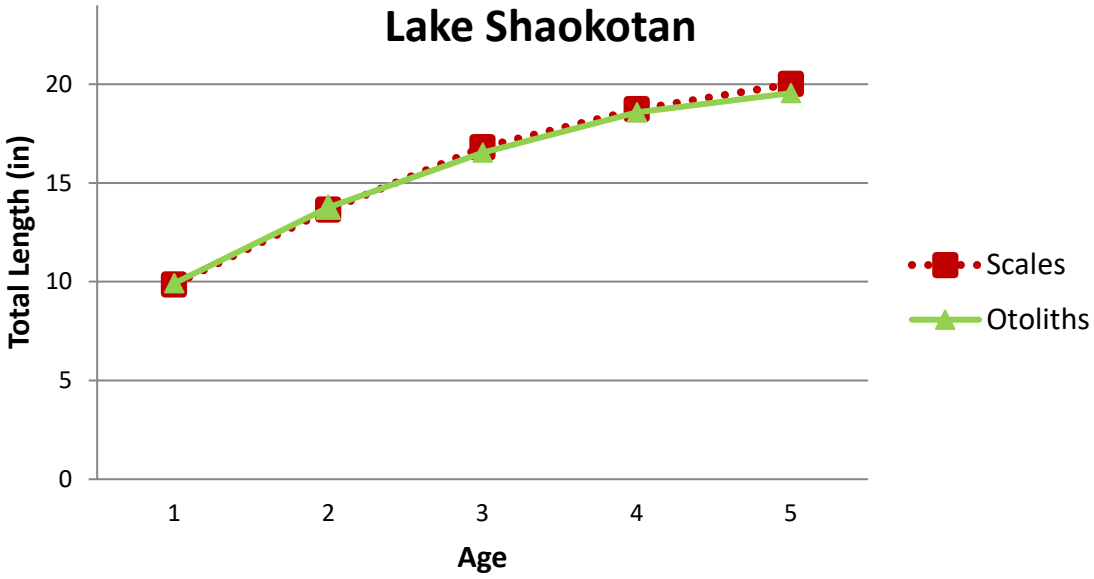
References for quality control

- (Beamish and Fournier 1981; Buckmeier 2002; Campana 2001; Chang 1982; Campana et al. 1995; Colombo et al. 2010; Hoenig et al. 1995; Francis et al. 2010; Maceina et al. 2007; Morison et al. 2005) (Richards et al. 1992)

Back Calculation and Increment Measurements

Any structure that grows in proportion to body size can be back-calculated to obtain estimates of earlier growth (Figure 30). However, scales are often more precise than other structures for back-calculation. Back-calculation of bony structures is useful for increasing sample size, to compare growth across lakes sampled at different times, obtaining historical growth information, and overcoming age biased sampled gears. Several methods of back-calculation are used to estimate length-at-age, including the direct proportion method (Dahl-Lea method), the intercept corrected direct proportion method (Fraser-Lee method), regression method (linear, nonlinear, polynomial methods), and the Weisburg method (Weisberg et al. 2010)(Box 1). The Dahl-Lea (mainly otoliths) and the Fraser-Lee (mainly scales) are the most widely used and validated back-calculation methods. Back calculation of older fish using otoliths may require the use of an equation that takes into account the decoupling of somatic growth and otolith growth. These equations incorporate an age effect (Finstad 2003). Back-calculated lengths can often times underestimate previous lengths-at-age (Lee phenomenon) due to effects of differing somatic growth during different stages of life and due to incorrect use of back-calculation methods. Environmental changes in an aquatic system and elemental differences in aging structures can also have effects upon annuli and structure formation. Back-calculation of lengths-at-age to only the most recently formed annuli (i.e. last 2 years of growth for a 5 year old fish) is another way to reduce the effect of environmental changes and reduce the Lee phenomenon. In addition, biologically derived intercepts as opposed to statistically derived regression based intercepts are more accurate for back-calculation equations while using scales. Biologically derived intercepts can be gained from literature or from fish production and particular attention should be focused on potential regional effects and potential unique stocks (see Table 3). Statistically derived intercepts can be used, but sometimes the biological interpretations of the results are limited. Ultimately, back-calculated lengths are estimates that contain inherent error and fisheries managers need to determine the amount of error they are willing to accept in these estimates. In addition, it is important to select the most reliable aging structure and methods based on availability of that structure and how the data will be used.

Figure 30. Comparison of back-calculated lengths using scales and otoliths for Walleye from Lake Shaokotan.



Which equation do I use?

Maceina et al. (2007) state that a single computational method for back-calculating growth does not nor should exist. They go on to state that fishery biologists should be cognizant of the factors that influence back-calculation and select the most appropriate method for the data. Additionally, methods that work for one species or aging structure, may not be appropriate for another (i.e. slow vs. fast growth). Maceina et al. (2007) offer a suite of questions that should be asked and answered before back-calculating; they are as follows:

- Which hard part will be used to estimate age?
- Can ages accurately be assigned to that hard part?
- Can annular increments accurately be measured?
- Along what axis should measurements be taken?
- Is the body length-to-hard part relation linear?
- Which back-calculation formula (method) should be used?

Box 1. Equations for back calculating fish length.

Direct Proportion (Dahl-Lea): $L_i = \left(\frac{SR_i}{SR}\right)L_c$

Regression: $L_i = b_0 + b_i SR_i$

Fraser-Lee: $L_i = b_0 + (L_c - b_0)\frac{SR_i}{SR}$

Where:

L_i = Length at time interval *i*

L_c = Length at capture

SR_i = Structure radius at time interval *i*

SR = Structure radius at time of capture

b_0 = y-intercept or "length of fish at age structure formation"

- often set at a constant

b_i = slope parameter

Table 3. Fraser-Lee constants used in the MNDNR lake Survey program.

Species	b_0 (mm)	Species	b_0 (mm)
Brook Trout	46	Northern Pike	53
Black Crappie	20	Pumpkinseed	25
Bluegill	20	Rainbow Trout	33
Brown Trout	30	Rock Bass	25
Carpsucker spp.	23	Sauger	25
Freshwater Drum	20	Smallmouth Bass	36
Green Sunfish	10	Tullibee	36
Hybrid Sunfish	25	Walleye	28
Lake Trout	30	White Crappie	20
Lake Whitefish	36	White Sucker	30

Species	b_0 (mm)	Species	b_0 (mm)
Largemouth Bass	20	Yellow Perch	30
Muskellunge	76		

References for back calculation

- Scales (Casselman 1990; Howells et al. 1995; Klumb et al. 1999; Klumb et al. 1999; Klumb et al. 2001; Ricker 1992)
- Otoliths (Ashworth et al. 2017; Beamish 1979; Blackwell and Kaufman 2012; Campana 1990; Clayton and Maceina 1999; Howells et al. 1995; Klumb et al. 2001; Finstad 2003; Li et al. 2008; Michaletz et al. 2009) (Morita and Matsuishi 2001; Taubert and Tranquilli 1982; Vigliola et al. 2000)
- Fin rays/spines (Borkholder and Edwards 2001; Michaletz et al. 2009)
- Methods (Rypel 2008)
- Benefits/pitfalls (Heidinger and Clodfelter 1987; Klumb et al. 2001; Francis 1990; Johdal et al. 2001; Wahl et al. 2009)

Growth Metrics

Before generating any growth or age metrics, unaged fish from the sample need to be assigned an age. This can be done using an age-length key, a growth curve, or a mixed distribution model.

Mean length at age

Unaged fish must be assigned an age and this is often done through the FSM. Comparisons for some species and lake class can be found in Appendix .

Mean age at length

Generating mean age at length is useful when you only need a general idea of whether your population is stunted or fast growing. It allows you to target a specific size of fish and gives you a reliable estimate of growth using a small sample size. It also provides an intuitive statistic to report to anglers, as many anglers are curious as to "How old is a 15" Walleye?". The following lengths were chosen based on the availability in our sampling gear and sizes anglers find acceptable to harvest (Table 4). When collecting fish for age structures, collect fish within 10 mm of the recommended length. For example, take age structures from 20 Bluegill between 168 and 188mm. The mean age at length estimate will be more precise the closer you are to the recommended lengths.

Table 4. Recommended lengths by species for calculating mean age at length.

