

 <p>Environment and Sustainable Resource Development</p>	<p><b>Title:</b>  <b>STANDARD FOR AGEING WALLEYES  IN ALBERTA (Public version)</b></p>	<p><b>Date:</b>  <b>May-2013</b></p>
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**Principal Issue:**

Fisheries biologists rely on calcified structures from walleyes (*Sander vitreus*) to estimate age so that productivity, growth, mortality, year-class strength, length-at-age and age-at-maturity can be calculated. For decades Alberta has primarily used pelvic fins to age walleyes. There are many inherent problems with this structure or associated techniques that may contribute to inaccurate ageing of walleyes. Accurate fish ages are especially important for Alberta's walleyes as they are typically slower growing, longer lived, and less fecund than more southern populations. To provide reliable information for management purposes it is imperative that walleyes are aged accurately.

**Application:**

All who submit walleye aging information to the Fish and Wildlife Information Management System must now follow the ageing methodologies for walleyes outlined in the supporting documentation.

When ageing walleyes;

- 1) Otoliths are the preferred ageing structure;
- 2) However, if otolith to pelvic fin age agreement ( $(\sum(\text{otolith-pelvic fin age}))/n$ ) is greater than 90% for a specific waterbody, pelvic fins may be used to support non-lethal sampling strategies. (Ricker 1975).

Supporting documentation attached:

Watkins, Owen B. and Stephen C. Spencer 2009. Collection, preparation and ageing of walleye otoliths. Fish and Wildlife Division, Alberta Sustainable Resource Development. 14pp.

Watkins, Owen B. and Stephen C. Spencer 2009. Collection, Preparation and Ageing of Walleye Pelvic Fin Rays. Fish and Wildlife Division, Alberta Sustainable Resource Development 25pp.

Spencer, Stephen C., Owen B. Watkins, Michael G. Sullivan and A. Lee Foote 2013 Non-lethal ageing methods and ageing error identified for Alberta Walleyes: management consequences for slow growing fishes. *In prep.*

**Implications:**

Before non-lethal sampling can take place (i.e., using pelvic fins) biologists will need to validate pelvic fin ages at each waterbody being sampled. A minimum of two different experienced agers, using a blind test where the agers are unaware of any biological data in relation to the size of the fish and which waterbody and season were sampled, should be used to interpret paired samples of otolith and pelvic fin ages to validate pelvic fin ages. Fish lengths may be used where back-calculations are necessary to determine the location of the first annulus. Since otoliths are a poor source of suitable deoxyribonucleic acid (DNA) for genotyping, pelvic fins should still be collected to provide a backup ageing structure and DNA for any future genetic work.

**Developed by:**

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**Suggested citations:**

Watkins, O.B., and S.C. Spencer. 2009. Collection, preparation, and ageing of walleye otoliths. Fish and Wildlife Division, Alberta Sustainable Resource Development. 15pp.

Watkins, O.B., and S.C. Spencer. 2009. Collection, preparation, and ageing of walleye pelvic fin rays. Fish and Wildlife Division, Alberta Sustainable Resource Development. 26pp.

**Approved by (Executive):** \_\_\_\_\_ **Original Signed by:** \_\_\_\_\_

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**Executive Director of Fisheries Management**

**Date:** \_\_\_\_\_

*Nov 4 / 13*

# Collection, preparation and ageing of walleye otoliths

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## Table of Contents

Table of Contents .....	2
List of Figures .....	3
Introduction .....	4
Methodology .....	4
Collection.....	4
Otolith preparation.....	5
Identifying Annuli .....	8
Locating the First Annulus.....	9
Discussion .....	10
References .....	11
Appendices.....	13

## List of Figures

- Figure 1. a) Otolith physiology (*Photo courtesy of Susan Mann, Ontario Ministry of Natural Resources, 2008*) (b) walleye otolith sectioned transversely through the nucleus. ....5
- Figure 2. A 1.70 ml centrifuge tube and cap. The cap is filled with Plasticine™ to be used as a mounting medium for sectioned otoliths. ....6
- Figure 3. A sectioned and burnt otolith mounted in Plasticine™ in the centrifuge tube cap. ....7
- Figure 4. A sectioned, cracked and burned otolith exposing annuli. Measurement from the focus to distal edge indicates the location of the first annulus of an age-7 walleye collected from Lac Ste. Anne, Alberta, September, 2006. .... 10

## **Introduction**

Otoliths are the standard ageing structure for many fish species and are used for percids by 29 of the 38 surveyed North American jurisdictions (Maceina et al. 2007). Until recently, Alberta's preferred ageing structure for walleyes was pelvic fins (Mackay et al. 1990); as the need arose for precise fish ages, otoliths, which are considered the most accurate structure for ageing (Maceina et al. 2007, Spencer et al. 2009), was adopted for walleyes. Here we demonstrate the methods for extraction, preparation and interpretation of walleye otoliths.

## **Methodology**

### Collection

There are several different methods to extract otoliths from walleyes: split the head laterally through the eyes exposing the otoliths, remove the head from the body and extract the otoliths through the posterior of the brain, or remove the dorsal section of the skull and brain to access the otoliths (Fielder 2002).

We prefer accessing the otoliths through the roof of the walleye's mouth. First, using wire cutters, cut laterally through the isthmus, simultaneously cutting between the first and second gill-arch on the ventral side of the head (Appendix 1). Second, place the blades of the wire cutters on either side of the parasphenoid (where the first gill-arches join the roof of the mouth) perpendicular to the length of the fish's body with the flat edge directed posterior, and cut  $\frac{3}{4}$  the way through the parasphenoid. Third, using hands, forcefully crack the head backwards at the incision through the parasphenoid to expose the otoliths. This method requires practice, and for larger fish the sampler may need to crack the spinal column against a firm stationary object (e.g. sample table) subsequent to cutting the parasphenoid.

With practise, exposed otoliths should be readily apparent within membranous sacs on either side of the mid-line at the posterior ventral portion of the brain cavity. The membranous sac can be removed before storage. Membrane removal is easily done by rubbing the otolith between the thumb and forefinger or on a paper towel. Fine tipped forceps are useful in extracting the otoliths from the fish. Otoliths are then placed in sample envelopes (or equivalent) and stored until dried.

### Otolith preparation

Otoliths are sectioned transversely and burned (the crack and burn method) (Barber *et al.* 1987). The otolith is placed on a hard-plastic surface with the concave side up and a flat scalpel blade is placed on the focus perpendicular to the longitudinal axis over the nucleus. With thumb and index finger of the free hand placed lightly upon the posterior and anterior edges of the otolith for stability (and to prevent the sections from flying apart when sectioning), a slight downward sawing action is applied to break the otolith into two sections through the nucleus and the dorsoventral axis (Figure1). An incision not made directly through the nucleus, can contribute to missed annuli and misinterpretation of age.

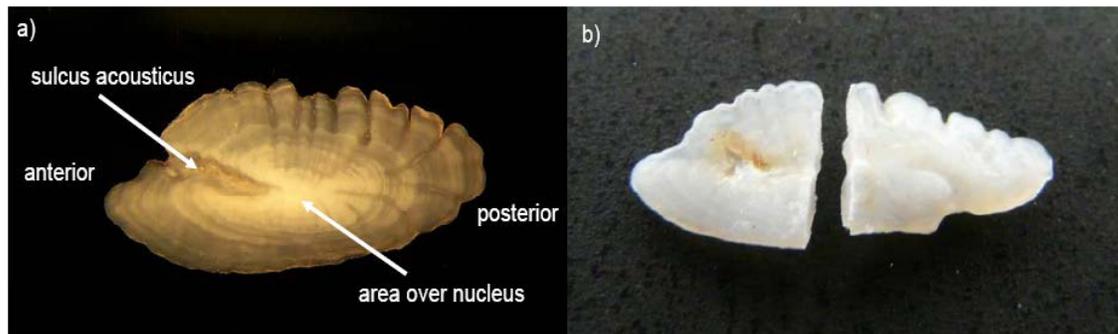


Figure 1. a) Otolith physiology (Photo courtesy of Susan Mann, Ontario Ministry of Natural Resources, 2008) (b) walleye otolith sectioned transversely through the nucleus.

A 1.70ml centrifuge tube is used for mounting and storage of the otolith sections. The cap is removed from the centrifuge tube and filled level with Plasticine™ (Figure 2).



Figure 2. A 1.70 ml centrifuge tube and cap. The cap is filled with Plasticine™ to be used as a mounting medium for sectioned otoliths.

