Polar Biol
DOI 10.1007/s00300-015-1848-z

ORIGINAL PAPER

A comparison of different structures and methods for estimating age of northern-form Dolly Varden *Salvelinus malma malma* from the Canadian Arctic

Colin P. Gallagher[1] · Kimberly L. Howland[1] · Rick J. Wastle[1]

Received: 23 February 2015 / Revised: 20 November 2015 / Accepted: 22 November 2015

Abstract Assessment of anadromous northern-form Dolly Varden *Salvelinus malma malma* in the Western Canadian Arctic requires reliable methods for estimating ages. Additionally, conservation efforts warrant determining whether fin rays provide a non-lethal alternative to otoliths. Precision and bias of whole and sectioned otoliths, and sectioned pectoral and pelvic fin rays were examined. Two age readers with different levels of experience ageing this species read each structure three times. Coefficient of variation (CV) was calculated to measure precision, and age bias plots were created for each method of preparation/structure within and between readers. The experienced reader demonstrated the highest precision with sectioned otoliths (CV = 1.6 %) followed by whole otoliths (CV = 4.2 %) while pectoral and pelvic fins were the lowest, CV = 7.7 % and 7.5 %, respectively. The age bias plot showed little difference between whole and sectioned otoliths, although greater imprecision/bias was evident for whole otoliths at age ≥9. Compared to otoliths, fin rays produced younger age estimates starting at 5 years; however, pelvic fins were more biased towards younger estimates than pectoral fins. The less experienced reader had greater inconsistencies, tending to overage younger and underage older samples for all methods compared to the more experienced reader, underscoring the importance of experience when estimating age for this species. We conclude that both types of fin rays are a poor non-lethal alternative to otoliths for fish ≥5 years and recommend an experienced ager could use whole otoliths up to age 8 and sectioned otoliths for fish ≥9 years (>500 mm fork length).

Keywords Ageing bias · Ageing precision · Dolly Varden · Otolith · Pectoral fin rays · Pelvic fin rays

✉ Colin P. Gallagher
colin.gallagher@dfo-mpo.gc.ca

[1] Fisheries and Oceans Canada, 501 University Crescent, Winnipeg, MB R3T-2N6, Canada

Introduction

Age is an important parameter used to characterize the biology of many species. Age estimation for fishes is based on the examination of incremental deposition in calcified structures (e.g. otolith, scale, fin ray, spine, opercle, cleithrum and vertebrae) that correspond to a daily or annual timescale (Campana 2001) using various preparation methods (e.g. whole otoliths; break and burn of otoliths; thin sectioning of otoliths, fin rays and spines; staining of various structures; and baking vertebrae) (Chilton and Beamish 1982; Penttila et al. 1988). Unless validated to determine whether a structure or preparation method provides accurate results (Beamish and McFarlane 1983), age estimates from any structure are limited because formation of annuli may not occur over the lifetime of the fish and because of the subjectivity of ageing as annuli are visually interpreted by the age reader (Campana 2001). In addition to errors that affect accuracy (closeness to true value), the level of precision (reproducibility of repeated measurement) (Kalish et al. 1995) by a reader for a particular structure is an issue that further complicates age estimation. In fisheries research, examples of the application of age information include understanding life history (e.g. age-at-smoltification, age-at-maturity, longevity, length-at-age, natural mortality and fishing mortality) and the dynamic of a population in response to a hypothesized harvest level (e.g. predicted change in birth, death and

Published online: 26 December 2015

growth rates). Age-based population assessment models (e.g. statistical catch-at-age) require accurate age information in order to make reliable scientific recommendations to managers of fisheries.

Northern-form Dolly Varden *Salvelinus malma malma* (Walbaum) is a relatively short-lived (modal age = ~5–7 years, maximum age = ~15 years) iteroparous salmonid that spawns, rears and overwinters in shallow mountainous streams with perennial groundwater sources (Armstrong and Morrow 1980). In Canada, some populations have an anadromous life history and undertake annual migration to the Beaufort Sea, and these are valued by Inuvialuit and Gwich'in harvesters in Yukon and North-west Territories, Canada, and Inupiat in Arctic Alaska, for subsistence. Life history and environmental characteristics that could influence growth of ageing structures in anadromous Dolly Varden include age-at-smoltification of approximately three to five years (Rat River stock; unpublished data) and a seaward migration that begins approximately mid- to late June and a return migration starting late July. The sub-Arctic climate is characterized by short summers and long winters with air temperatures averaging ~11 °C in July along the Yukon coast and ~−30 °C in January in the Mackenzie Delta. Nearshore sea surface temperatures of the Canadian Shelf of the Beaufort Sea west of the Mackenzie Delta in the summer (August) are ~6–12 °C (Eert et al. 2015). The sun remains above the horizon for 24 h from the end of May until the third week of July and is below the horizon for 24 h from early December to early January.

The population assessment of anadromous Dolly Varden in Canada is conducted by Fisheries and Oceans Canada to help guide co-management partners in recommending subsistence harvest levels for various stocks. Assessments have relied on age information from the reading of whole otoliths. Concerns over declining population abundance in some Canadian stocks (COSEWIC 2010) and a desire to find an alternative to dead sampling for small populations with conservation concerns prompted this age comparison study. Examples in other fish species where an alternative to lethal sampling was possible were observed using pelvic (Howland et al. 2004; Zymonas and McMahon 2009) or pectoral (Muir et al. 2008) fins which did not demonstrate considerable, if any, difference when compared to otoliths. Stolarski and Sutton (2013) evaluated the bias and precision of scales, sectioned otoliths and pectoral fin rays of northern-form Dolly Varden from Alaskan rivers and concluded scales and pectoral fin rays (in fish ≥6 years of age) were poor alternatives to dead sampling; however, the study did not test for differences in otolith preparation methods or evaluate pelvic fin rays.