Species	Length (in)	Length (mm)	Sample Size
Black crappie	9	229	
Bluegill	7	178	20
Largemouth bass	12	305	
Walleye	15	381	

Weisberg growth model

The Weisberg mixed effects growth model is useful for separating out age versus year effects. The model assumes three random and one fixed source of variation in growth. Random effects include year to year environmental effects (e.g., water temperature), individual fish effects (e.g., genetics), and residual (unexplained) variation. The fixed effect is age (e.g., old fish grow slower than young fish). Growth increment data is needed to run these models. These models can also incorporate additional fixed and random effects (e.g., gear bias, measured environmental conditions). For more information on these models see (Weisberg et al. 2010).

For R and SAS code used to run these models see link to Weisberg mixed effects models.

Age Structure

The age structure of a population is used to estimate mortality and identify year class strength. Similar to growth metrics, an aged subsample needs to be extrapolated to the entire catch before further analyses. Using just the aged sample will result in severe bias. Oftentimes, 300-400 fish should be sampled and measured for length with 100 of those being aged for an unbiased and accurate representation of the population.

Length Frequency Analyses

Length frequency histograms

Oftentimes a simple length frequency histogram can be used to determine age-0 fish from the rest of the sample. Length frequency analysis has been used to assign ages to fish since the late 1800's. While certainly the least expensive and time consuming, it can lead to inaccuracies when dealing with long lived and slow growing species. Several techniques have been used for length frequency analysis (Macdonald 1987). The easiest is visually selecting modes from a length frequency histogram of sampled fish. When distinct modes are present, this method can produce satisfactory results.

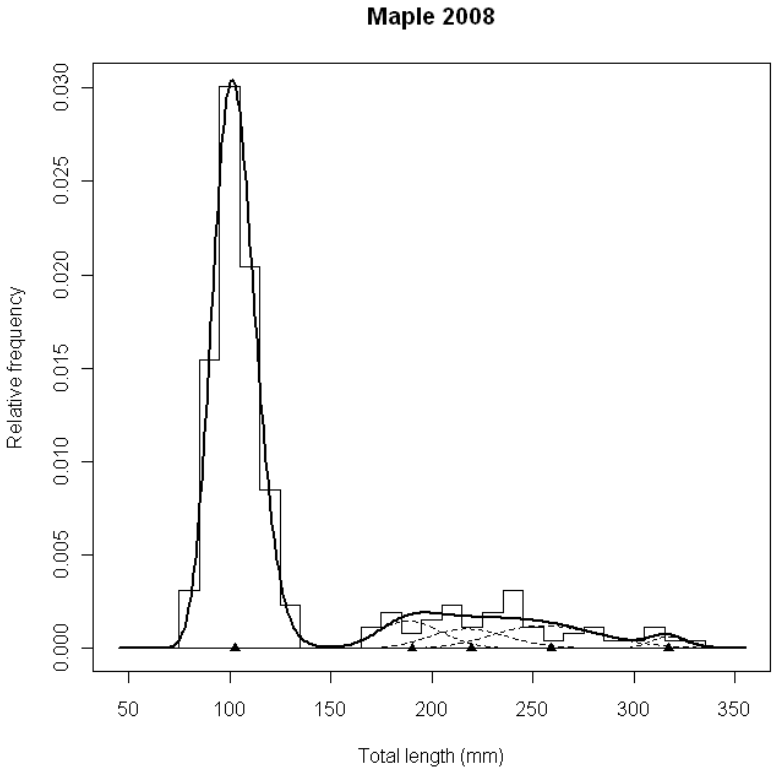
Mixed Distribution models

Another, more quantitative, technique involves using statistical models such as a mixed distribution model (Macdonald and Pitcher 1979). These are useful when the length frequency histogram does not have very distinct length groups, and can incorporate known-age data from other sources on some individuals. Several different software programs have been developed to analyze length frequency data (e.g., ELEFAN I, Pauly and David 1981; MIX, Macdonald and Green 1988; MULTIFAN, Fournier et al. 1990). In particular, the freely available mixdist package in the statistical software program R is very useful when analyzing length frequency data (Figure 31).

References for length frequency analyses

- Length Frequency (Fournier et al. 1998) (Schwarz and Runge 2009) (MacDonald 1987)
- Mixed Distribution models (Kimura and Dorn 2006) (Zhu et al. 2013) (Francis and Campana 2004)

Figure 31. Mixed distribution model results for Brook Trout in Maple Creek.



Marked Fish

Fish can be marked at a known age (most often at age 0) and recaptured at a later date for growth information. These fish are important in terms of measuring individual growth and in developing a population of known age fish for future age validation studies. Fish that are batch marked cannot be used for individual growth estimates, but more fish can be batch marked than individually marked.

Index

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Appendix

Appendix A. Purchasing Supplies

Acetate -

Coin Envelopes – Available through the DNR Warehouse

Epoxy for sectioning otoliths and fin rays - EpoxiCure 2 and EpoKwick link to Buehler Website

Microscopes and equipment

Meiji EMZ-5TRD Microscope. It is important that whatever scope you buy, has click stops for specific magnification. This is essential for calibrating the microscope at various magnifications.

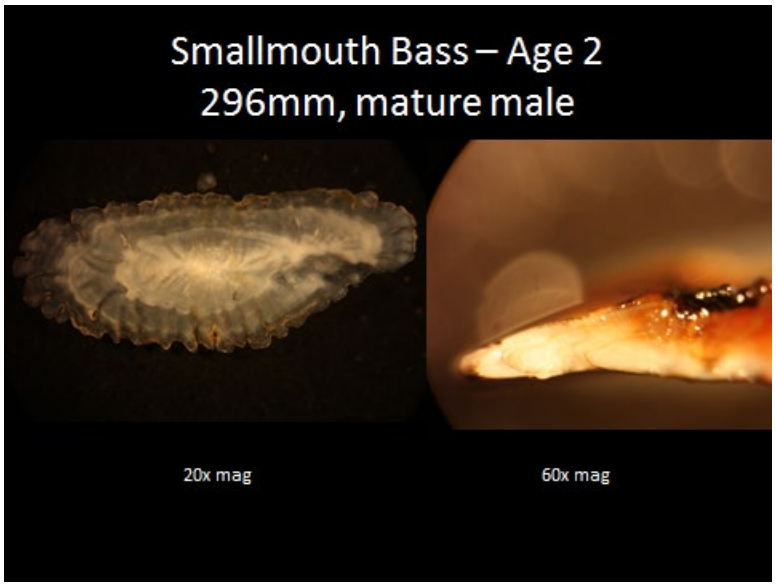
Canon EOS Rebel XSi with AC power converter. This camera package allows live imaging on your computer.

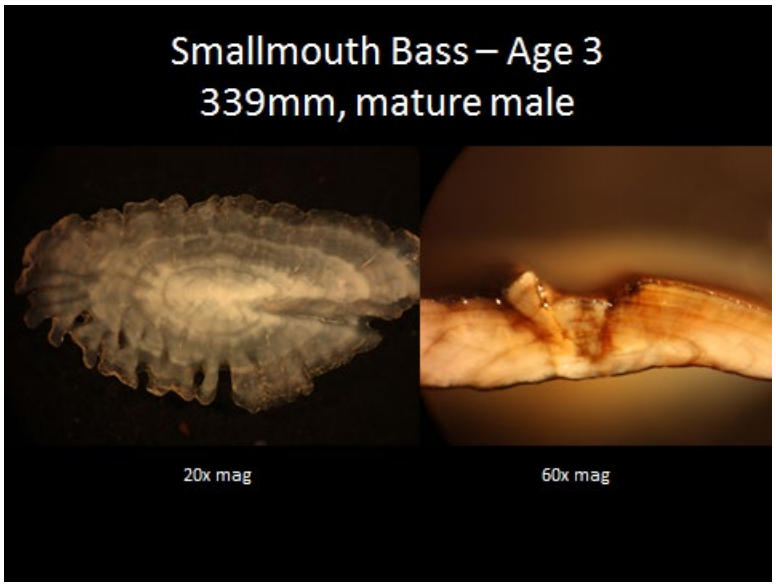
Adapter for camera and microscope.

Otolith molds - Tedpella.com (link to Tedpella Website).