Plasticine does not readily dry out like some other products that become hard and brittle over time, making it an appropriate medium (in our opinion) for mounting and holding otolith cross-sections for long-term storage. To enhance the annuli within the otolith, cross-sections should be heated with a flame (candle flame, alcohol burner or diethylene glycol). Use fine-tipped forceps to hold the base of the otolith exposing the sectioned surface to the flame. The placement of the otolith within the flame can be important to prevent carbon deposition on the open face which can obscure annuli. Alternatively, otoliths can also be heated on a hot plate to help avoid carbon deposition. The otolith must be positioned to ensure equal exposure across the open face to the flame. The otolith should be held to the hottest part of the flame (blue) approximately halfway up the wick (however, remain 1-2 mm back from the flame). Burning time is dependant upon the temperature of the heat

source, along with the size of the structure and the density of calcium. Approximately 7-10 seconds of burning results in the otolith section turning from white to amber to ash-grey. Note, juvenile or young walleye (small) otoliths take less time to burn. If the otolith section is exposed to the heat source for a longer period, the sectioned piece will completely burn, turning black and making it unreadable. In some instances, the sectioned piece can crumble into smaller pieces. When the otolith reaches the amber to ash-grey stage (dark caramel), place it horizontally, open face up, into the Plasticine™ (Figure 3). Avoid touching the burnt surface with fingers or forceps as the surface can be quite fragile. The hot otolith will melt the plasticine to secure its position within the centrifuge cap.



Figure 3. A sectioned and burnt otolith mounted in Plasticine™ in the centrifuge tube cap.

To enhance the readability of the annuli on the burnt, open face of the structure, apply a small amount of oil (cooking or mineral) to the surface with a fine tipped paintbrush and examine under a dissection or compound microscope at 25-32X magnification with reflected light. Once the surface of the otolith has been oiled, avoid re-burning the otolith as carbon will build up and obscure the annuli. The mounted otoliths can remain in the centrifuge cap and secured back into the centrifuge tube for long-term storage. Often a single otolith is sufficient for ageing so the remaining otolith can be stored dry within the centrifuge tube.

### Identifying Annuli

Annular formation (annuli) occurs due to the effects of two growing periods, fast summer growth and slow winter growth with the combined period constituting one year. Summer growth is referred to as the opaque zone which inhibits light passage when viewed under reflected light. The winter growth is a distinct translucent band known as the hyaline zone alternating with the opaque zone throughout the structure (Chilton and Beamish 1982). The opaque 'summer' zone is the period during which growth is most prominent in calcified structures where the fish lays down a heavy band consisting of minerals that is white when viewed under reflected light. The hyaline 'winter' zone is the period when growth tends to slow and a less dense band of minerals is laid down. The hyaline zone is generally narrower than the opaque zone and viewed as dark after burning. Under reflected light the reader counts the outside edge of the hyaline annuli as one year of growth (Appendix 2).

When counting annuli, the reader should follow a radial axis starting from the focus and moving outward towards the distal edge that shows clear and consistent annuli. If an area or pattern of inconsistent annuli is encountered the reader should follow a distinct annulus just prior to the inconsistent area to another well defined area or annulus. Counting these annuli for accurate age assignment requires significant

experience from the reader. It is important to identify and eliminate false annuli found in the sample while recognizing true annuli. Intra-annual variability (false annuli) can be created during periods of stress or life cycle change that a fish endures (Isley and Grabowski 2007). Generally, the most prominent increments are annuli found before sexual maturity when somatic growth is highest. Mature walleyes may exhibit a pattern of annular growth where distances between annuli become narrower at sexual maturity. At this point somatic growth decreases and reproductive growth tends to increase. Immature walleyes do not exhibit this type of growth pattern and false annuli may be mistaken for true annuli resulting in an over-age estimate.

### Locating the First Annulus

Because there can be false annuli in otolith cross-sections beginning at the time of otolith formation that are associated with growth (and change in diet), it may be difficult to distinguish the point at which annular growth or year one begins from these marginal increases. To determine the location at which the first annulus begins ( $O_a$ ), the following back-calculation formula (1) can be applied to an otolith cross-section prior to age assignment. To determine length at age-1 ( $L_1$ ) it is important to have independent knowledge of the walleye'(s) length-at-age-1 from its particular body of water:

$$(1) O_a = (OR \times L_1) / L_c$$

where  $O_a$  is the radius distance from the nucleus/focus to the 1st annulus of the otolith cross-section (mm) OR is the otolith radius (mm)  $L_1$  is the length at age-1 (mm), and  $L_c$  is the length of the fish at capture (mm). Length at age-1 ( $L_1$ ) was calculated from a mean length collected from Fall Walleye Index Netting (FWIN) data collected in September throughout Alberta (Morgan 2002). Generally,  $144 \pm 15$ mm will work in most cases if length of walleyes approaching age-1 is unavailable. It is

important to remain consistent with measurement calculations, using either fork length or total length for both  $L_1$  and  $L_c$ , but not interchangeably.

It is recommended that a graduated ocular micrometer (10:1 mm) in a dissection microscope at 25X magnification is used to measure distances from the focus to the distal edge of the age structure, measured along the longest plane where all the annuli are most discernable. Within a shorter plane, annuli tend to become compressed and measurement errors are more likely to occur. The reader's measurement ( $O_a$ ) from the focus should locate the age-1 hyaline zone or within a few increments within this zone (Figure 4).

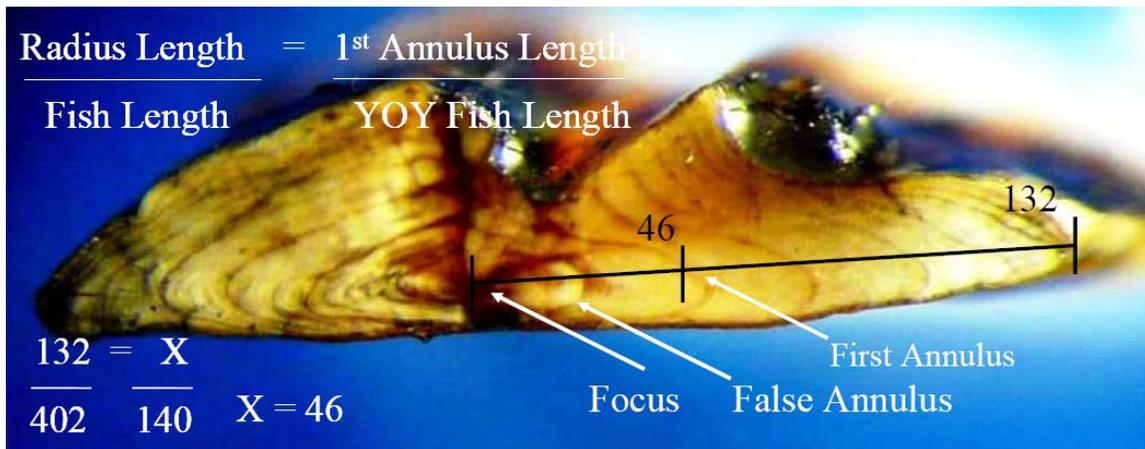


Figure 4. A sectioned, cracked and burned otolith exposing annuli. Measurement from the focus to distal edge indicates the location of the first annulus of an age-7 walleye collected from Lac Ste. Anne, Alberta, September, 2006.

## Discussion

Where possible, it is recommended to use a stereo or dissection microscope and digital camera with the capability of capturing, storing, and enhancing digital images of otolith structures in order to quantify and identify growth features on the structures. Captured digital images allow for enhancement of images and viewing by multiple agers simultaneously for interpretation or interpretation of discrepancies.

Additionally, images can be sent electronically to experienced agers for verification without having to send the actual structures.

The described method provides a consistent and repeatable means of ageing walleye otoliths. Although otolith collection takes more time in the field than the collection of most other age structures, the laboratory preparation is faster and easier than pelvic fin ray preparation. Otoliths are easily interpreted and the described crack and burn technique is a precise and efficient method for age estimation of walleye otoliths (Spencer et al., 2009).

