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MANAGEMENT BRIEF

Precision Analysis of Three Aging Structures for Amphidromous Dolly Varden from Alaskan Arctic Rivers

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Abstract
The accuracy of population statistics and the validity of management actions they motivate are in part dependent on the acquisition of quality age determinations. Such data for northern-form Dolly Varden *Salvelinus malma* have been traditionally garnered using otoliths, despite little research investigating the consistency of this or alternative nonlethal techniques. To address these gaps, the precision of age determinations generated from scales, otoliths, and fin rays was examined for 126 amphidromous Dolly Varden collected from two Arctic rivers. Three independent readers, age-bias plots, coefficients of variation (CVs), and percent agreement (PA) were used to estimate bias and precision for among-reader, within-structure comparisons and within-reader, among-structure comparisons. Among-reader, within-structure tests of CVs suggested that otoliths produced more precise age determinations than fin rays. Furthermore, the CV for scales was intermediate to and not significantly different from those for otoliths and fin rays. Age-bias plots suggested that, scales consistently underestimated age relative to otoliths beginning at age 6. Underestimation was also apparent, but less distinct, within fin ray–otolith and scale–fin ray comparisons. Potential sources of error and management implications are discussed. Because scale and otolith ages exhibited little bias within cohorts younger than age 6, age may be determined nonlethally in these cohorts using scales; otoliths should be used otherwise.

Northern-form Dolly Varden *Salvelinus malma*, hereafter referred to as Dolly Varden, are distributed along the Arctic coast of North America from the Mackenzie River in Canada, west and south through Alaska to the Seward Peninsula (Reist et al. 1997). Throughout their range, populations are largely organized by major river basin, which may contain both resident and sea-run individuals (McCart 1980; Everett et al. 1997). Amphidromous fish are generally larger and more abundant than resident fish and support one of the largest and most important traditional subsistence fisheries in the Arctic coastal communities of Alaska (McCart 1980; Pedersen and Linn 2005). Concerns regarding the potential ecological impacts of oil and gas exploration and climate change in the Arctic have strengthened the need for sound management and monitoring practices (Hachmeister et al. 1991; Reist et al. 2006). The validity of these practices depends in part upon the acquisition of quality age determinations, as they are often developed using age-specific biological data.

Northern fish species, such as Dolly Varden, are typically aged using calcified structures due to their longevity and slow rates of growth (McCart 1980; Howland et al. 2004). For a structure to be useful for age determination, it must produce ages that are both accurate (not addressed herein) and precise. Dolly Varden age is almost exclusively estimated by using otoliths, either viewed whole or broken through the nucleus (Heiser 1966; Yoshihara 1973; Armstrong 1974; McCart 1980; Underwood et al. 1995). Scale techniques have thus far been largely disregarded because research within Arctic Char *S. alpinus* suggests that scale circuli patterns are unreliable predictors of fish age (Barber and McFarlane 1987; Baker and Timmons 1991); fin ray techniques have rarely been used (Heiser 1966; Barber and McFarlane 1987). Nonlethal techniques using scales and fin rays conserve fish and allow age data to be collected from a greater proportion of individuals within a population. This may be particularly advantageous when aging Dolly Varden as the length

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ranges of successive cohorts typically display considerable overlap (Underwood et al. 1995). Such overlap can contribute to error in age-specific biological statistics when extrapolating age data from a subsample to a larger population, as is often the case when using age–length keys. However, before nonlethal techniques can be employed, the precision of age determination generated from lethal (otoliths) and nonlethal (scales and fin rays) techniques must be compared.

In the only study we could find that investigated the reproducibility of age determinations for Dolly Varden, Barber and McFarlane (1987) concluded that otoliths generally produced older age determinations relative to those based on pectoral and anal fin rays. However, this research did not use scales to determine age; used only a single reader, which limited analyses to comparisons among structures; and was conducted on pooled samples containing both Dolly Varden and Arctic Char (Reist et al. 1997). As a result, the precision of Dolly Varden aging techniques both within and among structures remains poorly defined. The objective of the present study was to address this data gap by estimating the precision of scale, otolith, and fin ray age determinations for within- and among-structure comparisons.