The objectives of this study were to have two age readers conduct multiple readings of whole otoliths, thin-sectioned otoliths, and sectioned pectoral and pelvic fin rays of Dolly Varden to (1) quantify the level of precision and bias for each structure/method within and between readers; (2) determine whether the whole otolith age estimation method used for assessment purposes provides the most consistent results; and (3) determine a range of ages where either thin-sectioned pectoral or pelvic fin rays could be used as a non-lethal alternative to otoliths.

Methods

Sample collection

Dolly Varden were collected from the Rat River Harvest Monitoring Program (Harwood et al. 2009; Roux et al. 2012). Using Gwich'in harvesters, the program collects annual harvest, catch-effort, biological and environmental data using a standardized methodology. Dolly Varden from the Rat River stock were captured during their migration from the Beaufort Sea to the spawning/overwintering area situated in Fish Creek (N65°45′, W136°17′), a tributary to the Rat River (Fig. 1). Fish were captured using single mesh gill nets varying in size between 102 and 127 mm (stretched mesh), and a sub-sample of the catch was retained for dead sampling. A total of 120 Dolly Varden were dead-sampled among the three monitoring sites (Big Eddy, mouth of the Rat River and Destruction City) between early August and early September in both 2007 and 2008 (Fig. 1). Each dead-sampled fish was assigned a sample number and was measured for fork length (±1 mm) and weight (±1 g) and assessed for sex and maturity. From each Dolly Varden, the sagittal otoliths were removed, cleaned and placed in a dry labelled envelope. A sub-sample was randomly chosen ($n = 60$ in 2007 and $n = 110$ in 2008; total $n = 170$) for removal of the left pectoral and pelvic fins which were clipped at the base of the fin using bone cutters, placed in separate labelled envelopes, and dried. The sample size of 170 used in the age comparison study was slightly reduced for sectioned otoliths and fin rays because some structures were either damaged during preparation or unreadable (Table 1). The mean ± SD fork length of the sample of Dolly Varden selected for the ageing study was 457 ± 58 mm and ranged between 282 and 698 mm.

Preparation and ageing of structures

One otolith was kept for whole age estimates, while the other was thin-sectioned on the transverse plane (0.5 mm thick). For the sectioning of otoliths, ColdCure Epoxy Resin (Industrial Formulators of Canada Ltd.) was poured in the shape of an ellipse on a piece of labelled Parafilm® (4 × 4 cm) in a fume hood. An otolith was embedded at

Fig. 1 Map of the Canadian Western Arctic showing the location of the Rat River and sampling locations of Dolly Varden (*A* Big Eddy, *B* mouth of the Rat River and *C* Destruction City)

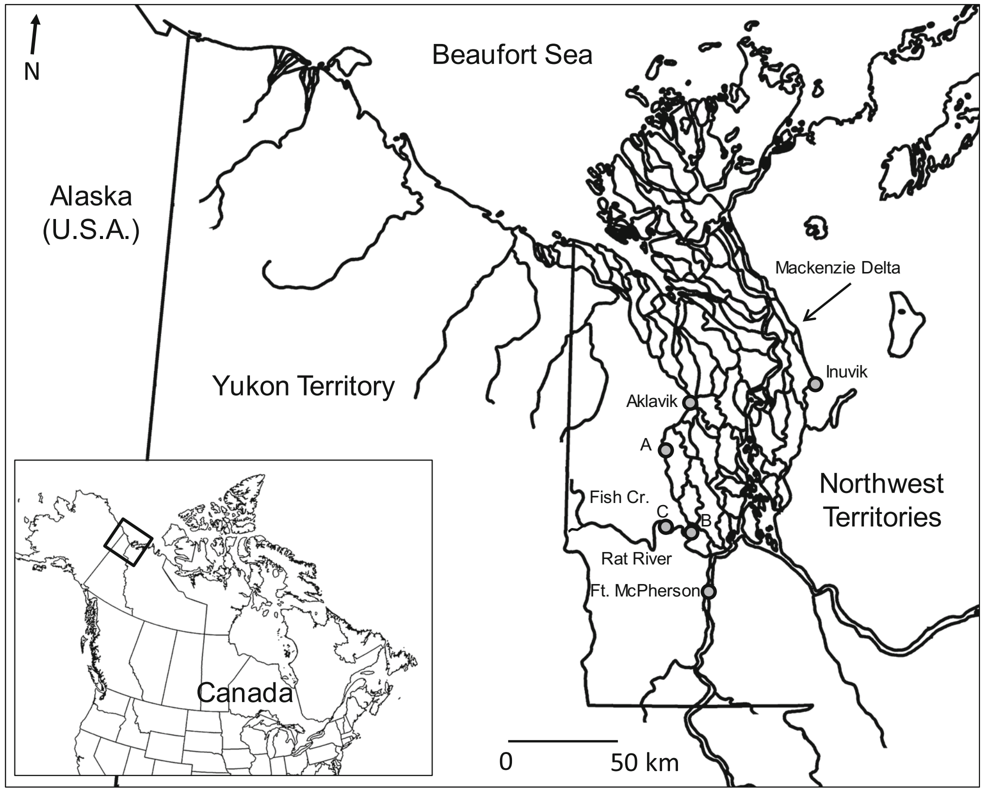

Table 1 Sample size of ageing structures and corresponding preparation method used for Dolly Varden captured at the sampling sites used for the Rat River Harvest Monitoring Program in 2007/2008