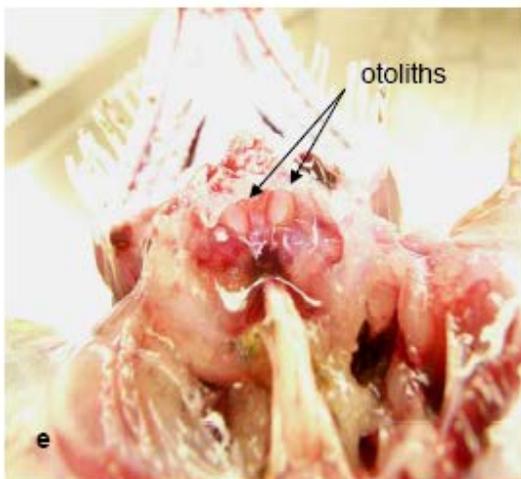
## References

- Barber, W. E., and G.A. McFarlane. 1987. Evaluation of three techniques to age arctic char from Alaskan and Canadian waters. *Transactions of the American Fisheries Society* 116; 874-881.
- Chilton, D. E. and Beamish, R. J. 1982. Age determination methods for the fishes studied by the groundfish program at the Pacific Biological Station. – *Can. Spec. Publ. Fish. Aquat. Sci.* 60: 1-102.
- Fielder, D. G. 2002. Methodolgy for immersion marking walleye fry and fingerlings in oxytetracycline hydrochloride and its detection with fluorescence microscopy. Michigan Department of Natural Resources Technical Report 2002-1, 2002.
- Isley, J. J., and T. B. Grabowski. 2007. Age and growth. Pages 187-228 *in* C.S. Guy and M. L., editors. *Analysis and interpretation of freshwater fisheries data*. American Fisheries Society, Bethesda, Maryland.
- Maceina, M. J., J. Boxrucker, D. L. Buckmeier, R. S. Gangl, D. O. Lucchesi, D. A. Isermann, J. R. Jackson, and P. J. Martinez. 2007. Current status and review of freshwater fish aging procedures used by state and provincial fisheries agencies with recommendations for future directions. *Fisheries* 32(7): 329-340.
- Mackay, W. C., G. R. Ash, and H. J. Norris (eds.). 1990. Fish ageing methods for Alberta. R.L. & L. Environmental Services Ltd. in assoc. with Alberta Fish and Wildl. Div. And Univ. of Alberta, Edmonton. 113p.
- Morgan, G. E. 2002. Manual of instructions — fall walleye index netting (FWIN). Diagnostics and Sampling Standards Working Group, Peterborough, Ontario.

Spencer, Stephen C., Owen B. Watkins, Michael G. Sullivan and A. Lee Foote 2009.  
Non-lethal ageing methods and ageing error: management consequences for  
slowing fishes. *In prep.*

## **Appendices**

Appendix 1. Removal of otoliths from a walleye.



- a. Ventral side of walleye - cutting through isthmus and gill arches with wire cutters.
- b. Roof of mouth - exposing the parasphenoid and gill arches
- c. Cutting through parasphenoid bone.
- d. Cracking head backwards through parasphenoid bone to expose otoliths.
- e. Location of exposed otoliths.

Appendix 2. Numbered dots indicating annuli on an otolith collected from a walleye from Buck Lake, Alberta, September, 2006. This otolith was assessed at age-10.



# Collection, preparation and ageing of walleye pelvic fin rays

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## Abstract

Accurate fish age is important for calculating parameters such as productivity, growth, mortality, estimating year-class strength, length-at-age and age-at-maturity for management and conservation purposes. Fish ages are especially important in Alberta for walleye (*Sander vitreus*) management, they are difficult to assess accurately because walleye are slower growing, longer lived, less fecund than more southern fish populations. Until recently, Alberta's preferred ageing method for walleyes was the examination of pelvic spines (Mackay et al. 1990) in order to minimize lethal sampling. In most jurisdictions across North America otoliths are the preferred ageing structure for walleyes (Maceina et al. 2007). To increase ageing accuracy for walleye using pelvic fins, we refined the techniques for pelvic fin collection and preparation. It is determined that pelvic fins need to be cut and sectioned within 3mm of the attachment to the walleye to prevent missed or lost annuli. This method is not a lethal collection for walleye specimens, unlike otolith collections. We recommend cutting the pelvic fin within 0.3mm to 0.5mm sections with a jeweler's saw and mounting the cross-sections consecutively. We describe how a back-calculation formula aids in locating the first annulus and address an inaccuracy in Fish Ageing Methods for Alberta's last ageing manual (Mackay et al. 1990). Finally, we demonstrate how these refinements in collection, preparation and ageing increase reader consistency and accuracy.

## Table of Contents

Abstract .....	2
Table of Contents .....	3
List of Figures .....	4
Introduction .....	5
Methods .....	6
Collection .....	6
Preparation .....	6
Interpreting Annuli .....	10
Identifying Annuli .....	10
Allometric Ratios .....	12
Pelvic fin Development .....	13
Results and discussion .....	14
Recommendations .....	17
References .....	18
Appendices .....	20

## List of Figures

Figure 1. Walleye pelvic fin ray cross-sections prior to mounting. ....	7
Figure 2. Back calculated fork lengths from an age-6 walleye pelvic fin ray. Thirty-five cross-sections were cut and back calculated from the first ray of a walleye pelvic fin. Dashed vertical lines depict recommended area for cross-section reading. ....	8
Figure 3. An example of walleye pelvic fin rays cross-sections mounted in series from left to right.....	9
Figure 4. 1st annulus landmarked on a cross-section of a walleye pelvic fin ray from Buck Laker, Alberta, September 2006. ....	12
Figure 5. An allometric relationship between walleye fork length and fin ray radius. Fish sizes ranged from 160-466mm with 14 age-classes represented (0-13 years).....	13
Figure 6. Age-0 walleye lengths captured during the summer months. By September, the average length of age-0 walleyes from eight Alberta lakes was 125mm. As the fish will continue to grow throughout the winter and by annulation in the following spring we estimate young-of-the-year walleyes will average 144mm fork length.....	14
Figure 7. Cross-section of a walleye pelvic fin ray viewed under transmitted light a) false annulus b) age-1 annulus and c) age-2 annulus.....	15
Figure 8. A twelve-slide test of pelvic fin cross-sections were given to a group of 8 fisheries staff of varying degrees of fish ageing experience (0 to 30 years). Error bars depict standard deviation from the mean.....	16

## Introduction

In Alberta, ageing walleyes by means of pelvic fin ray examination has been the common practice in fisheries management for over 30 years. They are non-lethal, provide a deoxyribonucleic acid (DNA) sample and, are easy to collect and prepare for ageing. We offer several techniques to improve pelvic fin accuracy and age agreement. Additionally, we believe that Mackay et al. (1990) misidentified the first annulus, giving all examined walleyes a tendency to be over-aged. Howland et al. (2003) found the pattern within the center area of fin rays to be inconsistent making it hard to distinguish the first year's growth. Howland et al. (2003) speculated that details in the core area of fin ray cross-sections will be lost if improper collection techniques are used (i.e. removal of the fin too far from the body). Although there have been many ways to identify and count structural annuli, interpretation errors are associated with a lack of objectivity in identifying annuli. If the first annulus is not identified correctly, fish age will be underestimated or overestimated; this procedure leads to errors in calculations of the growth and natural mortality coefficients, with drastic implications in fish stock management (Leaman & Nagtegaal 1987; Casey & Natanson 1992). To promote objectivity and accuracy among readers, we have developed a simple back-calculation model to identify the first annulus in walleye pelvic fin ray cross-sections based upon the average length of young-of-year walleyes approaching age-1.

## **Methodology**

### Collection

In walleyes, pelvic fins are located on either side of the fish located ventrally and posterior of pectoral fins. Pelvic fin rays are a bony structure in which mineralized layers of different density are deposited during annular formation (MacKay 1990). Any field collection of fin samples from mortalities should be excised as close to the body as possible with wire cutters or garden sheers, taking the entire fin. In the case of live fish, only the first three fin rays should be severed and peeled away from the remaining portion of fin through the membrane. Caution should be taken when removing pelvic fins from live fish; to ensure survival of released walleyes, pelvic fins should be excised no closer than 3mm from their articulation to the body. This will prevent excessive blood loss by the fish, yet provide an adequate sample for ageing. At the time of collection, pelvic fins should be placed in scale envelopes and dried to prevent rotting or frozen to process at a later date. If frozen, they should be thawed and dried prior to sectioning.

### Preparation

To prepare a pelvic fin for ageing it must be sectioned. We used a jeweler's saw; Grobet 1/3 h.p. motor, model number 34.995 with a flexible shaft drive connected to a Grobet handsaw with a variable speed control foot pedal. A 19mm x 0.1mm saw blade attached to a 45mm mandrel is used to section the pelvic fins. A section of X-Acto foam board (13mm x 8mm) was used as a cutting surface to minimize excessive blade wear, dust and debris that may obscure pelvic fin cross-sections. Pelvic fin cross-sections were mounted on a clear acrylic slide measuring 75mm x 20mm x 1mm labeled with a sample number corresponding to the original scale envelope to prevent mismatching biological data.

