Age estimation comparison between whole and thin-sectioned otoliths and pelvic fin-ray sections of long-lived lake trout, *Salvelinus namaycush*, from Great Bear Lake, Northwest Territories, Canada

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Received: 21 October 2020 / Revised: 26 March 2021 / Accepted: 8 June 2021 / Published online: 16 July 2021
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Abstract
Studies to determine precision and bias of both methods and age-readers are important to evaluate reliability of age data used for developing fisheries management objectives. We assessed within-reader, between-reader, and between-method precision (coefficient of variation, CV%) and bias of age estimations for long-lived lake trout, *Salvelinus namaycush*, from Great Bear Lake using three readers with different levels of experience. The assessment used independent age estimates (*n* = 3 per reader) from whole and transverse-sectioned otoliths (range = 1–67 years), and pelvic fin-ray sections (range = 3–26 years). We also examined between-method differences in assigned confidence scores. Within readers, age estimates from sectioned otoliths were more precise (2.6–3.0%) than whole (3.6–4.5%) otoliths. Between whole and sectioned otoliths, precision of age estimates was 5.4% and bias was low up to age 20. Age was typically under-estimated from whole otoliths compared to sections for fish ≥ 34 years. Increased reader confidence was correlated with greater precision and younger age estimates, particularly for whole otoliths, but less so for fin rays. Age was estimated with higher confidence from otolith sections than other methods. The least experienced reader estimated age with the lowest precision, and between-reader bias was evident among older ages. Age was consistently under-estimated and less precise from pelvic fins compared to sectioned otoliths, and are therefore an unsuitable non-lethal alternative. Sectioned otoliths revealed longevity was greater (67 years) than historically documented using whole otoliths (53 years) for these fish. Our findings contribute to those relying on otoliths or pelvic fin rays to estimate ages of long-lived lake trout populations, which are a key component of freshwater fauna in polar North America.

Keywords Bias · Precision · Otoliths · Pelvic fin rays · Confidence

Introduction
Age-based research into life-history, and population demographics and dynamics of teleost fishes typically relies on calcified structures (e.g., rays, spines, otoliths) to estimate age (Chilton and Beamish 1982; Kerns and Lombardi-Carlson 2017). Assessing and reducing age estimation error (process error and observation error; Campana 2001) is essential to determine the accuracy and precision of age information. Process error is a result of inconsistent patterns in the deposition of growth zones in a particular calcified structure that does not reflect the period of interest (e.g., annual) and produces inaccurate age estimates. Observation error is a result of uncertainty for a reader with the interpretation of growth zones associated with a particular age estimation method. This can lead to different age estimates from repeated independent counts (Campana 2001; McBride 2015).

Even if a structure and preparation method has been validated (using known-age fish or confirming annuli periodicity: Buckmeier et al. 2017), age comparison studies, either within-reader, between-reader, or between-method, based on multiple estimates from individual structures, are necessary to evaluate repeatability (i.e., precision) of age estimates.
These age comparison studies also allow identification of bias, and selection of the most optimal structure and preparation method to minimize age estimation error (Campana et al. 1995). Estimating ages of long-lived species can be particularly challenging and complex because of their slower growth after sexual maturity that results in smaller spacing between annuli (Andrews et al. 2005; Campana et al. 2008; Hamel et al. 2015). Another factor influencing the quality of age data is reader experience, where less-experienced readers tend to produce less precise estimates, particularly with species or structures that are difficult to read (Campana and Moksness 1991; Rude et al. 2013; Oele et al. 2015). To address issues related to experience, readers can subjectively codify their personal level of certainty using pre-determined criteria for each age estimate with a reader confidence (i.e., readability) score to identify an estimate that may be biased or less accurate (Fitzgerald et al. 1997; Spiegel et al. 2010). Using inaccurate or imprecise age data could result in biased population parameter estimates, and the resulting consequences on management decisions can have detrimental effects on the sustainability of a fishery and/or population (Lai and Gunderson 1987; Reeves 2003; Tyszko and Pritt 2017). This is particularly true for slow-growing, late-maturing, and long-lived species that would face a longer recovery period after significant declines in population status (Juan-Jordá et al. 2015).

The lake trout (Salvelinus namaycush) is a large, slow-growing, long-lived and mainly lacustrine salmonid adapted to cold and oxygen-rich habitats, and whose endemic distribution ranges between ~41 and 74°N (Scott and Crossman 1973). The species is important for commercial, recreational, and Indigenous subsistence fisheries, and many populations have been or are currently being examined to assess population status and characterize life history (e.g., Hansen et al. 2008; Nieland et al. 2008; McDermid et al. 2010; Chavarie et al. 2013). Lake trout are particularly vulnerable to overharvest as a result of their life history characteristics (Healey 1978; Schuter et al. 1998; Post et al. 2002; Kaufman et al. 2009). Given the species’ importance to various fisheries, accurate and precise age estimates are necessary for science advice to support fisheries management and conservation objectives.

