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MANAGEMENT BRIEF

Investigating Nonlethal Age Estimation in a Lentic Brook Trout Population

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Abstract
Routine lethal sampling on small, conservation, or threatened populations may be unsustainable. Therefore, determining if alternative, nonlethal structures can provide accurate age data is important. We evaluated nonlethal structures (scales and pectoral fin rays) in conjunction with otoliths collected from lentic Brook Trout *Salvelinus fontinalis* in Washington State. We used age-bias plots, percent agreement, and coefficient of variation to determine how scale or fin ray age estimates compared with otolith estimates, which had been validated in a previous study. Prior experience with aging specific structure types was the primary variable affecting age agreement with validated otolith-determined age among three age readers. In general, fin rays and scales did not consistently reflect otolith age. Between-structure coefficient of variation was 17.8–19.1% for scales and fin rays, and between-structure percent agreement (within 1 year) was 80–82%. Individual percent agreement within 1 year was highest (92% and 86% for fin rays and scales, respectively) and coefficient of variation was lowest (9.0% and 14.8%, respectively) for the most experienced reader. Agreement of age estimates between readers improved with experience aging specific structures. Our study suggests that otolith data remains the preferred option.

Fisheries managers must have a firm understanding of population dynamics—characterized by a combination of vital rates (growth, mortality, recruitment)—all of which depend heavily on age data (e.g., Cattanéo et al. 2002; Berkeley et al. 2004; Venturelli et al. 2010). As a result, managers can interpret age-structured populations to create successful, science-based regulations and goals (Arlinghaus et al. 2016; Kerns and Lombardi-Carlson 2017). Unless a fish has been monitored for its entire life cycle, knowing for certain that age estimates match the "true" age of the fish is difficult. Age validation studies aim to gauge whether the estimated age matches the true age of the fish. Such studies are important because age estimate disparities exist between structures from the same fish and across species (e.g., Quist et al. 2007; Herbst and Marsden 2011; Watkins et al. 2015). Structures should also be verified (age verification) to evaluate how precise measurements are across structures and individuals estimating fish age (hereby referred to as "readers"). Both validation and verification studies are important when used in sustaining small, conservation, or threatened fisheries because obtaining some calcified structures (e.g., otoliths, vertebrae) is lethal, and managers might be restricted to use nonlethal structures. Fisheries managers who utilize age structures that have not been validated or verified risk mismanaging valuable resources (e.g., Yule et al. 2008; Harry 2018).

Brook Trout *Salvelinus fontinalis* is a species that could benefit from nonlethal age estimation studies. In the

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eastern United States, Brook Trout are a species of concern (Hudy et al. 2008; DeWeber and Wagner 2015) and efforts are underway to restore and conserve their populations in this region (Poplar-Jeffers et al. 2009; EBTJV 2018).

Brook Trout have been aged using scales (Stolarski and Hartman 2008), and otolith age estimates have been validated using mark–recapture techniques (Hall 1991). One study found that ages estimated from lotic Brook Trout scales match otolith age for young fish (≤ age 2) but not older fish (Stolarski and Hartman 2008), whereas other studies have found conflicting results. For example, Magnan and Fitzgerald (1983) identified 92% agreement between scale and otolith ages, but Kozel and Hubert (1987) documented 27% agreement. Additionally, Stolarski and Hartman (2008) found that fin rays produced more variable age estimates than scales and suggested that more research be conducted on Brook Trout fin rays, particularly due to success with other salmonid species (e.g., Shirvell 1980; Silkstrom 1983). Most recently, Parsley (2017) identified between-reader agreement of age estimates of 77% and 78% for both otoliths and fin rays, respectively. Evidently, past verification studies on scales, otoliths, and fin rays provide inconsistent conclusions.

One consistent factor across all studies is that lotic Brook Trout were evaluated. Many factors, including geographic location, longevity, spawning, and growth rates, can influence annulus formation in calcified structures in fishes (Phelps et al. 2017). For salmonids, lentic populations typically grow faster than their lotic counterparts, which may be influenced by differences in productivity or the mean prey size available (Keeley and Grant 2001; Stolarski and Hartman 2010). Consequently, annulus formation mechanisms can cause precision to vary, especially across structures, readers, and fish populations (Phelps et al. 2017). To date, age estimates on lentic Brook Trout have not been evaluated.

The objectives of this study were to (1) determine the efficacy of nonlethal structures (fin rays and scales) to age lentic Brook Trout and (2) investigate the precision and readability of nonlethal structures compared with otoliths (grinding along sagittal plane) among readers with varying experience levels. Moreover, evaluating nonlethal aging and sampling alternatives may provide a more sustainable approach than lethal methods towards managing Brook Trout fisheries.