STUDY SITE

This study was conducted at spawning and overwintering habitats of amphidromous Dolly Varden on the Ivishak and Hulahula rivers, located on the coastal plain of the Alaskan Arctic (Figure 1; Daum et al. 1984; Viavant 2005). The Ivishak River is a north-flowing tributary of the Sagavanirktok River, the second largest basin on the North Slope of Alaska. Both rivers originate in the Brooks Mountain range and drain into the Beaufort Sea: the Sagavanirktok River at Prudhoe Bay and the Hulahula River near the coastal community of Kaktovik. Both rivers contain resident and amphidromous populations of Dolly Varden.

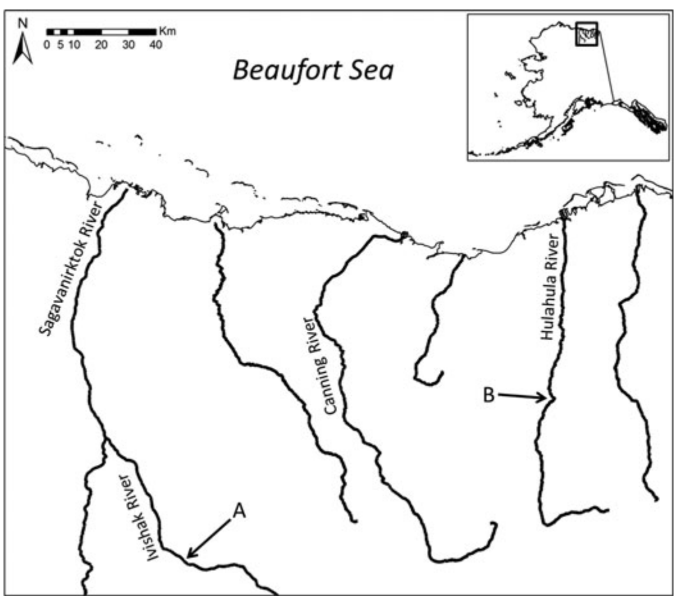

FIGURE 1. Map of the Eastern North Slope of Alaska with arrows indicating the general locations where postsmolt (**A**) and presmolt (**B**) Dolly Varden were sampled from the Ivishak and Hulahula rivers, respectively.

METHODS

Fish sampling.—Postsmolt Dolly Varden were captured via angling from the Ivishak River during sampling events in early September of 2009, 2010, and 2011. Presmolt fish were collected using minnow traps from the Hulahula River during August 2011. Sampling exclusively within habitats known to be frequented by large numbers of amphidromous fish minimized the likelihood of capture and inclusion of resident fish into the study. Upon capture, individuals were killed, weighed to the nearest 1 g, and measured for fork length to the nearest 1 mm. Each fish was individually labeled, wrapped in plastic, and transported to University of Alaska Fairbanks, where they were frozen. In the laboratory, scales were sampled with a scalpel from an area posterior to the dorsal fin and above the lateral line and stored on waterproof paper (DeVries and Frie 1996). The right pectoral fin was removed from each fish, rinsed in water, and stored on waterproof paper in a well-ventilated area to facilitate drying. Sagittal otoliths were removed using the "open hatch" method of Secor et al. (1992), rinsed in water, dried, and stored dry in individually labeled plastic vials.

Structure preparation.—Fifteen to 20 scales from each fish were wet-mounted on a glass slide and viewed with a compound microscope under transmitted light at 40× magnification. After the subsample was screened for the presence of regenerated scales, an image of a representative scale was captured using a microscope-mounted 3.3 megapixel digital camera (Quantitative Imaging Co., Burnaby, British Columbia).

Fin rays were embedded in Epoxycure epoxy resin (Buehler, Lake Bluff, Illinois) following methods outlined in Koch and Quist (2007). Multiple transverse sections, each 0.5–0.75 mm thick, were cut using an Isomet low-speed saw (Buehler), equipped with a 102-mm-diameter diamond wafering blade rotating at 240 revolutions per minute. Care was taken to assure that the first thin section encompassed or was slightly posterior to the inflection point of the ray (Beamish 1981). Sections were affixed to a glass slide using Crystalbond thermoplastic cement (Structure Probe, Inc., West Chester, Pennsylvania) and viewed with a compound microscope. A digital image was captured of a representative fin ray at 20× and 40× magnifications under transmitted light.