Otolith Vials
- Cole-Parmer 2.0ml clear tubes; Catalog # C-06333-70
- Sycamore Life Sciences: Globe Scientific 1.5 mL Microcentrifuge Tube, Natural SKU 111558

Appendix B. Reference Photos





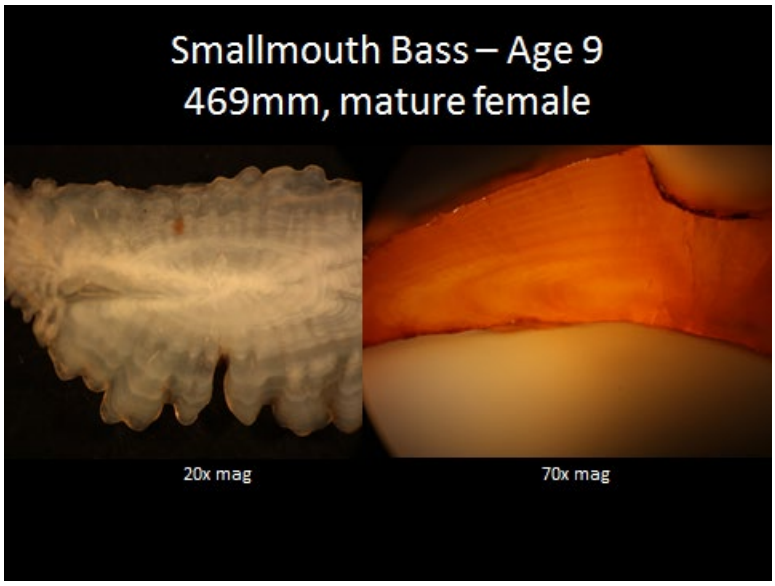


Figure 32. Age 4 Bluegill whole otolith.

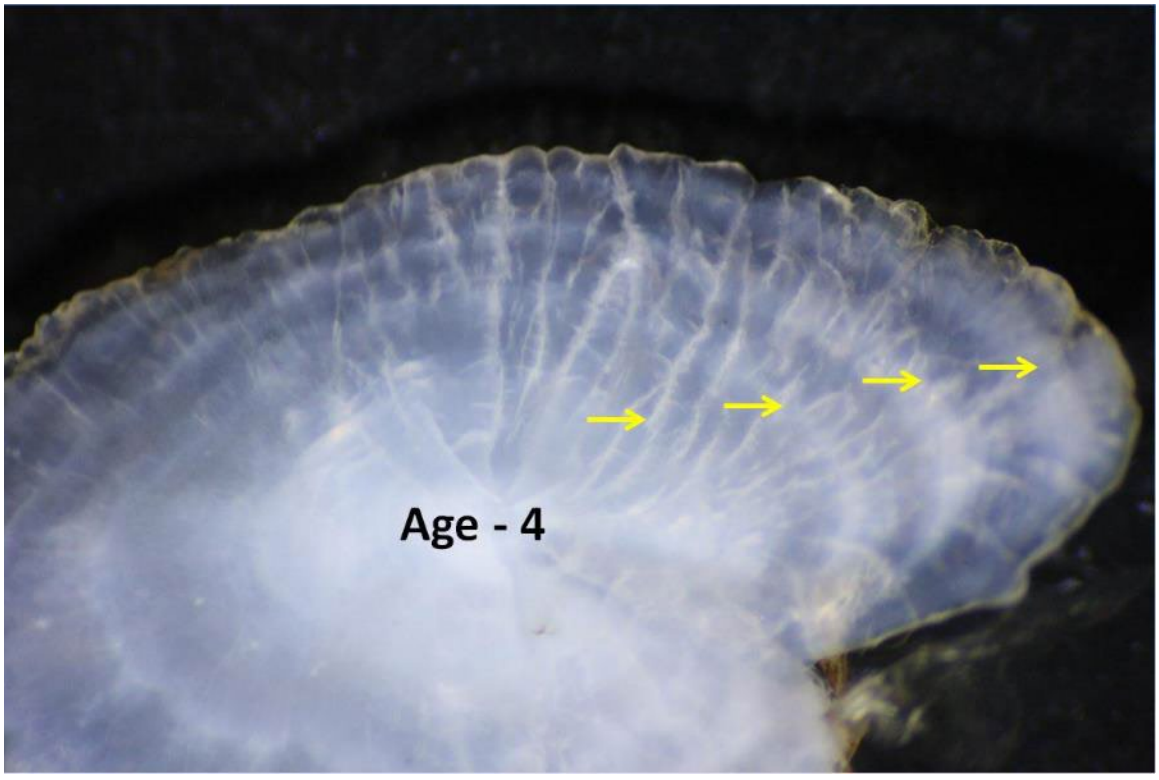


Figure 33. Crack and burned otolith from Age 5 Largemouth bass.

Appendix C. Instructions for taking digital photographs with Cannon EOS and back-calculation measurements using Image J

1. Turn camera on – (EOS Utility will pop-up)
2. "Click" on **Camera Settings/Remote Shooting** – EOS Digital Rebel Box comes up
3. On bottom of box scroll over to **Remote Live view Shooting** – (little box with lashes)
4. Create a new folder with the Lake name and year (ex., Pulaski 2010). This has to be done outside of the EOS Box.
5. In the EOS box "click" on the file folder symbol (in upper right next to the file name)
6. Under the destination tab "click" **Browse**. Find your file name under the appropriate species "click" **OK.**
7. "click" on the **File Name tab**. Under file prefix, type in the lake name species and serial number (ex., Pulaski BLG ser_no0035)
8. In the same tab under **assign sequence number** leave the # of digits at 4. Change the **Start number** to whatever serial number you are on. This will increase by one with each picture so if you have a sequence of numbers you do not need to change the start number, however if there is a break in the sequence you will need to change the start number each time.
9. Now you are ready to take pictures. Fill out the paper data sheet with Lake name, serial number, TL, last annuli and zoom power (Figure 1).
10. Place whole otolith in alcohol (it is what image J was calibrated with and also decreases the amount of bubbles). Center the otolith and zoom to the lowest power that you can, that still contains the whole otolith (usually 1.5 or 2.0)
11. Take picture by "clicking" on the big button in the upper right of the EOS Box.
12. Go on to the next otolith.
13. If the otolith is broken do not take a picture.

Calibrating Image J for Back Calculation estimates

1. Bring scale image in and change "Type" to 16-bit
2. Select "Straight Line" tool and measure from 0-1 on scale image
3. Under "Analyze" select "Set Scale". Distance in pixels should be set from previous measurement.
4. Change "Known Distance" to 1.0
5. Leave aspect ratio alone
6. Change "Unit of length" to mm
7. Check "Global"
8. Click "ok"
9. Scale Set. You will need to do this for each time you change magnification.

Approximate distance in pixels by magnification in alcohol. These can be manually entered in during step 3 in the **Analyze - Set Scale** step instead of performing step 2.

Magnification	Pixels
0.7	120
1.0	168
1.5	248
2.0	336
2.5	414
3.0	502
3.5	580
4.0	661
4.5	746

Instructions for performing Back Calculations in Image J.

1. *Make sure you have the right magnification scale set before you start measuring.
2. *When taking images put Lake, Species and serial# in the name (ex. Maple_Lake_wae_ser_no_ 0001)
3. Load Image J
4. Open image file in Image J
5. Under “Analyze” set your scale for the magnification used
6. Measure from the nucleus to the outer edge using the “Straight Line” tool
7. Click “Analyze and select “measure” or Control M
8. Scroll over the line and move the line back to the Annuli you wish to measure and repeat step 5.
9. Record measurements on Data sheet.

