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Precision of age estimates using three different aging methods for walleye (*Sander vitreus*) in Cedar Bluff Reservoir, Kansas

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We evaluated the precision among age estimates obtained from sagittal otolith cross-sections, whole-view sagittal otoliths, and scales from a sample of 95 walleye (*Sander vitreus*). Precision was evaluated by comparing age estimates between two readers, and the consistency of estimates from one reader among hard structures. Agreement between readers was greatest for otolith cross-sections (91.6%) compared to whole-view otoliths (52.6%), and scales (34.7%). Agreement between structures was greatest for whole-view otoliths and scales for reader one 34.7% and reader two 37.9%. Using scales and whole-view otoliths, both readers tended to under-estimate the age of older fish compared to age estimated with the otolith cross-sections. Agreement between age estimates made with scales and otolith cross-sections was 23.2% for reader one and 34.7% for reader two. Agreement between age estimates made with whole-view and otolith cross-sections was 32.6% for reader one and 37.9% for reader two. Due to the greatest precision in age estimates we recommend the use of cross-sectioned sagittal otoliths for estimating the age of walleye. If the sample is restricted to fish age-4 and younger and time efficiency is a priority, whole-view otoliths can be used to obtain precise age estimates.

Introduction

High-quality age estimations are essential to age structure analyses (Maceina et al. 2007). Age data are used to estimate a variety of fish population characteristics including: growth rates, recruitment, year-class strength and mortality, ranking it among the most influential of biological variables (Campana 2001). Fish age is frequently estimated using calcified structures (Devries and Frie 1996) and scales and sagittal otoliths were the most commonly used calcified structures used by state agencies in a 2006 review paper (Maceina et al. 2007). Historically, scales have been the most often used aging structure used by Kansas researchers and managers due to the non-lethal collection process (Maceina et al. 2007).

Walleye (*Sander vitreus*) is one of the most popular sportfish in Kansas (Steffen 2015). Additionally, it is one of the most abundantly stocked and managed fish in the state (Steffen 2015). Throughout the walleye range, a considerable amount of research has been conducted to validate age estimation methods and verify accuracy and precision of age estimates. For example, Erickson (1983) validated annulli formation in scales and otoliths and reported otoliths to be more accurate in estimating age. Koenigs (2015), expanded validation to age 10 and again reported superior accuracy of otoliths compared to other calcified structures. Many studies also report otolith age estimates to have superior precision compared to other structures (Campbell and Babaluk 1979; Marwitz and Hubert 1995; Kocoysky and Carline 2000; Isermann et al. 2003). For example, Koenigs (2013) suggested scales and dorsal spines, although inaccurate, might be acceptable to use for general trends in age and growth in young, fast growing fish populations. Additionally, Dembkowski (2017) suggests dorsal spines be used to estimate ages for shorter walleye <450 mm total length, and otoliths for ≥450 mm.

Accuracy of age estimates was not evaluated in this study. Accuracy of fish age estimates can only be evaluated with known age fish (Devries and Frie 1996). The primary objective of this study was to quantifying precision of age estimates between readers and among scales, whole-view otoliths, and cross-sectioned sagittal otoliths. In Kansas, precision and correlation of age estimations made with sagittal otoliths and scales have not been formerly evaluated. However, precision of age estimates was recently evaluated using cross-sectioned sagittal otoliths, scales, and dorsal fin spines obtained from saugeyes (Walleye *Sander vitreus* X Sauger *S. Canadensis*) (Koch et al. 2018). Koch (2018) recommended, when fish sacrifice is not a concern, to use cross-sectioned sagittal otoliths to estimate saugeye age. Therefore, a secondary objective of this study was to evaluate whether cross-sectioned sagittal otoliths provide walleye age estimates similar to the more time efficient preparation techniques as reported by Isermann (2003), such as whole-view otoliths and scales.

Methods

Cedar Bluff Reservoir is an impoundment of the Smoky-Hill River in northwest Kansas. The watershed is approximately 6,928km^2 (Kansas Department of Health and Environment [http://www. kdheks.gov/tmdl/ss/CedarBluff. Pdf] accessed 16 January 2012) and the landcover types within the watershed are almost exclusively rangeland and row crops (Data Access and Support Center, Kansas land cover, 2012). At conservation pool Cedar Bluff Reservoir has a surface area of 2,678 ha, a mean depth of 7.8 m and is marginally eutrophic. The reservoir ranged from 3.7 to 5.0 m below conservation pool during the study period (U.S. Bureau of Reclamation, (http://www.usbr.gov/gp-bin/arcweb_cbks.pl, accessed 16 January 2012). Temperature and dissolved oxygen concentrations rarely stratify at Cedar Bluff Reservoir (Dave Spalsbury, pers. comm. 2018).