In earlier years (~1940s–1970s), scales were commonly used to estimate age of lake trout, but were eventually shown to substantially under-estimate age of older fish compared to otoliths (Miller and Kennedy 1948; Dubois and Langeux 1968; Sharp and Bernard 1988). Examples of maximum age obtained reading lake trout scales was 23 years (“with reasonable certainty”) up to 37 years (with low certainty) in Great Bear Lake, Northwest Territories, Canada (Miller and Kennedy 1948), 12 years in Lake Mistassini, Québec, Canada (Dubois and Langeux 1968), and 19–20 years in Summit Lake, Alaska, U.S.A. ( Sharp and Bernard 1988). Power (1978) examined whole otoliths (lateral surface) from long-lived Arctic lake trout where for larger otoliths a lateral grind was performed to expose the inner rings and a hotplate was occasionally used to bake otoliths to clarify the outer rings. In some instances, these extra preparation steps helped to produce age estimates > 50 years. Historical between-method comparison studies mainly focused on evaluating scales and otoliths that were either read whole (Sharp and Bernard 1988; Burnham-Curtis and Bronte 1996; Schram and Fabrizio 1998; and references therein) or thin-sectioned along the transverse plane (Casselman and Gunn 1992). Annual periodicity of growth increments for lake trout has been validated for otolith sections to an age of at least 50 years (Campana et al. 2008) and maxilla sections between ages 3 and 27 (Wellenkamp et al. 2015).

For lake trout, the precision and bias of age estimation structures and preparation methods have rarely been evaluated based on one or more reads of a single sample using multiple age-readers (see Sharp and Bernard 1988; Murphy et al. 2018). However, an age comparison study using a wide range of multiple paired reads from individual fish that includes age estimates > 30 years using whole and thin-sectioned otoliths, and pelvic fin-ray sections has not been conducted. Campana et al. (2008) compared age estimates of sectioned lake trout otoliths, which included very old fish (> 50 years), to assess bias and precision between dissecting (reflected light at 16–40x magnification) and compound (transmitted light at magnifications up to 160x) microscopes based on a single read.

This study used samples from Great Bear Lake, a location where lake trout can attain ages > 50 years (Falk et al. 1974; Chavarie et al. 2016). Multiple age-readers produced three independent age estimates from whole otoliths, and transverse thin-sectioned otoliths and pelvic fins. The objectives of our study were to: (1) determine if whole otoliths provide a reliable alternative to thin-sectioned otoliths, particularly at younger ages, and (2) determine if pelvic fins provide a suitable non-lethal age estimation alternative to thin-sectioned otoliths. We sought to achieve our objectives by evaluating precision and bias, and assessing confidence: (1) between otolith preparation methods and between age-readers, and (2) between thin-sectioned otoliths and pelvic fins and between age-readers. This study is relevant for the management and conservation of Great Bear Lake’s exceptional intraspecific diversity of lake trout, which supports a subsistence and world-class trophy fishery (mainly catch and release) (Howland and Tallman 2005; Chavarie et al. 2013). Furthermore, the findings should be pertinent for all laboratories, stock assessment programs, research facilities, and fisheries management agencies that have relied on otoliths or pelvic fin rays (e.g., Mills et al. 2002) to estimate ages of long-lived lake trout populations.
Materials and methods

Study area

Great Bear Lake, located on the Canadian Arctic Circle between 65° and 67°N latitude, is a five-armed, cold, and ultra-oligotrophic freshwater lake (Fig. 1). The lake's volume (> 2200 km³), surface area (> 31,000 km²) and depth (> 400 m), make it the largest lake entirely within Canada and the fourth largest lake in North America (Johnson 1975a; Rao et al. 2012). The lake is typically ice covered between November and June (Rao et al. 2012). The predominant fish species encountered in gill net fisheries in Great Bear Lake are lake trout, lake whitefish (Coregonus clupeaformis), and lake cisco (Coregonus artedi) (Johnson 1975b, Howland unpublished data). Other large-bodied species include walleye (Sander vitreus), burbot (Lota lota), northern pike (Esox lucius), longnose sucker (Catostomus catostomus), Arctic grayling (Thymallus arcticus), and round whitefish (Prosopium cylindraceum) typically comprise < 5% of gill net catches (Johnson 1975b; Howland unpublished data). Located in the Sahtu Settlement Area, Great Bear Lake is considered to be a relatively pristine ecosystem with only one community situated on its shores (Délı̨nę; population 576) (Evans 2000; Howland and Tallman 2005; GNWT 2020).

Sample collection

Lake trout were collected as part of ongoing fisheries assessment studies on Great Bear Lake during 2000–2016 using overnight gillnet sets (see Howland et al. 2013; Chavarie et al. 2018 for details of net configuration and procedures). Sagittal otoliths and pelvic fin rays were collected from captured fish sampled for comprehensive biological...
information. Otoliths were removed, cleaned, and stored dry in labeled envelopes. The first three rays of the left pelvic fin were cut at the base using bone cutters, excised, and left to dry in labeled envelopes.

