Canadian Journal of Zoology

Note

Challenges in fish aging: the role of otolith preparation technique and experience level in aging lake whitefish (*Coregonus clupeaformis*)

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Abstract

Lake whitefish (*Coregonus clupeaformis* (Mitchill, 1818)) is an important commercial and recreational species in the Great Lakes. Precise age estimates are important for management, and two widely used techniques for otolith preparation are thin-section and crack-and-burn, which have not been compared for lake whitefish. Sagittal otoliths were collected from 92 lake whitefish in Green Bay and Lake Michigan and aged using thin-section and crack-and-burn techniques. Otoliths were aged independently by three individuals (two novices and one expert) to assess repeatability in estimated ages. Our investigation highlights the inherent difficulty of aging lake whitefish, where thin-section produced significantly older estimated ages (6–30 years) compared to crack-and-burn (5–26 years). Percent agreement of estimated ages between preparation techniques was low for all readers within ±0 years but increased when tolerance buffers were applied. Mean coefficient of variation values from both experience levels (>10.8%) exceeded the acceptable range reported in the literature (5%–7%); however, species longevity and nature of the structure must be considered when establishing target values. Variation in estimated ages is attributed to the experience level and interpretation of structural features. Species-specific training and establishing an objective framework to identify annuli will improve precision metrics.

Key words: lake whitefish, *Coregonus clupeaformis*, otolith, aging

Introduction

Age determination is a fundamental component of fish management (Hoxmeier et al. 2001), serving as a backbone of population models used to establish harvest policies (Mohr and Nalepa 2005; DeBruyne et al. 2008). Calcified structures, including scales, fin rays, and otoliths, are used to age fish by revealing the succession of various density bands that represent growth rings called annuli (Campana 2001; Muir et al. 2008*a*, 2008*b*). Furthermore, different preparation techniques exist for visualizing annuli and estimating ages depending on the structure (Edwards et al. 2011). For example, otolith preparation techniques include but are not limited to "thin-section", where structures are embedded in epoxy and cut into thin cross sections, and "crack-and-burn", where the otolith is simply cracked in half and scorched to enhance the contrast in annuli. Aging method and preparation technique are recognized to cause variation in age estimation within species (Edwards et al. 2011) and require consideration for population-wide management actions. The implications of inaccurately aging or interpreting aging structures can result in misrepresenting stock demographics and misunderstanding recruitment, longevity, and numbers surviving (Yule et al. 2008; Edwards et al. 2011; Lackmann et al. 2019).

The lake whitefish (*Coregonus clupeaformis* (Mitchill, 1818)) metapopulation in Lake Michigan is managed by international, federal, state, and tribal agencies. The metapopulation has fluctuated in abundance over the last 100 years, with many stocks reaching historic lows in the early 1900s (Ebener et al. 2008). Lake whitefish populations have also experienced changes in age, growth, and body condition throughout the Great Lakes (Nalepa et al. 2005), motivating management actions based on data interpretation gathered from a variety of aging methodologies and structures over time (Mraz 1964; Patriarche 1977; DeBruyne et al. 2008; Ebener et al. 2008; Madenjian 2019). Lake whitefish in the Green Bay region are aged using otoliths prepared by thin-section and crack-and-burn techniques (S. Hansen, personal communication, 2021). Comparisons of lake whitefish ages among otoliths, scales, and fin rays are available (Mills et al. 2004; Muir et al. 2008*a*, 2008*b*; Herbst and Marsden 2011), but a comparison of the different preparation techniques for otoliths has never been conducted for lake whitefish nor have they been evaluated in the context of experience level. In the absence of true age validation methods, precision analyses are used to measure the repeatability of assigning age to a structure and for identifying potential biases, in each structure and within