Age estimations among hard structures (otoliths and scales) and otolith preparation procedure (whole and cross-sectioned) were assessed to determine the most precise aging method and at what length or age scales become imprecise. In spring 2010, during the collection of walleye gametes by KDWPT from Cedar Bluff Reservoir, walleye were collected with 25-mm mesh trap-nets, and 76-mm mesh gill nets. Each gill net measured 91-m X 1.8m. Paired samples of scales and sagittal otoliths were removed systematically in 20 mm length-groups in an attempt to collect three individuals of each sex per length group. Scales were removed between the lateral line and anterior portion of the dorsal fin and placed in coin envelopes labeled with fish length, sex, and date of capture (Devries and Frie 1996). In the lab, scales were cleaned with water to remove fish mucus and dirt. Several scales from each fish were pressed with an Ann Arbor roller press onto acetate impression slides (25-mm X 75-mm) to provide a permanent impression of the scale. Scale impressions were randomized, assigned a code number, and separated from length measurements to minimize reader bias (Campana 2001). Impressions were photographed by using an Olympus szx16 microscope and Altra 20 camera. Two readers independently estimated age from the same photograph of one scale per fish.

Sagittal otoliths were removed from each fish and placed in a vial with the same code as the corresponding fish scale impression (Devries and Frie 1996). A mixture of 50% glycerin and water was added to the vial to promote annulus visibility (Devries and Frie 1996). Once the otoliths had sufficiently cleared and annuli were visible, within 4 to12 days, photographs were taken with the same equipment used for the scales.

Once photographed, sagittal otoliths were mounted in Enviro Tex Lite epoxy© and cut in a transverse section with a Buehler Isomet low-speed saw on the posterior end near the core (Secor et al. 1991). The anterior side of the otolith was then mounted to a clear glass slide with Super Glue Liquid© and cut to a thin section of approximately 300 µm. The mounted thin sections were photographed with an Olympus BX51 microscope and an Olympus DP71 camera. Two readers independently estimated the age from the same photograph of one cross-sectioned otolith per fish. For all structures, two readers independently estimated age from the same photograph of one whole-view otolith per fish. Reader one had several years experience reading scales. Reader two had minimal experience reading scales. Reader one and two had minimal experience reading whole-view and cross-sectioned sagittal otoliths.