Sampling site	Otolith (whole)	Otolith (section)	Pectoral fin	Pelvic fin
Big Eddy	26/38	26/38	26/35	26/35
Rat River (mouth)	34/36	34/34	34/28	34/28
Destruction City	0/36	0/36	0/38	0/40
Total	170	168	161	163

one end of the epoxy (sulcus up and the rostrum pointing to that end) parallel to the long axis of the ellipse. After hardening for 1 week, the Parafilm® was removed and the embedded otolith was viewed (sulcus side down) under a dissecting microscope with a crosshairs micrometre eyepiece. The centre of the crosshairs was positioned over the nucleus, and a small dot was made there with an ultra-fine point Sharpie® marker. A section plane was chosen by selecting an area on the dorsal lobe of the otolith where annuli were clearly visible and widely spaced, close to the transverse plane. With the nucleus centred in the crosshairs, the eyepiece was rotated until the desired section plane was indicated along the crosshair, and then, a small dot was made on the epoxy on each side of the otolith. A Buehler® Isomet™ low-speed saw (Lake Bluff, Illinois, USA) with two Buehler® diamond wafering blades (101.6 mm × 0.3 mm) separated by a 0.5-mm spacer was used for sectioning. When looking down on the embedded otolith (sulcus side facing up) secured in the saw chuck, the two dots on both sides of the otolith, visible through the epoxy, were lined up directly over the right-side blade edge with the help of a magnifying lamp. The otolith block was then shifted slightly left so that the right blade cut the anterior half of the dots. Fin rays were embedded, sectioned (0.35 mm thick) and slide-mounted using methods consistent with Zhu et al. (2015).

Estimating ages from whole otoliths entailed placing the otolith in a small glass petri dish with distilled water. Annuli were read over a black background using a Leica (model MZ6) dissecting microscope (20–40× magnification) and reflected light. Annuli of otolith sections were read the same way as whole otoliths. Annuli of fin ray sections were read similarly, and only the slide was placed directly on the black base. Annuli were identified based on the criteria described in Chilton and Beamish (1982). Weak and incomplete translucent bands were considered to be false annuli (or "checks") (Chilton and Beamish 1982) and therefore not counted.

Two experienced age readers, one with more experience (reader 1) (22 years of ageing fish; previously aged >500 samples of Dolly Varden otoliths) than the other (reader 2) (6 years of ageing fish; no experience with Dolly Varden), both conducted three reads for each ageing structure. The only information available to the readers was date of capture, and each replicate read was done without knowledge of their prior ages or the other reader's ages.

Statistical analysis

The coefficient of variation (CV), a measure of precision (i.e. the reproducibility of the repeated measurement) expressed as a per cent, as described by Chang (1982):

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

where R is the number of times each sample was aged, X_j is the average age of the jth fish, and X_{ij} is the ith age estimate for the jth fish, was calculated for each age estimation method within and between readers. CV and the degree of precision are inversely related. Age bias plots were created to visualize bias within readers, between readers, and among pairs of age estimation methods separately for both readers. Age bias plots represent the mean age, with 95 % confidence intervals, of an age reader corresponding to each age category of a separate age reader, or a replicate from the first reader (Campana et al. 1995). CV values were not distributed parametrically and were therefore compared using a Mann–Whitney U test to determine whether there were significant differences between readers within preparation method/structures and within readers between preparation method/structures. Testing for differences between matched pairs of age estimates between readers for each age estimation method and among methods for each reader separately was done using the Wilcoxon rank test.

The average age estimate among three reads for each sample was calculated separately for both age readers among the four methods. Spearman's rank correlation (ρ) was used to quantify the comparability of age estimates among methods for each reader separately. Analysis of covariance (ANCOVA) was used to determine whether there were differences in the slope and intercept between readers for each method, and among methods for each age reader using the average value among reads. The slope and intercept were compared to those of a theoretical 1:1 line (complete agreement), where significant differences between slope and intercept would indicate inconsistency in the interpretation of annuli and a systematic difference between agers, respectively (Campana et al. 1995). Statistical tests were conducted using SPSS v.20, and results were considered significantly different if p values were <0.05.

Results

Within-reader and between-readers precision and bias

Summary statistics of the data among the age estimation methods demonstrated some differences both within and between readers, but overall otoliths tended to produce older age estimates compared to fin rays (Table 2). Otoliths had the highest precision for both readers; however, the level of precision varied by method and reader. For reader 1, age estimates from sectioned otoliths had higher mean precision (CV = 1.6 %) compared to whole otoliths (CV = 4.2 %) (Fig. 2). In contrast, for reader 2, precision was highest for whole otoliths (CV = 6.9 %) compared to sectioned otoliths (CV = 8.8 %). Fins had the lowest precision for both readers, with CV values ranging between 7.5 and 10.9 % (Fig. 2). The between-readers precision was always lower than within-reader precision, with the precision of reader 1 being significantly higher for all structures/preparation methods compared to reader 2 (all tests had $p = 0.005 < 0.0001$).