METHODS

Data collection.— Brook Trout were sampled from Owhi Lake, a mesoeutrophic lake (surface area = 202 ha, maximum depth = 23.5 m) located on the Confederated Tribes of the Colville Reservation in north-central Washington State. The Owhi Lake fishery consists of a naturally reproducing Brook Trout monoculture supplemented by hatchery stocking. Brook Trout in Owhi Lake range from 280 to 480 mm total length during summer and live up to 7 years (Taylor 2016).

Brook Trout were sampled in early November 2015 with gill nets and boat electrofishing to capture all length-classes. Total length (mm) and weight (g) were measured, and five fish per centimeter size-class were collected (minimum number suggested by Quist et al. 2012).

From each fish, sagittal otoliths, pectoral fin rays, and scales were removed for aging. Otoliths were collected following the Schneidervin and Hubert (1986) method, briefly cleaned in a bleach solution (Secor et al. 1992), and stored dry in microcentrifuge tubes. Otoliths were placed sulcus-side up, adhered to a microscope slide, sectioned via grinding, and viewed under a dissecting microscope at 30× magnification (Maceina 1988; Stolarski and Hartman 2008). Left pectoral fin rays were removed using flush cutters and placed in coin envelopes to air dry. Each cut was made proximal to the body and included the first three rays. When dried, fin rays were fixed in an epoxy resin similar to methods used by Koch and Quist (2007). A Buehler Isomet low-speed saw equipped with a diamond blade was used to section each sample (~0.725 mm thick). To improve clarity, each section was ground with 600 and 800 grit sandpaper, immersed in cedar oil contained within a viewing apparatus (Smith et al. 2016), and viewed with a dissecting microscope at 50× magnification. Scales were removed above the lateral line and behind the dorsal fin and wet-mounted on a microscope slide. Three nonregenerated scales were selected to determine fish age and viewed under a compound microscope at 45× magnification. Given that fish were sampled in the fall, annuli on the structure's edge were not counted.

All structures were photographed using a digital microscope camera and read one time by three readers (i.e., single read). Each image was edited in photo-editing software to sharpen and adjust light levels (Campana et al. 2016). To remove bias by matching structures derived from the same fish, all photos were randomized, which prevented readers from pairing structures from the same fish. Two of three readers produced all age estimates based on digital photographs. Additionally, length data were not provided to readers and readers were asked to age each structure independently. Hall (1991) validated otolith annulus formation for some age-classes, so we assumed otolith age to be reflective of true age for age-verification purposes. Given this assumption, any disagreements in otolith age estimates were reviewed together to discuss and finalize estimates. When comparing between-structure age estimates, otolith estimates were made independently first and then a consensus age estimate was assigned to make these comparisons similar to most aging estimation methods (e.g., Erhardt and Scarnecchia 2013;

Watkins et al. 2015). Readers were also asked to assign confidence ratings to each structure as a measure of readability (Spiegel et al. 2010). Ratings ranged between 0 (absolutely no confidence) and 3 (complete confidence).

Lastly, aging experience was documented in this study to determine if familiarity with specific structures introduced bias in results (e.g., Campana 2001; Faust et al. 2013). Reader 1 had experience aging fish from otoliths, scales, and fin rays, while readers 2 and 3 had experience aging only otoliths or scales, respectively. Experience level (i.e., time spent aging; seasons) varied substantially by reader. Reader 1 had approximately 11 seasons of experience estimating otolith age from Brook Trout and Hawaiian Goby *Lentipes concolor*, 1 season estimating fin ray age from Brook Trout, and 10 seasons for Brook Trout and kokanee *Oncorhynchus nerka* scale age. Reader 2 had approximately two seasons of experience estimating otolith age from Brook Trout and Rainbow Trout *Oncorhynchus mykiss*. Reader 3 had nine seasons of experience estimating scale age from kokanee. Based on previous studies documenting aging experience bias (Campana 2001; Faust et al. 2013), the reader's experience aging fin rays in this study is minimal (0–1 seasons); therefore, these results should be interpreted with caution.

