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Evaluating otolith preparation methods for anadromous Arctic Char: establishing an age estimation protocol and comparing historical with contemporary data

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Reliable age estimation of anadromous Arctic Char, *Salvelinus alpinus*, is important for assessment and management of populations. We examined precision (coefficient of variation-CV% and percent agreement) and bias of within-reader age estimates between whole and thin-section, and whole and grind otolith preparation methods based on single reads using samples from two populations of Arctic Char (Tatik Lake and Hornaday River, Northwest Territories) collected between 2010 and 2012. Additionally, standardized criteria were used to assess between-method differences in age reader confidence to determine whether perceived improvement in confidence resulted in different age estimates. Whole vs thin-section CV was 4.6% while exact percent agreement was 51–56%. Bias between whole and thin-section methods was evident among older age classes where the whole method tended to under-estimate compared to thin-sectioning. A combination of whole and thin-section otolith preparation methods is recommended to minimize sample preparation time while maintaining confidence in age estimates. The age estimation protocol for Tatik Lake and Hornaday River would use the whole method for ≤ age-12 and ≤ age-9, respectively and use the thin-section method thereafter. Any otolith, regardless of age, would be thin-sectioned if the confidence associated with the whole read was low as thin-sectioning produced a greater frequency of high-confidence age estimates compared to whole. While age estimates from whole otoliths read with low confidence were not significantly different when thin-sectioned and read with high confidence, thin-sectioning improved reader confidence for 50.5–58.4% of the whole reads with low confidence. Similar findings were observed between whole and grind otolith preparation methods. Finally, we evaluated bias between the whole method (used historically by one age reader) and the combination of whole and thin-section method (used recently by another age reader), to assess the comparability of historical and contemporary age data. Between-reader CV was 6.0% for Tatik Lake and 6.7% for Hornaday River, while exact percent agreement was 45% and 54%, respectively. The contemporary method tended to produce older age estimates resulting in differences in age frequency distributions, maximum age, and annual mortality estimates. However, there was no significant difference in von Bertalanffy growth parameters for either location. Researchers should consider the implication of these differences when working with both historic and contemporary data sets for these locations and any others with a similar difference in the methods used to assign age estimates.

INTRODUCTION

Age estimation is essential to characterize fish life history and population dynamics. Many assessments of fish populations use age data to evaluate the demographic structure and/or temporal trends in age-related parameters (e.g., growth and mortality). These assessments should strive to use age estimation structures and preparation methods that provide accurate (i.e., true age value) and precise (i.e., reproducibility of repeated measurements) estimates (Kalish et al. 1995). Ideally these methods would be validated (Campana 2001). Unreliable age data can produce inaccurate recommendations to fisheries managers, which can negatively impact the viability of fisheries and fish populations (Lai and Gunderson 1987, Reeves 2003).

In fishes, annual or daily growth can be interpreted using calcified structures, including fin rays, spines, scales, and otoliths. The otolith is one of the principal age estimation structures used in fisheries research (Secor et al. 1995, Campana and Thorrold 2001), with many examples where annual periodicity for this structure has been validated (Geffen 1992, Spurgeon et al. 2015). The otolith preparation method, which includes whole (lateral surface), break-and-burn, thin-section, and grind (lateral or transverse axes), can greatly affect the interpretation of annuli or 'readability' (Stransky et al. 2005, Snow et al. 2018, Winkler et al. 2019). Furthermore, age reader training and experience can have an important influence on the accuracy and precision of age estimates (Buckmeier et al. 2017).

This study examines age estimation of anadromous Arctic Char, *Salvelinus alpinus*, a cold-adapted salmonid species inhabiting mainly Arctic and sub-Arctic locations that exhibits complex phenotypic and life history diversity (Johnson 1980, Jonsson and Jonsson 2001, Klemetsen 2013). Early studies mainly evaluated scales and whole otoliths to estimate age (Grainger 1953, Nordeng 1961, Frost 1978, Johnson 1980, Barbour and Einarsson 1987). Scales, fin rays, and vertebrae were found to be of limited or no use, due to difficulty in interpreting annuli (Nordeng 1961, Barber and MacFarlane 1987). In subsequent studies, comparisons between otolith preparation methods have demonstrated the whole method tended to underestimate among older age groups compared to break-and-burn or thin-section methods (Barber and MacFarlane 1987, Kristofferson and Klemetsen 1991, Babaluk et al. 2007), although the accuracy of this conclusion can only be confirmed by a validation study.

Until 2009, assessments of western Canadian populations of Arctic Char used ages that were estimated using the whole otolith method, with annuli interpreted using criteria of Nordeng (1961) (see Kristofferson and Carder 1980, Carder 1995). Since 2009, a combination of whole and thin-section methods has been used along with a confidence index for each age estimate produced. Development of the protocol included comparisons between the whole otolith method (i.e., shortest preparation time) and transverse cross-section method (i.e., longer preparation time but higher quality and presumed accuracy for older fish) to determine the extent to which the whole and section methods could be used interchangeably to minimize preparation time without compromising quality (high confidence in the assigned age). Given this change in otolith preparation method, assessing the integrity of age-based time-series analysis for stocks that use both historic (whole reads only; prior to 2010) and contemporary age data (new protocol) is necessary (see DFO 2016a,b, Zhu et al. 2017).

OBJECTIVES

1. Compare the whole otolith preparation method with a transverse-section otolith method (thin-section or grind) for Arctic Char from Tatik Lake and Hornaday River; specifically:

- evaluate within-reader bias based on otolith preparation for A) whole vs. thin-section and B) whole vs. grind; and,
- examine whether perceived improvement in age reader confidence resulted in significantly different age estimates.

2. Evaluate bias between the whole (Reader 1) and whole-section (i.e., final age estimate based on combination of whole and thin-section methods) (Reader 2) methods to inform the comparability between historical and contemporary age data.

METHODS

DATA COLLECTION, OTOLITH PREPARATION, AND AGE ESTIMATION

Sagittal otoliths were collected during winter 2010, 2011, and 2012 (n = 579) from Arctic Char harvested from Tatik Lake, NT (N 71.2°, W 116.57°), near the hamlet of Ulukhaktok, using methods described in Harwood et al. (2013) (Figure 1, Table 1). Otoliths were also collected from Arctic Char (n = 708) captured during 2010, 2011, and 2012 summer coastal fisheries in eastern Darnley Bay, NT (N 69.4°, W 123.6°), at the mouth of Lasard Creek and Hornaday River near the hamlet of Paulatuk (Gallagher et al. 2017) (Figure 1, Table 1). Samples collected at Hornaday River (2010 and 2012) and Lasard Creek (2011 and 2012) were combined and will henceforth be referred to as "Hornaday River", given the majority of the fish from these locations originate from the Hornaday River (Harris et al. 2016).

The assessment of historical and contemporary age estimation methods was based on data generated by two age readers. Reader 1 read the samples using the whole otolith method that involved placing otoliths distal surface up in a petri dish over a black background, immersed in oil of wintergreen (clearing agent) and viewed using a dissecting microscope (Nikon SMZ-10A Stereozoom) and reflected light. Otoliths were read once with annuli interpreted according to Nordeng (1961). Reader 1 was able to estimate ages from 568 otoliths from Tatik Lake and 707 from the Hornaday River (Table 1). Note, Reader 1 had provided age estimates for Tatik Lake and Hornaday River on annual basis since the early 1990s.

Reader 2 first read the samples using the whole method (note, 3% of the total combined samples were not examined whole and prepared using the thin-section or grind method only; see paragraph below). Otoliths were placed distal surface up in a petri dish of distilled water over a black background and viewed using a dissecting microscope (Leica MZ 12.5 with 10 - 40 X magnification) and reflected light. Otoliths were read once with annuli interpreted according to Nordeng (1961). Reader 2's confidence in the readability of each sample was categorized using a confidence index (Poor, Fairly Poor, Fair, Fairly Good, and Good; Table 2). Reader 2 was able to estimate whole ages from 552 samples from Tatik Lake and 696 from Hornaday River (Table 1).

A sub-sample of otoliths examined whole was re-read by Reader 2 using either the grind (Zhu et al. 2015; although otoliths were not baked after grinding) or the thin-section (Gallagher et al. 2016) preparation method. In 2010, the objective was to compare the whole and grind methods, unless there was only one usable otolith available, in which case the otolith was thin-sectioned to preserve it for future use (e.g., microchemistry analysis) (Table 1). The otolith would sometimes break during grinding and the reading angle was inconsistent (R. Wastle, personal observation), therefore, in 2011 and 2012 the thin-section method was chosen as the preferred approach. All otoliths that were ground or thin-sectioned were read once with the same microscope and light source used for whole otoliths without knowledge of the whole age estimates. These age estimates were also assigned a confidence index rating (Table 2). For

Tatik Lake samples, the grind and thin-section methods were applied to 162 and 269 otoliths, respectively (Table 1). For Hornaday River samples, the grind and thin-section methods were applied to 123 and 259 otoliths, respectively (Table 1).

Reader 2 assigned a final age based on a combination of whole and thin-section age estimates (henceforth referred as the 'whole-section' method). When age estimates differed between methods, the final estimate selected by Reader 2 was based on the read that had the highest confidence rating. The thin-section or grind age estimate was chosen if the confidence index ratings were the same.

ANALYSIS

Otolith structure and annulus formation tend to be consistent over time within populations, and as the objectives of this study were to assess otolith preparation methods and reader bias, the age data were pooled among years (2010, 2011 and 2012) for both locations (Tatik Lake and Hornaday River), unless otherwise indicated.

Age bias plots, which represent the mean age estimate with 95% confidence intervals, of an age reader corresponding to each age category of the other age reader or age estimation method (Campana et al. 1995), were created to graphically examine bias: A) between whole and both thin-section and grind methods (Reader 2 only), B) between methods associated with a change in confidence (i.e., otoliths read whole with low confidence that when sectioned were read with high confidence; Reader 2 only), C) between methods associated with no change in confidence (i.e., otoliths read whole with high confidence that when sectioned were also read with high confidence; Reader 2 only), D) between readers for the whole method, and E) between readers for the whole method (Reader 1) and final age estimates assigned using the whole-section method (Reader 2). For the purpose of this study, confidence ratings of 'Poor', 'Fairly Poor' or 'Fair' were categorized as "low confidence" estimates while 'Fairly Good' or 'Good' were "high confidence" estimates.