Reader 1 demonstrated little bias among replicate reads for sectioned and whole otoliths, although an increased bias was observed among age estimates ≥10 for whole otoliths (Fig. 2). Bias plots for reader 2 indicated relatively consistent replication among all age classes with whole otoliths, while there was a tendency to produce older and younger age estimates among age classes 4–5 and ≥7, respectively, in replicate reads of sectioned otoliths (Fig. 2). Increased bias was apparent for both fin rays read

Table 2 Mean and standard deviation (SD), median, and range of age estimates determined by readers 1 and 2 among age estimation methods of Dolly Varden from the Rat River

Reader	Ageing method	Mean (SD)	Median	Range
1	Otolith (whole)	5.4 (1.09)	5	3–10
	Otolith (section)	5.3 (1.06)	5	3–10
	Pectoral fin	4.3 (0.90)	4	2–10
	Pelvic fin	3.8 (0.95)	4	2–9
2	Otolith (whole)	5.4 (0.90)	5	3–9
	Otolith (section)	5.7 (0.86)	6	3–10
	Pectoral fin	4.7 (0.85)	5	2–8
	Pelvic fin	4.6 (0.59)	5	2–8

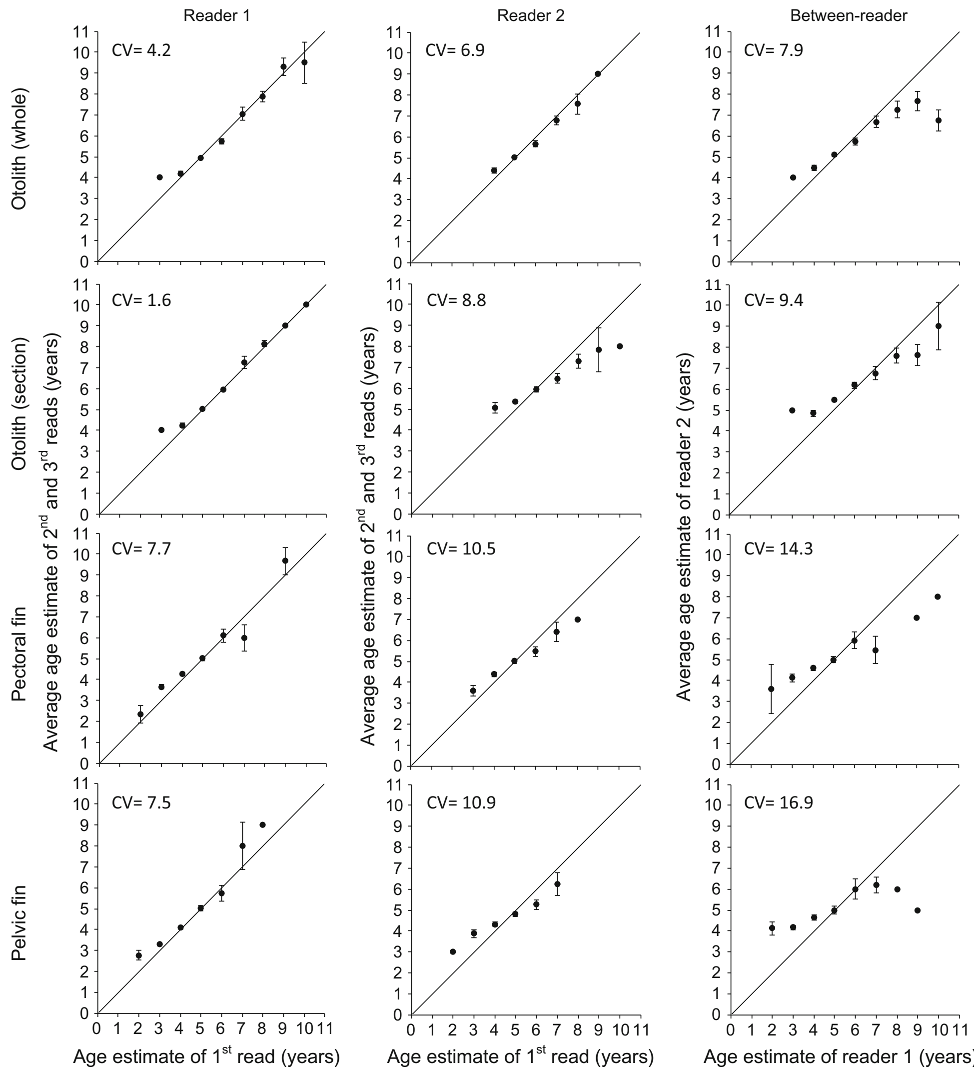

Fig. 2 Age bias plots within readers (reader 1 and reader 2) and between readers among age estimation methods. Each error bar represents 95 % confidence intervals from the average reading of the other reads (within-reader)/second age reader (between readers). *Solid line* indicates the 1:1 line. CV is the coefficient of variation

by reader 1 among age estimates ≥7; however, the average bias for pectoral fins did not appear to favour older or younger estimates, while a bias towards older estimates was observed for pelvic fins. The bias plots of reader 2 were similar between pectoral and pelvic rays where there was a tendency to obtain older and younger age estimates among age classes 2–4 and ≥6, respectively, in replicate reads (Fig. 2).

Bias plots used to infer age estimation difference between readers demonstrated that reader 2 consistently overaged younger (2–4 years) and underaged older (≥7–8 years) samples relative to reader 1 for all methods (Fig. 2). These biases were greater among older estimates. Pelvic fins appeared to have the largest degree of bias between readers regardless of age. Disparate results were obtained when testing for differences between readers for whole otoliths as the Wilcoxon rank test indicated that there were none ($Z = -0.39$, $p = 0.7$), while ANCOVA indicated higher age estimates by reader 1 based on significant differences in both the slope ($F = 77.9$, $p < 0.0001$) and intercept ($F = 73.1$, $p < 0.0001$). Results from the Wilcoxon rank test and ANCOVA showed that age estimates were significantly higher for reader 2 for sectioned otoliths, pectoral fins and pelvic fins (all tests had $p = 0.001 < 0.0001$).