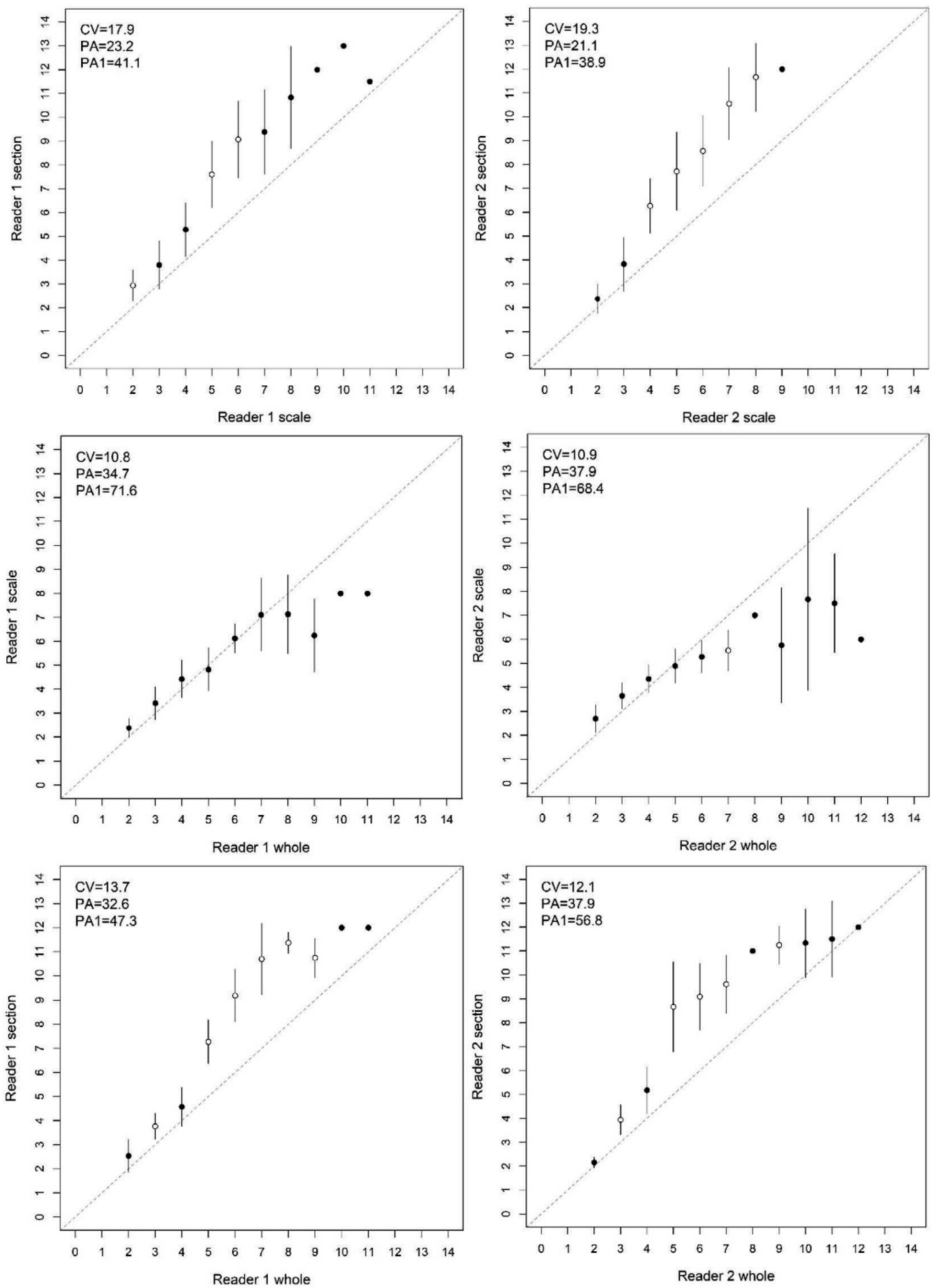
Age bias plots and coefficients of variation were produced to analyze precision in age determinations made with scales, whole-view otoliths, and otolith cross-sections (Campana 1995). Additionally, percent agreements (PA) and percent agreement within one year (PA1) in age estimates among hard structures and readers were calculated. Statistical bias in age estimates was evaluated with age–bias plots by using the ageBias function in R (Ogle 2015; R Core Team 2016). This function uses one-sample t-tests, corrected for multiple comparisons, to compare the mean age estimate values generated from the age bias plots to the 1:1 line of equivalency (complete agreement).

Results

In spring 2010, 95 walleye were collected in 25-mm mesh trap-nets and 76-mm mesh gill nets. Total length varied from 339 mm to 735 mm. Paired samples of scales and sagittal otoliths were removed from walleye in eighteen 20-mm length-groups. The oldest fish captured, estimated by the otolith section method, was age13 and the youngest was age 2.

Age bias plots (Fig. 1), were produced to visually assess precision in age estimations between readers. The direction of deviation from equivalence was the same for age estimates made with all structures for male and female walleye. Therefore, sexes were combined for analyses. Age bias plots revealed greatest precision between readers was derived with age estimates from otolith cross sections (Fig. 1). Age bias plot (Fig. 1) of whole-view otoliths indicate decreasing precision with age among the age estimates by two readers. Significant deviations from equivalence did not occur between readers for otolith cross sections or whole-view otoliths. However, agreement between readers was higher for otolith cross-sections 91.6% compared to 52.6% for whole-view otoliths. Additionally, CV was lower 0.9% for otolith cross-sections compared to 5.3% for whole-view otoliths. Consistency in age estimates between readers was lowest when estimates were based on scales (Fig. 1). Significant deviations from equivalence occurred at age two, and seven for scales. Agreement was lower 34.7% and CV was higher 11.4% for scales compared to whole-view otoliths and cross-sectioned otoliths. Similar to PA, PA1 was highest in otolith cross-sections 97.9%, compared to 84.2% with whole-view otoliths, and 72.6% with scales.

Using scales and whole-view otoliths, both readers tended to under-estimate the age of older fish compared to age estimated with the otolith cross-sections (Fig. 1). Agreement of scale age estimates with otolith cross-section was 23.2% (PA1=41.1%) for reader one and 19.3% (PA1= 38.9%) for reader two. CV was 17.9% for reader one and 19.3% for reader two using scales and cross-sectioned otoliths. Agreement of whole-view otolith age estimates with otolith cross-sections was 32.6% for reader one (PA1=47.3%) and 37.9% (PA1=56.8%) for reader two. CV was 13.7% for reader one and 12.1% for reader two using whole-view otoliths and otolith cross-sections. Significant deviation from equivalence was evident for two or more consecutive age estimates for both readers when scales and whole-view otoliths were compared to otolith cross-sections.