**Preparation of structures and age estimation**

Otoliths were read whole by placing them in a small water-filled glass petri dish, lateral side up, over a black background and interpreted under a Leica MZ12.5 or a Nikon SMZ1000 dissecting microscope at magnifications of 10–80x with reflected light. The lateral surface of lake trout otolith is convex, and consequently the centre area of larger ones is thicker and opaque. Therefore, a slight grind was performed by hand on this surface for medium-to-large otoliths only (i.e., > 6 mm long), using a 1000-grit wet stone filled glass petri dish, lateral side up, over a black back. The lateral surface of lake trout otolith is convex, and consequently the centre area of larger ones is thicker and opaque. Therefore, a slight grind was performed by hand on this surface for medium-to-large otoliths only (i.e., > 6 mm long), using a 1000-grit wet stone to expose the nucleus and inner few annuli. Also, to help clarify the remaining annuli, large otoliths were pre-soaked in water for 4 to 24 h.

Otolith and pelvic fin-ray sections were prepared as described by Chavarie et al. (2016) and Zhu et al. (2015), respectively. Otoliths were embedded with the sulcus side up in ColdCure Epoxy Resin (Industrial Formulators of Canada Ltd.). After hardening, the otolith was viewed with the sulcus side down under a dissecting microscope equipped with a cross-hair micrometer eyepiece, and the nucleus was demarcated using an ultra-fine tip marker. With the cross-hair centered on the nucleus, a section line was then chosen through the nucleus and the best area of the dorsal lobe, close to the transverse plane. Dots were placed on the epoxy above and below the otolith along that line. Using the dots on the epoxy as a guide, a Buehler Isomet low-speed saw (Lake Bluff, Illinois, U.S.A.) with two diamond wafering blades separated by a 0.5 mm spacer was used to obtain the section. These sections were examined in a manner similar to whole otoliths using the same microscopes (15–100x) and lighting. Pelvic fin rays were trimmed, embedded in Cold-Cure Epoxy Resin, and sectioned from the proximal end at a thickness of 0.35 mm with the low-speed saw. Three fin sections were typically taken and affixed to labeled microscope slides. Fin sections were also viewed using the same equipment as the whole otolith reads, at 15–80x magnification (although they were not immersed in water). Annuli were identified based on criteria described by Chilton and Beamish (1982).

**Study design**

To address the stated objectives, analyses were linked to one of two study designs. For objective 1, otoliths of lake trout (fork length range = 69–1136 mm) collected from the multiple arms of Great Bear Lake between 2008 and 2016 were used to compare bias and precision of the two otolith preparation methods for a wide range of age estimates (1 to ~67 years) using two experienced age-readers (Reader 1 and Reader 2). Otoliths from later sampling years were selected as these were directly accessible to both readers in the age estimation laboratory and also provided multiple samples of lake trout > 50 years of age. The samples used for this part of this study had been previously processed, which means that one otolith of each pair had already been sectioned. Consequently, only one otolith was available to the readers for whole reads (also referred as ‘surface reads’). Both readers read whole (n = 199) and sectioned otoliths (n = 252) three times each, blind to results from previous reads or estimates by the other reader. Sufficient time was allowed to pass between successive reads (several days to weeks) to ensure readers were not familiar with individual samples from one read to the next. Sample size differed between readers because either one age-reader was unable to provide an age estimate or the sample was lost between the readers (whole otolith: Reader 1 n = 189, Reader 2 n = 199; sectioned otolith: Reader 1 n = 250, Reader 2 n = 252). A confidence score was assigned to each age estimate by both age-readers: 1 (very good; no interpretation issues and would expect high repeatability every time), 2 (fairly good; few interpretation issues and would expect high repeatability most of the time), 3 (fair; some interpretation issues with moderate level of repeatability), 4 (fairly poor; interpretation is fairly difficult and low amount of repeatability), and 5 (poor; interpretation is difficult and very low amount of repeatability). Therefore, a high score implied reduced reader confidence in the accuracy of an age estimate.

For objective 2, sectioned otoliths and pelvic fins from lake trout (fork length range = 207–1075 mm) collected in 2000 were used to compare bias and precision between structures using two age-readers (Reader 1 and Reader 3). The range of the age estimates based on otolith reads was 6 to 41 years. Both readers read sectioned otoliths and pelvic fins three times each, again allowing sufficient time between successive reads to prevent reader familiarization of samples, blind to results from previous reads or estimates by the other reader. Sample size was not equal between age-readers because some samples were lost or damaged between the readers (sectioned otolith: Reader 1 n = 172, Reader 3 n = 175; pelvic fin: Reader 1 n = 194, Reader 3 n = 193). Only the confidence scores provided by Reader 1 for both structures were used as Reader 3 did not use a similar criteria in assessing confidence.