Analyses.— Age-bias and Bland–Altman plots were used to evaluate systematic bias between structure ages (Campana et al. 1995; Ogle 2016). These plots create a visual representation of age estimates from readers for each structure compared with the estimated true age and can be used to assess between-reader and between-structure bias. Bland–Altman plots were modified with a generalized additive model to explain potential nonlinear relationships (Wood 2017). Additionally, the Evans–Hoenig test was used to evaluate bias based on the main diagonal symmetry of an age-agreement table (Evans and Hoenig 1998). The Evans–Hoenig test performs well under most conditions (i.e., different sample sizes and types of asymmetry) and was therefore selected to test our data (McBride 2015). Precision was estimated using percent agreement (PA [0 or exact]; ±1-year agreement) and coefficient of variation (CV) metrics. As noted by Ogle (2016), CV was defined as

$$\mathrm{CV} = 100 \times \frac{\sum_{j=1}^{n} \frac{s_j}{\bar{x}}}{n},$$

where s_j is the standard deviation of R, R is the number of readers per age estimate for jth fish, and $\bar{x}_j$ is the mean age for jth fish. To assess differences in readability, the Kruskall–Wallis test was used to evaluate median confidence rating differences between structures ($\alpha = 0.05$), and if significant differences ($P < 0.05$) existed, a Dunn's post hoc multiple comparisons test was used to determine which structure's readability was significantly different.

RESULTS

A total of 157 Brook Trout were collected, ranging from 124 to 474 mm total length. All fish were aged using otoliths and fin rays, and one fish was removed from scale age estimation due to regenerated scales. Estimated ages ranged from 1 to 7 years for otoliths and fin rays and from 0 to 7 years for scales. Two of three readers did not estimate fish age below age 1.

For most readers, between-structure age-bias plots depicted deviations from the linear 1:1 relationship between fin rays and otoliths (Figure 1). An Evans–Hoenig test for bias was significant ($P \leq 0.05$) for fin ray estimates by readers 2 and 3. Reader 1 fin ray estimates remained near the 1:1 line for all age-classes as evident by the lack of a significant difference between fin ray and otolith age estimates across all ages. Percent agreement and CV were 62% (±1 year = 92%) and 9%, respectively. Both reader 2 and reader 3 fin ray estimates were more variable than reader 1 estimates, characterized by low PA (36–37%) and high CV (20–28%). Fin ray estimates for reader 2 were consistent with otolith age for fish < age 5 but underestimated otolith age for fish ≥ age 5, while reader 3 fin ray age overestimated otolith age for young fish (age 1 to age 3) and underestimated older fish age (age 6 through age 7).

For scales, age-bias estimates occurred for all readers. Scale age estimates for age-1 fish were higher than otolith age, and depending on the reader, age-2 and age-3 fish were also higher (Figure 1). All scale age estimates for fish ≥ age 5 underestimated otolith age. An Evans–Hoenig test for bias was only significant for reader 3 ($P \leq 0.05$). Scale PA was highest for reader 1 (47%, ±1 year = 86%), and scale CV was lowest for reader 1 (15%). Cumulatively, PA was marginally higher for scales than for fin rays and CV was lower for scales than for fin rays (Table 1).

The Evans–Hoenig test for between-reader bias was significant ($P \leq 0.05$) for all readers across nonlethal structures (Figure 2). In general, fin ray estimates were similar between readers 1 and 2 up to age 5, but reader 2 underestimated age for fish ≥ age 6 (Figure 2). Readers 1 and 2 fin ray PA was 41% (±1 year = 85%), and CV was 25%. All other between-reader fin ray comparisons were dissimilar and variable (Figure 2). Readers 2 and 3 exhibited lower PA (27%; ±1 year = 71%) and higher CV (27%) than other reader comparisons.

Between-reader age-bias plots for scales indicated that reader 1 and reader 3 had comparable age estimates, while other plots depicted strong biases between readers (Figure 2). Scale PA was highest for readers 1 and 3 (58%, ±1 year = 93%), and CV was 12%. As a whole, between-reader PA