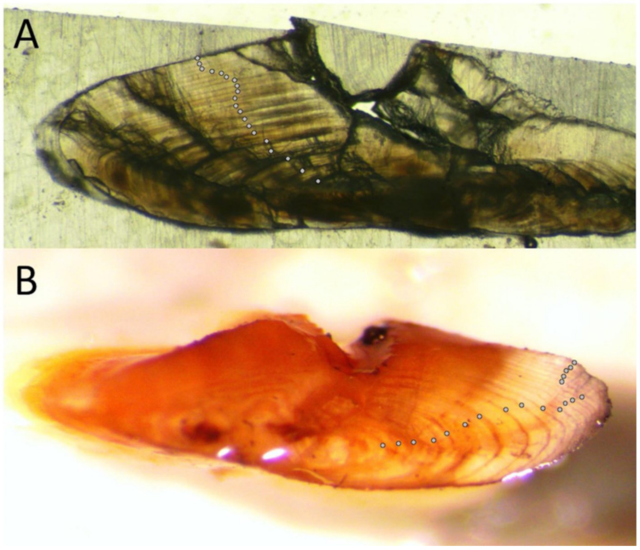


Fig. 1. Lake whitefish (*Coregonus clupeaformis*) sagittal otolith prepared for aging by (A) thin-section and (B) crack-and-burn techniques. Dots on otoliths indicate estimated annuli produced by the N1 reader.

each reader, which may lead to inaccurate age estimates (Campana 2001). These types of assessments are relevant due to the need to accurately monitor fish stocks. The objective of this study was to evaluate the precision of age estimates from lake whitefish otoliths using thin-section and crack-and-burn while understanding how estimates from these techniques are influenced by reader experience. We hypothesize agreement and precision metrics will increase with the thin-section preparation technique as well as the experience level.

Materials and methods

Lake whitefish were collected in the fall of 2017 through the winter of 2018, where paired sagittal otoliths were extracted from 92 lake whitefish ($n = 184$ otoliths) collected from northern Lake Michigan (70%) and northern (16%) and southern Green Bay (14%). Each individual lake whitefish provided one otolith to be prepared using the thin-section technique and the other prepared by the crack-and-burn technique. For thin-section (Fig. 1A), otoliths were placed in rubber molding cells with a layer of Loctite Quick Set Epoxy® on the bottom (filled ~50% full). Otoliths were set within individual cells, filled with epoxy, allowed to harden, and removed. Epoxy blocks were cut transversely with a PICO 155 precision saw using a diamond blade, where the sections were 0.62 μm thick. Cuts were made from the outer edge of the embedded otolith moving toward the origin (Edwards et al. 2011). Thin sections were sanded to remove scratches and imperfections from the cutting process, to improve the clarity of the otolith's origin and annuli. For the crack-and-burn technique (Fig. 1B), otoliths were cut along the transverse edge, convex side up, through the origin with a razor blade. The cut surface was placed over a flame burner until the surface turned dark brown (~20 s), set in putty burned-face up, and coated in immersion oil to enhance annuli appearance (Edwards et al. 2011).

Otolith samples were viewed with a Lecia DM 2500 microscope with ocular mounted camera and photographed using Amscope FMA050 software to clearly interpret annuli from the core to the outer edge. Images were evaluated by three individuals of varying experience aging fish in a randomized study design, where the biological attributes of the fish were unknown. Individuals aged each otolith once and did not know which fish they were aging or the corresponding otolith for the other technique. Two experience levels were examined, novice and expert — the first novice (N1) had no prior experience of aging, the second novice (N2) had 3 years of broad taxonomic experience but none aging lake whitefish, and a third reader was considered an expert (E) and had been aging for over 30 years with emphasis on lake whitefish and bloater chubs (*Coregonus hoyi* (Milner, 1874)).