The coefficient of variation (CV) (Chang (1982)) was calculated for each comparison:

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

where R is the number of times each sample was read, X_j is the average age estimate of the j^{th} fish, and X_{ij} is the i^{th} age estimate for the j^{th} fish. The lower the CV the higher the precision. Coefficient of variation is influenced by age reader experience, species, and preparation method. While there is not an established threshold, Campana (2001) suggests a CV of 5% to delineate precise estimates for age estimation laboratories. We calculated a CV for each of the comparisons described above for the bias plots. We also calculated percent agreement (PA), which is another measure of precision to compare age data. The percentage of samples that had exact agreement between method or reader (PA0) and the percentage that differed by ± 1-year (PA1) was also calculated.

The Wilcoxon rank test was used to test for differences between matched pairs of age estimates. A chi squared test was used to evaluate differences in the frequency of confidence index categories assigned by Reader 2. Age frequency distribution, mean length-at-age, and annual mortality (Robson and Chapman 1961) were compared for the whole method (Reader 1) and the final age estimates from the whole-section method (Reader 2). A Mann-Whitney test (independent samples) was used to test for differences in the age distributions for each sampling year for both locations.

Finally, we tested for differences in growth using age estimates from the whole method (Reader 1) and the final estimates from the whole-section method (Reader 2). The von Bertalanffy growth model:

$$L_t = L_\infty \ (1 - e^{-K\,(t-t_0)})$$

where L_t is the expected length at time t, L_∞ is the asymptotic average length, K is the Brody growth rate coefficient, and t_0 is the modelling artefact representing time or age when the average length was zero (Ricker 1975), was generated using the R (R Core Team 2017) package 'FSA' (Simple Fisheries Stock Assessment Methods) detailed in Ogle (2016). To determine whether parameters L_∞, K, and t_0 were significantly different between readers, pairs of candidate models of the same type were compared in a hierarchical framework where complex models were tested against simpler models with fewer parameters using an *F*-test on the residual sum of squares (RSS) (Ogle 2016). Comparisons were conducted until a simpler model did not fit the data as well as a more complex one and the model with the lowest RSS was selected if multiple models with the same parameters fit the data as well as a more complex model. All statistical results were considered significant for *P* values ≤ 0.05.

RESULTS

WITHIN-READER BETWEEN-METHODS

Tatik Lake

Reader 2's paired whole and thin-sectioned otolith age estimates were significantly different (Z = -3.27, *P* = .001) (Table 3A). No bias was observed for age estimates ≤ 11 (Figure 2A). However, the whole estimates were on average about one year younger than thin-section ages 13 to 15 and two to six years younger than thin-section ages 16 to 21. Maximum age was 17 and 21 years using whole and thin-section methods, respectively. The CV was 4.6%, while PA0 and PA1 were 50.8% and 87.7%, respectively (Table 3A). A statistically significant difference in the frequency of low and high confidence levels was observed between whole and thin-sectioned otolith reads (X^2 = 48.6, *P* < .05). The combined frequency of 'Fairly Good' and 'Good' increased substantially from 46 to 126 when otoliths were thin-sectioned and Reader 2's confidence improved for ages assigned using the thin-section method compared to the whole method for 58.4% of the samples (104 of 178) (Table 4A).

The whole and grind methods match-pairs age estimate comparison was not significantly different (Z = -0.11, *P* = .91). The CV was 1.5% and the PA and PA1 was 85.6% and 95%, respectively (Table 3A). Grinding otoliths improved Reader 2's confidence in 91.3% of the samples (42 of 46) that had a low confidence rating when read whole (X^2 = 28.1, *P* < .05) (Table 4B). After grinding, readability improved for just over half (33 of 60) of 'Fairly Good' and did not change for nearly all (47 of 54) of the 'Good' otoliths (Table 4B). There were a few instances where readability decreased with grinding (n = 10).

Otolith age estimates that received high confidence ratings for both the whole and thin-section methods were not significantly different (Z = -0.71, *P* = .48) (Figure 4A). Whole otolith reads that received a low confidence index rating also did not differ (Z = -1.35, *P* = .17) from corresponding thin-section age estimates that were assigned a high confidence score (Table 3, Figure 5A). However, precision was higher for the comparison between whole and thin-section age estimates (both with high confidence ratings) (CV = 1.5%, PA0 = 86.5%, PA1 = 97.3%) than for thin-section age estimates with high confidence and whole estimates with low confidence (CV = 4.1%, PA0 = 51.7%, PA1 = 93.3%).

Hornaday River

The comparison of paired age estimates from whole and thin-section otoliths (Reader 2) was significantly different (Z = -4.5, $P < .001$). No bias was observed between ages 5 and 9. However, whole age estimates were approximately 1 year younger than thin-section ages 10 to 13 with a difference of 3 years for a single sample with a thin-section estimate of 13 years (Figure 2B).The CV was 4.6% and the PA0 and PA1 were 56.4% and 93.8%, respectively (Table 3B). A statistically significant difference in the frequency of low and high confidence levels was observed between methods ($X^2 = 101.2$, $P < .05$). The combined frequency of 'Fairly Good' and 'Good' increased from 25 to 128 when otoliths were thin-sectioned and Reader 2's confidence improved for ages assigned using the thin-section method compared to the whole method for 50.5% of the samples (110 of 218) (Table 5A).

The comparison of age estimates from the whole and grind methods was not significant (Z = -1.73, $P = .08$) (Table 3B) (Figure 3B). The CV between whole and grind methods was 2.1% and percent agreement was high (PA0 = 80.5% and PA1 = 99.2%) (Table 3B). Grinding otoliths improved Reader 2's confidence in 76.2% of the samples (16 of 21) that received a low confidence score when read whole ($X^2 = 6.7$, $P < .01$) (Table 5B). After grinding, confidence increased for most (16 of 21) of the 'Fair' and approximately half (22 of 47) of the 'Fairly Good' whole reads and did not change for nearly all (48 of 55) of the 'Good' otoliths (Table 5B).

Age estimates that received high confidence index ratings for both whole and thin-section methods were significantly different (Z = -2.13, $P = .03$) (Figure 4B). Whole otolith age estimates that received a low confidence index rating did not differ (Z = -1.12, $P = .26$) from corresponding thin-section age estimates deemed easier to interpret (Figure 5B). Precision was generally higher for the comparison of thin-section and whole age estimates both with high confidence ratings (CV = 3.0%, PA0 = 73.3%, PA1 = 93.3%) than between thin-section with high confidence and whole age estimates with low confidence (CV = 4.0%, PA0 = 61.3%, PA1 = 95.3%) (Table 3B).

The frequency of low and high confidence scores between locations was significantly different for whole otolith age estimates ($X^2 = 9.4$, $P = .002$) where a greater prevalence of lower confidence was detected for Tatik Lake compared to Hornaday River (75% vs. 69%). However, no significant difference was detected between sites for the thin-section ($X^2 = .05$, $P = .8$) or grind ($X^2 = .08$, $P = .78$) methods.

BETWEEN-READERS

Tatik Lake

Paired whole otolith age estimates between Readers 1 and 2 were statistically different (Z = -3.6, $P < .001$). The bias plot demonstrated relatively close agreement between ages 8 and 11, but Reader 1 produced estimates that were older by about one year for ages 4 to 7 and younger by one to two years after age 12 (Figure 6A). The CV between readers was 5.7% while PA0 and PA1 were 44.0% and 88.2%, respectively (Table 3A).

A comparison between the whole method (Reader 1) and the final age assigned using the whole-section method (Reader 2) was not significantly different (Z = -1.34, $P = .18$). However, on average Reader 1 obtained estimates that were older by about 0.5 years from ages 5 to 7, while Reader 2 obtained estimates that were on average 1 to 2 years older starting at age 11

(Figure 7A). The CV was 6.0%, and PA0 and PA1 were 44.6% and 88.4%, respectively (Table 3A).

Age frequency distribution was significantly different between methods/readers in each sampling year (all $P \leq .02$). Among years, the distributions had a similar shape but the whole-section method applied by Reader 2 produced a greater proportion of ages >12 years and an older maximum age (21 vs. 17 years) compared to the whole method of Reader 1 (Figure 8). Annual mortality followed a similar trend across years for both methods/readers. However, annual mortality values were consistently lower for the whole-section method (Reader 2; mean = 0.36 range = 0.47 to 0.30) compared to the whole method (Reader 1; mean = 0.47 range = 0.55 to 0.39) (Figure 9A). Mean length-at-age did not differ substantially across ages common to both readers (Figure 9B) and there was no significant difference in von Bertalanffy growth model parameters ($P > 0.1$) (Table 6).

Hornaday River

Whole otolith age estimates from Readers 1 and 2 were statistically different (Z = -12.6, $P < .001$) (Table 3B). Whole otolith age estimates were similar between readers for ages 4 to 7 but beyond age 8, Reader 2 obtained ages that were on average 1-4 years older than Reader 1, with the exception of a single sample where there was agreement at 14 years of age (Figure 6B). The CV was 5.8% while PA0 and PA1 were 55.5% and 90.1%, respectively.

There was a significant difference between the whole method (Reader 1) and the whole-section method (Reader 2) (Z = -14.0, $P < .001$) (Table 3B). Results were similar for ages 5 to 7, but beyond age 8 Reader 2 estimated older ages except for one fish (Figure 7B). The CV was 6.7% while the PA0 and PA1 were 53.6% and 86.2%, respectively.

Age frequency distribution was significantly different between methods/readers in each sampling year (all $P \leq .01$). The distribution for all years combined had a similar mode (age-7) but there was a higher proportion of ages ≥ 8 for the whole-section method used by Reader 2 and a higher proportion of ages ≤ 6 for the whole method used by Reader 1 (Figure 10). The maximum estimated ages were 14 for the whole method (Reader 1) and 23 for the whole-section method (Reader 2) (note, Reader 2 assigned one sample an age of 23 years that was labelled by Reader 1 as 'unreadable' and was not included in the comparison of matched pairs). Annual mortality values followed a similar trend across years for both methods/readers. However, mortality based on the final age estimates from the whole-section method (Reader 2) were consistently lower (mean = 0.61 range = 0.44 to 0.70) compared to mortality based on the whole method (Reader 1) (mean = 0.77 range = 0.57 to 0.88) (Figure 11A). Mean length-at-age was lower for the whole-section method (Reader 2) compared to the whole method (Reader 1) (Figure 11B) although no significant differences in von Bertalanffy growth model parameters were detected between readers ($P > 0.2$) (Table 6).