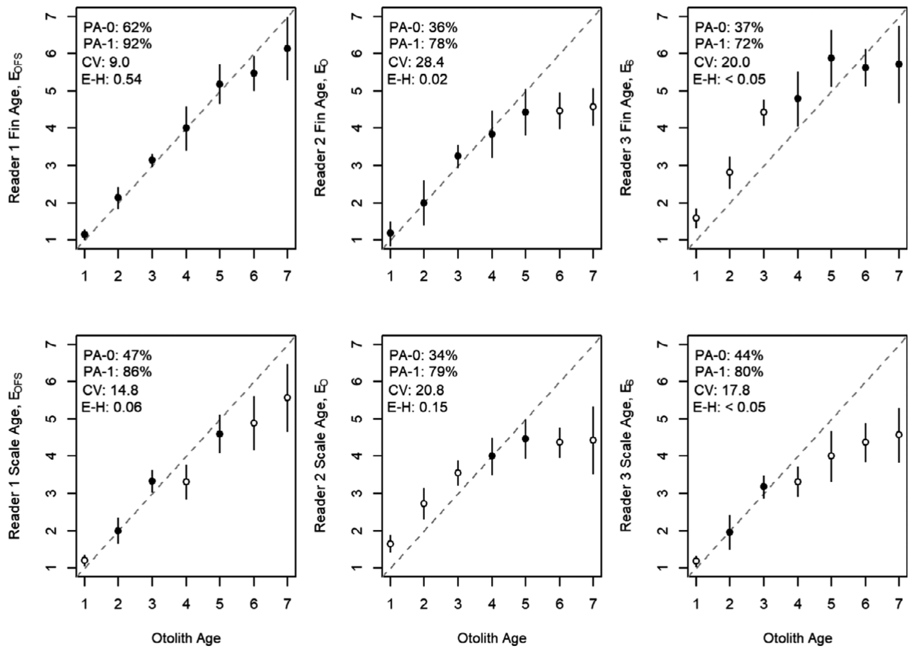

FIGURE 1. Age-bias plots comparing consensus otolith age versus estimated fin ray age (top) and scale age (bottom) for three different readers (left, middle, and right columns) with 95% confidence intervals. The gray dashed line indicates a 1:1 unbiased relationship between structures. Solid points indicate no significant difference between nonlethal structure to otolith age; open circles indicate a significant difference ($\alpha = 0.05$). Abbreviations are as follow: PA-0 = exact age agreement, PA-1 = ±1-year agreement, CV = coefficient of variation, E-H = P for Evans–Hoenig test for symmetry, E = experience type (O = otolith, F = fin ray, S = scale).

was highest for scales. Across all structures, average between-reader bias was lowest for scales and highest for otoliths (Table 1). Scale PA-0 was 49%, while otolith PA-0 was 28%.

Reader's confidence in estimating age across all structures was not significantly different ($\chi^2 = 1.34$, df = 2, $P = 0.51$), suggesting that readers were equally confident in reading and estimating age across structures. However, reader's confidence for otolith age estimates was significantly different ($\chi^2 = 104.6$, df = 2, $P < 0.05$). Reader 2 confidence (mean = 2.14; SD = 0.58) was significantly lower than that for reader 1 (mean = 2.71; SD = 0.62) and reader 3 (mean = 2.47; SD = 0.84). Scale confidence ratings were not significantly different across readers ($\chi^2 = 3.8$, df = 2, $P = 0.15$), with means ranging from 2.51 to 2.67. Fin ray reader confidence was significantly different across readers ($\chi^2 = 42.7$, df = 2, $P < 0.05$). Confidence of reader 3 (mean = 2.15; SD = 0.93) in estimating age from fin rays was lower than that for reader 1 (mean = 2.69; SD = 0.61) and reader 2 (mean = 2.69; SD = 0.49).

TABLE 1. Average percent agreement (PA) and coefficient of variation (CV) for each comparison and Brook Trout age structure. Abbreviations are as follows: PA-0 = exact age agreement and PA-1 = ±1-year agreement; values in parentheses are the standard error of mean estimates. Individual estimates can be found on Figures 1 and 2.

Comparison	Structure	PA-0	PA-1	CV
Between otolith	Fin ray	45 (8.5)	80 (5.9)	19.1 (5.6)
Between otolith	Scale	42 (4.0)	82 (2.2)	17.8 (1.7)
Between reader	Fin ray	35 (3.9)	78 (4.1)	23.4 (2.3)
Between reader	Scale	49 (4.1)	90 (1.6)	15.1 (1.8)
Between reader	Otolith	28 (1.6)	66 (1.5)	28.2 (2.0)

DISCUSSION

A comparative review of precision studies from many aging laboratories suggested that a CV ≤ 5% is typical for fishes of moderate longevity and complex structure readings, but median CV may be as high as 7.6% (Campana 2001). In our study, reader CV was 9–28% for fin rays and 15–21% for scales. These ranges suggest that fin rays and scales are beyond the reported average CV guidelines for precision studies and should not be used instead of otoliths as an alternative age structure given the variability around age estimates. While fin ray PA-1 was high for the most experienced reader (92%, reader 1), short-lived species of conservation concern should be evaluated with PA-0, which was low for all readers (36–62%). Age estimate variability was partially explained by readability (Table A.1 in the Appendix) and age-class differences (Table A.2). In general, confidence ratings declined with increasing age for readers 1 and 3, while reader 2 was equally