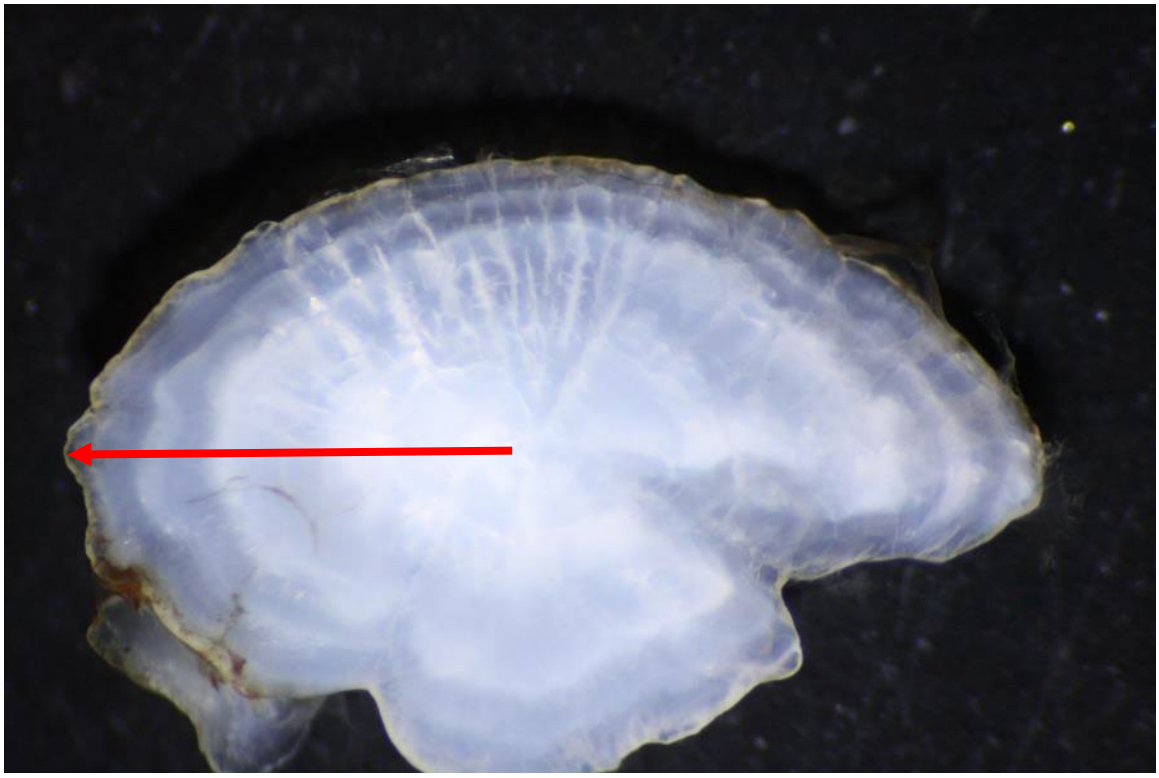
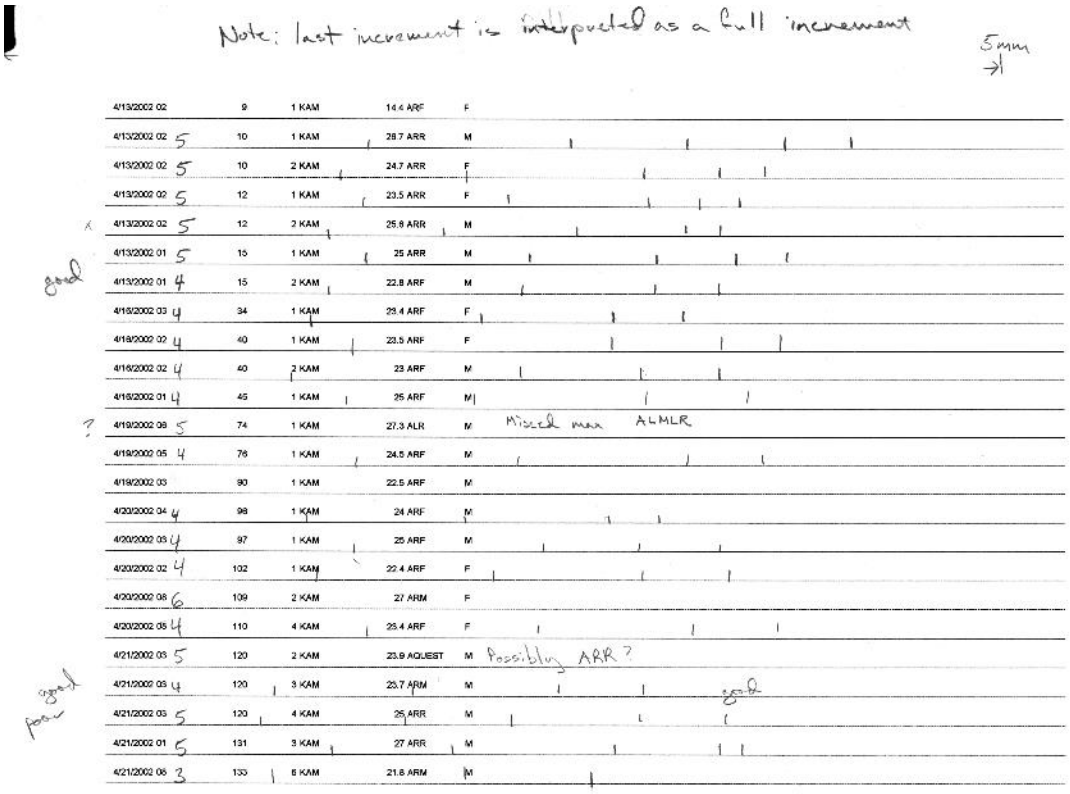


Figure 34. Radius measurement on Bluegill otolith

Appendix D. Instructions for measuring scanned scale measurements

1) Compile growth marks on a lined paper form.
   a) Use a lined sheet of paper in landscape orientation to compile scale increment data from multiple fish.
   b) Include species, serial number and length on the form. See figure. The figure should also have magnification recorded.



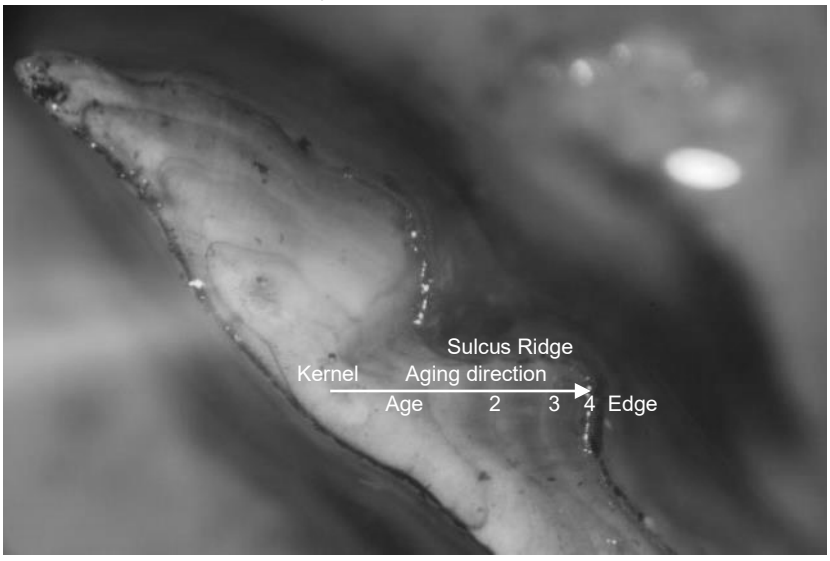
   c) Forms may be computer generated using various outside software.
2) Record reference distance.
   a) Place stage micrometer on the stage of the microfiche reader using the same magnification that you will use to make the structure measurements.
   b) Choose the appropriate distance that corresponds to a length slightly longer than the longest radial measurement that you will make for the group of fish that you are working on. This will depend on the size of the structures aged, but will range from 1 to 10mm in most cases.

c) Transcribe the known distance to the lined form marking the ends with thin vertical lines in a horizontal orientation while the form is in landscape orientation.
d) Record the distance on the form and the magnification.