Statistical analysis

Agreement and precision are common metrics for assessing the repeatability of age data and evaluating estimates of age across experience levels, techniques, and aging structures (Campana 2001). Percent agreement (PA; Beamish and Fournier 1981; Campana 2001) for each reader was used to assess differences in estimated ages produced by each preparation technique. PA was calculated by dividing the number of identical age estimates by the total number of individuals aged (Campana 2001). A PA approaching 100% indicates full agreement between techniques for all samples. The mean coefficient of variation (CV; Chang 1982; Campana 2001) is expressed as a ratio of the standard deviation over the mean, denoted as

$$\mathrm{CV}_j = 100\% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{\left(X_{ij} - X_j\right)^2}{R-1}}}{X_j}$$

where X_{ij} is the ith age estimation of the jth fish, X_j is the mean estimated age calculated for the jth fish, and R is the number of times each fish is aged. A smaller CV value indicates higher precision for paired age estimates for all fish. CV and PA for each reader were calculated using the FSA package in R (Ogle 2013). A repeated measures ANOVA analysis was performed in SPSS, where the mean otolith age was compared against the preparation technique while accounting for experience level using the mixed-effect procedure.

Ethics approval

The collection of lake whitefish for this study was conducted under the Wisconsin Department of Natural Resources laws and regulations.

Results

Estimated ages of lake whitefish ranged from 6 to 30 years for thin-section and from 5 to 26 years for crack-and-burn,

Can. J. Zool. 101: 1–6 (2023) | dx.doi.org/10.1139/cjz-2022-0055

Table 1. Estimated ages of lake whitefish (*Coregonus clupeaformis*) produced using two otolith preparation techniques, thin-section (Thin; $n = 92$) and crack-and-burn (Burn; $n = 92$), by three readers of various experience levels, where N1 and N2 are novice and E is expert.

Technique	Experience level	Lake whitefish estimated age (years)			
		Minimum	Maximum	Mean	SD
Thin	N1	6	24	12.9	4.5
Thin	N2	6	30	15.5	5.2
Thin	E	6	23	13.6	4.0
Burn	N1	5	22	11.9	3.9
Burn	N2	5	26	14.0	4.9
Burn	E	6	20	11.9	3.3

Fig. 2. Estimated ages of lake whitefish (*Coregonus clupeaformis*) using two otolith preparation techniques, where blue is thin-section (T) and red is crack-and-burn (B), by three readers of various experience levels; E is expert and N1 and N2 are novice. The box plot shows the interquartile range of the data, while the horizontal black line along each box plot represents the estimated mean age of that technique for each reader.

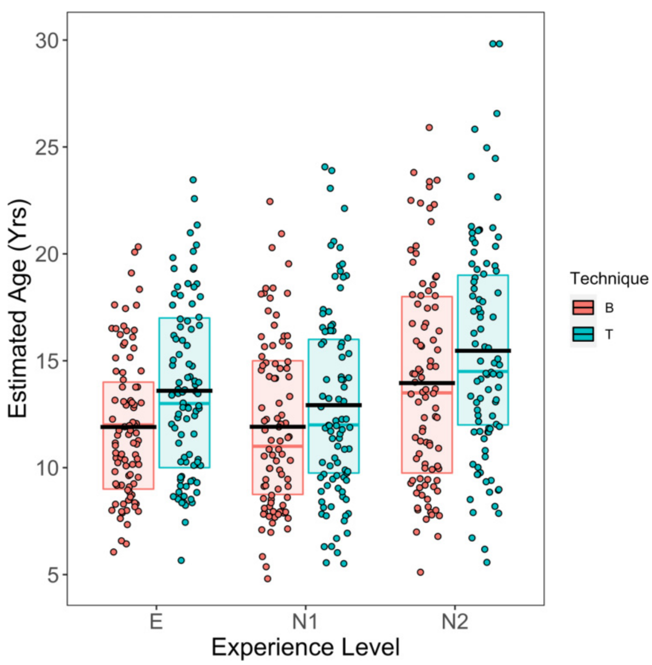
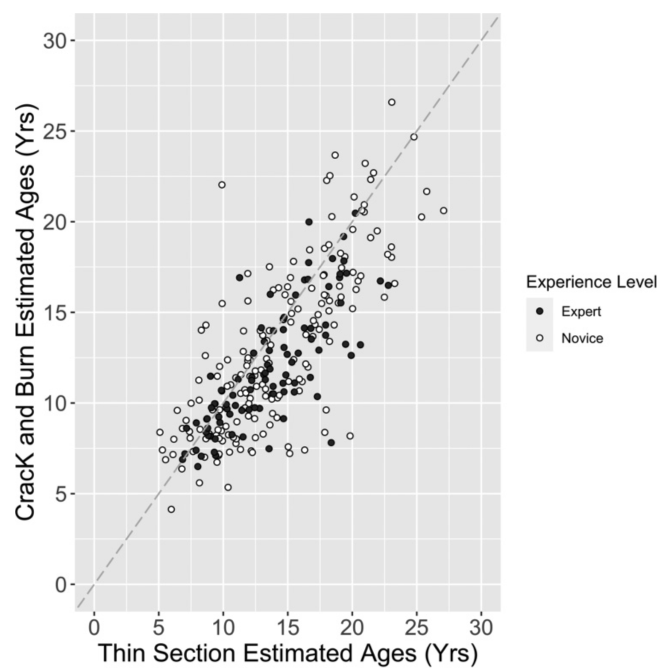