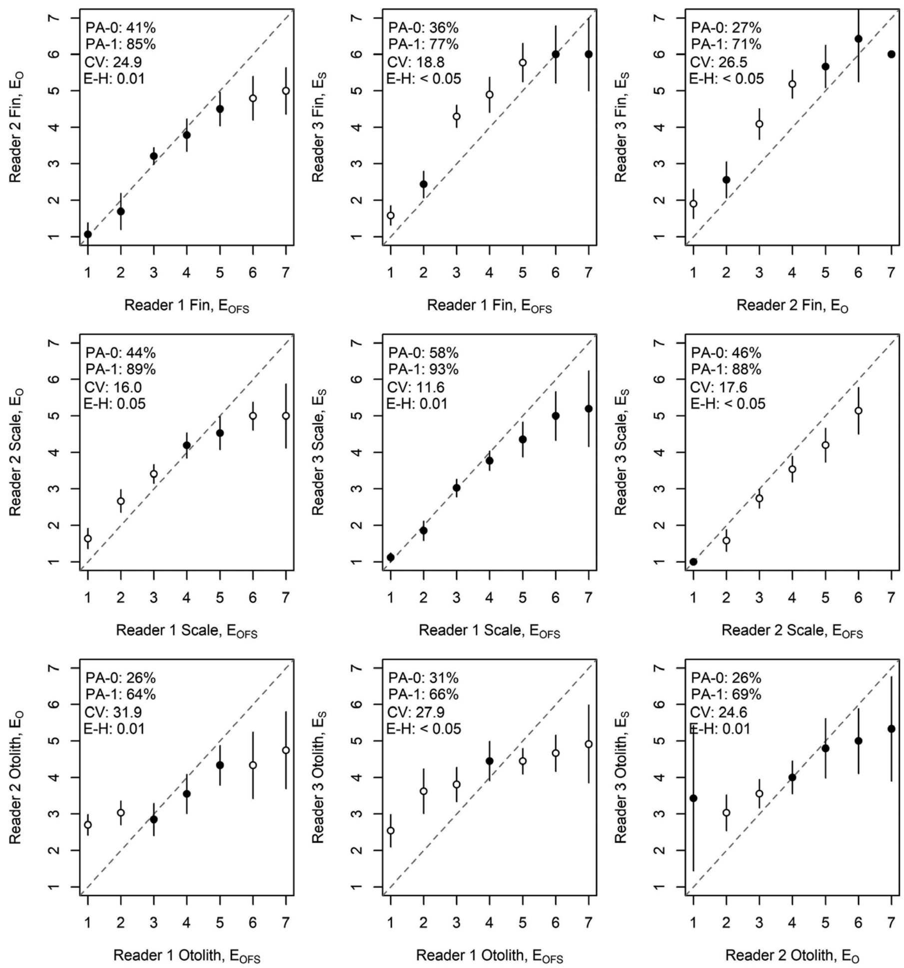

FIGURE 2. Age-bias plots comparing between-reader bias for fin rays (top), scales (middle), and otoliths (bottom) with 95% confidence intervals. The gray dashed line indicates a 1:1 unbiased relationship between structures. Solid points indicate no significant difference between reader age estimates; open circles indicate a significant difference ($\alpha = 0.05$). Abbreviations are as follow: PA-0 = exact age agreement, PA-1 = ±1-year agreement, CV = coefficient of variation, E-H = P for Evans–Hoenig test for symmetry, E = experience type (O = otolith, F = fin ray, S = scale).

confident across all age-classes, excluding otoliths. For PA-0 and CV, young (≤ age 2) and old (≥ age 6) fish had the highest CV and lowest PA-0 across all readers, especially readers 2 and 3. Young and old fish are typically the most difficult to age (Campana 2001), and these difficulties may result in underestimating or overestimating age-classes, ultimately inflating the metrics (e.g., mortality, growth, age at maturity) used to dictate management objectives (Buckmeier et al. 2017). The effects from using PA-1 on short-lived species compared to long-lived species would be more impactful since fewer age-classes are used to calculate vital rates. However, by removing older fish, PA-0 in our study only increased 4–6%, thereby suggesting the relative effect to analysis was minimal. Therefore, young fish may have influenced age estimate variability the most due to difficulties in identifying the first annuli.

A potential problem some readers had aging structures can be explained by reviewing between-reader bias for otoliths. Brook Trout otoliths were validated by Hall (1991) and are assumed to represent true age (fish size <225 mm fork length, ages 2–10, lentic system; Hall 1991). Despite using a structure that has been selected to reflect the true age of the fish, our results suggest a lack of complete agreement in otolith age estimates. Average CV was highest for otoliths (all reader comparisons were >24%; Table 1), and age estimates from readers experienced with otoliths (readers 1 and 2) were not comparable. In fact, reader 2 has the highest CV for all structures when age estimates were compared with consensus age (Table A.2). Two seasons of experience aging otoliths may not provide sufficient experience given the high degree of variation and low confidence in age estimates.