Comparability

Comparisons among age estimation methods revealed a consistent pattern that was similar for both readers (Table 3; Fig. 3). Whole and sectioned otoliths were most comparable as there was relatively little bias between methods given that age estimates were highly correlated for reader 1 ($\rho = 0.89$) with a low CV (5.2 %), albeit moderately correlated ($\rho = 0.58$) with a relatively high CV (13.4 %) for reader 2. Wilcoxon and ANCOVA tests demonstrated significant differences (all test had $p = 0.01 < 0.0001$) among all possible pair-wise comparisons of age estimation methods within both readers.

Table 3 Mean coefficient of variation (CV), p value of Mann–Whitney U statistic, and Spearman's rank correlation coefficient (ρ) among pair-wise comparison of age estimation methods for Dolly Varden from the Rat River from two age readers

Comparison	Reader 1			Reader 2		
	CV (%)	p value	ρ	CV (%)	p value	ρ
Otolith (whole) versus otolith (section)	5.2	<0.0001	0.89	13.4	0.007	0.58
Otolith (whole) versus pectoral fin	19.5	0.0007	0.51	18.2	<0.0001	0.22
Otolith (whole) versus pelvic fin	26.1	0.0007	0.39	18.7	0.0004	0.22
Otolith (section) versus pectoral fin	18.6	<0.0001	0.54	20.3	0.14	0.29
Otolith (section) versus pelvic fin	28.0	<0.0001	0.43	23.7	0.07	0.28
Pectoral fin versus pelvic fin	40.6	0.93	0.61	34.9	0.62	0.55

Compared to sectioned otoliths, whole otoliths examined by reader 1 produced similar age estimates among all age classes observed with the exception of age 10 where younger estimates were observed using sectioned otoliths (Fig. 3). Reader 2 had more instances of bias between sectioned and whole otoliths among age classes, with age estimates from sectioned otoliths being older for ages 3–5 and younger for ages 8 and 9 relative to whole otoliths. Similar to reader 1, variation was highest for the oldest age class (9 years) observed from whole otoliths by reader 2.

Both whole and sectioned otoliths were poorly comparable to either type of sectioned fin ray as indicated by the low ρ values (reader 1: between 0.43 and 0.54; reader 2: between 0.28 and 0.29), high CV values (Table 3) and degree of bias (Fig. 3). Additionally, a considerable and significant bias towards younger age estimates was apparent for both fin rays relative to otoliths among ages $\geq$5 for both age readers (Fig. 3). Pelvic and pectoral fin rays were also not very comparable to one another, with pelvic fins being biased towards younger age estimates starting at age 5 for reader 1 (Table 3, Fig. 3).

Discussion

The results from our study revealed three key findings:

1. Otoliths had an overall better precision and smaller bias than fin rays, particularly for estimates $\geq$5 years of age, indicating otoliths are a better structure for estimating age and that fins are a poor non-lethal alternative for adult Dolly Varden.
2. There was little bias between otolith preparation methods for age estimates between 3 and 9 years for the more experienced age reader, although precision was lower for otoliths aged whole that were 9 years, suggesting either whole or thin-sectioned otoliths can be used up to this age.
3. Precision for all methods/structures was higher for reader 1 who had experience interpreting annuli from Dolly Varden otoliths, suggesting that training is an important prerequisite for estimating age for this species, even for those who are experienced with ageing other fish species.

Previous studies have shown that annuli read from the otoliths of other species, some of which are closely related to Dolly Varden and share similar life history characteristics, provide accurate age estimates (Beamish and McFarlane 1983; Hall 1991; Hining et al. 2000; Ross et al. 2005; DeCicco and Brown 2006; Campana et al. 2008; Zymonas and McMahon 2009). Assuming otolith age estimates for Dolly Varden are accurate, it is a positive outcome that precision was highest for otoliths, regardless of preparation method, for both age readers. Preparation method for otoliths has been shown to influence age estimation results with studies demonstrating sectioned otoliths produced older age estimates compared to whole (Beamish 1979; Chilton and Beamish 1982; Campana 1984; Stuby 2008). This is contrary to our results, based on reader 1 (the more experienced reader), where a higher frequency of older age estimates (10 years) was observed from whole otoliths ($n = 4$) compared to sectioned otoliths ($n = 3$), which is also evident when comparing the mean estimate from multiple reads of sectioned otoliths (8.25 years) among age 10 samples read using whole otoliths (Fig. 3). However, the wide 95 % confidence intervals of the within-method comparison for whole otoliths estimated at 10 years suggest low reproducibility/poor precision and are contrary to the high degree of precision/low bias observed for sectioned otoliths at this age (Fig. 2, reader 1).