The pelvic fin was placed on an appropriate cutting surface and held down (use fingers or masking tape, etc.) to minimize vibration (great caution must be taken as the blade cuts very close to your hand). We recommend cutting off the ragged edge (1mm section) from where the fin was excised during field collection. The 1st and subsequent cuts should be made perpendicular and vertical to the first three pelvic fins with the saw at maximum speed (Figure 1). Slight adjustments in the saw's position will need to be made to correct for the changing angle of the pelvic fins as sections are cut towards the distal end of the pelvic fin. Cross-section thickness is important to maximize reflected or transmitted light through the sections; too thick and fine annuli may be obscured, too thin and annuli may not be discernable. All sections must be 0.3mm to 0.5mm wide and should include the first three branches of the first fin ray. Sections are cut approximately  $\frac{2}{3}$  to  $\frac{3}{4}$  through the width of the entire fin to prevent cross-sections from being lost. Considerable fine dust that can irritate eyes and mucous membranes is created when cutting through pelvic fins. A dust mask and safety glasses may not be sufficient. We found that fins cut in a fume hood with the glass door partially lowered provided improved comfort and safety.



Figure 1. Walleye pelvic fin ray cross-sections prior to mounting.

Our aim was to determine the best location to section a pelvic fin for age assignment. We were concerned that excise the pelvic fin too close to the walleye would injure the fish while cross-sectioning too far down the pelvic fin would make age assignment difficult. One walleye pelvic fin was continuously cross sectioned from its attachment to the fish, to 20 mm along the length of the pelvic fin (Figure 2). We assigned ages and backcalculated size at age to provide information on the suitability of the location of the cross-section. We found that sections should be cut from 3 to 8 mm of the attachment to the walleye. Please note, this method was followed for only one sample and should be replicated with additional samples to ensure the validity of our result.

Multiple cross-sections allow the reader to cross-reference annuli from one cross-section to another. As cross-sections are cut towards the distal end of the fin, the diameter of the fin ray decreases progressively. Annuli are compressed or disappear along the margin.

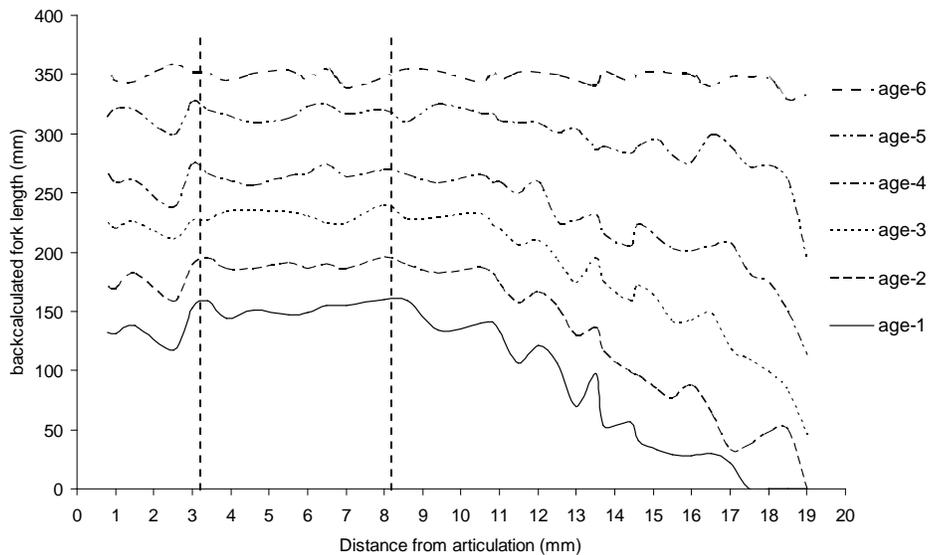


Figure 2. Back-calculated fork lengths from an age-6 walleye pelvic fin ray. Thirty-five cross-sections were cut and back-calculated from the first ray of a walleye pelvic fin. Dashed vertical lines depict recommended area for cross-section reading.

Sections were removed from the fin with fine-tipped forceps and placed in order of cut on the slide (Figure 3). Cytoseal XYL™ (low viscosity) was the preferred mounting media. Because of potential health risks to the reader refer to the Material Safety and Data Sheets for proper handling, Appendix 1). Use was restricted to the inside of a functional fume hood only (where no fume hoods are available a water-based polyurethane can be used). A bead of mounting media was run over all cross-sections. To ensure proper cross-section placement and media coverage, the sample was viewed under low power through a dissection microscope. A needle-tipped handle was used to push all cross-sections flat against the slide's surface to ensure reflected light was not lost between the slide and the bottom of the cross-section. Care was taken to minimize the movement of the needle tip within the Cytoseal to prevent excess bubbles. The structures can be aged at this point, but the slide should be exposed to the air for 24hrs to ensure complete drying before storing in an appropriately labeled scale envelope.

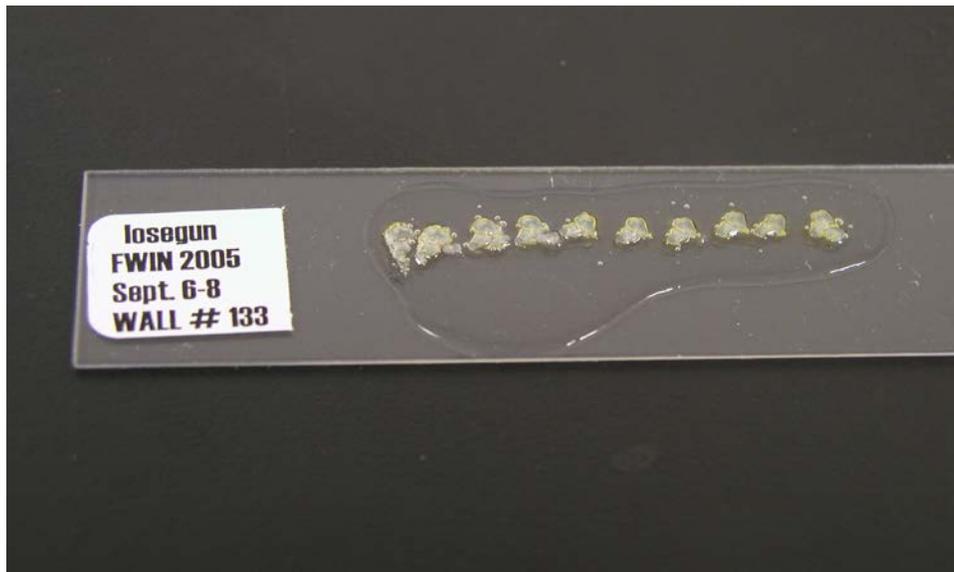


Figure 3. An example of walleye pelvic fin rays cross-sections mounted in series from left to right.

## Interpreting Annuli

Annular formation (annuli) results from a combination of two growing periods, fast summer growth and slow winter growth with the combined period constituting one year. Summer growth is referred to as the opaque zone which inhibits light passage when viewed by a reader under reflected light. The winter growth is distinguished by a translucent band known as the hyaline zone alternating throughout the structure (Chilton and Beamish 1982). The opaque 'summer' zone is the period during which growth is most prominent in calcified structures where the fish lays down a heavy band of minerals which is white when viewed under reflected light. The hyaline 'winter' zone is the period when growth tends to slow and a less dense band of minerals is laid down. The hyaline zone is generally narrower than the opaque zone and viewed as black under reflected light (with a black background). Under reflected light the reader counts the outside edge of the hyaline annuli as one year of growth. Counting these annuli for accurate age assignment requires significant experience by the reader. It is important to identify and eliminate false annuli or supernumerary checks found in the sample while recognizing true annuli. Intra-annual variability can be created during periods of stress or life cycle change that a fish may endure (Isley and Grabowski 2007). Generally, the most prominent increments are annuli found before sexual maturity when somatic growth is highest. Following sexual maturity, annular spacing becomes narrow, the annuli are often compressed and can become over lapped towards the margin.

## Identifying Annuli

There are visible marginal increases (false annuli) in fin ray cross-sections beginning at the time of fin formation that are associated with growth, making it difficult to distinguish the point at which annular growth or year one begins from these marginal

increases. Back-calculations from annular lengths are an important tool to describe fish growth. Growth back-calculations in bony structures are commonly used to determine size-at-age based on proportional differences between the measured structure and fish length (Campana 1990). To determine the point at which the first annulus begins ( $Sc$ ), the following back-calculation formula (1) can be applied to a pelvic fin ray cross-section prior to age assignment. To determine length at age-1 ( $L_1$ ) it is important to have independent knowledge of the walleye's length-at-age-1 from its particular body of water:

$$(2) Sc = (PFRR \times L_1) / Lc$$

where  $Sc$  is the radius distance from focus to 1st annulus of fin ray cross-section (mm),  $Lc$  is the length of fish at capture (mm), and PFRR is the pelvic fin ray radius (mm). Length at age-1 ( $L_1$ ) was calculated from a mean length collected from 8 Alberta lakes using the Fall Walleye Index Netting (FWIN) protocol (Morgan 2002). In September young-of-the-year (YOY) walleyes were found to have a mean fork length of 135mm. Growth of YOY walleyes from fall until first annulation has a mean fork length of 150mm. If length at age-1 is unknown, 150mm  $\pm$  20mm will work for most Alberta lakes.