DISCUSSION

OTOLITH PREPARATION

Otolith preparation method used had an effect on the age data that was produced for both locations. Tatik Lake and Hornaday River differed in the age at which the thin-section (age-13 versus age-10, respectively) and grind (age-10 versus age-8, respectively) methods began to diverge from the whole method. Previous Arctic Char studies comparing age estimates between whole and break-and-burn (Barber and MacFarlane 1987, Kristofferson and Klemetsen 1991, Babaluk et al. 2007), and whole and thin-section (Babaluk et al. 2007) methods also found the whole method tended to underestimate among older age classes. The age estimate (whole) when the bias appeared was at approximately age 4 for Nauyuk Lake and age 10 for Ekalluk River in Barber and MacFarlane (1987) (note, we are excluding their observations for Beaufort Sea samples given these were subsequently identified as Dolly Varden, *S. malma*; see Reist et al. 1997), age 10 for Lake Ellasjøen ("lower mode charr" only) (Kristofferson and Klemetsen 1991), and approximately age 14 for Lake Hazen (Babaluk et al. 2007). The authors of the previous studies indicated that the deviation between methods increased with increasing age. Our findings suggest the readability of otoliths may differ between populations, which is supported by the higher proportion of lower confidence reads for whole otoliths from Tatik Lake presented here. The difference could be a result of the distinct life histories between populations. Anadromous Arctic Char from Tatik Lake rear and overwinter in lacustrine habitat and are typically longer-lived while those from the Hornaday River rear and overwinter in riverine habitat and tend to be shorter-lived (see Jensen 1994). Otoliths from longer lived fish are known to be more difficult to interpret using the whole method, as growth slows and annuli are laid down closer to each other at the edge (Power 1978).

The measures of precision (CV, PA0, PA1) from the whole otolith-otolith section and whole otolith-otolith grind comparisons in this study suggest that the grind method may be better than the thin-section method for these Arctic char otoliths, but this is likely an artefact of the narrower age range, with fewer older samples, encountered in the whole-grind comparison portion. It is plausible to hypothesize that grinding otoliths may be a suitable alternative to thin-sectioning given both methods examine annuli along the transverse plane and produce high confidence age data. Grinding takes less time as it does not require embedding otoliths in epoxy, which requires a few days to harden. However, the grind method is more difficult to learn, tends to split the otolith down the sulcus, and is more likely to produce an inconsistent reading angle on the transverse plane (R. Wastle, personal observation). Therefore, it is recommended that Arctic Char otoliths be thin-sectioned rather than ground.

Our assessment of the confidence index ratings and perceived improvement in readability found a significant difference between whole and thin-section age estimates when both were assigned high confidence scores for Hornaday River ($P < 0.05$), but not Tatik Lake. However, the low sample sizes for both tests (30 and 37 fish) could have influenced the outcome. The comparison of whole ages assigned a low confidence and the corresponding thin-section ages assigned a high confidence was not significant for both locations (sample sizes of 89 and 106 fish). These results indicate that it may not be necessary to thin-section otoliths with low confidence ratings for ages assigned using the whole method (see Age Estimation Protocol below). However, the increased confidence that thin-sectioning provides to the age reader may be a reasonable justification for this additional step. Furthermore, it is likely that over time reader experience will increase the prevalence of high confidence scores for whole reads, and therefore the number of otoliths requiring sectioning would go down.

AGE ESTIMATION PROTOCOL

The development of the age estimation protocol for anadromous Arctic Char adopted by the Department of Fisheries and Oceans (DFO) Freshwater Institute in the late 2000s was based on the results presented here for Tatik Lake and Hornaday River, as well as other Arctic Char stocks examined by the laboratory. The protocol uses the whole and thin-section otolith preparation methods in conjunction with the confidence index. An additional quality assurance and quality control procedure requires that a second age reader in the lab, trained and experienced with age estimation of Arctic Char, randomly selects and re-reads 15% of the samples. The target is to achieve a relative percent difference (RPD, %) of < 10% for a single sample between readers and an average of < 5% for the total combined samples that were re-read:

$$RPD = \frac{|Reader\ 1\ age - Reader\ 2\ age|}{mean\ of\ pooled\ age}\ x\ 100$$

All samples with an RPD of > 10% will be re-examined by both readers to establish a consensus age estimate.

Determining the age at which the whole otolith method and the thin-section or grind method begin to diverge appears to be stock-specific (based on the work presented here and other unpublished comparisons). Therefore, a between-method age comparison study should be conducted on a stock-by-stock basis to determine the appropriate age at which to switch from the whole method to a cross-section method. Furthermore, additional work is required to determine the within-reader precision (repeatability) of the protocol.

The following age estimation protocols are recommended for Tatik Lake and Hornaday River Arctic Char:

Tatik Lake:

- Whole method if ≤ age 12;
- Thin-section ≥ age 13 (whole);
- If confidence in the whole age estimate ≤ age-12 is low, the otolith would be thin-sectioned to confirm or re-assess the assigned age.

Note, the bias observed at age 12 with the whole method using all samples (Figure 2A) is not present when examining whole estimates with high confidence (Figure 4A).

Hornaday River:

- Whole method for ≤ age 9;
- Thin-section ≥ age 10 (whole);
- If confidence in the whole age estimate ≤ age 9 is low, the otolith would be thin-sectioned to confirm or re-assess the assigned age.

Based on whole age estimates produced by Reader 2 and following the second bullet point of the proposed age estimation protocols, 14.4% of samples from Tatik Lake and 4.4% of samples from Hornaday River would need to be thin-sectioned. However, to maximize confidence in age estimates, thin-sectioning whole otoliths ≤ 12 years from Tatik Lake and ≤ 9 years from Hornaday River with low confidence (third bullet point) would increase the percentages requiring sectioning to 50.3% and 45.6%, respectively. Ultimately, producing high confidence age data requires a considerable investment (i.e., cost and time) in otolith preparation (embed and sectioning) and repeated reading.

HISTORICAL VERSUS CONTEMPORARY AGE DATA

The comparison of whole otolith age data between age readers was significantly different for both locations. Bias plots suggest the readers interpreted some annuli differently. Possible explanations include inconsistency between readers in the interpretation of the age 1 annulus and issues with interpretation at the margin of the otolith where tighter spacing of annuli can make reading more challenging. On average, Reader 1 consistently estimated ages older than Reader 2 among younger individuals. The extent of differences between readers increased among older age groups, with Reader 2 producing older age estimates.

Statistical comparisons of the whole and section methods were significant for Tatik Lake but not for Hornaday River, perhaps due to the larger number of older fish in the Tatik Lake sample.

Bias plots comparing the whole method (Reader 1) with the whole-section method (Reader 2) had a similar pattern as the whole method comparison but with somewhat greater age differences among the older age groups. In addition, growth parameters of the von Bertalanffy model did not differ substantially between methods/readers, likely because of the wide range of sizes observed among age classes, which is typical for this species (Johnson 1980) (Figures 9 and 11).

If the ages of Arctic Char have been under-estimated for the older age classes by Reader 1 using the whole method, the proportion of older age classes in the population would have been underestimated and the annual mortality of the population overestimated, by about 0.1 for both Tatik Lake and Hornaday River.

If it is determined that the difference in age between methods/readers would impact the outcome of a stock assessment, then it may be necessary to re-read some of the historic samples. Based on results presented here, the proportion of whole method otoliths read by Reader 1 that would need to be re-examined to make the data more comparable is 22.5% for Tatik Lake (i.e., ≥ age-12 and ≤ age-7; 52 + 76, respectively out of 568) and 20.4% for Hornaday River (≥ age-8; 144 out of 707).

The potential for bias and the corresponding implications when comparing historical data (whole otolith method) with contemporary age estimates (whole-section method) should be evaluated for other locations in the Canadian Arctic as well. Also, it should not be assumed that the extent of between-reader bias observed for Tatik Lake and Hornaday River during 2010 to 2012 would be observed for earlier years or for other populations. The passage of time could impact a reader's interpretation of annuli (known as age reader "drift"), which introduces error related to age estimation consistency (Campana et al. 1995). While speculative, the discrepancies we observed between readers in this study could have been at its maximum given this comparison occurred at the end of Reader 1's decades-long fish age estimation career.

CONCLUSION

The evaluation of between-method precision, bias, and reader confidence revealed that a combination of otolith preparation methods (whole and thin-sectioning) used for an age estimation protocol produced highly precise age information. Otoliths would be examined whole up to a certain age and then thin-sectioned thereafter. The difference observed when sectioning would occur for Tatik Lake and Hornaday River indicates that protocols for Arctic Char are stock-specific. Therefore, a between-method age comparison study should be conducted on a stock-by-stock basis to determine the appropriate age at which to start applying a thin-section method. While this method has not been validated, it is considered to provide high confidence age estimates for Arctic Char. Associating a level of confidence using standardized criteria to each age estimate in conjunction with a target average RPD of < 5% when a second reader re-reads a sub-sample, provides the basis to track quality control for the DFO Freshwater Institute's age estimation laboratory. While our findings suggest that it may not be necessary to thin-section otoliths with a low confidence rating for the age assigned using the whole method, this may not hold true for other stocks and could be examined as part of the stock-specific comparison. Furthermore, periodically examining a reference collection for Arctic Char and tracking the consistency in assigned confidence could enhance quality control measures for the laboratory (see Campana 2001).

Comparing historical (whole otolith method) and contemporary (whole-section method) age data and age-based population parameter estimates for Tatik Lake and Hornaday River, and other populations where the contemporary protocol has been introduced should be done cautiously. Also, it should not be assumed that the degree of bias observed for Tatik Lake and Hornaday

River between 2010 and 2012 would be observed for other locations. Therefore, it would be prudent to re-read some samples from earlier years for populations with a long time-series of ages assigned using the whole otolith method. It will be important to weigh the implications to the stock assessment against the time and cost of re-reading historic samples. This work is a first step towards providing guidance on this topic.