The right sagittal otolith of each fish was affixed to a glass slide using Crystalbond thermoplastic cement perpendicular to the long axis of the otolith. Each otolith was ground to the core in the transverse plane using a thin-section machine (Hillquist, Inc., Denver, Colorado) and remounted to the slide flat side down before being ground to a final thickness of approximately 0.3 mm. The otolith was hand-polished with a 1-μm diamond abrasive and viewed with a compound microscope under transmitted light. Digital images were captured at 20× and 40× magnifications. If the mounted otolith section was deemed inadequate for age determination, the left sagitta was processed in the same fashion.

Age determination.—Age determinations were produced by three independent readers trained in annulus identification. Each reader estimated fish age from scales, otoliths, and fin rays with knowledge of the capture date of the fish, but not the fish length. Scale annuli were identified as areas of greater circuli density or when successive circuli cut over each other. Annuli in fin ray and otolith sections were identified as alternating opaque and hyaline zones (DeVries and Frie 1996). Fin ray–based age estimates were derived from the first or second ray. Images were organized into separate libraries by structure, and no reader was allowed to determine age from multiple libraries within a single day. To our knowledge, scale, otolith, and fin ray age determinations for Dolly Varden have yet to be validated.

Statistical analysis.—Age-bias plots were used to assess among-reader, within-structure and within-reader, among-structure bias (Campana et al. 1995). Age-bias plots depict the mean age of fish determined by one reader that have been assigned a given age by a second reader. Cohorts displaying complete agreement among ages assigned by each reader will fall on the 1:1 line of equivalence. Thus, bias is detected visually as persistent (lasting more than two successive years) deviations of 95% confidence intervals surrounding each mean from the line of equivalence. Detection of among-reader, within-structure bias is important as it indicates whether readers are using unified criteria to identify and count annuli. If one or multiple readers consistently over- or underestimate age relative to other readers, precision within that structure will reflect variability in aging methods rather than the reproducibility of age determinations. If bias is detected, the criteria by which annuli are identified and counted must be revisited and agreed upon by readers, and ages must be redetermined. Alternatively, within-reader, among-structure bias can be used to evaluate the relative strength of any under- or overestimation of ages between the techniques. Precision of among-reader, within-structure and within-reader, among structure comparisons was estimated with percent agreement (PA), percent agreement to within 1 year (PA1), and coefficient of variation (CV). Percent agreement statistics were calculated as the number of pairwise comparisons in which age was in total agreement (in the case of PA) or the number of pairwise comparisons in which age was in agreement to within 1 year (in the case of PA1), divided by the total number of comparisons made. Percent agreement statistics, once the predominant means of accessing the precision of aging structures are slowly being replaced with statistics such as CV, as the latter measures do not account for age structure variation between species (Beamish and Fournier 1981). As such, these statistics are mentioned only briefly and included primarily as a means of comparison with past research. Coefficient of variation was calculated as

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

where X_{ij} is the ith age determination for the jth fish, X_j is the mean age of the jth fish, and R is the number of times the age of the fish is estimated (Chang 1982). Coefficient of variation was averaged across all fish for each structure in the case of among-reader, within-structure comparisons and across specific comparisons (i.e., scales versus fin rays) for within-reader, among-structure comparisons. Potential differences among structures and comparisons were tested using analysis of variance (ANOVA) with a post hoc Tukey's honestly significant difference test when significant differences were detected. All statistical analyses were conducted using the statistical software package R (R development Core Team 2012) and evaluated at an $\alpha = 0.05$.

RESULTS

Of the total 143 pre- and postsmolt Dolly Varden collected over the 3 years of sampling, 126 were included into the final analyses. Individuals ranged in fork length from 63 to 680 mm and encompassed ages 0–14 years (Figure 2). Annuli were identified from digital images for all three structures (Figure 3). Scale circuli patterns varied substantially between the freshwater and marine periods of growth, with the marine phase exhibiting far greater spacing between successive circuli. Visual examination of among-reader, within-structure age-bias plots showed little persistent (i.e., did not last more than two consecutive years) deviation from the 1:1 equivalence line, indicating that readers used similar standards in identifying and counting annuli (Figure 4). Mean PA and PA1 of among-reader, within-structure comparisons were similar among structures (Table 1). However, mean PA did not exceed 55% for any structure, while mean PA1 exceeded 90% for all structures (Table 1). Age-bias plots of within-reader, among-structure comparisons indicated that scales began to underestimate fish age relative to otoliths beginning at age 6 (Figure 5); moreover, errors increased with age. Age-bias plots also indicated that fin rays tended to underestimate age relative to otoliths and that scales tended to underestimate age relative to fin rays. For both of these comparisons, underestimation generally began at age 6 and remained somewhat constant as age increased (Figure 5). However, trends within scale–fin ray and otolith–fin ray comparisons were less distinct relative to scale–otolith comparisons. Mean PA and PA1 of within-reader, among-structure comparisons were similar among comparisons but were generally lower than among-reader, within-structure estimates (Table 1). No differences in CV were detected for within-reader, among-structure