A graduated ocular micrometer (10:1mm) was used in a Zeiss stereomicroscope (dissection microscope) to measure distances from the focus to the outer edge of each age structure. These measurements should be taken along the longest plane where all the annuli are most discernable. Within a shorter plane, the annuli tend to become compressed and measurement errors may occur. Pelvic fin rays were measured from the focus to the distal edge of the largest lobe on the third cross-section of the first pelvic fin ray under 25-32X magnification (Figure 4). The reader's measurement from the focus should locate the age-1 hyaline zone or within a few increments within this zone to identify the first annulus.

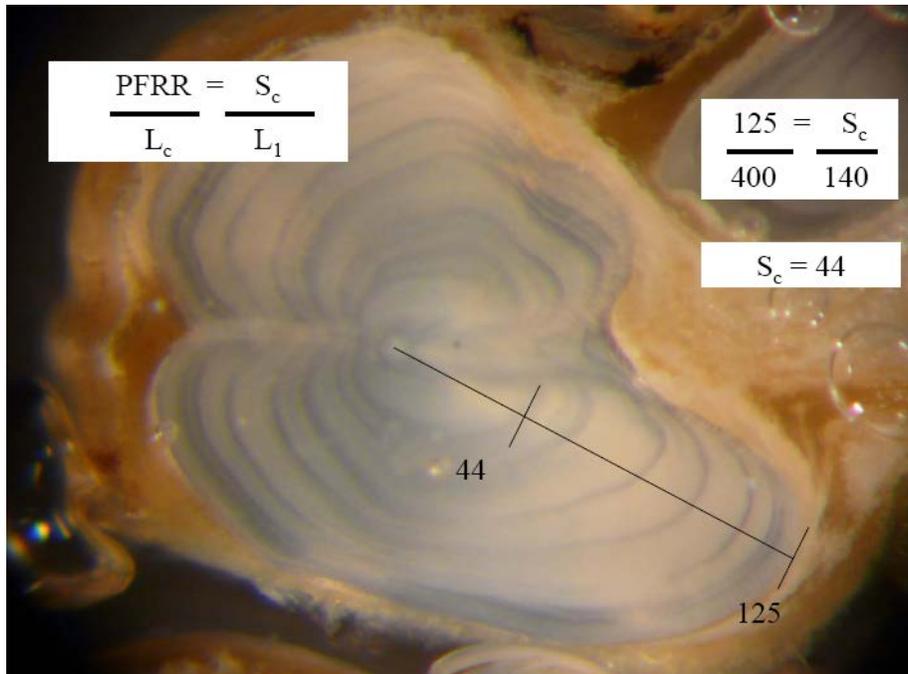


Figure 4. 1st annulus landmarked on a cross-section of a walleye pelvic fin ray from Buck Lake, Alberta, September, 2006.

If age-1 ( $L_1$ ) average length-at-age data is unavailable, another approach is to back-calculate (2) to the suspect annulus (hyaline zone).

$$(3) L_1 = (S_c \times L_c) / \text{PFRR}$$

where  $S_c$  is the radius distance from focus to 1st annulus of fin ray cross-section (mm),  $L_c$  is the length of fish at capture (mm); PFRR is the pelvic fin ray radius (mm).

### Allometric Ratios

To determine the validity of the back-calculation formula, a relationship between first annular formation and fish length, an allometric ratio, was performed in 2008 on 143 walleye pelvic fin rays under constant 32X magnification (Figure 5). Regression analysis resulted in the following slope equation:

$$y = 0.0178 x - 0.2564$$

where:

$y$  = fin ray radius (mm)

$x$  = fork length (mm)

This relationship was significant ( $P > 0.07$ ,  $R^2 = 0.89$ ). After examination of residuals and in the absence of any contradictory evidence, it was posited that this allometric relationship was linear.

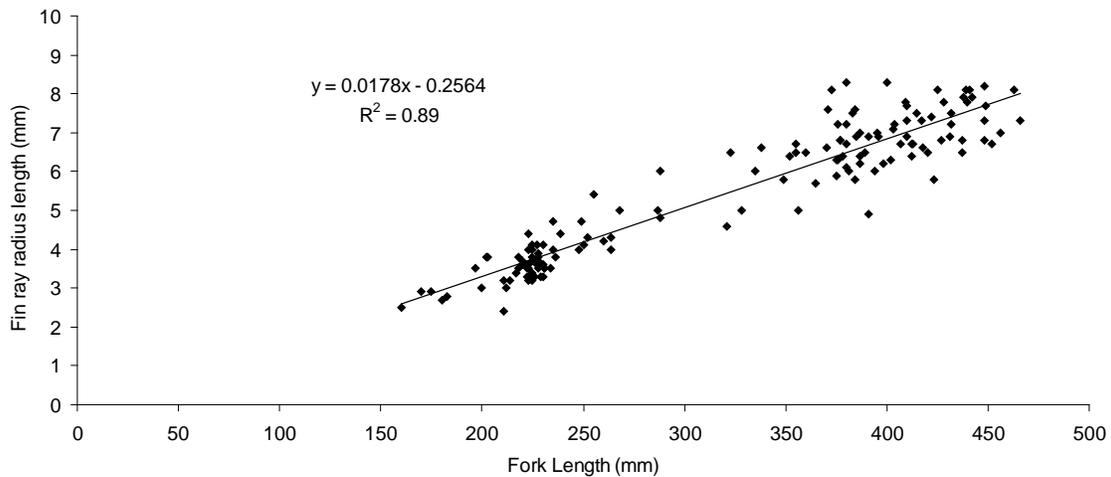


Figure 5. An allometric relationship between walleye fork length and fin ray radius. Fish sizes ranged from 160-466mm with 14 age-classes represented (0-13 years). Both sexes were used.

### Fin Ray Development

We believe that fin ray growth is analogous to tree growth where the most recent growth is found on the margins while the structure increases in diameter and length. To help us determine the length of Alberta walleyes at their first annulation ( $L_1$ ), we compiled young of the year walleye lengths from fourteen different drainages throughout the summer months starting in June and ending in mid September (Figure 6). Based on this analysis, we estimated that by their first annulation (May-June) walleyes have a mean fork length of 144mm (95% C.I. = 137-149mm). We found this length to vary depending on growing conditions and waterbody

productivity; however a 145mm fork length provides an appropriate approximation for the first annulus calculation.

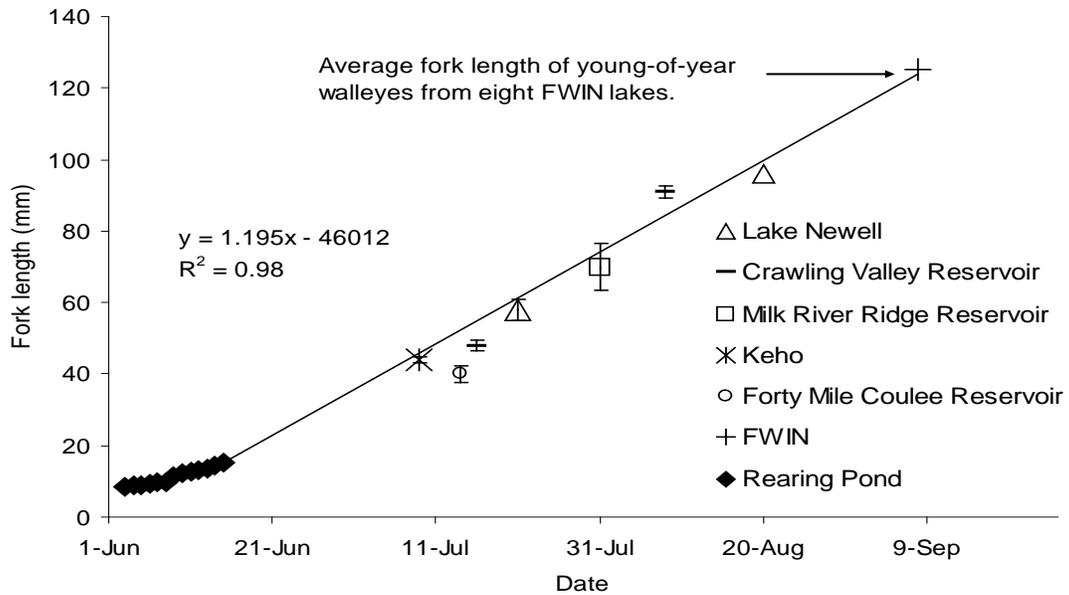


Figure 6. Age-0 walleye lengths captured during the summer months. By September, the average length of age-0 walleyes from eight Alberta lakes was 125mm. As the fish will continue to grow throughout the winter and by annulation in the following spring we estimate young-of-the-year walleyes will average 144mm fork length.