The higher precision in age estimates from otoliths compared to fin rays in Dolly Varden, with rays producing younger ages than otoliths, is consistent with results from previous studies that have examined ages between both structures in other species (Sikstrom 1983; Sylvester and Berry 2006; Stolarski and Hartman 2008). Both readers had similar results in regard to the direction of the bias between otoliths and fin rays and also between pectoral and pelvic fin rays. Compared to both whole and sectioned otoliths, a linear bias was evident for both types of fin rays towards younger ages, particularly among estimates $\geq$5 years (based on results from reader 1), with instances among older age estimates where the difference was >4 years

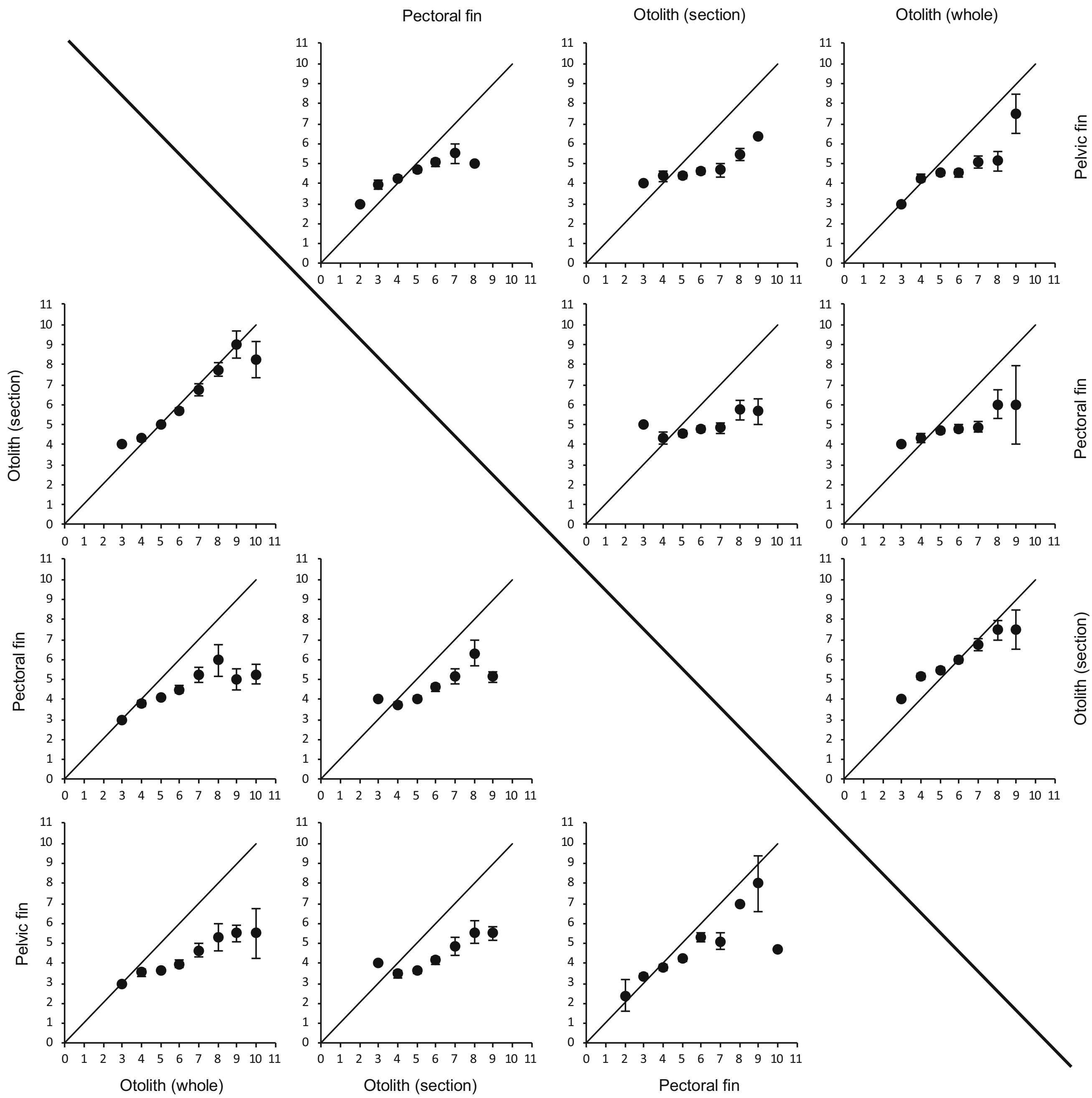

Fig. 3 Pair-wise comparison of average age estimates (years) and 95 % confidence intervals obtained from readers 1 (*below diagonal*) and 2 (*above diagonal*) among age estimation methods. *Solid line* indicates the 1:1 line

(Fig. 3), which is considerable for a relatively short-lived species such as Dolly Varden. Interestingly, results from both readers showed a large difference between pectoral and pelvic fin rays with pectoral rays producing older age estimates among most age classes. This suggests that annulus formation differs between fins and/or that annuli in pelvic fins are more difficult to interpret. These results suggest multiple rays should be examined simultaneously (e.g. Belanger and Hogler 1982) when attempting to validate ages from either pectoral or pelvic fin rays, or other fin rays. For Dolly Varden, we conclude that pectoral and pelvic fin rays are a poor alternative to otoliths and should not be used to estimate the age of fish ≥5 years (~≥390 mm fork length).

Our findings are corroborated by Stolarski and Sutton (2013) who conducted an age comparison study on Dolly Varden using thin-sectioned otoliths (0.3 mm) and pectoral fin rays (0.5–0.75 mm) and found that otoliths had the highest precision. Our results are also consistent with Barber and McFarlane (1987), who assessed age estimates among whole and break and burn otoliths, and anal fin rays from anadromous charr captured along the North Slope of Alaska [originally identified as Arctic charr *S. alpinus* (Linnaeus) which have since been confirmed as Dolly Varden (Reist et al. 1997)] (thin sections of rays were 0.5–1 mm), and Stolarski and Sutton (2013) who reported that pectoral fin rays generally resulted in lower age estimates compared to otoliths. Stolarski and Sutton (2013) reported otolith sections and pectoral fin ray sections were not comparable for Dolly Varden ≥6 years, a 1-year difference from our results (≥5 years).