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TABLES AND FIGURES

Table 1. Sample size among otolith preparation methods utilized by two age readers to estimate the ages of Arctic Char captured from Hornaday River and Tatik Lake, Northwest Territories. Note, samples collected from Lasard Creek and Hornaday River mainly originate from the same stock.*

Location	Year	Otolith sample size	Reader 1	Reader 2		
			Whole	Whole	Thin-section	Grind
Tatik L.	2010	199	196	194	41	162
	2011	184	183	175	114	0
	2012	196	189	183	114	0
Total	-	**579**	**568**	**552**	**269**	**162**
Hornaday R.	2010	170	170	163	45	123
Lasard Cr.	2011	284	283	283	92	0
Hornaday R.	2012	154	154	153	73	0
Lasard Cr.	2012	100	100	97	48	0
Total		**708**	**707**	**696**	**259**	**123**

* Differences in the number of whole otoliths examined between readers are either due to misplacement of otoliths between reads or instances when one reader provided an estimate while the other did not.

Table 2. Confidence index used by the Fisheries and Oceans Canada, Freshwater Institute age estimation lab (adapted from S. MacLellan, unpublished report on age estimation procedures used by Fisheries and Oceans Canada's Pacific Biological Station).

Confidence index (abbreviation)	Qualitative meaning (Pattern clarity)	Quantitative meaning (Repeatability)	Age and comments examples
Good (G)	Pattern is very clear with no interpretation problems.	Reader would always get the same age.	10+(G), 38+(G)
Fairly good (FG)	Pattern is clear with a few easy interpretation problems.	Reader would get the same age most of the time for fish < 20 years, within 1 year for fish 20–40 years, etc.	7+(FG), 33+ (FG)
Fair (F)	Pattern is fairly clear with some areas presenting easy and moderate interpretation problems.	Reader would be within 1 year most of the time for fish < 20 years and 2–3 years for fish 2–40 years, etc.	9+ (F) – 1st year is unknown
Fairly poor (FP)	Pattern is fairly unclear presenting a number of difficult interpretation problems.	Reader would be within 2–3 years most of time for fish < 20 years and 4–5 years for fish 20–40 years, etc.	19+(FP) – may be 1 more between 3 and 4; outer rings are close together and weak.
Poor (P)	Pattern is very unclear presenting significant interpretation problems.	Reader has little confidence in repeatability of age within 5–10 years, or more in the case of older fish.	36+(P) – wrong section plane and part crystalline.

Table 3. Coefficient of variation (CV), Wilcoxon rank test (matched pairs) (Z statistic and P-value), exact percent agreement (PA0), percent agreement within one year (± 1 year) (PA1), and otolith sample size (n) for paired age comparisons of Arctic Char age data from Tatik Lake and Hornaday River, Northwest Territories, between methods and readers (R1 and R2)†.

A) Tatik Lake

Comparison	Preparation method	CV (%)	Z	PA0 (%)	PA1 (%)	n
Within Reader 2	Whole vs thin-section	4.6	-3.27, *P* =.001	50.8	87.7	244
	Whole vs grind	1.5	-0.11, *P* = .91	85.6	95.0	160
	Whole (P, FP, F)* vs thin-section (FG, G)*	4.1	-1.35, *P* = .17	51.7	93.3	89
	Whole (FG, G)* vs thin-section (FG, G)*	1.5	-0.71, *P* = .48	86.5	97.3	37
Between-reader	Whole	5.7	-3.6, *P* < .001	44.0	88.2	543
	Whole (R1) vs whole-section (R2)	6.0	-1.34, *P* = .18	44.6	88.4	561

B) Hornaday River

Comparison	Preparation method	CV (%)	Z	PA0 (%)	PA1 (%)	n
Within Reader 2	Whole vs thin-section	4.6	-4.5, *P* < .001	56.4	93.8	249
	Whole vs grind	2.1	-1.73, *P* = .08	80.5	99.2	123
	Whole (P, FP, F)* vs thin-section (FG, G)*	4.0	-1.12, *P* = .26	61.3	95.3	106
	Whole (FG, G)* vs thin-section (FG, G)*	3.0	-2.13, *P* = .03	73.3	93.3	30
Between-reader	Whole	5.8	-12.6, *P* < .001	55.5	90.1	695
	Whole (R1) vs whole-section (R2)	6.7	-14.0, *P* < .001	53.6	86.2	703

* age reader confidence index: P = 'poor', FP = 'fairly poor', F = 'fair', FG = 'fairly good', and G = 'good'

Table 4. Frequency among confidence index categories (P= 'Poor', FP= 'Fairly Poor', F= 'Fair', FG= 'Fairly Good', and G= 'Good') assigned to whole otolith age estimates of Arctic Char from Tatik Lake, Northwest Territories and the change observed after A) thin-sectioning, and B) grinding. Note, color added to highlight cases when confidence did not change (yellow), improved (green), or declined (red) after otoliths were thin-sectioned or ground (n=sample size).

A)

n	**Whole CI**	**Change after thin-sectioning**				
		P	**FP**	**F**	**FG**	**G**
1	P	0	0	0	1	0
7	FP	0	0	15	10	2
170	F	0	1	93	56	20
37	FG	0	0	9	16	12
9	G	0	0	0	4	5
244	-	0	1	117	87	39

B)

n	**Whole CI**	**Change after grinding**				
		P	**FP**	**F**	**FG**	**G**
2	P	1	0	0	0	1
5	FP	0	0	2	2	1
39	F	0	0	3	27	9
60	FG	0	1	2	24	33
54	G	0	0	1	6	47
160	-	1	1	8	59	91

Table 5. Frequency among confidence index categories (P= 'Poor', FP= 'Fairly Poor', F= 'Fair', FG= 'Fairly Good', and G= 'Good) assigned to whole otolith age estimates of Arctic Char from Hornaday River, Northwest Territories and the change observed after A) thin-sectioning, and B) grinding. Note, colour added to highlight cases when confidence did not change (yellow), improved (green), or declined (red) after otoliths were thin-sectioned (n=sample size).

A)

n	**Whole CI**	**Change after thin-sectioning**				
		P	**FP**	**F**	**FG**	**G**
0	P	0	0	0	0	0
5	FP	0	0	5	0	0
213	F	0	0	108	90	15
12	FG	0	0	2	7	3
13	G	0	0	0	4	9
243	-	0	0	115	101	27

B)

n	**Whole CI**	**Change after grinding**				
		P	**FP**	**F**	**FG**	**G**
0	P	0	0	0	0	0
0	FP	0	0	0	0	0
21	F	0	0	5	11	5
47	FG	0	0	3	22	22
55	G	0	0	0	7	48
123	-	0	0	8	41	75

Table 6. von Bertalanffy growth model parameters (L_∞, K, and t_0) with 95% confidence intervals for Arctic Char from Tatik Lake and Hornaday River, Northwest Territories, calculated using age estimates from the whole method (Reader 1) and the final estimate from the combined whole-section method (Reader 2).

Location	Method(Reader)	L_∞	K	t_0
Tatik Lake	Whole (Reader 1)	702 (665–805)	0.23 (0.11–0.35)	0.44 (-3.69–2.3)
	Whole-section (Reader 2)	680 (661–705)	0.32 (0.24–0.42)	2.14 (0.95–3.07)
Hornaday River	Whole (Reader 1)	635 (620–656)	0.54 (0.38–0.74)	2.07 (0.82–2.84)
	Whole-section (Reader 2)	666 (637–740)	0.35 (0.18–0.55)	0.4 (-2.44–1.75)

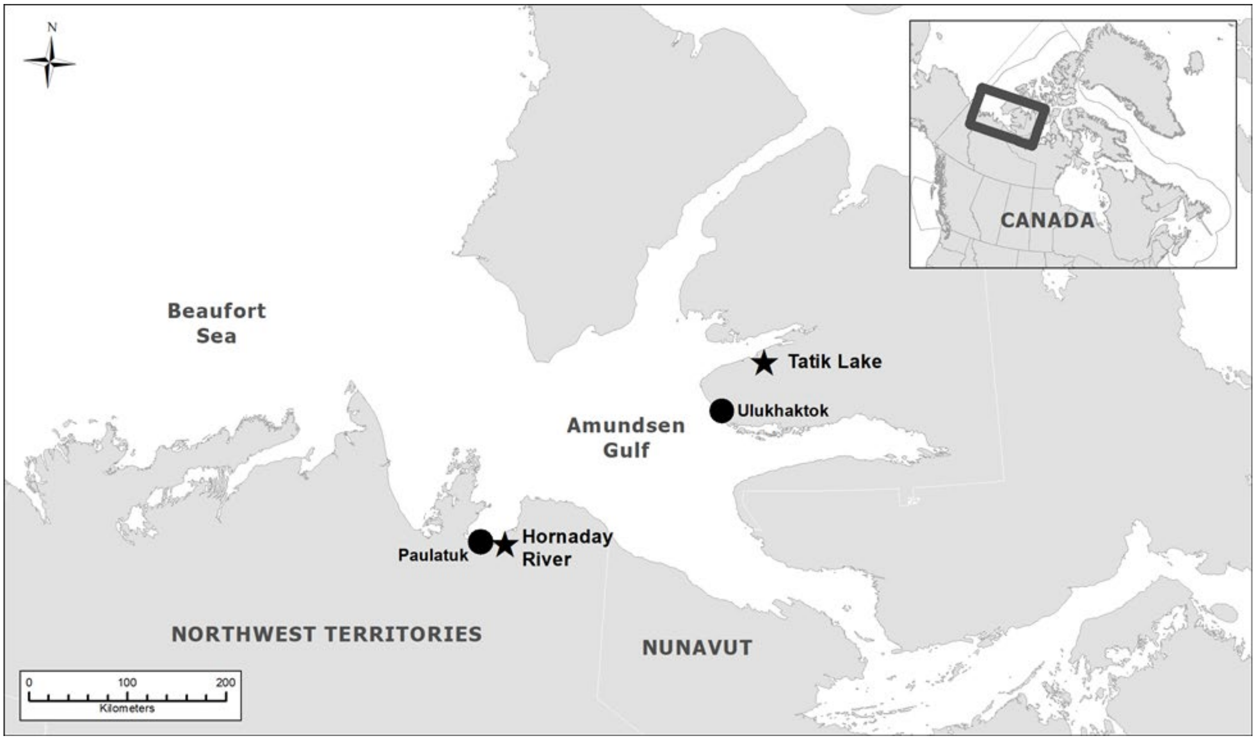

Figure 1. Location of Tatik Lake and Hornaday River, Northwest Territories, Canada.