3) Proceed with aging and recording growth increments as in the past by using paper strips and then transcribing measurements to the form.
   a) Moving on to another form or another group of fish: if magnification and microfiche machine have not changed and the group of structures is of similar size the reference distance may be transcribed from a previous sheet. However, the user must be certain that magnification and microfiche are identical before transcribing the reference distance from another form. It is a good practice to check this distance with the micrometer and record the measurement if your aging session has been discontinuous.
      i) Record the reference measurement, distance, and magnification on each form.
4) Scan the forms.
   a) Individual scanner settings will vary; so make notes of what works and what doesn't for your particular scanning device. Guidelines for scanning include:
      i) Use landscape orientation.
      ii) Grayscale or binary color may be adequate and help limit file size.
      iii) Save the scan in JPEG format.
   b) Reduce the scanned image to size compatible with the Aging Module. Currently the digitizing import screen can only handle JPEG images that are less than 2000 pixels in the horizontal dimension.
      i) Lock the aspect ratio of the image when resizing.
      ii) Set the horizontal, usually width, distance to 2000 pixels.
      iii) Save the image as a JPEG and rename.
         (1) May have to decrease the image width to less than 2000 pixels. The functional limit may be closer to 1000 horizontal depending on other characteristics of the image, so experiment and keep a record of what settings worked with your scanner and image processing software.
5) Import scanned image.
   a) Click the Options button and select Import Scanned Marks.
   b) Navigate to the location where you have saved your resized images.
      i) Select and open scanned image.
         (1) If the image failed to be displayed but the file path is shown, it may be too large.
            (a) Resize the image to a smaller horizontal pixel distance using an outside image processing software, save and try importing again.
   c) Maximize the size of the image in the digitizing field using the Zoom In and Zoom Out options. The reference distance recorded on the scanned image should fill the maximum horizontal extent of the digitizing field and the measurements for each fish should also be visible.
6) Calibrate the scanned image as described above using the Calibrate Option.
7) Digitize marks as described above.
8) Transparencies may also be scanned to produce an image of the transparency that may be imported using the Import Scanned Marks option.

Appendix E. Instructions for measuring images in the Fisheries Survey Module

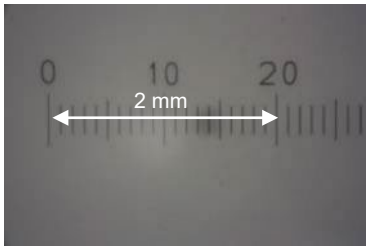
1. You are going to need to have a dissecting microscope that has the ability to zoom to 45x. Higher magnifications may be desired, but at a minimum, otoliths and scales viewed from 10 to 45x have good readability. Pictures are taken of each scale or otolith with a naming convention using the serial number, the three letter species code, the age, and the magnification (ex. 145_WAE_age3_45x.JPG). NOTE: A microscope with magnification detents is preferred but one without detents can be used if you are careful not to bump the magnification knob while processing structure.
2. For otoliths using the crack-and-burn methodology, take pictures of the plane where the otolith was cracked and burned; for scales, press the scales in acetate and take a picture of a scale that has the best display of annuli.



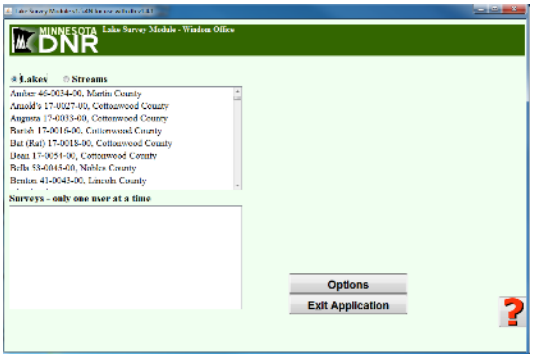
   i. The Sulcus ridge is the best location to take measurements for digitizing back-calculated length-at-age information. In addition, the picture of the otolith should be done so that the Kernel is on the left and the direction of back-calculation is from left to right (see image below). This aids in making the best digitization of structures within the Fisheries Survey Module (FSM) program because digitization of length-at-age is done from left to right in a straight line.

b. Methodology for scales
   i. Press scales between 2 microscope slides or press scales with scale press and use acetate impressions.
   ii. View scales or impressions under microscope at best magnification and lighting to fully read all annuli and to get the best images.
   iii. Take a picture of the scale or impression so that the direction of back-calculation is from left to right (See image below).

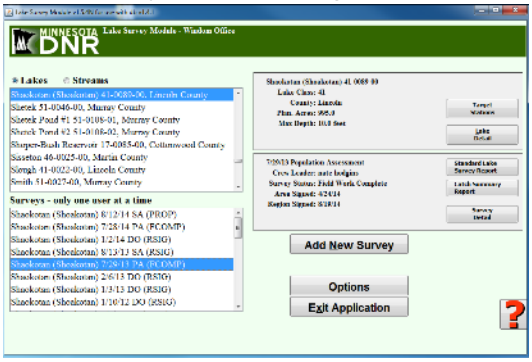
3. Calibration reference for each magnification is done using a calibration slide that came with the microscope. A picture of the calibration guide is taken at each magnification used to import into FSM while back-calculating structures. The image below is an example of a 20 micrometer (2mm) calibration guide viewed at 45x.



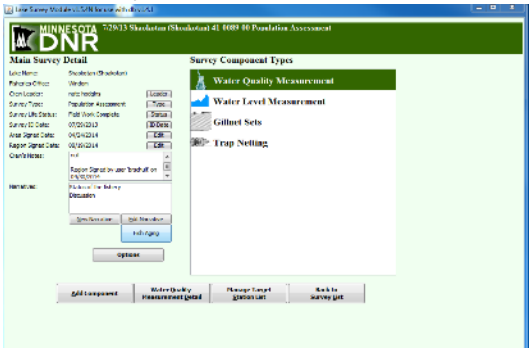
4. When all structures have been aged and pictures taken the images can be uploaded into FSM and the images can be digitized to back-calculate the length-at-age information.
    a. NOTE: FSM will only allow images to be uploaded that are under 2000 pixels wide. Therefore, you will need to use a software program to compress the images to less than 2000 pixels wide. While compressing the images, it is of utmost importance that you maintain the aspect ratio of the image so distortion does not affect the back-calculation of the annuli. Digital Photo Professional 3.4.0.0 has a batch process function that will compress all images and maintain aspect ratios at the same time. As an example, final images are oriented in a landscape direction and are 1024 pixels wide by 683 pixels tall.
    b. It is helpful to use the extended memory version of FSM that will allow you to upload more images to the program without the program crashing due to excessive memory usage.
5. How to digitize (back-calculate) from an otolith or scale within FSM
    a. Start the Extended Memory FSM program



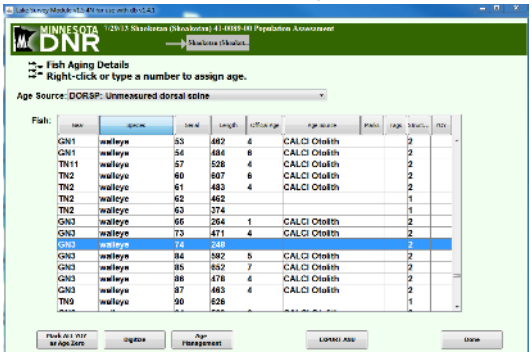
b. Select lake you are working with and open survey



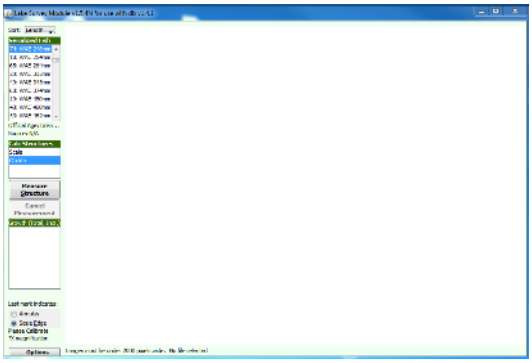
c. Enter Fish Aging option (only available while survey life status is “Field Work Complete”)



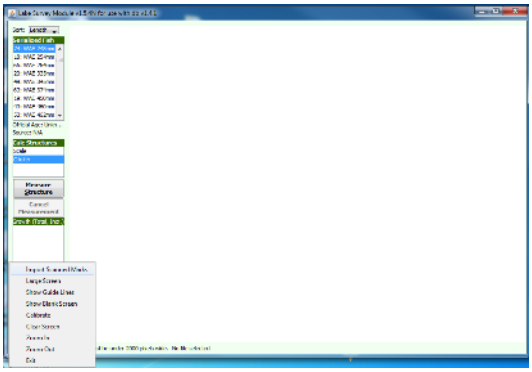
d. Select fish you want to age and digitize otolith or scale from the Fish Aging Details list, then select digitize.