TABLE 1. Coefficient of variation (CV), mean percent agreement (PA), and mean percent agreement to within 1 year (PA1) of among-reader, within-structure and within-reader, among-structure comparisons of age determinations based on scales, otoliths, and fin rays for Dolly Varden sampled from the Ivishak and Hulahula rivers. Within each comparison type, CV estimates with different lowercase letters are significantly different ($P < 0.05$) following ANOVA with a post hoc Tukey's test.

Comparison type	Structures	CV	Mean PA	Mean PA1
Among-reader, within-structure	Scales	9.08 zy	55.91	94.35
	Otoliths	7.91 y	55.02	94.18
	Fin rays	11.91 z	52.38	94.18
Within-reader, among-structure	Scale–otolith	14.28 z	33.87	81.18
	Scale–fin ray	14.11 z	40.05	81.74
	Otolith–fin ray	13.59 z	33.07	83.33

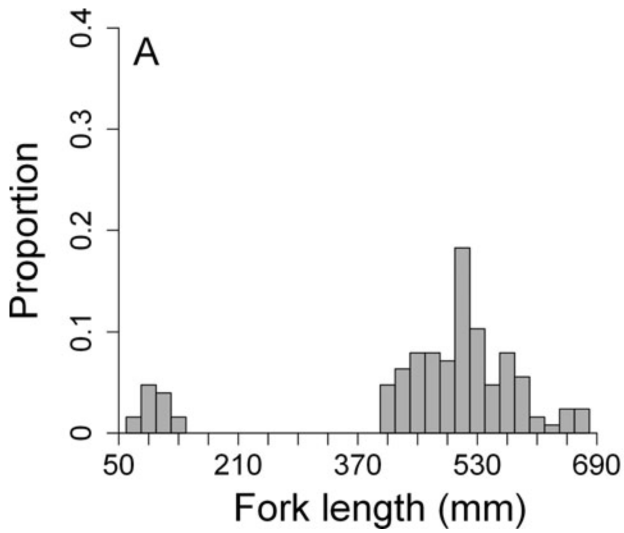

FIGURE 2. Composite length (**A**) and age (**B**) data plotted as a function of sample proportion for Dolly Varden collected from the Ivishak and Hulahula rivers between 2009 and 2011. Age data in **B** were derived from otoliths.