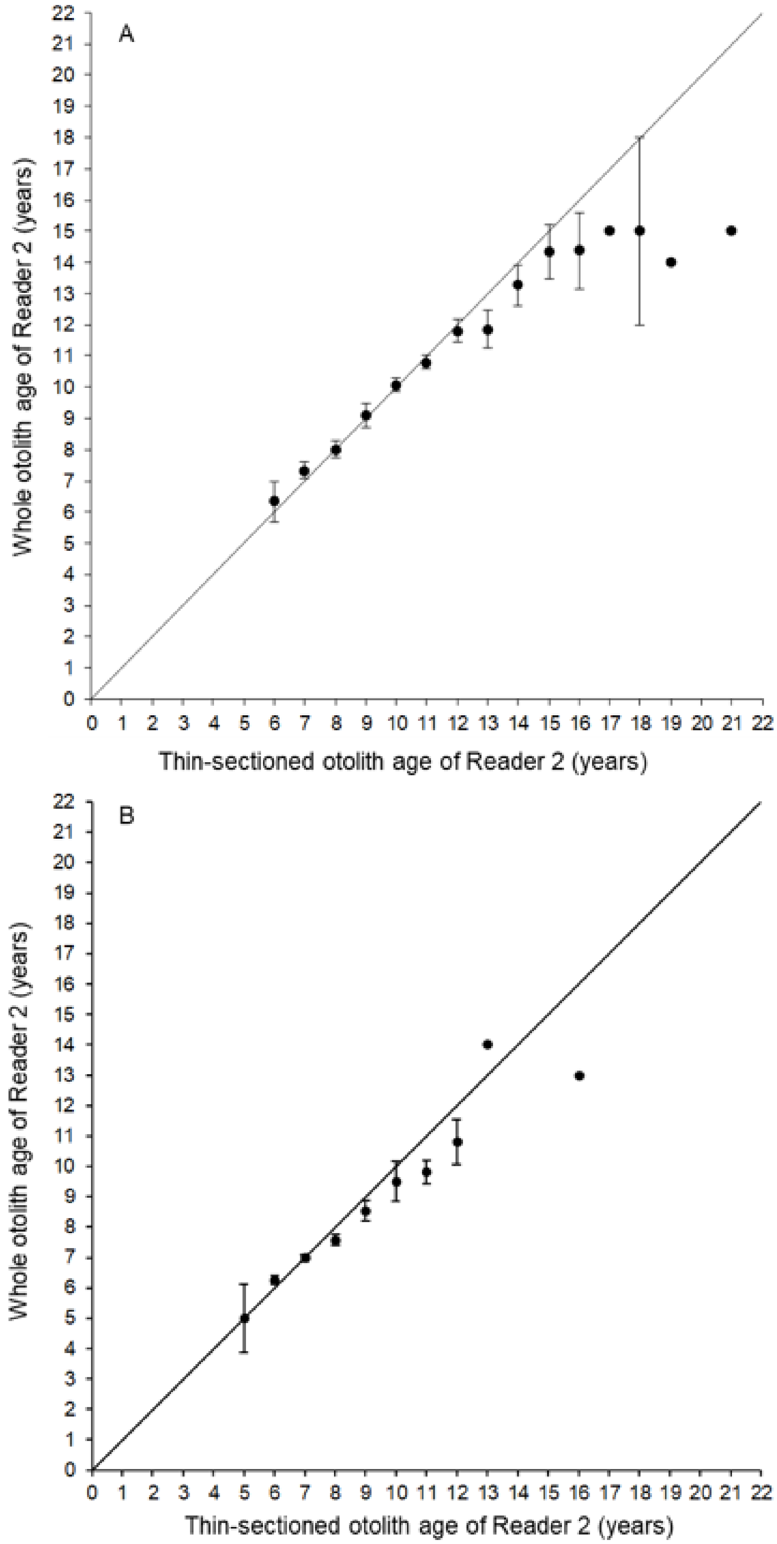

Figure 2. Bias plots for Reader 2 whole and thin-section otolith method age estimates for Arctic Char from A) Tatik Lake and B) Hornaday River, Northwest Territories. Each error bar represents 95% confidence intervals for the average age estimate obtained using the whole otolith method for all estimates assigned using the section method. Solid line indicates the 1:1 line.

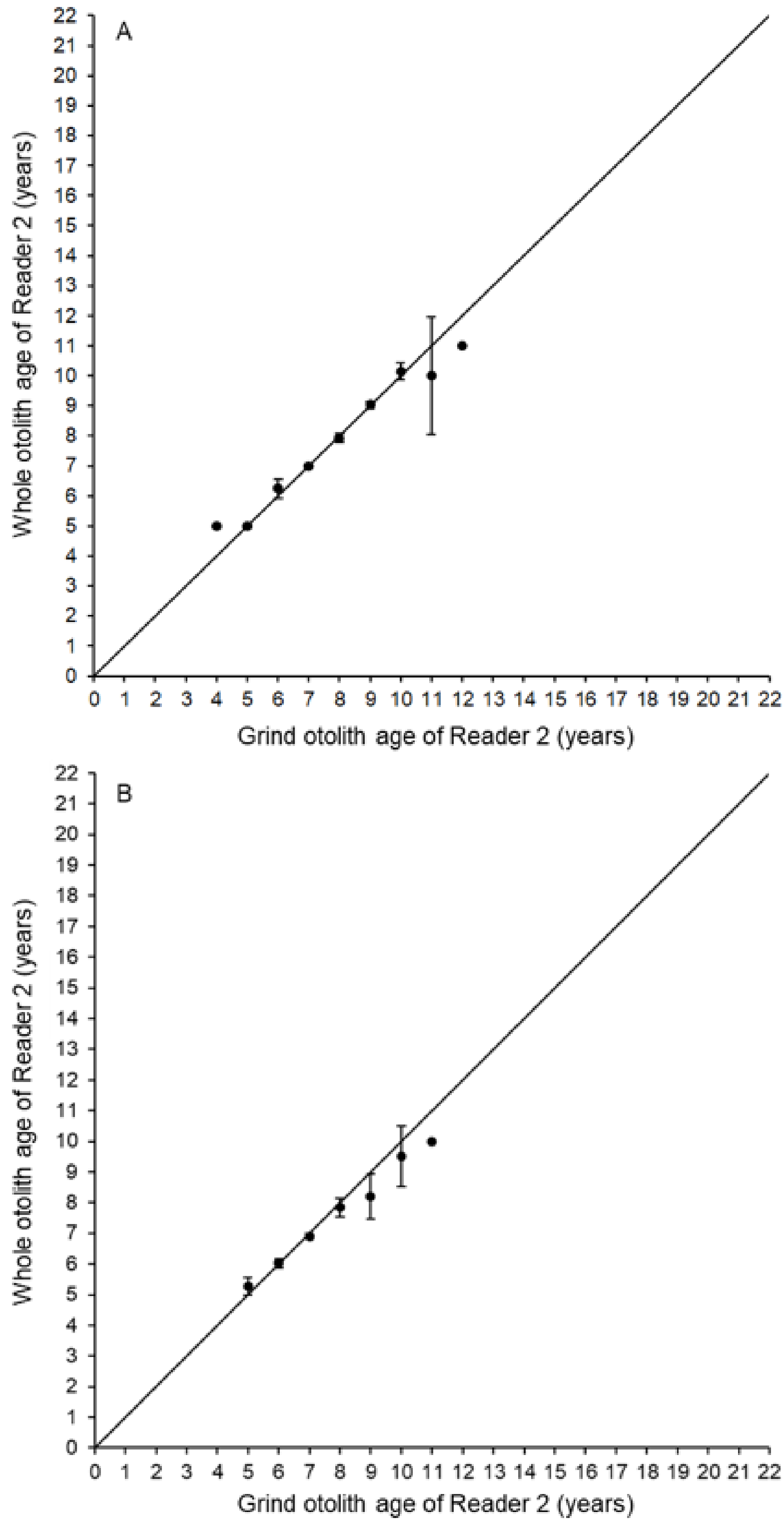

Figure 3. Bias plots for Reader 2 whole and grind otolith method age estimates for Arctic Char from A) Tatik Lake (≤ 12 years only) and B) Hornaday River (≤ 11 years only), Northwest Territories. Each error bar represents 95% confidence intervals from the average age estimate obtained using the whole otolith method for all estimates assigned using the grind method. Solid line indicates the 1:1 line.