Fig. 3. Lake whitefish (*Coregonus clupeaformis*) age estimations of paired sagittal otoliths by preparation technique and experience level. The gray dashed line shows a 1:1 reference line. The black points represent the expert experience level, while the white points represent the combination of N1 and N2 for the novice experience level.



with the expert reader having the narrowest estimated age ranges (Table 1, Fig. 2). The ANOVA model showed a statistical difference in mean estimated age according to otolith preparation technique ($F_{[1,135]} = 60.97$, $p < 0.001$) and experience level ($F_{[2,135]} = 27.79$, $p < 0.001$). Thin-sectioned otoliths produced higher age estimates ($\bar{x} = 12.3 \pm 0.14$ SE) than crack-and-burn ($\bar{x} = 10.8 \pm 0.14$ SE) for all readers, but estimated ages between paired otoliths were, at times, highly variable (Table 1, Figs. 2 and 3). PA was low (<23%) within ±0 years but increased as tolerance buffer was applied (Table 2). The expert reader exhibited the highest PA values across the buffers, and PA values for all experience levels aligned once a buffer of ±5 years was applied. The expert was also the most precise (CV = 10.8%), with novice N2 being the least precise (CV = 14.5%, Table 2, Figs. 2 and 3).

Discussion

The proper identification of annuli is the fundamental principle for age estimation using calcified structures. The PA and CV values of our work underscore the inherent difficulty in aging lake whitefish (see also Muir et al. 2008*a*, 2008*b*). PA values within 0 years were poor; however, PA im-

Table 2. Precision measurements for lake whitefish (*Coregonus clupeaformis*) age estimation between thin-section and crack-and-burn otolith preparation techniques.

Experience level	CV (%)	PA of estimated ages (%)					
		±0 years	±1 year	±2 years	±3 years	±4 years	±5 years
N1	13.6	16.3	42.4	64.1	78.2	84.7	90.2
N2	14.5	13.0	32.6	53.3	68.4	83.7	90.2
E	10.8	22.8	50.0	75.0	81.5	86.9	90.2

Note: Mean coefficient of variation (CV) and percent agreement (PA) with a 1 to 5 year buffer are listed for each reader across two experience levels, where N1 and N2 are novice and E is expert.

proved with experience level and when the stringency on precision was reduced (e.g., ±5 years). This is evidence of subjectivity between readers that originates with the preparation and the interpretation of features showcased on the calcified structures, which can vary among readers and laboratories (Campana 2001). It should be noted that PA values reported in this study are not to be used as a proxy for accuracy of true age, but rather assessing ease of use for a particular structure and technique, as well as the measure of repeatability of an individual's age estimation and comparison of experience level (Campana 2001). Arguably, PA values have been scrutinized as a valid measure of precision due to variation among species and within ages. Allowing for a tolerance buffer in PA values may be appropriate and non-deleterious in longer-lived species, but this requires confirmation of life history traits and species longevity (Beamish and Fournier 1981; Ralston and Ianelli 1997; Yule et al. 2008; Edwards et al. 2011).