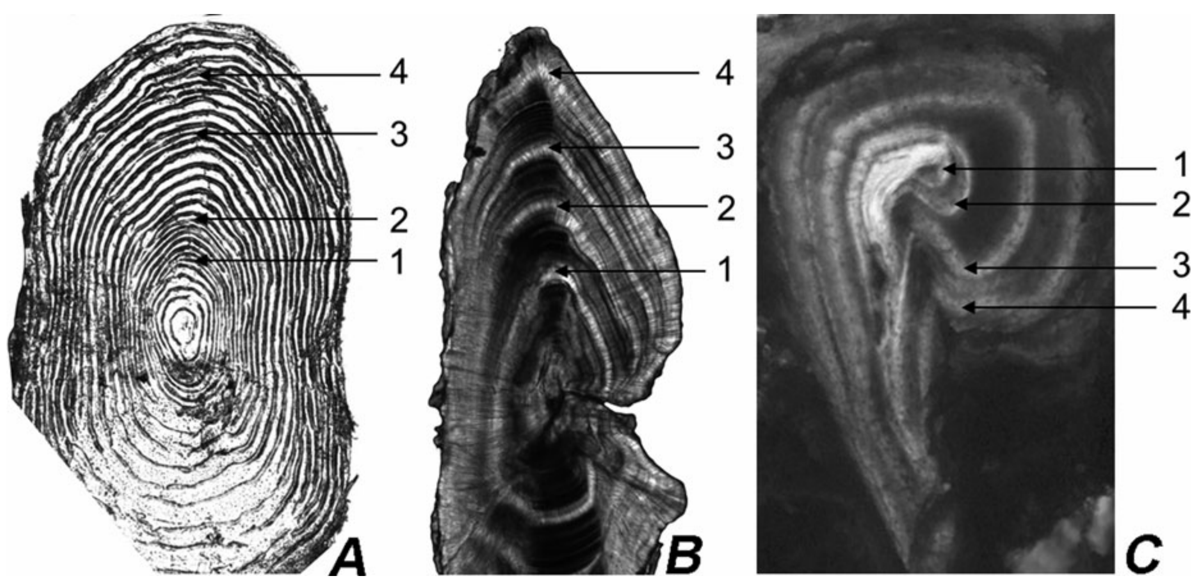

FIGURE 3. Digital image of an amphidromous, northern-form Dolly Varden scale (**A**), otolith (**B**), and fin ray (**C**) collected from the Ivishak River, Alaska. Each structure depicts four annuli. Note the "cutting over" of scale circuli at labeled annuli and the proximity of the first and second annuli on the fin ray.