## Results and discussion

We take the position that the first annulus described by Mackay et al. (1990) was too close to the focus and is likely a false annulus or supernumerary. Because of this interpretation the sample (Figure 7) is over aged by one year.

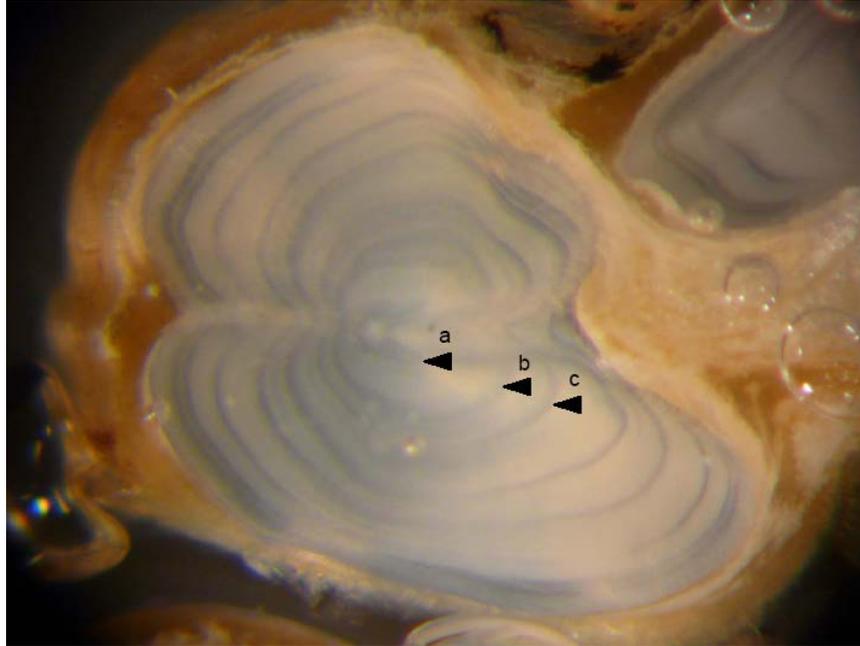


Figure 7. Cross-section of a walleye pelvic fin ray viewed under transmitted light: a) false annulus, b) age-1 annulus and c) age-2 annulus.

Otoliths are the standard ageing structure in jurisdictions outside Alberta and are used for many species (Campana 2001; Isermann et al 2003). Unfortunately, otolith collections are lethal and are slower to collect than other structures such as pelvic fin rays. The pelvic fin ray preparation technique can easily produce a slide every four minutes while, otolith preparation and reading takes approximately half the time. Koch and Quist (2007) recommend mounting fin rays in epoxy to provide a convenient means to read and archive. There is no fin size bias (e.g. age-1 vs. age-10 fin ray) when sectioning rays with a jeweler's saw. In addition, we have found it unnecessary to mount YOY spines and fin rays in epoxy to increase overall fin ray diameter prior to sectioning. With a sharp saw blade YOY fin rays can easily be sectioned without further preparation. The time factor can be reduced with the refinement of present procedures.

Although we have used the collection and preparation techniques to age pelvic fin rays from northern pike (*Esox lucius*) and yellow perch (*Perca flavescens*) with repeatable results, we have not verified the validity of the described back-calculation technique for these two fish species. The present measurement model has been applied extensively with proven results to locate the first annulus in walleye operculae, otoliths and dorsal spines.

To test the validity and ease of the back-calculation model we tested eight fisheries staff with varying degrees of expertise (0 to 30 years). We presented 12 slides of walleye pelvic fin cross-sections of various ages (ages 1-10) to the eight staff members to test and re-test the variability between agers (Figure 8). In every case, the average specimen age decreased by at least one year for every sample after the re-test.

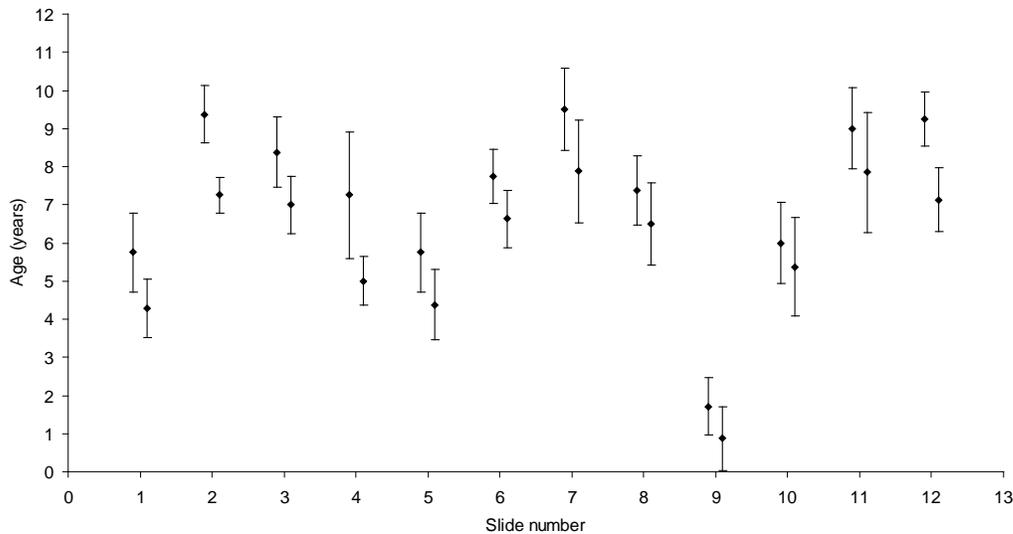


Figure 8. A twelve-slide test of pelvic fin cross-sections were given to a group of 8 fisheries staff of varying degrees of fish ageing experience (0 to 30 years). Error bars depict standard deviation from the mean.

## Recommendations

Inexperienced readers must spend time with an accomplished reader in a lab setting to ensure that their preparation and ageing techniques are appropriate. Paired sample sets of pelvic fin rays and otoliths of known ages, encompassing a broad range of age-classes, should be used to train the new reader. Comparison of age estimates between structures is an alternative technique to validation that may provide useful information on the accuracy and bias of age estimating structures (Sylvester 2006). Subsamples of otoliths of known ages can be used to verify pelvic fin ages.

We prefer the technique outlined in this paper because of the ease of preparation, low cost and highly detailed samples. We find that epoxy is unnecessary and Cytoseal provides a convenient means to archive pelvic fin cross-sections with minimal discoloration and cracking over time.

We caution that pelvic fins, especially those from slower and older populations, may provide spurious results unless verified against otolith ages. Therefore, age comparisons among paired otolith/pelvic fin samples should be made prior to pelvic fin age assignment (Spencer *et al.*, 2008 *in prep*).