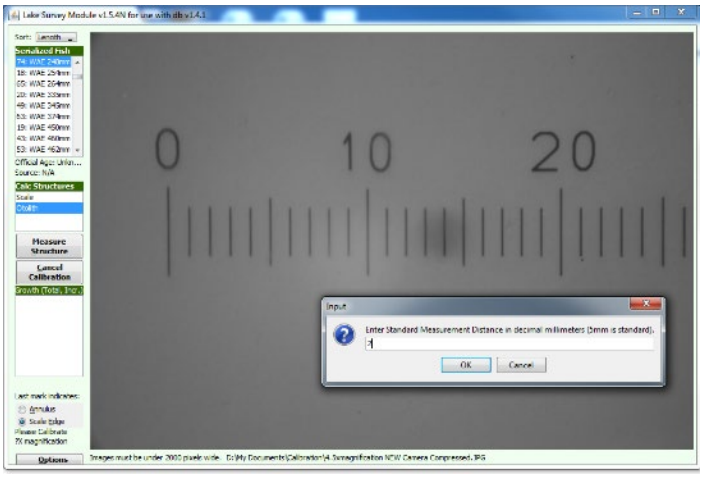
e. Select calcified structure that you are aging from “Calc Structures” list and then make sure that the “Last Mark Indicates” option is set to “Scale edge”.



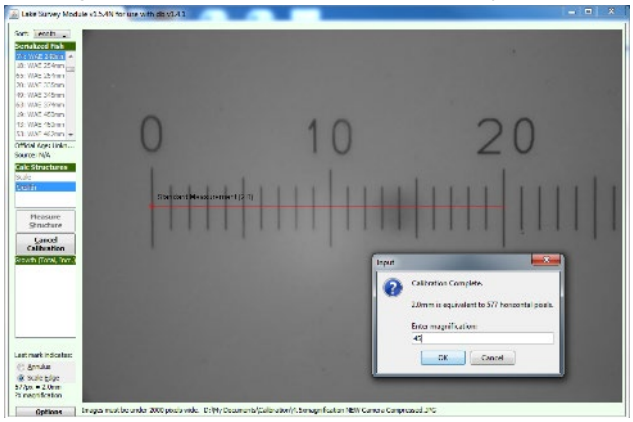
f. Click “Options” in the lower left corner and then click “Import Scanned Marks”.



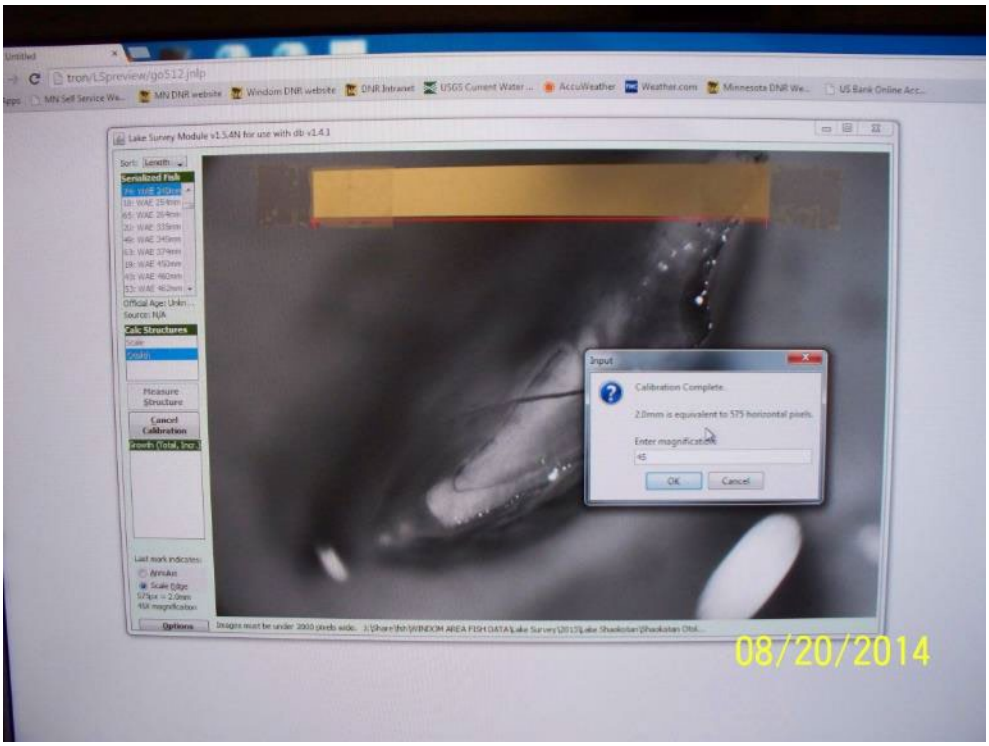
g. Navigate to file where your pictures are saved and click on the calibration file to open the image of your calibration guide. Import the calibration image of the magnification that was used to take pictures of otoliths or scales. The “Enter Standard Measurement Distance in decimal millimeters (5mm is standard)” prompt will appear and you will need to enter your standard. In the example below it is 2mm. Then click “OK”. Then click “OK” again.



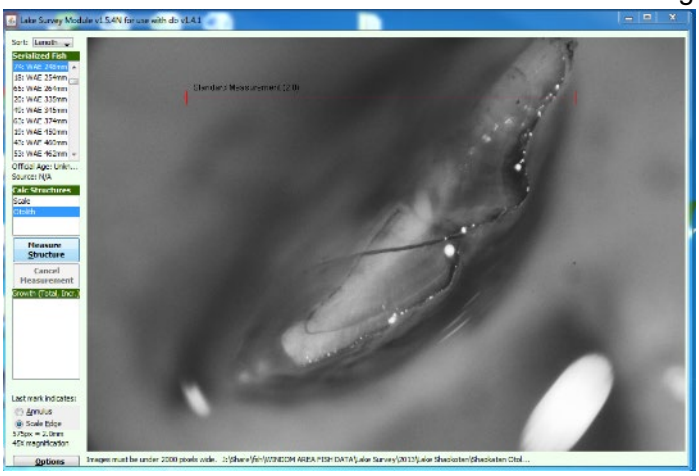
h. Then you will click on the starting point of your standard measurement, in this case 0. Then you will click on the ending point to your standard measurement, in this case 20. It will then prompt you to enter your magnification. In this case it is 45x. Therefore, 2mm is equal to 577 horizontal pixels in this instance. Now take a strip of paper and cut it to the length of your standard mark. Tape the strip of cut paper within the upper photo viewing portion of the computer screen (see i below). Then click “OK”.



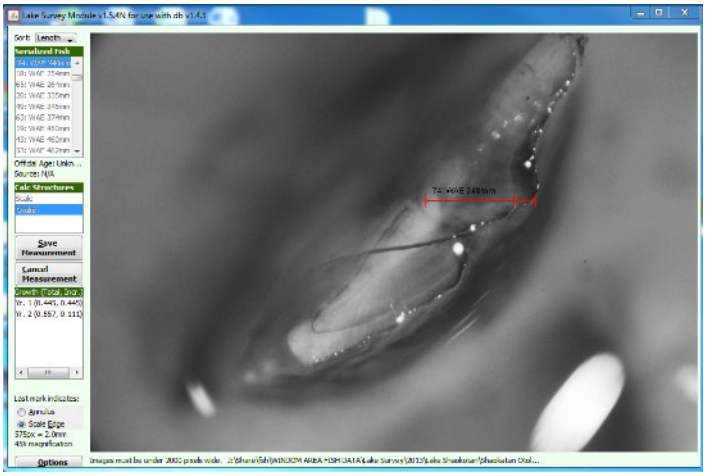
i. Now you will click on “Options” and “Import Scanned Marks” and then import the image of your otolith or scale. It will prompt you to “Enter Standard Measurement in decimal millimeters (5mm is standard)”. You will be able to enter your standard, in this case 2mm, and then click “OK”. Then click “OK” when it says “Now, measure the mark that represents your 2.0mm Standard”. You will now be able to measure the strip of paper that is taped to the screen. Then enter your magnification, in this case 45x, and click “OK”.