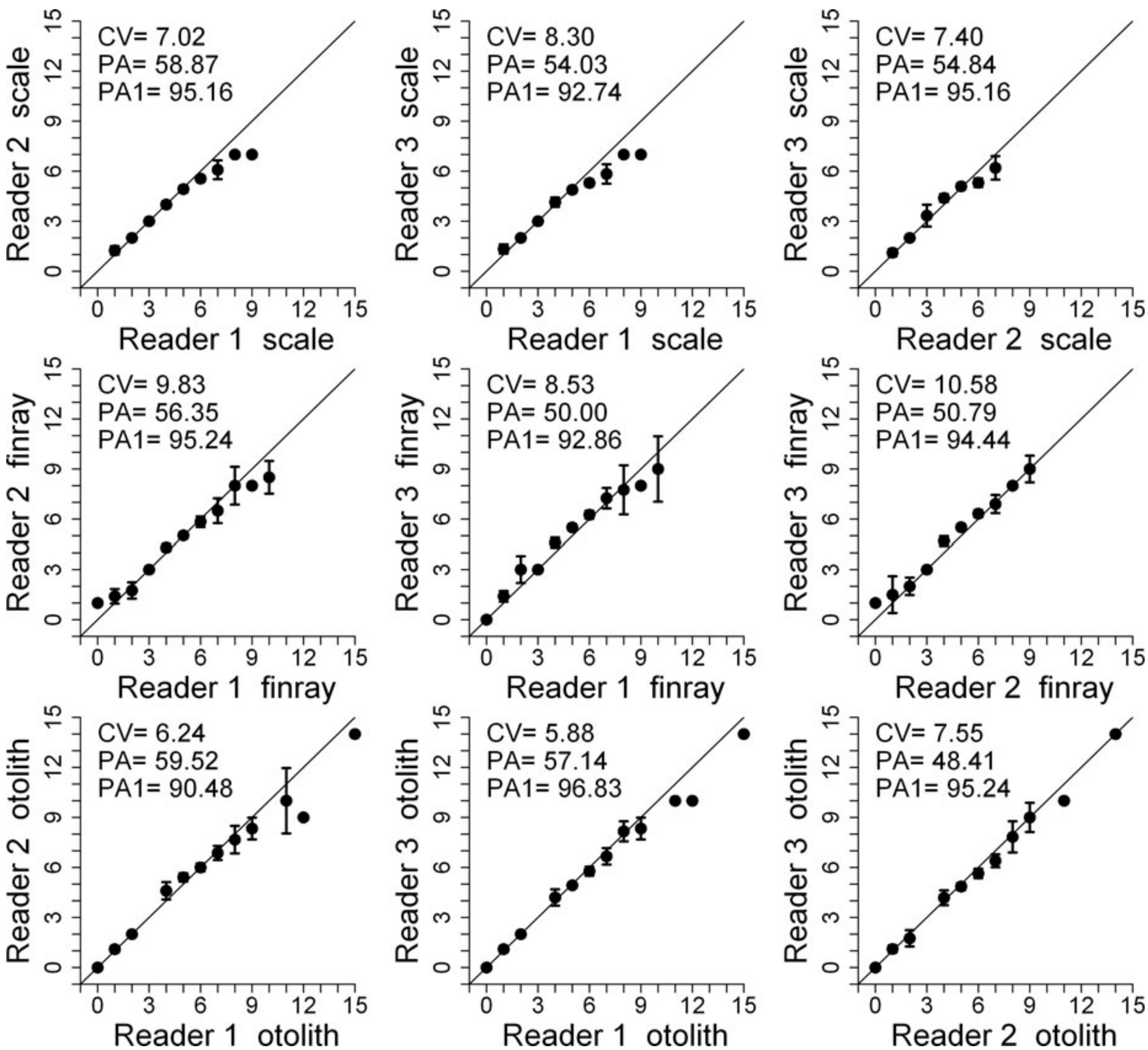

FIGURE 4. Age-bias plots with pairwise estimates of coefficient of variation (CV), percent agreement (PA), and percent agreement to within 1 year (PA1) for among-reader, within-structure comparisons of scale, otolith, and fin ray age determinations for Dolly Varden collected from the Ivishak and Hulahula rivers. Error bars represent 95% confidence intervals (for points with multiple observations) around the mean age assigned by one reader relative to all fish assigned a given age by a second reader (Campana et al. 1995).

comparisons (Table 1; $F_{2,373} = 0.347$, $P = 0.707$). Coefficient of variation of among-reader, within-structure comparisons differed among structures (Table 1; $F_{2,373} = 3.143$, $P = 0.044$). The post hoc Tukey test indicated that otoliths were more precise predictors of Dolly Varden age than fin rays were.

DISCUSSION

This research contributes to a growing body of literature indicating that scales typically underestimate fish age relative to otoliths (Sikstrom 1983; Hubert et al. 1987; Sharp and Bernard 1988; Graynoth 1996; Kruse et al. 1997; DeCicco and Brown 2006; Stolarski and Hartman 2008). The onset of scale underestimation corresponded well with estimates of the age at first reproduction for Dolly Varden, suggesting that underestimation was a result of ontogenetic reductions in growth and the formation of a “dense edge” on the scale margins (Nordeng 1961; Yoshihara 1973; Craig and Halderson 1981). A similar artifact was often present in the interior of the scale, probably a result of slow presmolt growth while in freshwater (McCart 1980). These features highlight the importance of training readers in identification of both freshwater and marine annulus because scale morphology and annuli appearance will change depending upon the growth rate of the fish (Carlander 1974).

Within-reader comparisons of fin ray and otolith age determinations suggested that fin rays underestimated age relative to otoliths, beginning at age 6. Barber and McFarlane (1987) noted similar results studying age determinations from a mixed sample