Although scale age was more consistent across readers, we found a nonlinear relationship existed; age was consistently underestimated for fish > age 5 compared with the otolith-derived estimated age (Figure 1; Figure A.1 in the

Appendix). Two of the three readers agreed that age-2 and age-3 fish could be aged with scales, but most other age-classes did not match otolith estimates. These results are congruent with Stolarski and Hartman (2008), who suggested that scale age matched otolith age for young fish (≤ age 2) and fin ray estimates had more variability than scales or otoliths. Considering our results relative to similar studies, we conclude that scales and fin rays produce variable age estimates relative to otolith age and researchers should not use either structure to estimate Brook Trout age. However, these results may be influenced by reader bias.

To our knowledge, four studies have reported Brook Trout age precision and bias (Magnan and Fitzgerald 1983; Kozel and Hubert 1987; Stolarski and Hartman 2008; Parsley 2017). Parsley (2017) found that between-reader average CV was lowest for fin rays and otoliths and highest for scales (~6% and 10%, respectively). Exact percent agreement between readers ranged from 62% to 78%. Stolarski and Hartman (2008) reported similar ranges of average CV and PA, though Parsley (2017) assessed more age-classes. Our study reports a between-reader average CV larger than these authors and a PA-1 similar to Stolarski and Hartman (2008). Parsley (2017) did not report PA-1 for comparison. We identify two main differences between these studies and ours: (1) we assessed fish spanning more size-classes (<475 mm total length) and (2) our study focused on Brook Trout in a lentic environment. This gives rise to potential differences in annulus formation (i.e., longevity and growth rate) and may influence variability in age estimates. Salmonid growth is typically faster in lakes than in streams (Keeley and Grant 2001; Stolarski and Hartman 2010), and growth differences in other species occupying lotic and lentic habitats have been also been observed (Freshwater Drum *Aplodinotus grunniens*, Paukert and Fisher 2001; Paddlefish *Polyodon spathula*, Rypel et al. 2006). Growth rate differences can influence annulus formation in calcified structures in fishes (Phelps et al. 2017); therefore, age precision differences from previous studies to the present study seem reasonable, especially since precision can vary between readers (Hurley et al. 2004) and fish populations (Edwards et al. 2005). However, published literature on how environmental factors influence annulus formation is limited and should be further researched (Phelps et al. 2017). As demonstrated here, reader experience with Brook Trout calcified structures in these studies may also have contributed to observed differences between previous studies.

With respect to measured CV differences, the recommendation of 5.0–7.6% median CV by Campana (2001) was consistent with published age precision and bias studies across species (Quist et al. 2007; Zymonas and McMahon 2009; Herbst and Marsden 2011; Erhardt and Scarnecchia 2013; Watkins et al. 2015), including Brook Trout (Stolarski and Hartman 2008; Parsley 2017). Our results do not fall within this range, and the differences may be explained by reader's past experience and/or the inability to correctly interpret annuli. Fin ray age estimates relative to consensus otolith estimates depicted a significant bias. Reader 1, who was more familiar with aging Brook Trout structures, had the highest PA and the least variation around mean estimates. Reader 2 was more familiar with aging otoliths but only had two seasons of aging experience. The highest PA for scales was between reader 1 and reader 3; both readers documented at least nine seasons of aging salmonid scales. Our results suggest that readers with a similar experience aging a particular structure (e.g., readers 1 and 3, scales) tend to have more agreement than those who did not have prior experience.

Therefore, we emphasize that when conducting an age precision and bias study, the readers should have previous experience with all the structures being aged. Buckmeier et al. (2017) suggested that comprehensive training programs for age estimation were rare, and agencies often assign inexperienced readers to age fish. Readers need to interpret hundreds to thousands of calcified structures to produce accurate age estimates, and this needs to occur with prepared, referenced structures to provide immediate feedback (Morison et al. 2005; Buckmeier et al. 2017). Although readers in this study had variable experience aging, they may not have had enough to provide accurate age estimates. This is evident when reviewing between-reader age-bias plots for otoliths (CV > 25%, PA-0 > 31%), and these results could be expected with agencies that do not have comprehensive training programs. Nonetheless, the variable aging experience between readers was an inherent weakness of this study.