CV=17.9
PA=23.2
PA1=41.1
Reader 1 section
Reader 1 scale
CV=19.3
PA=21.1
PA1=38.9
Reader 2 section
Reader 2 scale
CV=10.8
PA=34.7
PA1=71.6
Reader 1 scale
Reader 1 whole
CV=10.9
PA=37.9
PA1=68.4
Reader 2 scale
Reader 2 whole
CV=13.7
PA=32.6
PA1=47.3
Reader 1 section
Reader 1 whole
CV=12.1
PA=37.9
PA1=56.8
Reader 2 section
Reader 2 whole

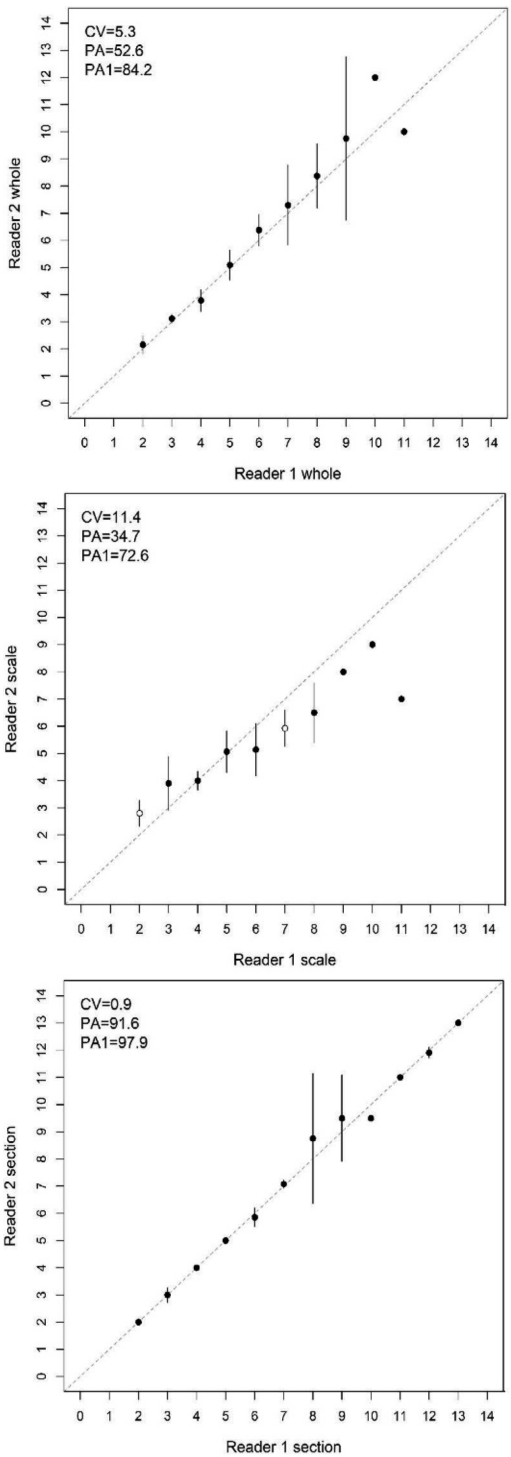


Figure 1. (LEFT) Age–bias plots, with estimates of the mean coefficient of variation (CV; %), between-reader and between-structure percent agreement (PA), and percent agreement within 1 year (PA1), for two readers of cross-sectioned sagittal otoliths, whole-view sagittal otoliths, and scales from walleyes collected from Cedar Bluff Reservoir, Kansas in 2010. Error bars represent 95% confidence intervals around the mean age (years) assigned by one reader relative to fish assigned an age by a second reader, or second structure. Open circles represent significant deviation from the line of equivalency (i.e., the dashed line in each panel).

Agreement of whole-view otoliths age estimates with scales was 34.7% (PA1=71.6%) for reader one and 37.9% (PA1=68.4%) for reader two. CV was 10.8 % for reader one and 10.9% for reader two using whole-view otoliths and scales. One significant deviation from equivalence was evident for reader two when whole-view otoliths were compared to scales. For each reader, age estimates comparing whole-view otoliths and scales were more precise than other structure comparisons (Fig. 1). Age estimates from scales and whole-view otoliths had bias in the same direction relative to age estimates using otolith cross-sections.