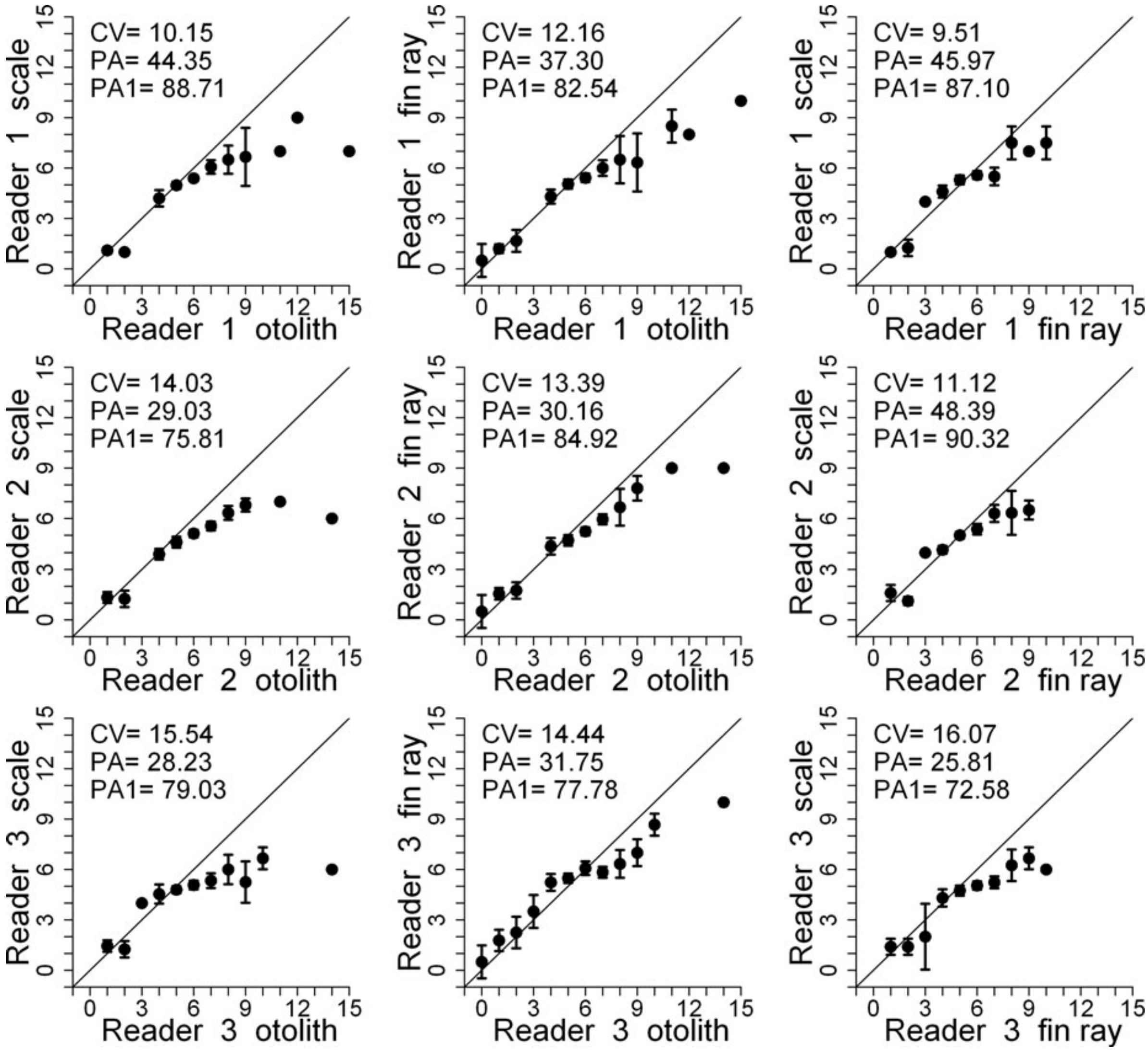

FIGURE 5. Age-bias plots with pairwise estimates of coefficient of variation (CV), percent agreement (PA), and percent agreement to within 1 year (PA1) for within-reader, among-structure comparisons of scale, otolith, and fin ray age determinations for Dolly Varden collected from the Ivishak and Hulahula rivers. Error bars represent 95% confidence intervals (for points with multiple observations) around the mean age assigned by one reader relative to all fish assigned a given age by a second reader (Campana et al. 1995).

of Dolly Varden and Arctic Char. Fin ray underestimation has also been reported in Arctic Grayling *Thymallus arcticus* (Sikstrom 1983), Rainbow Trout *Oncorhynchus mykiss* and Brown Trout *Salmo trutta* (Graynoth 1996), Cutthroat Trout *O. clarkii* (Hubert et al. 1987), and Brook Trout *Salvelinus fontinalis* (Stolarski and Hartman 2008). However, Zymonas and McMahon (2009) reported no bias between comparisons of ages derived from pelvic fin rays and those derived from otoliths of Bull Trout *Salvelinus confluentus*. Chronic misidentification of the first few annuli in fin ray sections is commonly cited as a potential cause of underestimation (Sikstrom 1983; Hubert et al. 1987). Working with a population of slow-growing White Suckers *Catostomus commersonii*, Beamish (1973) noted that the first fin ray annulus was often too closely associated with the ray center to be consistently identified, particularly within older fish. For Dolly Varden, interior annuli clarity was often diminished within older fish and declined as the location where the fin ray section was cut moved further from the inflection point of the ray. The effect of the latter phenomenon was minimized by deriving ages from one of the first three fin ray sections of the series. However, evidence of more constant errors within older fish suggests that misidentification of freshwater annuli could be occurring within these cohorts.

Within-reader comparisons of scale and fin ray age determinations suggested that scales often underestimated fish age, again beginning at age 6. However, this relationship was less pronounced relative to scale–otolith and fin ray–otolith comparisons. Previous research involving similar comparisons has been generally inconsistent, with some studies confirming (Sikstrom 1983; Stolarski and Hartman 2008) and others refuting (Hubert et al. 1987; Copeland et al. 2007) our results. Given suspected sources of error within each of the two structures (see above), the lack of a consistent trend could be a function of the proportion of instances in which reader errors are isolated within a single structure versus when errors occur simultaneously in both.