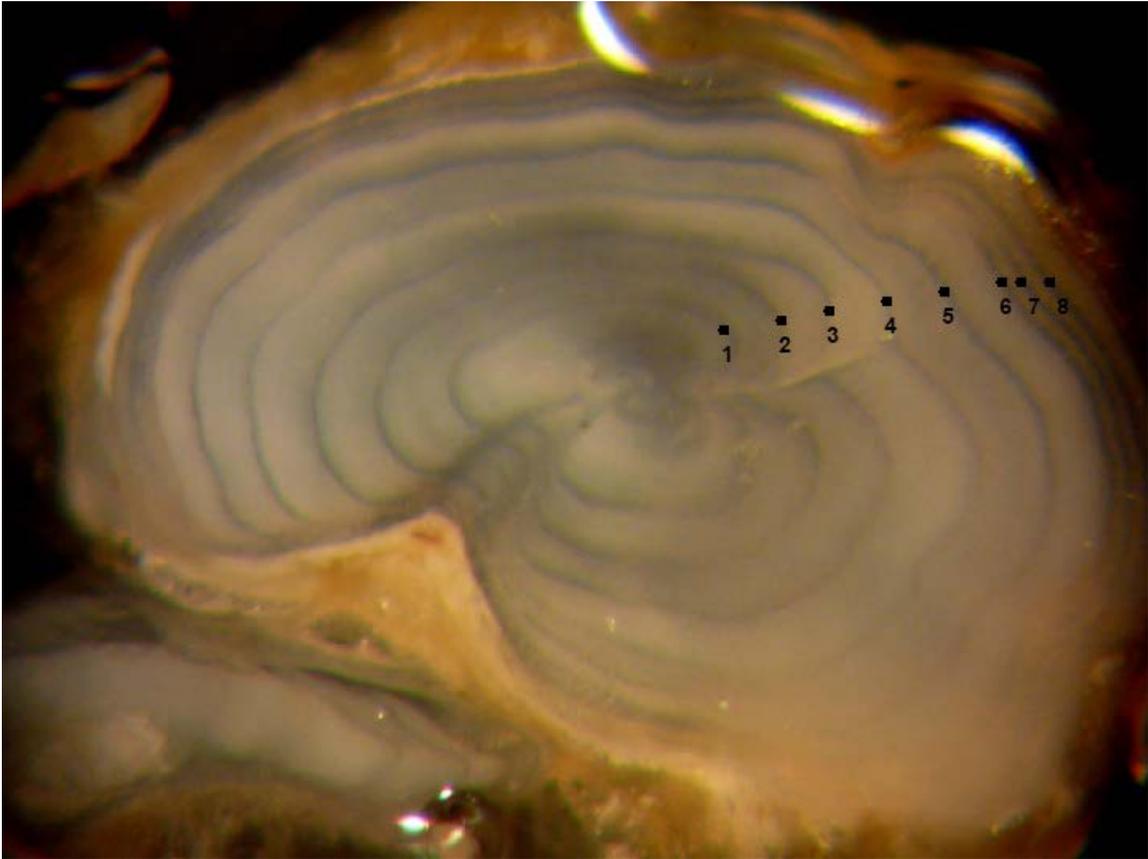
## References

- Baccante, D. A., and P. J. Colby. 1996. Harvest, density and reproductive characteristics of North American walleye populations. *Annales Zoologici Fennici* 33(3-4):601-615.
- Campana, S. E. 1990. How reliable are growth back-calculations based on otoliths? *Can. J. Fish. Aquat. Sci.* 47: 2219-2227.
- Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including the review of use and abuse of age validation methods. *Journal of Fish Biology* 2001(59):197-242.
- Casey, J. G. and L. J. Natanson (1992) Revised estimates of age and growth of the sandbar shark (*Cacharhinus plumbeus*) from the western North Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences* 49, 1474-1477.
- Chilton, D. E. and Beamish, R. J. 1982: Age determination methods for the fishes studied by the groundfish program at the Pacific Biological Station. – *Can. Spec. Publ. Fish. Aquat. Sci.* 60: 1-102.
- Isermann, D. A., J. R. Merbeek, G. D. Scholten, and D. W. Willis (2003) Evaluation of three different structures used for walleye age estimation with emphasis on removal and processing times. *North American Journal of Fisheries Management* 23:625-631, 2003.
- Howland, K. L., Gendron, M., Tonn, W. M. & Tallman, R. F. 2004: Age determination of a long lived coregonid from the Canadian North: comparison of otoliths, fin rays and scales in inconnu (*Stenodus leucichthys*). – *Ann. Zool. Fennici* 41 : 205-214.
- Isley, J. J., and T. B. Grabowski. 2007. Age and growth. Pages 187-228 in C.S. Guy and M. L., editors. *Analysis and interpretation of freshwater fisheries data*. American Fisheries Society, Bethesda, Maryland.
- Koch, J. D. and M. C. Quist (2007) A technique for preparing fin rays and spines for age and growth analysis. *North American Journal of Fisheries Management* 27:782-784.
- Leaman, B.M. and D. A. Nagtegaal (1987) Age validation and revised natural mortality rate for yellowtail rockfish. *Transactions of the American Fisheries Society* 116, 171-175.
- Maceina, M. J., J. Boxrucker, D. L. Buckmeier, R. S. Gangl, D. O. Lucchesi, D. A. Isermann, J. R. Jackson, and P. J. Martinez. 2007. Current status and review of freshwater fish ageing procedures used by state and provincial fisheries agencies with recommendations for future directions. *Fisheries* 32(7): 329-340.

- Mackay, W. C., G. R. Ash, and H. J. Norris (eds.). 1990. Fish ageing methods for Alberta. R.L. & L. Environmental Services Ltd. In assoc. with Alberta Fish and Wildl. Div. And Univ. of Alberta, Edmonton. 113p.
- Morgan, G. E. 2002. Manual of instructions — fall walleye index netting (FWIN). Diagnostics and Sampling Standards Working Group, Peterborough, Ontario.
- Spencer, Stephen C., Owen B. Watkins, Michael G. Sullivan and A. Lee Foote 2008. Non-lethal ageing methods and ageing error: management consequences for slowing fishes. *In prep.*
- Sullivan, M. G. 2003. Active management of walleye fisheries in Alberta: Dilemmas of managing recovering fisheries. *North American Journal of Fisheries Management* 23(4):1343-1358.
- Sylvester, R. M., and C. R. Berry. 2006. Comparison of white sucker age estimates from scales, pectoral fins rays, and otoliths. *North American Journal of Fisheries Management* 26:24-31.

## Appendices

Appendix 1. Numbered dots indicating annuli on an age-8 pelvic fin ray collected from a female walleye (417mm fork length) from Buck Lake, Alberta, September, 2006.



## Appendix 2. Material Safety Data Sheet (MSDS) for Cytoseal XYL

Richard-Allan Scientific Cytoseal™ XYL

1 Revision April 2002

### **MATERIAL SAFETY DATA SHEET**

**CYTOSEAL™ XYL**

**RICHARD-ALLAN SCIENTIFIC**

**4481 Campus Drive**

**Kalamazoo, MI 49008 CHEMTREC (800) 424-9300**

800-522-7270 U.S.A. 8:00 a.m. - 5:00 p.m. EST 24 hours Everyday

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### **1. SUBSTANCE IDENTIFICATION**

SUBSTANCE: **Cytoseal™ XYL**

CATALOG NUMBER: 8312-4

TRADE NAMES/SYNONYMS: Cytoseal™ XYL Mounting Medium

CHEMICAL FAMILY: Hydrocarbon, aromatic

### **2. COMPOSITION AND INGREDIENTS INFORMATION**

Total aromatic content:

Xylenes CAS# 1330-20-7 65%

Acrylic Resin CAS# 28262-63-7 35%

Antioxidant CAS# 128-37-0

Butyl Benzyl Phthalate CAS# 85-68-7

### **3. HAZARDS INFORMATION**

NFPA RATINGS (SCALE 0-4): Health=2 Fire=3 Reactivity=0

Cytoseal™ XYL is a mixture of acrylic resin suspended in xylene. The xylene content is approximately 65%. Cytoseal™ XYL is a colorless, viscous solution with a characteristic aromatic odor. Cytoseal™ XYL can be toxic if swallowed. Systemic effects by inhalation are most commonly seen. Symptoms from mild exposure may include dizziness, weakness, euphoria, headache, nausea and vomiting. Repeated or prolonged exposure increases the toxic effects.

Primary Routes of Exposure: Inhalation, Ingestion, Skin and Eye contact.

Acute Effects: Acute effects due to inhalation or ingestion range from headache, nausea, vomiting, tightness of the chest and staggering due to visual blurring, tremors, shallow and rapid respiration and ventricular irregularities. Kidney or liver damage may occur.

Chronic Effects: Repeated or prolonged exposure to toluene may cause headaches, loss of appetite, drowsiness, nervousness and pallor. Continued repeated inhalation of toluene to the point of euphoria has caused irreversible encephalopathy with ataxia, tremulousness, emotional liability and diffused cerebral atrophy.

Potential Health Effects:

- *Inhalation: May cause dizziness, headache, nausea or vomiting.*
- *Eye contact: May cause severe irritation and damage to eyes.*

- *Skin contact: May cause skin irritation.*
- *Ingestion: Harmful, may be fatal if swallowed. May cause nausea or vomiting.*

#### **4. FIRST-AID PROCEDURES**

Inhalation: Remove from exposure area to fresh air immediately. If breathing has stopped, give artificial respiration. If breathing is difficult, give oxygen. Keep affected person warm and at rest. Get medical attention immediately.

Eye Contact: Wash eyes immediately with large amounts of water, occasionally lifting upper and lower lids, until no evidence of chemical remains (at least 15-20 minutes). Get medical attention immediately.

Skin Contact: Remove contaminated clothing and shoes immediately. Wash affected area with soap or mild detergent and large amounts of water until no evidence of chemical remains (at least 15-20 minutes). Get medical attention if irritation persists.