Age frequency distributions for each structure as estimated by reader one are presented in (Fig. 2). The age frequency distributions derived from age estimates using scales and whole-view otoliths suggest a more productive or younger population with frequency declining with estimated age compared to the age frequency distribution derived from age estimates using otolith cross-sections. Also, age frequency distributions by using scales and whole-view otoliths suggest little variation in year-class strength. In the age frequency distribution from otolith sections a pattern of strong year-class production was identified.

Discussion

In a review of fish aging procedures, Maceina et al. (2007) suggested precision of age estimates should be assessed in all aging

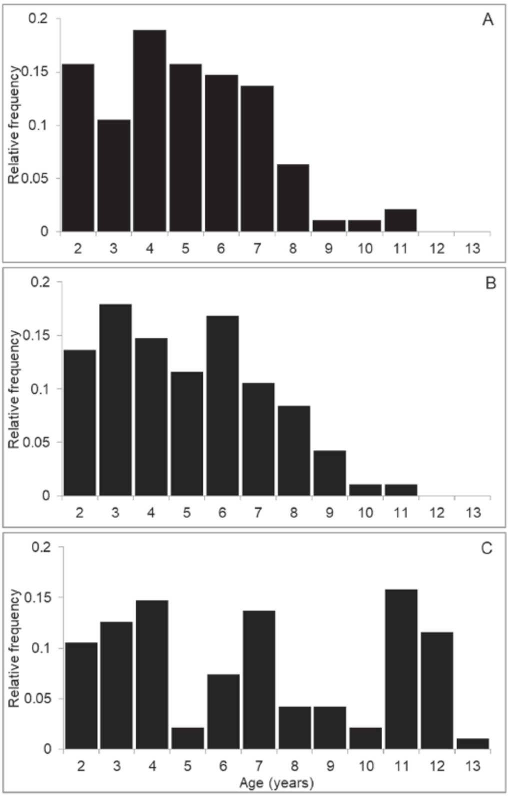


Figure 2. Age (years) frequency distribution of (n=95) walleye sampled during gamete harvest at Cedar Bluff Reservoir in the spring of 2010. Age was estimated by reader one using (A) scales, (B) whole-view otoliths, (C) cross-sectioned otoliths.

studies. Inaccurate age estimates can lead to erroneous population assessment and mismanagement (Beamish and McFarlane 1995). The accuracy of age estimates (validation) was not evaluated in this study. However, otolith sections were determined, both graphically and statistically, to produce the most precise age estimations. Age estimations derived from whole-view otoliths also were precise between readers and a bias was not evident. Although, whole-view otolith age estimates had more error and lower agreement compared to otolith cross sections. Scale based age estimations had low precision between readers as indicated by significant deviations from equivalence.

Precision between readers in age estimates derived from whole-view otoliths decreased with fish age. Age estimates from more experienced readers might be different, but reader experience did not appear to effect precision in age estimates from otolith sections. Results from this study agree with Isermann et al. (2003) and Dembkowski (2017), that whole-view otoliths produce age estimates with high precision between readers for fish age-4 and younger.

Reader experience potentially influenced age estimates using scales; however, results from this study agree with results from other studies (Campbell and Babaluk 1979; Marwitz and Hubert 1995; Kocovsky and Carline 2000; Isermann et al. 2003) that indicate scales produce less precise age estimations relative to sagittal otoliths. Both readers tended to underestimate the age of older fish using scales and whole-view otoliths compared to age estimated with otolith cross-sections. Bias was not evident for comparisons between scales and whole-view otoliths for reader one. One age estimate for reader two was deviated from equivalence for the same comparison.

This bias produces an age frequency distribution derived from scales and whole-view otoliths that suggests a younger more productive population with higher mortality rates compared to age frequency distribution derived from age estimates using otolith cross-sections. Additionally, reader agreement was low (34.7%) using scales and whole-view otoliths (52.6%), indicating strong year-classes might be incorrectly identified, or might be assigned to multiple age-classes and not identified (Fig. 2).

To obtain precise age estimates of walleye at Cedar Bluff Reservoir, we recommend cross-sectioned otoliths be used to estimate age. If the sample is restricted to fish age-4 and younger and time efficiency is a priority, whole-view otoliths can be used to obtain precise age estimates. Sampling using overnight sets of gill nets typically results in walleye mortality. Otolith removal from these fish, will increase the amount of information collected with no additional mortality.