Percent agreement of among-reader, within-structure comparisons was generally low compared with previous research reports (Graynoth 1996; Zymonas and McMahon 2009). However, such interspecies comparisons are made difficult by the fact that PA varies as a function of the age structure of the species in question (Beamish and Fournier 1981). The low PA seen here could be a result of the age structure of the sample (Beamish and Fournier 1981; Zymonas and McMahon 2009), the use of multiple readers instead of multiple reads by the same reader (Ihde and Chittenden 2002), the relatively slow growth rates of high-latitude fishes (Sikstrom 1983), or some combination of the three. Percent agreement of among-reader, within-structure comparisons of scales, otoliths, and fin rays for species with similar age structures such as Arctic Grayling and Bull Trout range between 50% and 67% and are comparable with PA observed in Dolly Varden (Sikstrom 1983; Zymonas and McMahon 2009). Despite these contentions, PA1 was greater than 90% for all structures, suggesting that gross disagreements in Dolly Varden age were infrequent.

Tests of among-reader, within-structure CV suggested that otoliths were more precise estimators of Dolly Varden age than fin rays. Similar results have been reported in other species and are likely a result of misidentification of interior annuli, as previously discussed (Graynoth 1996; Stolarski and Hartman 2008; Zymonas and McMahon 2009). However, the mean CV of age determinations garnered from scales and otoliths were found to be similar, which is contrary to the findings of numerous studies reporting that, among all samples, scale-based age determinations are less precise indicators of fish age than are otoliths (Sikstrom 1983; Kruse et al. 1997; DeCicco and Brown 2006; Zymonas and McMahon 2009; Schill et al. 2010). This finding may be a direct result of the intensity and duration in which Dolly Varden grow throughout the year. Dolly Varden acquire nearly 100% of their annual energy budget during the short Arctic summers, spending the remainder of the year overwintering in freshwater, where little to no food is consumed (Craig 1984; Boivin and Power 1990). Before reaching reproductive age, the intensity of growth within these periods and the consistency with which they occur probably contribute to the distinctiveness of scale annuli. The annulus formed following the first migration to the sea is particularly distinguishable due to the contrast between it and the adjacent freshwater annuli. The consistency of annulus formation in scales stemming from this seasonal pattern probably rivals that of otoliths over the same time interval and contributes to the similarity in precision observed between those two structures. However, if our sample had contained a larger proportion of older fish, our results might have differed.

This research suggests that Dolly Varden age may be determined nonlethally using scales within individuals age 5 and younger. Our assertion is a result of our findings that bias of within-reader, among-structure comparisons of scales and otoliths is minimal within cohorts younger than age 6. Furthermore, no statistical differences in CV calculated from among-reader, within-structure comparisons of scale and otolith age determinations were detected. However, beyond age 5, otoliths should be used to generate age determinations for Dolly Varden. The majority of Dolly Varden research and monitoring projects have been conducted within nearshore coastal areas using fyke nets. These catch data suggest that Dolly Varden distribute themselves along the shore according to size, with the smaller individuals occupying shallower habitats closer to shore (Craig and Halderson 1981; Hachmeister et al. 1991; Underwood et al. 1995; Fechhelm et al. 1997; Brown 2008). Age data collected from random subsets of this catch indicate that up to 70% of the individuals are younger than age 6 (Underwood et al. 1995). While age composition probably varies over time and space, it is reasonable to assume that many Dolly Varden captured in nearshore fyke nets can be aged nonlethally using scales. Scale-based age determination may be particularly valuable for identifying first year smolts. This demographic is often pooled for analysis purposes and can be easily and quickly identified using scales because of the contrast between freshwater and marine circuli patterns (Fechhelm et al. 1997). Smolt identification has been previously accomplished using graphical methods; however, these techniques are not as successful when sampling locations are distant from river mouths (Fechhelm et al. 1997; Brown 2008). Nonlethal age determination will also allow age data to be collected from a greater proportion of the population, which may increase the precision of age-specific statistics. However, it is always important to independently verify the consistency of scale- and otolith-based age determinations in the field within a subset of fish prior to implementation of a particular technique.

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