The literature demonstrating the validity of otoliths for age estimation in many species, combined with the high precision observed with otoliths from Dolly Varden, supports their continued use. The more experienced reader (reader 1) in this study had the highest precision with sectioned otoliths, even among the oldest age classes, suggesting that this method would be the most favourable for estimating age. However, our data showed similar results between both otolith preparation methods for age estimates 3–9 for reader 1 based on the low level of bias and narrow 95 % confidence intervals (excluding age 9), while this was only evident among age estimates 5–8 for reader 2 (Fig. 3). Based on these observations, combined with the increased amount of time and cost required to embed and section otoliths (Hoyer et al. 1985; Long and Fisher 2001), we recommend that age readers could use whole otoliths for estimating age of Dolly Varden up to 8 years, but should embed and section otoliths for fish ≥9 years (>500 mm fork length). Given that Dolly Varden tend to be a relatively short-lived species, sectioning otoliths should not add significantly to the time and cost of estimating age (in the case of this study, fish ≥9 years of age only made up 2.4 and 0.39 % of the 510 reads of whole otoliths examined by reader 1 and reader 2, respectively).

Consistent differences between both readers suggest experience with this species is an important consideration when estimating the age of Dolly Varden, given that CVs were consistently lower for the experienced reader and among structures/preparation methods were greater between readers than within. The linear bias revealed that age readers were interpreting annuli differently, mainly among older age estimates for otoliths (≥8 and ≥9 years for whole and sectioned otoliths, respectively), and both young (<4 years) and older age estimates (approximately ≥7 years) for pectoral and pelvic fins (Fig. 2). The disparate results between Wilcoxon ranks and ANCOVA for whole otoliths between readers is a result of nearly equally distributed positive and negative differences between matched pairs from a sample where it appears that under- and overageing is occurring at opposite ends of the age range (Fig. 2), which limits the effectiveness of the test to demonstrate differences (Campana et al. 1995). In this situation, Campana et al. (1995) states that differences are more easily detected using regression (slope/intercept) analysis and examining bias plots, which, in our case, showed a significant difference. Given the level of experience, lower precision values and higher bias among replicate reads for reader 2 (excluding whole otoliths), we would be more confident using the results from reader 1. Our results reiterate the importance of experience when it comes to estimating age (Campana and Moksness 1991; Long and Fisher 2001; Edwards et al. 2011) and that age readers who plan to age anadromous Dolly Varden should not only ensure they have a consistent approach in regard to their interpretation of annuli but consult with an experienced reader and practice with a reference collection to identify possible biases.

In conclusion, the highest precision was observed using sectioned otoliths, rather than whole otoliths used in population assessments, by an experienced age reader suggesting this method would be optimal in estimating ages of Dolly Varden. However, bias plots indicate that either whole or sectioned otoliths could be used between ages 4 and 9 and that greater precision would be achieved by sectioning otoliths for fish ≥9 years (>500 mm fork length). Fin rays are a poor alternative to otoliths for Dolly Varden ≥5 years (∼≥390 mm fork length) and are consistently biased towards younger age estimates, although further study is required to confirm this, as a weakness of this study is the lack of samples of from ages 0 to 2 (based on results from otoliths) from this population. Assuming otoliths are an accurate ageing structure, our results show that the use of pectoral and, particularly, pelvic fin rays would yield higher estimates of mortality and length-at-age, and younger-age-at-maturity, producing flawed scientific advice for co-management partners. Further work is required in order to properly assess the use of whole versus sectioned otoliths (e.g. broader range of younger and older fish) in Dolly Varden and to validate annuli. Ideally, future sampling and comparison of ageing structures and techniques should be conducted on an additional population that is harvested less intensively than the Rat River in order to increase the probability of acquiring a better representation of older fish.

Acknowledgments We would like to thank John Carmichael, Selwyn Kay and Billy Wilson for collected the ageing structures for this study, and Kevin Bill (Fisheries and Oceans Canada, Inuvik) for assisting with administering the sampling program. Funding for this research was provided by the Department of Fisheries and Oceans.

Compliance with ethical standards

Conflict of interest None of the authors have a financial or non-financial conflict of interest regarding this research.

Human and animal rights The fish that were sampled were handled based on guidelines approved by the Canadian Council of Animal Care.

References

Armstrong RH, Morrow JE (1980) The dolly Varden charr, *Salvelinus malma*. In: Balon EK (ed) Charrs: salmonid fishes of the genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, pp 99–140

Barber WE, McFarlane GA (1987) Evaluation of three techniques to age Arctic char from Alaskan and Canadian waters. Trans Am Fish Soc 116:874–881

Beamish RJ (1979) Differences in the age of Pacific hake (*Merluccius productus*) using whole otoliths and sections of otoliths. J Fish Res Board Can 36:141–151

Beamish RJ, McFarlane GA (1983) The forgotten requirement for age validation in fisheries biology. Trans Am Fish Soc 112:735–743

Belanger SE, Hogler SR (1982) Comparison of five ageing methodologies applied to walleye (*Stizostedion vitreum*) in Burt Lake, Michigan. J Great Lakes Res 8:666–671

Campana SE (1984) Comparison of age determination methods for the starry flounder. Trans Am Fish Soc 113:365–369

Campana SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J Fish Biol 59:197–242

Campana SE, Moksness E (1991) Accuracy and precision of age and hatch date estimates from otolith microstructure examination. ICES J Mar Sci 48:303–316

Campana SE, Annand MC, McMillan JI (1995) Graphical and statistical methods for determining the consistency of age determinations. Trans Am Fish Soc 124:131–138