Reader 1 had the most experience estimating the age of lake trout among the three readers (> 30 years of experience; 22 years with lake trout otoliths). Reader 1 trained Reader 2 (6 years of experience including ~ 200 annual lake trout otolith samples) and both worked in the same age estimation laboratory. Reader 3 worked in an independent laboratory.
Statistical analysis

We used age bias plots (Campana et al. 1995) to illustrate the consistency of age estimates between readers for each preparation method (otolith) and structure, and between preparation methods (otolith) and structures for each reader. The bias plots indicate the 95% confidence interval around the mean age estimate assigned by one reader for all fish assigned an age by a second reader where confidence intervals that are either equal to zero or very narrow indicates consistency in repeated age estimates (Campana 1995). Additionally, we used the Evans-Hoenig test to evaluate bias based on the diagonal symmetry of an age-agreement table where a significant P-value indicated that differences among reads (i.e., between readers and methods for this study) were due to bias and not random error (Evans and Hoenig 1998; McBride 2015). However, the Evans-Hoenig symmetry test cannot reliably detect bias when precision is low; therefore, the test was not performed in instances when the coefficient of variation (see below) was > 10 (McBride 2015). The Evans-Hoenig test was designed to detect signed differences (i.e., +1 vs. −1 year) for paired-age data across multiple age classes and performs well compared to other tests of symmetry (McBride 2015). Precision (i.e., repeatability of reads and expressed as %) was measured by calculating the coefficient of variation (CV) (Chang 1982; Campana 2001):

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CV_j = 100 \times \sqrt{\frac{\sum_{i=1}^{R} (X_{ij} - \bar{X}_j)^2}{R - 1}} \]

where \( R \) is the number of times each sample was read, \( X_{ij} \) is the average age estimate of the jth fish, and \( \bar{X}_j \) is the ith age estimate for the jth fish. Higher CV values reflect decreased precision. Data used to create age bias plots and to calculate Evans-Hoenig tests of symmetry and CV were generated using the FSA (Fisheries Stock Assessment) package in R (Ogle 2016). To evaluate within-reader repeatability among ages, the median age of each triplicate reading was plotted against mean CV for that age and a Spearman rho (\( \rho \)) nonparametric correlation was used to test whether CV was positively correlated with age.

We also tested for differences between methods (otolith preparation, and sectioned otoliths vs. pelvic fins) and between readers using non-parametric Wilcoxon signed-rank tests (V) for paired data. To assess differences in reader confidence scores, we used a chi-square test to evaluate frequencies of median confidence scores between otolith preparation methods and between sectioned otoliths and pelvic fins separately for each reader (except Reader 3). We did not test for differences in confidence between readers given the subjective nature of assigning confidence. For Reader 1 and Reader 2, Spearman rho nonparametric correlation was used to determine if reader confidence scores and CVs were correlated, and if reader confidence scores and age estimates were correlated. All statistics were performed in RStudio (version 1.1.442) with R (version 3.5.0; R Core Team 2018) and considered significant if \( \rho < 0.05 \).

Results

Whole versus sectioned otoliths

Age estimates were more precise and more confident from sectioned otoliths than whole otoliths for both readers. Age estimates from whole otoliths were more precise for Reader 2 (CV = 3.6%) than Reader 1 (CV = 4.5%) (Table 1). Confidence scores for estimated ages (mean ± SD) from whole otoliths were similar between readers (Reader 1 = 2.4 ± 0.93; Reader 2 = 2.0 ± 0.94) and were strongly and significantly positively correlated with both CV (Reader 1 \( \rho = 0.73 \); Reader 2 \( \rho = 0.67 \)) and age estimates (Reader 1 \( \rho = 0.72 \); Reader 2 \( \rho = 0.78 \)) (Table 2). Whole otolith age and CV were significantly positively correlated for both readers (Reader 1 \( \rho = 0.73 \); Reader 2 \( \rho = 0.67 \)) with relatively high CV values (>5%) predominantly observed in age estimates ≥20 years for Reader 1 and ≥23 years for Reader 2 (Table 2; Fig. 2). Age estimates from sectioned otoliths were more precise for Reader 1 (CV = 2.6%) than Reader 2 (CV = 3.0%) (Table 1). Confidence scores for estimated ages (mean ± SD) from sectioned otoliths were similar between readers (Reader 1 = 2.0 ± 0.59; Reader 2 = 2.0 ± 0.67) and significantly positively correlated to both CV (Reader 1 \( \rho = 0.53 \); Reader 2 \( \rho = 0.50 \)) and age estimates (Reader 1 \( \rho = 0.43 \); Reader 2 \( \rho = 0.61 \)) (Table 2). Sectioned otolith age and CV were significantly positively correlated for both readers (Reader 1 \( \rho = 0.68 \); Reader 2 \( \rho = 0.51 \)) with relatively high CV values becoming more prevalent among ages >35 years for both readers (Table 2; Fig. 2).

Between-reader comparisons for both whole and sectioned otoliths revealed bias among older ages where agreement was highest from sectioned otoliths. Ages estimated from whole and sectioned otoliths by both readers generally agreed between ages 1 and 37 (Fig. 3). For lake trout >37 years from whole and sectioned otoliths methods, age estimates by Reader 1 were consistently older than Reader 2, with average differences of 10–17 years observed from whole otoliths (Fig. 3). Variability in repeated age estimates from sectioned otoliths increased after age 38, although bias was low up to age 58 (Fig. 3). At ages ≥60 years using sectioned otoliths, Reader 1 tended
to estimate older ages than Reader 2 (Fig. 3). The Evans-Hoenig test between readers was statistically significant for sectioned otoliths only, indicating high symmetry between readers for whole otoliths, while precision was higher for sectioned otoliths (CV = 3.3%) compared to whole otoliths (CV = 4.7%) (Table 1). The Wilcoxon matched-pairs tests demonstrated significant differences in age estimates between readers for both methods (Table 3).