Ingestion: If swallowed, do not induce vomiting. If vomiting does occur, insure victim's head is lower than hips in order to prevent aspiration. Call a physician immediately.

ANTIDOTE: No specific antidote. Treat symptomatically and supportively.

#### **5. FIREFIGHTING PROCEDURES**

FIRE AND EXPLOSION HAZARD: Flammable Liquid

FLASH POINT: 66                      ~~UPPER~~ EXPLOSIVE LIMIT: 6.0%

LOWER EXPLOSIVE LIMIT: For Xylene 1.0%

FIRE FIGHTING MEDIA: DRY CHEMICAL, CARBON DIOXIDE, WATER SPRAY OR REGULAR FOAM (1993 Emergency Response Guidebook, DOT P 5800.5). FOR LARGER FIRES, USE WATER SPRAY, FOG OR REGULAR FOAM (1993 Emergency Response Guidebook, DOT P 5800.5).

**FIRE RESPONSE PROCEDURES:** Provide respiratory protection by wearing a self-contained breathing apparatus. Use water spray to reduce vapors and keep fire-exposed containers cool.

**UNUSUAL FIRE AND EXPLOSION HAZARDS:** Vapors form explosive mixtures with air. Vapor may travel a considerable distance to a source of ignition and flash back. Not soluble with water.

#### **6. ACCIDENTAL RELEASE MEASURES**

SMALL SPILL: Remove all ignition sources. Wear protective equipment, appropriate gloves, safety glasses and apron. Ventilate area of spill or leak. Stop leak if you can do it without risk. Take up with sand or other absorbent material and place into containers for later disposal.

LARGE SPILL: Wear an approved respirator. Follow the above procedure and dike far ahead of spill for later disposal. Keep unnecessary people away; isolate hazard area and restrict entry. No smoking, flames or flares in hazard area. If spill is very large call fire department immediately. Use water spray to reduce vapors

#### **7. HANDLING AND STORAGE**

General Handling: FLAMMABLE: Store in a cool, dry place away from heat, sparks and open flames. Vapors may be explosive. Do not get into eyes. Avoid contact with skin and clothing. Avoid breathing vapor. Keep containers tightly closed and in an upright position to prevent leakage. Wash hands thoroughly after handling. Containers of this material may be hazardous when empty. Since emptied containers retain product residues, assume emptied containers to have the same hazard qualities as full containers.

#### **8. EXPOSURE CONTROL (PERSONAL PROTECTION)**

VENTILATION: Provide local exhaust or general dilution ventilation to meet published exposure limits. Ventilation equipment must be explosion-proof.

RESPIRATOR: In the event of a very large spill, an appropriate respirator should be worn for clean up procedures.

## **FOR FIRE FIGHTING AND OTHER IMMEDIATELY DANGEROUS TO LIFE OR HEALTH CONDITIONS:**

Any self-contained breathing apparatus that has a full-face piece and is operated in a pressure-demand or other positive-pressure mode. Any supplied-air respirator that has a full-face piece and is operated in a pressure-demand or other positive-pressure mode in combination with an auxiliary self-contained breathing apparatus operated in pressure-demand or other positive-NEED pressure mode.

**CLOTHING:** Employee should wear protective outer garment when spill or splattering is likely.

**GLOVES:** Employee must wear resistant gloves for prolonged or repeated contact with this substance.

**EYE PROTECTION:** Employee must wear splash-proof or dust-resistant safety goggles to prevent eye contact with this substance.

**EMERGENCY EYE WASH:** Where there is any possibility that an employee's eyes and/or skin may be exposed to this substance, the employer should provide an eye wash fountain and quick drench shower within the immediate work area for emergency use. Protective eye equipment should meet the requirements for protective clothing and equipment in 29 CFR 1910.1048(H).

### **EXPOSURE GUIDELINES**

100 ppm (434 mg/m<sup>3</sup>) OSHA TWA

150 ppm (651 mg/m<sup>3</sup>) OSHA STEL

100 ppm (434 mg/m<sup>3</sup>) ACGIH TWA

150 ppm (651 mg/m<sup>3</sup>) ACGIH STEL

100 ppm (434 mg/m<sup>3</sup>) NIOSH recommended TWA

150 ppm (651 mg/m<sup>3</sup>) NIOSH recommended

100ppm (440 mg/m<sup>3</sup>) DFG MAKs TWA

## **9. PHYSICAL AND CHEMICAL PROPERTIES**

**DESCRIPTION:** Light colored or colorless mobile liquid with an aromatic odor.

**SPECIFIC GRAVITY:** 0.864 Kg/l **BOILING POINT:** 231°F (110°C)

**VAPOR PRESSURE:** Approx. 6.7mm/Hg @21°C **SOLUBILITY IN WATER:** Insoluble

**VAPOR DENSITY:** Heavier than air **EVAPORATION RATE (Butyl Acetate = 1):** Slower than ether **FLASHPOINT:** 66°F (19°C)

## **10. STABILITY AND REACTIVITY INFORMATION**

**REACTIVITY:** Cytoseal™ Mounting Medium is stable in closed containers under normal temperatures and pressures; Toluene may evaporate on exposure to air.

**INCOMPATIBILITIES:** Strong Acids, Oxidizing materials. Xylene may cause an explosive reaction with acetic acid + air; 1,3-dichloro-5, 5-dimethyl-2, 4-imidazolididione; nitric acid + pressure; Can react violently with oxidizing materials.

**DECOMPOSITION:** Thermal decomposition products may release acrid smoke and irritating fumes.

**POLYMERIZATION:** Hazardous polymerization has not been reported to occur under normal temperatures and pressures.

## **11. TOXICOLOGICAL INFORMATION**

**XYLENES:** inh-hmn TCl<sub>o</sub>: 200ppm: NOSE, EYE, PUL

inh-man LCl<sub>o</sub>: 10000ppm/6H

orl-hmn LDLo: 50mg/kg

eye-hum: 200ppm

skn-rbt: 500mg/24H MOD

orl-rat LD50: 4300mg/kg

**Carcinogenicity:** Xylene is an experimental teratogen (IARC 3)

## **12. ECOLOGICAL INFORMATION**

**Acute Effects:** Acute toxic effects may include the death of animals, birds, or fish, and death or low growth rate in plants. Acute effects are seen two to four days after

animals or plants come in contact with a toxic chemical substance. Xylene has high acute toxicity to aquatic life. Xylene causes injury to various agricultural and ornamental crops. Insufficient data are available to evaluate or predict the short-term effects of xylene to birds or land animals.

Chronic Effects: Chronic toxic effects may include shortened lifespan, reproductive problems, lower fertility, and changes in appearance and behavior. Chronic effects can be seen long after first exposure(s) to a toxic chemical. Xylene has high chronic toxicity to aquatic life. Insufficient data are available to evaluate or predict the long-term effects of xylene to plants, birds or land animals

### **13. DISPOSAL GUIDELINES**

Dispose mounting media as toluene, an EPA hazardous waste. Hazardous waste numbers: U239 (toxic), D001 (ignitable). Follow local state and federal regulations.

### **14. TRANSPORT INFORMATION**

Proper shipping name: XYLENES, SOLUTIONS

Hazard class or Division: 3

Identification Numbers: UN1307

Packing Group: II

Label(s) required (if not excepted): None, Exception 1 liter or less (LTD. QTY.)

Special Provisions: Packageing authorizations: Exceptions: 173.150

Non-bulk packageing: 173.202

Quantity Limitations: Passenger aircraft or railcar: 5L

Cargo aircraft only: 60L

### **15. REGULATORY INFORMATION**

SARA TITLE III (Superfund Amendment and Reauthorization Act)

SECTION 302 AND 304: Extremely Hazardous Substance List (40 CFR 355)- Not Listed

SECTION 311: Hazard Categorization (40 CFR 370)- Acute, Chronic, and Fire

SECTION 313: Toxic Chemicals Listing (40 CFR 372.65)- Listed as a toxic chemical

CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act)

SECTION 102(A) Hazardous Substances (40 CFR 302.4)- Listed

Reportable Quantity - 1,000 pounds.

SECTION 101(14) Reportable Quantity: 1,000 lbs.

RCRA (Resource Conservation and Recovery Act.)

40 CFR 261.21 Hazardous Waste Number: D001 or appropriate Spent Solvent Number.

NJ-RTK (New Jersey- State Right To Know)

Environmental Hazardous Substance List: Listed, Substance # 2014

TSCA (Toxic Substance Control Act)

Xylene is listed on the TSCA Inventory.

### **16. OTHER INFORMATION**

Cytoseal™ XYL, as manufactured by Richard-Allan Scientific, is intended for legal use in laboratories and manufacturing environments.