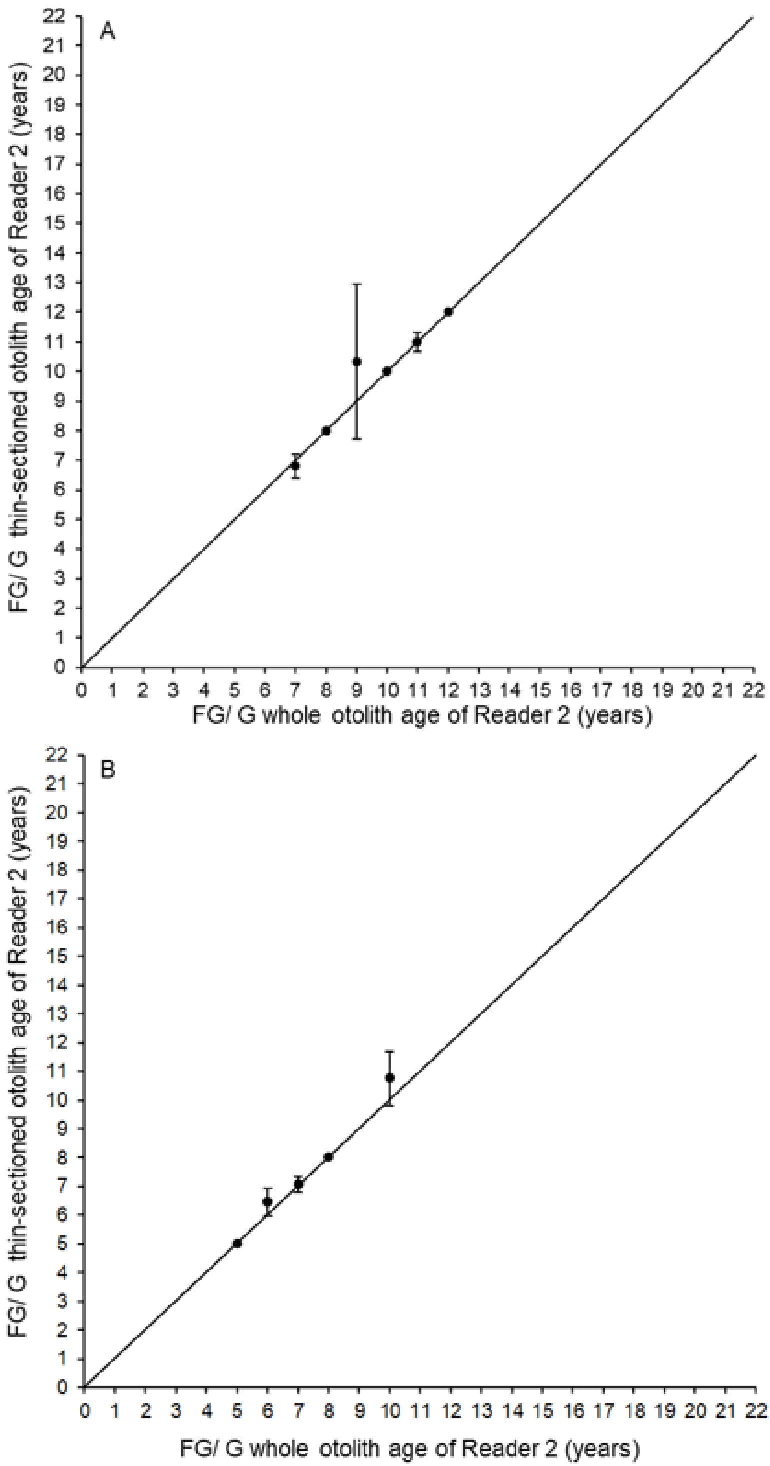

Figure 4. Bias plots for Reader 2 whole otolith method age estimates assigned a confidence index rating of 'Fairly Good' or 'Good' (i.e., high confidence) and the same otoliths after being thin-sectioned and assigned a confidence index rating of 'Fairly Good' or 'Good' for Arctic Char from A) Tatik Lake and B) Hornaday River, Northwest Territories. Each error bar represents 95% confidence intervals from the average age estimate obtained using the sectioned otolith method for all estimates assigned using the whole method. Solid line indicates the 1:1 line.

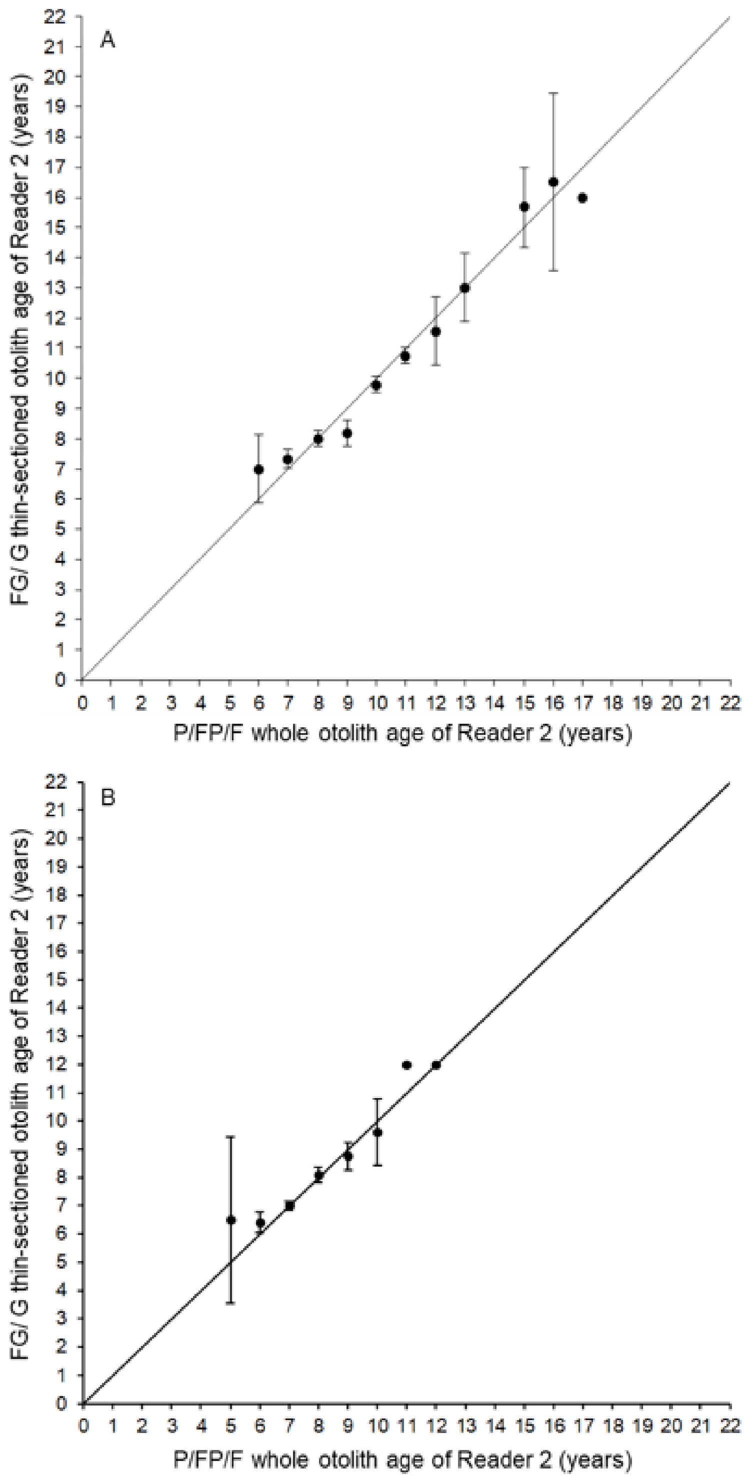

Figure 5. Bias plots for Reader 2 whole otolith method age estimates assigned a confidence index rating of 'Poor', 'Fairly Poor' or 'Fair' (i.e., low confidence) and the same otoliths after being thin-sectioned and assigned a confidence index rating of 'Fairly Good' or 'Good' (i.e., high confidence) for Arctic Char from A) Tatik Lake and B) Hornaday River, Northwest Territories. Each error bar represents 95% confidence intervals from the average age estimate obtained using the otolith section method for all estimates assigned using the whole method. Solid line indicates the 1:1 line.

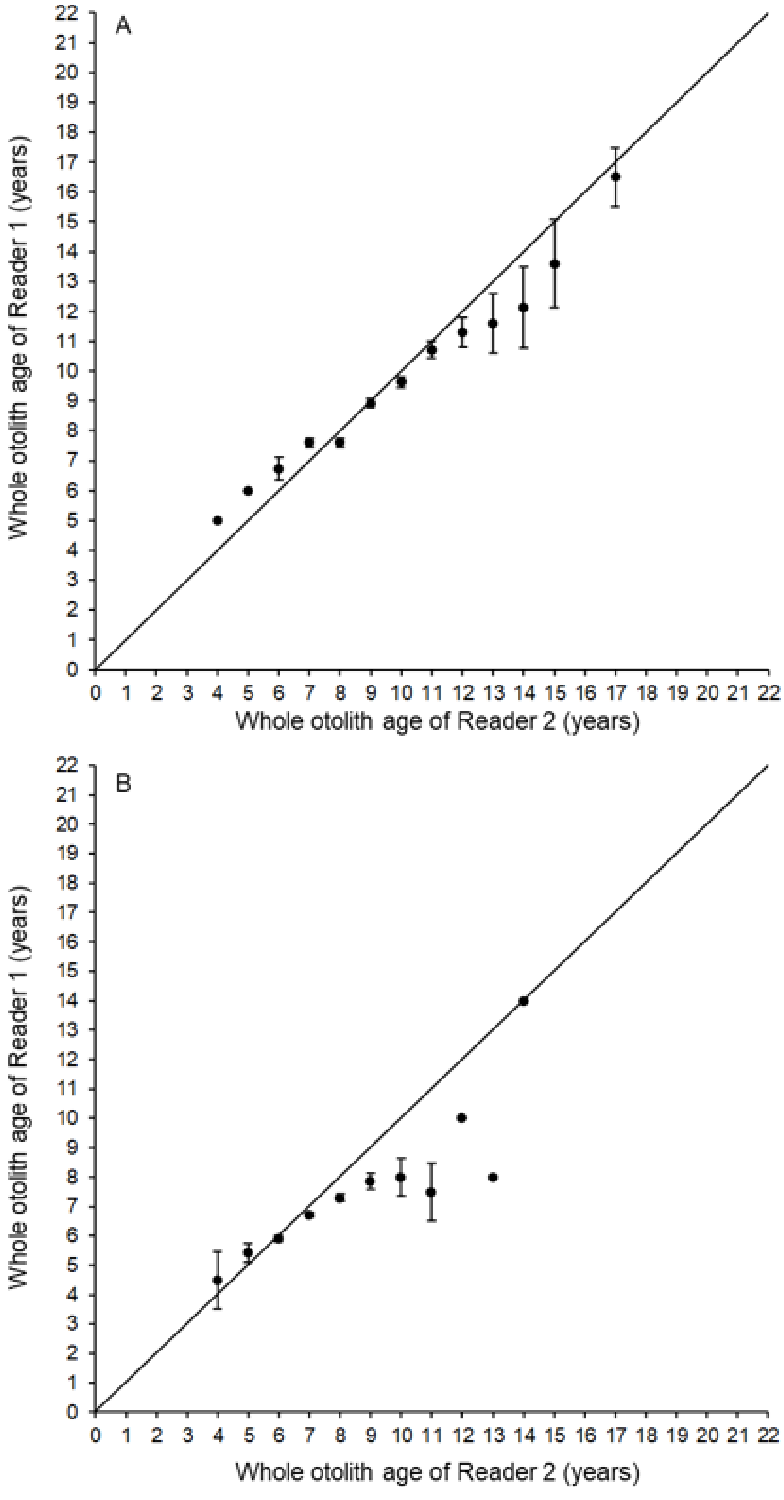

Figure 6. Between-reader bias plots for the whole otolith method age estimates for Arctic Char from A) Tatik Lake and B) Hornaday River, Northwest Territories. Each error bar represents 95% confidence intervals from the average whole age estimates obtained from Reader 1 for all whole estimates assigned by Reader 2. Solid line indicates the 1:1 line.