Campana SE, Casselman JM, Jones CM (2008) Bomb radiocarbon chronologies in the Arctic, with implications for the age validation of lake trout (*Salvelinus namaycush*) and other Arctic species. Can J Fish Aquat Sci 65:733–743

Chang WYB (1982) A statistical method for evaluating the reproducibility of age determination. Can J Fish Aquat Sci 39:1208–1210

Chilton DE, Beamish RJ (1982) Age determination methods for fishes studied by the groundfish program at the Pacific Biological Station. Can Spec Publ Fish Aquat Sci 60:1–102

COSEWIC (2010) COSEWIC assessment and status report on the Dolly Varden *Salvelinus malma malma* (Western Arctic populations) in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa

DeCicco AL, Brown RJ (2006) Direct validation of annual growth increments on sectioned otoliths from adult Arctic grayling and a comparison of otolith and scale ages. North Am J Fish Manage 26:580–586

Edwards WH, Stapanian MA, Stoneman AT (2011) Precision of two methods for estimating age from burbot otoliths. J Appl Ichthyol 27(suppl. 1):43–48

Eert JE, Michel C, Niemi A, Reist J, Williams WJ (2015) Physical, chemical and biological oceanographic data from the Beaufort Regional Environmental Assessment: Marine Fishes Project, August–September 2012. Can Data Rep Hydrogr Ocean Sci 197: 12p

Hall DL (1991) Age validation and aging methods for stunted brook trout. Trans Am Fish Soc 120:644–649

Harwood LA, Sandstrom S, Linn E (2009) Status of anadromous Dolly Varden (*Salvelinus malma*) of the Rat River, Northwest Territories, as assessed through sampling of the subsistence fishery (1995–2007). Can Manange Rep Fish Aquat Sci 2891: 52p

Hining KJ, West JL, Kulp MA, Neubauer AD (2000) Validation of scales and otoliths for estimating age of rainbow trout from southern Appalachian streams. North Am J Fish Manage 20:978–985

Howland KL, Gendron M, Tonn WM, Tallman RF (2004) Age determination of a long-lived coregonid from the Canadian North: comparison of otoliths, fin rays, and scales in inconnu (*Stenodus leucichthys*). Ann Zool Fenn 41:205–214

Hoyer MV, Shireman JV, Maceina MJ (1985) Use of otoliths to determine age and growth of largemouth bass in Florida. Trans Am Fish Soc 114:307–309

Kalish JM, Beamish RJ, Brothers EB, Casselman JM, Francis RICC, Mosegaard H, Panfili J, Prince ED, Thresher RE, Wilson CA, Wright PJ (1995) Glossary for otolith studies. In: Secor DH, Dean JM, Campana SE (eds) Recent developments in fish otolith research. University of South Carolina Press, South Carolina, pp 723–729

Long JM, Fisher WL (2001) Precision and bias of largemouth, smallmouth, and spotted bass ages estimated from scales, whole otoliths, and sectioned otoliths. North Am J Fish Manage 21:636–645

Muir AM, Sutton TM, Peeters PJ, Claramunt RM, Kinnunen RE (2008) An evaluation of age estimation structures for lake whitefish in Lake Michigan: selecting an ageing method based on precision and a decision analysis. North Am J Fish Manage 28:1928–1940

Penttila J, Nichy F, Ropes J, Dery L, Ambrose J (1988) Methods and equipment. In: Penttila J, Dery LM (eds) Age determination methods for northwest Atlantic species. NOAA/National Marine Fisheries Service, NOAA Technical Report NMFS 72, pp 7–16

Reist JD, Johnson JD, Carmichael TJ (1997) Variation and specific identity of char from Northwestern Arctic Canada and Alaska. Am Fish Symp 19:250–261

Ross JR, Crosby JD, Kosa JT (2005) Accuracy and precision of age estimation of crappies. North Am J Fish Manage 25:423–428

Roux M-J, Howland KL, Gallagher CP, Tallman RF (2012) Synthesis of biological and harvest information used to assess populations of northern form Dolly Varden (*Salvelinus malma malma*) in Canada. Part I: Rat River. DFO Can Sci Adv Sec Res Doc 2011/132

Sikstrom CB (1983) Otolith, pectoral fin ray, and scale age determinations for Arctic grayling. Progress Fish-Cult 45:220–223

Stolarski JT, Hartman KJ (2008) An evaluation of the precision of fin ray, otolith, and scale age determinations for brook trout. North Am J Fish Manage 28:1790–1795

Stolarski JT, Sutton TM (2013) Precision analysis of three aging structures for amphidromous Dolly Varden from Alaskan Arctic rivers. North Am J Fish Manage 33:732–740

Stuby L (2008) An evaluation of precision in age assessment of thin-sectioned and whole otoliths and the efficacy of using oxytetracycline as an aid in validating otolith annuli in burbot. In: Paragamian VL, Bennett DH (eds) Burbot ecology, management, and culture. American Fisheries Society Symposium 59, Bethesda, Maryland, pp 235–247

Sylvester RM, Berry CR (2006) Comparison of white sucker age estimates from scales, pectoral fin rays, and otoliths. North Am J Fish Manage 26:24–31

Zhu X, Wastle RJ, Howland KL, Leonard DJ, Mann S, Carmichael TJ, Tallman RF (2015) A comparison of three anatomical structures for estimating age in a slow-growing subarctic population of lake whitefish. North Am J Fish Manage 35:262–270

Zymonas ND, McMahon TE (2009) Comparison of pelvic fin rays, scales and otoliths for estimating age and growth of bull trout, *Salvelinus confluentus*. Fish Manage Ecol 16:155–164