A CV value of 5%–7% is commonly acceptable for species classified as moderate longevity and aging complexity (Campana 2001). Our results produced CV values above this range; therefore, we recognize that this would suggest poor precision and repeatability of estimated ages following the metric provided by Campana (2001). In a similar study looking at only thin-sectioned lake whitefish otoliths, experienced readers were able to produce CV values of 6.4%, but increased to 13.6% when aged by two less experienced readers (Casselman et al. 2019). Similarly, our CV values increased when aging was conducted by novice readers (13.58% and 14.46%). However, our expert reader produced a CV value of 10.83% that was still higher than the published acceptable range of 5%–7% (Campana 2001), again demonstrating the difficulty in consistently aging lake whitefish. Campana (2001) explains that no "a priori" level of precision can be designated as a target level because precision is highly influenced by the species and not just the reader. Other studies focusing on aging longer-lived species often document CV values >10% and suggest that this may not be problematic in some cases and may be caused by the physical nature of the aging structure (Campana 2001 and references therein).

The long lifespan of lake whitefish (30+ years) can produce crowded growth rings with increased age that may become obscured and provide challenges when precisely aging older specimens (Campana 2001; Mills et al. 2004; Herbst and Marsden 2011). Thin-section was generally more time consuming than crack-and-burn, which tilts in favor of using the crack-and-burn technique when time and support staff are reduced, but thin-sectioning archival was easier and produced subjectively clearer images making annuli easier to identify. However, while performing the crack-and-burn technique, otoliths could quickly be "over-burnt" resulting in a loss of outer-edge annuli. For instance, our estimated mean, minimum, and maximum ages across experience levels were lowest for crack-and-burn, which can be a direct result of the otolith preparation. When aging long-lived fish, this may have large implications by under-aging a fish species. This conclusion mirrors results from a similar study examining burbot (*Lota lota* (Linnaeus, 1758)), where thin-section was a preferred technique, produced clearer annuli, and resulted in better precision values over the crack-and-burn technique (Edwards et al. 2011). As lake whitefish get older and more annuli are present, it becomes challenging to visualize all annuli equally as they become crowded at the outer edge (Campana 2001) and the core may become blurry resulting in misidentifying the first annuli (see Aldanondo et al. 2015). The potential for false annuli can influence age estimations (see Morales-Nin et al. 1998), especially for inexperienced readers.

Our results suggest that at a minimum the type of otolith preparation technique should be communicated between agencies that are co-managing the lake whitefish metapopulation in the Great Lakes. Uncertainty in age determination will likely remain a challenge for the species (Muir et al. 2008*a*, 2008*b*), and both otolith preparation techniques will result in variable ages. However, our examination points to thin-section as the preferred technique in favor of increased precision across reader experience and otolith clarity, especially at the outer edges of the structure. The variation we observed across experience levels further highlights the importance of species-specific training and establishing an objective framework to appropriately identify annuli. Increased experience level paired with training will improve estimate of age and standardization between agencies. Agencies should be wary of delegating fish aging to technicians with limited experience for whitefish specifically; however, training demonstrations with a focus on the otolith preparation technique can improve precision and produce more reliable data (Dembkowski et al. 2019). Future steps for this work would include measuring the accuracy with age validation to improve our understanding of the difficulties to consistently identify annuli.

Acknowledgements

The authors thank Scott Hansen from the Wisconsin Department of Natural Resources for providing otoliths and tools to make this project possible.

Article information

History dates

Received: 29 April 2022
Accepted: 16 August 2022
Accepted manuscript online: 26 September 2022
Version of record online: 15 November 2022

Data availability

The primary author has the research data and the data that support the research of this study can be available upon reasonable request.

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Project administration: MM
Resources: PF
Software: PF
Supervision: ST, PF
Validation: TK
Visualization: MM
Writing – original draft: MM, ST, AR, PF
Writing – review & editing: ST, PF

Competing interests

All authors that are affiliated with the research have no conflicts of interest financially or otherwise to disclose.

Funding information

There are no funders to report for this submission.

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