Ages estimated by both readers from whole and sectioned otoliths were relatively free of bias and highly precise from ages 1 to ~20 (Fig. 4). Variation between readers increased slightly between age ~21 and 38 (section reads). Whole otolith reads beyond ~33 years were consistently younger than sectioned otolith reads in most cases. Average differences between methods were approximately 8 years, although differences of over 20 years were sometimes observed for individuals with sectioned otolith age estimates ≥50 years (Fig. 4). The Evans-Hoenig test of symmetry between methods was significant for both readers indicating systematic bias in reading sectioned otoliths multiple times; however, no such bias was observed for whole otoliths. The table below presents the results of the symmetry tests between readers and methods/structure combinations, as well as the correlation between age-confidence scores and the coefficient of variation (CV):
Precision was similar for both readers (CV ~ 5.4%) (Table 1). The Wilcoxon matched-pairs tests demonstrated significant differences in age estimates between methods for both readers (Table 3). Reader confidence differed significantly between preparation methods for Readers 1 (Chi-square test, $\chi^2 = 113.5, P < 0.0001$) and 2 (Chi-square test, $\chi^2 = 62.9, P < 0.0001$) with greater frequencies of high confidence scores (i.e., 1–2) from age estimates based on otolith sections (Table 4).

**Sectioned otoliths versus sectioned pelvic fins**

Age estimates were more precise and more confident from sectioned otoliths than pelvic fins for Reader 1, while reader experience had an important effect on precision of ages for both structures. Precision of age estimates from otolith sections was higher for Reader 1 (CV = 2.4%) than Reader 3 (CV = 7.9%) (Table 1). The confidence score (mean ± SD) for sectioned otoliths for Reader 1 was 2.3 ± 0.79, and a weak statistically significant positive correlation was observed between the reader’s confidence score and CV ($\rho = 0.43$) as well as age ($\rho = 0.30$) (Table 2). Precision of age estimates from pelvic fins was higher for Reader 1 (CV = 4.3%) than Reader 3 (CV = 7.9%) (Table 1). The confidence score (mean ± SD) for pelvic fins for Reader 1 was 2.5 ± 0.96, and a weak significant positive correlation was observed between the reader’s confidence score and CV ($\rho = 0.32$) and age ($\rho = 0.38$) (Table 2). While a weak yet statistically significant positive correlation was also detected between sectioned otolith age and CV for Reader

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**Fig. 2** Mean and standard deviation of coefficient of variation (CV, %) plotted against median age estimated from three independent reads of the same otolith for two age-readers examining whole and transversely thin-sectioned otoliths of lake trout from Great Bear Lake, Northwest Territories, Canada.
no statistically significant relationship was observed for Reader 3 ($\rho = -0.20$) (Table 2; Fig. 5).

Age estimate comparisons between readers from sectioned otoliths were relatively unbiased between ages 8 and 24, whereas those by Reader 3 tended to be slightly older for ages 6–7 and considerably younger for ages ≥ 25 years (Fig. 6). Ages estimated by Reader 3 from pelvic fins were consistently younger than those estimated by Reader 1 among all ages, and bias increased after age 10 (Fig. 6). Between-reader precision was higher for sectioned otoliths (CV = 7.5%) than pelvic fins (CV = 12.0%). Significant differences were observed between readers in the test of symmetry for sectioned otoliths only and for matched-pairs of age estimates for both structures (Tables 1 and 3).

Age was consistently under-estimated from pelvic fins by both readers when compared to otolith sections (Fig. 7). The bias was parallel to the 1:1 line up to age ~ 20 (otolith) when the relationship changed and the bias plots demonstrated the inability of pelvic fin sections to reasonably estimate age for older lake trout (Fig. 7). CV values were the poorest among all comparisons in this study with Reader 1 having greater precision (CV = 14.7%) compared to Reader 3 (CV = 24%). The matched-pairs tests indicated statistically significant differences between methods for both readers (Table 3). Reader confidence differed significantly between structures for Reader 1 (Chi-square test, $X^2_8 = 119.2$, $P < 0.0001$) with greater frequencies of low confidence scores (i.e., 3–5) observed for pelvic fin sections (Table 4). A weak statistically significant positive and negative correlation was detected between pelvic fin age and CV for Reader 1 ($\rho = 0.38$) and Reader 3 ($\rho = -0.27$), respectively (Table 2; Fig. 5). CV tended to be relatively high among pelvic fin ages > 20 years for Reader 1 and considerably higher among ages < 11 years for Reader 3 (Fig. 5).