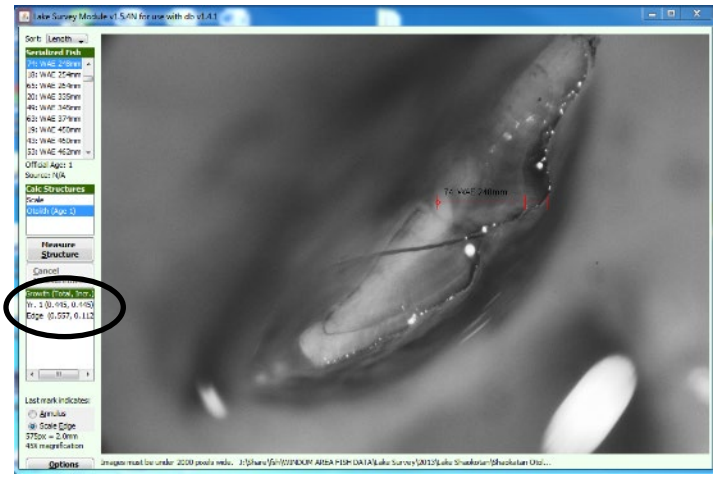


j. Then click on “Measure Structure” to the left of the image.



k. Your first click will be on the kernel of the otolith or nucleus of the scale. Next, click on each annuli from left to right. Then click on the edge of the otolith and <u>stop clicking</u> within the photo viewing window. In the example below the Walleye, serial number 74, was only 1 year old and the edge is labeled “Yr. 2” to the left of the image. You will then click on “Save Measurement” to the left of the photo viewing window, when you do that it will automatically change the last measurement, the edge, to be labeled as an edge and not “Yr. 2” in the example below (circled in the lower image).





l. Repeat the above steps for each fish using the appropriate magnification standard and photo of the structure.
m. Close program when done.

Appendix F. Growth rates of Minnesota Fishes

Table 5. Mean length (mm) at capture for Walleye collected in standard gill nets during **June**. Lake Class means and standard errors were generated by using individual surveys from 2006-2013 as replicates.

Lake Class		Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12
5	**Mean Length**	**194.1**	**288.2**	**378.4**	**426.9**	**460.2**	**509.2**	**536.0**		**567.7**		**650.0**	
	Number	2	3	2	2	1	5	1		1		1	
	SE	0.6	12.3	13.7	1.9		16.9						
8	**Mean Length**			**300.4**		**445.7**	**479.8**	**486.6**					
	Number			4		1	3	1					
	SE			7.7			3.8						
12	**Mean Length**		**242.5**	**308.3**	**367.5**	**393.4**	**449.9**	**466.6**		**547.0**			
	Number		3	4	4	6	2	2		1			
	SE		26.6	18.8	9.0	6.3	36.6	25.4					
16	**Mean Length**	**203.0**	**268.8**	**319.6**	**424.8**	**439.0**	**428.4**	**473.1**	**497.7**	**453.7**			
	Number	1	2	3	2	4	1	4	2	1			
	SE		30.2	20.5	28.0	18.0		16.8	2.7				
22	**Mean Length**	**218.1**	**284.7**	**357.0**	**412.2**	**477.5**	**522.1**	**537.2**	**546.7**	**572.6**	**564.5**	**588.7**	**616.4**
	Number	5	7	12	11	11	10	10	6	5	4	3	2
	SE	24.2	11.5	11.7	11.3	9.3	14.6	8.0	12.3	18.3	12.3	7.7	5.6
23	**Mean Length**		**299.7**	**354.7**	**396.7**	**458.2**	**490.4**	**545.7**	**553.5**			**617.0**	
	Number		1	9	8	6	6	4	3			1	
	SE			12.5	19.7	25.5	15.9	30.3	31.7				

Lake Class		Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12
24	**Mean Length**	**245.0**	**299.4**	**355.9**	**421.3**	**470.0**	**499.0**	**544.2**	**557.2**	**564.7**	**666.0**		**569.3**
	Number	2	11	16	13	13	8	6	3	2	1		1
	SE	0.0	10.2	15.7	15.7	15.2	9.0	14.6	22.7	50.3			
25	**Mean Length**	**204.3**	**301.3**	**347.1**	**398.1**	**452.7**	**482.4**	**518.8**	**533.1**	**541.5**	**587.3**	**619.5**	**603.1**
	Number	5	6	22	20	20	14	8	7	11	4	1	1
	SE	2.5	12.8	8.4	9.2	11.4	8.8	11.0	13.7	10.8	20.0		
27	**Mean Length**	**213.5**	**273.5**	**339.8**	**404.6**	**447.0**	**480.2**	**512.4**	**523.6**	**541.8**	**561.6**	**589.7**	**545.4**
	Number	12	25	28	32	27	23	24	14	14	12	4	5
	SE	5.5	4.5	6.1	5.7	5.5	6.2	6.2	7.5	6.7	11.2	9.8	5.8
28	**Mean Length**		**268.3**	**327.4**	**385.3**	**465.4**	**454.5**	**521.3**	**570.8**	**559.5**	**676.7**		
	Number		1	2	4	2	3	1	1	1	1		
	SE			32.7	20.3	1.2	56.0						
29	**Mean Length**		**282.4**	**364.0**	**435.2**	**471.1**	**530.3**	**608.0**	**506.5**	**568.6**	**591.3**		
	Number		2	5	5	4	3	1	1	2	1		
	SE		26.8	17.7	22.8	31.0	25.9			1.1			
31	**Mean Length**	**208.2**	**276.2**	**373.3**	**419.9**	**479.5**	**495.1**	**537.9**	**546.1**		**559.7**		**587.4**
	Number	2	5	11	8	6	10	6	5		2		2
	SE	16.8	9.9	10.7	11.2	15.1	10.0	16.2	5.0		20.0		17.2
34	**Mean Length**	**213.9**	**308.9**	**377.8**	**423.8**	**482.4**	**499.4**	**532.9**	**546.1**	**551.4**	**560.8**	**551.0**	
	Number	7	12	11	8	9	6	4	4	3	3	2	
	SE	8.4	11.8	14.6	17.4	5.3	17.3	10.4	9.2	20.8	13.3	5.6	

Lake Class		Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12
35	**Mean Length**	**206.8**	**276.7**	**341.1**	**364.7**	**442.5**	**465.1**						
	Number	1	3	4	2	2	3						
	SE		13.2	14.1	46.7	42.5	18.9						
38	**Mean Length**	**262.2**	**340.0**	**415.8**	**401.9**	**491.4**	**518.0**	**588.7**	**541.4**	**605.0**	**616.3**		
	Number	5	5	6	3	4	3	1	1	1	1		
	SE	43.9	25.7	30.1	47.3	19.5	56.8						
39	**Mean Length**	**199.5**		**356.4**	**425.8**	**455.8**	**525.5**	**490.8**	**539.8**	**583.0**	**563.0**		
	Number	1		4	7	7	3	3	2	3	2		
	SE			30.8	18.6	15.7	35.0	14.5	19.8	37.6	28.7		
40	**Mean Length**	**225.0**	**279.7**	**404.1**	**495.8**	**485.8**	**506.6**				**569.7**		
	Number	1	2	3	2	1	1				1		
	SE		8.3	32.5	7.6								
41	**Mean Length**	**228.6**	**312.4**	**392.7**	**413.6**	**474.5**	**520.1**	**580.9**	**553.0**	**595.4**	**578.4**	**623.9**	**618.5**
	Number	6.0	14	14	9	8	6	3	1	3	2	1	2
	SE	13.1	13.2	14.0	17.8	21.2	14.8	59.1		40.9	17.2		67.8
42	**Mean Length**	**215.3**	**288.5**	**384.2**	**452.8**	**470.0**	**563.0**	**516.5**		**596.0**			
	Number	1	6	6	7	3	1	2		1			
	SE		23.1	25.2	22.0	23.2		84.8					
43	**Mean Length**	**239.7**	**350.4**	**415.8**	**452.6**	**480.2**	**519.4**	**590.3**	**590.0**	**557.4**		**604.3**	
	Number	18	33	24	20	12	4	1	1	1		1.0	
	SE	6.8	5.9	8.6	11.3	13.1	16.1						

Table 6. Mean length (mm) at capture for Walleye collected in standard gill nets during July. Lake Class means and standard errors were generated by using individual surveys from 2006-2013 as replicates.