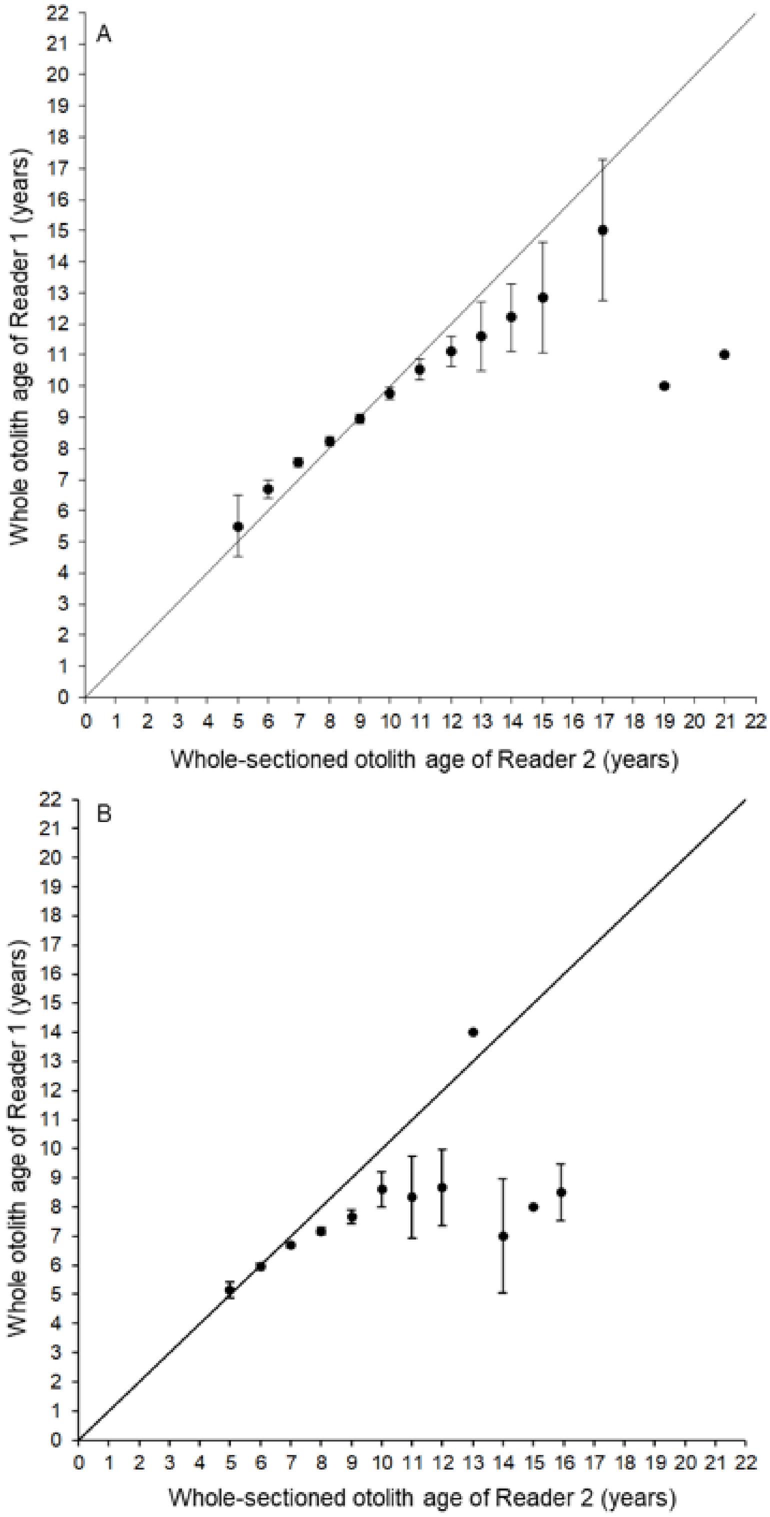

Figure 7. Between-reader bias plots for the whole otolith method age estimates (Reader 1) and the final age estimates from the whole-section method (Reader 2) for Arctic Char from A) Tatik Lake and B) Hornaday River, Northwest Territories. Each error bar represents 95% confidence intervals from the average age estimates obtained from Reader 1 for all estimates assigned by Reader 2. Solid line indicates the 1:1 line.

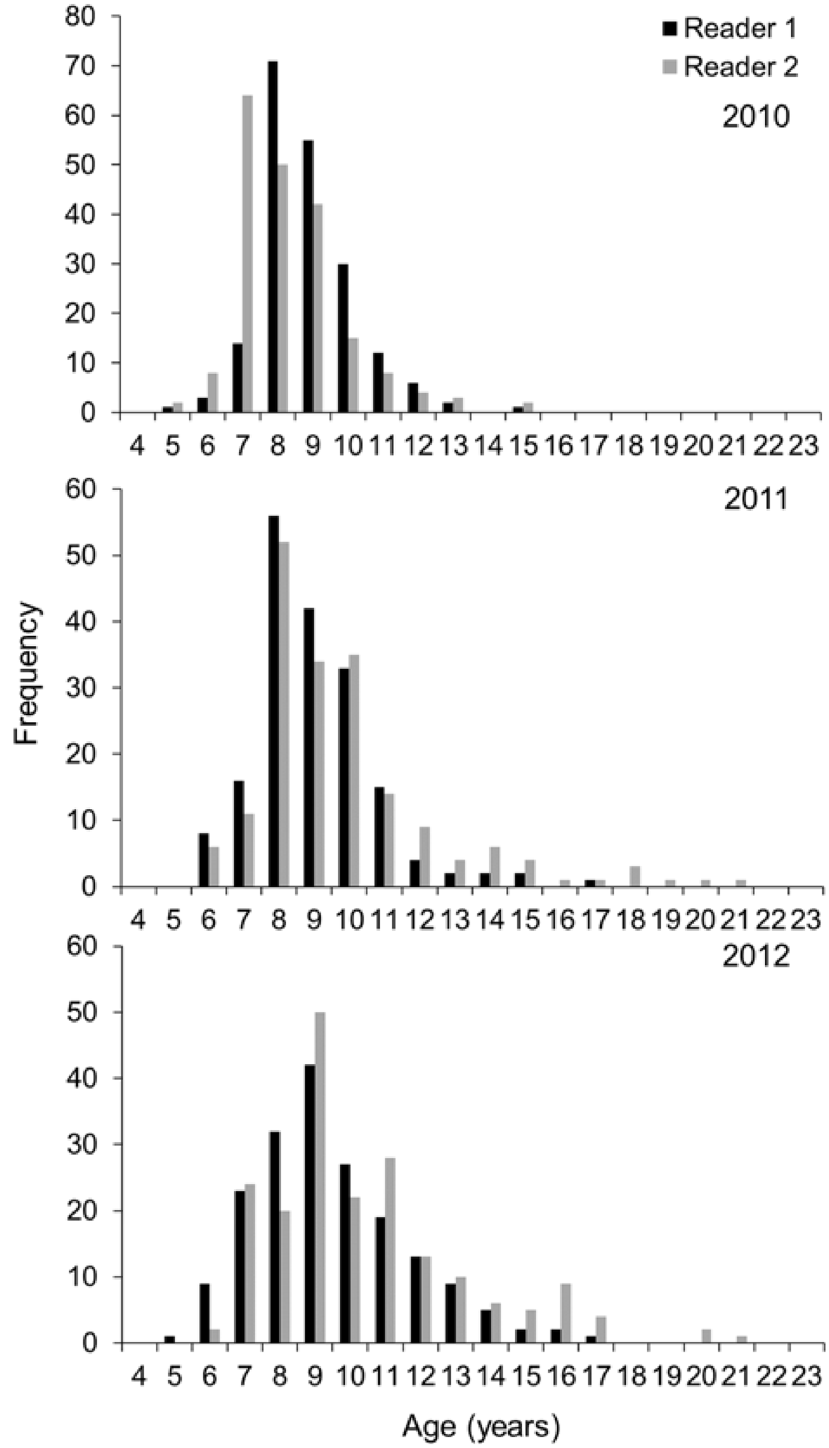

Figure 8. Age frequency distribution based on estimated ages derived from the whole otolith method (Reader 1) and the final estimates from the whole-section method (Reader 2) for Arctic Char from Tatik Lake, Northwest Territories captured between 2010 and 2012.