### Discussion

We demonstrated that the otolith preparation method used affected bias, precision, and reader confidence, particularly for older fish (≥ ~ 34 years; whole method), when estimating ages of lake trout from Great Bear Lake. We also found that pelvic fin-ray sections were a poor non-lethal alternative to otoliths throughout the age range examined. Sectioning was the superior otolith preparation method that produced the greatest precision within and between readers with relatively low CV across a wide range of ages. We note, however, that readers only had one otolith from each pair available for whole otolith reads, where the previously sectioned otolith may have been the preferred one for these reads at times.
Ideally, both otoliths from each fish would be available for whole reads before sectioning. The transverse otolith section was the structure and preparation method used to validate age of long-lived lake trout in Arctic lakes (Campana et al. 2008) and our results confirm the method’s suitability to provide accurate, precise, and confident age estimates for lake trout with proper training (e.g., mentorship and use of reference collection) and adequate experience.

Nonetheless, our study demonstrated that the whole otolith method could provide accurate, precise, and confident age estimates for lake trout over a relatively young age range. The whole method provides robust and repeatable age estimates that are almost identical to the thin-sectioned method between ages 1 and 20 for experienced readers (Fig. 4). Therefore, a proposed age estimation protocol for lake trout populations inhabiting ultra-oligotrophic or slightly more productive lakes would be to use experienced readers to read otoliths using the whole method up to age 20 and section any beyond that age. If this protocol was applied to the otolith data from this study, the combined-method CV from both experienced readers would be similar to that obtained from sections alone (3.1% and 2.1% compared to 2.6% and 3.0%, respectively). Furthermore, we suggest that any otoliths ≤ 20 years (whole) with low confidence should also be sectioned, given the increased likelihood of generating age estimates with higher confidence. Using a protocol that allows for not having to section every otolith would save preparation time and money for consumables (e.g., epoxy; Muir et al. 2008; Williams et al. 2015). Based on all the lake trout age data accumulated from the Great Bear Lake fisheries-independent sampling conducted by Fisheries and Oceans Canada between 2008 and 2016 (years examined for otolith preparation comparison; n = 2114 samples), about half of the age estimates could have been from whole otoliths. Although other studies are required to determine if age 20 is an appropriate threshold for when to section otoliths from other lake trout populations, we posit that that the threshold is likely to be very similar for other populations inhabiting ultra-oligotrophic or slightly more productive Arctic and subarctic systems.

Fig. 4 Between-method age bias plots of whole and transversely thin-sectioned otolith preparation methods for two different age-readers using lake trout from Great Bear Lake, Northwest Territories, Canada. Each error bar represents 95% confidence intervals. Dashed diagonal line is the 1:1 line. Coefficient of variation (CV, %) in the top left of each panel

Table 4 Percent frequency of confidence scores assigned by two age-readers (Reader 1 and 2) to age estimates obtained from whole and transversely thin-sectioned otoliths, and thin-sectioned otoliths and pelvic fin rays (Reader 1 only) using lake trout from Great Bear Lake, Northwest Territories, Canada

| Score | Confidence | Reader 1 (%) | Reader 2 (%) | Reader 1 (%) |
|-------|------------|--------------|--------------|--------------|
|       | Whole      | Section      | Whole        | Section      | Pelvic*       |
| 1     | Very good  | 19.0         | 16.8         | 16.1         | 19.0          | 20.3          | 16.1          |
| 2     | Fairly good| 34.4         | 72.0         | 44.7         | 63.5          | 32.0          | 34.4          |
| 3     | Fair       | 39.7         | 10.8         | 23.1         | 17.5          | 47.1          | 34.4          |
| 4     | Fairly poor| 6.9          | 0.4          | 16.1         | 0             | 0.6           | 14.1          |
| 5     | Poor       | 0            | 0            | 0            | 0             | 0             | 1.0           |
| Total sample size | 198 | 250 | 199 | 252 | 170 | 192 |
*Total n = does not equal to Table 1 as confidence score was not assigned for two samples
Our conclusion that age of older lake trout cannot be estimated accurately from whole otoliths agrees with Campana et al. (2008), who discussed how earlier studies likely underestimated lake trout ages using this method. Similar conclusions regarding whole versus sectioned otolith methods have been made for other long-lived species (Beamish 1979; Winkler et al. 2019). Experienced readers in our study (Readers 1 and 2) clearly interpreted annuli differently toward the margin of whole otoliths for lake trout ≥ 35 years (Fig. 3). Although neither reader had extensive experience with whole otoliths in this age range, they were both challenged by outer annuli that cannot be attributed to experience level alone. Confidence and precision were both strongly correlated with lake trout age that decreased as age estimates increased. This observation underscores the uncertainty associated with ages estimated from whole otoliths for older ages. Sectioning otoliths produced over twice as many age estimates ≥ 50 years compared to the whole method (5.5% vs 2.2% of all age estimates) and a greater maximum age (67 vs 59 years).

The estimated longevity of lake trout of 67 years from Great Bear Lake in this study is greater than previously thought. Historically, longevity was estimated to be 53 years based on studies conducted in the 1970s and 1980s that only used laterally ground whole otoliths viewed under a dissecting microscope (Falk et al. 1974; Yaremchuk 1986), which likely underestimated population longevity. Our study had some of the oldest age estimates for lake trout ever recorded (see McDermid et al. 2010 (suppl.); Hansen et al. 2021) and had a similar maximum age to the lake trout from Great Bear Lake, Northwest Territories, Canada.