One noteworthy characteristic of both age-bias and Bland–Altman plots is that they often fit linear regressions to potentially nonlinear relationships. For example, departure from the 1:1 relationship for older fish was observed (Figure 1), especially for reader 2. When coupled with Bland–Altman plots, generalized additive models may be more applicable in explaining overall relationships as opposed to age-class differences between age comparisons (D. Ogle, Northland College, personal communication; Wood 2017). Bland–Altman plots evaluate bias and precision of an entire sample as opposed to specific age-classes (e.g., age-bias plots) and are considered an alternative to age-bias plots (McBride 2015). With the addition of a generalized additive model, bias is not assumed to be constant across age-classes and can be modeled with a smoothed nonlinear relationship (Ogle, personal communication). Our modified Bland–Altman plots for between-structure comparisons depicted similar results to our age-bias plots (Figures 1 and A.1). Departures from zero (i.e., the difference between nonlethal structures and otoliths) were still present for the same age-classes identified by age-bias plots. Therefore, modified Bland–Altman plots can be used reasonably as an alternative to Bland–Altman and age-bias plots.

Reader 1 estimates were based directly on structures via microscope. Noting reader 1 CV for scales and fin rays between structures, their estimates were less variable and more precise than other readers. Buckmeier et al. (2017) summarized the difficulties in using digital media, noting reduced resolution and an inability to manipulate lighting, focal plane, and structure orientation. Decreased precision and higher variability between a reader's age estimates were likely caused by an interplay between reader experience and the use of digital media.

Managers overseeing conservation, subsistence, or sport fisheries cannot afford mismanagement resulting from aging errors (e.g., Yule et al. 2008). Brook Trout fin rays may be an alternative to otoliths if readers have considerable experience aging fin rays. Yet, with the high reader bias in this study, further studies to address this issue will be necessary before definitive methodology is implemented. Fin rays should be revisited once readers have ample training and experience aging all Brook Trout age structures, especially given previous success aging salmonids with fin rays (e.g., Shirvell 1980; Silkstrom 1983). The current body of research suggests that Brook Trout otoliths remain the only viable age structure. Moreover, differences in between-reader estimates may be linked to reader familiarity with structure types, experience, and limitations caused by using digital media. Age-precision studies have infrequently quantified reader's experience, often stating "experienced readers were used," "readers had several years of experience," or previous experience was not mentioned (Stolarski and Hartman 2008; Spiegel et al. 2010; Herbst and Marsden 2011; Erhardt and Scarnecchia 2013; Koch et al. 2018). Moreover, few studies clarify reader's past experience by structure type. Brook Trout should be aged using recommendations made by Buckmeier et al. (2017), especially using a known-age age structure library to train and evaluate readers. Future studies should consider the following prior to evaluating Brook Trout age precision: (1) select readers with ample aging experience (hundreds to thousands of age structures) and determine which specific structure types and fish species readers previously evaluated, (2) quantitatively document reader's experience levels to evaluate performance (e.g., total structures, weeks, months, years aging), (3) obtain access to reference (i.e., known age) samples to intermix with unknown-age samples to gauge reader's accuracy, and (4) compare reader's accuracy with reference samples to determine if reader's experience influenced results. Furthermore, a study documenting a reader's accuracy with increasing levels of instruction and how precision metrics change as a result of increased training is warranted. Monitoring a reader's accuracy aging structures with increasing levels of training would allow agencies to estimate how much training is required to obtain a certain range in accuracy and precision.