Lake Class		Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12
1	**Mean Length**	**195.1**	**268.8**	**325.2**	**387.1**	**440.8**	**478.0**	**485.4**	**520.7**	**560.0**	**584.0**		
	Number	2	4	7	7	6	6	3	4	2	1		
	SE	0.6	14.9	7.7	11.8	9.8	13.4	16.0	8.8	35.6			
2	**Mean Length**	**195.1**	**257.3**	**339.9**	**356.6**	**452.1**	**453.9**	**488.1**	**421.3**	**588.0**		**688.0**	
	Number	5	4	5	4	5	3	4	2	2		1	
	SE	7.0	15.9	23.8	22.5	28.6	22.5	22.2	27.3	50.0			
3	**Mean Length**	**198.7**	**256.3**	**339.6**	**396.7**	**449.4**							
	Number	1	2	5	2	3							
	SE		3.7	13.3	45.0	31.0							
5	**Mean Length**	**208.7**	**257.7**	**317.1**	**364.3**	**423.5**	**447.3**	**461.4**	**521.0**	**521.8**	**513.4**		
	Number	1	6	5	8	5	3	5	1	1	3		
	SE		15.3	22.3	12.0	33.0	32.5	25.1			35.0		
6	**Mean Length**	**179.3**	**245.3**	**289.4**	**329.3**	**380.8**	**410.2**	**397.7**	**501.3**	**461.7**		**542.7**	
	Number	3	8	7	5	6	7	3	1	1		1	
	SE	0.9	6.9	10.0	12.6	20.6	17.6	15.0					
7	**Mean Length**	**209.2**	**275.7**	**290.2**	**357.4**	**404.2**	**449.9**	**476.7**			**510.8**		
	Number	2	5	2	2	3	3	2			1		
	SE	18.4	23.8	3.2	45.1	41.0	26.4	58.0					
10	**Mean Length**	**195.9**	**276.6**	**348.4**	**404.3**	**407.8**	**515.7**				**534.3**		

Lake Class		Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12
	Number	3	3	2	4	3	1				1		
	SE	12.9	6.1	30.4	19.3	28.4							
12	**Mean Length**	**200.3**	**265.5**	**323.6**	**357.7**	**376.9**	**418.4**	**454.4**	**495.5**				
	Number	8	9	12	10	7	3	4	4				
	SE	8.9	8.1	9.1	10.4	8.2	16.6	12.3	9.8				
13	**Mean Length**	**270.6**	**331.1**	**390.3**	**411.7**	**468.4**	**462.9**	**474.0**	**481.6**		**502.4**		**533.0**
	Number	1	6	4	4	1	2	1	3		1		1
	SE		15.7	13.6	21.3		9.4		2.4				
16	**Mean Length**	**195.0**	**262.7**	**321.1**	**376.7**	**419.9**	**438.8**	**454.8**	**498.1**	**489.9**	**537.0**	**517.3**	**529.5**
	Number	9	12	15	12	9	10	5	4	5	2	3	2
	SE	3.7	6.1	6.0	7.0	10.9	10.0	12.1	10.5	9.6	10.3	18.4	17.5
17	**Mean Length**	**185.4**	**288.2**	**349.2**	**398.6**	**401.1**	**383.8**	**549.3**					
	Number	1	3	3	3	2	1	1					
	SE		26.3	32.4	32.4	34.6							
22	**Mean Length**	**215.6**	**287.4**	**351.9**	**403.4**	**451.1**	**496.9**	**514.4**	**552.6**	**549.1**	**576.6**	**612.7**	**652.5**
	Number	38	58	62	57	52	39	30	22	21	10	4	2
	SE	4.0	4.0	4.6	4.9	4.3	5.5	7.7	8.5	9.6	11.8	27.8	11.8
23	**Mean Length**	**233.1**	**317.1**	**374.6**	**434.7**	**488.1**	**499.8**	**523.4**	**548.2**	**581.9**	**588.3**	**551.0**	**628.3**
	Number	8	16	20	12	14	13	7	8	4	1	1	1
	SE	8.7	8.0	8.7	7.4	10.4	7.0	9.2	11.0	18.9			
24	**Mean Length**	**246.6**	**313.5**	**395.8**	**458.5**	**494.7**	**522.0**	**551.4**	**587.3**	**599.0**	**606.7**	**577.2**	**641.0**

Lake Class		Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12
	Number	14	28	41	38	38	24	23	8	4	7	1	1
	SE	9.9	8.9	6.3	7.5	7.5	7.0	9.0	10.2	16.2	12.1		
25	**Mean Length**	**219.6**	**304.0**	**367.8**	**424.4**	**468.3**	**496.6**	**521.9**	**537.2**	**578.7**	**565.9**	**558.0**	**626.5**
	Number	24	25	36	34	40	28	18	12	10	12	6	5
	SE	4.4	6.5	6.3	7.6	6.9	9.7	7.2	8.0	10.6	8.4	5.3	21.8
27	**Mean Length**	**216.6**	**293.3**	**351.4**	**402.2**	**449.4**	**489.6**	**513.6**	**548.9**	**555.8**	**573.1**	**590.3**	**626.2**
	Number	29	59	54	56	41	40	22	16	13	8	3	6
	SE	3.3	3.5	3.8	3.9	6.5	5.5	7.5	10.3	13.0	21.9	47.1	15.8
28	**Mean Length**		**283.4**	**388.0**	**478.7**			**533.7**					
	Number		3	5	1			1					
	SE		10.9	18.4									
29	**Mean Length**		**311.2**	**383.5**		**506.5**	**538.9**	**555.8**	**552.6**			**493.3**	**591.3**
	Number		4	6		3	2	1	1			1	1
	SE		18.6	10.7		23.8	13.7						
31	**Mean Length**	**235.0**	**291.6**	**360.7**	**421.9**	**467.0**	**511.1**	**521.2**	**534.1**	**540.1**	**591.0**	**571.8**	**592.2**
	Number	6	13	15	22	19	15	8	4	5	4	3	2
	SE	5.5	11.8	10.8	10.6	12.4	10.2	15.0	17.0	10.1	8.9	11.4	67.8
34	**Mean Length**	**230.7**	**308.4**	**383.1**	**433.6**	**488.0**	**471.4**	**572.5**	**536.1**	**534.5**	**638.6**		
	Number	7	12	11	11	9	5	2	3	2	2		
	SE	16.4	13.0	11.3	15.2	12.8	19.5	68.5	29.4	31.3	13.9		
35	**Mean Length**	**217.2**	**280.3**	**338.8**	**406.4**	**459.8**	**476.6**	**524.8**	**504.8**	**559.6**	**487.0**		**598.7**

Lake Class		Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12
	Number	3	5	5	5	3	2	2	2	1	1		1
	SE	13.0	11.4	13.1	17.1	26.2	27.4	18.3	30.2				
38	**Mean Length**	**259.0**	**315.4**	**429.2**	**468.0**	**509.5**	**523.6**	**542.2**	**646.8**	**568.0**	**596.4**	**592.2**	**601.9**
	Number	1	9	7	9	10	6	3	1	3	3	1	2
	SE		21.1	19.3	13.6	8.4	16.8	24.3		25.0	40.9		19.8
39	**Mean Length**	**199.8**	**273.8**	**347.4**	**419.1**	**471.3**	**503.2**	**513.0**		**605.8**	**505.8**	**565.0**	
	Number	1	2	5	4	5	4	2		1	2	1	
	SE		10.4	12.9	20.2	27.5	16.5	47.8			29.6		
41	**Mean Length**	**247.5**	**319.6**	**409.0**	**427.8**	**492.8**	**495.6**	**588.5**	**560.2**	**548.9**	**508.5**	**536.3**	
	Number	18	21	21	18	15	7	3	3	3	3	1	
	SE	5.6	10.2	11.6	13.0	12.5	16.6	26.6	25.1	26.1	3.4		
42	**Mean Length**	**359.4**	**350.5**	**407.1**	**456.9**	**514.2**	**493.4**			**620.0**			
	Number	1	6	7	2	4	1			1			
	SE		19.1	20.1	32.9	19.5							
43	**Mean Length**	**244.5**	**342.9**	**420.6**	**452.7**	**490.3**	**549.0**	**600.2**					
	Number	16	17	19	15	8	2	1					
	SE	7.9	11.0	9.5	11.0	8.6	11.3						