Fig. 5 Mean and standard deviation of coefficient of variation (CV, %) plotted against median age estimated from three independent reads of the same otolith for two age-readers examining transversely thin-sectioned otoliths and pelvic fin rays of lake trout from Great Bear Lake, Northwest Territories, Canada.
Bear Lake examined by Chavarie et al. (2016) (60 years) and Chavarie et al. (2018) and Hansen et al. (2021) (68 years) who also used sectioned otoliths.

The readability of annuli on otolith sections of older lake trout can be affected by many factors, including microscope type and magnification, section polishing (Campana et al. 2008), choice of section plane, cutting precision, and the positioning and lighting of the section (R. Wastle, personal observation). Clarity of annuli of older age classes examined along the ventral sulcus edge (described as ‘dorsal’ in the text) of sectioned otoliths was improved when polished and viewed under a compound microscope (at 160x) with transmitted light (Campana et al. 2008). We focused on choosing the best section plane through the dorsal lobe of the otoliths, close to the true transverse plane, to create sections that were readable out to the dorsal tip, which is the longest transect from nucleus to edge (Online Resource 1). A longer transect means greater separation between outer annuli of older samples. Consequently, readers were able to read these samples beyond age 60 with reasonable confidence using dissecting microscopes (up to 100x) and reflected light.

Our findings indicate that pelvic fin-ray sections, prepared and viewed as in our study, should not be used to estimate the ages of lake trout from Great Bear Lake. Age estimates from pelvic fin-ray sections yielded age estimates of the lowest

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Fig. 6 Between-reader age bias plots of transversely thin-sectioned otoliths and pelvic fin rays for two different age-readers using lake trout from Great Bear Lake, Northwest Territories, Canada. Each error bar represents 95% confidence intervals. Dashed diagonal line is the 1:1 line. Coefficient of variation (CV, %) in the top left of each panel.

Fig. 7 Between-method age bias plots of transversely thin-sectioned otoliths and pelvic fin rays for two different age-readers using lake trout from Great Bear Lake, Northwest Territories, Canada. Each error bar represents 95% confidence intervals. Dashed diagonal line is the 1:1 line. Coefficient of variation (CV, %) in the top left of each panel.
average reader confidence (Reader 1) and yet were relatively reproducible within readers (higher CV than sectioned otoliths, except for Reader 3; as discussed below). Interestingly, reproducibility of age estimates improved among older ages of pelvic fins examined by Reader 3, which suggests that the reader had difficulties delineating annuli in younger (<11 years) lake trout. The weak correlation between Reader 1 confidence and both CV and age estimates of pelvic fin sections indicated that higher confidence demonstrated by an experienced age-reader was not strongly associated with better precision, and that generally lower confidence values were observed among all age classes. Comparison of otolith and pelvic fin-ray sections age estimates produced considerably higher CV values than the CV = 5% precision target threshold suggested by Campana (2001). Pelvic fin-ray sections consistently underestimated age relative to otolith sections up to ~20 years and could not be used to reliably discern older age classes. Similarly, pelvic and pectoral fin rays produced less precise and younger age estimates than whole or sectioned otoliths for Dolly Varden (Salvelinus malma) (Gallagher et al. 2016). Reader confidence, precision and maximum age estimate (<12 years) were also lower for pectoral fin rays than for broken and burnt otoliths of lake whitefish from Great Slave Lake (Zhu et al. 2015). Pectoral and anal fin rays also tended to underestimate ages compared to whole otoliths in Arctic Grayling (Sikstrom 1983), while anal and pectoral fin rays produced quite variable age estimates that were typically younger than broken and burnt otoliths of Arctic char (Salvelinus alpinus) and Dolly Varden (Barber and McFarlane 1987). Alternatively, pectoral fin rays and broken and burnt otoliths produced very similar age estimates in incomna (Stenodus leucichthys) (Howland et al. 2004). In temperate North American locations, dorsal fin rays were less precise than broken and burnt otoliths of lake whitefish from Lake Champlain (Herbst and Marsden 2011) and pectoral fin rays were less precise than whole otoliths of brook trout (Salvelinus fontinalis; Stolarksi and Hartman 2008). Ages estimated from sectioned otoliths did not differ from those of pelvic fin rays of lake whitefish from Lake Michigan (age range = 5–13 years) although pelvic fins tended to under-estimate ages ≥10 years (Muir et al. 2008). Similarly, ages estimated from pelvic fin rays were similar to those of sectioned otoliths of lake whitefish from the Experimental Lakes Area (age range = 3–19 years), where fin rays have long been used to estimate ages of various species including lake trout (Mills et al. 2002; Mills and Chalanchuk 2004). In Europe, high agreement and low bias was observed between pectoral fin rays and thin-sectioned otoliths (age range = 2–6 years) of Peipsi whitefish (Coregonus maraenoides) from Bulgaria (Uzunova et al. 2020). Although further age validation research is required, it is possible that fin rays may provide a suitable alternative to otoliths among a wider range of relatively younger ages for some species from faster-growing and shorter-lived populations in more temperate climates.