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Appendix: Additional Data

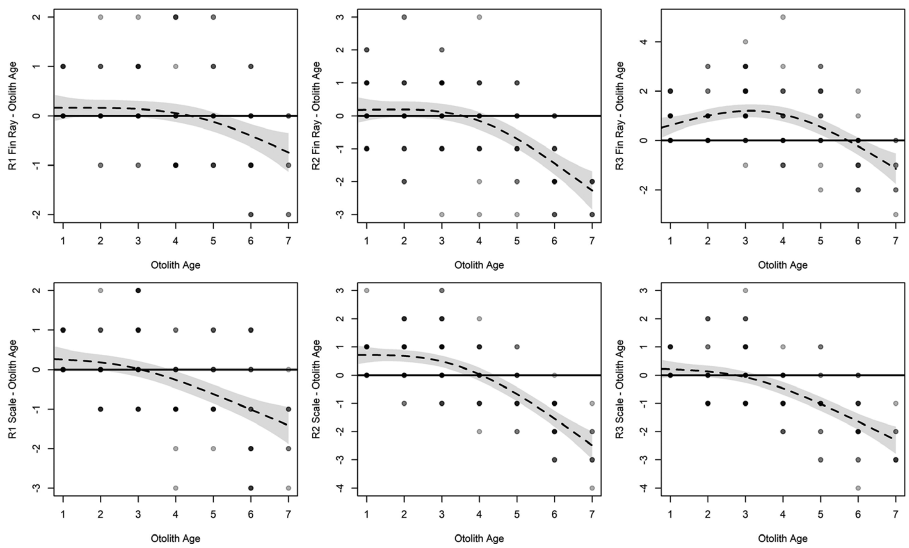

FIGURE A.1. Modified Bland–Altman plots depicting differences between fin ray (top) and scale (bottom) estimated age and otoliths for three readers (R1–R3; left, middle, and right columns, respectively). The solid black line depicts no difference in age estimates, the dashed line indicates the general trend created by a generalized additive model, the gray area depicts the 95% confidence region for the age estimates, and the point color represents overlapping points (i.e., darker colors indicate more overlapping points).

TABLE A.1. Confidence ratings for lentic Brook Trout by reader, structure, and age-class. Values in parentheses are standard deviations.

Structure	Age-class	*n*	Reader 1 confidence	Reader 2 confidence	Reader 3 confidence
Otolith	1	34	2.91 (0.29)	2.33 (0.48)	2.85 (0.44)
	2	22	2.95 (0.21)	2.41 (0.59)	2.23 (0.92)
	3	40	2.55 (0.78)	2.03 (0.48)	2.43 (0.93)
	4	19	2.79 (0.54)	2.00 (0.67)	2.26 (0.93)
	5	16	2.69 (0.60)	2.19 (0.54)	2.50 (0.73)
	6	19	2.37 (0.83)	1.89 (0.66)	2.32 (0.95)
	7	7	2.57 (0.79)	2.00 (0.82)	2.57 (0.79)
Fin ray	1	34	2.94 (0.24)	2.71 (0.52)	2.26 (1.05)
	2	22	2.91 (0.29)	2.73 (0.46)	2.32 (0.99)
	3	40	2.65 (0.62)	2.73 (0.45)	2.25 (0.84)
	4	19	2.79 (0.42)	2.68 (0.48)	2.05 (0.97)
	5	16	2.38 (0.72)	2.56 (0.51)	1.63 (0.89)
	6	19	2.37 (0.90)	2.68 (0.58)	2.11 (0.88)
	7	7	2.29 (0.95)	2.71 (0.49)	2.00 (0.82)
Scale	1	34	2.94 (0.24)	2.53 (0.61)	2.79 (0.54)
	2	22	2.95 (0.21)	2.45 (0.67)	2.45 (0.67)
	3	40	2.58 (0.50)	2.68 (0.47)	2.50 (0.64)
	4	19	2.79 (0.42)	2.84 (0.37)	2.58 (0.51)
	5	15	2.25 (0.58)	2.69 (0.48)	2.53 (0.64)
	6	19	2.42 (0.51)	2.53 (0.51)	2.26 (0.73)
	7	7	2.14 (0.38)	2.29 (0.38)	1.71 (0.76)

TABLE A.2. Exact percent agreement (PA-0) and coefficient of variation (CV) for lentic Brook Trout by reader, structure, and age-class.

Structure	Age-class	*n*	Reader 1 PA-0	Reader 2 PA-0	Reader 3 PA-0	Reader 1 CV	Reader 2 CV	Reader 3 CV
Otolith	1	34	94	9	24	2.8	59.6	48.2
	2	22	73	45	27	12.0	21.2	31.0
	3	40	55	48	45	11.9	16.8	17.5
	4	19	63	42	42	6.5	13.4	11.1
	5	16	63	25	25	6.0	18.8	13.0
	6	19	47	32	5	7.1	24.4	23.9
	7	7	86	43	0	1.6	32.3	26.4
Fin ray	1	34	85	44	56	6.9	50.6	24.3
	2	22	73	41	45	10.3	38.5	20.1
	3	40	78	40	13	5.3	17.1	26.2
	4	19	26	37	37	16.4	17.6	15.9
	5	16	44	31	31	9.3	15.5	15.6
	6	19	32	21	53	10.9	22.1	8.8
	7	7	43	0	29	9.8	30.0	15.4
Scale	1	34	79	41	82	9.7	28.8	8.3
	2	22	59	32	36	16.7	25.3	28.3
	3	40	53	48	50	13.1	14.7	13.7
	4	19	26	37	37	19.2	13.4	16.8
	5	15	27	33	13	12.3	12.7	21.8
	6	19	16	5	16	21.7	23.3	24.0
	7	7	14	0	0	16.9	32.9	30.3