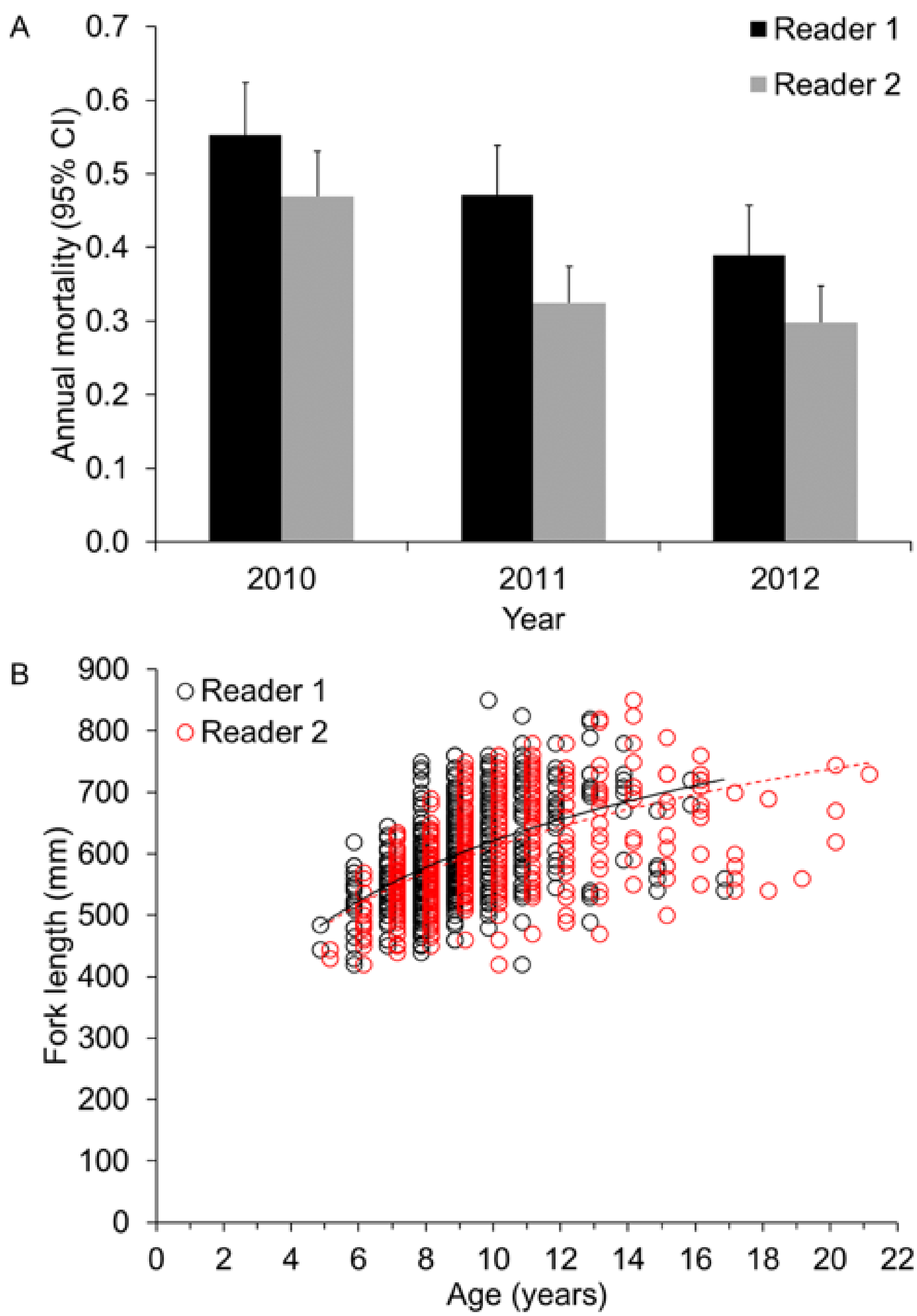

Figure 9. A) Annual mortality and B) length-at-age based on estimated ages derived from the whole otolith method (Reader 1) and the final estimates from the whole-section method (Reader 2) for Arctic Char from Tatik Lake, Northwest Territories captured between 2010 and 2012.

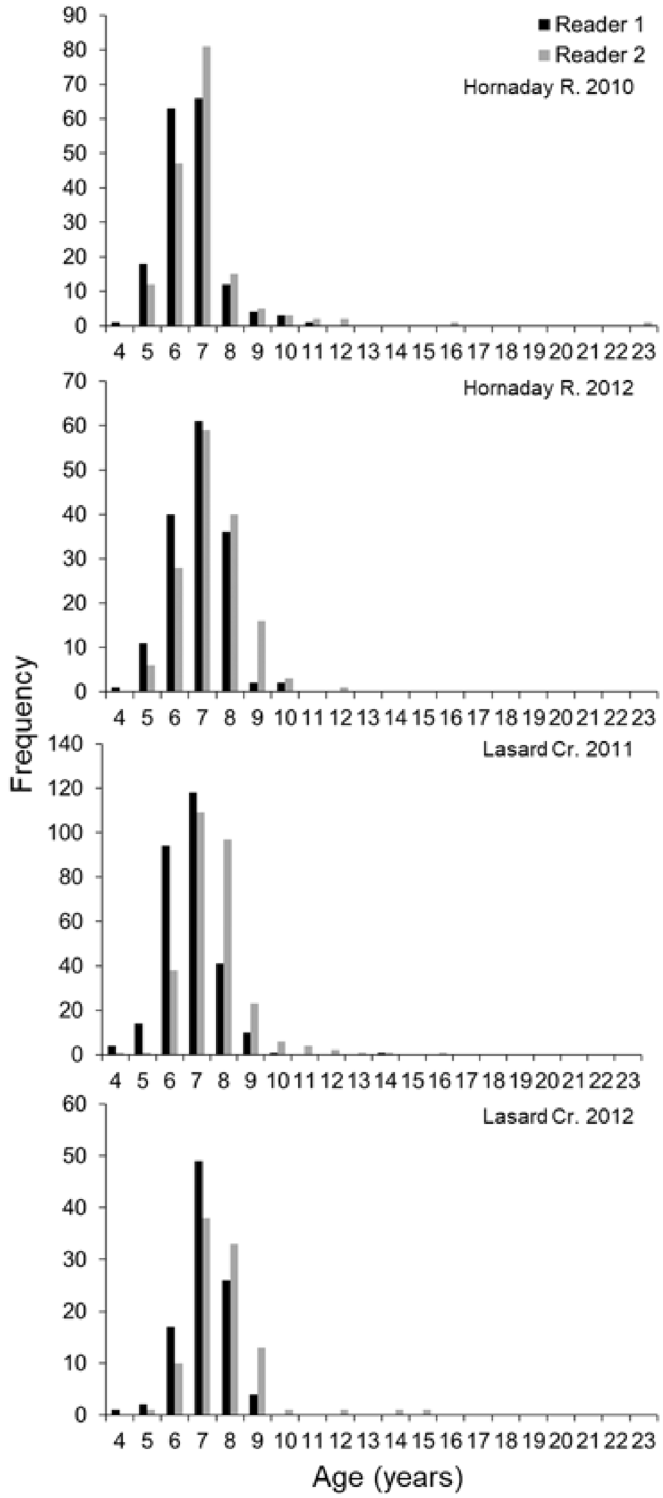

Figure 10. Age frequency distribution based on estimated ages derived from the whole otolith method (Reader 1) and the final estimates from the whole-section method (Reader 2) for Arctic Char from Hornaday River/Lasard Creek, Northwest Territories captured between 2010 and 2012.

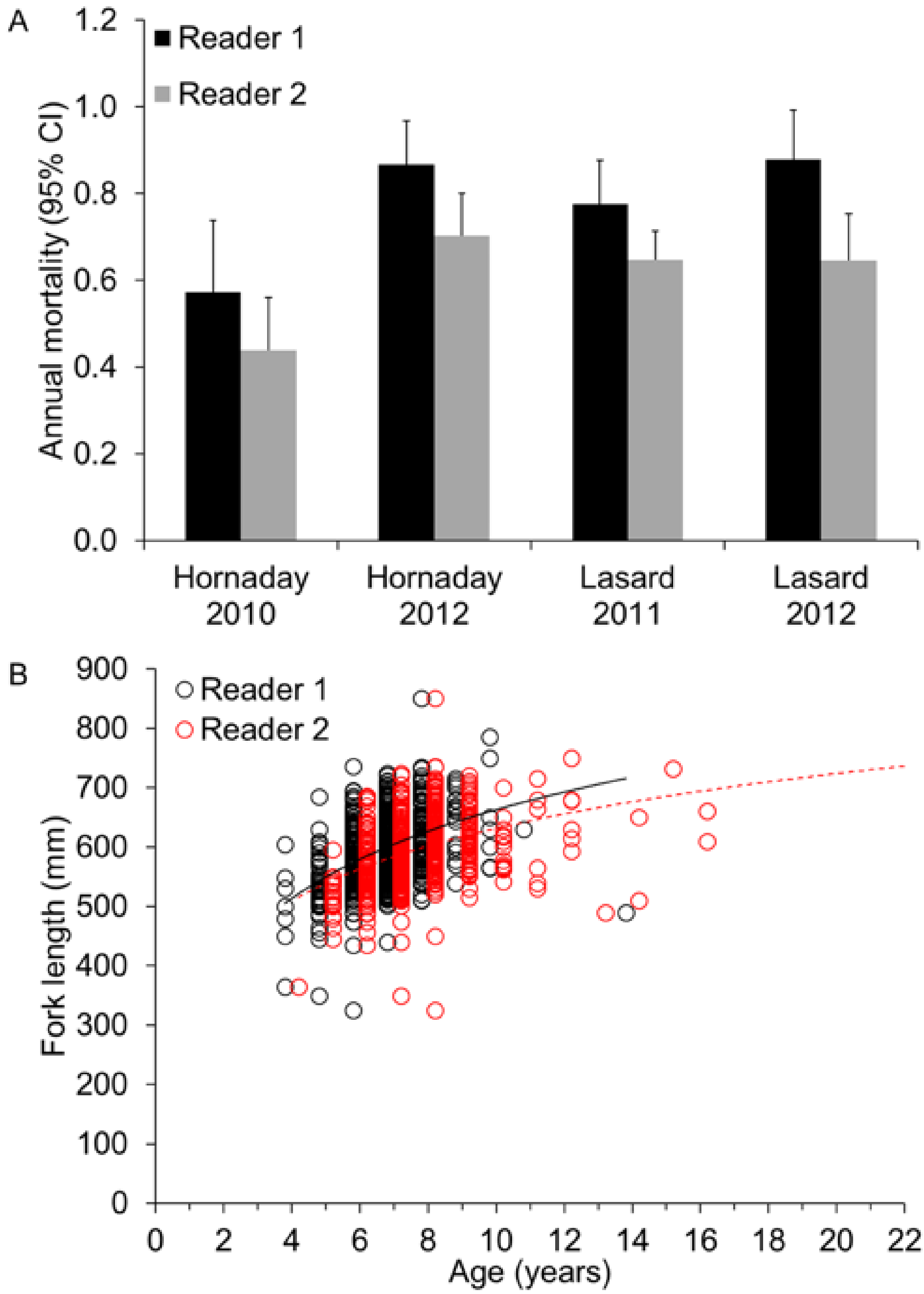

Figure 11. A) Annual mortality and B) length-at-age based on estimated ages derived from the whole otolith method (Reader 1) and the final estimates from the whole-section method (Reader 2) for Arctic Char from Hornaday River/Lasard Creek, Northwest Territories captured between 2010 and 2012.