Reader experience affected precision and bias of lake trout age estimates. Reader 3 had the least experience and the lowest precision for sectioned otoliths and pelvic fins compared to highly experienced Reader 1. One might expect better precision with sectioned otoliths compared to fin rays given the within-reader results of the two highly experienced readers, and the multiple examples of between-structure comparisons cited above; however, this was not the case for the less-experienced Reader 3. Challenges in the reproducibility of sectioned otolith reads were evident for Reader 3, particularly among older age classes. Clearly, readers 1 and 3 were interpreting otolith annuli differently for ages 6 to 7 and >20 where mean differences of >5 years were observed in older age classes. Similarly, between-reader bias of pelvic fin section reads revealed the readers were interpreting annuli differently among all sampled age classes, with a mean difference of 1 year between ages 3 and ~10, which gradually increased thereafter. These results emphasize the need for proper training and sharing of reference material among laboratories (Campana 2001; Buckmeier et al. 2017), particularly for long-lived species where extremely small increments between outer annuli of older fish can challenge accurate and precise age estimation.

Our study revealed that a highly experienced reader can produce precise age estimates over a wide range of otolith ages for lake trout from Great Bear Lake, which has the greatest longevity recorded for this species. While sectioning was the best overall otolith preparation method, the most efficient protocol without sacrificing quality would be to use a combination of whole and section methods, where whole otoliths are used to estimate age of fish up to age 20 and sections are used for older fish. Multiple lake trout ecotypes that inhabit Great Bear Lake exhibit differences in adult growth, age- and size-at-maturity, fecundity, and longevity (Chavarie et al. 2016) that could influence the spacing and readability of otolith annuli. Therefore, evaluating precision, bias, and age-reader confidence among ecotypes would be beneficial to advance conservation objectives for these lake trout. The development of ecotype-specific age estimation protocols could benefit other populations as phenotypic and life history diversity of lake trout is prevalent in large and deep lakes throughout its range (Chavarie et al. 2021). Assessing the impacts of fisheries in Great Bear Lake requires reliable age estimates that would also ideally minimize sampling mortalities of the very large (> ~900 mm) and presumably old lake trout (we note that very large lake trout are not necessarily the oldest; Chavarie et al. 2016) from both the trophy fishery that primarily targets large fish, and fishery-independent surveys (using gill nets) that target a wide range of sizes. While such an approach to sampling would encourage the sustainability of the trophy fishery and
support the request from Indigenous co-management partners to limit mortality of large fish that are still alive when gill nets are retrieved, our results suggest pelvic fins are not a suitable non-lethal alternative. Until such an alternative is discovered, otoliths are currently the best-known structure to produce reliable age estimates for population assessment and life history studies of long-lived lake trout inhabiting Great Bear Lake. The findings of our study on lake trout from Great Bear Lake are pertinent for all laboratories that have relied on otoliths or pelvic fin rays to estimate ages of slow-growing and long-lived lake trout populations, which are a key component of the freshwater fauna across much of polar North America.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00300-021-02901-9.

Acknowledgements We appreciate the financial support provided by the Fisheries and Oceans Canada (DFO) Sahtu Implementation Fund, Sahtu Renewable Resources Board, Aboriginal Affairs and Northern Development Canada/Government of Northwest Territories Cumulative Impact Monitoring Program. We acknowledge the Dě́̀ǹh Renewable Resources Council for their continued support over the years. We also sincerely appreciate the involvement of various technicians and camp support from Dě́̀ǹh (Darryl Betsidea, Isodore Betsidea, Maurice Betsidea, Bruce Kenny, Darren Kenny, Greg Kenny, George Menacho, Bobby Modeste, Brent Taniton, Allison Tatti, Gerald Tutcho, Archie Vital, Barbara Yukon, Charity Yukon, Chris Yukon, Cyre Yukon, Mary Rose Yukon), staff from DFO (Kristin Adair, Dave Boguski, Heath Clark, Les Harris, Kristin Hynes, Michel LeClaire, Brendan Malley, Simon Wiley) and volunteers (Jean-Guy Chavarie) that collected biological data. Logistical support was provided by the Polar Continental Shelf Program. The age-readers used for this study were Rick Wastle, Lenore Vandenbyllaardt, and Jason Friesen. The map in figure 1 was produced by Adriana Rivas Ruis (DFO). We appreciate comments and editorial suggestions from Michael Hansen, Heidi Swanson, and an anonymous reviewer that improved the manuscript.

Author contributions KLH, CPG and RJW conceived and designed the study. KLH, LC, and CPG contributed to the data acquisition in the field. CPG and JRM analyzed the data. CPG, JRM, RJW and KLH interpreted results. KLH led proposals to secure funding for the study. CPG, JRM, and RJW wrote the manuscript, and all authors reviewed and edited it.

Declarations Conflict of interest None of the authors have a financial or non-financial conflict of interest regarding this research. The fish that were sampled were handled based on guidelines approved by the Canadian Council of Animal Care.

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