Management Implications

Similar to results of many previous studies at other latitudes, ages estimated from otoliths were determined to be more precise than ages estimated from scales. Using scales, reader agreement was 34.7%. Using whole-view otoliths, variation in age estimation was considerably lower but increased with age. Cross-sectioned otoliths produced age estimations with the highest agreement and lowest variation and therefore, produced the highest quality age estimates. These data produced markedly different estimates of year-class strength relative to data generated for other hard structures (Fig. 2). Accordingly, the periodic sacrifice, of a sample of walleye for otolith removal, might be justified to obtain high quality age structure information, if recruitment is evaluated or if growth parameters are needed for population growth or harvest models.

Literature Cited

Beamish, R.J. and McFarlane, G.A. 1995. A discussion of the importance of aging errors, and an application to walleye Pollock: the world's largest fishery. Pp. 545-565 in Secor, D.H., Dean, J.M. and Campana, S.E. (eds.), *Recent developments in fish otolith research*. University of South Carolina Press, Columbia.

Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59:197-242.

Campana, S.E., Annand, M.C. and Mcmillan, J.I. 1995. Graphical and statistical methods for determining the consistency of age determinations. Transactions of the American fisheries Society 124:131–138.

Campbell, J.S., and Babaluk, J.A. 1979. Age determination of walleye *Stizostedion vitreum vitreum* (Mitchill), based on the examination of eight different structures. Western Region Fisheries and Marine Service, Department of Fisheries and the Environment, Technical Report 849, Winnipeg, Manitoba.

Dembkowski, D.J., Isermann, D.A., Koenigs, R.P. 2017. Walleye age estimation using otoliths and dorsal spines: preparation techniques and sampling guidelines based on sex and total length. Journal of Fish and Wildlife Management 6:474-486.

Devries, D.R., and Frie, R.V. 1996. Determination of age and growth. pp. 483-512 in Murphy, B.R. and Willis, D.W. (eds.), *Fisheries Techniques*, second edition. American Fisheries Society, Bethesda, MD.

Erickson, C.M. 1983. Age determination of Manitoban walleyes using otoliths, dorsal spines, and scales. North American Journal of Fisheries Management 3:176-181.

Isermann, D.A., Meerbeek, J.R., Scholten, G.D and Willis, D.W. 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.

Kansas Department of Health and Environment. 2003. Smoky Hill/Saline River basin total maximum daily load water body: Cedar Bluff Lake water quality impairment: eutrophication. [www.kdheks.gov/tmdl/ss/CedarBluff. Pdf - Accessed 16 Jan. 2012]

Koch, J., Neely, B. Sowards, B. 2018. Precision of three structures for saugeye age estimation. North American Journal of Fisheries Management 38:31-38.

Kocovsky, P.M. and Carline, R.F. 2000. A comparison of methods for estimating ages of unexploited walleyes. North American Journal of Fisheries Management 20:1044-1048.

Koenigs, R. P., Bruch, R. M, Stelzer, R. S. and Kamke, K. K. 2015. Validation of otolith ages for walleye (Sander vitreus) in the Winnebago System. Fisheries Research 167:13-21.

Koenigs, R.P., Bruch, R.M. and Kamke, K.K. 2013. Impacts of Aging Error on Walleye Management in the Winnebago System. North American Journal of Fisheries Management 33:900-908.

Maceina, M.J., Boxrucker, J., Buckmeier, D.L. Gangl, R.S., Lucchesi, D.O., Isermann, D.A., Jackson, J.R. and Martinez, P.J. 2007. Current status and review of freshwater fish aging procedures used by state and provincial fisheries agencies with recommendations for future directions. Fisheries 32:329-340.

Marwitz, T.D. and Hubert, W.A. 1995. Precision of age estimates of Wyoming walleyes from different calcified structures. Prairie Naturalist 27:41-48.

Ogle, D.H. 2015. *Introductory fisheries analyses with R.* CRC Press, Boca Raton, Florida.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Secor, D.H., Dean, J.M. and Laban, E.H. 1991. Manual for otolith removal and preparation for microstructural examination. University of south Carolina, Baruch Institute for Marine Biology and Coastal Research Technical Report 91-1, Columbia.

Steffen, S.F. 2015 *2013 Kansas Licensed Angler Survey Report.* Project Report FW-2015-7A. Emporia, KS: Kansas Department of Wildlife, Parks and Tourism, Fisheries and Wildlife Division, Research and Survey Section.